

Editors' Note

SPECIAL ISSUE HONORING PROFESSOR NINA BEROVA

We are happy to publish this special issue dedicated to Professor Nina Berova. It not only celebrates her receiving the 2007 Chirality Medal award but also appreciates and acknowledges four decades of dedication to the promotion of organic stereochemistry and chiroptical spectroscopy in the broadest sense. Without going into details, Nina started her academic career in Bulgaria before moving to Columbia University in New York some twenty years ago. Over the years, Nina has contributed significantly to advancement and broader application of organic stereochemistry and chiroptical, particularly circular dichroism (CD) spectroscopy from both practical and theoretical points of view. Highlights include ECCD methods development with emphasis on microscale protocols, CD reporter groups including elegant molecular tweezers, theoretical simulation and fluorescence detection. Nina's uncompromising, passionate commitment to absolutely outstanding service to the community is legendary—as editor, organizer, advisor, teacher and longstanding friend to many of us. Early on, she co-organized the First International Conference on CD in Sofia, the prototype of today's international symposia on chiroptical spectroscopy. Since then, Nina has played an active role in many occasions, acting for example also as co-organizer of the 5th CD conference (Colorado, 1993) and the 16th ISCD (New York, 2004). Nina has been co-editor of *Chirality* since 1999. She is also the co-editor and co-author of the by now classical book “Circular Dichroism: Interpretation and Applications” (Wiley). Many thanks for all that, Nina!

The overwhelming response to our invitations to contribute to this Special Issue illustrates the appreciation of Nina's leadership in the community better than all words of praise. We are delighted to present more than forty contributions from top-quality groups in more than twelve countries. The many topics covered reflect perfectly both Nina's broad interests and involvement and the scope of *Chirality*. Living up to the occasion, the special issue is centered around a rich and diverse collection on CD spectroscopy. Topics covered reach from computational predictions of CD spectra of the benzene chromophore reported by Pescitelli and coworkers to DNA binding studies by Corradini. CD spectroscopy of G-quartets, planar chromophores in dichromophoric molecules, diglycosyl dichalcogenides, distorted tetracyanoanthraquinonedimethanes and, in an excellent review, chiral molecular self-assemblies is covered in contributions from the groups of Gray, Botkus, Kurtan, Inoue, Spada and Gottarelli. Related methods covered include VCD described by the Monde and coworkers using phenyl glycosides as examples. Chiroptical luminescence is described by Hopkins, chiral NMR discrimination by Wenzel and optical rotation by Vaccaro, focusing on solvent and conformational effects with substituted epoxides.

The determination of absolute configuration, one of Nina's favorite topics, is addressed in contributions from the groups of Polavarapu, Kacprzak, and Stephens. Chiral discrimination is addressed by Gawronski in an elegant study on chiral derivatization agents for amino acids based on trans-1,2-diaminocyclohexane (another of Nina's favorite molecules). Enantiomeric resolution is illustrated by Tamura. An excellent collection of contributions on chromatographic enantioseparation includes new chitosan, crown ether, and fluoruous phases contributed by the groups of Okamoto, Hyun, and Mikami.

Asymmetric synthesis as another key topic is addressed, among others, by Wipf and coworkers, reporting on ligand optimization for transmetalation aldehyde addition reaction. The Lacour group elaborates on sweetened hexacoordinated phosphates as chiral auxiliaries, Duddeck on atropisomeric oxazolidinone ligands, Rosini on enantioselective sulfoxidations, and Proni on the synthesis of enantiopure fulleryl amino acids.

Last but not least, there is an exciting collection of contributions focusing on topics related to supramolecular chirality and chiral nanotechnology, one of the more recent topics of interest of Nina. Highlights include Aida's porphyrin dimers that can stereoselectively recognize chiral fullerenes and Yashima's helical poly(phenylacetylene)s chirality sensors, here decorated with chiral pyrene pendants. Saigo reports on chiral hydrogen-bonded networks, Purello on the chiral memory of porphyrin stacks, and Burgi and Huang on the chirality of golden and magnetic nanoparticles, respectively.

We hope you enjoy reading this firework on chirality and warmly thank all authors for their contributions which make this tribute to Nina such a great success.

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Synthesis of Enantiopure 2-Aryl-2-methoxypropionic Acids and Determination of Their Absolute Configurations by X-Ray Crystallography

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT Racemic 2-aryl-2-methoxypropionic acids were enantioresolved by the use of (S)-(-)-phenylalaninol **4**. For instance, racemic 2-methoxy-2-phenylpropionic acid ($\pm$)-**7** was condensed with phenylalaninol (S)-(-)-**4** yielding a diastereomeric mixture of amides, which was easily separated by HPLC on silica gel affording the first-eluted amide (-)-**13a** and the second-eluted amide (+)-**13b**: $\alpha = 3.19$, $R_s = 3.49$. The absolute configuration of amide (-)-**13a** was determined to be (R,S) by X-ray crystallography by reference to the S configuration of the phenylalaninol moiety. Amide (R,S)-(-)-**13a** was converted to oxazoline (R,S)-(-)-**14a**, from which enantiopure 2-methoxy-2-phenylpropionic acid (R)-(-)-**7** was recovered. Other 2-aryl-2-methoxypropionic acids, (R)-(-)-**8**, (R)-(-)-**9**, (R)-(+)-**10**, (R)-(-)-**11**, and (R)-(-)-**12**, were similarly prepared in enantiopure forms with the use of phenylalaninol (S)-(-)-**4**, and their absolute configurations were clearly determined by X-ray crystallography or by chemical correlation. *Chirality* 20:251–264, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: 2-methoxy-2-phenylpropionic acid; 2-aryl-2-methoxypropionic acids; phenylalaninol; diastereomeric amides; HPLC separation on silica gel; absolute configurations; X-ray crystallography; oxazolines

INTRODUCTION

The M α NP acid method^{1–7} is very powerful for the preparation of enantiopure alcohols and the simultaneous determination of their absolute configurations by the ¹H NMR anisotropy effect. For example, racemic alcohol ($\pm$)-**2** is esterified with M α NP acid, 2-methoxy-2-(1-naphthyl)propionic acid (R)-(-)-**1** (Fig. 1a), yielding diastereomeric M α NP esters, which are easily separable by HPLC on silica gel. The ¹H NMR signals of the first-eluted ester (R;X)-**3a** and those of the second-eluted ester (R;-X)-**3b** are assigned by ¹H-¹H COSY, ¹³C, HMQC, HMBC, and HSQC-TOCSY methods leading to the calculation of $\Delta\delta$ values [$\Delta\delta = \delta(R;X) - \delta(S;X) = \delta(R;X) - \delta(R;-X) = \delta(1st\ Fr.) - \delta(2nd\ Fr.)$], where R and S denote the absolute configurations of the M α NP acid parts, respectively, and X and -X are those of the alcoholic parts of the first-eluted ester and the second-eluted ester, respectively. By applying the M α NP sector rule,^{1–7} the absolute configuration X of the first-eluted ester **3a** can be determined. The solvolysis of the first-eluted ester (R;X)-**3a** yields enantiopure alcohol (X)-**2** together with the recovery of M α NP acid (R)-(-)-**1**. From the second-eluted ester (R;-X)-**3b**, enantiopure alcohol (-X)-**2** is obtained. M α NP acid **1** is thus useful for the preparation of enantiopure alcohols and the simultaneous determination of their absolute configura-

tions as a chiral ¹H NMR anisotropy reagent (CAR) with resolving power. It should be noted that when M α NP acid (S)-(+)-**1** is used, $\Delta\delta$ is defined as $\Delta\delta = \delta(R;X) - \delta(S;X) = \delta(2nd\ Fr.) - \delta(1st\ Fr.)$.^{1–7}

To obtain enantiopure alcohol **2**, it is necessary to use enantiopure M α NP acid **1**. Recently we reported a large-scale preparation of enantiopure M α NP acid **1** by the use of (S)-(-)-phenylalaninol **4**.⁸ In this, racemic M α NP acid ($\pm$)-**1** was condensed with amine (S)-(-)-**4** yielding diastereomeric amides **5a** and **5b** (Fig. 1b). It was surprising to find that these diastereomeric amides were effectively separable by HPLC on silica gel with a large separation factor $\alpha = 4.53$. In addition, these amides have the advantage of crystallizing as prisms, and the absolute configuration of the first-eluted amide (-)-**5a** was determined to be R by X-ray crystallography by reference to the S absolute

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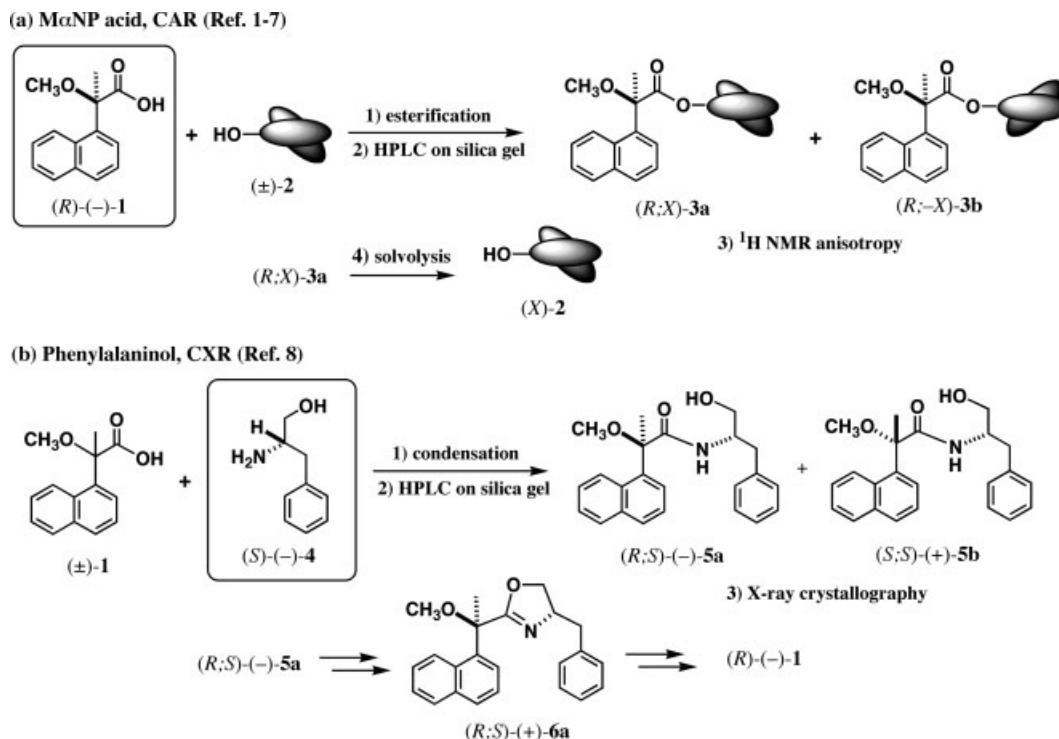


Fig. 1. (a) Enantioresolution of alcohols and determination of their absolute configurations by the use of M α NP acid, a chiral ^1H NMR anisotropy reagent (CAR) with resolving power. (b) Enantioresolution of M α NP acid and determination of its absolute configuration by X-ray crystallography by the use of (S)-(-)-phenylalaninol, a chiral X-ray reference reagent (CXR) with resolving power.

configuration of the phenylalaninol part. The first-eluted amide (R:S)-(-)-5a was converted to oxazoline (R:S)-(+)-6a, from which enantiopure M α NP acid (R)-(-)-1 was recovered.⁸

This methodology is generally applicable to the enantioresolution of other carboxylic acids with a quaternary stereogenic center and the determination of their absolute configurations by X-ray crystallography. We have recently succeeded in the preparation of enantiopure 2-aryl-2-methoxypropionic acids 7–12, the absolute configurations of which were unambiguously determined as shown in Figure 2 by X-ray crystallography and/or by chemical correlation. Phenylalaninol (S)-(-)-4 prepared from L-phenylalanine is thus very useful as a chiral X-ray reference reagent (CXR) with resolving power.^{8,9} We report here the results.

EXPERIMENTAL SECTION

General Procedures

Melting points are uncorrected. IR spectra were obtained as KBr disks or neat on a Jasco FT/IR-410 spectrophotometer. ^1H NMR spectra were recorded on a Jeol JNM-LA400 (400 MHz) or a Jeol JNM-LA600 (600 MHz) spectrometer. ^{13}C NMR spectra were obtained on a Jeol JNM-LA400 (100 MHz) or a Jeol JNM-LA600 (150 MHz) spectrometer. All NMR data are reported in ppm (δ) downfield from TMS. Specific rotations $[\alpha]_D$ were measured on a Jasco DIP-1000 spectropolarimeter. Silica gel 60 F₂₅₄ precoated plates on glass from Merck were used for thinlayer chromatography (TLC). HPLC separation was performed using prepacked glass columns (22 ϕ $\times$ 100 mm, or 22 ϕ $\times$ 300 mm) of silica

gel (particle size, 5–10 μm) from Kusano, Tokyo. The purities of the title compounds were shown to be $\geq 99\%$ by ^1H NMR, TLC, HPLC, and/or elemental analysis.

X-Ray Crystallography

A single crystal was selected for data collection and mounted on a Mac Science MXC18 automated four-circle diffractometer: radiation, Cu K α (1.54178 Å); monochromator, graphite crystal. The crystal system, space group, unit cell parameters, and orientation matrix were determined. Data collection was carried out using a 2 θ - θ scan: tempera-

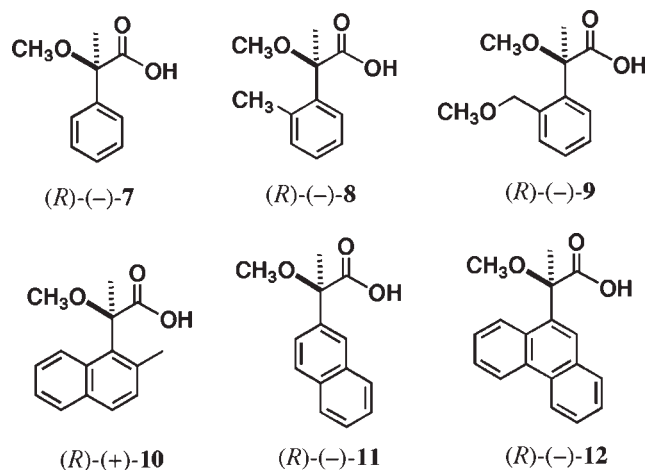


Fig. 2. Chiral 2-aryl-2-methoxypropionic acids.

TABLE 1. X-ray crystallographic data of amides prepared from 2-aryl-2-methoxypropionic acid and phenylalaninol (S)(-)-4

Compound	(-)- 13a ^a	(+)- 13b ^a	(+)- 15b ^a	(+)- 16b ^a	(-)- 17a ^a	(-)- 18a ^a
Formula	C ₁₉ H ₂₃ NO ₃	C ₁₉ H ₂₃ NO ₃	C ₂₀ H ₂₅ NO ₃	C ₂₁ H ₂₇ NO ₄	C ₂₄ H ₂₇ NO ₃	C ₂₃ H ₂₅ NO ₃
Formula weight (amu)	313.40	313.40	327.42	357.44	377.48	363.46
Solvent	EtOH	EtOAc	EtOAc	Et ₂ O	EtOH	EtOH
Crystal dimension (mm)	0.45 × 0.26 × 0.16	0.29 × 0.24 × 0.23	0.27 × 0.26 × 0.25	0.35 × 0.31 × 0.20	0.23 × 0.22 × 0.17	0.33 × 0.29 × 0.22
Crystal system	Monoclinic	Orthorhombic	Orthorhombic	Monoclinic	Monoclinic	Orthorhombic
Space group	<i>P</i> ₂ ₁ (#4)	<i>P</i> ₂ ₁ 2 ₁ 2 ₁ (#19)	<i>P</i> ₂ ₁ 2 ₁ 2 ₁ (#19)	<i>P</i> ₂ ₁ (#4)	<i>P</i> ₂ ₁ (#4)	<i>P</i> ₂ ₁ 2 ₁ 2 ₁ (#19)
<i>a</i> (Å)	12.203 (2)	12.177 (2)	12.330 (2)	12.915 (3)	8.912 (2)	11.137 (1)
<i>b</i> (Å)	11.953 (2)	15.746 (3)	15.459 (3)	6.795 (1)	15.607 (3)	17.332 (3)
<i>c</i> (Å)	6.137 (1)	9.127 (2)	9.615 (2)	11.614 (2)	7.519 (2)	10.380 (2)
β (°)	99.56 (1)	—	—	99.82 (2)	103.61 (2)	—
Vol (Å ³)	882.7 (3)	1750.0 (6)	1832.7 (6)	1004.2 (3)	1016.4 (4)	2003.5 (5)
<i>Z</i>	2	4	4	2	2	4
<i>D</i> _{calcd} (g/cm ³)	1.179	1.189	1.187	1.182	1.233	1.205
<i>D</i> _{obsd} (g/cm ³)	1.170	1.186	—	1.177	1.223	1.197
No. of independent reflections, <i>F</i> ₀ > 3.0σ (<i>F</i> ₀)	1699	1832	1912	1994	1929	2095
No. of variables	300	300	317	343	361	325
Goodness of fit <i>S</i>	1.053	1.112	1.092	1.068	1.051	1.066
Absolute configuration	<i>R,S</i>	<i>S,S</i>	<i>S,S</i>	<i>S,S</i>	<i>R,S</i>	<i>R,S</i>
<i>R</i> and <i>R</i> _w	0.0390 (0.0977)	0.0407 (0.0982)	0.0391 (0.0957)	0.0325 (0.0818)	0.0492 (0.1277)	0.0704 (0.1890)
Flack <i>χ</i>	0.1 (3)	-0.1 (3)	-0.7 (3)	0.1 (2)	0.0 (3)	1.0 (5)

^aThe crystallographic data of compounds were deposited with the Cambridge Crystallographic Data Center: Ref. Number CCDC 634324 for (-)-**13a**; 634327 for (+)-**13b**; 634328 for (+)-**15b**; 634329 for (+)-**16b**; 634325 for (-)-**17a**; 634326 for (-)-**18a**. The data can be obtained free of charge via www.ccdc.cam.ac.uk.

ture, 20°C; scan speed, 14°/min; scan range, 1.75–1.87° + 0.2° tan θ ; 2 θ scan limits, 2°–136°; standard reflections, 3 per 100 reflections; crystal stability, no indication of standard reflection decay during data collection. The density of crystals was measured by flotation, using CCl₄-hexane or aqueous KBr solution.

The structures were solved by the direct method and successive Fourier syntheses. Some hydrogen atoms were placed at the calculated positions. Full matrix least-squares refinement of positional and thermal parameters, including anomalous scattering factors of oxygen, nitrogen, and carbon atoms, led to the final convergence with *R* values listed in Table 1. The absolute configurations of chiral amides were determined using the phenylalaninol moiety as the internal reference of absolute configuration. The absolute configurations were also checked by the Flack parameter.

Synthesis of Diastereomeric Amides (R;S)-(–)-13a and (S;S)-(+)-13b from 2-Methoxy-2-phenylpropionic acids (±)-7 and Phenylalaninol (S)-(–)-4, and Their HPLC Separation

To a solution of racemic acid (±)-7 (100 mg, 0.55 mmol) in CH₂Cl₂ (1.0 ml), were added 1,3-dicyclohexylcarbodiimide (DCC, 137 mg, 0.67 mmol), 1-hydroxybenzotriazole (HOBt, 91 mg, 0.67 mmol), *N,N*-diisopropylethylamine (DIEA, 0.10 ml, 0.55 mmol), 10-camphorsulfonic acid (CSA, 13 mg, 0.055 mmol), and phenylalaninol (S)-(–)-4 (83 mg, 0.55 mmol), and the mixture was stirred at room temperature overnight. After addition of aqueous saturated NH₄Cl, the mixture was stirred for a few hours to quench the reaction. After removal of solid material by filtration, the filtrate was diluted with EtOAc. The organic layer was washed with saturated aq. NH₄Cl, saturated aq. NaHCO₃, water, and brine, successively. After being dried over anhydrous Na₂SO₄, the organic layer was evaporated under reduced pressure, and the residue obtained was subjected to a short column chromatography on silica gel (hexane/EtOAc 1:1) yielding a diastereomeric mixture of amides. The mixture was easily separated by HPLC on silica gel (hexane/EtOAc 1:1), as listed in Table 2, yielding the first-eluted amide (R;S)-(–)-13a and the second-eluted one (S;S)-(+)-13b.

Amide (R;S)-(–)-13a: yield 83 mg, 48%; colorless prisms from EtOH; $[\alpha]_D^{26}$ –68.1 (*c* 1.01, CHCl₃); IR (film) $\nu_{\max}$ 3400, 2931, 1653, 1520, 1447, 1149, 1069, 1049, 731,

699 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 1.67 (3H, s), 2.82 (1H, dd, *J* = 13.9, 7.9 Hz), 2.92 (1H, dd, *J* = 13.9, 6.8 Hz), 3.01 (1H, dd, *J* = 4.9, 4.7 Hz), 3.05 (3H, s), 3.50 (1H, ddd, *J* = 10.9, 4.9, 4.9 Hz), 3.58 (1H, ddd, *J* = 10.9, 4.7, 4.7 Hz), 4.07 (1H, m), 7.14 (1H, br. d, *J* = 7.6 Hz), 7.18–7.34 (8H, m), 7.38–7.41 (2H, m); ¹³C NMR (100 MHz, CDCl₃) δ 20.2, 36.9, 51.0, 52.9, 64.2, 81.8, 126.0, 126.5, 127.8, 128.3, 128.5, 129.2, 137.7, 140.7, 174.2; UV (EtOH), $\lambda_{\max}$ 258.6 nm (ϵ 400), 253.0 (300), 206.6sh (19,600); CD (EtOH), λ_{ext} 229.0 nm ($\Delta\epsilon$ +2.6), 216.0 (–0.5). Anal. Calcd for C₁₉H₂₃NO₃: C, 72.82; H, 7.40; N, 4.47. Found: C, 72.85; H, 7.48; N, 4.53.

Amide (S;S)-(+)-13b: yield 80 mg, 46%; colorless prisms from EtOAc; $[\alpha]_D^{26}$ +41.6 (*c* 1.15, CHCl₃); IR (film) $\nu_{\max}$ 3406, 2938, 1657, 1519, 1447, 1147, 1071, 732, 699 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 1.76 (3H, s), 2.76 (1H, dd, *J* = 13.9, 8.3 Hz), 2.90 (1H, dd, *J* = 13.9, 6.9 Hz), 3.10 (3H, s), 3.59 (1H, dd, *J* = 11.1, 5.6 Hz), 3.69 (1H, dd, *J* = 11.1, 3.8 Hz), 4.11 (1H, m), 7.12–7.28 (10H, m); ¹³C NMR (100 MHz, CDCl₃) δ 20.0, 36.8, 51.0, 52.8, 64.9, 81.7, 126.1, 126.5, 127.7, 128.9, 128.5, 129.2, 137.5, 140.2, 174.3; UV (EtOH), $\lambda_{\max}$ 258.4 nm (ϵ 400), 252.6 (300), 206.8sh (18,500); CD (EtOH), λ_{ext} 233.4 nm ($\Delta\epsilon$ –1.7), 221.0 (–3.0). Anal. Calcd for C₁₉H₂₃NO₃: C, 72.82; H, 7.40; N, 4.47. Found: C, 72.62; H, 7.44; N, 4.46.

Preparation and HPLC Separation of Diastereomeric Amides (R;S)-(–)-15a and (S;S)-(+)-15b

In a similar way, racemic acid (±)-8 (*R* = 2-tolyl, 800 mg, 4.12 mmol) was condensed with phenylalaninol (S)-(–)-4 (622 mg, 4.12 mmol) giving a diastereomeric mixture of amides. The mixture was easily separated by HPLC on silica gel (hexane/EtOAc 1:1) as shown in Table 2, yielding the first-eluted amide (R;S)-(–)-15a and the second-eluted one (S;S)-(+)-15b.

Amide (R;S)-(–)-15a (*R* = 2-tolyl): yield 584 mg, 43%; colorless amorphous solid; $[\alpha]_D^{23}$ –67.7 (*c* 1.09, CHCl₃); IR (film) $\nu_{\max}$ 3407, 2934, 1655, 1519, 1066, 738 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 1.63 (3H, s), 2.31 (3H, s), 2.82 (1H, dd, *J* = 13.7, 7.8 Hz), 2.97 (3H, s), 2.98 (1H, dd, *J* = 13.7, 7.9 Hz), 3.61 (1H, ddd, *J* = 10.8, 5.4, 5.4 Hz), 3.71 (1H, ddd, *J* = 10.8, 5.4, 3.7 Hz), 4.21 (1H, m), 7.10–7.35 (10H, m); ¹³C NMR (100 MHz, CDCl₃) δ 20.4, 21.9, 37.1, 50.3, 53.0, 64.8, 82.2, 125.6, 126.6, 128.3, 128.45, 128.54, 129.2, 131.8, 136.7, 137.4, 137.5, 175.1.

Amide (S;S)-(+)-15b (*R* = 2-tolyl): yield 618 mg, 46%; colorless prisms from EtOAc; $[\alpha]_D^{23}$ +64.6 (*c* 1.03, CHCl₃); IR (film) $\nu_{\max}$ 3407, 2936, 1655, 1517, 1066, 738 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 1.78 (3H, s), 1.97 (3H, s), 2.83 (1H, dd, *J* = 13.7, 8.6 Hz), 2.96 (1H, dd, *J* = 13.7, 6.5 Hz), 2.97 (3H, s), 3.29 (1H, dd, *J* = 5.6, 5.6 Hz), 3.63 (1H, ddd, *J* = 11.0, 5.6, 5.6 Hz), 3.73 (1H, ddd, *J* = 11.0, 5.6, 3.4 Hz), 4.13 (1H, m), 7.04–7.06 (1H, m), 7.15–7.36 (9H, m); ¹³C NMR (100 MHz, CDCl₃) δ 20.0, 22.3, 36.8, 50.3, 53.7, 65.4, 82.2, 125.5, 126.7, 128.3, 128.5, 128.6, 129.2, 131.8, 136.4, 137.4, 137.6, 175.6.

Preparation of Diastereomeric Amides (R;S)-(–)-16a and (S;S)-(+)-16b

Racemic acid (±)-9 (*R* = 2-methoxymethylphenyl, 800 mg, 3.57 mmol) was similarly condensed with phenylalani-

TABLE 2. HPLC separation of diastereomeric amides formed from phenylalaninol (S)-(–)-4

Entry	Diastereomers	Solvent ^a	α	<i>R</i> _s	1st-eluted Fr.
1 ^b	13a/13b	H/EA = 1/1	3.19	3.49	(R;S)-(–)-13a
2 ^b	15a/15b	H/EA = 1/1	2.53	2.86	(R;S)-(–)-15a
3 ^c	16a/16b	H/EA = 1/2	1.57	2.75	(R;S)-(–)-16a
4 ^c	17a/17b	H/EA = 1/1	2.43	7.18	(R;S)-(–)-17a
5 ^b	18a/18b	H/EA = 1/1	3.52	3.92	(R;S)-(–)-18a
6 ^b	19a/19b	H/EA = 1/1	2.80	2.77	(R;S)-(–)-19a

^aH, hexane; EA, ethyl acetate.

^bGlass column (22 ϕ × 100 mm) of silica gel (particle size 5–10 μ m).

^cGlass column (22 ϕ × 300 mm) of silica gel (particle size 5–10 μ m).

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nol (S)-(-)-**4** (539 mg, 3.57 mmol) yielding the first-eluted amide (R;S)-(-)-**16a** and the second-eluted one (S;S)-(+)-**16b**.

Amide (R;S)-(-)-16a (R = 2-methoxymethylphenyl): yield 623 mg, 49%; colorless amorphous solid; $[\alpha]_D^{22}$ -68.2 (*c* 1.16, CHCl₃); IR (film) $\nu_{\max}$ 3409, 2983, 2934, 2829, 1660, 1517, 1454, 1194, 1145, 1095, 1065, 747, 702 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.63 (3H, s), 2.81 (1H, dd, *J* = 13.8, 7.9 Hz), 2.93 (1H, dd, *J* = 13.8, 7.0 Hz), 2.97 (3H, s), 3.24 (3H, s), 3.48–3.53 (2H, m), 3.68–3.74 (1H, m), 4.17 (1H, m), 4.37 (1H, d, *J* = 12.7 Hz), 4.84 (1H, d, *J* = 12.7 Hz), 6.98 (1H, br. d, *J* = 7.8 Hz), 7.20–7.33 (7H, m), 7.37–7.43 (2H, m); ¹³C NMR (100 MHz, CDCl₃) δ 22.3, 37.2, 50.4, 52.5, 57.4, 63.6, 71.9, 82.2, 126.5, 127.6, 128.1, 128.4, 128.8, 129.2, 130.1, 136.3, 137.0, 137.7, 174.2.

Amide (S;S)-(+)-16b (R = 2-methoxymethylphenyl): yield 618 mg, 48%; colorless prisms from Et₂O; $[\alpha]_D^{22}$ +27.0 (*c* 1.210, CHCl₃); IR (film) $\nu_{\max}$ 3408, 2983, 2933, 2829, 1657, 1516, 1453, 1145, 1096, 1065, 746, 702 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.75 (3H, s), 2.87 (1H, dd, *J* = 13.8, 7.9 Hz), 2.91 (1H, dd, *J* = 13.8, 7.2 Hz), 3.01 (3H, s), 3.24 (3H, s), 3.50 (1H, m), 3.57–3.68 (2H, m), 4.05 (1H, m), 4.33 (1H, d, *J* = 13.2 Hz), 4.40 (1H, d, *J* = 13.2 Hz), 6.99 (1H, br. d, *J* = 7.1 Hz), 7.19–7.37 (8H, m), 7.49 (1H, d, *J* = 7.3 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 22.4, 36.6, 50.5, 53.7, 58.0, 64.1, 71.4, 82.2, 126.6, 127.2, 128.40, 128.44, 128.6, 128.9, 129.1, 136.3, 137.3, 137.7, 174.8.

Preparation of Diastereomeric Amides

(R;S)-(-)-17a and (S;S)-(-)-17b

Racemic acid (±)-**10** (R = 2-methyl-1-naphthyl, 800 mg, 3.27 mmol) was condensed with phenylalaninol (S)-(-)-**4** (494 mg, 3.27 mmol) giving the first-eluted amide (R;S)-(-)-**17a** and the second-eluted one (S;S)-(-)-**17b**.

Amide (R;S)-(-)-17a (R = 2-methyl-1-naphthyl): yield 470 mg, 38%; colorless prisms from EtOH; $[\alpha]_D^{24}$ -13.4 (*c* 1.08, CHCl₃); IR (film) $\nu_{\max}$ 3407, 3028, 2935, 2830, 1660, 1510, 1455, 1137, 1084, 1054, 908, 812, 735, 701 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.76 (1H, dd, *J* = 5.9, 5.9 Hz), 1.88 (3H, s), 2.64 (3H, s), 2.66 (1H, dd, *J* = 13.9, 8.2 Hz), 2.82 (1H, dd, *J* = 13.9, 6.8 Hz), 2.99 (3H, s), 3.38 (1H, ddd, *J* = 11.0, 5.9, 5.9 Hz), 3.53 (1H, ddd, *J* = 11.0, 5.9, 3.6 Hz), 4.16 (1H, m), 6.16 (1H, br. d, *J* = 7.7 Hz), 7.11–7.13 (2H, m), 7.17–7.28 (4H, m), 7.34–7.40 (2H, m), 7.65 (1H, d, *J* = 8.3 Hz), 7.73–7.77 (1H, m), 8.24–8.27 (1H, m); ¹³C NMR (100 MHz, CDCl₃) δ 21.8, 24.5, 37.0, 50.1, 52.9, 63.8, 84.5, 124.5, 124.7, 125.5, 126.5, 128.4, 128.5, 128.7, 129.1, 131.78, 131.83, 132.7, 134.3, 136.5, 137.6, 175.1; UV (EtOH), $\lambda_{\max}$ 284.6 nm (ϵ 6,600), 228.6(68,500); CD (EtOH), λ_{ext} 238.0 nm ($\Delta\epsilon$ +3.5), 222.2 (-6.5), 206.8 (+5.4).

Amide (S;S)-(-)-17b (R = 2-methyl-1-naphthyl): yield 600 mg, 49%; colorless amorphous solid; $[\alpha]_D^{26}$ -5.6 (*c* 1.05, CHCl₃); IR (film) $\nu_{\max}$ 3407, 3028, 2935, 2830, 2246, 1660, 1511, 1455, 1137, 1085, 1055, 909, 812, 735, 702 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.95 (3H, s), 2.49 (3H, s), 2.63 (1H, dd, *J* = 13.8, 7.2 Hz), 2.69 (1H, dd, *J* = 13.8, 6.7 Hz), 2.93 (1H, dd, *J* = 5.2, 5.2 Hz), 3.18 (3H, s), 3.49 (1H, ddd, *J* = 10.7, 5.2, 5.2 Hz), 3.58 (1H, ddd, *J* = 10.7, 5.2, 5.2 Hz), 4.12 (1H, m), 6.34 (1H, d, *J* = 7.6 Hz),

6.89 (1H, m), 7.13–7.18 (1H, m), 7.28 (1H, ddd, *J* = 8.3, 6.8, 1.4 Hz), 7.34 (1H, ddd, *J* = 8.0, 6.8, 1.6 Hz), 7.63 (1H, d, *J* = 8.3 Hz), 7.73 (1H, dd, *J* = 7.8, 1.2 Hz), 8.21 (1H, d, *J* = 8.8 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 22.4, 24.1, 36.3, 50.3, 53.3, 64.7, 84.6, 124.5, 125.3, 125.5, 126.6, 128.4, 128.51, 128.54, 129.0, 131.5, 132.0, 132.9, 133.9, 136.2, 137.2, 175.6; UV (EtOH), $\lambda_{\max}$ 285.0 nm (ϵ 6,100), 228.8(68,300); CD (EtOH), λ_{ext} 237.0 nm ($\Delta\epsilon$ -4.4), 224.4 (+15.1).

Preparation of Diastereomeric Amides

(R;S)-(-)-18a and (S;S)-(+)-18b

Racemic acid (±)-**11** (R = 2-naphthyl, 800 mg, 3.48 mmol) was condensed with phenylalaninol (S)-(-)-**4** (525 mg, 3.48 mmol) giving the first-eluted amide (R;S)-(-)-**18a** and the second-eluted one (S;S)-(+)-**18b**.

Amide (R;S)-(-)-18a (R = 2-naphthyl): yield 606 mg, 48%; colorless prisms from EtOH; $[\alpha]_D^{24}$ -95.2 (*c* 1.07, CHCl₃); IR (film) $\nu_{\max}$ 3405, 2937, 1656, 1518, 1147, 1131, 1066, 1047, 747, 701 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.78 (3H, s), 2.75 (1H, dd, *J* = 5.5, 5.5 Hz), 2.83 (1H, dd, *J* = 13.7, 8.1 Hz), 2.92 (1H, dd, *J* = 13.7, 6.6 Hz), 3.09 (3H, s), 3.54 (1H, ddd, *J* = 10.1, 5.5, 5.5 Hz), 3.63 (1H, ddd, *J* = 10.1, 5.5, 3.7 Hz), 4.09 (1H, m), 7.17–7.30 (1H, m), 7.45–7.51 (3H, m), 7.79–7.85 (3H, m), 7.88 (1H, d, *J* = 1.0 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 20.4, 36.9, 51.2, 53.2, 64.5, 82.0, 123.8, 125.4, 126.1, 126.2, 126.6, 127.5, 128.2, 128.3, 128.5, 129.2, 132.9, 133.0, 137.7, 138.2, 174.2.

Amide (S;S)-(+)-18b (R = 2-naphthyl): yield 660 mg, 50%; colorless amorphous solid; $[\alpha]_D^{24}$ +56.8 (*c* 0.98, CHCl₃); IR (film) $\nu_{\max}$ 3405, 2936, 1656, 1518, 1130, 1045, 749, 701 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.87 (3H, s), 2.77 (1H, dd, *J* = 13.9, 8.3 Hz), 2.86 (1H, dd, *J* = 5.0, 5.0 Hz), 2.91 (1H, dd, *J* = 13.9, 6.3 Hz), 3.15 (3H, s), 3.63 (1H, ddd, *J* = 10.7, 5.0, 5.0 Hz), 3.73 (1H, ddd, *J* = 10.7, 5.0, 5.0 Hz), 4.12 (1H, m), 7.10–7.21 (7H, m), 7.46–7.49 (2H, m), 7.70 (1H, d, *J* = 8.5 Hz), 7.78–7.81 (3H, m); ¹³C NMR (100 MHz, CDCl₃) δ 20.2, 36.9, 51.1, 52.9, 65.1, 81.9, 123.7, 125.5, 126.1, 126.2, 126.6, 127.4, 128.2, 128.3, 128.6, 129.1, 132.9, 133.0, 137.4, 137.7, 174.3.

Preparation of Diastereomeric Amides

(R;S)-(-)-19a and (S;S)-(+)-19b

Racemic acid (±)-**12** (R = 9-phenanthryl, 100 mg, 0.36 mmol) was condensed with phenylalaninol (S)-(-)-**4** (54 mg, 0.36 mmol) giving the first-eluted amide (R;S)-(-)-**19a** and the second-eluted one (S;S)-(+)-**19b**.

Amide (R;S)-(-)-19a (R = 9-phenanthryl): yield 69 mg, 47%; colorless amorphous solid; $[\alpha]_D^{26}$ -108.3 (*c* 1.05, CHCl₃); IR (film) $\nu_{\max}$ 3394, 2931, 1652, 1518, 1453, 1142, 1088, 910 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.87 (3H, s), 2.43 (1H, dd, *J* = 5.6, 5.6 Hz), 2.87 (1H, dd, *J* = 13.9, 7.8 Hz), 3.00 (1H, dd, *J* = 13.9, 7.6 Hz), 3.64 (1H, ddd, *J* = 11.1, 5.6, 5.6 Hz), 3.75 (1H, ddd, *J* = 11.1, 5.6, 3.7 Hz), 4.24 (1H, m), 7.21–7.32 (5H, m), 7.53 (1H, ddd, *J* = 8.3, 7.1, 1.2 Hz), 7.57–7.68 (3H, m), 7.86 (1H, s), 7.88 (1H, dd, *J* = 8.0, 1.5 Hz), 8.41 (1H, dd, *J* = 8.4, 0.9 Hz), 8.64 (1H, d, *J* = 8.3 Hz), 8.72 (1H, dd, *J* = 8.3, 0.7 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 22.3, 37.1, 50.7, 52.8, 64.5, 82.4, 122.4, 123.2, 125.6, 126.4, 126.5, 126.6, 126.7, 127.3, 128.6, 128.8, 129.1,

129.2, 130.1, 130.8, 131.0, 132.9, 137.5, 175.0; UV (EtOH), λ_{max} 259.4 nm (ϵ 11,100), 283.4 (10,400), 275.4 (13,300), 253.4 (63,700), 246.8 (49,100), 222.2 (25,200), 210.6 (38,800); CD (EtOH), λ_{ext} 259.8 nm ($\Delta\epsilon$ +2.7), 252.0 (+3.3), 226.4 (+1.6), 215.8 (−19.1). Anal. Calcd for $\text{C}_{27}\text{H}_{27}\text{NO}_3$: C, 78.42; H, 6.58; N, 3.39. Found: C, 78.21; H, 6.67; N, 3.35.

Amide (S,S)-(+)-19b (R = 9-phenanthryl): yield 73 mg, 50%; colorless amorphous solid; $[\alpha]_{\text{D}}^{25}$ +88.2 (c 1.02, CHCl_3); IR (film) ν_{max} 3403, 2934, 1660, 1516, 1142, 1090, 1055, 909, 729 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 2.01 (3H, s), 2.87 (1H, dd, J = 13.8, 8.2 Hz), 2.97 (1H, dd, J = 13.8, 6.7 Hz), 3.05 (3H, s), 3.65 (1H, dd, J = 11.1, 5.7 Hz), 3.73 (1H, dd, J = 11.1, 3.3 Hz), 4.15 (1H, m), 7.21–7.35 (6H, m), 7.56–7.61 (2H, m), 7.66 (1H, ddd, J = 8.2, 7.0, 1.3 Hz), 7.87 (1H, s), 7.87–7.90 (1H, m), 8.09 (1H, dd, J = 8.4, 0.7 Hz), 8.63 (1H, d, J = 8.3 Hz), 8.69 (1H, br. d, J = 8.3 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 22.7, 36.9, 50.8, 53.6, 65.1, 82.4, 122.4, 123.1, 125.4, 126.3, 126.6, 126.70, 126.74, 127.3, 128.7, 128.9, 129.1, 129.2, 130.0, 130.7, 130.8, 131.0, 132.7, 137.6, 175.6; UV (EtOH), λ_{max} 295.6 nm (ϵ 10,800), 283.6 (9,700), 275.6 (12,400), 253.6 (60,000), 246.8 (46,100), 222.8 (22,800), 211.2 (36,700); CD (EtOH), λ_{ext} 254.2 nm ($\Delta\epsilon$ −3.9), 215.2 (+31.4). Anal. Calcd for $\text{C}_{27}\text{H}_{27}\text{NO}_3$: C, 78.42; H, 6.58; N, 3.39. Found: C, 77.76; H, 6.71; N, 3.35.

Conversion of Amide (R,S)-(-)-13a to Oxazoline (R,S)-(-)-14a

To a mixture of amide (R,S)-(-)-13a (420 mg, 1.28 mmol) and toluene (5 ml), thionyl chloride (0.92 ml, 12.8 mmol) was added dropwise under stirring at room temperature, and the reaction mixture was stirred at 70°C for 2 h. After addition of saturated aq. NaHCO_3 under ice-cooling, the mixture was extracted with EtOAc several times. The combined organic layers were dried over anhydrous MgSO_4 , and evaporated under reduced pressure, yielding a crude product, to which a solution of 14% NaOMe/MeOH (10 ml) was added. The mixture was stirred at 70°C for 2 h, diluted with water, and extracted with CH_2Cl_2 . The organic layer was dried over anhydrous MgSO_4 and evaporated under reduced pressure. The residue was subjected to a short column chromatography on silica gel (hexane/EtOAc 4:1) and then to HPLC on silica gel (hexane/EtOAc 3:1), yielding oxazoline (R,S)-(-)-14a (382 mg, 97%): colorless syrup; $[\alpha]_{\text{D}}^{25}$ −0.8 (c 5.81, CHCl_3); IR (neat) ν_{max} 3061, 3027, 2988, 2935, 2830, 1655, 1495, 1449, 1370, 1239, 1193, 1131, 1101, 1074, 1049, 971, 767, 700 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.79 (3H, s), 2.75 (1H, dd, J = 13.8, 8.6 Hz), 3.17 (1H, dd, J = 13.8, 4.6 Hz), 3.20 (3H, s), 4.02 (1H, dd, J = 8.8, 7.6 Hz), 4.17 (1H, dd, J = 8.8, 8.8 Hz), 4.48 (1H, dddd, J = 8.8, 8.6, 7.6, 4.6 Hz), 7.19–7.36 (8H, m), 7.44–7.47 (2H, m); ^{13}C NMR (100 MHz, CDCl_3) δ 23.5, 41.3, 51.5, 67.2, 71.9, 77.9, 125.6, 126.5, 127.6, 128.2, 128.5, 129.5, 137.4, 141.8, 168.6; UV (EtOH), λ_{max} 257.8 nm (ϵ 400), 252.2 (300), 205.6 (18,600); CD (EtOH), λ_{ext} 205.8 nm ($\Delta\epsilon$ −8.7).

Conversion of Amide (R,S)-(-)-15a to Oxazoline (R,S)-(-)-20a (R = 2-Tolyl)

In a similar way, amide (R,S)-(-)-15a (500 mg, 1.52 mmol) was converted to the corresponding oxazoline

(R,S)-(-)-20a (374 mg, 79%): colorless syrup; $[\alpha]_{\text{D}}^{25}$ −9.5 (c 1.11, CHCl_3); IR (neat) ν_{max} 2936, 1655, 1455, 1092, 970, 702 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.82 (3H, s), 2.38 (3H, s), 2.67 (1H, dd, J = 13.8, 9.3 Hz), 3.16 (3H, s), 3.23 (1H, dd, J = 13.8, 4.4 Hz), 4.02 (1H, dd, J = 8.8, 7.8 Hz), 4.20 (1H, dd, J = 8.8, 8.8 Hz), 4.48 (1H, dddd, J = 9.3, 8.8, 7.8, 4.4 Hz), 7.14–7.31 (8H, m), 7.45–7.48 (1H, m); ^{13}C NMR (100 MHz, CDCl_3) δ 20.5, 22.9, 41.5, 50.6, 67.5, 72.1, 78.1, 125.7, 126.5, 126.7, 127.9, 128.6, 129.3, 131.9, 136.5, 137.7, 138.6, 168.8.

Conversion of Amide (R,S)-(-)-16a to Oxazoline (R,S)-(-)-21a (R = 2-Methoxymethylphenyl)

Amide (R,S)-(-)-16a (397 mg, 1.11 mmol) was similarly converted to oxazoline (R,S)-(-)-21a (377 mg, quantitative): colorless syrup; $[\alpha]_{\text{D}}^{25}$ −2.6 (c 0.98, CHCl_3); IR (neat) ν_{max} 2930, 1655, 1453, 1191, 1093, 969, 703 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.85 (3H, s), 2.66 (1H, dd, J = 13.7, 9.3 Hz), 3.15 (3H, s), 3.24 (1H, dd, J = 13.7, 4.4 Hz), 3.41 (3H, s), 4.02 (1H, dd, J = 8.9, 7.4 Hz), 4.20 (1H, dd, J = 8.9, 8.9 Hz), 4.48 (1H, dddd, J = 9.3, 8.9, 7.4, 4.4 Hz), 4.65 (1H, d, J = 12.9 Hz), 4.68 (1H, d, J = 12.9 Hz), 7.18–7.36 (7H, m), 7.42 (1H, dd, J = 7.8, 1.4 Hz), 7.58 (1H, dd, J = 7.6, 1.0 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 23.2, 41.4, 50.7, 58.4, 67.6, 71.5, 72.2, 78.4, 126.5, 126.7, 127.2, 128.2, 128.6, 128.9, 129.3, 137.3, 137.7, 137.9, 168.8.

Conversion of Amide (R,S)-(-)-17a to Oxazoline (R,S)-(+)-22a (R = 2-Methyl-1-naphthyl)

Amide (R,S)-(-)-17a (401 mg, 1.06 mmol) was converted to oxazoline (R,S)-(+)-22a (340 mg, 99%): colorless syrup; $[\alpha]_{\text{D}}^{22}$ +38.2 (c 1.18, CHCl_3); IR (neat) ν_{max} 3050, 3028, 2991, 2936, 2829, 1653, 1509, 1496, 1454, 1370, 1245, 1207, 1183, 1130, 1111, 1090, 1055, 1039, 969, 929, 910, 812, 784, 733, 702 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 2.09 (3H, s), 2.59 (3H, s), 2.73 (1H, dd, J = 13.7, 8.8 Hz), 3.25 (1H, dd, J = 13.7, 4.9 Hz), 3.26 (3H, s), 3.97 (1H, dd, J = 8.7, 7.8 Hz), 4.10 (1H, dd, J = 8.7, 8.7 Hz), 4.53 (1H, dddd, J = 8.8, 8.7, 7.8, 4.9 Hz), 7.19–7.38 (8H, m), 7.62 (1H, d, J = 8.5 Hz), 7.70–7.75 (1H, m), 8.39–8.42 (1H, m); ^{13}C NMR (100 MHz, CDCl_3) δ 23.3, 41.7, 50.9, 67.3, 72.0, 77.2, 79.8, 124.2, 124.7, 125.2, 126.5, 128.0, 128.5, 129.2, 131.2, 131.5, 132.7, 134.2, 134.4, 137.7, 168.6; UV (EtOH), λ_{max} 284.0 nm (ϵ 6,100), 228.4 (73,100); CD (EtOH), λ_{ext} 228.6 nm ($\Delta\epsilon$ +8.4), 208.8 (−11.5).

Conversion of Amide (R,S)-(-)-18a to Oxazoline (R,S)-(+)-23a (R = 2-Naphthyl)

Amide (R,S)-(-)-18a (400 mg, 1.10 mmol) was converted to oxazoline (R,S)-(+)-23a (343 mg, 90%): colorless syrup; $[\alpha]_{\text{D}}^{22}$ +5.3 (c 1.07, CHCl_3); IR (neat) ν_{max} 2934, 1655, 1242, 1182, 1008, 971, 820, 749, 702 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.89 (3H, s), 2.76 (1H, dd, J = 13.7, 8.5 Hz), 3.18 (1H, dd, J = 13.7, 4.6 Hz), 3.25 (3H, s), 4.02 (1H, dd, J = 8.8, 7.8 Hz), 4.16 (1H, dd, J = 9.5, 8.8 Hz), 4.50 (1H, dddd, J = 9.5, 8.5, 7.8, 4.6 Hz), 7.19–7.30 (5H, m), 7.44–7.49 (2H, m), 7.57 (1H, dd, J = 8.8, 2.0 Hz), 7.80–7.85 (3H, m), 7.95 (1H, d, J = 1.4 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 23.3, 41.3, 51.6, 67.2, 71.9, 77.2, 78.0,

123.8, 124.6, 126.03, 126.08, 126.5, 127.5, 128.0, 128.3, 128.5, 129.5, 132.8, 133.0, 137.4, 139.2, 168.6.

Conversion of Amide (R;S)-(-)-19a to Oxazoline (R;S)-(-)-24a (R = 9-Phenanthryl)

Amide (R;S)-(-)-**19a** (100 mg, 0.24 mmol) was converted to oxazoline (R;S)-(-)-**24a** (85 mg, 89%): colorless amorphous solid; $[\alpha]_D^{25}$ -28.3 (c 1.21, CHCl₃); IR (film) $\nu_{\max}$ 2939, 1655, 1453, 1119, 967, 750 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 2.09 (3H, s), 2.59 (1H, dd, *J* = 13.7, 9.3 Hz), 3.20 (3H, s), 3.23 (1H, dd, *J* = 13.7, 4.6 Hz), 3.90 (1H, dd, *J* = 8.7, 7.1 Hz), 4.11 (1H, dd, *J* = 8.7, 8.7 Hz), 4.47 (1H, m), 7.15–7.28 (5H, m), 7.55–7.66 (4H, m), 7.89 (1H, dd, *J* = 7.9, 1.1 Hz), 7.96 (1H, s), 8.65 (1H, d, *J* = 8.3 Hz), 8.69 (1H, dd, *J* = 8.3, 0.7 Hz), 8.73 (1H, dd, *J* = 8.3, 1.0 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 23.1, 41.4, 50.7, 67.6, 72.3, 78.1, 122.3, 123.0, 126.1, 126.3, 126.4, 126.7, 126.8, 127.2, 128.5, 129.2, 130.0, 130.6, 130.8, 131.0, 134.0, 137.8, 169.6; UV (EtOH), $\lambda_{\max}$ 296.2 nm (ϵ 10,300), 284.2(9,100), 275.8(11,500), 253.8(56,100), 247.2(43,600), 223.0(22,900), 211.4(35,600); CD (EtOH), λ_{ext} 262.6 nm ($\Delta\epsilon$ +3.5), 252.8 (+3.4), 244.8 (+1.9), 213.2 (-23.5). Anal. Calcd for C₂₇H₂₅NO₂: C, 82.00; H, 6.37; N, 3.54. Found: C, 81.67; H, 6.62; N, 3.50.

Conversion of Amide (S;S)-(+)-19b to Oxazoline (S;S)-(+)-24b (R = 9-Phenanthryl)

Amide (S;S)-(+)-**19b** (500 mg, 1.21 mmol) was converted to oxazoline (S;S)-(+)-**24b** (430 mg, 90%): colorless amorphous solid; $[\alpha]_D^{25}$ +7.6 (c 1.32, CHCl₃); IR (film) $\nu_{\max}$ 2936, 2829, 1657, 1496, 1453, 1251, 1153, 1141, 1119, 1052, 967, 910, 749, 729, 701 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 2.07 (3H, s), 2.66 (1H, dd, *J* = 13.7, 8.8 Hz), 3.16 (1H, dd, *J* = 13.7, 4.6 Hz), 3.23 (3H, s), 3.96 (1H, dd, *J* = 8.9, 7.3 Hz), 4.08 (1H, dd, *J* = 8.9, 8.9 Hz), 4.48 (1H, dddd, *J* = 8.9, 8.8, 7.3, 4.6 Hz), 7.10–7.12 (2H, m), 7.16–7.26 (3H, m), 7.56–7.67 (4H, m), 7.89 (1H, dd, *J* = 7.8, 1.5 Hz), 7.95 (1H, s), 8.64–8.67 (2H, m), 8.73–8.75 (1H, m); ¹³C NMR (100 MHz, CDCl₃) δ 22.9, 41.2, 50.8, 67.3, 72.3, 78.1, 122.3, 123.0, 126.1, 126.3, 126.5, 126.7, 126.8, 127.2, 128.5, 129.25, 129.29, 130.0, 130.6, 130.9, 131.0, 134.2, 137.6, 169.6; UV (EtOH), $\lambda_{\max}$ 295.8 nm (ϵ 9,700), 284.0 (8,600), 275.6(11,300), 253.6(52,500), 247.0(40,800), 221.8(21,700), 211.0(33,300); CD (EtOH), λ_{ext} 295.6 nm ($\Delta\epsilon$ +0.5), 261.4 (-3.2), 225.6 (-3.3), 213.4 (+12.4).

Recovery of 2-Methoxy-2-phenylpropionic Acid (R)-(-)-7 from Oxazoline (R;S)-(-)-14a

A mixture of oxazoline (R;S)-(-)-**14a** (300 mg, 1.02 mmol) and iodomethane (5 ml) was refluxed overnight, and excess iodomethane was removed by distillation. To the residue, MeOH (4 ml), water (1 ml), and NaOH (406 mg, 8.10 mmol) were added, and the mixture was refluxed under stirring overnight, and then evaporated under reduced pressure. The residue was partitioned with diethyl ether, and the aqueous layer was acidified with aqueous 6 M HCl under ice-cooling. The precipitate formed was collected by filtration and dried under reduced pressure, giving acid (R)-(-)-**7**. The aqueous filtrate was

extracted with EtOAc, and the organic layer was dried over anhydrous MgSO₄ and evaporated under reduced pressure yielding another crop of acid (R)-(-)-**7**: yield, 138 mg, 75%; colorless syrup; $[\alpha]_D^{25}$ -27.8 (c 1.29, CHCl₃); IR (film) $\nu_{\max}$ 2989, 1718, 1448, 1143, 1074, 698 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.85 (3H, s), 3.28 (3H, s), 7.17–7.29 (3H, m), 7.42 (2H, m); ¹³C NMR (100 MHz, CDCl₃) δ 20.6, 51.7, 81.3, 126.2, 128.5, 128.6, 138.8, 175.7.

Recovery of 2-Methoxy-2-(2-tolyl)propionic Acid (R)-(-)-8 from Oxazoline (R;S)-(-)-20a

In a similar way, oxazoline (R;S)-(-)-**20a** (300 mg, 0.97 mmol) was converted to acid (R)-(-)-**8** (156 mg, 83%): colorless solid; $[\alpha]_D^{23}$ -90.0 (c 1.08, CHCl₃); IR (film) $\nu_{\max}$ 3164, 2942, 2837, 1715, 1602, 1492, 1463, 1438, 1252, 1125, 1101, 1075, 1047, 1025, 758 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.88 (3H, s), 2.34 (3H, s), 3.09 (3H, s), 7.31–7.41 (3H, m), 7.48 (1H, dd, *J* = 7.6, 1.7 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 20.5, 51.2, 55.6, 78.9, 111.7, 120.5, 126.6, 128.0, 130.2, 157.1, 176.0.

Recovery of 2-Methoxy-2-(2-methoxymethylphenyl)propionic Acid (R)-(-)-9 from Oxazoline (R;S)-(-)-21a

Oxazoline (R;S)-(-)-**21a** (300 mg, 0.88 mmol) was converted to acid (R)-(-)-**9** (186 mg, 94%): colorless syrup; $[\alpha]_D^{20}$ -43.9 (c 1.06, CHCl₃); IR (neat) $\nu_{\max}$ 2988, 2938, 2830, 1739, 1452, 1228, 1194, 1140, 1097, 1066, 755 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.89 (3H, s), 3.16 (3H, s), 3.35 (3H, s), 4.44 (1H, d, *J* = 12.2 Hz), 4.78 (1H, d, *J* = 12.2 Hz), 7.32 (1H, ddd, *J* = 7.3, 7.3, 1.7 Hz), 7.35 (1H, ddd, *J* = 7.3, 7.3, 1.7 Hz), 7.43 (1H, dd, *J* = 7.3, 1.7 Hz), 7.47 (1H, dd, *J* = 7.3, 1.7 Hz), 9.13 (1H, br. s); ¹³C NMR (100 MHz, CDCl₃) δ 21.6, 51.1, 58.0, 72.7, 82.0, 127.8, 128.1, 128.7, 130.0, 136.1, 136.7, 175.5.

Recovery of 2-Methoxy-2-(2-methyl-1-naphthyl)propionic Acid (R)-(+)-10 from Oxazoline (R;S)-(+)-22a

Oxazoline (R;S)-(+)-**22a** (250 mg, 0.70 mmol) was converted to acid (R)-(+)-**10** (147 mg, 87%): colorless solid; $[\alpha]_D^{23}$ +27.1 (c 0.80, CHCl₃); IR (film) $\nu_{\max}$ 3050, 2991, 2940, 1708, 1134, 1096, 813, 743 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 2.03 (3H, s), 2.58 (3H, s), 3.25 (3H, s), 7.22 (1H, d, *J* = 8.5 Hz), 7.37–7.42 (2H, m), 7.68 (1H, d, *J* = 8.6 Hz), 7.76–7.79 (1H, m), 8.33–8.36 (1H, m); ¹³C NMR (100 MHz, CDCl₃) δ 23.4, 23.6, 52.0, 83.9, 124.6, 124.8, 125.9, 128.65, 128.70, 131.0, 131.8, 132.1, 132.9, 135.1, 176.9.

Recovery of 2-Methoxy-2-(2-naphthyl)propionic acid (R)-(-)-11 from Oxazoline (R;S)-(+)-23a

Oxazoline (R;S)-(+)-**23a** (280 mg, 0.77 mmol) was converted to acid (R)-(-)-**11** (154 mg, 83%): colorless solid; $[\alpha]_D^{22}$ -32.3 (c 0.49, EtOH); IR (film) $\nu_{\max}$ 3157, 3058, 2989, 1717, 1458, 1374, 1274, 1186, 1132, 1047, 858, 821, 748, 699 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.96 (3H, s), 3.30 (3H, s), 7.48–7.52 (2H, m), 7.56 (1H, dd, *J* = 8.8, 2.0 Hz), 7.81–7.88 (3H, m), 7.94 (1H, d, *J* = 1.7 Hz); ¹³C NMR

(100 MHz, CDCl_3) δ 20.5, 51.7, 81.5, 123.6, 125.8, 126.4, 126.6, 127.6, 128.3, 128.6, 133.0, 133.1, 136.0, 175.0.

Recovery of 2-Methoxy-2-(9-phenanthryl)propionic acid (R)-(-)-12 from Oxazoline (R;S)-(-)-24a

Oxazoline (R;S)-(-)-24a (320 mg, 0.81 mmol) was converted to acid (R)-(-)-12 (217 mg, 96%): colorless solid; $[\alpha]_D^{28}$ -100.3 (c 1.08, EtOH); IR (film) ν_{max} 3021, 1704, 1282, 1137, 909, 742 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 2.10 (3H, s), 3.11 (3H, s), 7.55–7.70 (4H, m), 7.89 (1H, s), 7.90 (1H, d, $J = 7.3$ Hz), 8.32 (1H, d, $J = 8.3$ Hz), 8.66 (1H, d, $J = 8.3$ Hz), 8.73 (1H, d, $J = 8.0$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 21.8, 51.0, 81.4, 122.5, 123.2, 125.3, 126.7, 126.9, 127.1, 127.7, 128.6, 129.2, 129.8, 130.6, 130.9, 131.0, 131.5, 176.2.

Preparation of Methyl 2-methoxy-2-(9-phenanthryl)propionate (R)-(-)-25

A mixture of acid (R)-(-)-12 (55 mg, 0.21 mmol), acetonitrile (2 ml), K_2CO_3 (300 mg), and iodomethane (0.2 ml) was stirred at room temperature overnight. After addition of saturated aq. NH_4Cl , the mixture was extracted with EtOAc several times. The combined organic layers were washed with water, dried over anhydrous MgSO_4 , and then evaporated under reduced pressure. The residue was subjected to a short column chromatography on silica gel (hexane/EtOAc 3:1) and then to HPLC on silica gel (hexane/EtOAc 3:1), yielding ester (R)-(-)-25 (87 mg, 99%): colorless solid; $[\alpha]_D^{28}$ -86.0 (c 1.88, EtOH); IR (film) ν_{max} 2990, 2950, 2830, 1749, 1450, 1257, 1129, 751, 732 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 2.07 (3H, s), 3.12 (3H, s), 3.64 (3H, s), 7.57–7.69 (4H, m), 7.89 (1H, s), 7.90 (1H, dd, $J = 7.8, 1.5$ Hz), 8.41 (1H, dd, $J = 8.3, 1.0$ Hz), 8.66 (1H, br. d, $J = 8.3$ Hz), 8.73 (1H, br. d, $J = 8.3$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 21.8, 50.8, 52.7, 81.4, 122.4, 123.0, 125.5, 126.5, 126.8, 127.2, 127.38, 127.41, 129.1, 130.1, 130.7, 130.8, 130.9, 133.2, 174.8; UV (EtOH), λ_{max} 296.0 nm (ϵ 11,300), 284.0(10,100), 275.6(12,700), 253.6(62,100), 246.8(48,100), 222.8(20,500), 212.6(29,100); CD (EtOH), λ_{ext} 296.0 nm ($\Delta\epsilon$ -1.6), 254.2 (-7.0), 245.6 (-7.3), 226.4 (+7.2), 217.4 (-2.8), 209.4 (+5.0). Anal. Calcd for $\text{C}_{19}\text{H}_{18}\text{O}_3$: C, 77.53; H, 6.16. Found: C, 77.28; H, 6.28.

Recovery of 2-Methoxy-2-(9-phenanthryl)propionic acid (S)-(+)-12 from Oxazoline (S;S)-(+)-24b

Oxazoline (S;S)-(+)-24b (320 mg, 0.81 mmol) was converted to ester (S)-(+)-25 (50 mg, 92%): colorless solid; $[\alpha]_D^{28}$ +98.1 (c 1.03, EtOH); IR, ^1H NMR, and ^{13}C NMR spectra agreed with those of acid (R)-(-)-12. Anal. Calcd for $\text{C}_{18}\text{H}_{16}\text{O}_3$: C, 77.12; H, 5.75. Found: C, 76.89; H, 5.92.

Preparation of Methyl 2-methoxy-2-(9-phenanthryl)propionate (S)-(+)-25

Acid (S)-(+)-12 (52 mg, 0.19 mmol) was converted to ester (S)-(+)-25 (50 mg, 92%): colorless solid; $[\alpha]_D^{28}$ +86.3 (c 1.74, EtOH); IR, ^1H NMR, ^{13}C NMR, and UV spectra agreed with those of ester (R)-(-)-25; CD (EtOH), λ_{ext} 296.8 nm ($\Delta\epsilon$ +1.6), 253.6 (+7.3), 246.4 (+7.4), 226.4

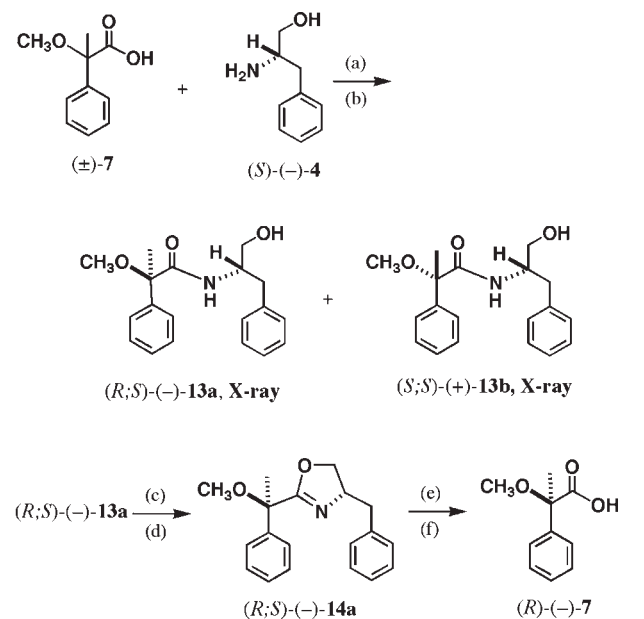
(-6.7), 216.2 (+2.8), 208.0 (-4.5). Anal. Calcd for $\text{C}_{19}\text{H}_{18}\text{O}_3$: C, 77.53; H, 6.16. Found: C, 77.46; H, 6.31.

RESULTS AND DISCUSSION

Preparation of Diastereomeric Amides from Racemic 2-Aryl-2-methoxypropionic Acids 7-12 and Phenylalaninol (S)-(-)-4, and HPLC Separation

The preparations of racemic 2-aryl-2-methoxypropionic acids **7-12** was previously reported.^{6,10-13} These racemic acids were condensed with phenylalaninol (S)-(-)-4 as follows. Racemic 2-methoxy-2-phenylpropionic acid ($\pm$)-**7** was treated with (S)-(-)-4, 1,3-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), 10-camphorsulfonic acid (CSA), and *N,N*-diisopropylethylamine (DIEA) in CH_2Cl_2 yielding a diastereomeric mixture of amides **13a/13b** in good yield (Scheme 1).

Diastereomeric amides **13a** and **13b** were easily separable by HPLC on silica gel (hexane/EtOAc = 1/1) as shown in Figure 3 and Table 2: $\alpha = 3.19$, $R_s = 3.49$. It should be noted that diastereomers **13a/13b** could be separated on a short column ($22\phi \times 100$ mm). This effective HPLC separation is an advantage of the enantioresolution method using amino alcohols, such as phenylalaninol (S)-(-)-4. The first-eluted fraction gave amide (-)-**13a** (48% yield), which was recrystallized from EtOH affording colorless prisms suitable for X-ray crystallography. The second-eluted fraction gave amide (+)-**13b** (46% yield), which was also recrystallized from EtOAc, giving colorless prisms suitable for X-ray analysis. Both amides thus provide single crystals ideal for X-ray crystallography, indicating that high probability of affording single crystals is



(a) DCC, HOBt, CSA, DIEA/ CH_2Cl_2 . (b) HPLC on silica gel. (c) SOCl_2 /toluene, Δ . (d) 28% NaOMe/MeOH , Δ . (e) CH_3I , Δ . (f) NaOH/MeOH , water, Δ .

Scheme 1. Enantioresolution of 2-methoxy-2-phenylpropionic acid by the use of phenylalaninol (S)-(-)-4.

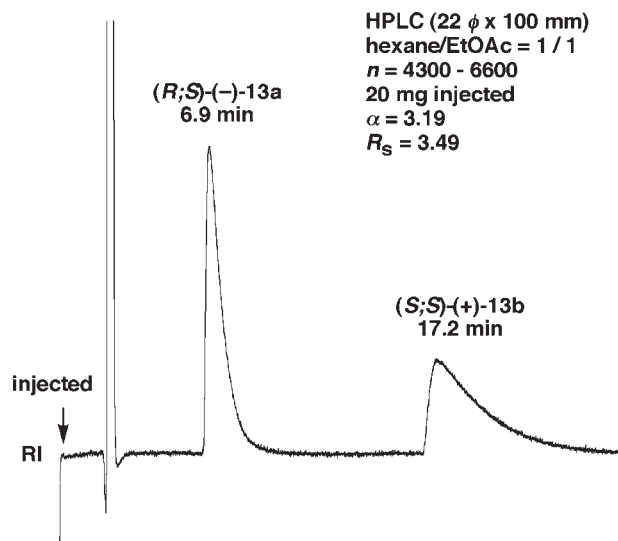


Fig. 3. HPLC separation of amides (*R,S*)-(-)-13a and (*S,S*)-(+)-13b.

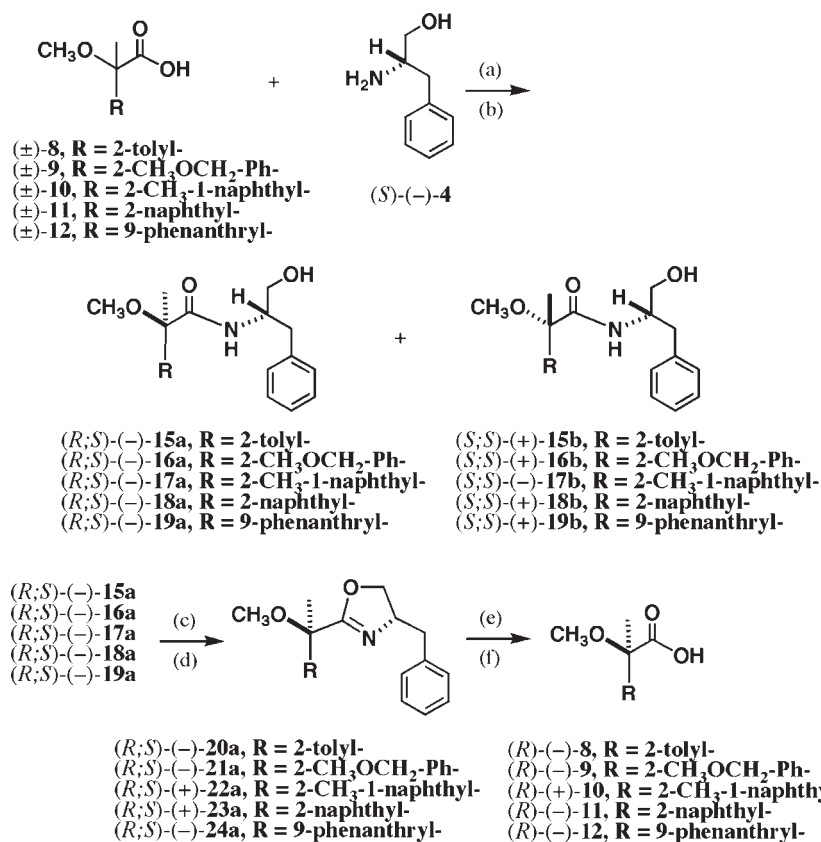
another advantage of amides prepared from phenylalaninol (*S*)-(-)-4.

In a similar way, 2-methoxy-2-(2-tolyl)propionic acid ($\pm$)-8 was condensed with (*S*)-(-)-4 giving diastereomeric

amides **15a/15b**, which were easily separated by HPLC on silica gel (hexane/EtOAc = 1/1): $\alpha = 2.53$, $R_s = 2.86$ (Scheme 2 and Table 2); the first-eluted fraction gave amide (-)-**15a** (43%), whereas the second-eluted one (+)-**15b** (46%). Although the crystallization of amide (-)-**15a** failed, the second-eluted amide (+)-**15b** could be obtained as single crystals suitable for X-ray crystallography, when recrystallized from EtOAc.

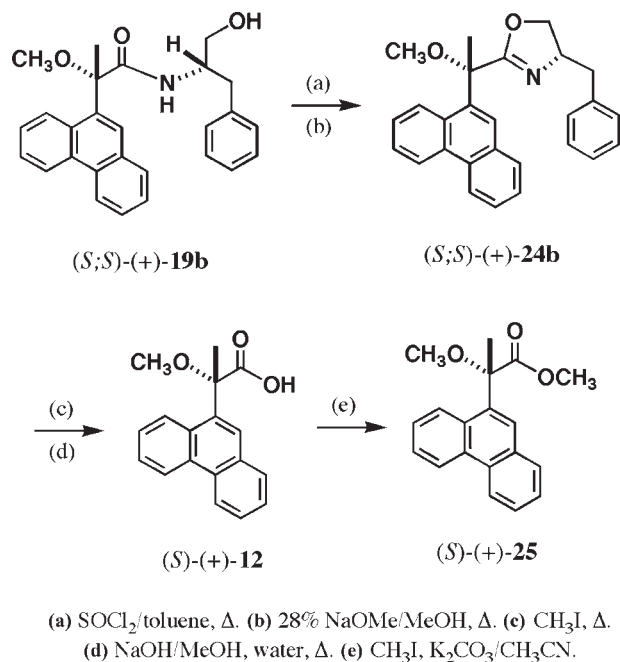
Racemic 2-methoxy-2-(2-methoxymethylphenyl)propionic acid ($\pm$)-**9** was similarly subjected to the reaction with (*S*)-(-)-4 yielding amides **16a/16b**, which were base-line separated by HPLC on silica gel (hexane/EtOAc = 1/2): $\alpha = 1.57$, $R_s = 2.75$ (Table 2); the first-eluted fraction gave amide (-)-**16a** (49%), whereas the second-eluted one (+)-**16b** (48%). It was interesting to find that amides **16a/16b** having a polar substituent in the aromatic group were less effectively separated by HPLC on silica gel, compared with other amides having a nonpolar aromatic group (Table 2). Amide (+)-**16b** was recrystallized from diethyl ether affording colorless prisms suitable for X-ray crystallography.

Racemic 2-methoxy-2-(2-methyl-1-naphthyl)propionic acid ($\pm$)-**10** was condensed with (*S*)-(-)-4 yielding amides **17a/17b**, which were easily separated by HPLC on silica gel (hexane/EtOAc = 1/1): $\alpha = 2.43$, $R_s = 7.18$ (Table 2);



(a) DCC, HOBt, CSA, DIEA/CH₂Cl₂. (b) HPLC on silica gel. (c) SOCl_2 /toluene, Δ .
(d) 28% NaOMe/MeOH, Δ . (e) CH_3I , Δ . (f) NaOH /MeOH, water, Δ .

Scheme 2. Enantioresolution of 2-aryl-2-methoxypropionic acids by the use of phenylalaninol (*S*)-(-)-4.



Scheme 3. Preparation of enantiopure acid (S)-(+)-12 and ester (S)-(+)-25.

the first-eluted fraction gave amide (–)-17a (38%), whereas the second-eluted one (+)-17b (49%). The recrystallization of the first-eluted amide (–)-17a from EtOH gave colorless prisms suitable for X-ray crystallography.

Racemic 2-methoxy-2-(2-naphthyl)propionic acid ($\pm$)-11 was subjected to the reaction with (S)-(-)-4 yielding amides 18a/18b, which were most effectively separated, as listed in Table 2, by HPLC on silica gel (hexane/EtOAc = 1/1): $\alpha = 3.52$, $R_s = 3.92$. The first-eluted fraction gave amide (–)-18a (48%), whereas the second-eluted one (+)-18b (50%). Amide (–)-18a was recrystallized from EtOH affording colorless prisms suitable for X-ray crystallography.

Racemic 2-methoxy-2-(9-phenanthryl)propionic acid ($\pm$)-12 was similarly condensed with (S)-(-)-4 yielding amides 19a/19b, which were easily separated by HPLC on silica gel (hexane/EtOAc = 1/1): $\alpha = 2.80$, $R_s = 2.77$ (Table 2); the first-eluted fraction gave amide (–)-19a (47%), whereas the second-eluted one (+)-19b (50%). We have attempted to crystallize amides 19a and 19b from various solvents. However, no ideal large single crystals suitable for X-ray diffraction experiments were obtained. Their absolute configurations were thus determined by the chemical correlations as shown in Schemes 2 and 3.

X-Ray Crystallographic Determination of the Absolute Configurations of Amides (–)-13a, (+)-13b, (+)-15b, (+)-16b, (–)-17a, and (–)-18a by the Use of the S-Phenylalaninol Moiety as an Internal Reference

A single crystal of the first-eluted amide (–)-13a described earlier was subjected to X-ray crystallography: monoclinic, $P2_1$. The structure was solved by the direct method and successive Fourier syntheses: $R = 0.0390$ and $R_w = 0.0977$; Flack $\chi = 0.1(3)$ (Table 1). As phenylalaninol (S)-(-)-4 was used as an internal reference, the absolute configuration of 2-methoxy-2-phenylpropionic acid part was automatically determined to be *R* from the stereostructure illustrated in Figure 4. The (*R*,*S*) absolute configuration of (–)-13a was confirmed also by the Flack parameter.

The second-eluted amide (+)-13b was similarly subjected to X-ray crystallography: orthorhombic, $P2_12_12_1$. The structure was solved by the direct method and successive Fourier syntheses: $R = 0.0407$ and $R_w = 0.0982$; Flack $\chi = -0.1(3)$ (Table 1). As the *S* absolute configuration of the phenylalaninol part was known, the absolute configuration of 2-methoxy-2-phenylpropionic acid part was determined to be *S* as shown in Figure 4. Of course, the present absolute configurational assignment is consistent with that of amide (–)-13a.

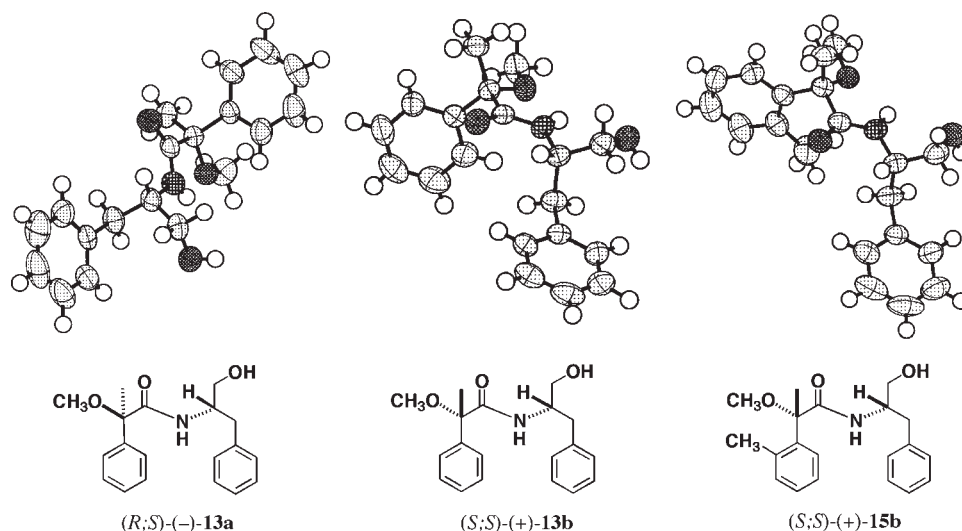


Fig. 4. X-ray crystallographic stereostructures of amides prepared from 2-aryl-2-methoxy-propionic acid and phenylalaninol (S)-(-)-4.

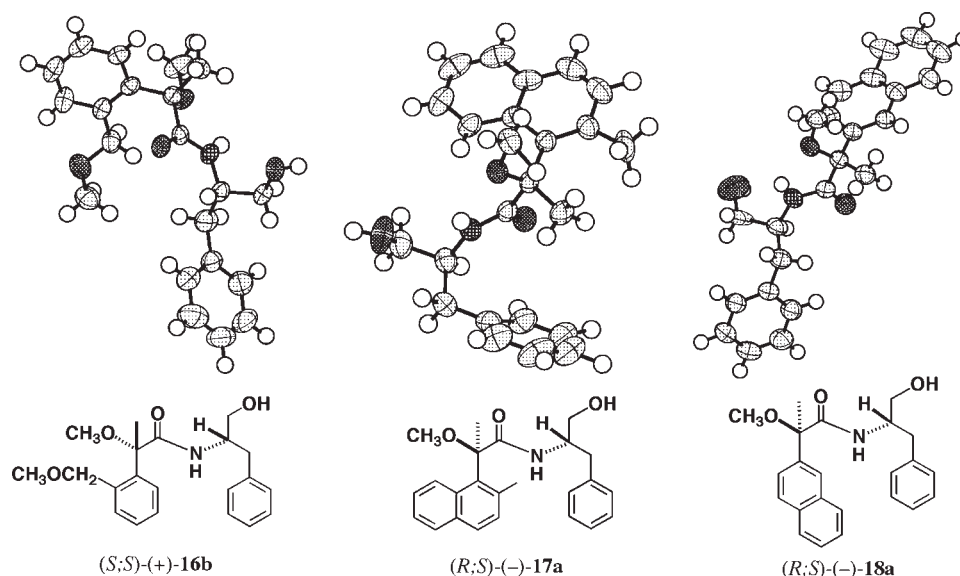


Fig. 5. X-ray crystallographic stereostructures of amides prepared from 2-aryl-2-methoxypropionic acid and phenylalaninol (S)-(-)-4.

The second-eluted amide (+)-**15b** was subjected to X-ray crystallography: orthorhombic, $P2_12_12_1$. The structure was solved by the direct method and successive Fourier syntheses: $R = 0.0391$ and $R_w = 0.0957$; Flack $\chi = -0.7$ (3) (Table 1). The absolute configuration of 2-methoxy-2-(2-tolyl)propionic acid part was determined to be *S* by reference to the *S* absolute configuration of the phenylalaninol part (Fig. 4).

The second-eluted amide (+)-**16b** was similarly subjected to X-ray crystallography: monoclinic, $P2_1$. The structure was solved by the direct method and successive Fourier syntheses: $R = 0.0325$ and $R_w = 0.0818$; Flack $\chi = 0.1$ (2) (Table 1). The absolute configuration of 2-methoxy-2-(2-methoxymethylphenyl)propionic acid part was determined to be *S* by reference to the *S* absolute configuration of the phenylalaninol part (Fig. 5).

The first-eluted amide (-)-**17a** was subjected to X-ray crystallography: monoclinic, $P2_1$. The structure was solved by the direct method and successive Fourier syntheses: $R = 0.0492$ and $R_w = 0.1277$; Flack $\chi = 0.0$ (3) (Table 1). The absolute configuration of 2-methoxy-2-(2-methyl-1-naphthyl)propionic acid part was determined to be *R* by reference to the *S* absolute configuration of the phenylalaninol part (Fig. 5). The (*R*;S) absolute configuration of (-)-**17a** was confirmed also by the Flack parameter.

The first-eluted amide (-)-**18a** was similarly subjected to X-ray crystallography: orthorhombic, $P2_12_12_1$. The structure was solved by the direct method and successive Fourier syntheses: $R = 0.0704$ and $R_w = 0.1890$; Flack $\chi = 1.0$ (5) (Table 1). The *R* absolute configuration of 2-methoxy-2-(2-naphthyl)propionic acid part was determined by reference to the *S* absolute configuration of the phenylalaninol part (Fig. 5). On the other hand, the Flack parameter $\chi = 1.0$ (5) indicates the opposite absolute configuration; the result may be due to the low crystallinity of amide (-)-**18a** as indicated by higher R and R_w values: $R = 0.0704$ and $R_w = 0.1890$. In such cases, the absolute configura-

tional assignment by the use of an internal reference, phenylalaninol (S)-(-)-**4**, is of course more reliable than that by the Flack parameter. The absolute configuration of a compound is unambiguously determined by the use of an internal reference even in the case of low crystallinity, if a relative configuration can be obtained. This is a great advantage of the internal reference method of X-ray crystallography.

As illustrated in Figures 4 and 5, the oxygen atom of the methoxyl group is antiperiplanar to the oxygen atom of the amide group in all cases, because of the hydrogen bond between the amide hydrogen atom and the oxygen atom of the methoxyl group. On the other hand, the amide oxygen atom is approximately synperiplanar to the methine hydrogen atom of the phenylalaninol part in all cases. Those are the general features of the crystalline state conformations of amides (-)-**13a**, (+)-**13b**, (+)-**15b**, (+)-**16b**, (-)-**17a**, and (-)-**18a**. Studies on the correlation between the conformations in crystalline state of these amides and those in solution state, which are related to ^1H NMR anisotropy effects, are in progress.

Recovery of Enantiopure 2-Aryl-2-methoxypropionic Acids from Amides via Oxazolines

To recover enantiopure M α NP acid (*R*)-(-)-**1** from amide (*R*;S)-(-)-**5a**, we have previously established a new route for converting amide (*R*;S)-(-)-**5a** to acid (*R*)-(-)-**1** via oxazoline (*R*;S)-(+)-**6a** (Fig. 1)⁸ (for the preparation of oxazolines and conversion to carboxylic acids)^{14,15} (for the mechanism of the hydrolysis of imidate salts).¹⁶⁻¹⁸ This method is generally applicable to amides formed from carboxylic acids with a quaternary stereogenic center and amino alcohols. We have therefore applied this method to amides (-)-**13a**, (-)-**15a**, (-)-**16a**, (-)-**17a**, (-)-**18a**, (-)-**19a**, and (+)-**19b** as shown in Schemes 1-3.

Amide (*R*;S)-(-)-**13a** was treated with SOCl_2 in toluene, and the crude product obtained was treated with NaOMe

in MeOH yielding oxazoline (*R*;S)-(-)-**14a** (97%), the structure of which was confirmed by ^1H and ^{13}C NMR spectra (Scheme 1). Oxazoline (*R*;S)-(-)-**14a** was treated with iodomethane, and after removal of excess iodomethane, the crude product was hydrolyzed with NaOH in MeOH/water giving enantiopure 2-methoxy-2-phenylpropionic acid (*R*)-(-)-**7** (75%), the IR, ^1H and ^{13}C NMR spectral data of which agreed with those of racemic acid ($\pm$)-**7**.⁶ acid (*R*)-(-)-**7**, $[\alpha]_{\text{D}}^{25} -27.8$ (*c* 1.29, MeOH) {lit.¹⁹ $[\alpha]_{\text{D}} -26$ (*c* 1, MeOH) for acid (*R*)-(-)-**7**}. The previous absolute configurational assignment¹⁹ of acid (*R*)-(-)-**7** by chemical correlation was thus confirmed by X-ray crystallography.

Amide (*R*;S)-(-)-**15a** was similarly converted to oxazoline (*R*;S)-(-)-**20a**, which was methylated and then hydrolyzed giving enantiopure 2-methoxy-2-(2-tolyl)propionic acid (*R*)-(-)-**8**: $[\alpha]_{\text{D}}^{23} -90.0$ (*c* 1.08, CHCl_3). The absolute configuration of acid (-)-**8** was thus first determined to be *R* by X-ray crystallography.

Amide (*R*;S)-(-)-**16a** was also converted to oxazoline (*R*;S)-(-)-**21a**, which was methylated and then hydrolyzed yielding enantiopure 2-methoxy-2-(2-methoxymethylphenyl)propionic acid (*R*)-(-)-**9**: $[\alpha]_{\text{D}}^{20} -43.9$ (*c* 1.06, CHCl_3). The *R* absolute configuration of acid (-)-**9** was first determined by X-ray crystallography.

In a similar way, amide (*R*;S)-(-)-**17a** was converted to oxazoline (*R*;S)-(+)-**22a**, which was methylated and then hydrolyzed giving enantiopure 2-methoxy-2-(2-methyl-1-naphthyl)propionic acid (*R*)-(+)-**10**: $[\alpha]_{\text{D}}^{23} +27.1$ (*c* 0.80, CHCl_3). The *R* absolute configuration of acid (+)-**10** was thus established by X-ray crystallography. It should be noted that although M α NP acid (*R*)-**1** and acid (*R*)-**10** are similar in structure to each other and have the same absolute configuration, they are opposite in the sign of optical rotation: (*R*)-(-)-**1**, $[\alpha]_{\text{D}}^{25} -73.8$ (*c* 1.03, CHCl_3). Namely, acid **10** has an extra methyl group at the 2-position of the naphthyl group, when compared with M α NP acid **1**. However, M α NP acid (*R*)-**1** is levorotatory, whereas acid (*R*)-**10** is dextrorotatory. The present result provides a striking example that the absolute configurational assignment by the comparison of $[\alpha]_{\text{D}}$ values is not always correct.

Amide (*R*;S)-(-)-**18a** was similarly converted to oxazoline (*R*;S)-(+)-**23a**, which was methylated and then hydrolyzed giving enantiopure 2-methoxy-2-(2-naphthyl)propionic acid (*R*)-(-)-**11**: $[\alpha]_{\text{D}}^{22} -32.3$ (*c* 0.49, EtOH) {lit.^{10,11} $[\alpha]_{\text{D}}^{31} -30$ (*c* 0.37, EtOH) for (*R*)-(-)-**11**}. The *R* absolute configuration of acid (-)-**11** was thus first established by X-ray crystallography, confirming the previous absolute configurational assignments of acid (-)-**11** made by ^1H NMR anisotropy.^{10,11}

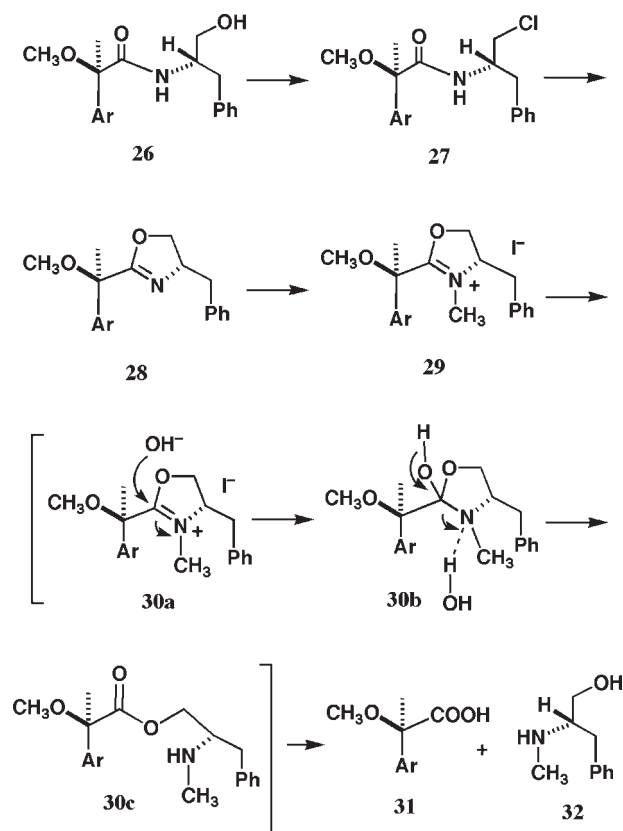
The first-eluted amide (-)-**19a**, to which X-ray crystallography could not be applied, was converted to oxazoline (-)-**24a**, $[\alpha]_{\text{D}}^{25} -28.3$ (*c* 1.21, CHCl_3). The methylation of (-)-**24a** and successive hydrolysis yielded enantiopure 2-methoxy-2-(9-phenanthryl)propionic acid (-)-**12**: $[\alpha]_{\text{D}}^{28} -100.3$ (*c* 1.08, EtOH). The observed $[\alpha]_{\text{D}}$ value agreed well with that of the authentic sample, (*R*)-(-)-**12**, $[\alpha]_{\text{D}}^{28} -100$ (*c* 0.914, EtOH),^{12,13} the absolute configuration of which had been determined by X-ray crystallography.^{12,13} Acid (-)-**12** was converted to methyl ester (-)-**25**, $[\alpha]_{\text{D}}^{28} -86.0$ (*c* 1.88, EtOH), the $[\alpha]_{\text{D}}$ value of which also agreed

well with that of the authentic sample, (*R*)-(-)-**25**, $[\alpha]_{\text{D}}^{28} -86$ (*c* 0.90, EtOH).^{12,13} These results clearly indicate that the acid (-)-**12** obtained here has an *R* absolute configuration. Therefore, the absolute configurations of amide (-)-**19a** and oxazoline (-)-**24a** were naturally assigned to (*R*;S) and (*R*;S), respectively, as shown in Scheme 2.

From the second-eluted amide (+)-**19b**, enantiopure 2-methoxy-2-(9-phenanthryl)propionic acid (+)-**12**, $[\alpha]_{\text{D}}^{27} +98.1$ (*c* 1.03, EtOH), was similarly prepared via oxazoline (+)-**24b**, $[\alpha]_{\text{D}}^{25} +7.6$ (*c* 1.32, CHCl_3) (Scheme 3). Acid (+)-**12** was methylated giving ester (+)-**25**, $[\alpha]_{\text{D}}^{27} +86.3$ (*c* 1.74, EtOH). The $[\alpha]_{\text{D}}$ values of acid (+)-**12** and ester (+)-**25** are opposite in sign to those of (*R*)-(-)-**12** and (*R*)-(-)-**25**, respectively, but their absolute values agree reasonably well with each other. Therefore, the absolute configurations of acid (+)-**12** and ester (+)-**25** were unambiguously determined to be *S* and *S*, respectively. These results led to the absolute configurational assignments of amide (*S*;S)-(+)-**19b** and oxazoline (*S*;S)-(+)-**24b** as shown in Scheme 3.

Short Remarks on the Reaction Mechanism for the Conversion of Amides to Acids via Oxazolines

In general, the direct hydrolysis of amides under basic conditions is difficult. For example, although we have tried the conversion of M α NP amide (*R*;S)-(-)-**5a** to acid (*R*)-(-)-**1** under various basic reaction conditions, all attempts



Scheme 4. The reaction mechanism for the conversion of amide **26** to acid **31** via oxazoline **28**.

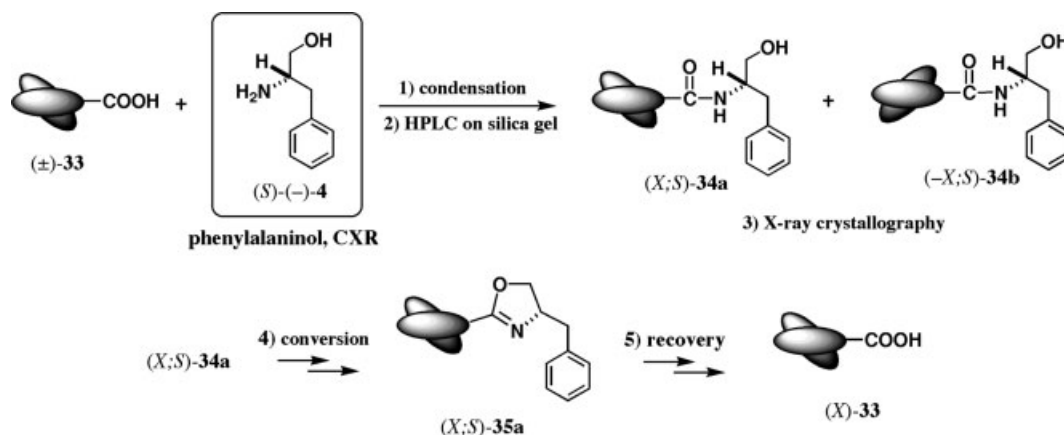


Fig. 6. A general scheme of the enantioresolution of carboxylic acids with a quaternary stereogenic center and the determination of their absolute configurations by X-ray crystallography by the use of (S)-(-)-phenylalaninol, a chiral X-ray reference reagent (CXR) with resolving power.

were unsuccessful.⁸ However, enantiopure acid (*R*)-(-)-**1** was recovered from amide (*R*;S)-(-)-**5a** via oxazoline (*R*;S)-(+)-**6a**.⁸ In addition, as discussed earlier, enantiopure acids (*R*)-(-)-**7** ~ (*R*)-(-)-**12** were also recovered from amides (*R*;S)-(-)-**13a** ~ (*R*;S)-(-)-**19a** via oxazolines (*R*;S)-(-)-**14a** ~ (*R*;S)-(-)-**24a**, respectively. Therefore, it is interesting to discuss the reaction mechanism of the conversion of amide **26** to acid **31** via oxazoline **28** (Scheme 4). Namely, why is the amide bond so easily cleaved in the present reactions?

It is known that amides having structures like **26** can be converted to oxazolines via chlorides (for the preparation of oxazolines and conversion to carboxylic acids).^{14,15} Namely, amide **26** was treated with SOCl_2 yielding chloride **27**, which was converted to oxazoline **28** by treatment with base, although we have not isolated chloride **27**. Oxazoline **28** was treated with CH_3I giving imide salt **29**, which was next treated with aqueous NaOH . The mechanism of the hydrolysis of imide salts has been well studied (for the mechanism of the hydrolysis of imide salts).^{16–18} For example, the hydrolysis of an imide salt with a mild base like aqueous NaHCO_3 yielded ester, but not amide, indicating that the C–N bond cleaves first (for the mechanism of the hydrolysis of imide salts).¹⁸ Therefore, as shown in Scheme 4, the reaction proceeds through the intermediate **30b**, in which the C–N bond cleaves first with the participation of water affording ester intermediate **30c**. This is a reason why the amide C–N bond cleaves easily. Ester **30c** was hydrolyzed finally yielding acid **31** and *N*-methyl-phenylalaninol **32**. In the case of $\text{M}\alpha\text{NP}$ amide (*R*;S)-(-)-**5a**, the structure of amino alcohol **32** was confirmed.

CONCLUSION

Racemic 2-aryl-2-methoxypropionic acids **7–12** with a quaternary stereogenic center were enantioresolved by the use of (S)-(-)-phenylalaninol **4** via their amide and oxazoline derivatives. For example, acid (±)-**7** was condensed with (S)-(-)-**4** yielding diastereomeric amides, which were easily separated by HPLC on silica gel giving the first-eluted amide (-)-**13a** and the second-eluted am-

ide (+)-**13b**. The absolute configuration of amide (-)-**13a** was unambiguously determined to be (*R*;S) by X-ray crystallography, where the *S* configuration of the phenylalaninol moiety was used as an internal reference. Amide (*R*;S)-(-)-**13a** was converted to oxazoline (*R*;S)-(-)-**14a**, from which enantiopure 2-methoxy-2-phenylpropionic acid (*R*)-(-)-**7** was recovered. This method has been successfully applied to the preparation of other enantiopure 2-aryl-2-methoxypropionic acids, (*R*)-(-)-**8**, (*R*)-(-)-**9**, (*R*)-(+)-**10**, (*R*)-(-)-**11**, and (*R*)-(-)-**12**, and their absolute configurations have been clearly determined by X-ray crystallography, except the case of (*R*)-(-)-**12**, the absolute configuration of which has been established by chemical correlation. (S)-(-)-Phenylalaninol **4** is thus a powerful chiral X-ray reference reagent (CXR) with resolving power,^{8,9} and this method should be applicable to various carboxylic acids with a quaternary stereogenic center, as shown in the general scheme of Figure 6.

The advantages of (S)-(-)-phenylalaninol **4** as a chiral X-ray reference reagent (CXR) with resolving power can be summarized as follows; (i) it is easily prepared from *L*-phenylalanine, a natural amino acid; (ii) diastereomeric amides formed from racemic acids with a quaternary stereogenic center and (S)-(-)-**4** are easily separable by HPLC on silica gel; (iii) the separated amides have a high probability of providing single crystals suitable for X-ray crystallography; (iv) in X-ray crystallography, the absolute configurations of the amides are clearly determinable by reference to the *S* absolute configuration of the phenylalaninol part; (v) enantiopure carboxylic acids with a quaternary stereogenic center are recovered from the amides via oxazoline derivatives. In this article, although we have focused on (S)-(-)-phenylalaninol **4**, other chiral α -amino alcohols derived from natural α -amino acids, e.g., (*S,S*)-(+)-isoleucinol,⁸ also should be useful as CXRs.

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Functionalization of Magnetic Nanoparticles with Organic Molecules: Loading Level Determination and Evaluation of Linker Length Effect on Immobilization

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT A general method is introduced to immobilize organic molecules on magnetic nanoparticles through silanization reactions and determine the maximum loading level by UV–vis spectroscopy. Loading levels of 1.5×10^{-3} mol per gram of nanoparticle were obtained with structurally diverse compounds such as rhodamine B and glucosamine. The length of the linker did not have a significant effect on loading as comparable maximum amounts of rhodamine B were immobilized on magnetic nanoparticles regardless of the linker length. Interestingly, rhodamine B derivatives lost conjugation during synthetic manipulations due to reversible spiroisobenzofuran formation. Full regeneration of conjugation was found to be slow with rhodamine B attached on magnetic nanoparticles. The results obtained from these studies will be useful for studying surface functionalization of MNPs in general. *Chirality* 20:265–277, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: glucosamine; linker length; magnetic nanoparticle; rhodamine B; silanization; UV–vis spectroscopy

INTRODUCTION

Magnetic nanoparticles are receiving increasing interest in recent years.^{1–3} Since many MNPs show biocompatibility and low toxicity,^{4,5} they have been intensively studied for biomedical applications^{6–9} such as targeted drug delivery,¹⁰ MRI contrast enhancement,^{11,12} cancer diagnosis,^{13,14} hyperthermic treatment of tumors,¹⁵ and magnetically mediated separation of biomolecules.¹⁶ In addition, MNPs serve as an attractive platform for catalyst immobilization.^{17–27} Because of the nanometer dimensions of MNPs, such catalysts have better reaction kinetics as compared to the traditional heterogeneous catalysts on mesoporous solid support. Moreover, the magnetic nature of these particles allows for facile recovery and recycling of catalysts. Chiral discrimination can also be achieved using MNPs. For example, chiral phosphines have been immobilized on MNPs to produce magnetically recoverable catalysts for asymmetric hydrogenation of aromatic ketones with high enantiomeric excess.²³ MNPs with a lipase attached have been used to resolve racemic carboxylic acids with improved long term stability compared with the free enzyme.²⁵

Key to the successful application of MNPs is the surface functionalization. There are two general surface modification approaches taken: (1) Molecules containing functional groups such as carboxylic acids or phosphoric acids are attached through noncovalent reversible absorption on MNPs;^{22,28,29} although this approach is conceptually simple, the presence of a functional group does not guarantee that a ligand will remain bound to the surface of a nanopar-

ticle at various stages of surface modification and during application. In fact, the loss of catalytic activities of MNP supported catalyst was attributed to the dissociation of catalysts from MNP surface.^{26,30} (2) Ligands are attached through a covalent linkage. This can be accomplished by first coating the surface of MNPs with a functionalized alkoxy silane reagent, such as APTES through the silanization reaction. The alkoxy silane crosslinks to form a passive layer of silica coating on the nanoparticle surface with amino moieties on the exterior. The amino group can be converted to a variety of functional groups such as carboxylic acid or aldehyde for linking the molecule of interest.^{31–35} This stepwise modification approach has been successfully

Abbreviations: Ac, acetyl; ACA, 6-aminocaproic acid; APTES, γ -aminopropyltriethoxy silane; Bn, benzyl; Boc, *t*-butoxycarbonyl; BOP, benzotriazol-1-yl-oxy-*tris*-(dimethylamino)phosphonium hexafluorophosphate; DCM, dichloromethane; DIPEA, *N,N*-diisopropylethyl amine; DMAP, 4-(*N,N*-dimethylamino)pyridine; DMF, *N,N*-dimethylformamide; EDC, *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride; ESI-MS, electron spray ionization-mass spectra; FTIR, Fourier transform infrared spectra; Lev, levuniloyl; MNPs, magnetic nanoparticles; MRI, magnetic resonance imaging; MS-AW, molecular sieves, acid washed; NHS, *N*-hydroxy succinimide; NMR, nuclear magnetic resonance; Phth, phthalimido; SEM-EDS, scanning electron microscopy-energy dispersive spectra; TEM, transmission electron microscopy; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; UV–vis, ultraviolet–visible; XRD, X-ray diffraction

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applied to attach enzymes, antibodies as well as small molecule ligands to MNPs.

The density and distribution of the immobilized molecules on nanoparticle surfaces can significantly affect their properties. Using the stepwise modification approach, some functional groups may be left unreacted at each stage, which can lead to defects in the nanoparticle and decrease of the overall loading level. Previous studies to determine the density of molecules loaded have relied upon thermogravimetric and elemental analyses.^{5,32,36,37} However, because of the small mass of surface molecules relative to the metal oxide core, these types of analyses are not very accurate in detecting the subtle changes of surface composition.³¹ Chemisorption isotherm analyses based on fluorescence or UV-vis spectroscopies have been reported, albeit for evaluation at later stages of multiple step surface modification.^{31,32,38}

Another important factor that can influence the modification of MNPs is the length of the linker used. Because of the small size of the MNPs, steric hindrance associated with bulky substrates can prevent efficient immobilization. The installation of a linker between the MNP and the substrate helps minimize the steric problem. However, there have not been systematic studies about the effects of linker length on immobilization efficiencies.

Herein we report our studies on functionalization of MNPs with organic molecules using a direct attachment approach. The amounts of rhodamine B loaded on MNPs were determined by UV-vis spectroscopy and the effect of linker length on immobilization was systematically evaluated. Our method can provide valuable quantitative information and is further applied to the determination of the amount of carbohydrate loaded on MNPs.

MATERIALS AND METHODS

General

¹H-NMR (400 or 600 MHz) and ¹³C-NMR (100 or 150 MHz) spectra were recorded at room temperature. Chemical shift (δ) values are given in ppm. Mass spectra were recorded on an ESQUIRE LC-MS using the ESI technique to introduce the sample. IR spectra were recorded as KBr pellets using a Perkin Elmer Spectrum GX FTIR with ATR capabilities. UV-vis spectra were recorded on a Beckman DU-50 spectrophotometer. Air- and moisture-sensitive liquids and solutions were transferred via syringes. Organic solutions were concentrated by rotary evaporation below 40°C at ca. 25 Torr. Flash column chromatography was performed employing 230–400 mesh silica gels. Thin-layer chromatography was performed using glass plates precoated to a depth of 0.25 mm with 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm). TLC plates were visualized with UV light and or by staining either with a yellow stain (containing Ce(NH₄)₂(NO₃)₆ (0.5 g) and (NH₄)₆Mo₇O₂₄·4H₂O (24.0 g) in 6% H₂SO₄ (500 ml) or a pink stain (containing KMnO₄ (3 g), K₂CO₃ (20 g), 5% aq. NaOH (5 ml) in 300 ml H₂O). The nanoparticles were characterized on carbon-coated Formvar support film 300 mesh copper grids with a TEM microscope (Philips, CM10 Mawah, NJ, software Image Pro Plus 6.0).

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For TEM sample preparation, MNPs were suspended in ethanol using sonication for 30 min and then drop-cast on a TEM grid. SEM-EDS spectra were obtained using JEOL JSM 6100 SEM with Link/eXL Energy dispersive X-ray spectrometer. Powder X-ray diffraction spectra were obtained using PAnalytical X'pert Pro MPD with CuK α radiation.

Preparation of Fe₃O₄ Nanoparticles

For MNP synthesis, 1 M ferric chloride and 1 M ferrous sulfate solutions were prepared by dissolving ferric chloride hexahydrate (FeCl₃·6H₂O (99%, 27.1 g) and ferrous sulfate heptahydrate (FeSO₄·7H₂O (99%, 27.8g) in 2 M HCl (100 ml) respectively. Degassed and deionized water was used to prepare all the solutions. To prepare Fe₃O₄ nanoparticles, 1 M aqueous FeCl₃ (10 ml) solution was mixed with 1 M aqueous FeSO₄ (5 ml) solution in a flask under a nitrogen atmosphere. Ammonium hydroxide solution (~30 ml) was slowly added with vigorous stirring until a pH between 11 and 12 was reached. Vigorous stirring was continued for another 30 min. The solution color could be seen to alter from orange to black, leading to black Fe₃O₄ nanoparticles. The MNPs were isolated by applying a permanent external magnet (Lifesept[®] 50SX magnetic separator). The supernatant was decanted and discarded. Deionized and degassed water was then added to wash the particles thoroughly and was repeated for 5 times to remove excess ions and salts. Finally particles were washed with ethanol twice and stored as dispersion in ethanol.

Preparation of MNPs Coated with Rhodamine B Derivatives and Loading Evaluations

To vials each containing MNPs (0.37 mg) were added respectively 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1, 1.5, 2, 2.5, and 3 mg of rhodamine B derivative in absolute ethanol (2 ml) under nitrogen. The vial sets were mechanically shaken for 24 h. Rhodamine B coated MNPs were isolated by positioning a permanent external magnet (Lifesept[®] 50SX magnetic separator) on the side of the vial. The supernatant was carefully collected by a pipette, the particles were washed with ethanol and redispersed by shaking. Several cycles of magnetically induced precipitation washing and redispersion were performed. All ethanol solutions were combined and diluted to a fixed volume, to which TFA (10 μ l) was added. UV-vis spectrum of the solution was measured, from which the amount of unattached dye was calculated based on its absorbance at 554 nm. The amount of dye loaded on MNPs was obtained by subtracting the amount of unattached dye from that added for the silanization reaction.

Preparation of MNPs Coated with Glucosamine 14 and Loading Evaluation of the Resulting Glyco-Nanoparticles

To vials each containing MNPs (0.37 mg) were added respectively 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1, 1.5, 2, 2.5, and 3 mg of glucosamine **14** in absolute ethanol (2 ml) under nitrogen. The vial sets were mechanically shaken for 24 h. The glyco-nanoparticles were isolated by positioning a permanent external magnet (Lifesept[®] 50SX magnetic

separator) on the side of the vial. The supernatant was carefully removed by pipette and particles were washed several times with ethanol. Subsequently, rhodamine B derivative **2** (1 mg) in absolute ethanol (2 ml) was added to nanoparticles in each vial. The vial sets were mechanically shaken for 24 h under nitrogen, after which nanoparticles were isolated by positioning a permanent external magnet on the side of the vial. The supernatant was carefully collected by a pipette, and the particles were washed with ethanol. All solutions were combined and diluted to a fixed volume, to which TFA (10 μ l) was added. UV-vis spectra of the solutions were recorded from which the amount of unattached dye was measured based on its absorbance at 554 nm ($\epsilon = 25,612$). The amount of dye immobilized on MNPs was calculated by subtracting the amount of unattached dye from its amount added. The amount of glucosamine **14** attached on MNPs was assumed to be 60% of the amount of **14** added for the silanization reaction. The sum of glucosamine **14** and rhodamine B **2** immobilized on MNPs equals 1.6 mmol/g, which is the maximum quantity of compounds that can be loaded on MNPs.

Rhodamine B APTES Conjugate (1)

To a mixture of rhodamine B **8** (500 mg, 1.04 mmol) and APTES (254 mg, 1.15 mmol) in dry DCM (20 ml), EDC (300 mg, 1.57 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. The solvent was evaporated and the product was purified by flash chromatography (15% methanol in DCM) in 70% yield. ^1H -NMR (400 MHz, $CDCl_3$) δ 7.88–7.90 (m, 1H), 7.36–7.42 (m, 2H), 7.02–7.04 (m, 1H), 6.46 (s, 1H), 6.44 (s, 1H), 6.37 (d, 2H, $J = 2.4$ Hz), 6.26 (dd, 2H, $J = 2.4$, 8.8 Hz), 3.64 (q, 6H, $J = 7.2$ Hz), 3.32 (q, 8H, $J = 7.2$ Hz), 3.09–3.14 (t, 2H, $J = 7.2$ Hz), 1.31–1.35 (m, 2H), 1.13–1.17 (t, 12H, $J = 7.2$ Hz), 1.11 (t, 9H, $J = 7.2$ Hz), 0.40–0.46 (m, 2H); ^{13}C -NMR (100 MHz, $CDCl_3$) δ 168.0, 153.9, 153.2, 148.6, 132.1, 131.3, 129.0, 127.8, 123.7, 122.6, 107.9, 105.9, 97.6, 64.8, 58.1, 44.3, 43.3, 21.7, 18.2, 12.6, 8.2; ESI-MS: m/z : calcd for $C_{37}H_{52}N_3O_5Si$: 646.4; found: 646.5 $[M + H]^+$.

N-Boc-diACA (5)

To a mixture of *N*-Boc-6-aminocaproic acid³⁹ (2 g, 8.65 mmol) and *N*-hydroxysuccinimide (0.995 g, 8.65 mmol) in dry DCM (75 ml), EDC (2.49 g, 13 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. The reaction mass was washed with saturated brine and organic layer was dried over anhydrous Na_2SO_4 . The solvent was evaporated to give succinimidyl activated ester as a white solid (2.56 g, 90% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 4.65 (bs, 1H), 3.00–3.10 (m, 2H), 2.80 (s, 4H), 2.57 (t, 2H, $J = 7.2$ Hz), 1.70–1.74 (m, 2H), 1.37–1.51 (m, 13H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 168.7, 156.2, 79.2, 40.4, 31.0, 29.7, 28.6, 26.1, 25.8, 24.4; ESI-MS: m/z : calcd for $C_{15}H_{24}N_2O_6$ Na: 351.1; found: 351.1 $[M + Na]^+$.

To a mixture of succinimidyl activated ester (1 g, 3.05 mmol) and 6-aminocaproic acid (0.4 g, 3.05 mmol) in dry DMF (30 ml), triethylamine (1.3 ml, 9.15 mmol) was added. The reaction mixture was stirred under a N_2 atmos-

phere at room temperature for overnight. DMF was removed under reduced pressure and diluted with ethyl acetate. The solution was washed with aqueous 1 N HCl solution. Organic layer was dried over anhydrous Na_2SO_4 . The solvent was evaporated, and the product **5** was obtained by recrystallization using 1:1 ethyl acetate: hexanes (0.97 g, 92% yield). ^1H NMR (400 MHz, CD_3OD) δ 3.15 (t, 2H, $J = 7.2$ Hz), 3.01 (t, 2H, $J = 7.2$ Hz), 2.29 (t, 2H, $J = 7.2$ Hz), 2.16 (t, 2H, $J = 7.2$ Hz), 1.57–1.64 (m, 4H), 1.27–1.51 (m, 17H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.3, 174.9, 157.4, 78.6, 40.0, 38.9, 35.8, 33.6, 29.5, 28.9, 27.6, 26.3, 25.6, 24.5; ESI-MS: m/z : calcd for $C_{17}H_{32}N_2O_5Na$: 367.2; found: 367.3 $[M + Na]^+$.

DiACA (6)

Compound **5** (1 g) was dissolved in 1:1 TFA/water (10 ml) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure to give oily product **6** (100% yield). Traces of water from the product were removed by coevaporation with toluene. ^1H -NMR (600 MHz, CD_3OD) δ 3.12–3.16 (m, 2H), 2.92 (t, 2H, $J = 7.2$ Hz), 2.26–2.31 (m, 2H), 2.19 (t, 2H, $J = 7.2$ Hz), 1.56–1.69 (m, 6H), 1.45–1.52 (m, 2H), 1.27–1.41 (m, 4H); ^{13}C -NMR (100 MHz, CD_3OD) δ 175.5, 175.8, 175.7, 175.6, 52.0, 40.5, 40.1, 36.6, 34.8, 34.6, 30.0, 29.9, 28.1, 27.4, 27.3, 26.8, 26.3, 25.6, 25.5; ESI-MS: m/z : calcd for $C_{12}H_{25}N_2O_3Na$: 268.2; found: 268.8 $[M + H + Na]^+$.

TetraACA (7)

To a mixture of *N*-Boc-diACA compound **5** (1.0 g, 2.9 mmol) and *N*-hydroxysuccinimide (368 mg, 3.2 mmol) in dry DCM (50 ml), EDC (834 mg, 4.4 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. The reaction mass was washed with saturated brine and organic layer was dried over anhydrous Na_2SO_4 . Solvent was evaporated to give succinimidyl activated ester as a white solid (1.18 g, 92% yield). ^1H -NMR (600 MHz, $CDCl_3$) δ 5.77 (s, 1H), 4.57 (s, 1H), 3.21–3.25 (m, 2H), 3.05–3.09 (m, 2H), 2.82, 2.83 (2s, 4H), 2.60 (t, 2H, $J = 7.2$ Hz), 2.14 (t, 2H, $J = 7.2$ Hz), 1.72–1.77 (m, 2H), 1.58–1.64 (m, 2H), 1.48–1.52 (m, 2H), 1.39–1.47 (m, 11H), 1.27–1.32 (m, 2H); ^{13}C -NMR (100 MHz, $CDCl_3$) δ 173.2, 169.5, 168.7, 79.2, 40.6, 39.1, 36.6, 31.0, 29.9, 29.1, 28.6, 25.9, 25.8, 25.5, 25.4. To a mixture of the succinimidyl activated ester (500 mg, 1.13 mmol) and diACA **6** (406 mg, 1.13 mmol) in dry DMF (15 ml), triethylamine (0.64 ml, 4.5 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. Reaction solvents were removed under reduced pressure and then diluted with ethyl acetate. The solution was washed with 1 N aqueous HCl solution. The organic layer was dried over anhydrous Na_2SO_4 . Solvent was evaporated, and the *N*-Boc protected tetraACA was obtained by recrystallization using 1:1 ethyl acetate: hexanes (595 mg, 92% yield). ^1H -NMR (400 MHz, CD_3OD) δ 3.12–3.16 (m, 6H), 2.99–3.13 (m, 2H), 2.12–2.17 (m, 6H), 1.26–1.65 (m, 35H); ^{13}C -NMR (100 MHz, CD_3OD) δ 176.2, 174.9, 174.8, 170.658, 157.356, 40.0, 39.0, 38.9, 35.9, 35.9, 35.8, 33.6, 30.3, 29.5, 28.9, 28.7, 27.6, 26.4, 26.3, 25.8, 25.6, 25.5, 25.3, 24.5, 24.2; ESI-MS: m/z : calcd for $C_{29}H_{53}N_4O_7$:

569.4; found: 569.2 $[M - H]^-$. *N*-Boc protected tetraACA (500 mg) was dissolved in 1:1 TFA/water (5 ml) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure to give oily product tetraACA **7** (100% yield). Traces of water from the product were removed by coevaporation with toluene. ^1H -NMR (400 MHz, CD_3OD) δ 3.12–3.18 (m, 6H), 2.86–2.93 (m, 2H), 2.26–3.32 (m, 2H), 2.14–2.22 (m, 6H), 1.58–1.70 (m, 10H), 1.46–1.54 (m, 6H), 1.30–1.43 (m, 8H); ^{13}C -NMR (100 MHz, CD_3OD) δ 176.3, 174.9, 174.6, 39.3, 39.0, 35.8, 35.4, 33.6, 28.9, 27.1, 26.3, 25.7, 25.5, 25.1, 24.5 ESI-MS: m/z : calcd for $\text{C}_{24}\text{H}_{45}\text{N}_4\text{O}_5$: 469.3; found: 469.2 $[M - H]^-$.

Rhodamine B ACA Conjugate (9)

To a mixture of rhodamine B **8** (2 g, 4.18 mmol) and *N*-hydroxysuccinimide (0.528 g, 4.6 mmol) in dry DCM (100 ml), EDC (1.21 g, 6.27 mmol) was added. The reaction mixture was stirred under N_2 atmosphere at room temperature overnight. Solvent was evaporated and the succinimidyl activated ester was purified by flash chromatography (1:10 methanol/DCM) in 90% yield. ^1H -NMR (600 MHz, CD_3OD) δ 8.42 (d, 1H, $J = 7.8$ Hz), 8.00 (t, 1H, $J = 7.8$ Hz), 7.90 (t, 1H, $J = 7.8$ Hz), 7.58 (d, 1H, $J = 7.8$ Hz), 7.14 (s, 1H), 7.12 (s, 1H), 7.03 (dd, 2H, $J = 6.0, 9.0$ Hz), 6.95 (d, 2H, $J = 1.8$ Hz), 3.66 (q, 8H, $J = 7.2$ Hz), 2.69 (s, 4H), 1.29 (t, 12H, $J = 7.2$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 168.9, 160.9, 157.9, 155.9, 155.8, 135.1, 134.6, 131.9, 131.2, 130.9, 125.5, 114.6, 113.6, 96.6, 77.1, 50.6, 25.8, 12.8; ESI-MS: m/z : calcd for $\text{C}_{32}\text{H}_{34}\text{N}_3\text{O}_5$: 540.3; found: 540.3 $[M - \text{Cl}]^+$. To a mixture of the succinimidyl activated ester (1.8 g, 3.12 mmol) and 6-aminocaproic acid (0.41 g, 3.12 mmol) in dry DMF (30 ml), triethylamine (1.32 ml, 9.37 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. DMF was removed under reduced pressure and the product **9** was purified by flash chromatography (15% methanol in DCM) in 96% yield. ^1H -NMR (600 MHz, CD_3OD) δ 7.95 (s, 1H), 7.82–7.84 (m, 1H), 7.47–7.50 (m, 2H), 6.99–7.01 (m, 1H), 6.40 (d, 2H, $J = 2.4$ Hz), 6.27–6.32 (m, 4H), 3.32–3.36 (q, 8H, $J = 7.2$ Hz), 3.02 (m, 2H), 2.03 (t, 2H, $J = 7.8$ Hz), 1.27–1.30 (m, 2H), 1.12 (t, 12H, $J = 7.2$ Hz), 1.02–1.06 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.8, 168.5, 163.7, 153.7, 153.6, 149.1, 132.8, 131.4, 128.7, 128.3, 123.9, 122.3, 108.3, 105.3, 97.8, 65.7, 44.2, 39.9, 35.9, 33.5, 30.6, 27.3, 26.4, 24.4; ESI-MS: m/z : calcd for $\text{C}_{34}\text{H}_{42}\text{N}_3\text{O}_4$: 556.3; found: 556.3 $[M + H]^+$.

Rhodamine B ACA APTES Conjugate (2)

To a mixture of rhodamine B ACA conjugate **9** (750 mg, 1.25 mmol) and APTES (305 mg, 1.38 mmol) in dry DCM (25 ml), EDC (360 mg, 1.88 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. The solvent was evaporated and the product **2** was purified by flash chromatography (15% methanol in DCM) in 65% yield. ^1H -NMR (400 MHz, CDCl_3) δ 7.87–7.89 (m, 1H), 7.40–7.43 (m, 2H), 7.05–7.08 (m, 1H), 6.37–6.43 (m, 4H), 6.26–6.28 (m, 2H), 6.07–6.12 (m, 1H), 3.81 (q, 6H, $J = 7.2$ Hz), 3.34 (q, 8H, $J = 7.2$ Hz), 3.18–3.23 (m, 2H), 3.08–3.14 (m, 2H), 2.01 (t, 2H, $J = 7.2$ Hz), 1.57–1.65 (m, 2H), 1.41–1.48 (m, 2H), 1.13–1.23 (m,

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24H), 0.63 (t, 2H, $J = 8.0$ Hz); ^{13}C -NMR (100 MHz, CDCl_3) δ 173.0, 168.1, 153.6, 153.3, 148.7, 132.2, 131.4, 128.9, 127.9, 123.7, 122.6, 108.1, 105.8, 97.8, 64.9, 58.4, 50.4, 44.4, 41.9, 39.9, 36.5, 27.7, 26.5, 25.0, 23.0, 18.3, 12.6, 7.8; ESI-MS: m/z : calcd for $\text{C}_{43}\text{H}_{63}\text{N}_4\text{O}_6\text{Si}$: 759.6; found: 759.8 $[M + H]^+$, calcd for $\text{C}_{43}\text{H}_{62}\text{N}_4\text{O}_6\text{SiNa}$: 781.4, found: 781.7 $[M + \text{Na}]^+$.

Rhodamine B triACA (12)

To a mixture of rhodamine B ACA conjugate **9** (367 mg, 0.612 mmol) and *N*-hydroxysuccinimide (77.5 mg, 0.674 mmol) in dry DCM (20 ml), EDC (176 mg, 0.919 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. The solvent was evaporated and the succinimidyl activated ester product was purified by flash chromatography (1:10 methanol/DCM) in 95% yield. ^1H NMR (400 MHz, CDCl_3) δ 7.88–7.90 (m, 1H), 7.42–7.44 (m, 2H), 7.07–7.10 (m, 1H), 6.43 (s, 1H), 6.41 (s, 1H), 6.38 (m, 2H), 6.25–6.28 (dd, 2H, $J = 6.0, 9.0$ Hz), 3.34 (q, 8H, $J = 7.2$ Hz), 3.12 (t, 2H, $J = 7.2$ Hz), 2.81 (s, 4H), 2.39 (t, 2H, $J = 7.2$ Hz), 1.50–1.53 (m, 2H), 1.16 (t, 12H, $J = 7.2$ Hz); ^{13}C -NMR (100 MHz, CDCl_3) δ 169.1, 168.5, 168.0, 153.4, 148.8, 132.2, 131.6, 129.0, 127.9, 123.7, 122.7, 108.0, 106.0, 97.7, 64.8, 44.4, 39.9, 30.6, 27.6, 26.3, 25.6, 24.1, 12.6; ESI-MS: m/z : calcd for $\text{C}_{38}\text{H}_{45}\text{N}_4\text{O}_6$: 653.3; found: 653.4 $[M + H]^+$.

To a mixture of succinimidyl activated ester (300 mg, 0.435 mmol) and amino acid **6** (0.41 g, 0.435 mmol) in dry DMF (5 ml), triethylamine (0.25 ml, 1.74 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. DMF was removed under reduced pressure and the product rhodamine B triACA **12** was purified by flash chromatography (15% methanol in DCM) in 84% yield. ^1H NMR (600 MHz, CD_3OD) δ 7.96 (s, 2H), 7.81–7.83 (m, 1H), 7.47–7.48 (m, 2H), 6.98–7.00 (m, 1H), 6.40–6.41 (d, 1H), 6.27–6.32 (m, 3H), 3.34 (q, 8H, $J = 7.2$ Hz), 3.12–3.15 (m, 2H), 3.07–3.10 (m, 2H), 3.01–3.05 (t, 2H), 2.25–2.30 (m, 2H), 2.13–2.16 (m, 2H), 1.94–1.97 (m, 2H), 1.56–1.61 (m, 4H), 1.43–1.50 (m, 4H), 1.27–1.34 (m, 6H), 1.10–1.13 (t, 12H, $J = 7.2$ Hz), 1.04–1.05 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.8, 168.6, 153.7, 153.7, 153.6, 150.7, 149.2, 132.8, 131.3, 128.5, 128.3, 123.9, 122.3, 109.9, 108.4, 105.3, 97.9, 65.9, 65.8, 39.9, 39.0, 37.9, 35.8, 33.7, 33.5, 29.0, 27.8, 26.6, 26.4, 26.3, 25.5, 25.2, 24.6, 24.5, 13.6, 11.8; ESI-MS: m/z : calcd for $\text{C}_{46}\text{H}_{64}\text{N}_5\text{O}_6$: 782.49; found: 782.9 $[M + H]^+$, calcd for $\text{C}_{46}\text{H}_{63}\text{N}_5\text{O}_6\text{Na}$: 804.5; found: 804.8 $[M + \text{Na}]^+$.

Rhodamine B TriACA APTES Conjugate (3)

To a mixture of **12** (200 mg, 0.244 mmol) and APTES (65 mg, 0.293 mmol) in dry DCM (10 ml), EDC (70 mg, 0.366 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. The solvent was evaporated and the product **3** was purified by flash chromatography (10% methanol in DCM) in 55% yield. ^1H -NMR (400 MHz, CDCl_3) δ 7.78–7.81 (m, 1H), 7.37–7.42 (m, 2H), 7.02–7.04 (m, 1H), 6.47–6.50 (bs, 1H), 6.16–6.38 (m, 5H), 3.76 (q, 8H, $J = 7.2$ Hz), 3.29 (q, 6H, $J = 6.8$ Hz), 3.02–3.20 (m, 10H), 2.23–3.36 (m, 1H), 2.09–2.15 (m, 4H), 1.95–1.99 (m, 2H), 1.53–1.62 (m, 6H),

1.34–1.49 (m, 7H), 1.03–1.33 (m, 28H), 0.56–0.60 (m, 2H); ^{13}C -NMR (100 MHz, CDCl_3) δ 173.4, 173.2, 171.1, 168.3, 168.2, 162.7, 132.5, 132.4, 129.0, 128.3, 128.2, 124.0, 122.6, 108.2, 105.8, 98.0, 77.0, 58.6, 50.6, 44.5, 42.2, 40.1, 39.4, 38.4, 36.7, 36.6, 35.2, 34.0, 31.6, 29.4, 27.8, 26.7, 26.6, 25.8, 25.6, 25.5, 25.2, 24.7, 23.8, 23.0, 18.5, 14.8, 12.7, 8.0; ESI-MS: m/z : calcd for $\text{C}_{55}\text{H}_{85}\text{N}_6\text{O}_8\text{Si}$: 985.6; found: 986.1 $[\text{M} + \text{H}]^+$.

Rhodamine B PentaACA (13)

To a mixture of rhodamine B ACA conjugate **9** (367 mg, 0.612 mmol) and *N*-hydroxysuccinimide (77.5 mg, 0.674 mmol) in dry DCM (20 ml), EDC (176 mg, 0.919 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. The solvent was evaporated and the succinimidyl activated ester product was purified by flash chromatography (1:10 methanol/DCM) in 95% yield. To a mixture of rhodamine B ACA succinimidyl ester (200 mg, 0.29 mmol) and amino acid **7** (170 mg, 0.29 mmol) in dry DMF (10 ml), triethylamine (0.16 ml, 0.116 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. DMF was removed under reduced pressure and the product **13** was purified by flash chromatography (15% methanol in DCM) in 69% yield. ^1H -NMR (400 MHz, CDCl_3) δ 7.82–7.85 (m, 1H), 7.42–7.45 (m, 2H), 7.14–7.18 (m, 1H), 7.06–7.09 (m, 3H), 6.67–6.72 (m, 1H), 6.36–6.40 (m, 4H), 6.24–6.29 (m, 2H), 3.42–3.46 (s, 1H), 3.30–3.36 (q, 8H, $J = 6.4$ Hz), 3.14–3.24 (m, 8H), 3.00–3.13 (m, 2H), 2.30–2.36 (m, 2H), 2.17–2.21 (m, 6H), 2.01–2.07 (m, 2H), 1.57–1.66 (m, 8H), 1.10–1.52 (m, 30H); ^{13}C -NMR (100 MHz, CDCl_3) δ 175.9, 173.8, 173.7, 173.6, 168.3, 153.6, 153.3, 148.8, 132.5, 131.1, 128.7, 128.1, 123.9, 122.5, 108.1, 105.5, 76.8, 65.2, 50.3, 44.4, 40.0, 39.2, 36.3, 36.2, 34.1, 29.2, 29.1, 29.0, 27.7, 26.5, 26.4, 26.3, 25.4, 25.2, 24.5, 12.6; ESI-MS: m/z : calcd for $\text{C}_{58}\text{H}_{84}\text{N}_7\text{O}_8$: 1007.65; found: 1007.0 $[\text{M} - \text{H}]^-$, calcd for $\text{C}_{58}\text{H}_{85}\text{N}_7\text{O}_8\text{Na}$: 1030.6; found: 1031.1 $[\text{M} + \text{Na}]^+$.

Rhodamine B PentaACA APTES Conjugate (4)

To a mixture of **13** (200 mg, 0.191 mmol) and APTES (47 mg, 0.211 mmol) in dry DCM (15 ml), EDC (55 mg, 0.287 mmol) was added. The reaction mixture was stirred under a N_2 atmosphere at room temperature overnight. The solvent was evaporated and the product **4** was purified by flash chromatography (15% methanol in DCM) in 70% yield. ^1H -NMR (600 MHz, CDCl_3) δ 7.81–7.83 (m, 1H), 7.42–7.44 (m, 2H), 7.07–7.09 (m, 1H), 6.99 (t, 1H, $J = 5.4$ Hz), 6.93–6.96 (m, 3H), 6.65 (t, 1H, $J = 5.4$ Hz), 6.54 (t, 1H, $J = 5.4$ Hz), 6.34–6.40 (m, 4H), 6.27 (dd, 2H, $J = 9.0$, 2.4 Hz), 3.81 (q, 6H, $J = 7.2$ Hz), 3.34 (q, 8H, $J = 7.2$ Hz), 3.16–3.22 (m, 10H), 3.10 (t, 2H, $J = 6.6$ Hz), 2.16–2.19 (m, 8H), 2.02 (t, 2H, $J = 7.2$ Hz), 1.58–1.66 (m, 8H), 1.46–1.54 (m, 8H), 1.38–1.44 (m, 2H), 1.28–1.36 (m, 8H), 1.21 (t, 9H, $J = 7.2$ Hz), 1.16 (t, 12H, $J = 7.2$ Hz), 1.07–1.14 (m, 6H), 0.63 (t, 2H, $J = 8.4$ Hz); ^{13}C -NMR (100 MHz, CDCl_3) δ 173.3, 173.2, 173.1, 168.1, 153.6, 153.3, 148.8, 132.4, 131.2, 128.8, 128.0, 123.9, 122.4, 108.0, 105.6, 97.8, 65.1, 58.4, 44.4, 42.0, 40.0, 39.2, 36.5, 36.4, 29.4, 29.3, 27.8, 26.7, 26.6, 26.5, 26.4, 25.4, 25.2, 23.0, 18.3, 12.6, 7.9; ESI-MS: m/z :

calcd for $\text{C}_{67}\text{H}_{106}\text{N}_8\text{O}_{10}\text{SiNa}$: 1233.8; found: 1234.0 $[\text{M} + \text{Na}]^+$.

p-Nitrobenzyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (16)

A mixture of 1,3,4,6-tetra-*O*-acetyl-2-phthalimido-2-deoxy-D-glucopyranoside (**15**) (3 g, 0.628 mmol), *p*-nitrobenzyl alcohol (1.06 g, 0.691 mmol) and activated MS-AW300 in DCM (50 ml) was stirred at 0°C under a N_2 atmosphere for 30 min. SnCl_4 (6.548 g, 2.51 mmol) was added dropwise using a syringe. Yellow coloration was seen. The mixture was stirred at room temperature overnight. The mixture was filtered through celite, washed successively with ice cold 2 M aqueous H_2SO_4 solution, saturated aqueous NaHCO_3 solution and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography using hexanes/EtOAc (1:1) gave the pure title compound in 86% yield. ^1H -NMR (600 MHz, CDCl_3) δ 7.91–7.93 (m, 2H), 7.7–7.78 (m, 4H), 7.20–7.28 (m, 2H), 5.76 (dd, 1H, $J = 9.6$, 10.8 Hz), 5.41 (d, 1H, $J = 8.4$ Hz), 5.18 (dd, 1H, $J = 9.6$, 9.6 Hz), 4.93 (d, 1H, $J = 13.2$ Hz), 4.59 (d, 1H, $J = 13.2$ Hz), 4.37 (dd, 1H, $J = 8.4$, 10.8 Hz), 4.32 (dd, 1H, $J = 4.8$, 12.0 Hz), 4.17 (dd, 1H, $J = 1.8$, 12.0 Hz), 3.86–3.88 (m, 1H), 2.1 (s, 3H), 2.0 (s, 3H), 1.84 (s, 3H); ^{13}C -NMR (100 MHz, CDCl_3) δ 170.9, 170.3, 169.7, 147.6, 144.4, 134.7, 131.4, 128.2, 123.8, 123.7, 98.0, 77.2, 70.7, 70.4, 68.9, 62.1, 54.8, 21.0, 20.8, 20.6; ESI-MS: m/z : calcd for $\text{C}_{27}\text{H}_{26}\text{N}_2\text{O}_{12}\text{Na}$: 593.1; found: 593.2 $[\text{M} + \text{Na}]^+$.

p-Nitrobenzyl 4,6-*O*-Benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranoside (17)

A solution of compound **16** (3 g, 5.25 mmol) in anhydrous DCM (30 ml) was cooled to -10°C under a nitrogen atmosphere. Freshly prepared sodium methoxide solution [sodium (48 mg, 2.10 mmol) in dry methanol (30 ml)] was added slowly to the reaction mixture and stirred at 0°C for 3 h. Reaction mixture was neutralized by adding Amberlyst IR 120 resin and filtered. The solvents were removed at reduced pressure to get *p*-nitrobenzyl 2-deoxy-2-phthalimido- β -D-glucopyranoside in 98% yield. ^1H -NMR (600 MHz, CD_3OD) δ 7.86–7.90 (m, 2H), 7.60–7.84 (m, 4H), 7.24–7.32 (m, 2H), 5.18 (d, 1H, $J = 9.0$ Hz), 4.92 (d, 1H, $J = 13.2$ Hz), 4.65 (d, 1H, $J = 13.2$ Hz), 4.23 (dd, 1H, $J = 9.0$, 10.8 Hz), 4.04 (dd, 1H, $J = 9.0$, 10.8 Hz), 3.94 (dd, 1H, $J = 2.4$, 12.0 Hz), 3.75 (dd, 1H, $J = 5.4$, 12.0 Hz), 3.38–3.46 (m, 2H); ^{13}C -NMR (100 MHz, CD_3OD) δ 148.3, 146.2, 135.3, 132.5, 129.1, 124.1, 98.9, 78.0, 72.2, 72.0, 70.5, 62.5, 58.2; ESI-MS: m/z : calcd for $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_9\text{Na}$: 467.1; found: 467.1 $[\text{M} + 23]^+$. *p*-Nitrobenzyl 2-deoxy-2-phthalimido- β -D-glucopyranoside (1.5 g, 0.031 mol) was azeotropically refluxed with toluene (100 ml) using a Dean-Stark apparatus for 3 h. The temperature was then lowered to 90°C . At this temperature, DL-camphorsulfonic acid (0.844 mmol) was added followed by benzaldehyde dimethylacetal (0.54 g, 0.035 mol). After 30 min, the temperature was raised to reflux temperature. The reaction was quenched by adding triethylamine. The solvents were removed under reduced pressure. Residue was extracted in ethyl acetate and washed with saturated brine. The organic layer was dried

over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography using hexanes/ethyl acetate (2:3) gave the pure compound **17** in 82% yield. ^1H NMR (400 MHz, CDCl_3) δ 7.86–7.90 (m, 2H), 7.68 (m, 4H), 7.46–7.49 (m, 2H), 7.33–7.35 (m, 3H), 7.20–7.26 (m, 2H), 5.56 (s, 1H), 5.27 (d, 1H, J = 8.8 Hz), 4.91 (d, 1H, J = 13.6 Hz), 4.57 (m, 2H), 4.38 (dd, 1H, J = 4.0, 10.4 Hz), 4.27 (dd, 1H, J = 8.8, 10.4 Hz), 3.79–3.85 (m, 1H), 3.56–3.65 (m, 2H), 2.98 (d, 1H, J = 4.0 Hz); ^{13}C -NMR (100 MHz, CDCl_3) δ 147.5, 144.6, 137.1, 134.6, 131.6, 129.7, 128.7, 128.1, 126.5, 123.8, 102.2, 98.7, 82.3, 70.3, 68.8, 68.7, 66.5, 56.7; ESI-MS: m/z : calcd for $\text{C}_{28}\text{H}_{24}\text{N}_2\text{O}_9$: Na: 555.1; found: 555.2 $[\text{M} + \text{Na}]^+$.

***p*-Aminobenzyl 3-(4-oxo-pentanoyl)-4,6-O-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranoside (18)**

A mixture of compound **17** (1 g, 1.32 mmol), 4-oxopentanoic acid (0.31 g, 2.64 mmol), dicyclohexylcarbodiimide (0.55 g, 2.64 mmol) and DMAP (32 mg, 0.26 mmol) in anhydrous DCM was stirred at room temperature overnight under a nitrogen atmosphere. The solvent was removed under reduced pressure and the residue was extracted in ethyl acetate. The precipitated dicyclohexyl urea was removed by filtration and ethyl acetate extract was washed with saturated brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography using hexanes/ethyl acetate (1:2) gave the pure compound *p*-nitrobenzyl 3-(4-oxo-pentanoyl)-4,6-O-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranoside in 81% yield. ^1H -NMR (600 MHz, CDCl_3) δ 7.85–7.92 (m, 1H), 7.66–7.86 (m, 4H), 7.43–7.45 (m, 2H), 7.32–7.36 (m, 3H), 7.20–7.28 (m, 1H), 5.91 (dd, 1H, J = 8.4, 10.8 Hz), 5.54 (s, 1H), 5.43 (d, 1H, J = 8.4 Hz), 4.93 (d, 1H, J = 13.8 Hz), 4.59 (d, 1H, J = 13.8 Hz), 4.42 (dd, 1H, J = 4.2, 10.2 Hz), 4.36 (dd, 1H, J = 8.4, 10.2 Hz), 3.85 (t, 1H, J = 9.6 Hz), 3.79 (t, 1H, J = 9.0 Hz), 3.72–3.78 (m, 1H), 2.50–2.56 (m, 1H), 2.34–2.46 (m, 3H), 1.85 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 206.0, 172.2, 147.5, 144.5, 137.0, 134.4, 129.4, 128.5, 128.1, 126.5, 123.7, 101.8, 98.7, 79.4, 70.6, 69.6, 68.8, 68.2, 66.6, 55.3, 37.9, 29.6, 28.0, 25.8; ESI-MS: m/z : calcd for $\text{C}_{33}\text{H}_{30}\text{N}_2\text{O}_{11}$: Na: 653.2; found: 653.3 $[\text{M} + \text{Na}]^+$. A round bottom flask was charged with palladium acetate (18 mg, 0.08 mmol), *p*-nitrobenzyl 3-(4-oxo-pentanoyl)-4,6-O-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranoside (1 g, 1.58 mmol) and dry THF (20 ml). The flask was sealed and purged with nitrogen. An aqueous solution of KF (184 mg, 3.17 mmol in 4 ml of degassed water) was added via a syringe. Triethylsilane (1.02 ml, 6.34 mmol) was slowly added dropwise with a syringe (caution: rapid addition of triethylsilane can result in uncontrollable gas evolution). The reaction mixture was stirred at room temperature for 1 h under nitrogen atmosphere. After completion of the reaction as indicated by TLC, flask was opened to air, diluted with diethyl ether (20 ml) and stirred for 5 min. The layers were separated and aqueous layer was back extracted with diethyl ether. The combined organic layers were filtered through a pad of celite. The filtrate was concentrated and purified by flash column chromatography using hexanes/ethyl acetate (1:2) gave the pure compound **18** in 76% yield. ^1H -NMR

(600 MHz, CDCl_3) δ 7.67–7.81 (m, 4H), 7.43 (d, 2H), 7.31–7.33 (m, 3H), 6.75–6.80 (m, 2H), 6.20–6.28 (m, 2H), 5.91 (t, 1H, J = 9.6 Hz), 5.52 (s, 1H), 5.35 (d, 1H, J = 9.6 Hz), 4.67 (d, 1H, J = 11.4 Hz), 4.40 (dd, 1H, J = 4.8, 11.4 Hz), 4.36 (d, 1H, J = 11.4 Hz), 4.29 (t, 1H, J = 9.6 Hz), 3.83 (t, 1H, J = 10.2 Hz), 3.68–3.76 (m, 2H), 3.54 (bs, 2H), 2.33–2.52 (m, 4H), 1.84 (s, 3H); ^{13}C -NMR (100 MHz, CDCl_3) δ 206.0, 172.1, 167.9, 146.6, 137.2, 134.1, 131.8, 129.7, 129.3, 128.5, 126.5, 126.4, 123.6, 114.9, 101.7, 97.6, 79.6, 71.8, 68.9, 68.9, 66.3, 55.4, 38.0, 29.6, 28.1; ESI-MS: m/z : calcd for $\text{C}_{33}\text{H}_{32}\text{N}_2\text{O}_9$: Na: 623.2; found: 623.4 $[\text{M} + \text{Na}]^+$.

***p*-(4-O-Benzoyloxycarbonyl-propionylamino)-benzyl 3-(4-oxo-pentanoyl)-4,6-O-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranoside (19)**

A mixture of compound **18** (1 g, 1.66 mmol), *O*-benzylsuccinate (416 mg, 2.0 mmol), BOP reagent (884 mg, 2.0 mmol) and DIPEA (0.87 ml, 5.0 mmol) in anhydrous DCM (40 ml) was stirred at room temperature for 48 h under a nitrogen atmosphere. The reaction mixture was washed with 1 N aqueous HCl and saturated brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography using hexanes/ethyl acetate (1:2) gave the pure compound **19** in 67% yield. ^1H -NMR (600 MHz, CDCl_3) δ 7.63–7.79 (m, 5H), 7.43–7.45 (m, 2H), 7.28–7.35 (m, 8H), 7.09–7.16 (m, 2H), 6.90–6.95 (m, 2H), 5.94 (dd, 1H, J = 9.0, 10.2 Hz), 5.53 (s, 1H), 5.35 (d, 1H, J = 7.8 Hz), 5.11 (s, 2H), 4.73 (d, 1H, J = 12.0 Hz), 4.40–4.44 (m, 2H), 4.32 (dd, 1H, J = 8.4, 10.2 Hz), 3.85 (t, 1H, J = 9.6 Hz), 3.71–3.78 (m, 2H), 2.72 (t, 2H, J = 6.6 Hz), 2.56 (t, 2H, J = 6.6 Hz), 2.34–2.53 (m, 4H), 1.85 (s, 3H); ^{13}C -NMR (100 MHz, CDCl_3) δ 206.2, 173.14, 172.2, 169.7, 138.0, 137.1, 135.9, 134.3, 132.3, 129.4, 128.9, 128.8, 128.6, 128.5, 128.4, 126.4, 123.7, 119.5, 101.7, 97.9, 79.5, 71.4, 69.8, 68.9, 66.9, 66.4, 55.3, 37.9, 32.0, 29.6, 29.5, 28.1 ESI-MS: m/z : calcd for $\text{C}_{44}\text{H}_{42}\text{N}_2\text{O}_{12}$: Na: 813.3; found: 813.5 $[\text{M} + 23]^+$.

***p*-[4-(N-(3-Triethoxysilylpropylamino)succinamido)]-benzyl 4,6-O-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranoside (14)**

A mixture of compound **19** (1g, 1.26 mmol) and hydrazine acetate (467 mg, 5.05 mmol) in DCM/MeOH (1:1, 100 ml) was stirred at room temperature for 3 h. Solvents were removed under reduced pressure and purified by flash column chromatography using hexanes/ethyl acetate (1:2) gave the pure compound *p*-(4-*O*-benzyloxycarbonyl-propionylamino-benzyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranoside in 90% yield. ^1H NMR (400 MHz, CDCl_3) δ 7.66–7.75 (m, 4H), 7.46–7.49 (m, 3H), 7.3–7.39 (m, 8H), 7.11–7.19 (m, 2H), 6.96–7.02 (m, 2H), 5.56 (s, 1H), 5.24 (d, 1H, J = 9.0 Hz), 5.13 (s, 2H), 4.76 (d, 1H, J = 12.6 Hz), 4.62 (dd, 1H, J = 9.0, 10.2 Hz), 4.44 (d, 1H, J = 12.6 Hz), 4.41 (dd, 1H, J = 4.2, 10.2 Hz), 4.26 (dd, 1H, J = 9.0, 10.2 Hz), 3.84 (t, 1H, J = 10.2 Hz), 3.59–3.65 (m, 2H), 2.77 (t, 2H, J = 6.6 Hz), 2.60 (t, 2H, J = 6.6 Hz); ^{13}C -NMR (100 MHz, CDCl_3) δ 173.1, 169.6, 168.1, 137.6, 137.0, 135.5, 134.1, 132.3, 131.3, 129.3, 128.6, 128.5, 128.3, 128.1, 126.3, 123.3, 119.3, 101.8, 97.8, 82.0, 70.9, 68.6, 68.3,

66.7, 66.1, 56.8, 31.7, 29.3; ESI-MS: m/z : calcd for $C_{39}H_{36}N_2O_{10}Na$: 715.2; found: 715.5 $[M + Na]^+$. A mixture of compound *p*-(4-*O*-benzyloxycarbonyl-propionylamino-benzyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranoside (600 mg) and palladium on activated charcoal (10%) (1.2 g) in dry methanol (50 ml) was stirred under a hydrogen atmosphere at room temperature for 30 min. The reaction mass was filtered through celite and concentrated to get the carboxylic acid in 98% yield. ^1H NMR (400 MHz, CD_3OD) δ 7.63–7.79 (m, 4H), 7.49–7.62 (m, 2H), 7.32–7.52 (m, 2H), 7.16 (d, 2H, $J = 8.4$ Hz), 6.94 (d, 1H, $J = 8.4$ Hz), 5.62 (s, 1H), 5.22 (d, 1H, $J = 8.4$ Hz), 4.68 (d, 1H, $J = 12.4$ Hz), 4.50–4.55 (m, 1H), 4.43 (d, 1H, $J = 12.4$ Hz), 4.37 (dd, 1H, $J = 4.4, 10.4$ Hz), 4.08 (dd, 1H, $J = 8.8, 10.4$ Hz), 3.83–3.88 (m, 1H), 3.58–3.63 (m, 2H), 2.62 (s, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.7, 168.3, 138.5, 137.8, 134.4, 132.6, 131.5, 128.8, 128.6, 127.9, 126.4, 123.1, 119.3, 101.8, 98.2, 81.9, 71.1, 68.5, 68.0, 66.7, 65.4, 57.8, 53.7, 31.6, 29.6; ESI-MS: m/z : calcd for $C_{32}H_{30}N_2O_{10}Na$: 625.2; found: 625.3 $[M + Na]^+$. A mixture of the carboxylic acid obtained (500 mg, 0.83 mmol), APTES (275 mg, 1.24 mmol) and EDC (239 mg, 1.24 mmol) in anhydrous DCM (15 ml) was stirred at room temperature under a nitrogen atmosphere for 5 h. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (10% methanol in DCM) producing the pure compound **14** in 77% yield. ^1H NMR (600 MHz, $CDCl_3$) δ 8.77 (s, 1H), 7.63–7.70 (m, 4H), 7.46–7.47 (m, 2H), 7.32–7.34 (m, 3H), 7.14–7.19 (m, 2H), 6.88–6.94 (m, 2H), 6.23 (m, 1H), 5.55 (s, 1H), 5.23 (d, 1H, $J = 8.4$ Hz), 4.73 (d, 1H, $J = 12.0$ Hz), 4.61 (m, 1H), 4.40 (t, 2H, $J = 9.0$ Hz), 4.24 (t, 1H, $J = 9.0$ Hz), 3.73–3.85 (m, 8H), 3.61–3.63 (m, 2H), 3.38 (s, 1H), 3.14–3.16 (m, 2H), 2.59 (t, 2H, $J = 5.4$ Hz), 2.48 (t, 2H, $J = 5.4$ Hz), 1.53–1.58 (m, 2H), 1.18 (t, 9H, $J = 7.2$ Hz), 0.57 (t, 2H, $J = 7.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 170.5, 168.1, 137.9, 137.1, 134.1, 132.2, 131.5, 129.3, 128.5, 128.3, 126.3, 123.4, 119.4, 101.9, 97.8, 82.1, 71.0, 68.7, 68.4, 66.2, 58.6, 58.5, 56.9, 42.1, 32.8, 31.5, 2.7, 18.3, 7.8 ESI-MS: m/z : calcd for $C_{41}H_{51}N_3O_{12}SiNa$: 828.3; found: 828.7 $[M + Na]^+$.

RESULTS AND DISCUSSION

Preparation of MNPs

Magnetic nanoparticles can be synthesized via either aqueous or organic solution methods. A hydrothermal reaction of $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$ in the presence of hydrazine produces Fe_3O_4 particles of about 70 nm.⁴⁰ Other aqueous solution syntheses such as coprecipitation of ferrous and ferric ions by thermal decomposition of alkaline solution of iron(III) chelate in the presence of hydrazine and by sonochemical decomposition of hydrolyzed iron(II) salt, have also been developed in recent years.^{36,41–43} An organic solution phase decomposition of $Fe(cup)_3$, $Fe(acac)_3$, or $Fe(CO)_5$ followed by oxidation led to γ - Fe_2O_3 nanoparticles.^{44,45} Surfactants such as oleic acid are often utilized to coat the surface of iron oxide nanoparticles in order to prevent particle aggregation. However, it

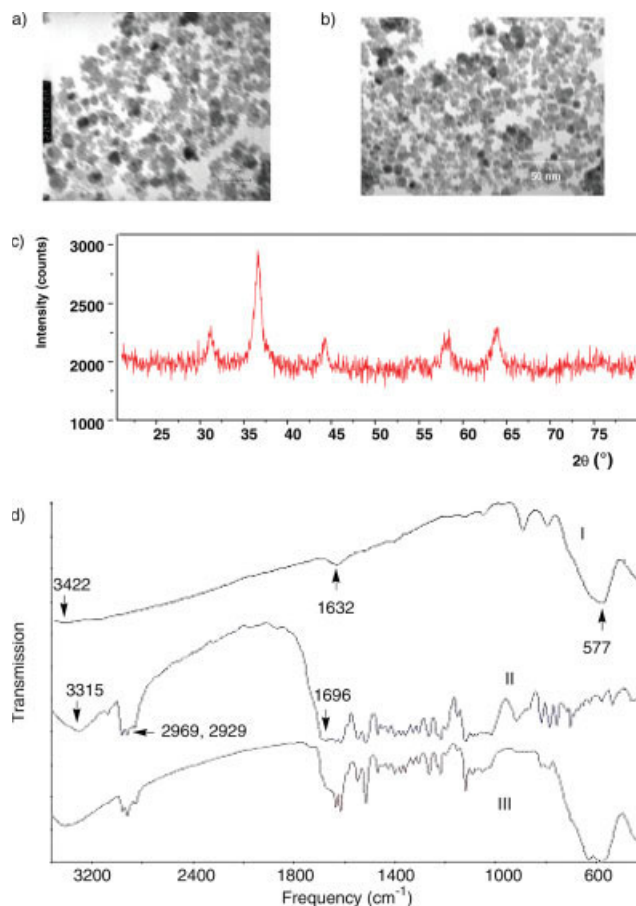
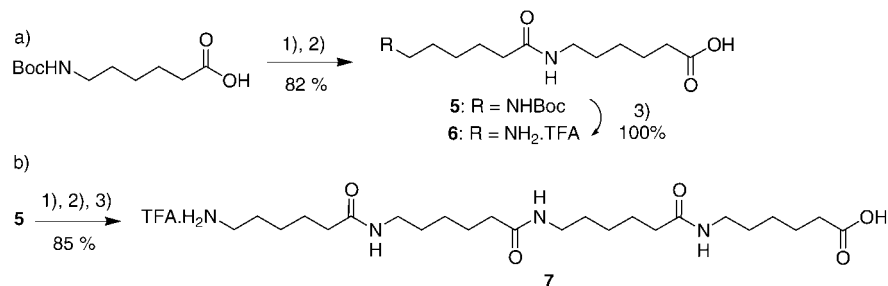


Fig. 1. (a) TEM image of uncoated MNPs. (b) TEM image of MNPs coated with rhodamine B 2. (c) XRD spectrum of uncoated MNPs. (d) IR spectra of uncoated MNPs (trace I), rhodamine 2 (trace II), and rhodamine 2 coated MNPs (trace III). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

is difficult to completely remove all surfactants for functionalization of the nanoparticle surface.

We have investigated the aqueous coprecipitation method without the use of any surfactants to obtain iron oxide nanoparticles. Several salts including $FeCl_3$, $Fe_2(SO_4)_3$ as sources for iron(III) and $FeCl_2$, $FeSO_4$ and $Fe(NO_3)_2$ for iron(II) were studied. NaOH and NH_4OH were used as the base to adjust the final pH ranging from 9 to 13. Out of all the possible combinations of iron(III) and iron(II) salts screened, most reproducible results were obtained through the titration of $FeCl_3$ (0.67 M) and $FeSO_4$ (0.33 M) in 2 M aqueous HCl solution with NH_4OH until the final pH reached between 11 and 12. Under these conditions, stable magnetic nanoparticle dispersions were consistently produced with a narrow size distribution around 10 nm as determined by TEM (Fig. 1a). Powder XRD patterns confirmed the nanocrystalline structure of Fe_3O_4 particles (Fig. 1c).³⁴ IR spectra of these MNPs showed characteristic O–H stretching vibration at 3422 cm^{-1} , O–H deformed vibration at 1632 cm^{-1} and Fe–O stretching vibration at 577 cm^{-1} (Fig. 1d, trace I).⁴¹



Reagents and Conditions: (1) *N*-hydroxysuccinimide, *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC), CH₂Cl₂, rt; (2) triethylamine, dimethylformamide, rt, ACA for compound **5**, diACA **6** for compound **7**; (3) TFA/H₂O (1:1), rt

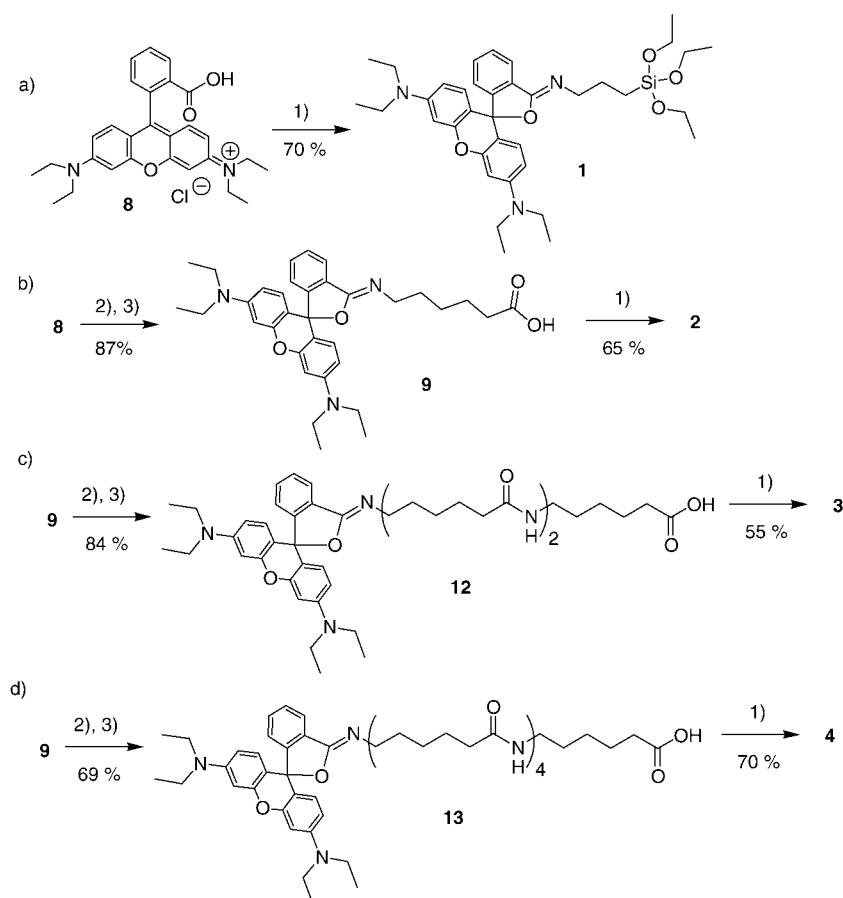
Scheme 1.

The MNPs were stable above pH 7 and could be oxidized to γ -Fe₂O₃ by air.⁴⁶

Syntheses of Rhodamine B Derivatives Containing APTES

Rhodamine B serves as a good model to study immobilization efficiencies because of its large size and its red-

shifted absorbance. Instead of adopting the stepwise approach of coating MNP with APTES first, we chose to link rhodamine B with APTES and then directly immobilize the full conjugate on to MNP in a single step. This will allow us to quantitatively determine the maximum loading capacity of MNPs. Moreover, it has been reported that coating of silica nanoparticles by APTES led to heteroge-



Reagents and Conditions: (1) APTES, EDC, CH₂Cl₂, rt; (2) NHS, EDC, CH₂Cl₂, rt; (3) triethylamine, dimethylformamide, rt, ACA for compound **9**, amino acid **6** for compound **12**, amino acid **7** for compound **13**.

Scheme 2.

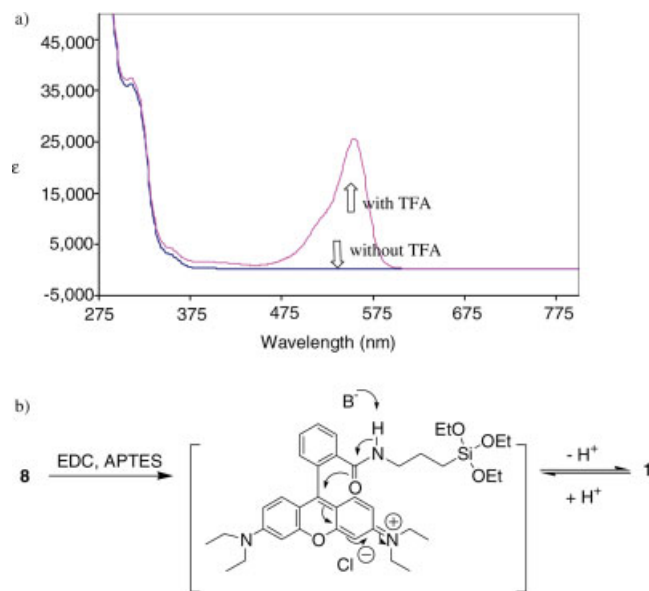
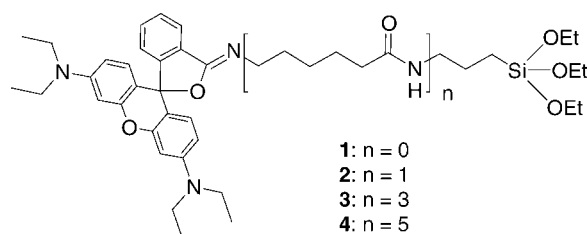


Fig. 2. (a) UV-vis spectra of rhodamine B **2** before and after addition of TFA; (b) spiro isobenzofuran formation of rhodamine B. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

neous surface coverage due to hydrogen bonding of the APTES amino group, which was alleviated by first protecting the amino group of APTES prior to silanization.⁴⁷ Derivatization of APTES first with rhodamine B may prevent hydrogen bonding and lead to more homogeneous distribution of ligands of MNP surfaces. Therefore, we synthesized rhodamine B APTES conjugates **1** to **4** with the linker length between the dye and alkoxyisilane systematically varied.



In order to prepare the linker, *N*-Boc-6-aminocaproic acid³⁹ was first converted to the NHS active ester using EDC, which was subsequently coupled to ACA to form *N*-Boc diACA **5** (Scheme 1a). Treatment of the acid **5** with TFA removed the *N*-Boc group, yielding amino acid diACA **6** quantitatively. Amino acid tetraACA **7** was synthesized in a similar fashion in 85% overall yield by coupling acid **5** with diACA **6** (Scheme 1b).

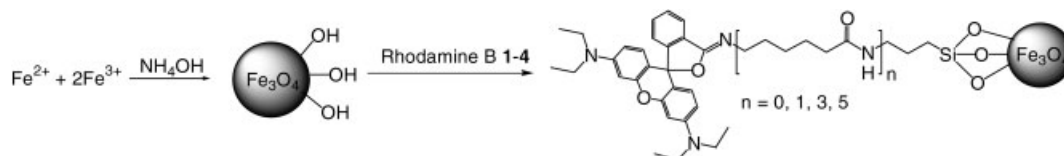
Rhodamine B **8** was directly coupled with APTES forming compound **1** in 70% yield (Scheme 2a). In order to install the linker, rhodamine B **8** was first converted to NHS active ester, which was coupled to ACA producing acid **9** in 87% yield. Amidation of **9** with APTES led to rhodamine B conjugate **2** with one ACA unit between rhodamine B and APTES (Scheme 2b). Repetition of this process with linkers **6** and **7** gave rhodamine B derivatives **3** and **4** containing three and five ACA units between rhodamine B and APTES in good overall yields (Schemes 2c and 2d). The triethoxysilane moieties in compounds **1** to **4** are hydrolytically unstable. For all reactions involving triethoxysilanes, anhydrous reaction conditions were maintained to prevent hydrolysis. The desired products were purified by quick silica gel column chromatography without any aqueous workup and are stable at room temperature when stored under a nitrogen atmosphere.

Because of its fully conjugated structure, rhodamine B **8** has the characteristic intense red color with the maximum absorbance at 554 nm. Interestingly, derivatives **1–4** are almost white, with their UV-vis spectra devoid of significant absorbance bands above 400 nm (Fig. 2a). We attribute this to the loss of full conjugation due to a side reaction, i.e., cyclization of the amide onto the center ring of the xanthene moiety under the reaction condition to form the spiro isobenzofuran (Fig. 2b). This was supported by ¹³C-NMR, as a new signal around 97 ppm was observed corresponding to the quaternary spiro carbon.⁴⁸ These rhodamine B derivatives fully regained the conjugation and color when TFA was added to the solution as evident from UV-vis spectra (Fig. 2a).⁴⁹

Immobilization of Rhodamine B Derivatives on MNPs and Surface Coverage Evaluations

We immobilized various rhodamine B conjugates on to MNPs through the silanization reaction to form a core-shell structure (Scheme 3). Upon completion of the reaction, an external magnetic field was applied to the reaction mixture separating the nanoparticles from the supernatant. Multiple cycles of redispersion, magnetically induced precipitation on nanoparticles and washing were performed to remove all rhodamine B derivatives not covalently linked to the surface of nanoparticles.

Immobilization of rhodamine B derivatives on MNPs was confirmed by SEM-EDS and IR spectrum. SEM-EDS spectrum of immobilized MNPs indicated the presence of Fe, Si, C, N, and O. IR spectrum of **2** showed NH stretching vibration at 3315 cm⁻¹, C–H vibration at 2969, 2929 cm⁻¹ and C=O stretching at 1696 cm⁻¹ (Fig. 1d, trace II), while IR of rhodamine B **2** coated MNPs contained all the characteristic absorptions due to **2** along with additional



Scheme 3.

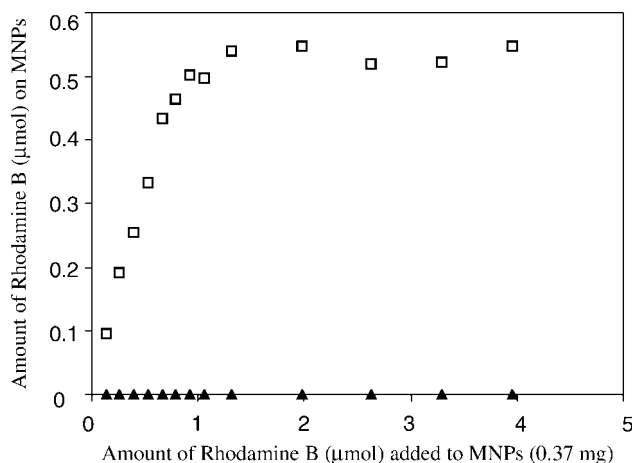


Fig. 3. The amount of rhodamine B immobilized on MNPs (0.37 mg) as calculated from subtracting the amount of un-immobilized dye from the total amount added for the silanization reaction ($\square$ for compound **2**, $\blacktriangle$ for rhodamine B **8**).

bands resulting from MNPs (Fig. 1d, trace III). TEM images of rhodamine B coated MNPs demonstrated no significant morphology changes upon immobilization (Fig. 1b).

To determine the maximum level of rhodamine B that could be loaded on an MNP surface, varying amounts of the rhodamine B APTES conjugates were added to a fixed quantity of MNP. The amount of rhodamine B attached to MNPs was first determined indirectly by subtracting the amount of dye left in the solution after the silanization reaction from the amount added. The dye concentrations were calculated from its absorbance at 554 nm after first establishing a standard calibration curve in the presence of TFA. The amount of compound immobilized on to MNP linearly increased at low concentrations of added rhodamine B until surface saturation of nanoparticles was reached as indicated by the plateau in absorbance (Fig. 3). Interestingly, maximum loading levels around 1.5×10^{-3} mol/g of MNPs were achieved for rhodamine B derivatives **1** to **3** containing 0 to 3 ACA linker units, while for rhodamine B **4**, slightly less compound (1.1×10^{-3} mol/g of MNPs) was attached (Table 1). These results indicated that linker length does not play a significant role in determining immobilization efficiencies, which alleviates concerns of possible steric effects of immobilized molecules on MNPs.

TABLE 1. Maximum loading levels of rhodamine B derivatives 1 to 4 on MNPs

Rhodamine B derivative	Maximum loading level (mol/g)	Minimum amount (mol/g) needed for surface saturation
1	1.44×10^{-3}	2.79×10^{-3}
2	1.50×10^{-3}	2.78×10^{-3}
3	1.53×10^{-3}	2.13×10^{-3}
4	1.11×10^{-3}	1.74×10^{-3}

The loading levels we obtained are approximately two orders of magnitudes higher than those previously determined by a colorimetric assay of APTES coated rhombic MNPs.³¹ The discrepancy can be explained by the smaller size and the spherical shape of our MNPs and the direct MNP derivatization method we used instead of the step-wise modification approach. In all of our studies, $\sim 60\%$ of the rhodamine B derivatives added were immobilized on nanoparticles before reaching the saturation point with the remaining compounds presumably consumed by the competitive hydrolysis reaction.³¹

In order to exclude the possibility of nonspecific binding, rhodamine B **8** devoid of the trialkoxysilane moiety was incubated with MNPs under identical conditions for silanization, and the amount of rhodamine B **8** absorbed on the MNP's surface was determined by UV-vis measurement in the same manner as described above. Even though rhodamine B **8** contains a negatively charged carboxylic acid moiety, its binding with the MNP was minimal (Fig. 3) under our experimental conditions.

It is interesting to note that while rhodamine B derivatives **1** to **4** quickly regained their characteristic red color in solution within a few seconds of TFA (20 μ L/mg) addition (Fig. 2a), UV-vis spectra of the MNP coated with rhodamine B derivatives were devoid of absorbance above 400 nm when the same amount of TFA was applied. To confirm that the unrecovered dyes were indeed present on the MNPs, we dissolved the MNP core by adding large amount of acid. Under highly acidic conditions, MNPs disintegrate releasing free rhodamine B into solution. Upon addition of excess TFA (200 μ L/mg) to MNPs containing rhodamine B **2**, the red color fully recovered only after one day. UV-vis measurement of the amount of rhodamine B released from MNPs (Fig. 4) matched that determined using the indirect method (Fig. 3). The much slower regeneration of full conjugation of rhodamine B dye on MNPs may be due to the slow dissolution of the basic iron oxide core of MNPs.

Rhodamine family dyes are popular fluorophores, which have been attached to nanoparticles for cellular imaging

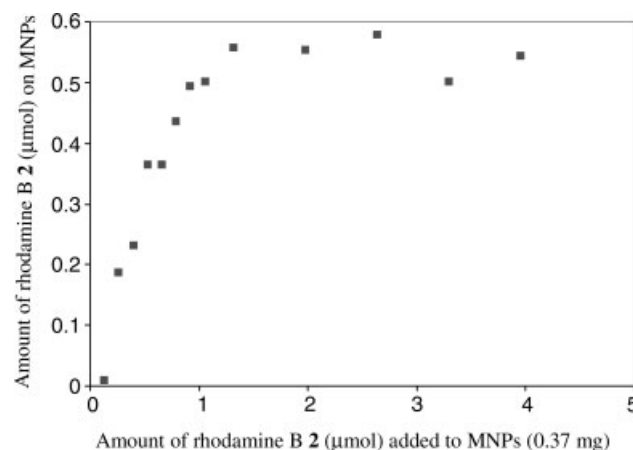
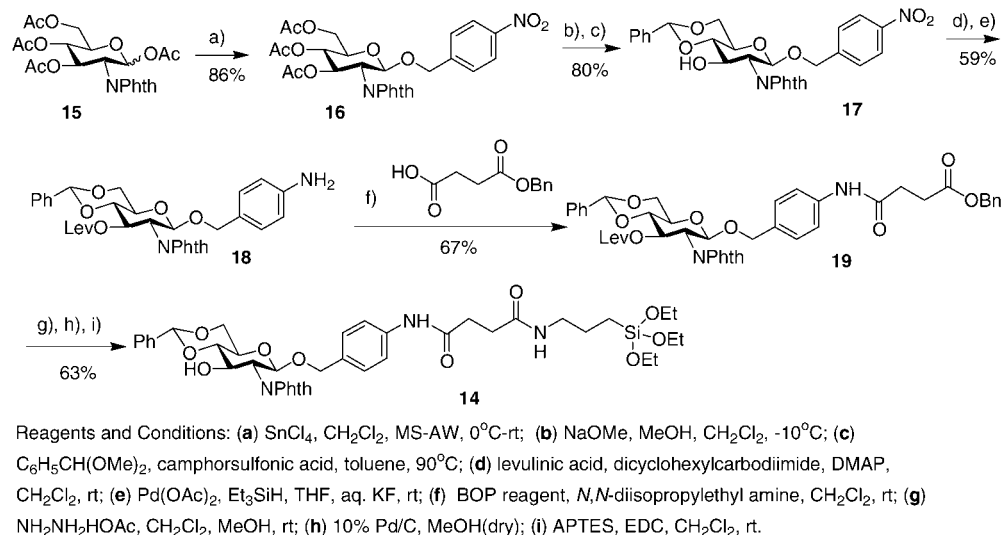


Fig. 4. The amount of rhodamine B **2** immobilized on MNPs (0.37 mg), as directly determined from UV-vis ($\lambda_{\text{max}} = 554$ nm, $\epsilon = 25,612$) after dissolving the coated MNPs with TFA.



Scheme 4.

and immunoassays.^{50,51} Our results indicate that for quantitative studies by UV-vis or fluorescence using rhodamine on nanoparticles, the formation of the spiro isobenzofuran and slow regeneration of conjugation, which can dramatically reduce its absorbance in the visible region, must be taken into consideration.

Synthesis of Carbohydrate-Modified APTES

Carbohydrates play important roles in many biological processes.⁵² Glyco-nanoparticles, i.e., nanoparticles with carbohydrates immobilized on the exterior, are emerging as promising tools for glycobiological studies.^{53–59} It is known that the density of carbohydrates on a surface is crucial for their biological functions.^{60,61} As a model for our glyco-nanoparticle studies, we chose to immobilize glucosamine derivative **14** because of important biological properties of glucosamine derivatives.⁶² The synthesis of **14** started from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-*N*-phthalimido- β -D-glucopyranoside **15**,⁶³ which was efficiently converted to β -nitrobenzyl glycoside **16** using SnCl_4 as the Lewis acid catalyst (Scheme 4). The three acetates in **16** were removed by the treatment with NaOMe at -10°C , followed by benzylidene protection leading to acetal **17** in 80% yield for the two steps. Levulinoyl protection and selective reduction of the nitro moiety⁶⁴ produced amine **18** (59% yield). Reaction of compound **18** with succinic anhydride using DMAP as the nucleophilic catalyst even at reflux conditions did not produce any desired amide due to the low nucleophilicity of anilinic nitrogen in **18**. Amidation of compound **18** by *O*-benzylsuccinate with EDC/DMAP failed as well. Finally amide **19** was produced in 67% yield using BOP reagent/DIPEA at room temperature for 48 h. Deprotection of the levulinoyl group and subsequent controlled hydrogenation to generate the carboxylic acid followed by amidation of APTES afforded target compound **14** in 63% yield. The compound **14** is stable at room temperature when stored under a nitrogen atmosphere.

Immobilization of Sugar-Modified APTES on MNPs and Surface Coverage Evaluations

In order to determine the maximum amount of glucosamine derivative **14** that can be loaded on MNPs, increasing concentrations of **14** were added to a fixed quantity of uncoated MNPs. Upon completion of the silanization reaction (Fig. 5a), an external magnetic field was applied to the reaction mixture separating the nanoparticles from the supernatant, followed by multiple cycles of redispersion, magnetically induced precipitation of MNPs and washing. TEM images of the resulting glyco-nanoparticles indicated no significant morphology changes. SEM-EDS demonstrated the presence of N, C, Fe, Si, and O on MNPs. IR spectrum of **14** showed characteristic broad absorption at 3316 cm^{-1} due to N–H and O–H stretching vibrations,

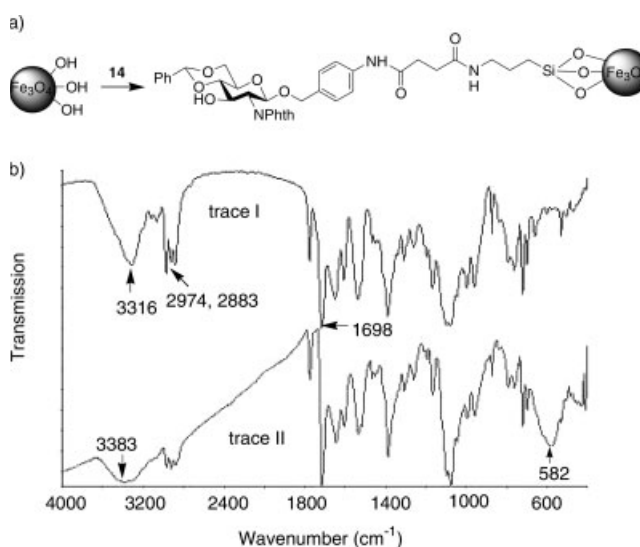


Fig. 5. (a) Attachment of glucosamine **14** to MNPs through the silanization reaction; (b) IR spectra of glucosamine **14** (trace I) and glucosamine **14** coated MNPs (trace II).

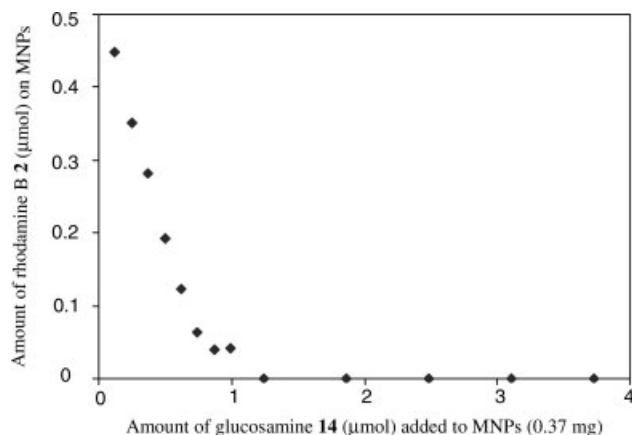


Fig. 6. With increasing amounts of glucosamine **14** used for silanization reaction, the amounts of rhodamine **B 2** that could be attached on glyco-nanoparticles decreased.

C—H stretching vibration at 2974, 2883 cm^{-1} and C=O stretching at 1698 cm^{-1} (Fig. 5b, trace II). Glyco-nanoparticles showed all IR absorptions due to **14** along with characteristic O—H stretching vibration at 3383 cm^{-1} and Fe—O stretching vibration at 582 cm^{-1} from MNPs (Fig. 5b, trace I), thus confirming the immobilization of **14** on MNPs.

Because glucosamine **14** lacks chromophores absorbing in the visible region, it is difficult to directly determine its loading level on glyco-nanoparticles by UV-vis spectroscopy due to interference from the light scattered by MNPs. In order to quantify carbohydrates on glyco-nanoparticles, a fixed amount of rhodamine **B 2** was added to glyco-nanoparticles. For glyco-nanoparticles prepared with increasing concentrations of glucosamine **14**, the additional amount of rhodamine **B 2** that could be immobilized on to these nanoparticles decreased, which reduced to zero after 1.0 μmol of glucosamine **14** was added to MNPs (0.37 mg) (Fig. 6). This indicated that the maximum loading level of glucosamine **14** on MNPs was 1.6×10^{-3} mol/g assuming 60% of **14** added was immobilized. The maximum amount of **14** that can be attached on MNPs is similar to that of rhodamine **B**, thus confirming that the silanization reaction is not influenced by the identity of the molecules to be loaded. Furthermore, the presence of a free hydroxyl group in glucosamine **14** does not interfere with loading. This signifies that the silanization approach may be used as a general method to functionalize MNPs.

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Chiroptical Properties and Racemization Behavior of Highly Distorted Donor-Acceptor Tetracyanoanthraquinodimethane with Interconvertible Planar Chirality

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT Absolute configuration of optically active 7,12-bis(dicyanomethylene)-7,12-dihydrobenz[a]anthracene (**1**) with interconvertible planar chirality was determined by a comparison of experimental and theoretical circular dichroism (CD) spectra. Upon standing at ambient temperatures, **1** was found to spontaneously racemize, the rates of which were assessed through the separation profiles of chiral HPLC at 5–20°C and also from the CD spectral decay profiles at the same temperatures. Mechanism of the racemization was discussed. *Chirality* 20:278–281, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: planar chirality; tetracyanoquinodimethane; circular dichroism; TD-DFT calculation; charge-transfer interaction; absolute configuration; racemization

INTRODUCTION

Chiral interconvertible molecules have widely been investigated as potential molecular devices that utilize heat or photon as a driving force to change their molecular conformation.^{1–3} Tetracyanoanthraquinodimethane (TCNAQ), which possesses unique planar chirality, is one such molecule, but its enantiomers are known to rapidly equilibrate to each other in solution at ambient temperatures. Although butterfly-shaped TCNAQs with chiral amide auxiliary were recently employed in asymmetric photoreactions,⁴ the chiroptical properties of such derivatives have not yet been reported.⁵ Our continued interest in chiral donor-acceptor systems^{6–10} prompted us to investigate the chiroptical properties of 7,12-bis(dicyanomethylene)-7,12-dihydrobenz[a]anthracene (**1**) (Scheme 1). The large steric hindrance between one of the cyano groups and the naphthalene moiety is expected to slow the racemization process.

MATERIALS AND METHODS

A racemic mixture of 7,12-bis(dicyanomethylene)-7,12-dihydrobenz[a]anthracene ((*R,R*)/(*S,S*)-**1**) was prepared by double dicyanomethylation of the corresponding 1,4-benzoquinone.¹¹ The enantiomers were separated by temperature-controlled chiral HPLC equipped with a Daicel Chiralpack IB (5.0 μ m, 4.6 $\times$ 250 mm) under the following conditions: flow rate: 0.35 ml/min; eluent: methanol (Wako, HPLC grade); column temperature: 5°C, detection wavelength (UV and CD): 330 nm. Under these conditions, a pair of enantiomerically pure **1** eluted at 21 and 35 min.

Electronic absorption (UV-vis) and circular dichroism (CD) spectra were measured in a conventional 1-cm quartz

cell on JASCO V-560 and J-820 spectrometers with a Unisoku temperature controller.

All the calculations were performed on LINUX-PCs using TURBOMOLE 5.6 program suite.¹² The resolution of identity (RI) approximation was employed in all geometry optimizations, and the corresponding auxiliary basis sets were taken from the TURBOMOLE basis set library. Structures were fully optimized at the DFT-D-BLYP/TZV2P level with an increased numerical grid (m4), and the CD (and UV-vis) spectra were simulated at the TD-DFT-BH-LYP/TZV2P level.^{13–15}

RESULTS AND DISCUSSION

The optical resolution of (*R,R*)/(*S,S*)-**1** was carried out by chiral HPLC at 5°C. Despite the steric hindrance between the cyano group and the naphthalene moiety, racemization of **1** occurred rapidly at an ambient temperature as demonstrated by the reduced CD intensity of separated **1**, but was practically suppressed at temperatures of <0°C. Thus, enantiopure **1**, kept at <0°C, was examined by UV-vis and CD spectroscopy immediately after HPLC separation (Fig. 1). The mirror-imaged CD spectra thus obtained indicate the successful separation of both enantiomers of

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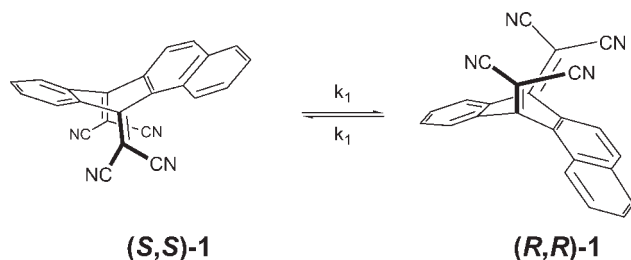
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Scheme 1. Racemization of 7,12-bis(dicyanomethylene)-7,12-dihydrobenz[a]anthracene (**1**).

1. In addition to the typical L_a and L_b bands that appeared at 240–310 and 310–400 nm, respectively, the broad CT transition was observed at 400–500 nm.

Absolute configuration of **1** was determined by a comparison of the experimental and the theoretical CD spectra (Fig. 2). The geometry optimization of **1** turned out to give two local-minimum structures **1a** and **1b**, the former of which is much more stable ($\Delta E > 50$ kJ/mol). Comparison of the simulated CD spectrum of **1a** with experimental data allows us to safely assign the second HPLC fraction eluted from Chiralpack IB as the (S,S)-enantiomer. In view of the significantly unstable nature of **1b** (with a 50 kJ/mol higher energy) and the steric hindrance between the naphthalene moiety and one of the cyano groups, it is rea-

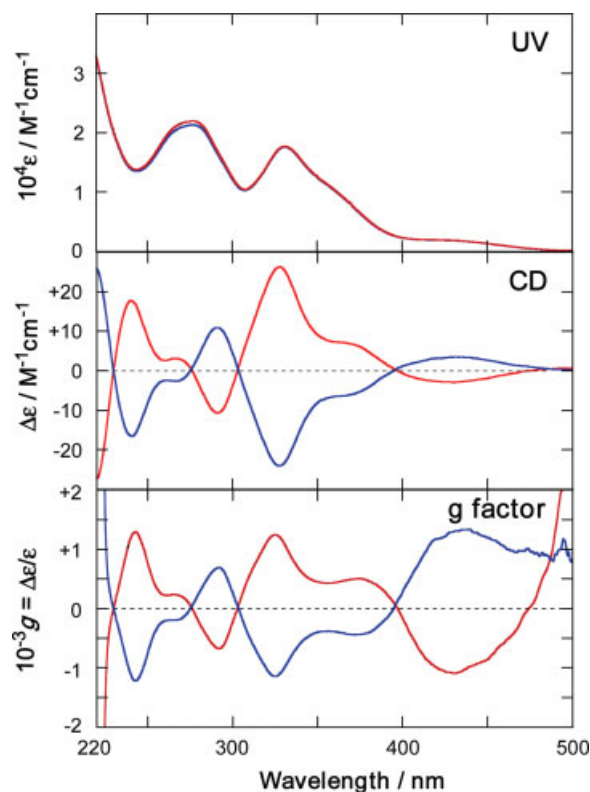


Fig. 1. UV-vis and CD spectra and g factor of (R,R)/(S,S)-**1** in methanol at 5°C (red: (R,R)-**1**, blue: (S,S)-**1**). For an assignment of absolute configuration, see text. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

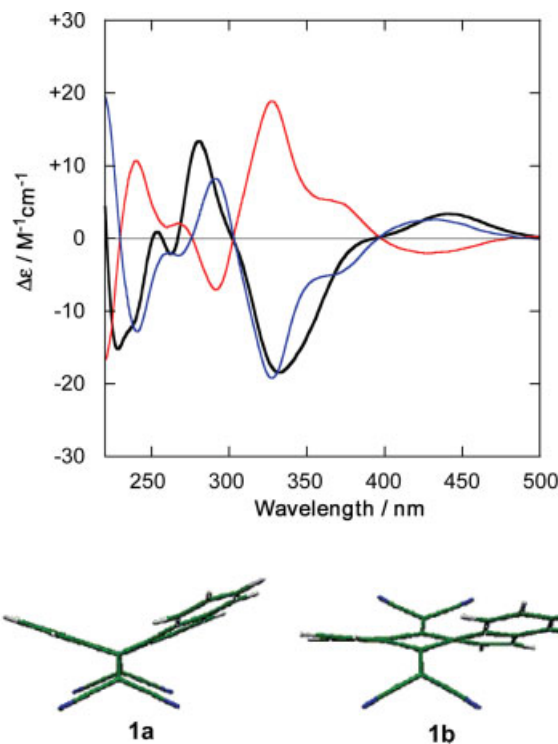


Fig. 2. Comparison of the experimental (blue: 2nd elute) and the calculated (black: for **1a**) CD spectra. Theoretical spectrum was red-shifted by 0.5 eV and scaled to 1/3. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sonable to consider that **1a** racemizes not through the flip-flop mechanism but via a biradicaloid or zwitterionic intermediate (Fig. 3).^{16,17}

When chiral HPLC was performed at ambient temperature, enantiomeric (R,R)/(S,S)-**1** were not completely separated and a characteristic plateau appeared between the two peaks due to the racemization on the column (Fig. 4). The column temperature critically influenced the chiral HPLC separation and the plateau between the peaks became higher at higher temperatures, as a result of accelerated interconversion of enantiomeric **1**. The rate of racemization can be determined through the theoretical treatment of these elution profiles as reported by Trapp et al.¹⁸ and Trapp and Schurig.¹⁹ All the parameters required for evaluating the racemization rate constants (k_1) are listed in Table 1.

The logarithms of the rate constants were plotted against the reciprocal temperature (Fig. 5) to give the activation parameters for racemization of **1**: $\Delta H^\ddagger = 92.5$ kJ/mol, $\Delta S^\ddagger = 16.5$ J/molK. The activation enthalpy obtained is much smaller than those reported for hexahelicene derivatives ($\Delta H^\ddagger = 146$ –172 kJ/mol). Intriguingly, the racemization of **1** is entropically favored ($\Delta S^\ddagger > 0$), which is in sharp contrast to the activation parameters reported for most racemization processes.²⁰ Such a positive activation entropy may be explained in terms of the extensive desolvation of methanol from CT-interacting **1** upon racemization.

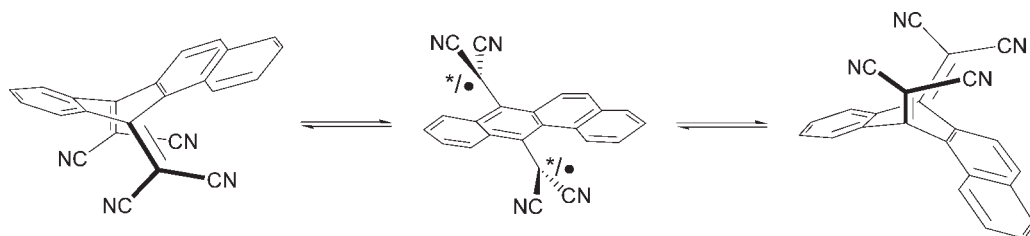


Fig. 3. Postulated mechanism of the racemization of **1**.

Racemization rate constants were also estimated by the decay profiles of CD spectra. The logarithm of the CD intensity monitored at 330 nm was plotted against time (Fig. 6), and the rate constants of racemization were obtained by fitting to be $(39 \pm 2) \times 10^4 \text{ s}^{-1}$. The rate

thus obtained was nearly 3 times larger than that obtained by HPLC (Table 1). This large discrepancy may be ascribed to the photoactivation of **1** with the routine CD instrument, which accelerates the racemization process.^{21,22}

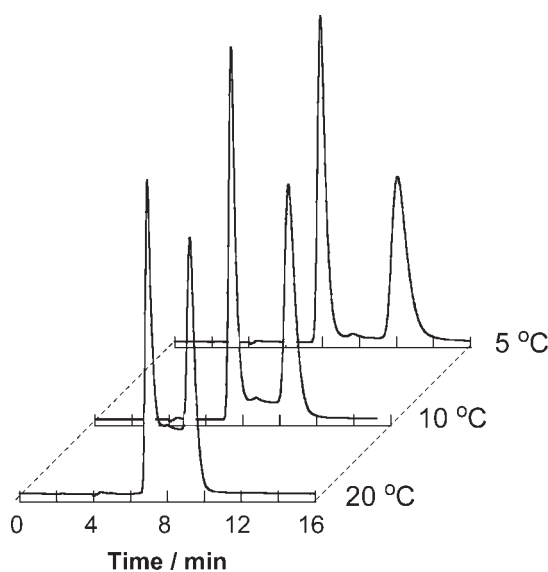


Fig. 4. Separation profiles of **1** on chiral HPLC analyses over a Daicel Chiralpack IB column with methanol as eluent at several temperatures.

TABLE 1. Experimental parameters extracted from the HPLC elution profiles of **1** and the racemization rate constants determined therefrom^a

Temp (°C)	t_1 (min)	t_2 (min)	$\Delta t = t_1 - t_2$	w_1 (s)	w_2 (s)	H_p (%)	k_1 (10^4 s^{-1})
5	21.1	34.6	13.6	33.3	78.9	1.8	1.5
10	19.9	31.1	11.2	30.9	63.0	5.7	3.9
15	19.0	28.2	9.2	28.2	50.4	13	7.2
20	18.1	25.6	7.5	27.6	43.8	33	13

^aObtained upon chiral HPLC analyses under the following conditions. Column: Daicel Chiralpack IB; flow rate: 0.35 ml/min; eluent: methanol (Wako, HPLC grade); column temperature: 5°C; detection wavelength (UV and CD): 330 nm. Parameters are defined after Schurig and Trapp^{18,19}; t_1 and t_2 : retention times of the respective enantiomers of **1**; w_1 and w_2 : widths at half-height of the peaks for the respective enantiomer of **1**; H_p : the plateau height at the mean retention time; k_1 : calculated rate of racemization according to Ref. 18.

Chirality DOI 10.1002/chir

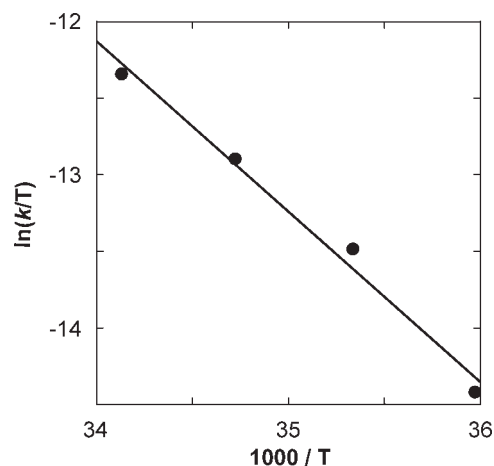


Fig. 5. Eyring plot for the racemization of **1** in methanol.

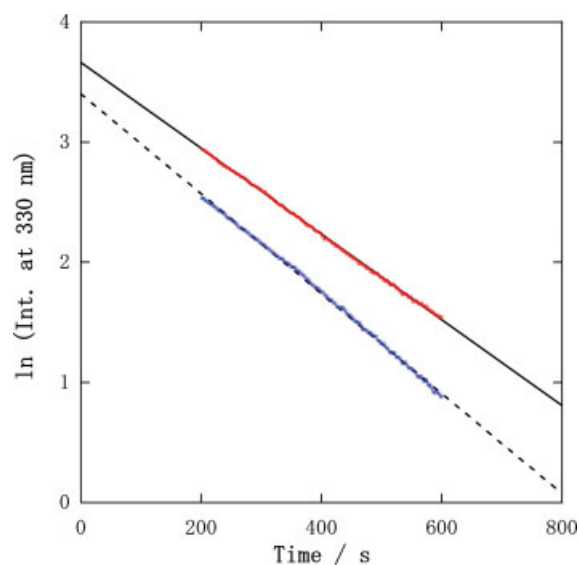


Fig. 6. CD decay profiles of the enantiomeric pair of **1** (red: 1st fraction, blue: 2nd fraction) in methanol at 20°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CONCLUSIONS

In the present study, we have successfully resolved the enantiomeric pair of 7,12-bis(dicyanomethylene)-7,12-dihydrobenz[a]anthracene (**1**) to reveal their chiroptical properties and determined the absolute configuration through comparison of the experimental CD spectra with the theoretical ones. Chiral **1** turned out to undergo fast racemization at an ambient temperature, the rate of which was determined at various temperatures from the chiral HPLC profiles. Despite the large steric hindrance between one of the cyano substituents and the naphthalene ring, the racemization was found to be significantly fast: $k_1 = 1.3 \times 10^5 \text{ s}^{-1}$ at 20°C, probably through the diradicaloid or zwitterionic intermediate. The activation parameters for this process were also determined ($\Delta H^\ddagger = 92.5 \text{ kJ/mol}$, $\Delta S^\ddagger = 16.5 \text{ J/molK}$) from the Eyring plot; the unusual positive activation entropy may be rationalized by extensive desolvation upon racemization of **1** in methanol.

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Partial Resolution of Racemic *trans*-4-[5-(4-Alkoxyphenyl)-2,5-dimethylpyrrolidine-1-oxyl-2-yl]benzoic Acids by the Diastereomer Method with (*R*)- or (*S*)-1-Phenylethylamine

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT The partial resolution is described of a series of racemic *trans*-4-[5-(4-alkoxyphenyl)-2,5-dimethylpyrrolidine-1-oxyl-2-yl]benzoic acids (**1**), which are the key intermediates for the synthesis of chiral organic radical liquid crystalline compounds and are crystallized to give racemic compounds. Racemic acid **1** [(±)-**1**] with a long alkyl chain (C7 to C13) could be resolved by the conventional diastereomeric salt formation using (*R*)- or (*S*)-1-phenylethylamine (**2**) as the resolving agent, whereas resolution of (±)-**1** with a short alkyl chain (C4 to C6) was unsuccessful. Use of six equiv of (*R*)- or (*S*)-**2** for the initial diastereomeric salt formation of (±)-**1** with a C7–C13 alkyl chain, followed by recrystallization of the resulting salts once or twice, gave 2*S*,5*S*- or 2*R*,5*R*-enriched **1**, respectively, in an *ee* range of 75–92% and with an overall recovery of 11–27%, based on the original quantity of (±)-**1**. *Chirality* 20:282–287, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: diastereomer method; optical resolution; chiral nitroxide; crystal structure; 1-phenylethylamine; racemic compound; resolving agent; chiral amine; diastereomeric salt

INTRODUCTION

Paramagnetic rod-like liquid crystals have attracted great interest as soft materials to enhance the effect of magnetic field on the optical and electric properties of liquid crystals.^{1,2} In this context, we reported the synthesis and the electric and magnetic properties of chiral all-organic radical liquid crystalline (LC) compounds, racemic and enantiomerically enriched *trans*-5-alkoxyphenyl-2-[4-(4-alkoxybenzenecarbonyloxy)phenyl]-2,5-dimethylpyrrolidine-1-oxyls (**3**)^{3–5} and 4-alkoxyphenyl *trans*-4-[5-(4-alkoxyphenyl)-2,5-dimethylpyrrolidine-1-oxyl-2-yl]benzoates (**4**) which contain a polar and chiral cyclic-nitroxide unit in the mesogen core and show nematic (N) or chiral nematic (N*), smectic C (SmC) or chiral smectic C (SmC*), and/or smectic A (SmA) or chiral smectic A (SmA*) phases, respectively (Scheme 1). Although these racemic samples were easily prepared from the readily available racemic nitroxide **5** (Scheme 2),^{6,7} the synthesis of a large quantity of (*R*)-**5** was troublesome because the process includes the less efficient enantioselective reduction of 2,5-hexanedione with baker's yeast,⁸ and furthermore a different procedure must be used for the synthesis of the (*S*)-**5** enantiomer.⁹ To obtain both (*R*)- and (*S*)-**3** or **4** efficiently, enantiomeric resolution of any of the racemic intermediates is supposed to be appropriate. Nowadays, diastereoselective enantiomeric resolution methods have significantly advanced; a number of resolving agents are listed^{10,11} and

the logical improvement and extension for efficient resolution, such as the Dutch resolution method,¹² the dielectrically controlled resolution (DCR) method,^{13,14} and the host-guest inclusion complexation method,¹⁵ have emerged.

Here we report the successful enantiomeric resolution of a series of racemic *trans*-4-[5-(4-alkoxyphenyl)-2,5-dimethylpyrrolidine-1-oxyl-2-yl]benzoic acids [(±)-**1**], which are the key intermediate carboxylic acids for the synthesis of enantiomerically enriched **4**, by the conventional diastereomeric salt formation using a chiral amine as the resolving base.

MATERIALS AND METHODS

Infrared spectra were recorded by the diffuse reflectance method using KBr. HPLC analysis was carried out by using a chiral stationary phase column (Daicel Chiralcel OD-H, 0.46 cm × 25 cm), a mixture of hexane, 2-propa-

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center (CCDC-639695).

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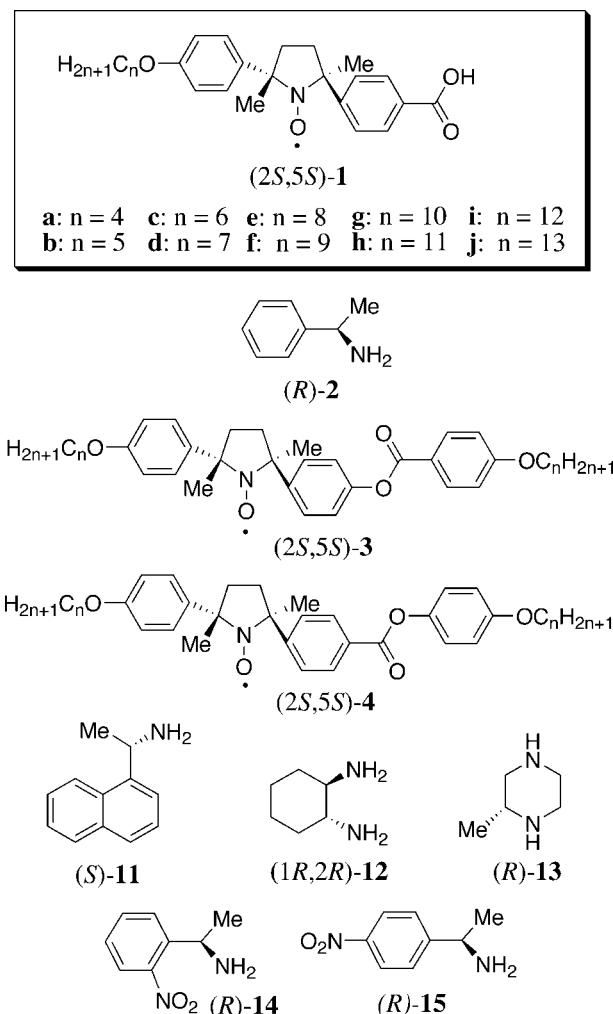
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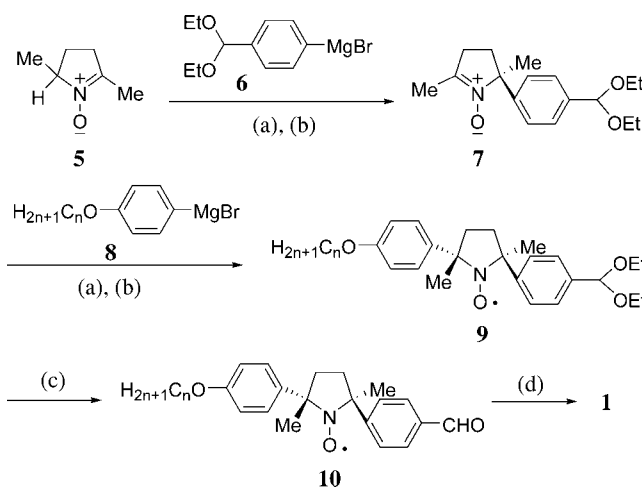
Scheme 1. Molecular structures.

sol, and trifluoroacetic acid (180:20:1) as the mobile phase at a flow rate of 0.5 ml min⁻¹, and a UV-vis spectrometer (254 nm) as the detector. X-band electron paramagnetic resonance (EPR) spectra were recorded in tetrahydrofuran (THF). X-ray diffraction (XRD) patterns were recorded at continuous scanning rate of 2° 2θ min⁻¹ and at a heating or cooling rate of 4°C min⁻¹ using Cu Kα radiation (40 kV, 40 mA), with the intensity of diffracted X-rays being collected at intervals of 0.02° 2θ. Unless otherwise noted, solvents and reagents were reagent grade and used without further purification. THF which is used for EPR spectral measurement or Grignard reactions was distilled from sodium/benzophenone ketyl under argon.

General Synthetic Procedure for (±)-1

Synthesis of (±)-1 was carried out by referring to the published procedure for analogous compounds (Scheme 2).⁷ To a solution of racemic nitron 5⁶ (5.0 mmol) in THF (10 ml) was slowly added a solution of the freshly prepared Grignard reagent 6 (10 mmol) in THF under argon at -78°C. The reaction mixture was slowly warmed to room temperature, and stirring continued overnight. The

reaction mixture was poured into saturated aqueous NH₄Cl (50 ml) solution, and then extracted with CH₂Cl₂ (2 × 50 ml). The combined organic extract was dried over MgSO₄ and evaporated. The residual oil was dissolved in methanol (20 ml). To this solution were added aqueous NH₃ solution (25 wt %, 1.4 ml) and cupric acetate monohydrate (0.160 g, 0.80 mmol). Oxygen was bubbled through the yellow solution until a persistent deep blue color was observed. The solvent was then removed under reduced pressure and the crude product was dissolved in CH₂Cl₂ (50 ml). This solution was washed with saturated aqueous NaHCO₃ (50 ml) solution and dried over MgSO₄, and the solvent was removed under reduced pressure. The residue was dried by azeotropic removal of water with benzene, followed by removal of the remaining benzene at 3 mm Hg. The crude acetal 7 was dissolved in THF (10 ml) and reacted with the freshly prepared Grignard reagent 8 (the same quantity and conditions as described above). After a similar workup procedure, the crude product was again oxidized by O₂/cupric acetate. The crude nitroxide 9 was purified by column chromatography (hexane/ether) on silica gel. To a solution of 9 in THF (2 ml) was added aqueous 5% H₂SO₄ solution (0.50 ml) and the mixture was stirred for 8 h at 25°C. Water (20 ml) and CH₂Cl₂ (50 ml) were then added. The organic phase was separated and dried over MgSO₄, and the solvent was evaporated under reduced pressure to provide 2-(4-alkoxyphenyl)-5-(4-formylphenyl)-2,5-dimethylpyrrolidine-1-oxyl (10). To a vigorously stirred suspension of Ag₂O (463 mg, 2.0 mmol) in aqueous 10% NaOH solution (4 ml) was added the crude 10 in THF (1.5 ml) at 60°C, and the mixture was stirred under reflux for 40 min. After cooling, the reaction mixture was acidified with aqueous 5% H₂SO₄ solution to pH 2, and extracted with CHCl₃ (3 × 10 ml). The organic phase was dried over MgSO₄ and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (CH₂Cl₂/ether) to give *trans*-4-[5-(4-alkoxyphenyl)-2,5-dimethylpyrrolidine-1-oxyl-2-yl]benzoic acid [(±)-1] as a yellow powder.



Scheme 2. Reaction (a): THF, -78°C. Reaction (b): Cu(OAc)₂, O₂. Reaction (c): H₂SO₄, H₂O, THF. Reaction (d): Ag₂O, NaOH, H₂O, THF, reflux.

(±)-**1a**: mp 172.8–173.0°C; EPR $g = 2.0058$, $a_N = 1.33$ mT. IR 2927, 2867, 2671, 2551, 1949, 1685, 1604, 1570, 1506, 1315, 1279, 1242, 1180, 1121, 1067, 1015, 973, 864, 845, 779, 712 cm^{-1} . Anal. Calcd. for $\text{C}_{23}\text{H}_{28}\text{NO}_4$: C, 72.23; H, 7.38; N, 3.66; Found: C, 72.05; H, 7.47; N, 3.49.

(±)-**1b**: mp 170.5–171.5°C; EPR $g = 2.0056$, $a_N = 1.34$ mT. IR 2937, 2872, 2656, 2549, 1902, 1689, 1607, 1510, 1424, 1289, 1247, 1185, 1126, 1051, 1016, 987, 839, 780, 707 cm^{-1} . Anal. Calcd. for $\text{C}_{24}\text{H}_{30}\text{NO}_4$: C, 72.70; H, 7.63; N, 3.53; Found: C, 72.89; H, 7.62; N, 3.48.

(±)-**1c**: mp 168.4–169.3°C; EPR $g = 2.0059$, $a_N = 1.33$ mT. IR 2949, 2859, 2655, 2542, 1903, 1684, 1606, 1505, 1424, 1290, 1247, 1182, 1075, 1017, 867, 839, 780, 708 cm^{-1} . Anal. Calcd. for $\text{C}_{25}\text{H}_{32}\text{NO}_4$: C, 73.14; H, 7.86; N, 3.41; Found: C, 73.04; H, 7.81; N, 3.31.

(±)-**1d**: mp 168.3–169.2°C; EPR $g = 2.0055$, $a_N = 1.34$ mT. IR 2919, 2853, 2656, 2540, 1903, 1685, 1607, 1570, 1424, 1285, 1247, 1185, 1127, 1074, 1008, 949, 840, 780, 707 cm^{-1} . Anal. Calcd. for $\text{C}_{26}\text{H}_{34}\text{NO}_4$: C, 73.55; H, 8.07; N, 3.30; Found: C, 73.69; H, 8.01; N, 3.22.

(±)-**1e**: mp 165.3–166.2°C; EPR $g = 2.0059$, $a_N = 1.34$ mT. IR 2914, 2853, 2667, 2542, 1908, 1700, 1507, 1315, 1180, 1126, 1051, 1016, 961, 867, 844, 780, 708 cm^{-1} . Anal. Calcd. for $\text{C}_{27}\text{H}_{36}\text{NO}_4$: C, 73.94; H, 8.27; N, 3.19; Found: C, 73.90; H, 8.21; N, 3.16.

(±)-**1f**: mp 160.5–161.5°C; EPR $g = 2.0060$, $a_N = 1.34$ mT. IR 2937, 2851, 2676, 2553, 1903, 1695, 1607, 1576, 1507, 1288, 1249, 1180, 1129, 1118, 1019, 859, 838, 777, 705 cm^{-1} . Anal. Calcd. for $\text{C}_{28}\text{H}_{38}\text{NO}_4$: C, 74.30; H, 8.46; N, 3.09; Found: C, 74.52; H, 8.36; N, 3.02.

(±)-**1g**: mp 160.0–161.2°C; EPR $g = 2.0060$, $a_N = 1.34$ mT. IR 2937, 2851, 2676, 2553, 1903, 1695, 1607, 1576, 1507, 1288, 1249, 1180, 1129, 1118, 1019, 859, 838, 777, 705 cm^{-1} . Anal. Calcd. for $\text{C}_{29}\text{H}_{40}\text{NO}_4$: C, 74.64; H, 8.64; N, 3.00; Found: C, 74.83; H, 8.65; N, 2.90.

(±)-**1h**: mp 157.5–158.3°C; EPR $g = 2.0060$, $a_N = 1.33$ mT. IR 2915, 2849, 2676, 2553, 1910, 1685, 1607, 1471, 1424, 1290, 1244, 1178, 1127, 1113, 1072, 948, 843, 780, 708 cm^{-1} . Anal. Calcd. for $\text{C}_{30}\text{H}_{42}\text{NO}_4$: C, 74.96; H, 8.81; N, 2.91; Found: C, 75.23; H, 9.00; N, 3.04.

(±)-**1i**: mp 157.8–158.7°C; EPR $g = 2.0061$, $a_N = 1.33$ mT. IR 2914, 2849, 2658, 2553, 1910, 1685, 1606, 1505, 1471, 1424, 1290, 1247, 1179, 1127, 1073, 1017, 950, 867, 845, 780, 708 cm^{-1} . Anal. Calcd. for $\text{C}_{31}\text{H}_{44}\text{NO}_4$: C, 75.27; H, 8.97; N, 2.83; Found: C, 75.44; H, 9.07; N, 2.77.

(±)-**1j**: mp 155.8–157.2°C; EPR $g = 2.0060$, $a_N = 1.33$ mT. IR 2915, 2849, 2661, 2541, 1910, 1685, 1620, 1607, 1507, 1472, 1424, 1290, 1243, 1179, 1072, 1051, 867, 843, 780 cm^{-1} . Anal. Calcd. for $\text{C}_{32}\text{H}_{46}\text{NO}_4$: C, 75.55; H, 9.11; N, 2.75; Found: C, 75.56; H, 9.34; N, 2.71.

General Procedure for Enantiomeric Resolution of (±)-**1** by the Diastereomer Method

To a solution of (±)-**1** (1.00 mmol) in Et_2O (50 ml) was added (*R*)- or (*S*)-**2** (6.00 mmol) and the mixture was allowed to stand for 24 h at 25°C. The resulting fine powder of **1·2** salt (1:1 ratio) was collected and recrystallized from Et_2O (50 ml). The deposited powder was again recrystallized from Et_2O (50 ml). The obtained powder was hydrolyzed with aqueous 2 N HCl solution and the

aqueous mixture was extracted with CH_2Cl_2 (3×30 ml). The combined organic phase was dried over MgSO_4 , filtered, and concentrated to give enantiomerically enriched **1** as a yellow powder.

(2*S*,5*S*)-**1j·(R)-2** (1:1) salt: Anal. Calcd. for $\text{C}_{40}\text{H}_{57}\text{N}_2\text{O}_4$: C, 76.27; H, 9.12; N, 4.45; Found: C, 76.02; H, 8.87; N, 4.20.

(2*S*,5*S*)-**1d** (83.9% *ee*): mp 125.2–126.1°C; IR 2917, 2854, 2656, 2540, 1903, 1685, 1606, 1570, 1424, 1286, 1247, 1184, 1128, 1071, 1007, 950, 840, 781, 708 cm^{-1} . $[\alpha]_D^{25} = -112.1^\circ$ (c 0.116, THF).

(2*R*,5*R*)-**1e** (72.8% *ee*): mp 134.1–135.6°C; IR 2914, 2853, 2540, 1906, 1701, 1507, 1315, 1184, 1125, 1054, 1017, 961, 868, 841, 782, 709 cm^{-1} . $[\alpha]_D^{25} = +117.1^\circ$ (c 0.158, THF) for (2*R*,5*R*)-**1e** of 90.5% *ee*.

(2*S*,5*S*)-**1f** (64.1% *ee*): mp 145.4–147.2°C; IR 2932, 2853, 2672, 2554, 1902, 1704, 1608, 1576, 1510, 1288, 1249, 1184, 1125, 1118, 1021, 858, 842, 780, 705 cm^{-1} . $[\alpha]_D^{25} = -90.8^\circ$ (c 0.201, THF) for (2*S*,5*S*)-**1f** of 75.2% *ee*.

(2*R*,5*R*)-**1g** (76.8% *ee*): mp 156.5–157.2°C; IR 2944, 2852, 2674, 2555, 1910, 1685, 1606, 1570, 1510, 1281, 1246, 1177, 1129, 1111, 1067, 867, 838, 780, 708 cm^{-1} . $[\alpha]_D^{25} = +109.3^\circ$ (c 0.226, THF) for (2*R*,5*R*)-**1g** of 91.7% *ee*.

(2*R*,5*R*)-**1h** (77.6% *ee*): mp 138.9–140.1°C; IR 2916, 2848, 2676, 2555, 1908, 1685, 1607, 1472, 1424, 1291, 1244, 1177, 1128, 1116, 1072, 948, 843, 781, 708 cm^{-1} . $[\alpha]_D^{25} = +130.9^\circ$ (c 0.167, THF).

(2*R*,5*R*)-**1i** (77.6% *ee*): mp 135.4–137.3°C; IR 2916, 2849, 2685, 2554, 1911, 1685, 1607, 1507, 1472, 1424, 1291, 1247, 1181, 1129, 1072, 1016, 954, 866, 843, 781, 709 cm^{-1} . $[\alpha]_D^{25} = +150.4^\circ$ (c 0.500, THF) for (2*R*,5*R*)-**1i** of 91.2% *ee*.

(2*S*,5*S*)-**1j** (82.3% *ee*): mp 118.3–119.4°C; IR 2913, 2848, 2660, 2541, 1911, 1685, 1621, 1607, 1507, 1472, 1424, 1291, 1247, 1181, 1072, 1053, 864, 843, 782 cm^{-1} . $[\alpha]_D^{25} = -165.2^\circ$ (c 0.120, THF) for (2*S*,5*S*)-**1j** of 80.7% *ee*.

Crystallography

For the X-ray crystallographic analysis of (±)-**1a**, the single crystal was mounted in a glass capillary. The crystal data are summarized below. The data collection was performed at 293 K on a Rigaku RAXIS RAPID diffractometer with graphite-monochromated Mo K α radiation to $2\theta_{\text{max}}$ of 54.9° . The crystallographic calculations were performed with the CrystalStructure software package of Rigaku and Rigaku/MSO. The crystal structure was solved by direct and refined full-matrix least-squares methods. All non-hydrogen atoms were refined anisotropically and all hydrogen atoms were fixed at calculated positions*.

(±)-**1a**: $\text{C}_{23}\text{H}_{28}\text{O}_4\text{N}$, $M_w = 382.48$, yellow needle, $0.40 \times 0.25 \times 0.25$ mm, monoclinic, space group $P2_1/a$ (No. 14), $a = 8.4879$ (7) Å, $b = 17.7605$ (15) Å, $c = 13.5429$ (14) Å, $\beta = 98.995$ (3) $^\circ$, $V = 2016.5$ (3) Å ^{3} , $Z = 4$, $\rho_{\text{calcd}} = 1.260$ g cm $^{-3}$, MoK α ($\lambda = 0.71075$ Å), $\mu = 0.855$ cm $^{-1}$, ω scan, 18817 independent reflections, 4537 observed reflections.

*Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: + 44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

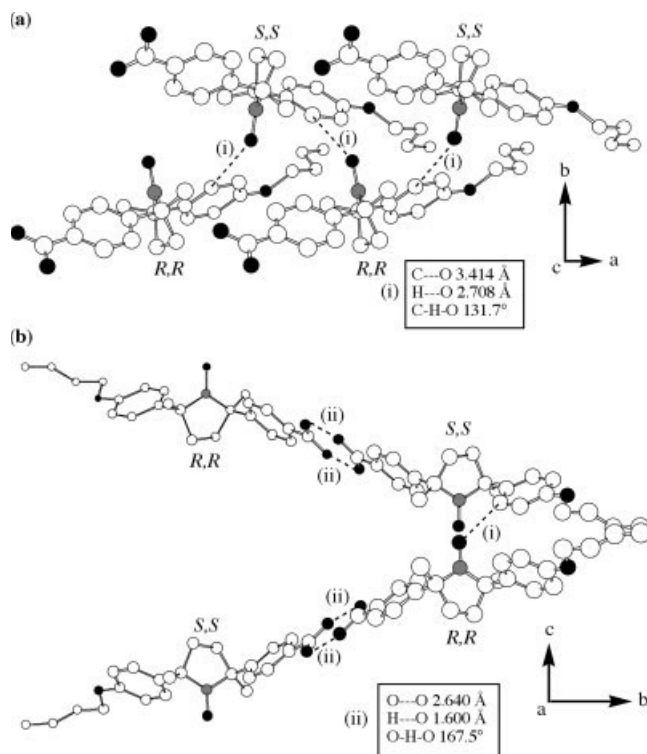


Fig. 1. Crystal structure of (±)-1a viewed down (a) the *c* axis and (b) the *a* axis. (i) C(sp²)H...O-N interaction. (ii) Cyclic dimer between two carboxyl groups. Carbon, nitrogen, and oxygen atoms are denoted by white, gray, and black circles, respectively.

tions ($I > 2.00\sigma(I)$), 339 parameters, $R_1 = 0.092$, $R_w = 0.1559$, $\rho_{\max} = 0.79 \text{ eÅ}^{-3}$, $\rho_{\min} = -0.44 \text{ eÅ}^{-3}$.

RESULTS AND DISCUSSION

The synthesis of (±)-1 is illustrated in Scheme 2. The initial step was the addition of Grignard reagent **6** to the racemic nitrone **5**, followed by Cu(II)-catalyzed oxidation with O₂, to give **7**.⁷ The obtained nitrone **7** was then subjected to the addition reaction of Grignard reagent **8**, followed by Cu(II)-catalyzed oxidation with O₂. The acetal of the resulting nitroxide **9** was hydrolyzed to give aldehyde **10**. Subsequent oxidation of **10** with Ag₂O in an aqueous NaOH solution gave carboxylic acid **1**.

To determine whether a series of (±)-1 exist as a racemic crystal or a conglomerate in the crystalline state and to confirm the molecular structure, X-ray crystallographic analysis was performed for (±)-1a, for which the single crystal of good quality was obtained by crystallization from CH₂Cl₂. As shown in Figure 1, this was a racemic compound (space group *P*₂₁/*a*). The crystal structure was characterized by two intermolecular interactions; one is the C(sp²)H...O-N interaction between the H(2) atom in the 4-butoxyphenyl group and the nitroxyl oxygen atom (type i), and the other is the heterochiral cyclic dimer structure [Graph set: R₂²(8)] by the hydrogen bonds between two carboxyl groups (type ii), eventually forming a heterochiral 1D chain along the *a* axis. Thus, preferential crystallization turned out to be unusable. Observation of

characteristic C=O stretching vibrations between 1684 and 1704 cm⁻¹ in the IR spectra of (±)-1 indicates that this cyclic dimer structure [R₂²(8)] between two carboxyl groups is present in all of (±)-1 synthesized. The XRD pattern simulated from the crystallographic data of (±)-1a was identical to the experimental XRD pattern of the bulk powder sample of (±)-1a obtained by crystallization from Et₂O, indicating no polymorphism.

As resolving agent candidates for the enantiomeric resolution of the typical carboxylic acid (±)-1j (*n* = 13) by the diastereomer method, four commercially available, optically-active amines namely (*R*)-1-phenylethylamine (**2**), (*S*)-1-(1-naphthalenyl)ethylamine (**11**), (1*R*,2*R*)-1,2-diaminocyclohexane (**12**), and (*R*)-2-methylpiperazine (**13**) were examined (Scheme 1). To a saturated ethereal solution of (±)-1j was added 1 equiv of the optically active amine, and the resulting deposited diastereomeric salts were collected and hydrolyzed with aqueous 2 N HCl to give free **1j**. The quantity and *ee* value of the recovered **1j** were measured. The diastereomeric salt was deposited as a fine powder when (*R*)-**2**, (1*R*,2*R*)-**12**, and (*R*)-**13** were used, whereas no precipitation was observed by the use of (*S*)-**11**. Among the three resolving amines, (*R*)-**2** gave the best *ee* value (17.5%) of 2*S*,5*S*-enriched **1j** with overall recovery of 36.5% based on the original quantity of (±)-1j, while 0% *ee* and 3.7% *ee* of 2*S*,5*S*-enriched **1j** were noted for (1*R*,2*R*)-**12** and (*S*)-**13**, respectively.

Then the solvent appropriate for the diastereoselective salt formation between (±)-1j and (*R*)-**2** was examined. Among typical solvents such as MeOH, EtOH, acetone, toluene, Et₂O, CH₂Cl₂, and hexane, the diastereomeric crystals were deposited only when Et₂O, hexane, or a mixture of hexane and CH₂Cl₂ (v/v 1:1) was employed. The *ee* values of 2*S*,5*S*-enriched **1j** obtained after hydrolysis were 17.5% (from Et₂O), 15.2% (hexane/CH₂Cl₂), and 10.2% (hexane). Thus, Et₂O was used as the solvent for further studies.

To improve the recovery and *ee* value of 2*S*,5*S*-enriched **1j**, the molar ratio of (*R*)-**2** to (±)-1j was optimized (Table 1). As the amount of (*R*)-**2** increased, the quantity and *ee* value of the recovered **1j** improved. A 6:1 ratio of (*R*)-**2** to (±)-1j gave the best results, 32.8% *ee* and 63.5% recovery

TABLE 1. Optimization of the ratio of (*R*)-**2** to (±)-1j^a

(<i>R</i>)- 2 vs. (±)-1j, molar ratio	1j , recovery (%) ^b	1j , <i>ee</i> (%) ^c
1:1	36.5	17.5
2:1	40.1	17.3
3:1	52.3	23.5
4:1	51.7	25.4
5:1	59.4	29.7
6:1	63.5	32.8
8:1	90.4	2.9
12:1	99.7	0.2

^aA solution of (±)-1j (1.00 mmol) and (*R*)-**2** in Et₂O (50 ml) was allowed to stand for 24 h at 25°C. The resulting diastereomeric salt crystals were collected and hydrolyzed with aqueous 2N HCl to give free 2*S*,5*S*-enriched **1**.

^bBased on the original quantity of (±)-1j.

^cDetermined by HPLC analysis using a chiral stationary phase column (Daicel Chiralcel OD-H).

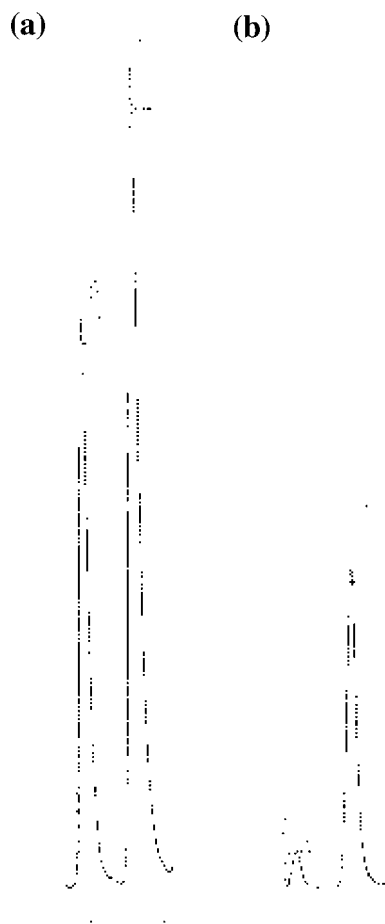


Fig. 2. Determination of the *ee* value of **1j** by HPLC analysis using Daicel Chiralcel OD-H (0.46 cm $\times$ 25 cm) and a mixture of hexane, EtOH, and CF₃COOH as the mobile phase; (a) racemic sample and (b) 2*S*,5*S*-enriched sample of 80.8% *ee*.

of **1j**. These results indicate the existence of a fairly small equilibrium constant in the acid–base reaction between ($\pm$)-**1j** and (*R*)-**2** in Et₂O as well as the relatively large difference in the stability or solubility between the resulting two diastereomeric crystals; the crystal of (2*S*,5*S*)-**1j**·(*R*)-**2** salt is more stable than that of (2*R*,5*R*)-**1j**·(*R*)-**2** salt. But the use of more than 6 equiv of (*R*)-**2** resulted in the drastic decrease in the *ee* value together with the substantial increase in the recovery of **1j**. Furthermore, by repeating recrystallization of the obtained diastereomeric crystals from Et₂O twice, the *ee* value of **1j** improved to 80.8% and the overall recovery of **1j** was 18.5% (Fig. 2). The 1:1 ratio of (*R*)-**2** to **1j** in the salt was confirmed by elemental analysis (See Materials and Methods).

In addition, application of the Dutch resolution method using (*R*)-**2** together with a 1:1 mixture of (*R*)-1-(2-nitrophenyl)ethylamine (**14**) and (*R*)-1-(4-nitrophenyl)ethylamine (**15**) as the families of resolving agents to ($\pm$)-**1j** was attempted.¹⁶ This method was not effective for the resolution of ($\pm$)-**1j**; as the amount of the mixture of (*R*)-**14** and (*R*)-**15** increased, the *ee* value of **1j** drastically decreased.

Since the enantiomeric purity of 70% to 80% *ee* is enough for the LC materials to show the individual chiral LC phases, the above optimum conditions using (*R*)- or (*S*)-**2** were applied to the resolution of a series of carboxylic acids ($\pm$)-**1**. Results are summarized in Table 2. Resolution of ($\pm$)-**1d–1j** with a long alkyl chain (*n* = 7–13) was accomplished to give 2*R*,5*R*- or 2*S*,5*S*-enriched **1d–1j** in an *ee* range of 75–92% and with the overall recovery of 11–27% based on the original quantity of ($\pm$)-**1**. The excess (*R*)- or (*S*)-**2** used was easily recovered from the ethereal solution after filtration and used for further resolution.

In contrast, the resolution by this protocol of ($\pm$)-**1a–1c** with a short alkyl chain (*n* = 4–6) was unsuccessful, even if the ratio of (*R*)-**2** to ($\pm$)-**1** and/or the solvent was changed. In these cases, recrystallization of the initially de-

TABLE 2. Enantiomeric resolution of ($\pm$)-**1** by the diastereomer method using (*R*)- or (*S*)-**2**

Compound ^a	Diastereomeric salt ^b		1st recrystallization ^c		2nd recrystallization ^c	
	Yield (%) ^d	1 , <i>ee</i> (%) ^e	Recovery (%) ^d	1 , <i>ee</i> (%) ^e	Recovery (%) ^d	1 , <i>ee</i> (%) ^e
1a (<i>n</i> = 4) ^f	47.6	4.6	27.4	1.7	–	–
1b (<i>n</i> = 5) ^f	52.3	7.4	34.9	0.8	–	–
1c (<i>n</i> = 6) ^f	37.4	6.5	6.0	4.9	–	–
1d (<i>n</i> = 7) ^f	55.0	52.3	19.0	83.9	–	–
1e (<i>n</i> = 8) ^g	87.1	49.1	43.1	68.1	21.6	90.5
1f (<i>n</i> = 9) ^f	87.1	7.9	50.6	39.1	22.8	75.2
1g (<i>n</i> = 10) ^g	80.0	11.5	44.7	60.7	13.6	91.7
1h (<i>n</i> = 11) ^g	59.8	46.2	53.2	50.3	26.6	77.6
1i (<i>n</i> = 12) ^g	73.4	38.0	42.3	69.1	11.3	91.2
1j (<i>n</i> = 13) ^f	65.4	31.4	48.3	70.7	18.5	80.8

^aRacemic samples.

^bA solution of ($\pm$)-**1** (1.00 mmol) and (*R*)- or (*S*)-**2** (6.00 mmol) in Et₂O (50 ml) was allowed to stand for 24 h at 25°C. The resulting diastereomeric salt crystals were collected.

^cRecrystallized from Et₂O.

^dBased on the original quantity of ($\pm$)-**1**.

^eDetermined by HPLC analysis using a chiral stationary phase column (Daicel Chiralcel OD-H) after hydrolysis with aqueous 2*N* HCl to give free **1**.

^fBy using (*R*)-**2**, 2*S*,5*S*-enriched **1** was obtained.

^gBy using (*S*)-**2**, 2*R*,5*R*-enriched **1** was obtained.

posited diastereomeric salts from Et₂O gave nearly racemic **1** after hydrolysis, indicating the very small difference in the stability or solubility between the resulting two diastereomeric crystals, compared with the cases of **1d–1j**. Since X-ray crystal structure of the diastereomeric salt between **1** and (*R*)-**2** has not been obtained yet due to the inaccessibility of the single crystals, further discussion is impossible at present.

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Review Article

Enantioseparation Using Urea- and Imide-Bearing Chitosan Phenylcarbamate Derivatives as Chiral Stationary Phases for High-Performance Liquid Chromatography

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT Completely deacetylated chitosan was prepared by the treatment of commercial chitosan with 50% aqueous NaOH, and then derivatized into several new chitosan phenylcarbamate derivatives having a urea and an imide moiety at the 2-position of the glucosamine ring by the reaction with isocyanate and phthalic anhydride/isocyanate, respectively. The chitosan derivatives were coated on macroporous silica gel and evaluated as chiral stationary phases (CSPs) for high-performance liquid chromatography. The chiral recognition ability of the chitosan derivative was improved using the completely deacetylated chitosan. Among the novel chitosan derivatives, the 3,5-dimethyl-, 3,5-dichloro-, and 3,4-dichlorophenylcarbamate derivatives were found to possess relatively high chiral resolution abilities. The CSPs based on the chitosan phenylcarbamate-urea and -imide derivatives were stable in the presence of chloroform and ethyl acetate as a component of the eluents, and some racemates were better resolved by such eluents. The dichlorophenylcarbamate-imide derivatives showed a high chiral recognition for metal acetylacetonate complexes. The enantiomerization of Al(acac)₃ was performed on the chitosan 3,5-dichlorophenylcarbamate-imide derivative CSP and the resulting chromatogram showed a 26% (+)-isomer enrichment. *Chirality* 20:288–294, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: polysaccharide; HPLC; resolution; chiral separation; deacetylation; enantiomerization; aluminum acetylacetonate

INTRODUCTION

Chromatographic enantioseparation by high-performance liquid chromatography (HPLC) has become the most powerful method not only for analyzing enantiomers, but also for obtaining them in a pure form. Advances in this method depend on the development of chiral stationary phases (CSPs) capable of effective chiral recognition, and until now, about 200 CSPs have been commercialized. These CSPs can be classified into two types: one consists of small chiral molecules which are usually attached to a support and the other is derived from optically active polymers which can be used as a porous gel or with silica gel.^{1–4} Chiral recognition by small molecule CSPs depends mainly on that of the small molecules themselves, but polymeric CSPs often show a chiral recognition that depends on the higher order structure of the polymers. Therefore, for the polymeric CSPs, a regular high-ordered structure of a polymer is an important factor for efficient chiral recognition.^{5,6} Among the many polymeric CSPs, the phenylcarbamates and benzoates of cellulose and amylose are

known to show a high chiral recognition to a broad range of chiral compounds.^{6–12} Cellulose and amylose are stereoregular polymers consisting of D-glucose, although their chiral recognition abilities are not high enough to be used as CSPs. However, their hydroxy groups can be readily converted to phenylcarbamates and benzoates through reaction with phenyl isocyanates and benzoyl chlorides, respectively. These derivatives show much better chromatographic and enantioselective properties than the native polysaccharides, and their chiral recognition abilities are significantly influenced by the substituents introduced on the phenyl group.¹³ The introduction of an electron-donating methyl group or an electron-withdrawing

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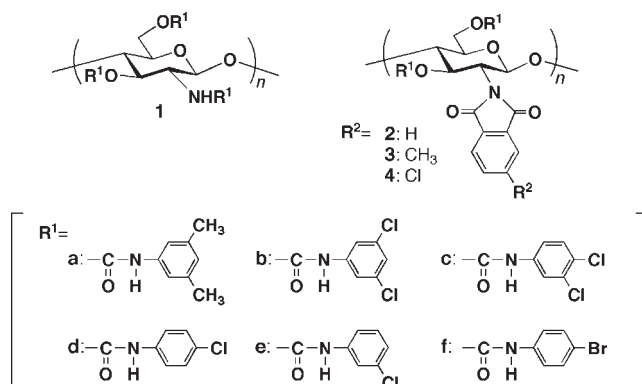


Fig. 1. Structures of carbamate-urea (1) and carbamate-imide derivatives (2–4) of chitosan.

halogen at the *m*- and/or *p*-position of the phenyl ring often improves the chiral recognition ability. Among the cellulose and amylose derivatives, the 3,5-dimethyl and 3,5-dichlorophenylcarbamates are particularly attractive derivatives.^{13–15}

For other polysaccharides, such as chitin, chitosan, xylan, and dextran, the 3,5-dimethyl- and 3,5-dichlorophenylcarbamates have also been evaluated as CSPs for HPLC.^{16–18} Their enantioselectivity significantly depends on the sugar units.¹⁶ Among them, chitosan has the β -(1 $\rightarrow$ 4) linkage as well as cellulose, but it has an amino group at the 2-position instead of the hydroxy group of cellulose. Therefore, various novel derivatives different from cellulose derivatives can be prepared from chitosan.

Chitosan is typically obtained by the deacetylation of chitin, which has an acetyl group at the 2-position of the glucosamine ring. However, commercial chitosan still contains around 20% of the acetyl group, and we used this in our previous study.¹⁶ In the present study, commercial chitosan was first treated with 50% aqueous NaOH to prepare completely deacetylated chitosan, which was then used for the subsequent reaction with various phthalic anhydrides and/or phenyl isocyanates. The chitosan derivatives obtained were evaluated as CSPs for HPLC using various solvents as the eluents. In addition, the enantiomerization of aluminum(III) tris(acetylacetonate) resolved with high α value was performed on the CSP of the chitosan carbamate-imide derivative.

MATERIALS AND METHODS

Materials

Chitosan was purchased from Wako (Osaka, Japan). Sodium hydroxide, acetic acid, and phthalic anhydride were from Kishida (Osaka, Japan). 3,5-Dimethylphenyl isocyanate was kindly supplied by Daicel Chemical (Tokyo, Japan). The other phenyl isocyanates and phthalic anhydrides were purchased from Tokyo Kasei (Tokyo, Japan). Porous spherical silica gels (Daiso gel SP-1000) with a mean particle size of 7 μ m and a mean pore diameter of 100 nm were kindly supplied from Daiso Chemical (Osaka, Japan). Pyridine and *N,N*-dimethylformamide were from Kanto Chemical (Tokyo, Japan) and Kishida, respectively,

as anhydrous reagents. The racemates were commercially available or were prepared by the usual methods.¹⁹

Deacetylation of Chitosan

The deacetylation of chitosan was performed according to a reference.²⁰ Chitosan was suspended in 50% aqueous NaOH and refluxed at 120–130°C for 2 h. After cooling, the treated chitosan was filtered off and washed with water. This procedure was repeated three times, and the product was then washed with water, ethanol, and hexane, and dried at 60°C in vacuo. The deacetylation was confirmed by IR spectroscopy, which confirmed the decrease in the peak intensity assigned to the amide group around 1655 cm^{-1} .

Synthesis of Carbamate-Urea Derivatives

The carbamate-urea derivatives (1a–f) (Fig. 1) were prepared by the reaction of chitosan with the corresponding phenyl isocyanates in dry pyridine at 80°C. To a suspended deacetylated-chitosan in pyridine, an excess of a phenyl isocyanate (1.5 equiv of the total of hydroxy and amino groups) was added, and the mixture was heated under nitrogen at 80°C with stirring. After at least 24 h, the resulting carbamate-urea derivatives were isolated as the methanol-insoluble fraction (yield 50–95%). The ¹H NMR and elemental analysis (Table 1) of the derivatives indicated that the hydroxy and amino groups of the chitosan were almost quantitatively converted into the carbamate and urea moieties, respectively. **1a**: ¹H NMR (pyridine-*d*₅, 80°C): δ 2.1–2.3 (CH₃ (18H)), 3.3–6.0 (br, glucose protons (7H)), 6.5–11.1 (aromatic (9H), NH (4H)). **1b**: ¹H NMR (pyridine-*d*₅, 80°C): δ 3.3–6.4 (br, glucose protons (7H)), 6.8–11.2 (aromatic (9H), NH (4H)). **1c**: ¹H NMR (pyridine-*d*₅, 80°C): δ 3.4–6.7 (br, glucose protons (7H)), 6.9–11.2 (aromatic (9H), NH (4H)). **1d**: ¹H NMR (pyridine-*d*₅, 80°C): δ 3.2–6.5 (br, glucose protons (7H)), 6.5–10.5 (aromatic (9H), NH (4H)). **1e**: ¹H NMR (pyridine-*d*₅,

TABLE 1. Elemental analysis of chitosan derivatives

	Calculated (%)			Found (%)		
	C	H	N	C	H	N
1a	65.77	6.36	9.30	65.69	6.49	9.36
1b	44.72	2.78	7.73	44.71	2.55	7.56
1c	44.72	2.78	7.73	44.71	2.56	7.53
1d	52.15	3.73	9.01	52.15	3.66	8.93
1e	52.15	3.73	9.01	52.15	3.64	9.01
1f	42.94	3.07	7.42	42.93	2.92	7.28
2a	65.63	5.34	7.18	65.80	5.14	7.23
2b	50.40	2.87	6.30	50.44	2.09	6.58
2c	50.40	2.87	6.30	50.48	2.73	6.56
3a	66.10	5.55	7.01	66.28	5.52	7.03
3b	51.12	3.11	6.17	51.12	3.09	5.98
3c	51.12	3.11	6.17	51.15	3.09	6.38
4a	61.99	4.88	6.78	63.06	4.82	7.26
4b	47.92	2.59	5.99	47.34	2.22	5.72
4c	47.92	2.59	5.99	47.23	2.12	5.82

Estimated based on a repeated glucosamine unit.

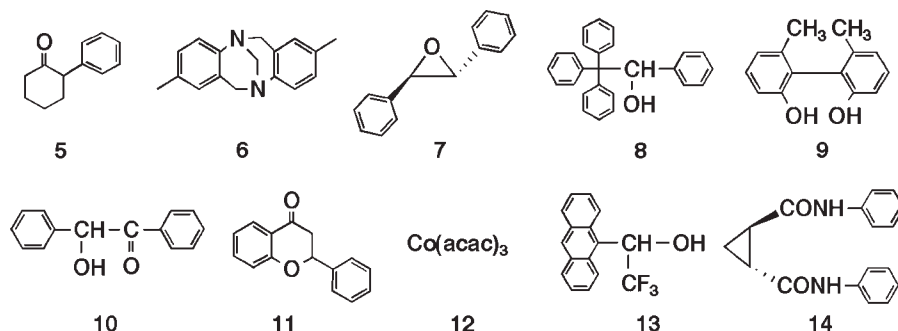


Fig. 2. Structures of racemates.

80°C): δ 3.0–6.2 (br, glucose protons (7H)), 6.5–10.6 (aromatic (12H), NH (3H)). **1f**: ^1H NMR (pyridine- d_5 , 80°C): δ 3.0–6.2 (br, glucose protons (7H)), 6.5–10.6 (aromatic (12H), NH (3H)).

Synthesis of Carbamate-Imide Derivatives

The carbamate-imide derivatives (**2**–**4**) (Fig. 1) were synthesized by the reaction of chitosan with the corresponding phthalic acid anhydride and isocyanates.²¹ The deacetylated-chitosan was added to *N,N*-dimethylformamide/water (95/5, v/v) containing a phthalic acid anhydride (3 equiv to the amino group of chitosan), and the mixture was heated at 120°C with stirring. After 8 h, the resulting gelatinous mixture was poured into water, filtered, washed with methanol, and dried at 60°C in vacuo (yield 85–90%). To the dispersion of the obtained phthaloyl chitosan in dry pyridine, the corresponding phenyl isocyanate (1.5 equiv to the total of hydroxyl groups of chitosan) was added, and the mixture was heated under nitrogen at 80°C with stirring. After 24 h or more, the carbamate-imide derivatives were isolated as the methanol-insoluble fraction (yield > 80%). The resulting carbamate-imide derivatives were confirmed by ^1H NMR and elemental analysis (Table 1). **2a**: ^1H NMR (pyridine- d_5 , 80°C): δ 2.2, 2.3 (CH_3 (12H)), 3.9–5.9 (br, glucose protons (7H)), 5.8–7.7 (aromatic (10H)), 9.1, 9.8 (NH (2H)). **2b**: ^1H NMR (pyridine- d_5 , 80°C): δ 3.7–5.8 (br, glucose protons (7H)), 6.7–7.8 (aromatic (12H)), 10.3, 10.4 (NH (2H)). **2c**: ^1H NMR (pyridine- d_5 , 80°C): δ 3.6–6.0 (br, glucose protons (7H)), 6.8–7.7 (aromatic (12H)), 10.1, 10.3 (NH (2H)). **3a**: ^1H NMR (pyridine- d_5 , 80°C): δ 2.0–2.3 (CH_3 (15H)), 3.9–5.9 (br, glucose protons (7H)), 6.4–7.6 (aromatic (10H)), 9.1, 9.8 (NH (2H)). **3b**: ^1H NMR (pyridine- d_5 , 80°C): δ 2.1 (CH_3 (3H)), 3.5–5.8 (br, glucose protons (7H)), 6.7–7.5 (aromatic (9H)), 10.2, 10.4 (NH (2H)). **3c**: ^1H NMR (DMSO- d_6 , 80°C): δ 2.1 (CH_3 (3H)), 2.1–5.6 (br, glucose protons (7H)), 6.5–7.4 (aromatic (9H)), 9.1, 9.4 (NH (2H)). **4a**: ^1H NMR (pyridine- d_5 , 80°C): δ 2.0, 2.3 (CH_3 (12H)), 3.9–5.9 (br, glucose protons (7H)), 6.4–7.7 (aromatic (10H)), 9.2, 9.9 (NH (2H)). **4b**: ^1H NMR (pyridine- d_5 , 80°C): δ 3.6–5.8 (br, glucose protons (7H)), 6.7–7.5 (aromatic (9H)), 10.3, 10.9 (NH (2H)). **4c**: ^1H NMR (pyridine- d_5 , 80°C): δ 3.6–6.0 (br, glucose protons (7H)), 6.8–7.6 (aromatic (9H)), 10.2, 10.4 (NH (2H)).

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Preparation of Stationary Phase

The packing materials were prepared by coating the chitosan derivatives on macroporous silica gel, which had been treated with a large excess of 3-aminopropyltriethoxysilane as previously described.¹³ As a coating solvent, tetrahydrofuran (THF) was used, while for only **3b**, dimethylsulfoxide (DMSO) was used, because of its low solubility in THF. The prepared packing materials were packed in a stainless steel column (250 mm $\times$ 4.6 mm i.d.) by a conventional high-pressure slurry packing technique using a Model CCP-085 Econo packer pump (Chemco). The theoretical plate numbers of the columns were 2300–6400 for benzene with hexane-2-propanol (90:10) as the eluent at a flow rate of 0.5 ml/min. 1,3,5-Tri-*tert*-butylbenzene was used as the nonretained compound for estimating the dead time (t_0).²²

Apparatus

The chromatographic experiments were performed using a JASCO PU-980 Intelligent HPLC pump equipped with UV (JASCO 970-UV) and polarimetric (JASCO OR-990) detectors at room temperature. A solution (1–10 μl) of a racemate was injected into the chromatographic system by a Rheodyne Model 7125 injector. The ^1H NMR spectra were recorded in pyridine- d_5 or DMSO- d_6 at 80°C using a Varian Gemini-2000 NMR spectrometer (400 MHz). The IR spectra were measured by a JASCO Fourier transform IR-7000 spectrometer as a KBr pellet.

RESULTS AND DISCUSSION

HPLC Resolution

The HPLC resolution on the chitosan derivative CSPs was examined for the following 10 racemates: 2-phenylcyclohexanone (**5**), Tröger base (**6**), *trans*-stilbene oxide (**7**), 1,2,2,2-tetraphenylethanol (**8**), 2,2'-dihydroxy-6,6'-dimethylbiphenyl (**9**), benzoin (**10**), flavanone (**11**), cobalt(III) tris(acetylacetonate) (**12**), 1-(9-anthryl)-2,2,2-trifluoroethanol (**13**), and *trans*-cyclopropanedicarboxylic acid dianilide (**14**) (Fig. 2). Figure 3 shows a chromatogram of the resolution of **12** on the column packed with chitosan bis(3,5-dichlorophenylcarbamate)-4-methylphthalimide (**3b**). The enantiomers eluted at the retention times of 10.1 and 13.0 min, showing complete separation. The retention factors, $k_1' [= (t_1 - t_0)/t_0]$ and $k_2' [= (t_2 - t_0)/t_0]$, were 0.52

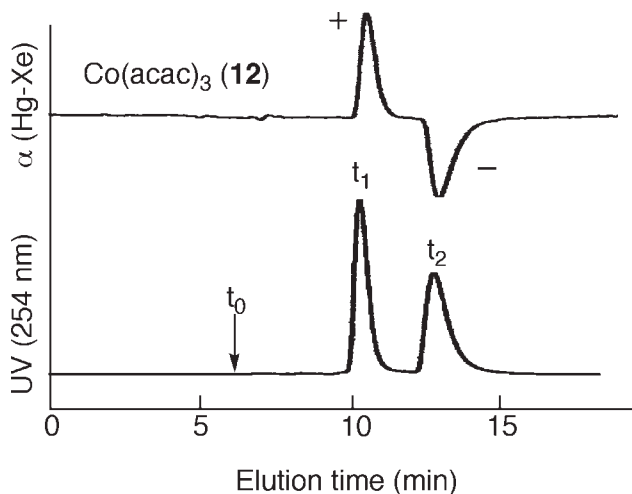


Fig. 3. Resolution of cobalt(III) tris(acetylacetonate) (**12**) on chitosan carbamate-imide derivative (**3b**).

and 0.95, respectively, and the separation factor $\alpha [= k_2'/k_1']$ was 1.84.

Completely Deacetylated Chitosan

Chitosan is usually prepared from chitin by deacetylation, and around 20% acetyl groups still remain as the acetamide group at the 2-position on the glucosamine ring. This indicates that the chitosan derivative obtained through the reaction with an isocyanate has an irregular structure bearing both acetamide and urea moieties at the 2-position. However, for the polymeric CSPs, a regular high-ordered structure is an important factor for efficient chiral recognition.^{5,6} In this study, the purchased chitosan was treated with 50% aqueous NaOH to remove the remaining acetyl groups, resulting in completely deacetylated chitosan. Figure 4 shows the NMR spectra of two kinds of **2c** prepared from the untreated and the deacetylated chitosans. In the spectrum of **2c** from the untreated chitosan (Fig. 4a), the methyl proton signal assigned to an acetamide group appeared around 2 ppm. Based on the in-

tensity of the methyl protons, the remaining acetyl group was estimated to be 17%. On the other hand, in the spectrum of **2c** from the deacetylated chitosan (Fig. 4b), the methyl proton signal was not observed, indicating that the complete deacetylation was achieved by the treatment with 50% aqueous NaOH.

These two **2c** were coated on silica gel and evaluated as CSPs to examine the influence of the remaining acetyl group on the chiral recognition ability (Table 2). The overall tendencies of the chiral recognition were similar between the two CSPs, while the separation factors for the racemates except for **6** on **2c** from the deacetylated chitosan were higher than those from the untreated chitosan. The elution orders of the enantiomers were the same. The improved chiral recognition ability may be ascribed to the more regular high-ordered structure of the derivative **2c** obtained from the deacetylated chitosan. Therefore, all the chitosan derivatives subsequently discussed in this study were prepared using the deacetylated chitosan.

Carbamate-Urea Derivatives

The 3,5-disubstituted and 4-substituted phenylcarbamate-urea chitosan derivatives (**1a–1f**) bearing methyl or halogen groups were prepared and their chiral recognition abilities as CSPs were compared for the 10 racemates (Table 3). The six carbamate-urea derivatives exhibited chiral recognition for many racemates. Among the derivatives, the 3,5-disubstituted phenylcarbamate-ureas **1a–1c** showed high resolving abilities, and the 3,5-dimethylphenyl derivative **1a** resolved all 10 racemates. The retention factors k_1' on **1a** were different from those of the other derivatives **1b–1f** having one or two halogens on the phenyl group. For example, the k_1' s of racemates **8**, **9**, and **13** on **1a** are larger than those on **1b–1f**. On the other hand, those of **5**, **6**, **7**, **10**, and **11** on **1a** are significantly lower than those on **1b–1f**. These differences in k_1' are caused by the electronic effect of the substituents introduced on the phenyl group of the carbamate and urea moieties, which are the main chiral recognition sites for polar racemates. Figure 5 shows the schematic interaction

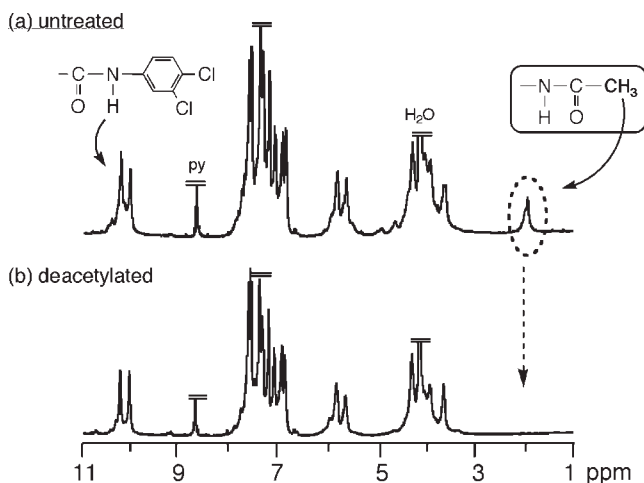


Fig. 4. NMR spectra of carbamate-imide derivatives (**2c**) prepared from the untreated (a) and the deacetylated chitosans (b).

TABLE 2. Separation factors of racemates on carbamate-imide derivatives **2c** derived from untreated and deacetylated chitosans

	Deacetylated	Untreated
5	~1 (–) ^a	~1 (–)
6	1.09 (+)	1.19 (+)
7	1.15 (+)	~1 (+)
8	~1 (+)	1.00
9	1.18 (+)	1.08 (+)
10	1.08 (–)	1.07 (–)
11	1.05 (–)	1.04 (–)
12	1.54 (+)	1.51 (+)
13	1.00	1.00
14	1.71 (+)	1.62 (+)

Column: 25 × 0.46 cm (i.d.); flow rate: 0.5 ml/min; eluent: hexane/2-propanol = 90/10.

^aThe signs in parentheses represent the optical rotation of the first-eluted enantiomer.

TABLE 3. Resolution of racemates on carbamate-urea derivatives of chitosan

	1a: 3,5-(CH ₃) ₂		1b: 3,5-Cl ₂		1c: 3,4-Cl ₂		1d: 4-Cl		1e: 3-Cl		1f: 4-Br	
	<i>k</i> ₁ '	α	<i>k</i> ₁ '	α	<i>k</i> ₁ '	α	<i>k</i> ₁ '	α	<i>k</i> ₁ '	α	<i>k</i> ₁ '	α
5	0.77 (–) ^a	1.29	2.38 (–)	1.18	2.25 (–)	1.05	1.94 (–)	1.08	1.99 (–)	1.11	1.93 (–)	1.08
6	0.39 (+)	1.51	0.90 (–)	1.09	0.74 (–)	1.12	0.88 (+)	~1	0.67 (+)	1.37	0.90 (+)	1.09
7	0.28 (+)	1.21	0.39 (+)	1.22	0.32 (+)	1.43	0.37 (+)	1.35	0.32 (+)	1.33	0.35 (+)	1.39
8	0.83 (+)	1.51	0.52 (+)	1.20	0.58 (+)	1.31	0.82 (+)	1.29	0.86 (+)	1.23	0.89 (+)	1.30
9	2.41 (+)	1.14	1.43 (–)	~1	1.23	1.00	1.65 (+)	~1	1.44 (+)	1.09	1.36	1.00
10	2.38 (–)	1.11	4.37 (–)	1.13	5.51 (–)	1.27	3.85 (–)	1.05	3.83 (–)	1.14	4.37 (–)	1.11
11	0.89 (–)	1.06	1.47 (–)	1.08	1.73 (–)	1.13	1.55 (–)	1.06	1.43 (–)	1.11	1.67 (–)	1.09
12	0.45 (+)	1.10	1.75 (+)	1.14	2.30 (+)	1.12	2.55 (+)	1.40	1.97 (+)	1.19	2.36 (+)	1.44
13	1.57 (–)	1.27	0.33 (–)	1.27	0.40 (–)	1.14	0.52 (–)	1.24	0.54 (–)	1.15	0.60 (–)	1.26
14	1.23 (–)	1.80	1.22 (+)	1.26	1.16 (+)	1.38	1.55	1.00	1.22 (–)	~1	1.61	1.00

Column: 25 × 0.46 cm (i.d.); flow rate: 0.5 ml/min; eluent: hexane/2-propanol = 90/10.

^aThe signs in parentheses represent the optical rotation of the first-eluted enantiomer.

between the carbamate residue and enantiomers. The electron-donating methyl groups introduced on the phenyl group may increase the electron density at the carbonyl oxygen atom of the carbamate and urea. Therefore, alcohols are more strongly adsorbed on **1a** through a hydrogen bonding interaction than the halogen-bearing **1b–1f**. On the other hand, the electron-withdrawing halogen groups on the phenyl group may enhance the acidity of the N–H protons, and the racemates containing ether or carbonyl oxygen interact more strongly with **1b–1f** than with **1a**. The same effect of the substituents on the phenyl groups of the CSPs on the *k*₁' values has been observed for the cellulose phenylcarbamates.¹³ The reversal of the elution order of the enantiomers was observed only for **6** on **1b** and **1c**.

For cellulose, the 3,5-dichlorophenylcarbamate derivative is soluble in a mixture of hexane and 2-propanol as a typical eluent. Therefore, it cannot be used as a practical CSP for HPLC, although it shows high chiral recognition. In contrast, the chitosan dichlorophenylcarbamates are not soluble in common solvents, and various eluents can be used for these CSPs. This will be discussed later.

Carbamate-Imide Derivatives

The chitosan carbamate-imide derivatives (**2–4**) were prepared by the phthaloylation of the 2-position and the subsequent carbamoylation of the 3- and 6-positions. The phthalimide moiety could be regioselectively and quantitatively introduced to the amino group at the 2-position by the one-step reaction using phthalic anhydrides in DMF/water without partial O-phthaloylation of the hydroxy groups at the 3- and 6-positions.²¹ The carbamoylation of the hydroxy group was performed using the 3,5-dimethyl, 3,5-dichloro, and 3,4-dichlorophenyl isocyanates, which yielded the carbamate-imide derivatives with high chiral recognition abilities. The nine carbamate-imide derivatives obtained were evaluated as CSPs for HPLC (Table 4). Comparing the chiral recognition abilities of the 3,5-dimethylphenylcarbamate-phthalimides (**2a**, **3a**, **4a**) with those of the corresponding carbamate-urea (**1a**), a decrease in the α values and the reversal of the elution order were observed for the carbamate-imides. Although

1a could resolve the 10 racemates, **2a** and **4a** resolved six racemates and **3a** resolved only three racemates. The side chains introduced on the 2-position were found to significantly affect chiral recognition. On the other hand, the 3,5-dichloro- and 3,4-dichlorophenylcarbamate-imide derivatives showed no significant deterioration of the chiral recognition, and some racemates, particularly for **12** and **14**, were better resolved by these derivatives than the corresponding carbamate-urea derivatives. The resolution of some other metal acetylacetonates was also examined on **3b**, and the chromium and aluminum complexes were found to be completely separated using hexane/2-propanol (90/10) as the eluent; Cr(acac)₃: *k*₁' = 0.44 (+), α = 1.51 at 20°C and Al(acac)₃: *k*₁' = 0.93 (+), α = 1.66 at 0°C (Fig. 6). The metal acetylacetonates are known to racemize depending on the kind of metal, temperature, and solvent.²³ Al(acac)₃ rapidly racemizes, and reports on its resolution are rather limited. So far, only partial resolutions at low temperature on D-lactose with isopentane/ethyl acetate²⁴ and on poly(triphenylmethyl methacrylate) with methanol²⁵ have been reported. In this study, we succeeded with the baseline separation of the Δ and Λ enan-

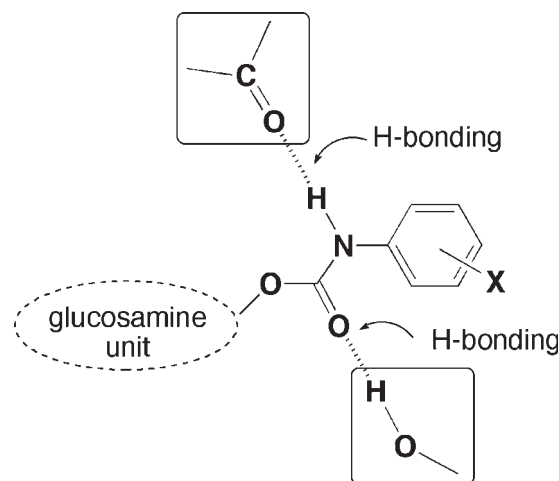


Fig. 5. Schematic interactions of enantiomers with the phenylcarbamoyl residue.

TABLE 4. Resolution of racemates on carbamate-imide derivatives of chitosan

	2a		3a		4a	
	k_1'	α	k_1'	α	k_1'	α
5	1.45 (+)	~1	1.18 (+)	1.07	1.21	1.00
6	1.15 (–)	1.23	0.88 (–)	~1	1.02 (+)	1.15
7	0.51 (+)	~1	0.34 (+)	~1	0.42 (+)	~1
8	1.26 (+)	1.19	1.14 (+)	~1	1.03 (+)	1.08
9	2.74 (+)	1.27	2.41 (+)	1.37	2.67 (+)	1.13
10	3.72 (+)	~1	3.65 (+)	~1	3.18 (–)	1.07
11	2.30 (–)	1.27	1.95 (–)	1.30	1.89 (–)	1.25
12	1.67 (+)	1.12	0.83 (+)	~1	0.97 (+)	1.25
13	1.79 (+)	1.12	1.81 (+)	~1	1.38	1.00
14	1.83 (+)	~1	1.56	1.00	1.41 (+)	~1

	2b		3b		4b	
	k_1'	α	k_1'	α	k_1'	α
5	1.25 (–)	1.11	1.01 (–)	1.09	2.39 (–)	~1
6	0.82 (+)	1.22	0.64 (+)	1.28	1.25 (+)	1.23
7	0.35 (+)	~1	0.22 (+)	1.20	0.48 (+)	1.10
8	0.98 (+)	~1	0.53 (+)	1.12	0.84 (+)	1.26
9	1.57 (+)	~1	2.01 (+)	~1	1.94 (+)	1.14
10	3.31 (–)	1.26	2.74 (–)	1.17	4.97 (–)	1.57
11	2.32 (–)	1.11	1.56 (–)	1.20	3.07 (–)	1.11
12	1.12 (+)	1.44	0.52 (+)	1.84	1.50 (+)	1.42
13	0.57	1.00	0.53	1.00	0.68	1.00
14	0.75 (+)	1.46	0.80 (+)	1.38	1.08 (+)	1.53

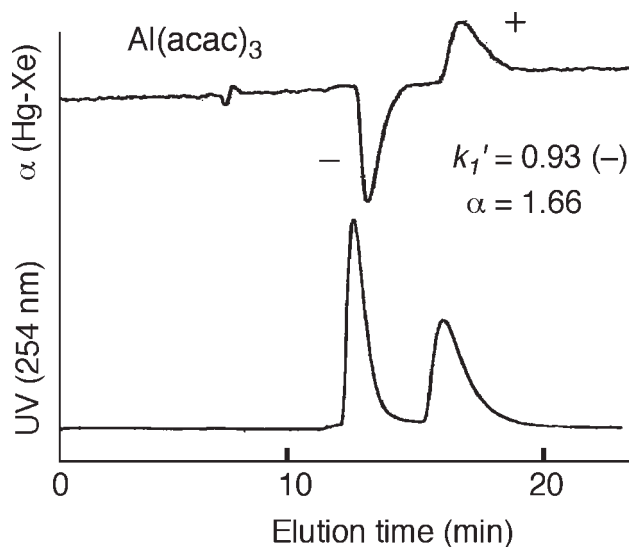
	2c		3c		4c	
	k_1'	α	k_1'	α	k_1'	α
5	1.67 (–) ^a	~1	1.69 (–)	~1	1.95 (–)	~1
6	0.80 (+)	1.09	0.71 (+)	1.18	0.92 (+)	1.17
7	0.30 (+)	1.15	0.33 (+)	3.26	0.38 (+)	~1
8	0.76 (+)	~1	0.75 (+)	~1	0.76 (+)	~1
9	0.82 (+)	1.18	0.96 (+)	1.07	1.69 (+)	1.09
10	2.92 (–)	1.08	3.34 (–)	1.14	3.69 (–)	1.12
11	1.88 (–)	1.05	1.92 (–)	1.05	2.22 (–)	1.10
12	1.53 (+)	1.54	0.96 (+)	1.45	1.64 (+)	1.42
13	0.66	1.00	0.85	1.00	0.63	1.00
14	0.88 (+)	1.71	0.93 (+)	1.78	0.89 (+)	1.63

Column: 25 × 0.46 cm² (i.d.); flow rate: 0.5 ml/min; eluent: hexane/2-propanol = 90/10.

^aThe signs in parentheses represent the optical rotation of the first-eluted enantiomer.

tiomers of Al(acac)₃ using the chitosan phenylcarbamate-imide derivative **3b**.

We also examined the racemization of Al(acac)₃ on the CSP **3b** according to our previous report about dynamic HPLC on CSPs.²⁶ The eluent was changed to hexane/benzene/2-propanol (100/100/0.2), in which Al(acac)₃ was resolved with the higher α value of 2.02 at –10°C. Al(acac)₃ was injected on to the CSP **3b**, and after 30 sec, the HPLC pump was stopped to allow it to racemize on the CSP. The column temperature was kept at 20°C for 10 min and then decreased to –10°C in about 20 min. After stopping of eluent run for a total of 80 min, the HPLC pump was started again, and the resulting chromatogram showed a 26% enrichment of the (+)-isomer.

**Fig. 6.** Resolution of aluminum(III) tris(acetylacetonate) on **3b** with hexane/2-propanol (90/10) at 0°C.

Effect of Eluent Composition on Enantioseparation

Usually, chiral packing materials based on polysaccharide derivatives have been prepared by physical adsorption of the derivatives onto silica gel. Therefore, such packed columns cannot be used with eluents containing solvents such as THF and chloroform, which dissolve or swell the polysaccharide derivatives. However, sometimes it is highly desirable to use these solvents in the eluent, because their addition may improve separation and also solve the problem of the low solubility of a sample for preparative separation.^{27–29} Some of the chitosan derivatives prepared here are not soluble in chloroform, and therefore, eluents can be utilized with any ratio of chloroform without immobilizing the chitosan derivatives on silica gel. The enantioseparation of the racemates using chloroform as an eluent component was examined on **1c**,

TABLE 5. Effect of eluents on resolution of racemates on **1c**

	Hexane/ 2-propanol (90/10)		Hexane/CHCl ₃ / 2-propanol (90/10/1)		Hexane/CHCl ₃ / 2-propanol (50/50/1)	
	k_1'	α	k_1'	α	k_1'	α
5	2.25 (–) ^a	1.05	3.21 (–)	1.23	0.57 (–)	1.24
6	0.74 (–)	1.12	1.15 (–)	1.16	0.27 (–)	~1
7	0.32 (+)	1.43	0.49 (+)	1.60	0.17 (+)	1.33
8	0.58 (+)	1.31	1.62 (+)	1.40	0.30 (+)	1.34
9	1.23	1.00	6.90 (–)	1.06	2.35	1.00
10	5.51 (–)	1.27	13.6 (–)	1.36	2.30 (–)	1.39
11	1.73 (–)	1.13	2.58 (–)	1.10	0.45 (–)	~1
12	2.30 (+)	1.12	1.40	1.00	0.14 (+)	~1
13	0.40 (–)	1.14	3.50 (–)	1.18	0.86 (–)	1.05
14	1.16 (+)	1.38	– ^b	– ^b	– ^b	– ^b

Column: 25 × 0.46 cm (i.d.); flow rate: 0.5 ml/min.

^aThe signs in parentheses represent the optical rotation of the first-eluted enantiomer.

^bNot eluted.

and the k_1' and α values were compared with those using hexane/2-propanol (90/10) (Table 5).

The effect of the eluents on the resolution depends on the racemates; the highest α values were obtained for **11** and **12** with hexane/2-propanol (90/10), **6**, **7**, **8**, **9**, and **13** with hexane/ CHCl_3 /2-propanol (90/10/1), and **5** and **10** with hexane/ CHCl_3 /2-propanol (50/50/1). The k_1' values were also affected by the eluent components. For example, the k_1' values of **8**, **9**, **10**, and **13**, which have an OH group interacting with the CSP through hydrogen bonding, increased remarkably using hexane/ CHCl_3 /2-propanol (90/10/1) as the eluent. This may be because the 2-propanol prevents the adsorption of the enantiomers onto the CSP. On the other hand, hexane/ CHCl_3 /2-propanol (50/50/1), which is a good solvent for both the CSP and most racemates, and therefore, can reduce the interaction between them resulting in the decreased k_1' values. Racemate **14** was not eluted with the eluents containing a small amount of 2-propanol, probably due to the strong hydrogen bond between the CSP and the analyte. Besides chloroform, these CSPs could also be stably used in the presence of ethyl acetate as a component of the eluents.

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Review Article

Structures and Inclusion Properties of Racemic and Enantiopure Dimethyl and Diphenyl 9,9'-Bianthryl-2,2'-dicarboxylates

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT The authors prepared the dimethyl and diphenyl esters of 9,9'-bianthryl-2,2'-dicarboxylic acid in racemic and enantiopure (*M*) forms. The enantiopure dimethyl ester forms inclusion compounds with various organic compounds such as benzene, methanol, phenol, and aniline whereas the racemic form does this only with benzene. No guest molecules are included by the racemic and enantiopure diphenyl esters. These effects of substituents and homochirality on the inclusion properties are discussed on the basis of X-ray structures of some inclusion and guest-free compounds. *Chirality* 20:295–300, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: inclusion compound; X-ray crystallography; 9,9'-bianthryl; enantiopure host

INTRODUCTION

The molecular framework of 9,9'-bianthryl has been utilized for the design of biaryl type hosts in the host–guest chemistry: for example, compounds **1** and *rac*-**2** form inclusion crystals with a wide range of small organic molecules (Fig. 1).^{1–4} Other known examples of 9,9'-bianthryl derivatives are 2,2'-dicarboxylic acid (**3**) and its dimethyl ester (**4**), the resolution of which was reported in 1969 as the first enantiopure substituted 9,9'-bianthryl.^{5,6} Recently, we reported that the absolute stereochemistry and chiroptical properties (CD spectra) of these carboxylic derivatives as well as **2**: the levorotatory enantiomers are related to the *M* isomers for all the compounds.⁷ However, little is known about the application of these compounds as biaryl hosts. Therefore, we examined the inclusion properties of racemic and enantiopure **4** and its diphenyl ester analog **5** toward various organic compounds. The effects of ester substituents and homochirality on inclusion properties are discussed on the basis of X-ray structures.

RESULTS AND DISCUSSION

Synthesis and Chiroptical Properties

The synthesis of *rac*- and (–)-(*M*)-**4** was previously reported in the literature.^{5,6} The diphenyl esters, *rac*- and (*M*)-**5**, were prepared from the corresponding carboxylic acid **3** by treatment with oxalyl dichloride followed by sodium phenoxide in good overall yield (Scheme 1). The specific rotation of (*M*)-**5** was $[\alpha]_{27}^D -249.5$ in acetone, namely, the absolute stereochemistry was (–)-(*M*) or (+)-(*P*). The CD spectra of (*M*)-**4** and (*M*)-**5** are similar to each other as shown in Figure 2. A relatively strong Cotton effect at 242 nm and weak and complicated Cotton effects around 400 nm were seen, these being attributed to the β' and p bands of the anthracene chromophores,

respectively, according to a theoretical study of **4** and related compounds.^{7,8}

Formation of Inclusion Compounds

Four host candidates, *rac*-**4**, (*M*)-**4**, *rac*-**5**, and (*M*)-**5**, were used in the inclusion experiments with the 12 common organic compounds in Table 1. Crystals were grown from solutions in neat guests or from solutions containing guests in appropriate solvents. Formation and stoichiometry of inclusion compounds were checked by ¹H NMR spectroscopy.

The enantiopure dimethyl ester (*M*)-**4** readily forms inclusion compounds with seven organic guest molecules, and the host:guest ratio ranges from 1:1 to 1:4. The guest ratio tends to be large for small molecules such as methanol. As for acetone, the inclusion ratio was influenced by the concentration of solutions: the values changed from 1:2 to 1:1 and then to 1:0 as the concentration was lowered. This finding suggests that this host forms a few types of crystals depending of the conditions. In contrast to benzene and *p*-xylene, toluene was not included by this host. The racemic compound, *rac*-**4**, formed an inclusion compound only with benzene, its ratio being in the range of 3:1 to 1:2 depending on the concentration. The racemic and enantiopure diphenyl esters, *rac*-**5** and (*M*)-**5**, formed no inclusion compounds with any foreign compounds examined above. These results show that the inclusion

Crystallographic data for the structures reported in this paper have been deposited to the Cambridge Crystallographic Data Centre (CCDC 641224–641227).

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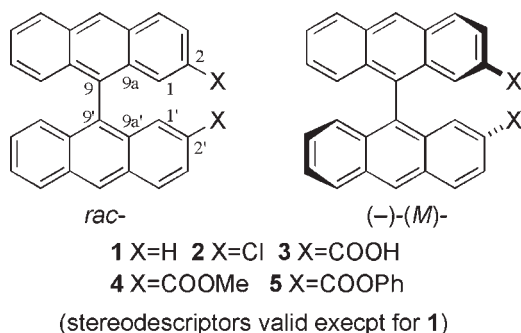


Fig. 1. 9,9'-Bianthryl derivatives.

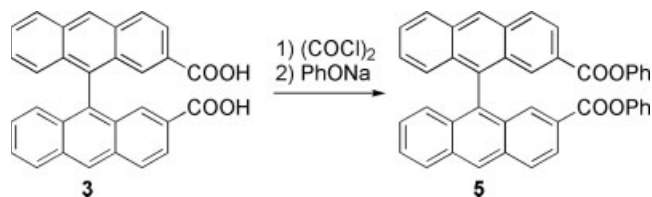
properties are considerably influenced by the homochirality, namely, enantiopure or racemic, of 9,9'-bianthryl molecules and the substituents in the ester moieties.

X-ray Structures

X-ray analyses were carried out for inclusion compounds of (*M*)-**4** with benzene and *p*-xylene and guest-free *rac*-**5** and (*M*)-**5**. ORTEP drawings of 9,9'-bianthryl moieties are shown in Figure 3. Two independent molecules are found in an asymmetric unit for (*M*)-**4**:benzene and (*M*)-**4**:*p*-xylene, and two anthracene moieties are related by a symmetric operation in *rac*-**5**. The selected dihedral angles are listed in Table 2. Packing structures are shown in Figure 4. We could not obtain a single crystal suitable for the analysis for *rac*-**4**.

In the all structures, two anthracene groups are nearly bisected each other in each bianthryl moiety at dihedral angles of $90^\circ \pm 10^\circ$. This structural feature is general for other simple 9,9'-bianthryl compounds. [We obtained ten X-ray structures of 9,9'-bianthryl derivatives in the Cambridge Crystallographic Database (Ver. 5.28). For simple derivatives, dihedral angles between two anthracene groups are as follows. 10,10'-Dibromo-9,9'-bianthryl (92.1° , 97.9°), 10,10'-diphenyl-9,9'-bianthryl (90.6°), 9,9'-bianthryl (81.9° , 71.6°), 9,9'-bianthryl:chlorocyclohexane (90.5°), 10-cyano-9,9'-bianthryl (82.9°).^{4,9–12} The ester moieties, $-\text{C}(=\text{O})-\text{O}-\text{C}$, are nearly coplanar with the attaching anthracene rings either in *anti* or *syn* form [$\text{C}(1)-\text{C}(2)-\text{C}(11)-\text{O}(2)$ is nearly 180° for *anti* and 0° for *syn*]. The methyl or phenyl groups are always *syn* to the carbonyl oxygen atoms as typical for ordinary esters.¹³

The structures of two independent molecules of (*M*)-**4**:benzene are similar to each other, where one of the ester groups is *syn* and the other is *anti* (*syn-anti*) in each 9,9'-bianthryl structure. As for (*M*)-**4**:*p*-xylene, two ester groups are *syn* (*syn-syn*) in Molecule A, whereas one of the ester groups is *anti* (*syn-anti*) in Molecule B. In the

Scheme 1. Synthesis of *rac*-**5** and (*M*)-**5**.

structure of *rac*-**5**, the ester groups are *syn-syn* and the phenyl groups are rotated by 46° from the coplanar conformation relative to the ester moiety. In the structure of (*M*)-**5**, the ester groups are *syn-anti* and the phenyl groups are rotated by 66° and 85° .

In (*M*)-**4**:benzene, a weak but significant $\pi-\pi$ contact is found between host molecules (about 3.4–3.5 Å) as indicated as a broken line in Figure 4. A similar stacking is also observed in the structure of (*M*)-**4**:*p*-xylene. There are no significant interactions between host and guest molecules in these inclusion compounds. Therefore, the $\pi-\pi$ interactions play an important role in building a network of host molecules, where guest molecules are included in the cavities without strong attractive interactions. The size and shape of cavities are adjustable within a range by changing the mode or area of $\pi-\pi$ overlapping and the conformation of ester moieties. As a result, this host system accommodates two benzene molecules per one host molecule, but only one *p*-xylene molecule. We consider that the racemic compound, *rac*-**4**, should form a tight packing system without cavities available for guest molecules. As for the phenyl ester, 9,9'-bianthryl moieties also form a lattice-like network both in crystals of *rac*-**5** and (*M*)-**5**. However, the phenyl groups occupy the cavities, and there is no room for guest molecules.

In summary, the enantiopure dimethyl ester (*M*)-**4** is found to be a good host for various guest molecules. This inclusion ability is attributed to the molecular network of 9,9'-bianthryl molecules with cavities for guest molecules. This enantiopure host is potentially available for enantiomeric resolution of racemic samples, and further studies will reveal the possibility of this application.

EXPERIMENTAL

General

^1H and ^{13}C NMR spectra were measured on a Varian Gemini-300 spectrometer. UV spectra were measured with

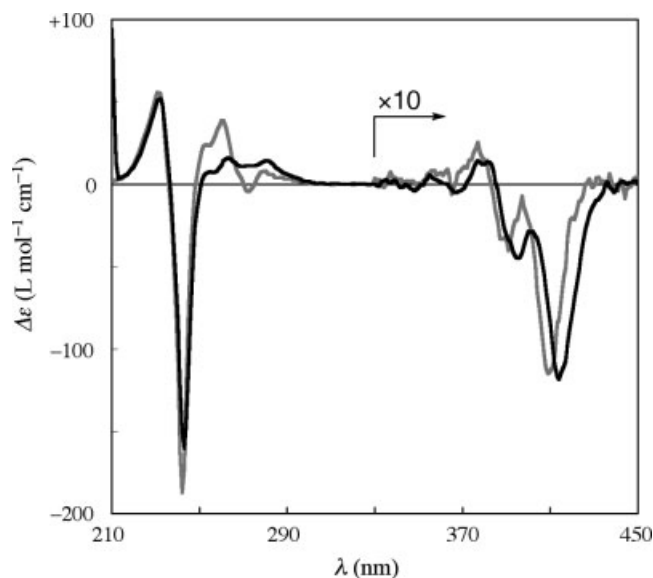
Fig. 2. CD spectra of (*M*)-**4** (gray) and (*M*)-**5** (black) in THF.

TABLE 1. Formation of inclusion compounds of (*M*)-4 and *rac*-4 with various guests molecules^a

Host	Benzene	<i>p</i> -Xylene	Methanol	2-Propanol	Acetone	Phenol	Aniline
(<i>M</i>)-4	1:2	1:1	1:4	1:1	1:2–1:1 ^b	1:1	1:1
<i>rac</i> -4	3:1–1:2 ^b	–	–	–	–	–	–

^aHost–guest molar ratios. Dash means formation of no inclusion compounds. None of the compounds included toluene, dichloromethane, chloroform, ethyl acetate, and THF upon crystallization.

^bRatio was variable depending on the conditions.

a Hitachi U-3000 spectrometer with a 10-mm cell. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter with a 3.5φ × 100 mm cell. CD spectra were measured on a JASCO J-810 polarimeter with a 1-mm cell.

Elemental analyses were performed by a Perkin-Elmer 2400 series analyzer. Compounds 3 and 4 in racemic and enantiopure forms were prepared by the literature method.^{5,6}

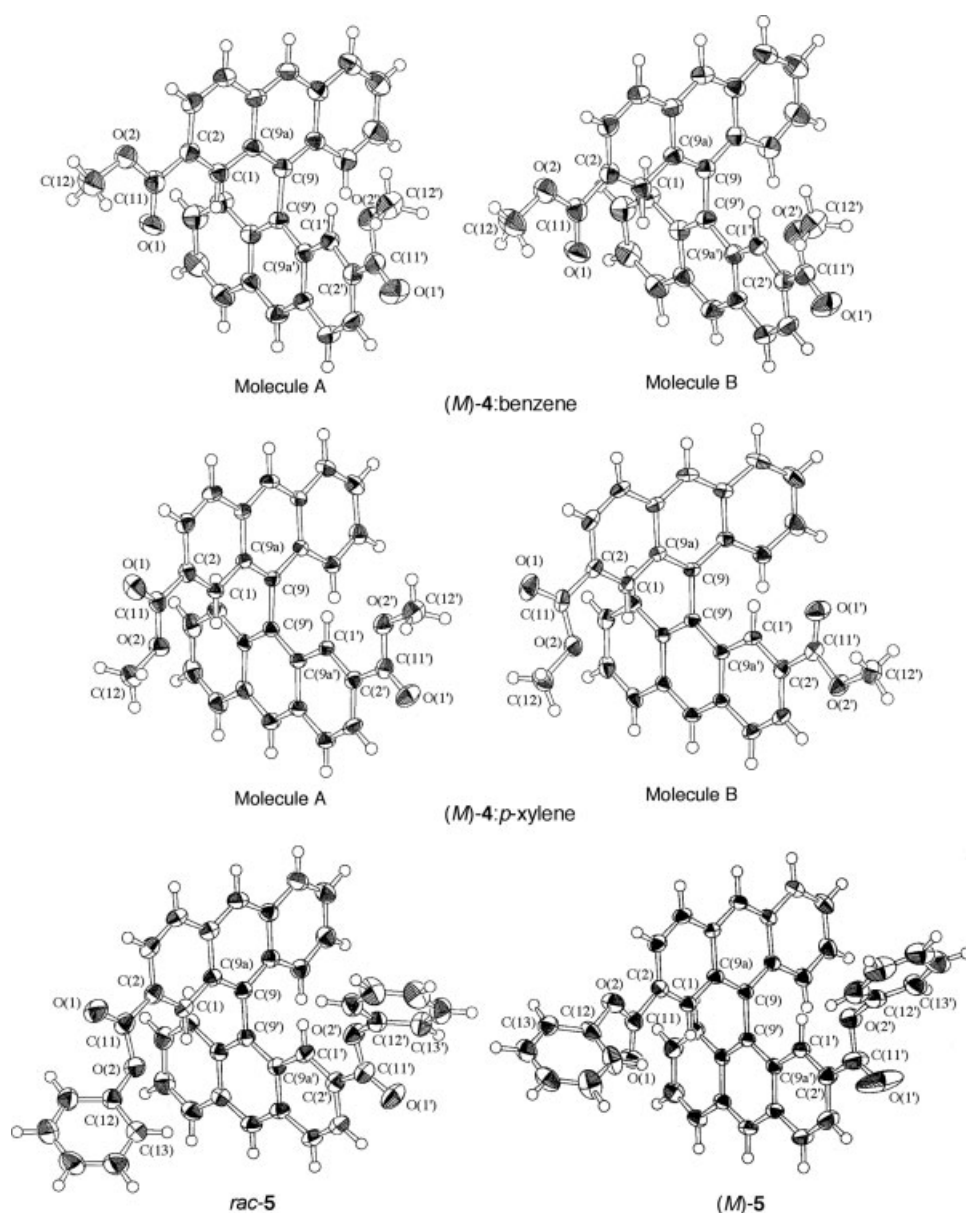


Fig. 3. X-ray structures of bianthryl moieties of (*M*)-4, *rac*-5, and (*M*)-5. (Guest molecules are omitted for 4. The stereochemistry was arbitrarily *M* for the structure of *rac*-5.)

TABLE 2. Selected dihedral angles for inclusion compounds of (*M*)-4 and guest-free *rac*-5 and (*M*)-5

	(<i>M</i>)-4: Benzene ^a		(<i>M</i>)-4: <i>p</i> -Xylene ^a		<i>rac</i> -5 ^b	(<i>M</i>)-5
	Molecule A	Molecule B	Molecule A	Molecule B		
C(9a)–C(9)–C(9')–C(9a')	–90.1(3)	–90.6(3)	–98.9(2)	–87.2(3)	–96.7(3)	–90.0(4)
C(1)–C(2)–C(11)–O(2)	174.8(3)	–175.2(3)	–169.4(3)	175.6(3)	8.8(3)	168.5(3)
C(2)–C(11)–O(2)–C(12)	178.7(3)	–179.6(3)	–179.0(2)	179.1(2)	175.0(2)	–173.1(3)
C(11)–O(2)–C(12)–C(13)	–	–	–	–	45.5(3)	66.1(5)
C(1')–C(2')–C(11')–O(2')	1.8(4)	0.2(4)	–3.5(4)	179.3(2)	8.8(3)	2.9(6)
C(2')–C(11')–O(2')–C(12')	–175.8(3)	177.0(3)	–180.0(2)	179.9(2)	175.0(2)	179.6(4)
C(11')–O(2')–C(12')–C(13')	–	–	–	–	45.5(3)	84.6(5)

^aTwo independent 9,9'-bianthryl molecules exist in an asymmetric unit.^bTwo anthryl moieties are symmetrically related.

Synthesis

(–)-(*M*)-Diphenyl 9,9'-bianthryl-2,2'-dicarboxylate (*M*)-5. To a suspension of 385 mg (0.84 mmol) of (–)-(*M*)-9,9'-

bianthryl-2,2'-dicarboxylic acid (**3**) in 25 ml of dry benzene were added 0.80 ml (10.5 mmol) of oxalyl dichloride and a drop of DMF. The mixture was stirred for 12 h to give a

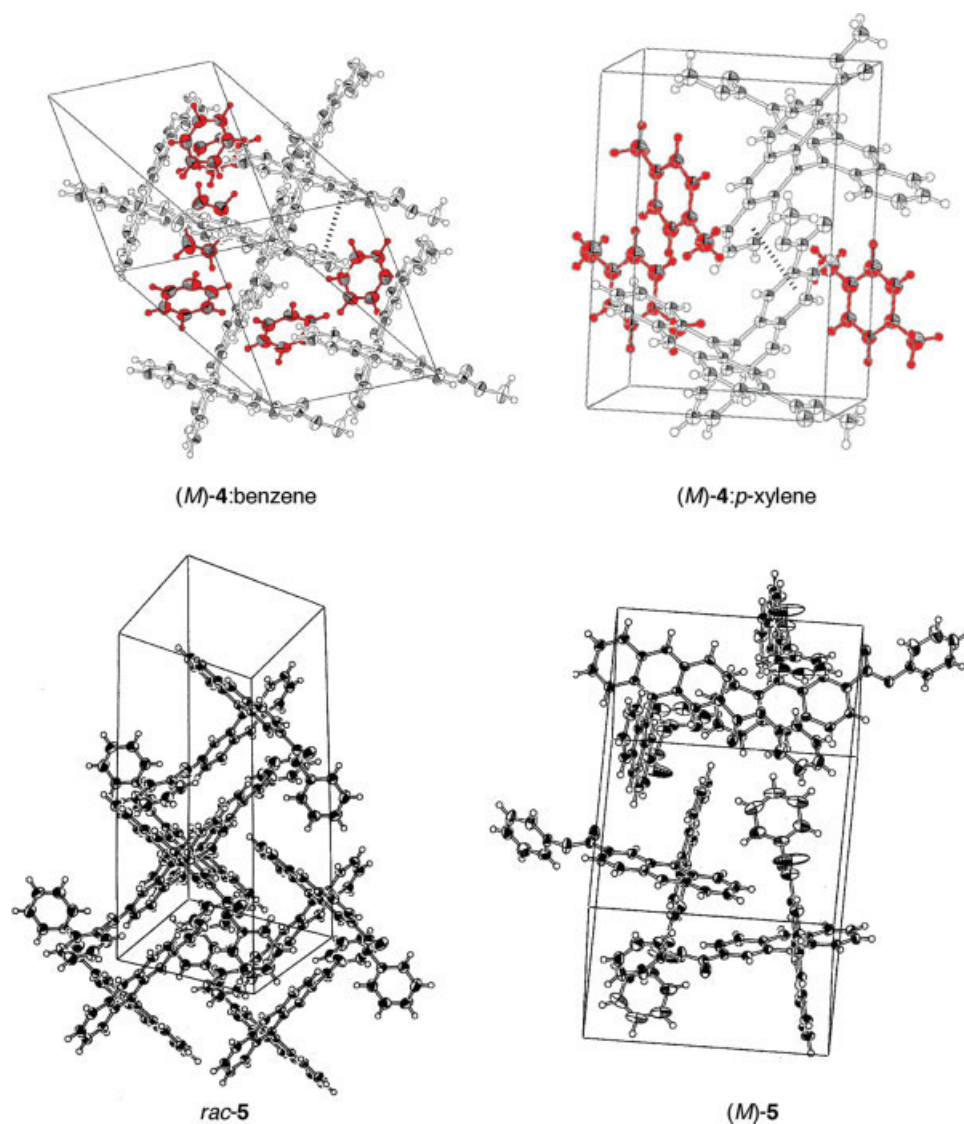


Fig. 4. Packing diagrams of inclusion compounds of (*M*)-4 and guest-free *rac*-5, and (*M*)-5. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 3. Crystallographic data of inclusion compounds of (*M*)-4 and guest-free *rac*-5 and (*M*)-5

	(<i>M</i>)-4:benzene	(<i>M</i>)-4: <i>p</i> -xylene	<i>rac</i> -5	(<i>M</i>)-5
Chemical formula	2(C ₃₂ H ₂₂ O ₄ ·2C ₆ H ₆)	2(C ₃₂ H ₂₂ O ₄ ·C ₈ H ₁₀)	C ₄₂ H ₂₆ O ₄	C ₄₂ H ₂₆ O ₄
<i>M</i>	1253.48	576.69 × 2	594.66	594.66
Crystal system	Triclinic	Triclinic	Monoclinic	Orthorhombic
Space group	<i>P</i> 1 (no. 1)	<i>P</i> 1 (no. 1)	<i>C</i> 2/ <i>c</i> (no. 15)	<i>P</i> 2 ₁ 2 ₁ 2 ₁ (no. 19)
α (Å)	11.952 (3)	11.6402 (5)	11.564 (6)	13.456 (3)
β (Å)	14.267 (2)	13.6246 (5)	22.142 (4)	22.223 (4)
γ (Å)	11.625 (3)	9.6139 (4)	11.960 (2)	10.375 (3)
α (°)	111.47 (1)	91.208 (2)	90	90
β (°)	113.52 (2)	92.501 (3)	109.43 (3)	90
γ (°)	90.22 (2)	84.896 (1)	90	90
<i>V</i> (Å ³)	1665.2 (8)	1517.0 (1)	2887 (1)	3102 (1)
<i>Z</i>	1	1	4	4
<i>D</i> _{calc} (g cm ^{−3})	1.250	1.262	1.368	1.273
<i>T</i> (K)	288	173	288	288
Reflections	7111	6444	3216	3779
<i>R</i>	0.070	0.060	0.063	0.077
<i>wR</i>	0.115	0.109	0.125	0.142

clear yellow solution. The volatile materials were removed by evaporation, and the residue was practically pure acid chloride, which was used for the next reaction without further purification. (*M*)-9,9'-Bianthryl-2,2'-dicarboxylic dichloride: ¹H NMR (300 MHz, CDCl₃) δ 7.05 (t, ³*J* = 8.4 Hz, 2H), 7.25 (m, 2H), 7.58 (m, 2H), 7.95–8.02 (m, 4H), 8.20–8.27 (m, 4H), 8.77 (s, 2H). To a solution of sodium phenoxide in 10 ml of benzene, prepared from 166 mg (1.77 mmol) of phenol and 47 mg (1.9 mmol) of sodium hydride, was slowly added a solution of the acid chloride in 20 ml of benzene. The whole was stirred for 30 min at room temperature. After the solvent was removed, the crude material was purified by chromatography on silica gel using hexane-dichloromethane (1:1) as eluent. Yellow powder; yield 0.43 g (86%); mp 246–247°C; ¹H NMR (300 MHz, CDCl₃) δ 6.99–7.06 (m, 6H), 7.14–7.33 (m, 8H), 7.52 (t, ³*J* = 7.6 Hz, 2H), 8.04 (s, 2H), 8.12–8.19 (m, 4H), 8.25 (d, ³*J* = 9.0 Hz, 2H), 8.74 (s, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 121.6, 124.1, 125.7, 126.7, 126.8, 126.9, 127.0, 127.9, 128.8, 129.2, 129.4, 130.6, 131.4, 132.3, 132.7, 133.1, 134.9, 150.8, 165.1; [α]_D²⁵ −249.5 (c 0.20, acetone). CD (THF): λ (Δε) 244 (−212), 277 (31), 418 (−13) nm. UV (THF): λ (ε) 242 (151000), 262 (361000), 345 (6600), 367 (8200), 390 (10000), 413 (12000) nm; Anal. Calcd for C₄₂H₂₆O₄: C, 84.83, H, 4.41%. Found C, 84.86, H, 4.31%.

rac-Diphenyl 9,9'-bianthryl-2,2'-dicarboxylate *rac*-5. The racemic compound was similarly prepared from *rac*-3. Yellow powder; yield 80%; mp 240–241°C; Anal. Calcd for C₄₂H₂₆O₄: C, 84.83, H, 4.41%. Found C, 84.63, H, 4.38%. The NMR data were identical with those of (*M*)-5.

Inclusion Experiments

About 20 mg of a bianthryl derivative was dissolved in a minimal amount of various organic solvents (benzene, toluene, *p*-xylene, acetone, dichloromethane, chloroform, ethyl acetate, THF, methanol, and 2-propanol). A host compound was treated in acetone for aniline and phenol as guest. The solutions were allowed to stand for several

days at room temperature until crystals were formed. The host–guest composition was determined by the integral intensity of ¹H NMR signals.

X-ray Crystallography

Single crystals used for the measurements were prepared by slow evaporation of solutions of benzene for the phenyl esters. Inclusion compounds diffraction data were collected on a Rigaku RAXIS-IV imaging plate diffractometer, using graphite monochromated Mo-*K*α radiation (λ = 0.71073 Å). The structures were solved by the direct method and refined by the full-matrix least squares on all data by Shelxt. Nonhydrogen and hydrogen atoms were refined anisotropically and isotropically, respectively. Selected crystal data are compiled in Table 3.

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Discrimination of Enantiomers of α -Amino Acids by Chiral Derivatizing Reagents from *trans*-1,2-Diaminocyclohexane

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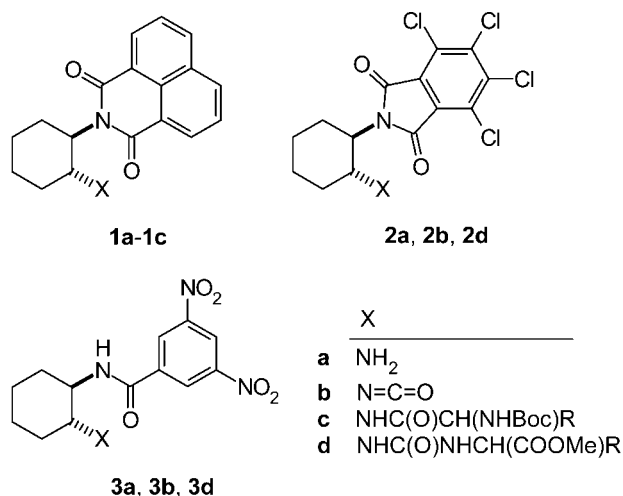
Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT New chiral derivatizing reagents (CDAs) derived from *trans*-1,2-diaminocyclohexane, having an electron-deficient aromatic substituent (either an aromatic imide or 3,5-dinitrobenzamide) and rigid structure (either an amide or a urea linker), are reported. Significant shift differences of diastereotopic protons in the ^1H NMR signals are observed for enantiomers of suitably protected α -amino acids, linked to CDA by a covalent bond. A simple, general model rationalizing the observed enantiomer discrimination and based on semiempirical conformational search is presented. *Chirality* 20:301–306, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: absolute configuration; α -amino acid derivatives; chemical shift; conformation; chiral derivatizing agent; *trans*-1,2-diaminocyclohexane; enantiomer excess

Chiral derivatizing reagents (CDAs) are of great importance as auxiliaries for the analysis of configuration^{1,2} and enantiomer excess^{3–6} of products of asymmetric synthesis. NMR spectroscopy is widely used as the analytical method of choice for both purposes. Starting with derivatives of *O*-methylmandelic acid (Mislow)⁷ and α -methoxy- α -trifluorophenylacetic acid (MTPA, Mosher),⁸ introduced four decades ago, many other CDAs have been developed.^{9–24} To act efficiently as a shift agent, the derivatizing reagent should contain an aromatic group, which provides anisotropy to diastereotopic substituents at the stereogenic center of the analyzed enantiomers. No less important appears the conformational rigidity of the chiral derivatizing agent (CDA), as well as of the product of derivatization. This secures higher $\Delta\delta$ values and allows for more accurate rationalization of the mode of action of CDA. Last, but not least, is the availability of the CDA from the pool of commercial enantiomerically pure compounds.

With these guidelines in mind we devised simple derivatives of *trans*-1,2-diaminocyclohexane (DACH) as chiral derivatizing agents for amines and carboxylic acids. These derivatives (**1c**, **2d**, **3d**) can be readily obtained from enantiomerically pure DACH by the method of monoimidation (**1a**, **2a**) with aromatic anhydrides, originally developed by us,²⁵ or by a selective monoacylation of DACH (**3a**, yield 92%) with 3,5-dinitrobenzoyl chloride, as described in the Experimental Section of this report. A clear antecedent for this group of CDAs is *trans*-2-phenylcyclohexanol, introduced by Riguera and coworkers as CDA for chiral carboxylic acids.¹³ In the course of our work Yang and coworkers described an NMR shift reagent based on the salt formation of **1a** with chiral carboxylic acids.²⁴



EXPERIMENTAL PROCEDURES

General

NMR spectra were recorded in deuteriochloroform (if not stated otherwise) on a Varian XL300 instrument and are reported in ppm with respect to TMS as a reference. MS were measured with a 604 AMD Intectra spectrom-

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ter. FTIR spectra were taken in KBr pellets on a Bruker IFS 113v spectrometer. Optical rotations were measured on a Perkin-Elmer 243B polarimeter. Melting points are uncorrected.

The structures of the representative derivatives: **D**- and **L-Ala-1c** as well as **D**- and **L-Ala-2d** were optimized by the PM3 method using the Gaussian 03 suite of programs.²⁵ Since the low-energy conformations of the imide derivatives of *trans*-1,2-diaminocyclohexane are well known,²⁷ the potential energy surfaces were obtained as a function of the amino acid torsion angles around the NH—C^{*}H and C^{*}H—C(=O) bonds. These bonds were rotated at the interval of 20° between 0° and ±180°.

Monoimides of DACH such as *N*-phthaloyl, *N*-(1,8-naphthaloyl) (**1a**) and *N*-tetrachlorophthaloyl (**2a**) derivatives were for the first time reported by us.²⁵ Later slightly different procedure for **1a** was described by the Chinese workers.²⁴

N-3,5-Dinitrobenzoyl-(1*R*,2*R*)-diaminocyclohexane (**3a**)

To a solution of (*R,R*)-1,2-diaminocyclohexane (114 mg, 1 mmol) in 10 mL CH₂Cl₂, stirred in an ice bath (0 to −5°C) for 15 min was added slowly 3,5-dinitrobenzoyl chloride (230 mg, 1 mmol) in 5 mL CH₂Cl₂. Stirring was continued for additional 3 h at room temperature, with the formation of a precipitate. The mixture was shaken thoroughly with dilute HCl, the aqueous phase was stirred with 1 N NaOH and extracted with dichloromethane. The organic solution was dried over MgSO₄ and evaporated. Yield 60%; m.p. 190–192°C; $[\alpha]_D^{20}$ −34 (*c* = 1, EtOH); ¹H NMR δ 1.23–1.58 (m, 4H), 1.78–1.98 (m, 2H), 2.01–2.18 (m, 1H), 2.27–2.42 (m, 1H), 2.61 (dt, *J* = 3.9, 10.4 Hz, 1H), 3.67 (ddd, *J* = 3.9, 8.9, 11.0 Hz, 1H), 6.77 (d, *J* = 5.8 Hz, 1H), 8.99 (d, *J* = 2.2 Hz, 2H), 9.16 (d, *J* = 2.2 Hz, 1H); ¹³C NMR δ (DMSO) 23.64, 24.23, 29.71, 31.20, 51.47, 52.81, 120.72, 127.85, 137.21, 147.76, 162.44; IR ν (cm^{−1}) 3340, 3101, 3031, 2937, 2872, 1550, 1536, 1344, 1192; EI MS: (*m/z*) 308.1, 291.1, 265.1, 248.1, 218.0, 195.0, 179.0, 149.1, 120.1, 97.1, 75.1, 56.1.

Isocyanates **1b**, **2b**, **3b**

A mixture of appropriate monoprotected diamine **1a**, **2a**, or **3a** (2.5 mmol), CH₂Cl₂ (25 mL), and saturated NaHCO₃ solution (25 mL) was stirred at −5°C for 20 min, then triphosgene (252 mg, 0.842 mmol) was added. Stirring was continued for additional 15 min in an ice bath (0 to −5°C). The mixture was extracted three times with dichloromethane and saturated NaHCO₃ solution. The organic solution was dried over MgSO₄ and evaporated to give crude product. After crystallization from benzene-Et₂O, the product was obtained as colorless crystals.

(1*R*,2*R*)-1-(1',8'-Naphthalimido)-2-cyclohexylisocyanate (1b**).** Yield 92%; m.p. 105–107°C; $[\alpha]_D^{20}$ −15 (*c* = 1, CHCl₃); ¹H NMR δ 1.43–1.68 (m, 6H), 2.23–2.29 (m, 1H), 2.40–2.54 (m, 1H), 4.70 (dt, *J* = 4.3, 10.7 Hz, 1H), 5.03 (dt, *J* = 3.8, 10.7 Hz, 1H), 7.74–7.80 (t, *J* = 7.1 Hz, 2H), 8.22 (d, *J* = 7.1 Hz, 2H), 8.61 (bs, 2H); ¹³C NMR δ 24.59, 25.50, 28.18, 35.65, 53.91, 58.11, 122.67, 126.87, 128.18, 131.37 (bs), 133.82, 164.58; IR ν (cm^{−1}) 2993, 2862, 2252, 1699,

1660, 1340, 1236, 1184; EI MS: (*m/z*) 320.3, 292.3, 277.2, 236.2, 236.2, 222.2, 198.2, 180.2, 152.1, 126.1, 99.1, 74.1, 59.1; EI HRMS: calcd. for C₁₉H₁₆O₃N₂ (M): 320.11609; found: 320.11496.

(1*R*,2*R*)-1-(Tetrachlorophthalimido)-2-cyclohexylisocyanate (2b**).** Yield 82%; m.p. 178–180°C; $[\alpha]_D^{20}$ −41 (*c* = 0.4, CHCl₃); ¹H NMR δ 1.36–1.59 (m, 3H), 1.78–1.89 (m, 3H), 2.07–2.19 (m, 1H), 2.24–2.29 (m, 1H), 4.01 (dt, *J* = 3.9, 10.5 Hz, 1H), 4.26 (dt, *J* = 3.9, 11.0 Hz, 1H); ¹³C NMR δ 24.29, 24.97; 28.69, 35.29, 54.01, 56.63, 123.28, 127.03, 129.74, 140.21, 163.25; IR ν (cm^{−1}) 2930, 2860, 2261, 1718, 1382, 1295, 1197; EI MS: (*m/z*) 408.0, 365.0, 338.9, 323.9, 285.9, 241.8, 213.9, 176.9, 142.0, 123.1, 97.1, 56.1; EI HRMS: calcd. for C₁₅H₁₀O₃N₂Cl₄ (M): 407.94159; found: 407.93880.

(1*R*,2*R*)-1-(3',5'-Dinitrobenzamido)-2-cyclohexylisocyanate (3b**).** Yield 78%; m.p. 273–276°C; $[\alpha]_D^{20}$ −24 (*c* = 0.8, CHCl₃); ¹H NMR δ 1.2–1.6 (m, 4H), 1.9–2.05 (m, 2H), 2.1–2.2 (m, 1H), 2.7–2.8 (m, 1H), 3.40 (dt, *J* = 4.12, 10.4 Hz, 1H), 3.70 (ddd, *J* = 3.8, 8.8, 10.4 Hz, 1H), 5.07 (s, 1H), 8.81 (d, *J* = 2.2 Hz, 2H), 9.16 (d, *J* = 2.1 Hz, 1H); ¹³C NMR δ (DMSO) 23.54, 24.11, 31.32, 33.39, 54.52, 57.38, 120.82, 127.27, 127.75, 136.59, 147.92, 161.88; IR ν (cm^{−1}) 3335, 3271, 3101, 2938, 2864, 2667, 2252, 1651, 1541, 1344; EI MS: (*m/z*) 334.1, 317.1, 291.1, 250.1, 212.0, 195.0, 139.1, 179.0, 139.1, 123.1, 96.1, 75.0; EI HRMS: calcd. for C₁₄H₁₄O₆N₄ (M): 334.09134; found: 334.09378.

Amides **1c**

To a solution of 29.4 mg (0.1 mmol) *N*-1',8'-naphthaloyl-(*R,R*)-1,2-diaminocyclohexane (**1a**)²⁵ and 0.1 mmol appropriate *N*-Boc amino acid in dichloromethane (7 mL) at −5°C was added DCC (20.6 mg, 0.1 mmol). The mixture was stirred in an ice bath (0 to −5°C) for 45 min and stirring was continued for additional 7 h at room temperature. The precipitated *N,N*-dicyclohexylurea was filtered and the filtrate evaporated to dryness. The crude product was dissolved in diethyl ether to precipitate a further amount of *N,N*-dicyclohexylurea. The product was then purified by extraction with dichloromethane and 1 N HCl, followed by washing with saturated NaHCO₃ solution, drying over MgSO₄ and evaporation.

D-Ala-1c. Yield 68%; m.p. 175–178°C; ¹H NMR δ 0.79 (d, *J* = 7.14 Hz, 3H), 1.04–1.91 (m, 6H), 1.32 (s, 9H), 2.14–2.18 (m, 1H), 2.80 (ddd, *J* = 5.1, 7.1, 13.1 Hz, 1H), 3.81–3.89 (m, 1H), 4.88–5.03 (m, 3H), 5.68 (d, *J* = 8.2 Hz, 1H), 7.73 (bs, 2H), 8.19 (d, *J* = 8.2 Hz, 2H), 8.54–8.61 (m, 2H).

D,L-Ala-1c. Yield 72%; m.p. 175–179°C; ¹H NMR δ 0.79 and 0.94 (d + bs, *J* = 7.14 Hz, 3H), 1.32 (s, 9H), 1.15–1.96 (m, 6H), 2.14–2.18 (m, 1H), 2.78–2.88 (m, 1H), 3.83–3.88 (m, 1H), 4.66 (bs, 1H), 4.91–4.95 (m, 2H), 5.74 and 5.98 (d + d, *J* = 8.2 Hz, 1H), 7.73 (t, *J* = 7.9 Hz, 2H), 8.19 (d, *J* = 8.2 Hz, 2H), 8.57 (d, *J* = 6.7 Hz, 2H).

D-Val-1c. Yield 76%; m.p. 185–187°C; ¹H NMR δ 0.22 (d, *J* = 6.6 Hz, 3H), 0.34 (d, *J* = 6.6 Hz, 3H), 1.22–2.0 (m, 7H), 1.36 (s, 9H), 2.16–2.20 (m, 1H), 2.78 (ddd, *J* = 5.7, 7.1, 13.9 Hz, 1H), 3.59–3.65 (m, 1H), 4.90–5.08 (m, 3H),

TABLE 1. Characteristic chemical shift differences $\Delta\delta_{(R-S)}$ (in bold) of diastereotopic protons in amide type amino acid derivatives **1c** (ppm, CDCl₃ solution)

Amino acid derivative	R	Config.	δ R	$\Delta\delta_{(R-S)}$	δ Boc	$\Delta\delta_{(R-S)}$
1c	Me	<i>R</i>	0.79	−0.16	1.33	0.11
		<i>S</i>	0.95		1.22	
1c	<i>i</i> -Pr	<i>R</i>	0.22; 0.34	−0.30; −0.40	1.36	0.19
		<i>S</i>	0.52; 0.74		1.17	
1c	Bn	<i>R</i>	6.72	−0.29	1.32	0.15
		<i>S</i>	7.01		1.17	

5.63 (d, J = 8.0 Hz, 1H), 7.75 (t, J = 8.2 Hz, 2H), 8.18 (d, J = 8.2 Hz, 2H), 8.58 (d, J = 8.1 Hz, 2H).

D,L-Val-1c. Yield 72%; m.p. 184–186°C; ¹H NMR δ 0.22 and 0.52 (d + bs, J = 6.6 Hz, 3H), 0.34 and 0.74 (d + d, J = 6.6 Hz, 3H), 1.16 and 1.36 (s + s, 9H), 1.22–1.67 (m, 4H), 1.80–1.91 (m, 3H), 2.16–2.23 (m, 1H), 2.78 (ddd, J = 5.3, 7.1, 13.6 Hz, 1H), 3.58–3.65 (m, 1H), 4.66 (bs, 0.5H), 4.90–5.08 (m, 2.5H), 5.62 and 5.87 (d + bs, J = 8.0 Hz, 1H), 7.75 (t, J = 8.2 Hz, 2H), 8.18 (d, J = 7.9 Hz, 2H), 8.58 (d, J = 7.3 Hz, 2H).

D-Phe-1c. Yield 70%, m.p. 221–223°C; ¹H NMR δ 1.21–1.52 (m, 5H), 1.32 (s, 9H), 1.84–1.88 (m, 2H), 2.16–2.20 (m, 1H), 2.56–2.63 (m, 1H), 2.70–2.77 (m, 1H), 3.97–4.04 (m, 1H), 4.74 (bs, 1H), 4.75–4.90 (m, 2H), 5.93 (d, J = 8.2 Hz, 1H), 6.56–6.87 (m, 5H), 7.33 (bs, 2H), 8.20 (d, J = 9.3 Hz, 2H), 8.52–8.58 (m, 2H).

D,L-Phe-1c. Yield 76%; m.p. 222–224°C; ¹H NMR δ 1.03–2.20 (m, 7H), 1.17 and 1.32 (s, 9H), 2.17–2.20 (m, 1H), 2.56–2.80 (m, 2H), 3.97–4.04 (m, 1H), 4.62 (bs, 1H), 4.74–4.96 (m, 2H), 5.66 and 5.93 (bs + d, J = 7.7 Hz, 1H), 6.56–6.87 and 6.97–7.05 (m + m, 5H), 7.73 (bs, 2H), 8.17 and 8.20 (d + d, 2H), 8.49–8.59 (m, 2H).

Ureas **2d**, **3d**

To a mixture of isocyanate **1b** or **3b** (0.20 mmol) and appropriate amino acid methyl ester hydrochloride (0.20 mmol) in dichloromethane (2 mL) was added triethylamine (0.1 mL). The mixture was stirred at room temperature for 1 h and stirring with refluxing was continued for additional 4–6 h. The solution was extracted with 1 N HCl followed by washing with saturated NaHCO₃ solution. The organic solution was dried over MgSO₄ and evaporated to give product **2d** or **3d**.

D-Ala-2d. Yield 87%; m.p. 185–189°C; ¹H NMR δ 1.10 (d, J = 7.1 Hz, 3H), 1.26–1.48 (m, 3H), 1.77–1.88 (m, 3H), 2.09–2.14 (m, 1H), 2.45 (ddd, J = 3.8, 6.7, 12.2 Hz, 1H), 3.62 (s, 3H), 3.89 (dt, J = 4.4, 12.3 Hz, 1H), 4.11–4.21 (m, 2H), 4.26 (d, J = 8.8 Hz, 1H), 4.79 (d, J = 7.1 Hz, 1H).

D,L-Ala-2d. Yield 81%; m.p. 189–191°C; ¹H NMR δ 1.10 and 1.21 (d, J = 7.1 Hz, 3H), 1.25–1.49 (m, 3H), 1.79–1.91 (m, 3H), 2.14–2.09 (m, 1H), 2.46 (ddd, J = 3.9, 6.7, 12.6 Hz, 1H), 3.42 and 3.62 (s + s, 3H), 3.91 (dt, J = 4.4, 12.6 Hz, 1H), 4.04–4.23 (m, 1H), 4.29 (dt, J = 3.6, 12.3 Hz, 1H), 4.47 (d, J = 8.8 Hz, 1H), 4.60 and 4.79 (d, J = 7.7 Hz, 1H).

D,L-Val-2d. Yield 78%, m.p. 218–224°C; ¹H NMR δ 0.55 and 0.66 (d + d, J = 6.8 Hz, 3H); 0.78 and 0.84 (d + d, J = 6.8 Hz, 3H), 1.2–1.6 (m, 3H), 1.8–2.0 (m, 3H), 2.00 (m, 1H), 2.05–2.15 (m, 1H), 2.45 (ddd, J = 3.3, 6.7, and 12.6 Hz, 1H), 3.50 and 3.64 (s + s, 3H), 3.92 (dt, J = 3.6, 12.7 Hz, 1H), 4.0–4.4 (m, 3H), 4.52 and 4.64 (d + d, J = 8.7 Hz, 1H).

L-Val-2d. Yield 75%, m.p. 244–251°C; ¹H NMR δ 0.78 (d, J = 6.8 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H), 1.2–1.6 (m, 3H), 1.8–2.0 (m, 3H), 2.01 (m, 1H), 2.11 (m, 1H), 2.46 (ddd, J = 3.3, 6.7, 12.6 Hz, 1H), 3.50 (s, 3H), 3.90 (m, 1H), 4.05 (m, 1H), 4.25 (m, 2H), 4.52 (d, J = 8.8 Hz, 1H).

D-Phe-2d. Yield 84%; m.p. 220–221°C; ¹H NMR δ 1.14–1.47 (m, 3H), 1.77–1.86 (m, 3H), 2.02–2.06 (m, 1H), 2.47 (ddd, J = 3.9, 6.7, 12.6 Hz, 1H), 2.75 (ddd, J = 4.2, 8.2, 13.2 Hz, 2H), 3.59 (s, 3H), 3.89 (dt, J = 4.1, 12.9 Hz, 1H), 4.19–4.22 (m, 2H), 4.46 (dd, J = 7.7, 13.7 Hz, 1H), 4.68 (d, J = 8.2 Hz, 1H), 6.95 (d, J = 7.8 Hz, 2H), 7.15–7.25 (m, 3H).

L-Phe-2d. Yield 76%; m.p. 219–222°C; ¹H NMR δ 1.14–1.47 (m, 3H), 1.77–1.86 (m, 3H), 2.07–2.11 (m, 1H), 2.47 (ddd, J = 3.9, 6.9, 12.6 Hz, 1H), 2.92 (ddd, J = 4.4, 8.8, 12.6 Hz, 2H), 3.45 (s, 3H), 3.88 (dt, J = 4.4, 12.6 Hz, 1H), 4.09–4.32 (m, 2H), 4.39–4.49 (m, 2H), 7.03 (d, J = 7.9 Hz, 2H), 7.20–7.25 (m, 3H).

D-Ala-3d. Yield 75%; dec. 295°C; ¹H NMR (DMSO) δ 1.04 (d, J = 7.3 Hz, 3H), 1.24–1.46 (m, 4H), 1.69–1.72 (m, 2H), 1.88–1.96 (m, 2H), 3.58 (s, 3H), 3.64–3.76 (m, 1H), 4.07–4.17 (m, 2H), 6.01 (d, J = 8.0 Hz, 1H), 6.31 (d, J = 7.6 Hz, 1H), 8.94–9.03 (m, 4H).

D,L-Ala-3d. Yield 71%; dec. 300°C; ¹H NMR (DMSO) δ 1.04 and 1.16 (d + d, J = 7.3 Hz, 3H), 1.25–1.42 (m, 4H), 1.69–1.73 (m, 2H), 1.88–1.96 (m, 2H), 3.26 and 3.58 (s + s, 3H), 3.64–3.76 (m, 1H), 4.10–4.18 (m, 2H), 6.01 and 6.06 (d + d, J = 8.0 Hz, 1H), 6.28 and 6.32 (d + d, J = 7.8 Hz, 1H), 8.95–9.03 (m, 4H).

D,L-Val-3d. Yield 54%, m.p. 253–256°C, ¹H NMR (DMSO) δ 0.51 and 0.59 (d + d, J = 6.8 Hz, 3H), 0.74 and 0.80 (d + d, J = 6.8 Hz, 3H), 1.2–1.6 (m, 5H), 1.6–1.8 (m, 2H), 1.8–2.0 (m, 2H), 3.22 and 3.60 (s + s, 3H), 3.3 (m, 1H), 3.58 (m, 1H), 3.71 (m, 1H), 4.0–4.15 (m, 1H), 6.05 (m, 1H), 6.22 (m, 1H), 8.96 (m, 3H).

L-Val-3d. Yield 58%, m.p. 229–231°C, ¹H NMR (DMSO) δ 0.74 (d, J = 6.8 Hz, 3H), 0.80 (d, J = 6.8 Hz, 3H), 1.2–1.6 (m, 5H), 1.7–2.0 (m, 4H), 3.22 (s, 3H), 3.2–3.4 (m, 1H),

TABLE 2. Characteristic chemical shift differences $\Delta\delta_{(R,S)}$ (in bold) of diastereotopic protons in urea type amino acid derivatives **2d**, **3d** (ppm, **2d** in CDCl_3 , **3d** in $\text{DMSO}-d_6$ solution)

Amino acid derivative	R	Config.	δ R	$\Delta\delta_{(R,S)}$	δ OMe	$\Delta\delta_{(R,S)}$
2d	Me	<i>R</i>	1.10	−0.11	3.62	0.20
		<i>S</i>	1.21		3.42	
2d	<i>i</i> -Pr	<i>R</i>	0.55; 0.66	−0.23; −0.18	3.64	0.14
		<i>S</i>	0.78; 0.84		3.50	
2d	Bn	<i>R</i>	2.75; 6.95; 7.15	−0.17; −0.08; −0.10	3.59	0.14
		<i>S</i>	2.92; 7.03; 7.25		3.45	
3d	Me	<i>R</i>	1.04	−0.12	3.58	0.31
		<i>S</i>	1.16		3.27	
3d	<i>i</i> -Pr	<i>R</i>	0.51; 0.59	−0.23; −0.21	3.60	0.38
		<i>S</i>	0.74; 0.80		3.22	
3d	Bn	<i>R</i>	2.72; 6.94	−0.12; −0.22	3.58	0.35
		<i>S</i>	2.84; 7.18		3.23	

3.58 (m, 1H), 3.70 (m, 1H), 4.09 (m, 1H), 6.06 (d, $J = 8.6$ Hz, 1H), 6.23 (d, $J = 9.0$ Hz, 1H), 8.96 (m, 3H).

D-Phe-3d. Yield 72%; m.p. 248–255°C; ^1H NMR (DMSO) δ 1.26–1.39 (m, 4H), 1.66–1.70 (m, 2H), 1.82–1.87 (m, 2H), 2.72 (ddd, $J = 5.5, 7.7, 13.7$ Hz, 2H), 3.58 (s, 3H), 3.53–3.59 (m, 1H), 3.64–3.74 (m, 1H), 4.41 (dd, $J = 5.4, 7.9$ Hz, 1H), 6.22 (d, $J = 8.2$ Hz, 1H), 6.29 (d, $J = 8.2$ Hz, 1H), 6.90–6.98 (m, 5H), 8.89–8.95 (m, 4H).

L-Phe-3d. Yield 74%; m.p. 238–242°C; ^1H NMR (DMSO) δ 1.24–1.45 (m, 4H), 1.67–1.69 (m, 2H), 1.83–1.85 (m, 2H), 2.84 (ddd, $J = 5.5, 7.7, 13.7$ Hz, 2H), 3.23 (s, 3H), 3.53–3.59 (m, 1H), 3.64–3.74 (m, 1H), 4.41 (dd, $J = 5.7, 7.9$ Hz, 1H), 6.17 (d, $J = 8.8$ Hz, 2H), 7.10 (d, $J = 7.7$ Hz, 2H), 7.19–7.27 (m, 3H), 8.94–8.97 (m, 4H).

RESULTS AND DISCUSSION

Among the monoamines **1a–3a** the 1,8-naphthaloyl derivative **1a** was found the most effective as CDA for the N-protected α -amino carboxylic acids in the form of amides (**1c**). Furthermore, the monoamines **2a**, **3a** were readily

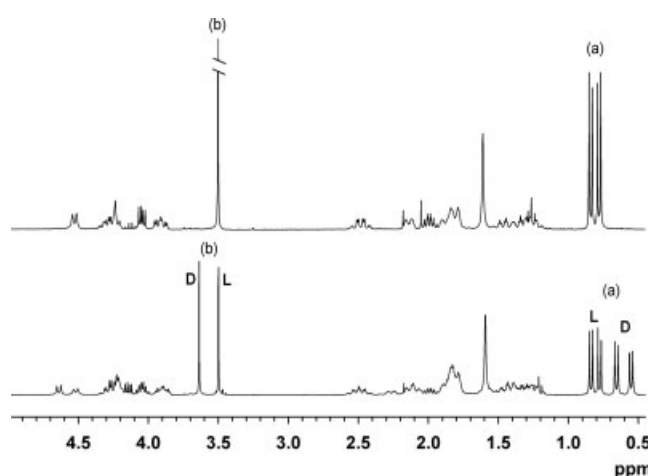


Fig. 1. ^1H NMR spectra of L-Val-**2d** (upper trace) and D,L-Val-**2d** (lower trace) in CDCl_3 solution. Diagnostic signals are due to the *i*-Pr groups (a) and the COOMe groups (b).

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converted to isocyanates in the reaction with triphosgene (yields 78–92%). The isocyanates **2b**, **3b** allowed simple derivatization of chiral α -amino acid esters via the urea linker (**2d**, **3d**). We note that $\Delta\delta$ increments²⁵ due to the isocyanate substituent in the ^1H NMR spectra of **1b**, **2b**, and **3b** are 0.8 ppm for C_αH and 0.2 ppm for C_βH . Attempts to derivatize esters of amino acids with **1b** were unsuccessful, yielding invariably the symmetrical urea derivative of **1a** as the main product.

The CDAs obtained from DACH are characterized by unique stereochemical features²⁸: (1) the cyclohexane ring locks the C–N bonds in equatorial positions, (2) the imide or 3,5-dinitrobenzamide substituents are planar and are locked approximately in the plane perpendicular to the plane of projection of the cyclohexane ring, (3) the aromatic rings in these substituents are electron-deficient, space-oriented and bring about diamagnetic effect, resulting in an upfield shift (shielding) of the proton signals of any group pointing in their direction, (4) the amide and

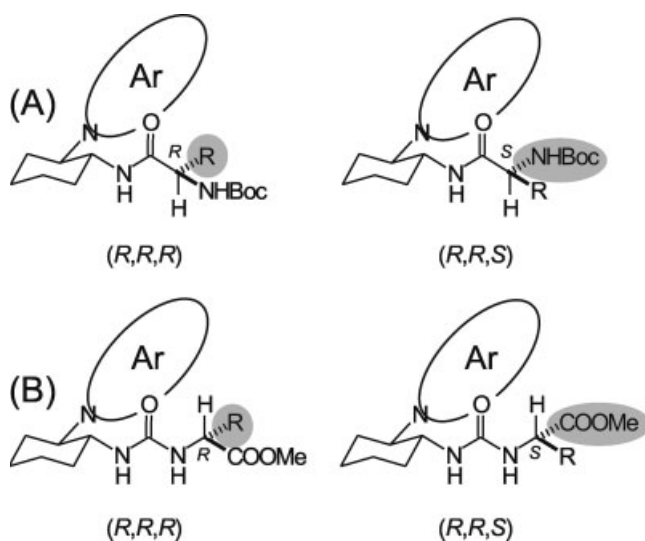


Fig. 2. Rationalization of the chemical shift differences in diastereomeric amide (A) and urea (B) derivatives of amino acids. The groups shown in gray spheres display upfield shift (shielding) of their signals in the ^1H NMR spectra.

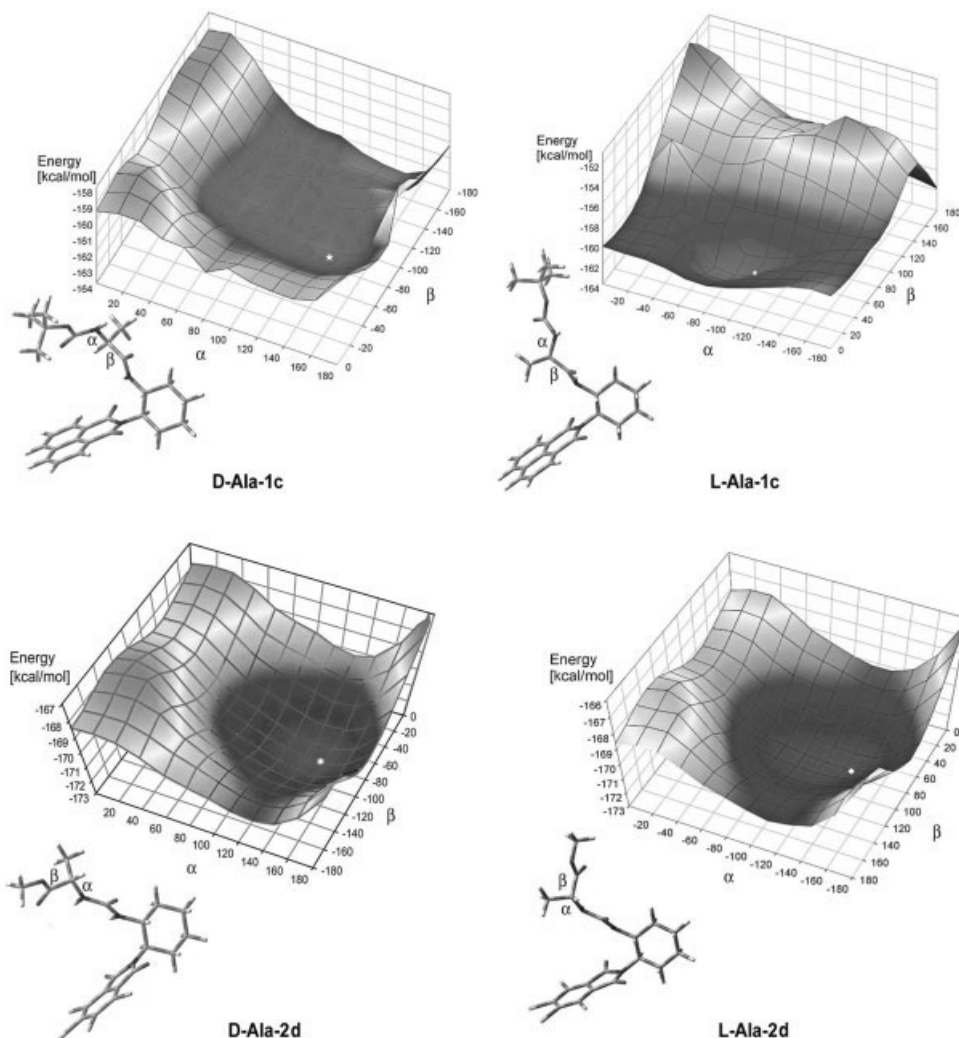


Fig. 3. Potential energy surfaces (PM3) calculated as a function of torsion angles α and β for diastereoisomers of **Ala-1c** and **Ala-2d**. Torsion angles are defined as Me-CH-NH-C(=O) (α) and Me-CH-C(=O)-NH (β) for amides **1c** and as Me-CH-NH-C(=O) (α) and Me-CH-C(=O)-O (β) for ureas **2d** and **3d**.

urea linkers are planar and conformationally restricted to *trans* NH-C(=O) bond arrangement.

We investigated the utility of the new CDAs for the discrimination of enantiomers of α -amino acids in their ^1H NMR spectra. The discrimination of diastereomeric amide derivatives of *N*-Boc-protected amino acids was found most effective with the use of **1a**; the representative ^1H NMR data for amides **1c** are collected in Table 1.

Methyl esters of α -aminoacids were efficiently discriminated as their urea derivatives **2d** and **3d**, see representative examples in Table 2. An example of the ^1H NMR spectra of diastereoisomeric derivatives **2d** is shown in Figure 1.

In all cases, the differences of chemical shifts ($\Delta\delta$) of diagnostic ^1H NMR signals, i. e. R group and *t*-Bu group in *N*-Boc-protected amino acids **1c** or R and Me group in methyl esters **2d**, **3d** were found significant (0.1–0.4 ppm), allowing baseline separation and easy integration of the respective signals and hence accurate determination of enantiomeric excess and/or amino acid configuration.

These differences could be readily rationalized by a simple general model, shown in Figure 2.

This model features a planar, *anti* orientation of the CH-NH-C(O)-CH or CH-NH-C(O)-NH-CH bonds in amide (A) or urea (B) derivatives, respectively. Such conformers are in fact of the lowest energy, according to PM3 computational search. Examples of potential energy surface determinations for diastereoisomeric alanine amide (**D**- and **L**-**Ala-1c**) and urea (**D**- and **L**-**Ala-2d**) derivatives are shown in Figure 3.

The coordinates of the lowest-energy conformer (marked with an asterisk) were calculated by a systematic conformational search, changing the angles α and β (see caption to Fig. 3 for assignment of α and β). The values of angles α and β for the lowest-energy conformers alter in sign for **D** and **L** diastereoisomers, enabling effective discrimination of amino acid enantiomers in their ^1H NMR spectra. Thus, groups close to the imide ring (shown as gray spheres in Fig. 2) would be shielded and the $\Delta\delta$ values are related to the absolute configuration of the amino acid under investi-

gation. Shielding was observed for the R groups in the derivatives of (*R,R*)-DACH and (*R*)-amino acid. On the other hand, shielding was observed for the *t*-butyl (Boc) group in **1c** or methyl ester group in the derivatives **2d**, **3d** of (*R,R*)-DACH and (*S*)-amino acid (note opposite-sign $\Delta\delta_{(R,S)}$ values in Tables 1 and 2). Since both enantiomers of DACH are readily available, the derivatization method with DACH CDAs can be used for the determination of configuration of the suitably protected α -amino acids.

It is of interest to note that significant chemical shift differences were also observed for NH protons in derivatives **1c**, **2d**, and **3d**. However, unequivocal assignment of these signals proved difficult and thus the use of their chemical shift differences for stereochemical purposes remains questionable.

CONCLUSIONS

In summary, we have developed efficient CDAs derived from (*R,R*)-DACH which can be useful in determining both the enantiomeric excess and the configuration of α -amino acids. Two methods of covalent attachment of the amino acid derivative to DACH derivative were used: either via the amide bond (for *N*-Boc amino acids) or via the urea bond (for esters of amino acids). Relatively large $\Delta\delta$ values for diastereotopic protons (up to 0.4 ppm) are accounted for by rigid structures of derivatives **1c**, **2d**, and **3d**, having limited number of low energy conformations.

Work is now in progress to apply these CDAs as chiral auxiliaries in diastereoselective synthesis.

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Chirality Sensing with Synthetic Pores

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ABSTRACT A concept to determine enantiomeric excess with synthetic multifunctional pores is introduced. To do so, the poor stereoselectivity of molecular recognition by stimuli-responsive pores is coupled with the stereospecificity of enzymes. With substrates as good and products as poor pore blockers, enzymatic conversion of one enantiomer is shown to readily reveal the concentration of the other one. Calculations suggest that high substrate/product discrimination by the synthetic pores may provide access to the accurate detection of the extreme enantiomeric excess that is of interest in chemistry, pharmacology, and medicine, but otherwise possibly problematic to detect. Validity of the introduced concept is experimentally confirmed with poly-L-glutamate and poly-D-glutamate as enantiomeric substrates with high blockage efficiency, L-glutamate and D-glutamate as enantiomeric products with poor blockage efficiency, subtilisin A as enzyme, and a classical rigid-rod β -barrel as synthetic pore. *Chirality* 20:307–312, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: chirality; enantiomeric excess; enzymes; molecular recognition; purity analysis; pores; stereoselectivity

INTRODUCTION

Synthetic multifunctional pores have been introduced as general optical transducers of chemical reactions, as detectors of the activity of many enzymes, and, in concert with enzymes as specific signal generators, as multianalyte sensors in samples from the supermarket.^{1–5} Contrary to the impractical⁶ stochastic detection of single enantiomer with bioengineered pores in planar bilayer conductance experiments,⁷ fluorometric detection with synthetic multifunctional pores in vesicles is unproblematic to use, also for the nonspecialist.^{1–5} On first view, this method appeared incompatible with chirality sensing because molecular recognition by stimuli-responsive synthetic pores is not stereoselective.² In combination with the stereospecificity of enzymes, however, this apparent limitation is expected to turn to an advantage, assuring broad applicability of the chirality sensor. Enzymes have been used previously for the successful determination of enantiomeric excess (ee) in combination with several different methods of detection other than pores.^{8–15} To explore the usefulness of enzymes for chirality sensing with synthetic pores, we selected subtilisin as enzyme,² polyglutamate as substrate, and rigid-rod β -barrel **1** as pore (Fig. 1).

Rigid-rod β -barrels such as pore **1** are composed of rigid-rod staves and β -sheet hoops.^{1–3} During self-assembly from monomeric *p*-oligophenyls such as **2**, the nonplanar staves help to roll the planar β -sheets into the cylindrical oligomers. The β -sheet hoops enable the introduction of functional groups at outer and inner pore surface to maximize interactions with the surrounding bilayer and molecules passing through the pore, respectively. In pore **1**, leucines (L) are placed at the outer surface as the ideal

compromise between β -propensity and bilayer affinity.¹⁶ The recognition of anionic molecules by the histidine-lysine dyads positioned at the inner barrel surface is expected to block the pore. Single-molecule images of pore blocker complexes are available,¹⁷ rigid-rod β -barrels with refined,¹⁸ contracted,¹⁹ and external²⁰ active sites exist.

The ability of synthetic pores similar to **1** to transduce the hydrolysis of poly-L-glutamate by various proteases for fluorometric “naked-eye” detection has been demonstrated.² Among a rich collection of enzymes compatible with synthetic pore transducers available today,^{1–5} this reaction was selected not only for convenience but also for the excellent discrimination factor *D* between the substrate as a potent blocker and the product as very poor blocker.

MATERIALS AND METHODS

General

Substrates, enzymes, lipids, fluorescent probes, buffers, salts, reagents, and solvents were from Fluka, Aldrich, Sigma, and Avanti Polar Lipids [poly-L-glutamate (pE): DP

Abbreviations: CF, 5(6)-carboxyfluorescein; DMSO, dimethylsulfoxide; EC, enzyme classification; ee, enantiomeric excess; EYPC LUVs, egg yolk phosphatidylcholine large unilamellar vesicles; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid); pE, poly-L-glutamate; pE₂, poly-D-glutamate.

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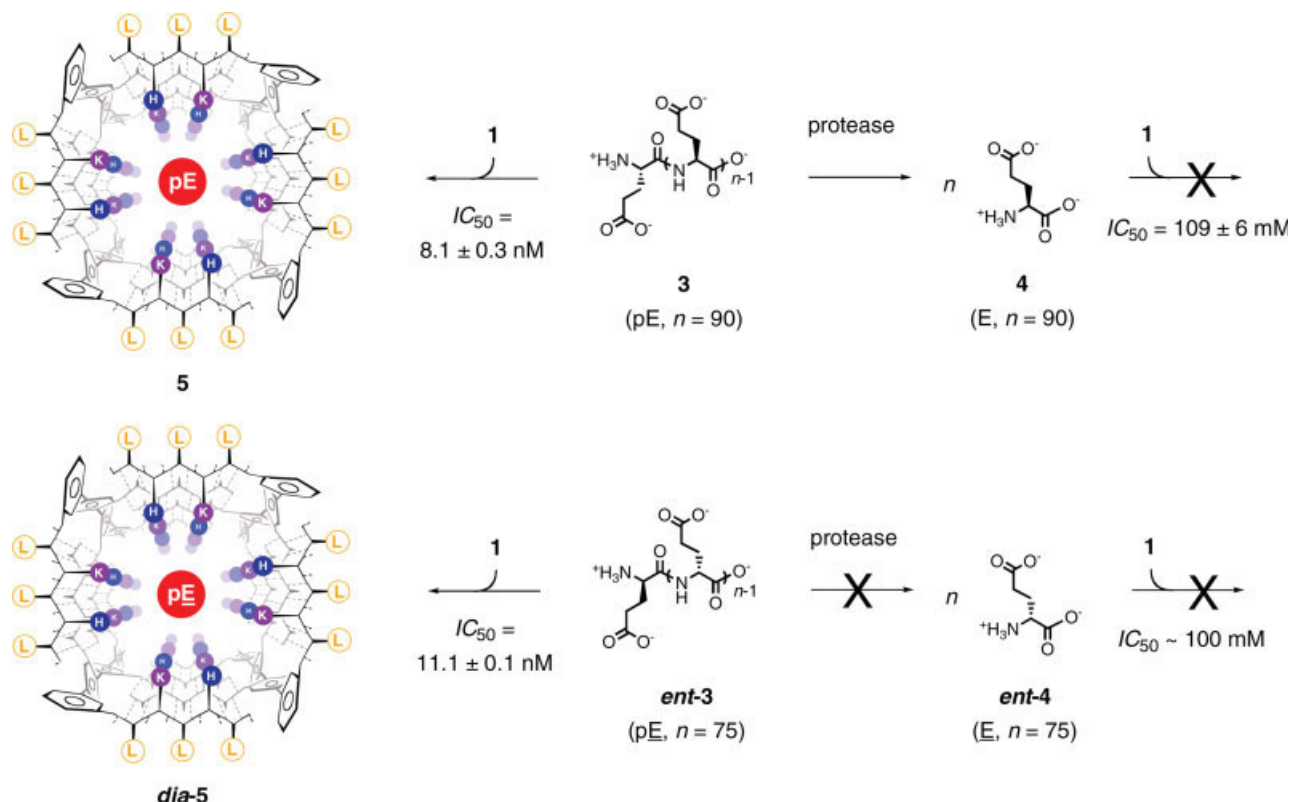


Fig. 2. The concept of chirality sensing with synthetic pores exemplified with poly-L-glutamate, pE **3** and poly-D-glutamate pE, *ent*-**3** as substrates, a protease as enzyme, and rigid-rod β -barrel **1** as pore. Stereospecific enzymatic conversion of a good blocker, here **3**, into a poor blocker, here **4**, provides access to the detection of the concentration of the enzyme-resistant substrate enantiomer, here *ent*-**3**, as long as (a) the discrimination of substrates and product by pore **1** is high and (b) the discrimination between the chiral substrates by pore **1** is negligible. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Enzyme Calibration

Mixtures of pE and pE (*ee* 0–100%, $100 \times c_{IN}$ total) were incubated with subtilisin A (0, 5, or 10 units/ml; Carlsberg, EC 3.4.21.62, from *B. licheniformis*) in buffer (10 mM HEPES, 200 mM NaCl, pH 5.0) at 37°C. In meaningful intervals (10–20 min), 20 μ l reaction mixture were taken, and the ability to block pore **1** was determined as described in the previous paragraph. The obtained fractional pore activities *Y* were plotted as a function of reaction time, and the obtained kinetics are shown in Results and Discussion section.

Blocker Calibration

The ability of pE and pE to block pore **1** was determined following the procedure described in Chirality Sensing section. The fractional pore activities *Y* obtained from data analysis with eqs. 1 and 2 were plotted as a function of concentration of the blockers ($c_{BLOCKER}$) pE and pE, and fitted to the Hill eq. 3

$$Y = Y_{\infty} + (Y_0 - Y_{\infty}) / \left\{ 1 + (c_{BLOCKER} / IC_{50})^n \right\}, \quad (3)$$

where Y_0 is *Y* without blocker, Y_{∞} is *Y* with excess blocker, IC_{50} the concentration for 50% pore blockage, and *n* the Hill coefficient.^{18,21} The obtained dose–response curves are shown in Results and Discussion section.

IC_{50} and *n* obtained for pE were used to simulate *ee* response curves for different initial total polyglutamate concentration c_{IN} , assuming complete enzymatic pE conversion and zero interference from the produced L-glutamate. The calculated dose–response curves are shown in Results and Discussion section, and the obtained effective ee_{50} defines the region of maximal accuracy of the pore sensor accessible with the corresponding c_{IN} .

RESULTS AND DISCUSSION

For chirality sensing with synthetic pores, we assumed, for example, an enzyme (here subtilisin A) that converts stereospecifically a good pore blocker (here poly-L-glutamate, pE, **3**) into a poor one (here L-glutamate, E, **4**; Fig. 2). In this situation, pore blockage experiments before enzymatic treatment will reveal the total concentration of chiral substrate (here pE and pE). After enzymatic treatment, the same blockage experiments will reveal the concentration of the enzyme-resistant enantiomer (here poly-D-glutamate, pE, *ent*-**3**). Similar concepts can naturally be formulated for similar situations such as enzymatic conversion of poor into good blockers, and so on.

This approach appeared attractive for pores that respond to chemical stimulation with poor stereoselectivity and high chemoselectivity. Stereoselective blockage, on the one hand, is undesirable because it will complicate the

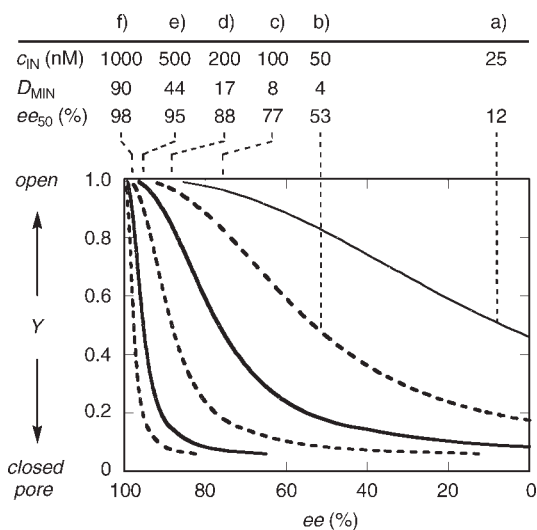


Fig. 3. Simulated dose-response curves for the blockage of pore **1** by mixtures of pE and pE as a function of ee after treatment of samples with initial concentration $c_{IN} = 25$ (a), 50 (b), 100 (c), 200 (d), 500 (e), and 1000 nM (f) with excess protease. Indicated are the effective enantiomeric excess ee_{50} and the minimal discrimination factor D_{MIN} . ee_{50} reveals the range of maximal accuracy of detection, $D_{MIN} = IC_{50}(\text{product, E})/IC_{50}(\text{substrate, pE}) = [c_{IN} - IC_{50}(\text{substrate, pE})]/IC_{50}(\text{substrate, pE})$, that is, the chemoselectivity needed to detect substrate pE without interference from excess product E obtained from enzymatic hydrolysis of pE at the given ee_{50} .

determination of the concentration of mixtures of enantiomers before enzymatic treatment. Chemoselective blockage, on the other hand, is essential for the determination of high $ee > 95\%$, an extreme region that is of particular interest in chemistry, pharmacology, and medicine and can be otherwise difficult to detect.

Chirality sensing with synthetic pores was simulated for pore blockage by chiral substrates with effective inhibitory constant $IC_{50} = 11$ nM and a Hill coefficient $n = 2.5$. These values apply for the blockage of pore **1** by pE **ent-3** under the experimental conditions selected for this study (see later). With a total polyglutamate concentration $c_{IN} = 25$ nM before enzymatic treatment, the 11 nM pE required for 50% pore blockage after enzymatic treatment will be obtained at an $ee = 12\%$ pE (Fig. 3a). This ee , the value detectable with highest accuracy, is referred in the following as effective ee_{50} . To measure with highest accuracy at an effective $ee_{50} \approx 50\%$, dose-response simulation readily revealed that an initial polyglutamate concentration of $c_{IN} = 50$ nM will be perfect (Fig. 3b). For $ee_{50} \approx 80\%$, the optimal initial concentration raises from $c_{IN} \approx 100$ nM (Fig. 3c), for $ee_{50} \approx 90\%$ to $c_{IN} = 200$ nM (Fig. 3d), and so on. These trends demonstrated that with increasing initial substrate concentration, increasingly high ee can be measured. The increasing slope of the simulated dose-response curves confirmed that accuracy of detection also increases with ee .

This finding was potentially attractive because it implied that chirality sensing with synthetic pores could provide access to the detection of high ee with high accuracy. To do so, high substrate recognition by the pore sensor is essential to avoid interference from the large excess of

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product obtained by enzymatic enantiomer removal. This essential substrate recognition is described as discrimination factor $D = IC_{50}(\text{product})/IC_{50}(\text{substrate})$. To measure at $ee_{50} \approx 95\%$ with an $IC_{50}(\text{substrate}) = 11$ nM, for example, an initial substrate concentration of $c_{IN} \approx 500$ nM will be needed (Fig. 3e). This suggested that a minimal discrimination factor of at least $D_{MIN} = [c_{IN} - IC_{50}(\text{substrate})]/IC_{50}(\text{substrate}) \approx 44$ will be required for interference-free substrate detection. To measure at $ee_{50} \approx 98\%$, this value rises to $D_{MIN} \approx 90$ (Fig. 3f), and so on. For polyglutamate with $IC_{50}(\text{substrate}) = 11$ nM and $n = 90$ monomers per polymer, this calculated to a minimal $IC_{50}(\text{product}) = n \times D_{MIN} \times IC_{50}(\text{substrate}) = 89$ μ M glutamate.

The response of synthetic pore **1** to chemical stimulation was determined in EYPC-LUVs $\supset$ CF. In the cheap, straightforward, sensitive, and reproducible CF assay, active pores are fluorogenic, because CF dilution by CF efflux from the vesicles reduces CF self-quenching. To determine molecular recognition with this assay on CF translocation, the change in fluorogenic pore activity is measured as a function of the concentration of the analyte of interest. The found increasing hindrance of CF efflux with increasing concentration of polyglutamate is exactly what is expected from polyglutamate recognition within pore **1** (Fig. 4). The sensitivity of polyglutamate recognition within pore **1** was confirmed to be in the nanomolar range, the enantioselectivity to be negligible. Glutamate was poorly recognized by pore **1** (Fig. 4, ■).

The same CF assay was used to detect the response of synthetic pore **1** to enzymatic treatment of mixtures of enantiomers **3** and **ent-3** (Fig. 5). The conversion of enantiopure **3** with subtilisin A was followed by taking aliquots of the reaction mixture in meaningful periods of time and measuring their ability to block pore **1**. Complete pore opening found with increasing reaction time was as expected for complete consumption of **3** (Fig. 5, ●). The situation changed in the presence of **ent-3**. At 90% ee of **3**,

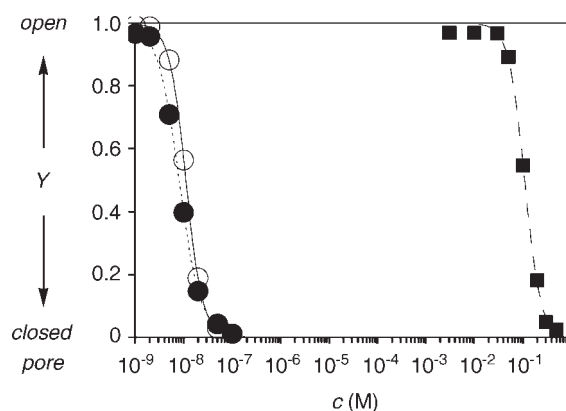


Fig. 4. Blockage of pore **1** by poly-L-glutamate pE (●), poly-D-glutamate pE (○), and L-glutamate E (■) determined from change in fractional CF emission intensity Y (λ_{ex} 492 nm, λ_{em} 517 nm) caused by addition of blockers (1 nM – 1 μ M) and monomer **2** (200 nM) to EYPC-LUVs $\supset$ CF (~ 125 μ M EYPC, 10 mM HEPES; inside: 50 mM CF, pH 7.4, 10 mM NaCl; outside: pH 6.5, 107 mM NaCl, calibrated with final addition of excess triton X-100).

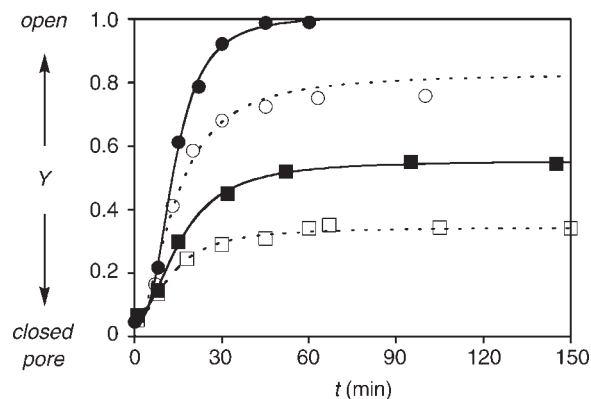


Fig. 5. Fractional activity Y of pore 1 in the presence of reaction mixtures of pE and pE (initial total concentration $c_{IN} = 100$ nM, $ee = 100\%$ (●), 90% (○), 80% (■), and 70% (□)) treated with subtilisin A as a function of reaction time. Determined from CF emission after addition of reaction mixture and pore 1 (Fig. 4).

pore opening during enzymatic blocker removal stopped at $\sim 80\%$ (Fig. 5, ○). With 80% ee , maximal pore opening was at $\sim 50\%$ (Fig. 5, ■), with 70% ee at $\sim 30\%$ (Fig. 5, □). Remarkably, the dependence of maximal fractional pore activity on ee was in excellent agreement with the expectations from simulations (Fig. 6).

Chirality sensing at reduced ee was explored by lowering the initial concentration of the mixture of enantiomers. The obtained dose–response curve revealed excellent fit with simulations (Fig. 7). These results confirmed that accuracy of detection decreases with decreasing effective ee_{50} because of the intrinsically decreasing slope of the dose–response curves (Fig. 3).

The most attractive situation, that is chirality sensing at high ee , was explored correspondingly by increasing the initial concentration of the mixture of enantiomers. The excellent fit of found and calculated dose–response curves demonstrated that the detection of ee up to 98% with pores is possible with comparably high accuracy of clearly less

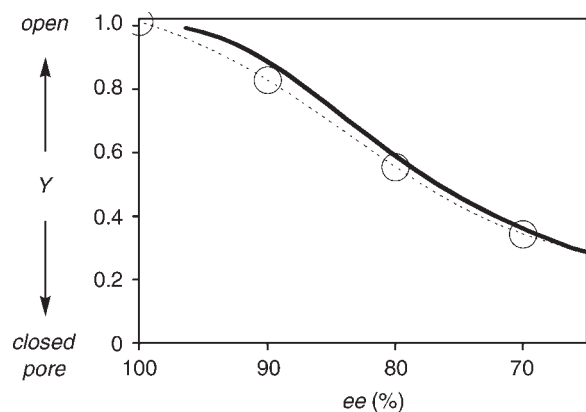


Fig. 6. Fractional activity Y of pore 1 as a function of intermediate enantiomeric excess ee of pE 3 in mixtures of pE 3 and pE *ent*-3 that were assayed for blockage efficiency after incubation with excess subtilisin. The found ee response (○, Fig. 5) is fitted to the Hill equation (dotted) and compared with expected curve from simulations (solid, Fig. 3, $c_{IN} = 100$ nM).

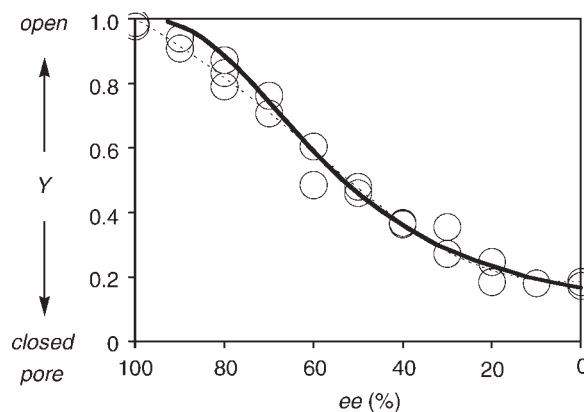


Fig. 7. Fractional activity Y of pore 1 as a function of low enantiomeric excess ee of pE 3 in mixtures of pE 3 and pE *ent*-3 that were assayed for blockage efficiency after incubation with excess subtilisin. The found ee response (○) is fitted to the Hill equation (dotted) and compared with expected curve from simulations (solid, Fig. 3, $c_{IN} = 50$ nM).

than $\pm 1\%$ (Fig. 8). This result was in support of the promise of chirality sensing with synthetic pores at the high ee that is of interest in chemistry, pharmacology, and medicine but otherwise possibly problematic to detect. As discussed earlier, this attractive characteristic originates from the increasing slope of dose–response curves for pore blockage with increasing ee (Fig. 3). This intrinsic effect occurs because the concentration of enzyme-resistant enantiomer required for pore blockage remains constant, whereas the concentration of the enzymatically removed enantiomer is naturally becoming extremely high at extremely high ee .

The theoretical limitation of chirality sensing at high ee is determined by the minimal discrimination D_{MIN} of substrate and product by the synthetic pores. It is readily predictable from the dependence of D_{MIN} on ee_{50} (Figs. 3 and 9). For example, a $D_{MIN} \approx 220$ can be extrapolated for detection at $ee = 99\%$, $D_{MIN} \approx 2400$ was found for $ee = 99.9\%$

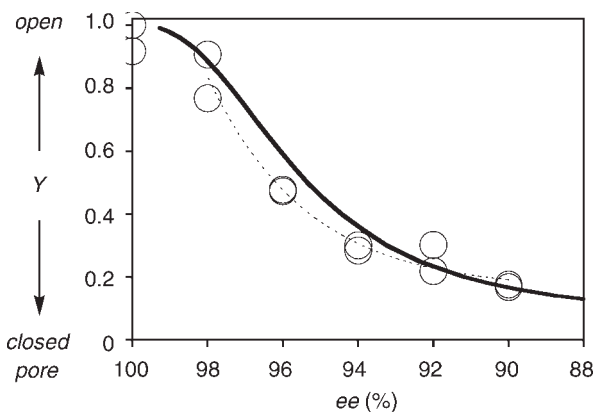


Fig. 8. Fractional activity Y of pore 1 as a function of high enantiomeric excess ee of pE 3 in mixtures of pE 3 and pE *ent*-3 that were assayed for blockage efficiency after incubation with excess subtilisin. The found ee response (○) is fitted to the Hill equation (dotted, values for 100% ee excluded) and compared with expected curve from simulations (solid, Fig. 3, $c_{IN} = 1000$ nM).

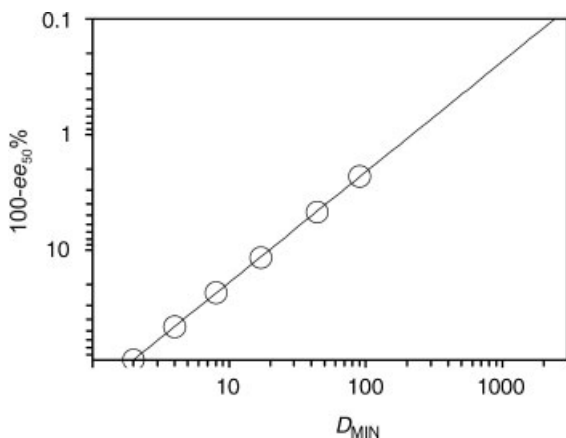


Fig. 9. Calculated minimal discrimination factor D_{MIN} of pore blockage by substrate rather than by product required for chirality sensing at extreme ee (data from Fig. 3).

(Figs. 3 and 9). For the present example with IC_{50} (substrate) = 11 nM, IC_{50} (product) = 109 mM (Fig. 4), and $n = 90$ products per substrate, $D = 1.1 \times 10^5$ calculated to a theoretical detectability of up to $ee \approx 99.9975\%$. Naturally, these theoretical predictions could not be confirmed in practice, additional parameters such as the stereoselectivity of the enzyme will have to suffice as well. We also caution that applicability of the chosen model system to identify stereochemical defects in polypeptides is very unlikely. The practical importance of the reported model study rather relates to the adaptability of one and the same pore transducer to many different enzymes that catalyze many different functional group transformations enantiospecifically.^{1–5} Additional recent breakthroughs on reactive signal amplification within advanced synthetic pores on the one hand,¹⁸ and cost-effective pore alternatives on the other²² suggest that reasonably extreme ee may become accessible by chirality sensing with pores in a quite general manner.

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Sugar Derived Hexacoordinated Phosphates: Chiral Anionic Auxiliaries with General Asymmetric Efficiency

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT Mannose-derived hexacoordinated phosphate anions, prepared in as few as two steps from methyl- α -D-mannopyranoside and tris(dimethylamino)phosphine, are chiral anionic auxiliaries with broad asymmetric efficiency. These chemically robust anions are effective NMR chiral solvating agents and efficient asymmetry-inducers, able to control the configuration of conformationally labile C_2 -symmetric monomethinium cations (organic, helical, charge 1+, *P* or *M* enantiomers) and D_3 -symmetric iron(II) trisdiimine complexes (metallo-organic, octahedral, charge 2+, Δ or Λ enantiomers). Diastereomeric control with often unmatched selective levels was achieved with the help of mannopyranoside backbone of the anions and the substituents on the [1,3]dioxane ring that play a key role in the recognition process, something not obvious at first sight. *Chirality* 20:313–324, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: asymmetric ion pairing; coordination complexes; helical dyes; NMR chiral solvating agent; octahedral geometry; Pfeiffer effect; stereoselective induction

INTRODUCTION

Many chemical reactions and processes involve cationic prochiral or chiral racemic reagents, intermediates, or products. To afford nonracemic or enantiopure adducts, an asymmetric ion pairing of the cations with chiral anions can be considered, the counterions behaving as asymmetric auxiliaries, ligands, or reagents.^{1–3} Recently, the chemistry of hexacoordinated phosphate anions has been rejuvenated for exactly this purpose, as the chiral TRISPHAT **1**^{4–6} and BINPHAT **2**⁷ anions (Λ or Δ enantiomers; Fig. 1) were shown to be valuable NMR chiral solvating, resolving, and asymmetry-inducing reagents.^{1,8} Recent results have indicated that **1** is efficient with metallo-organic and organometallic complexes,^{9–42} while **2** is primarily effective with organic compounds.^{43–53} Anion **2**, which contains a strained seven-membered ring, is however sensitive to acidic conditions and decomposes over time when associated with Brønsted or Lewis acidic cations. A family of enantiopure hexacoordinated phosphorus anions that would be (i) highly chemically stable, (ii) rapidly and stereoselectively synthesized, and (iii) asymmetrically efficient with both organic and metallo-organic cations was thus sought. In a preliminary report, we have shown earlier that mannose-derived hexacoordinated phosphate anion **3a** (Fig. 1 Λ diastereomer), easily prepared in two steps from commercially available materials, was such a derivative.⁵⁴ Herein, we detail the synthesis and applications of this and other sugar-derived phosphate anions and discuss the importance for chiral recognition processes of mannose as a structural

backbone and of the substituents on the ketal “protecting group,” something which is not so obvious at first sight.

EXPERIMENTAL SECTION

General Information

Most of the reactions were carried out under dry N_2 or Ar by means of an inert gas/vacuum double manifold line and standard Schlenk techniques with magnetic stirring, unless otherwise stated. Solvents were dried and distilled before use: toluene was freshly distilled from sodium, dichloromethane and hexane were freshly distilled from calcium hydride, and diethyl ether and tetrahydrofuran from sodium-benzophenone. $CHCl_3$, CH_2Cl_2 , $CDCl_3$, and CD_2Cl_2 (SDS) were filtered over basic alumina. Analytical thin-layer chromatography (TLC) was performed with Merck SIL G/UV₂₅₄ plates or Fluka 0.25 mm basic alumina (pH = 9.9) plates. Visualization of the developed chromatogram was performed by UV/VIS detection. Organic solutions were concentrated under reduced pressure on a Buchi or Heidolph rotary evaporator. Unless otherwise noted, column chromatography (silicagel 60, 40 μ m

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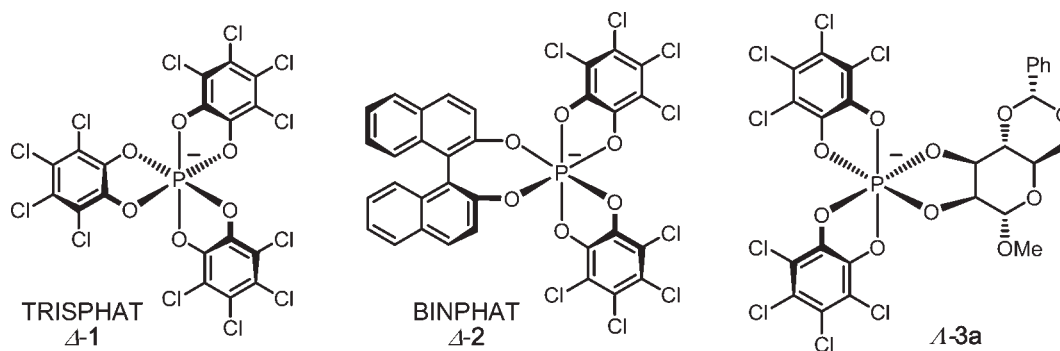


Fig. 1. Typical chiral hexacoordinated phosphate anions.

or Fluka basic alumina type 5016A) was performed in air and under pressure (1.1–1.3 bar). NMR spectra were recorded on Bruker AMX-400 at room temperature. ^1H NMR: chemical shifts are given in ppm relative to Me_4Si with the solvent resonance used as the internal standard. ^{31}P NMR: chemical shifts were reported in ppm relative to H_3PO_4 . ^{13}C NMR: chemical shifts were given in ppm relative to Me_4Si , with the solvent resonance used as the internal standard (CDCl_3 δ 77.0 ppm, $[\text{D}]_6\text{DMSO}$ δ 39.5 ppm, CD_2Cl_2 δ 53.8). Assignments may have been achieved using COSY, HETCOR, and/or NOESY experiments. ^{19}F NMR: chemical shifts were reported in ppm relative to C_6F_6 . IR spectra were recorded with a Perkin-Elmer 1650 FT-IR spectrometer using a diamond ATR Golden Gate sampling apparatus. Melting points (M.p.) were measured in open capillary tubes on a Stuart Scientific SMP3 melting point apparatus and were uncorrected. Electrospray mass spectra were obtained on a Finnigan SSQ 7000 spectrometer and EI-MS spectra were obtained on a Varian CH4 or SM1 spectrometer; ionizing voltage 70 eV; m/z (intensity in %) by the Department of Mass Spectroscopy of the University of Geneva. UV spectra were recorded on a CARY-1E spectrometer in a 1.0-cm quartz cell; λ_{max} are given in nanometers and molar adsorption coefficient ϵ in $\text{cm}^{-1} \text{dm}^3 \text{mol}^{-1}$. CD spectra were recorded on a JASCO J-715 polarimeter in a 1.0-cm quartz cell; λ are given in nm. Optical rotations were measured on a Perkin-Elmer 241 polarimeter in a thermostated (20°C) 10.0-cm long microcell with high pressure lamps of sodium or mercury and are reported as follows: $[\alpha]_D^{20}$ (c (g 100 mL^{-1}), solvent). Unless otherwise noted, all chemicals were obtained from Fluka or Aldrich and purified according to standard literature procedures.

Synthesis of Sugar-Derived Chiral Phosphates

Dimethylammonium (A)-bis[3,4,5,6-tetrachlorobenzene-1,2-diolo][(6*S*,7*S*,8*R*)-6-methoxy-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diolo]phosphate or salt $[\text{Me}_2\text{NH}_2][\text{A-3a}]$. Two hundred ninety-seven milligrams (1.2 mol, 1.2 equiv) of tetrachlorocatechol (recrystallized and sublimed) and a catalytic amount (~ 10 mol %) of NH_4Cl were mixed together in 5.0 ml of dry toluene under a nitrogen atmosphere. Two hundred seventy-three microliters (1.5 mmol, 1.5 equiv) of freshly distilled tris(dimethylamino)-

phosphine (HMPA) were slowly added. Dimethylamine evolved. The mixture was refluxed for 20 min, and then the solvent and excess HMPA were removed under reduced pressure. The white residue was carefully dried in vacuo. Two hundred ninety-five milligrams of *o*-chloranil (1.2 mmol, 1.2 equiv) was added followed by 5.0 ml of CH_2Cl_2 . The mixture was stirred for 3.5 h until a precipitate was formed from an orange solution. Then, 282 mg (1 mmol, 1 equiv) of (6*S*,7*S*,8*R*)-6-methoxy-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diol **4a** and 3.0 ml of CH_2Cl_2 were added. The mixture was stirred for 1 h and 1.0 ml of Et_2O was added. After 18 h, a further 10 ml of Et_2O was added. The precipitate was filtered in a Büchner funnel, washed with Et_2O , and dried under high vacuum to afford the title compound as a white solid (478 mg, 56%): M.p. $> 192^\circ\text{C}$ (dec).

^1H NMR (400 MHz, $\text{DMSO}-d_6$, δ): 2.54 (s, 6H, N— Me_2), 3.28 (s, 3H, OMe), 3.53 (td, 1H, $J_{9,10} = J_{10,4\text{ax}} = 10.1$ Hz, $J_{10,4\text{eq}} = 5.3$ Hz, H_{10}), 3.67 (t, 1H, $J_{4,4} = J_{10,4\text{ax}} = 10.4$ Hz, $\text{H}_{4\text{ax}}$), 3.98 (ddd, 1H, $J_{\text{H8,P}} = 31.6$ Hz, $J_{7,8} = 5.3$ Hz, $J_{8,9} = 8.6$ Hz, H_8), 3.99 (m, 1H, H_7), 4.28 (dd, 1H, $J_{10,4\text{eq}} = 5.0$, $J_{4,4} = 10.1$ Hz, $\text{H}_{4\text{eq}}$), 4.31 (dd, 1H, $J_{9,10} = 9.6$ Hz, $J_{8,9} = 8.8$ Hz, H_9), 4.85 (d, 1H, $J_{6,7} = 1.5$ Hz, H_6), 5.45 (s, 1H, PhCH), 7.21–7.24 (m, 2H, Ph), 7.32–7.35 (m, 3H, Ph), 8.26 (broad, 2H, NH_2). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$, δ): 34.3 (s, NMe), 54.3 (s, OMe), 60.1 (s, C_{10}), 68.4 (s, C_4), 69.5 (s, C_8), 70.9 (d, $J_{\text{C7,P}} = 4.1$ Hz, C_7), 78.0 (s, C_9), 98.3 (d, $J_{\text{C6,P}} = 21.6$ Hz, C_6), 101.7 (s, PhCH), 112.0 (d, $J_{\text{C,P}} = 19$ Hz, $\text{C}_{\text{catechol}}$), 112.17 (d, $J_{\text{C,P}} = 19$ Hz, $\text{C}_{\text{catechol}}$), 112.19 (d, $J_{\text{C,P}} = 19$ Hz, $\text{C}_{\text{catechol}}$), 112.3 (d, $J_{\text{C,P}} = 19$ Hz, $\text{C}_{\text{catechol}}$), 120.0 (s, $\text{C}_{\text{catechol}}$), 120.08 (s, $\text{C}_{\text{catechol}}$), 120.11 (s, $\text{C}_{\text{catechol}}$), 120.3 (s, $\text{C}_{\text{catechol}}$), 126.1 (s, C_{Ph}), 128.1 (s, C_{Ph}), 129.0 (s, C_{Ph}), 137.4 (s, C_{Ph}), 142.5 (d, $J_{\text{C,P}} = 5.8$ Hz, $\text{C}_{\text{catechol}}$), 142.82 (d, $J_{\text{C,P}} = 5.8$ Hz, $\text{C}_{\text{catechol}}$), 142.82 (d, $J_{\text{C,P}} = 5.8$ Hz, $\text{C}_{\text{catechol}}$), 143.0 (d, $J_{\text{C,P}} = 5.8$ Hz, $\text{C}_{\text{catechol}}$). $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, $\text{DMSO}-d_6$, δ): -80.66 (s). ^{31}P NMR (162 MHz, $\text{DMSO}-d_6$, δ): -80.65 (dd, $J_{\text{H8P}} = 30$ Hz, $J_{\text{H7P}} = 1.5$ Hz), MS (ES) (–) m/z : 803.1 (100), 799.1 (30), 766.9 (15). IR (neat): 3129, 1451, 1389, 1302, 1237, 1095, 993, 819, 746, 675, 633; $[\alpha]_D^{20} = +194$ (MeOH, 0.103). CD (MeOH, 1.67×10^{-5} M, 20°C) λ ($\Delta\epsilon$): 213 (-77), 222 (97), 248 (25).

Dimethylammonium bis[3,4,5,6-tetrachlorobenzene-1,2-diolato][(6*S*,7*S*,8*R*)-6-methoxy-2,2-dimethylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diolato]phosphate or salt [Me₂NH₂][A-3b]. Five hundred forty-five milligrams (2.4 mmol, 1.2 equiv) of tetrachlorocatechol (recrystallized and sublimed) and a catalytic amount (~10 mol %) of NH₄Cl were mixed together in 10 ml of dry toluene under a nitrogen atmosphere. Four hundred thirty-six microliters (3 mmol, 1.5 equiv) of freshly distilled HMPA was slowly added. Dimethylamine evolved. The mixture was refluxed for 20 min, and then the solvent and the excess of HMPA were removed under reduced pressure and the white residue was carefully dried in vacuo. Five hundred forty-one milligrams of *o*-chloranil (2.4 mmol, 1.2 equiv) was added followed by 10 ml of CH₂Cl₂. The mixture was stirred for 3 h until a precipitate was formed from an orange solution. Then, 468 mg (2 mmol, 1 equiv) of (6*S*,7*S*,8*R*)-6-methoxy-2,2-dimethylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diol **4b** and 8 ml of CH₂Cl₂ were added. The mixture was stirred for 30 min (brown solution) and 12 ml of Et₂O was added. After 16 h, the precipitate was filtered in a Büchner funnel and washed with Et₂O (20 ml) to afford the title compound as a white solid (977 mg, 61%).

¹H NMR (400 MHz, DMSO-*d*₆, δ): 1.23 (s, 3H, Me), 1.31 (s, 3H, Me), 2.53 (s, 6H, NMe₂), 3.24 (s, 3H, OMe), 3.35 (td, 1H, *J*_{9,10} = *J*_{10,4ax} = 10.1 Hz, *J*_{10,4eq} = 5.3 Hz, H₁₀), 3.64 (t, 1H, *J*₄₄ = *J*_{10,4ax} = 10.6 Hz, H_{4ax}), 3.77–3.88 (m, H₈ and H_{4eq}), 3.95 (d, 1H, *J*₇₈ = 5.8 Hz, H₇), 4.13 (dd, 1H, *J*_{9,10} = 10.1 Hz, *J*_{8,9} = 8.3 Hz, H₉), 4.80 (d, 1H, *J*_{6,7} = 1.3 Hz, H₆), 7.74 (bs, NH₂). ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 15.11 (s, Me), 18.83 (s, Me), 34.16 (s, NMe), 54.11 (s, OMe), 60.93 (s, C₁₀), 61.47 (s, C₄), 70.09 (s, C₈), 70.59 (d, *J*_{C7P} = 5.8 Hz, C₇), 70.81 (s, C₉), 98.42 (d, *J*_{C6P} = 20.7 Hz, C₆), 98.85 (s, C(Me)₂), 111.89, 111.96, 112.16, and 112.21 (4d, *J* = 19 Hz, C^{IV}), 119.93, 119.98, 120.03, and 120.20 (4s, C^{IV}), 142.43, 142.73, 142.81, 142.90 (4d, *J* = 5.8 Hz, C^{IV}). ³¹P{¹H} NMR (162 MHz, DMSO-*d*₆, δ): –80.50 (s). ³¹P NMR (162 MHz, DMSO-*d*₆, δ): –80.50 (dd, *J*_{H8P} = 29 Hz, *J*_{H7P} = 1.0 Hz). MS (ES) (–) *m/z*: 754.8. IR (neat): 3163, 2931, 1451, 1389, 1303, 1237, 1093, 991, 818, 749 cm^{–1}. UV–visible (MeOH, 1.35 × 10^{–5} M, 20°C) λ (log ε): 218 (5.02), 302 (3.88). CD (MeOH, 1.35 × 10^{–5} M, 20°C) λ (Δε): 214 (–69), 222 (81), 248 (20). [α]_D²⁰ = +163 (MeOH, *c* = 0.099).

Dimethylammonium bis[3,4,5,6-tetrachlorobenzene-1,2-diolato][(6*S*,7*S*,8*R*)-6-methoxy-2-methylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diolato]phosphate or salt [Me₂NH₂][A-3c]. Five hundred twenty-one milligrams (2.1 mmol, 1.2 equiv) of tetrachlorocatechol (recrystallized and sublimed) was mixed together in 10 ml of dry toluene under a nitrogen atmosphere. Four hundred seventy-three microliters (2.6 mmol, 1.5 equiv) of freshly distilled HMPA was slowly added. Dimethylamine evolved. The mixture was refluxed for 20 min, and then the solvent and the excess of HMPA were removed under reduced pressure and the white residue was carefully dried in vacuo. Five hundred thirteen milligrams of *o*-

chloranil (2.1 mmol, 1.2 equiv) was added followed by 10 ml of CH₂Cl₂. The mixture was stirred for 6 h until a precipitate was formed from an orange solution. Then, 383 mg (1.7 mmol, 1 equiv) of (6*S*,7*S*,8*R*)-6-methoxy-2-methylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diol **4c** and 6 ml of CH₂Cl₂ were added. The mixture was stirred for 10 min and 16 ml of Et₂O was added. After 16 h, the precipitate was filtered in a Büchner funnel and washed with Et₂O (20 ml) to afford the title compound as a white solid (676 mg, 49%).

¹H NMR (400 MHz, DMSO-*d*₆, δ): 1.14 (d, *J* = 5 Hz, 3H, Me), 2.55 (s, 6H, NMe₂), 3.25 (s, 3H, OMe), 3.38 (m, 2H), 3.85–3.96 (m, 2H), 4.01 (t, *J* = 8.6 Hz, 1H), 4.08 (dd, 1H, *J* = 3.3 and 8.8 Hz), 4.59 (q, *J* = 5 Hz, 1H), 4.80 (d, 1H, *J*_{6,7} = 1.3 Hz, H₆), 8.3 (bs, NH₂, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ): 20.86 (s, Me), 34.79 (s, NMe), 54.70 (s, OMe), 60.58 (s), 65.38 (s), 68.29 (s, C₄), 70.15 (s), 71.15 (d, *J*_{C7P} = 5 Hz, C₇), 98.84 (d, *J*_{C6P} = 21 Hz, C₆), 99.40 (s, CH(Me)), 112.42–11.85 (m, C^{IV}), 120.50–120.76 (m, C^{IV}), 143.08–143.18 (m, C^{IV}). ³¹P{¹H} NMR (162 MHz, DMSO-*d*₆, δ): –80.59 and –80.94 (2s). ³¹P NMR (162 MHz, DMSO-*d*₆, δ): –80.59 (d, *J*_{H8P} = 28 Hz), –80.94 (d, *J*_{H8P} = 28 Hz). MS (ES) (–) *m/z*: 740.9 (100), 736.9 (35). IR (neat): 3178, 2935, 1448, 1390, 1304, 1237, 1092, 991, 818, 746 cm^{–1}. UV–visible (MeOH, 1.31 × 10^{–5} M, 20°C) λ (log ε): 218 (5.05), 302 (3.92). CD (MeOH, 1.31 × 10^{–5} M, 20°C) λ (Δε): 213 (–55), 222 (68), 247 (17). [α]_D²⁰ = +187 (MeOH, *c* = 0.108).

Association with Monomethinium Cations

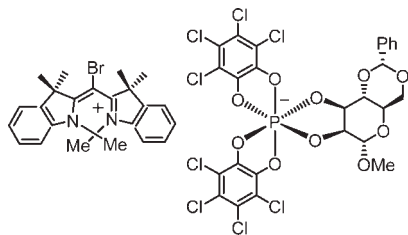
General procedure. One equivalent of the ammonium phosphate salt was dissolved in acetone (5 ml) and 1.2 equiv of the tetrafluoroborate salt of the monomethinium cation in acetone was added. The solvent was removed under reduced pressure. The crude reaction mixture was purified by chromatography over basic alumina using CH₂Cl₂ and CH₂Cl₂/AcOEt 50/50 as eluents.

α-Bromo-bis(1,3,3-trimethylindoline-2-yl)monomethinium bis[3,4,5,6-tetrachlorobenzene-1,2-diolato][(6*S*,7*S*,8*R*)-6-methoxy-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diolato]phosphate or salt [9d][A-3a].

TLC (DCM/AcOEt: 90/10) R_f = 0.53. MS (ES) (–) *m/z*: 803.2 (10), 113.7 (100). MS (ES) (+) *m/z*: 411.3 (95), 409.3 (100). IR (neat): 2931, 1557, 1483, 1455, 1383, 1358, 1305, 1096, 1080, 992, 924, 815, 748. CD (CHCl₃ filtered over basic alumina, 4.4 × 10^{–5} M, 20°C, *t* = 24 h) λ (Δε): 250 (13), 271 (17), 335 (3), 511 (–13). UV–visible (CHCl₃, 1.83 × 10^{–6} M, 20°C) λ (log ε): 270 (5.01), 513 (4.32). [α]_D²⁰ (CHCl₃, *c* = 0.022) = –651.

¹H NMR (400 MHz, CDCl₃, *t* = 0, δ): 1.76 (s, 3H, Me), 1.80 (s, 3H, Me), 1.89 (s, 3H, Me), 1.91 (s, 3H, Me), 2.84 (s, 3H, NMe), 2.94 (s, 3H, NMe), 3.39 (s, OMe, major diastereomer), 3.43 (s, OMe, minor diastereomer), 3.68 (td, H₁₀, major diastereomer), 3.69–3.82 (m, H₈ major, H₁₀ minor, H_{4ax} minor diastereomer), 3.88 (t, *J* = 10.1 Hz, H_{4ax} major diastereomer), 4.0 (m, H₃ minor diastereomer), 4.1 (m, H_{6eq} minor diastereomer), 4.26 (d, *J* = 5.3 Hz, H₂

major diastereomer), 4.31 (dd, $J = 5.3$ and 10.3 Hz, H_{4eq} major diastereomer), 4.62 (t, $J = 8.3$ Hz, H_9 minor diastereomer), 4.67 (d, $J = 5.8$ Hz, H_7 minor diastereomer), 4.71 (t, $J = 9.3$ Hz, H_9 major diastereomer), 4.93 (s, H_6 major



diastereomer), 4.97 (s, H_6 minor diastereomer), 5.33 (s, $PhCH$ minor diastereomer), 5.66 (s, $PhCH$ major diastereomer), 7.05–7.54 (m). ^1H NMR (400 MHz, $CDCl_3$, $t = 24$ h, δ): 1.74 (s, 3H, Me minor), 1.80 (s, 3H, Me major), 1.88 (s, 3H, Me major), 1.90 (s, 3H, Me minor), 2.84 (s, 3H, NMe minor), 2.93 (s, 3H, NMe major), 3.39 (s, OMe, major diastereomer), 3.42 (s, OMe, minor diastereomer), 3.69 (td, $J = 5.3$ and 10.3 Hz, H_{10} major diastereomer), 3.8 (m, H_8 major, H_{10} minor, H_{4ax} minor diastereomer), 3.89 (t, $J = 10.3$ Hz, H_{4ax} major diastereomer), 3.92 (m, H_8 minor diastereomer), 4.25 (dd, $J = 4.8$ and 9.8 Hz, H_{4eq} minor diastereomer), 4.31 (m, H_7 major diastereomer, H_{4eq} major diastereomer), 4.64 (m, H_5 minor diastereomer, H_7 minor diastereomer), 4.74 (t, $J = 9.1$ Hz, H_9 major diastereomer), 4.97 (s, H_6 major diastereomer), 5.35 (s, H_6 minor diastereomer), 5.40 (s, $PhCH$ minor diastereomer), 5.68 (s, $PhCH$ major diastereomer), 7.1–7.25 (m). $^{31}P\{^1H\}$ NMR (162 MHz, $DMSO-d_6$, δ): -81.82 and -82.01 (2s).

α -Bromo-bis(1,3,3-trimethylindoline-2-yl)monomethinium bis[3,4,5,6-tetrachlorobenzene-1,2-diols] [(6*S*,7*S*,8*R*)-6-methoxy-2,2-dimethylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diols]phosphate or salt [9d] [A-3b].

MS (ES) (–) m/z : 754.8. MS (ES) (+) m/z : 409.1 (50%), 411.1 (50%). IR (neat): 2932, 1555, 1482, 1454, 1383, 1358, 1305, 1236, 1120, 1080, 1034, 992, 925, 816, 745 cm^{-1} . CD ($CHCl_3$ filtered over basic alumina, 4.4×10^{-5} M, $20^\circ C$, $t = 19$ h) λ ($\Delta\epsilon$): 271 (35), 331.5 (5), 507 (–24). UV–visible ($CHCl_3$ filtered over basic alumina, 4.4×10^{-5} M, $20^\circ C$) λ ($\log \epsilon$): 512 (4.29), 303 (4.00), 241 (4.40). ^1H NMR (400 MHz, $CDCl_3$, $t = 0$, δ): 1.32 and 1.67 (2s, Me, 6H), 1.96 and 1.88 (2s, Me monomethinium, major helicity), 1.86 and 1.98 (2s, Me monomethinium, minor helicity), 3.04 (s, N–Me, minor helicity), 3.17 (s, N–Me, major helicity), 3.34 (s, OMe, 3H), 3.50–3.89 (m, 4H), 4.33 (d, $J = 5.1$ Hz, 1H), 4.70 (m, 1H), 4.93 (s, 1H, H_6), 7.20 (m, 2H), 7.40 (m, 4H), 7.86 (m, 2H). $^{31}P\{^1H\}$ NMR (162 MHz, $CDCl_3$, δ): -81.58 and -82.04 (2s).

Bis(1,3,3-trimethylindoline-2-yl)monomethinium bis[3,4,5,6-tetrachlorobenzene-1,2-diols] [(6*S*,7*S*,8*R*)-

6-methoxy-2,2-dimethylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diols]phosphate or salt [9a] [A-3b].

IR (neat): 2930, 1550, 1455, 1381, 1304, 1236, 1197, 1125, 1077, 1034, 992, 936, 816 cm^{-1} . CD ($CHCl_3$ filtered over basic alumina, 4.4×10^{-5} M, $20^\circ C$) λ ($\Delta\epsilon$): 254 (23), 439.5 (–13). UV–visible ($CHCl_3$ filtered over basic alumina, 4.4×10^{-6} M, $20^\circ C$) λ ($\log \epsilon$): 438 (4.51), 303 (3.99), 242 (4.42). ^1H NMR (400 MHz, $CDCl_3$, $t = 0$, δ): 1.31 (s, Me, 3H), 1.61 (s, Me, 3H), 1.62 and 1.64 (2s, Me monomethinium, 12H), 3.34 (s, O–Me, 3H), 3.46 (s, N–Me, 6H), 3.53 (m, 1H), 3.89 (m, 3H), 4.28 (d, $J = 5.1$ Hz, 1H), 4.60 (dd, $J = 8.3$ and 10.1 Hz, 1H), 4.93 (d, $J = 1.5$ Hz, 1H, H_6), 5.24 (s, H monomethinium, 1H), 7.42 (m, 8H). $^{31}P\{^1H\}$ NMR (162 MHz, $CDCl_3$, δ): -81.75 (s). ^{31}P NMR (162 MHz, $CDCl_3$, δ): -81.75 (d, $J = 29$ Hz).

α -Fluoro-bis(1,3,3-trimethylindoline-2-yl)monomethinium bis[3,4,5,6-tetrachlorobenzene-1,2-diols] [(6*S*,7*S*,8*R*)-6-methoxy-2,2-dimethylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diols]phosphate or salt [9b] [A-3b].

MS (ES) (+) m/z : 349.3 (M, 18), 319.3 (M – 30, 100). MS (ES) (–) m/z : 755.2. ^1H NMR (400 MHz, $CDCl_3$, $t = 0$, δ): 1.30 (s, 3H, Me), 1.68 (s, 3H, Me), 1.79 and 1.80 (2s, Me monomethinium, 12H), 3.24 and 3.32 (2s, N–Me, 6H, ratio = 4.3/1), 3.35 (s, O–Me, 3H), 3.53 (m, 1H, H_{10}), 3.93 (m, 3H), 4.34 (d, $J = 5.3$ Hz, 1H), 4.71 (m, 1H), 4.95 (m, 1H), 7.27 (t, $J = 7.6$ Hz, 2H), 7.43 (m, 4H), 7.62 (d, $J = 7.8$ Hz, 2H). $^{31}P\{^1H\}$ NMR (162 MHz, $CDCl_3$, $t = 0$, δ): -82.07 (s). ^{31}P NMR (162 MHz, $CDCl_3$, δ): -82.07 (d, $J = 29$ Hz). ^{19}F NMR (471 MHz, C_6D_6 , δ): -170.61 , -171.91 .

α -Chloro-bis(1,3,3-trimethylindoline-2-yl)monomethinium bis[3,4,5,6-tetrachlorobenzene-1,2-diols] [(6*S*,7*S*,8*R*)-6-methoxy-2,2-dimethylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diols]phosphate or salt [9c] [A-3b].

MS (ES) (–) m/z : 755.0. MS (ES) (+) m/z : 367.0 (M + 2, 13), 365.0 (M, 35), 352.0 (M + 2–15, 13), 350.1 (M – 15, 35), 337.0 (M + 2–30, 53), 335.0 (M – 30, 100), 299.1 (M – 30(2 × Me)–Cl, 70). ^1H NMR (400 MHz, $CDCl_3$, $t = 0$, δ): 1.31 (s, 3H, Me), 1.68 (s, 3H, Me), 1.85 and 1.91 (2s, Me monomethinium, major helicity), 1.83 and 1.93 (2s, Me monomethinium, minor helicity), 3.06 (s, N–Me monomethinium minor helicity), 3.17 (s, N–Me monomethinium, major helicity), 3.33 (s, O–Me, 3H), 3.51 (m, 1H), 3.82 (m, 1H, H_3), 3.89 (m, 2H), 4.34 (d, $J = 5.1$ Hz, 1H), 4.71 (d, $J = 9.1$ Hz, 1H), 4.91 (m, 1H), 7.22 (t, $J = 7.3$ Hz, 2H), 7.39 (m, 4H), 7.76 (d, $J = 7.8$ Hz, 2H). $^{31}P\{^1H\}$

NMR (162 MHz, CDCl_3 , $t = 0$, δ): -81.60 (s), -82.07 (s). ^{31}P NMR (162 MHz, CDCl_3 , $t = 0$, δ): -81.60 (d, $J = 31$ Hz), -82.07 (d, $J = 30$ Hz).

Association with Iron Complex 10

General procedure. In a 25-ml round bottom flask equipped with a condenser, a suspension of 30 mg (76.5 μmol , 1 equiv) of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ and 3 equiv of 1,10-phenanthroline- H_2O (230 μmol , 45.4 mg) in a mixture of water and ethanol (50:50, 5 ml) was heated at reflux (90°C) for 3 h. The resulting colored solution was cooled to 20°C , made up to 60 ml with water. To this was added a solution of ammonium phosphate salt (2 equiv) in 10% acetone/ CH_2Cl_2 (60 ml). The resulting biphasic mixture was stirred vigorously for 30 min. A quick transfer of the color from the aqueous to the organic layer was observed. The aqueous layer was separated and extracted with CH_2Cl_2 (30 ml). The combined organic layers were washed with water (2×30 ml), dried over MgSO_4 , and concentrated in vacuo to afford a deep colored solid. The crude was purified by chromatography over basic alumina (CH_2Cl_2 and $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ (80/20)).

[Iron(1,10-phenanthroline) $_3$] bis[3,4,5,6-tetrachlorobenzene-1,2-diolato][(6*S*,7*S*,8*R*)-6-methoxy-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diolato]phosphate or salt [10][Λ -3a]. Eighty-six milligrams of the title product was obtained after drying overnight in vacuo at 30°C . Yield 51%.

TLC ($\text{CH}_2\text{Cl}_2/\text{AcOEt}$: 90/10) $R_f = 0.27$. MS (ES) (–) m/z : 803.1. MS (ES) (+) m/z : 1219.2 ($[\text{Fe}(\text{phen})_2]^{2+}$ [phosphate] $^-$), 1397.2 ($[\text{Fe}(\text{phen})_3]^{2+}$ [phosphate] $^-$). CD (CHCl_3 filtered over basic alumina, 1×10^{-5} M, 20°C) λ ($\Delta\epsilon$): 263.5 (-388), 276 (462), 466.5 (17), 548.5 (-17). IR (neat): 2926, 1579, 1454, 1426, 1386, 1305, 1236, 1095, 1079, 991, 843, 814, 751, 722, 694. UV-visible (CHCl_3 , 3.77×10^{-6} M, 20°C) λ ($\log \epsilon$): 238 (5.11), 268 (5.23), 514 (4.19). $[\alpha]_D^{20}$ (CHCl_3 , $c = 0.008$) = -308 . ^1H NMR (400 MHz, 10 % $\text{DMSO}-d_6$ in CDCl_3 , δ): 3.08 (m, H_8 , 2H), 3.19 (s, OMe, 3H), 3.36 (m, H_{10} , 2H), 3.57 (t, $\text{H}_{4\text{ax}}$, $J = 10.6$ Hz, 2H), 3.92 (m, H_7 , 2H), 4.08 (m, $\text{H}_{4\text{eq}}$, 2H), 4.40 (t, $J = 9.4$ Hz, H_9 , 2H), 4.67 (s, H_6 , 2H), 5.43 (s, PhCH, 2H), 7.27 (m, $\text{H}_{3,8}$ major diastereomer), 7.38 (m, $\text{H}_{2,9}$ minor diastereomer), 7.38 (m, $\text{H}_{3,8}$ minor diastereomer), 7.71 (d, $\text{H}_{2,9}$ major diastereomer), 7.91 (s, $\text{H}_{5,6}$ minor diastereomer), 7.96 (s, $\text{H}_{5,6}$ major diastereomer), 7.99 (d, $\text{H}_{4,7}$ major diastereomer), 8.27 (d, $\text{H}_{4,7}$ minor diastereomer). $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, 10% $\text{DMSO}-d_6$ in CDCl_3 , δ): -81.48 , -81.68 .

[Iron(1,10-phenanthroline) $_3$] bis[3,4,5,6-tetrachlorobenzene-1,2-diolato][(6*S*,7*S*,8*R*)-6-methoxy-2,2-dimethylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diolato]phosphate or salt [10][Λ -3b]. The title product was obtained after drying overnight in vacuo at 30°C . Yield 78%.

MS (ES) (–) m/z : 754.8. MS (ES) (+) m/z : 1170.9 ($[\text{Fe}(\text{phen})_2]^{2+}$ [phosphate] $^-$), 1348.9 ($[\text{Fe}(\text{phen})_3]^{2+}$ [phos-

phate] $^-$). IR (neat): 2919, 1579, 1481, 1454, 1426, 1385, 1304, 1236, 1079, 1033, 992, 815, 747, 722, 694 cm^{-1} . CD (CHCl_3 filtered over basic alumina, 3.77×10^{-6} M, 20°C) λ ($\Delta\epsilon$): 548 (-19), 462 (14), 276 (435), 263.5 (-379), 240 (94). UV-visible (CHCl_3 filtered over basic alumina, 3.77×10^{-6} M, 20°C) λ ($\log \epsilon$): 516 (3.96), 268 (5.03), 238 (4.92), 237 (4.90). ^1H NMR (400 MHz, 5% $\text{DMSO}-d_6$ in CDCl_3 , δ): 0.70 and 0.91 (2s, Me $\times$ 2, 6H, minor and major diastereomers), 1.17 and 1.36 (2s, Me $\times$ 2, 6H, minor and major diastereomers), 3.12 (ddd, $J = 5.6$, 8.1 and 29 Hz, 2H, major diastereomer), 3.23 and 3.25 (2s, 2 $\times$ OMe, 6H, minor and major diastereomers), 3.30 (m, 2H), 3.68 (m, 4H), 4.00 (d, $J = 6.3$ Hz, 2H, major diastereomer), 4.26 (dd, $J = 8.6$ and 10.1 Hz, 2H, major diastereomer), 4.70 and 4.76 (2s, H_6 , minor and major diastereomers, 2H), 7.46 (m, $\text{H}_{3,8}$ major diastereomer), 7.96 (d, $J = 3.5$ Hz, $\text{H}_{2,9}$ major diastereomer), 8.08 (s, $\text{H}_{5,6}$ major diastereomer), 8.18 (d, $J = 7.8$ Hz, $\text{H}_{4,7}$ major diastereomer). $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, 5% $\text{DMSO}-d_6$ in CDCl_3 , δ): -81.48 and -81.56 (2s).

RESULTS AND DISCUSSION

Ligand Selection and Synthesis of Sugar Derived Hexacoordinated Phosphate Anions

Carbohydrates are probably the class of natural compounds with the most asymmetric centers and potential ligand atoms per molecule. As such, they have often been used in asymmetric synthesis and catalysis.^{55–57} From inexpensive commercially available sugars, interesting 1,2-, 1,3-, and 1,4-diols can be synthesized in a few steps. Their use as chiral ligands for the stereoselective synthesis of hexacoordinated phosphate anions was therefore tempting.

Of all possible compounds, (6*S*,7*S*,8*R*)-6-methoxy-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diol **4a**,⁵⁸ (6*S*,7*R*,8*R*)-6-methoxy-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diol **5**,⁵⁸ and (6*S*,7*R*,8*S*)-6-methoxy-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diol **6**^{59,60} were selected for an initial screening study (Fig. 2). These compounds were selected for the rigidity of their skeleton enforced by the *trans*-decaline like geometry. The *cis*-1,2-relationship of the free hydroxyl groups in diols **4a** and **6**, leading to sterically demanding axial-equatorial arrangements, was also perceived as a favorable factor for high diastereoselectivity at the P-atom of the subsequent phosphate anions.

Compounds **4a** and **5** were prepared in one step by the treatment of methyl- α -D-mannopyranose and methyl- α -D-glucopyranose, respectively, with dimethyl acetal benzaldehyde in an acidic medium. Methyl 4,6-*O*-benzylidene- α -D-allopyranoside **6** was obtained from **5** by (i) benzylic protection of the hydroxyl group at C7, (ii) oxidation of the alcohol at C8, and (iii) treatment with NaBH_4 to afford the axial alcohol at C8 and the concomitant deprotection of C7. With these diols in hands, the synthesis of the respective phosphate anions was attempted following the guidelines reported earlier in our group (Scheme 1).⁶¹ Anhydrous tetrachlorocatechol and $\text{P}(\text{NMe}_2)_3$ were refluxed in toluene. After concentration in vacuo, successive additions of *o*-chloranil (3,4,5,6-tetrachloro-3,5-cyclohexadiene-1,2-dione) and then of diols **4**, **5**, and **6** in CH_2Cl_2 were performed to achieve the preparation of the anions.

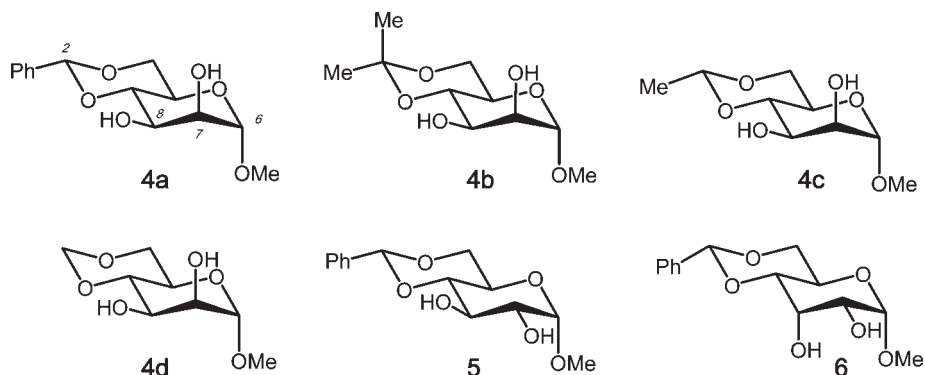


Fig. 2. Sugar diols used as chiral auxiliaries for hexacoordinated phosphate anion synthesis.

Using diol **4a**, this protocol yielded the desired dimethylammonium salt of phosphate **3a**, which precipitated upon addition of Et₂O (56%). Initial data further suggested the presence of only one diastereomer in the precipitate as a single set of signals was detected in ¹H, ¹³C, and ³¹P NMR. Crystals of (+)-[Me₂NH₂][**3a**] were obtained by diffusion of hexane into an acetone/EtOAc solution. Low temperature X-ray diffraction analysis confirmed the presence of a single diastereomer (Λ configuration; Fig. 3).⁵⁴ P—O bond lengths and O—P—O bond angles are similar to those measured for TRISPHAT **1** and BINPHAT **2**,^{4,7} with the two P—O bonds linked to the carbohydrate unit being shorter (~1.67 Å) than the others (~1.74 Å). The configurational assignment was further established in solution by circular dichroism (CD) analysis of salt (+)-[Me₂NH₂][**3a**] (MeOH, 1.67 × 10⁻⁵ M), which revealed strong exciton coupling in the π-π* region (Δε₂₁₃ = -77, Δε₂₂₂ = +97).⁶²

Diols **5** and **6** were then used in the aforementioned protocol. However, in these two cases, the synthesis of the resulting phosphate anions was unselective. Mixtures of diastereomers (1:1) were obtained in the precipitate fractions (double sets of signals detected in ¹H, ¹³C, and ³¹P NMR). Furthermore, the novel hexacoordinated phosphates isolated were contaminated with large amounts of TRISPHAT salts. Although low diastereoselectivity was expected for the phosphate anion derived from glucopyranoside **5** due to the poor sterically demanding trans-1,2-relationship of the free hydroxyl groups, the result was more surprising for the derivative of **6**; the lack of selectivity in this case is possibly because of the result of steric interactions with the axial MeO-substituent at C6.

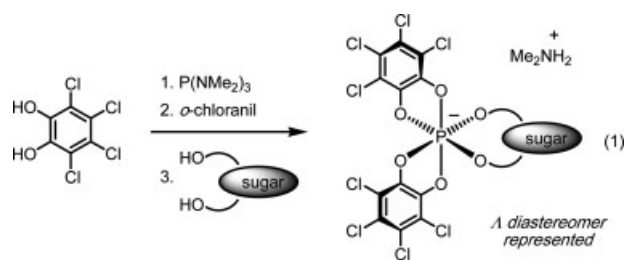
This quick survey showed us the importance of the mannopyranoside framework for the preparation of enantio- and, more importantly, diastereomerically pure sugar derived phosphate anions. All other attempted syntheses of sugar phosphates were thus made with mannopyranose derivatives, and **4b–4d** in particular (Fig. 2). Compounds **4b**, **4c**, and **4d** were prepared from methyl-α-D-mannopyranose using classical protecting group methodology.^{63,64} However, only **4b** and **4c** afforded the dimethylammonium salts of the corresponding phosphate anions **3b** and **3c** in high chemical purity and decent overall yields (61 and 49%, respectively). Anions **3b** and **3c** were characterized as sin-

gle stereoisomeric species by ¹H, ¹³C, and ³¹P NMR spectroscopy and isolated as Λ diastereomers as determined by CD analysis (Fig. 4). Unlike [Me₂NH₂][**2**], which decomposes in solution over time, salts (+)-[Me₂NH₂][**3a**], (+)-[Me₂NH₂][**3b**], and (+)-[Me₂NH₂][**3c**] were found to be stable. No evidence of decomposition of the phosphate moieties was found in the spectroscopic studies.

Interestingly, compound **4d** failed to yield the corresponding anion under the classical preparative conditions. At this stage, the reason for this lack of performance of **4d** remains obscure. One possible explanation is neighboring group participation of the sterically unencumbered oxygen atom O1 that would lead to the formation of a stable zwitterionic derivative (**7**) by reaction of **4d** with spiroposphorane intermediate **8** (Scheme 2). The establishment of such a dative bond between an oxygen atom and a Lewis acidic P(V) atom is well established in the literature and has already been observed by our group in another context.^{65–68}

Supramolecular Stereocontrol

To compare the asymmetric efficiency of anions Λ-**3a**, Λ-**3b**, and Λ-**3c**, and to judge them against **1** and **2**, chiral cationic systems **9a–9d** (organic, helical, charge 1+, *P* or *M* enantiomers; Fig. 5)⁶⁹ and **10** (metallo-organic, octahedral, charge 2+, Δ or Λ enantiomers; Fig. 7) were prepared and associated with the aforementioned anions. Compounds **9** and **10** are both configurationally labile in solution and their association with a chiral anion can be stereoselective if one diastereomeric ion pair (or ion triple) is thermodynamically favored over the other (e.g., [*M*-



Scheme 1. One-pot three-steps protocol for the preparation of hexacoordinated phosphate anions.

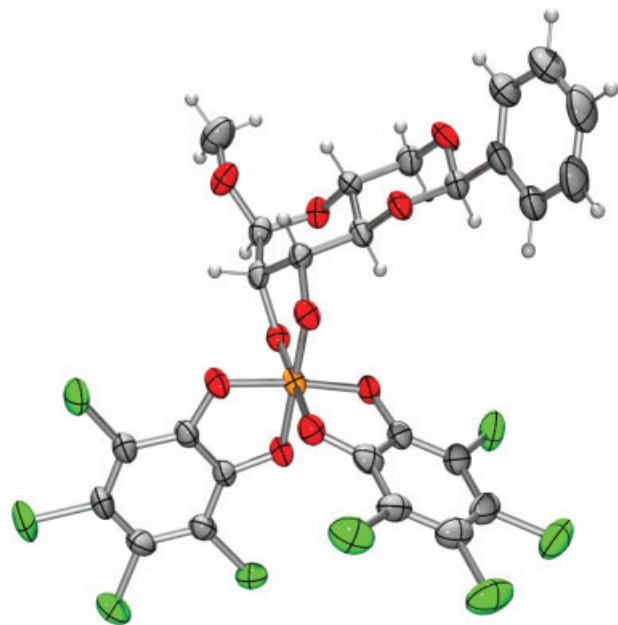


Fig. 3. X-ray crystal structure of (+)-[Me₂NH₂][**3a**]. Ellipsoids are represented with 50% probability level and the dimethylammonium cation omitted. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

9][**Δ-3a**] over [**P-9**][**Δ-3a**]). The characterization and quantification of the asymmetry-induction (Pfeiffer effect)^{70–77} is best performed by CD and NMR spectroscopy if the anionic auxiliary is, in addition, a good chiral NMR solvating agent.^{78–85} Before this study, BINPHAT **2** was the most effective auxiliary for cation **9d** (d.e. 27% in CDCl₃),⁷ whereas TRISPHAT **1** was by far the best with coordination complex **10** (d.e. > 98% in CDCl₃).¹⁷

As briefly mentioned, the monomethinium cations **9a–9d** adopt a helical conformation and these chiral dyes are configurationally labile in solution. As shown by Grahn et al.,⁶⁹ the rate of isomerization in solution between the *P* or *M* enantiomers depends essentially on the halogen substituent X positioned at the carbon connecting the hetero-

cycles. In the case of salts [**9a**][BF₄] and [**9d**][BF₄], X = H and Br, respectively, energy barriers for the enantiomerization ($\Delta G^\ddagger$) were previously determined by Grahn with values of 11.9 and 22.7 kcal mol^{−1}, respectively. For salts [**9b**][BF₄] and [**9c**][BF₄], X = F and Cl, the stereodynamic process was not characterized and variable temperature NMR experiments were thus carried out here to do so. These two salts were dissolved in CDCl₃ and DMSO-*d*₆ and analyzed by ¹H NMR spectroscopy at 298–320 K and 298–423 K, respectively. As expected, at lower temperature, the diastereotopic geminal methyl groups were magnetically nonequivalent and, upon increasing temperature, coalescence was seen at 308 and 413 K, respectively, indicative of the occurrence of the stereodynamic process. Values for the racemization barriers (16.5 and 21.4 kcal mol^{−1}) were then estimated using the relationship $\Delta G^\ddagger = RT_c(22.96 + \ln(T_c/\Delta\nu))$. At 25°C, the enantiomerization is thus fast on the NMR time scale for salt [**9a**][BF₄] and slow for compounds [**9b**][BF₄], [**9c**][BF₄], and [**9d**][BF₄].

The association of monomethinium cations **9** with enantiopure anions **1**, **2**, **3a**, **3b**, and **3c** was realized following previously reported methods.⁸⁶ For instance, solutions of salts [**9d**][BF₄] and of [cinchonidinium][**Δ-1**], [Me₂NH₂][**Δ-2**], or [Me₂NH₂][**Δ-3**] in CH₂Cl₂/acetone were prepared, mixed, concentrated, and purified by chromatography (on SiO₂ or Al₂O₃, CH₂Cl₂) to afford the desired ion pairs [**9d**][**Δ-1**], [**9d**][**Δ-2**], [**9d**][**Δ-3a**], [**9d**][**Δ-3b**], or [**9d**][**Δ-3c**] as the most eluted compounds. As anions **3** are more polar than **1** and **2**, EtOAc (0–10%) was usually added to the mobile phase to elute the corresponding [**9d**][**3**] salts.

In the case of **9d**, the conformational exchange was quite slow at 25°C, so it was necessary to wait several hours (>10 h) to obtain complete diastereomeric equilibration and measure the induced selectivity under thermodynamic conditions. ¹H NMR analysis of the salts containing anions **2** and **3a** then revealed two distinctive sets of signals for **9d** with rather large differences in chemical shifts between the *M* and *P* enantiomers (NMe signal, $\Delta\delta$ 0.083 and 0.086 ppm; Table 1). A moderate but definite

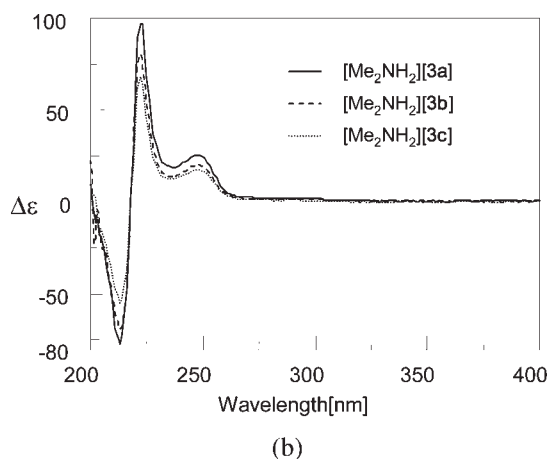
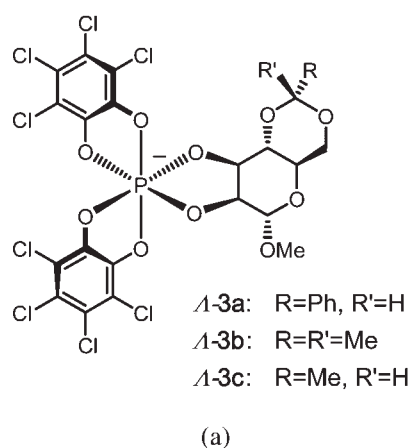
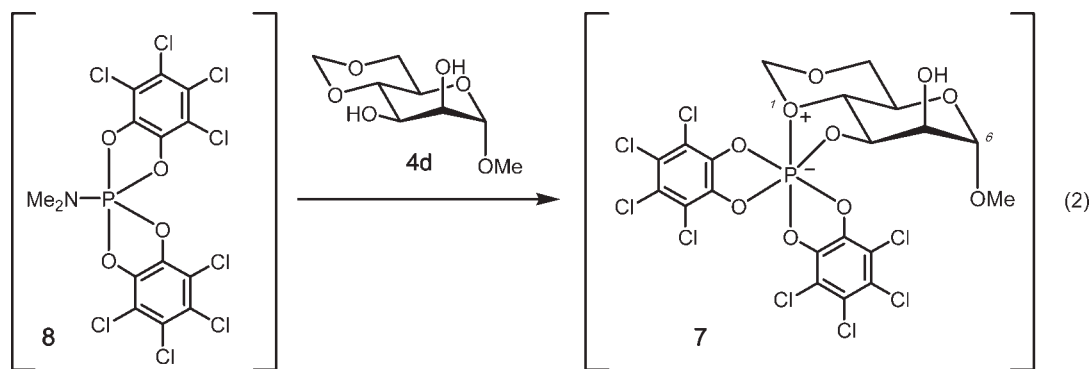


Fig. 4. CD spectra in MeOH of (+)-[Me₂NH₂][**3a**] (1.67×10^{-5} mol/L), (+)-[Me₂NH₂][**3b**] (1.35×10^{-5} mol/L) and (+)-[Me₂NH₂][**3c**] (1.31×10^{-5} mol/L).



Scheme 2. Possible explanation for the lack of performance of diol **4d**.

stereoselective induction was measured by integration of the respective signals (e.g., Fig. 6, spectrum b, d.e. 34% for **[9d][Λ -3a]**, and confirmed by CD analysis of **[9d][Λ -2]** and **[9d][Λ -3a]** ($\Delta\epsilon_{519} = +5$ and $\Delta\epsilon_{511} = -13$, CHCl_3 , 4.40×10^{-5} M). The positive and negative Cotton effects indicate that the helical dye adopts the *M* and *P* configurations in the presence of anions Λ -2 and Λ -3a, respectively.⁸⁷ A slightly better asymmetric efficiency was thus observed for anion **3a** over classical **2**. As expected, TRISPHAT **1** showed little effect.^{7,54}

More importantly, it was then observed that the nature of the substituents at carbon C2 of the [1,3]-dioxane ring modulates effectively chiral recognition between the sugar hexacoordinated phosphate anion and cationic helical dye **9d**, and the geminal dimethyl substituents of **3b** in particular. ^1H NMR analysis of salt **[9d][Λ -3b]** in CDCl_3 and C_6D_6 revealed two distinct sets of signals for **9d** with larger differences in chemical shifts between the *M* and *P* enantiomers (NMe signal, $\Delta\delta$ 0.116 and 0.338 ppm, respectively). Excellent values for the stereoselective induction (d.e. 69 and 77%) were measured by integration of the respective signals (e.g., Fig. 6, spectrum c) and confirmed by CD analysis ($\Delta\epsilon_{507} = -24$, CHCl_3 , 4.40×10^{-5} M), the negative Cotton effect indicated the preferred *P* configuration for **9d**. The better stereoselective induction in benzene over chloroform is most probably due to the lower polarity of the aromatic solvent and a result of stronger electrostatic interactions between the ions and less solvent competition. Compounds **[9d][Λ -2]** and **[9d][Λ -3c]** were then also analyzed in C_6D_6 but lower selectivity values resulted from the analyses (d.e. 39 and 34%, respectively, Table 1). Clearly, if one assumes that phosphates **3a**, **3b**, and **3c** possess the same geometrical framework, then the chiral recognition between the anions and cation **9d** is strongly influenced by the substituents at carbon C2

of the sugar moiety rather than by other elements of the skeleton. The asymmetric induction is stronger in the presence of an isopropylidene group, as opposed to benzyldiene or ethylidene moieties. NOESY experiments were attempted to shed some light on the recognition process, but no convincing correlations could be found. A HOESY experiment was performed on salt **[9b][Λ -3b]**, and no correlation was observed between the fluorine atom of **9b** and protons of the phosphate anion.

Interestingly, epimerization of all the phosphate anions occurs at the P-atom (**3a**, **3b**, and **3c**) upon their ion pairing to the helical cation, the anions being present in the salts as 2.9:1, 4.8:1, and 1.6:1 mixtures of Λ and Δ diastereomers (e.g., Fig. 6, OMe signals). One may then wonder if this epimerization might not be one of the reasons for the relative lack of asymmetric induction of anions **3a** and **3c** onto **9d** as the Λ/Δ ratio is quite better in the case of anion **3b**.

Finally, with the result of the better efficiency of **3b** over all the other anions in hand, it was decided to examine its association with cations **9a**, **9b**, and **9c** as well. The results of the NMR and CD experiments performed in chloroform are summarized in Table 1. In the case of salt **[9a][Λ -3b]**, it was necessary to measure the extent of the stereocontrol at low temperature (CDCl_3 , 213 K, d.e. 50%). The CD experiment was however performed at 20°C and confirmed the preferred *P* helicity for **9a** ($\Delta\epsilon_{440} = -13$, CHCl_3 , 4.40×10^{-5} M). Clearly, the larger the X atom (from H to Br), the higher is the asymmetric induction (d.e. from 50 to 69%, from X = H to Br).

We then turned our attention to the association of iron(II) tris(1,10-phenanthroline) complex **10** with counterions **1**, **2**, **3a**, and **3b**, which was attempted using the aforementioned metathesis/chromatographic purification method. Only salts **[10][Δ -1]₂**, **[10][Λ -3a]₂**, and **[10][Λ -**

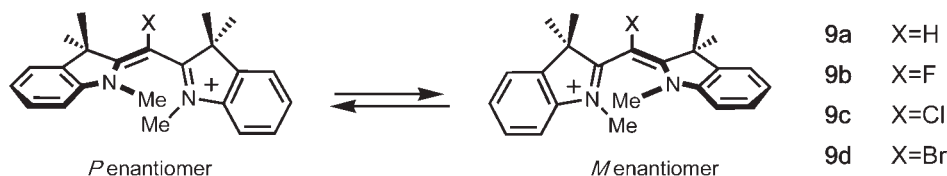


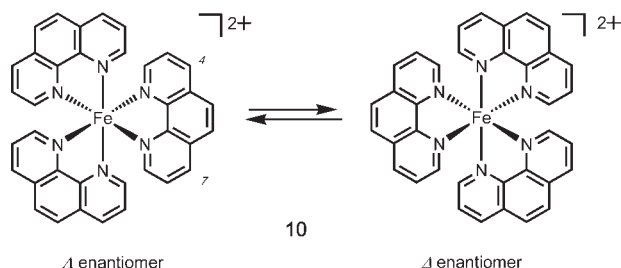
Fig. 5. Configurationally labile monomethinium cations **9a** to **9d**.

TABLE 1. Ion pairing of cations **9** and anions **3** (and **1** and **2**) and resulting diastereoselectivity

Salt	X	$\Delta\delta^a$	d.e. CDCl ₃ (%)	d.e. C ₆ D ₆ (%)
[9d][Λ - 3a]	Br	0.086	34	—
[9d][Λ - 3b]	Br	0.116	69	77
[9d][Λ - 3c]	Br	0.032	32	34
[9d][Λ - 1]	Br	0.007	0	—
[9d][Λ - 2]	Br	0.083	27	39
[9a][Λ - 3b]	H	0.027	50	—
[9b][Λ - 3b]	F	0.080	58	—
[9c][Λ - 3b]	Cl	0.100	64	—

^aNMe signal in CDCl₃, in ppm.

3b)₂ could be isolated (62, 51, and 78%, respectively), anion Δ -**2** decomposing upon ion pairing. Solutions of [**10**][Λ -**1**]₂, [**10**][Λ -**3a**]₂, and [**10**][Λ -**3b**]₂ were prepared in mixtures of CDCl₃ and DMSO-*d*₆ (2.5–30%). After diastereomeric equilibration (<30 min), ¹H NMR analyses revealed the enantiodifferentiation of cation **10**. In low polar media (lower % DMSO), large differences in chemical shifts were observed between analogous protons of the diastereomers ($\Delta\delta$, Table 2) and a high diastereoselectivity was measured by integration of the respective signals (d.e. upto 89, 86, and 97% salts of Λ -**3a**, Λ -**3b**, and Δ -**1**, respectively; Table 2). The increase in $\Delta\delta$ values and diastereoselectivity as the solvent polarity is reduced is interpreted as the result of stronger interactions between the ions. CD spectra analyses revealed positive and negative signals at the longer wavelengths for the complexes made of anions **1** and **3**, respectively, ([**10**][Δ -**1**]₂: $\Delta\epsilon_{557} = +13$; [**10**][Λ -**3a**]₂: $\Delta\epsilon_{549} = -17$, CHCl₃, 1.0×10^{-5} M and [**10**][Λ -**3b**]₂: $\Delta\epsilon_{548} = -19$, CHCl₃, 3.77×10^{-6} M), thus indicating induced absolute Δ and Λ configurations for **10**.^{88,89} An efficient homochiral [Δ -**10**][Δ -**1**]₂ and [Λ -**10**][Λ -**3**]₂ ion pairing is therefore preferred regardless of the lower C₁ symmetry of **3a** and **3b**. Interestingly, anions

**Fig. 7.** Iron (II) tris(1,10-phenanthroline) complex **10**.

3a (and **3b**) are more selective than **1** in more polar solvent conditions (DMSO/CHCl₃ > 25%).

In conclusion, mannose-derived anions **3** are essentially as efficient as TRISPHAT with the metallo-organic complex **10** and can be much more effective than BINPHAT with the organic cations **9**. The ease of the stereoselective synthesis and the chemical robustness of the anions ensures a general use in asymmetric anion mediated processes. Further studies are performed to better characterize the association between anions **3** and coordination complexes as discriminating interactions between carbohydrates and coordination complexes have recently been observed.^{90,91}

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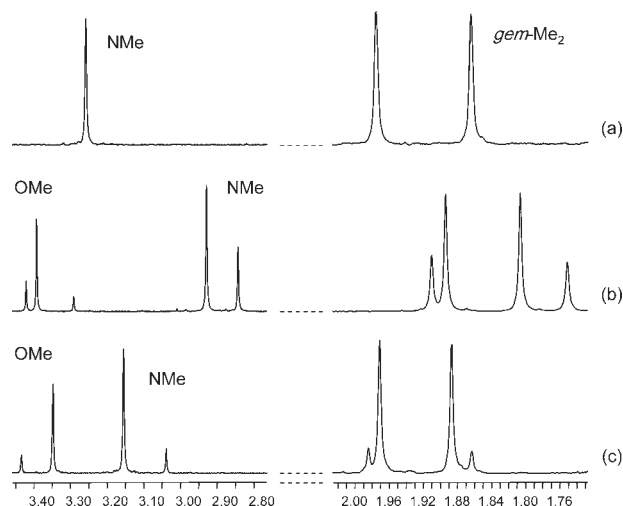
**Fig. 6.** ¹H NMR (400 MHz, CDCl₃) of (a) [**9d**][BF₄], (b) [**9d**][Λ -**3a**], and (c) [**9d**][Λ -**3b**].

TABLE 2. Association of iron(II) tris(1,10-phenanthroline) complex 10 with 1, 3a, and 3b

Solvent	[10][A-3a] ₂			[10][A-3b] ₂			[10][A-1] ₂		
	d.e. ^a	Δδ ^b	Conf ^c	d.e. ^a	Δδ ^b	Conf ^c	d.e. ^a	Δδ ^b	Conf ^c
CHCl ₃	89	−0.225	Λ	86	−0.276	Λ	97	^d	Δ
2.5% DMSO/CHCl ₃	89	−0.254	Λ	83	−0.278	Λ	97	^d	Δ
5.0% DMSO/CHCl ₃	87	−0.270	Λ	78	−0.277	Λ	95	−0.385	Δ
10% DMSO/CHCl ₃	81	−0.279	Λ	72	−0.256	Λ	82	−0.335	Δ
15% DMSO/CHCl ₃	70	−0.258	Λ	—	—	—	66	−0.249	Δ
20% DMSO/CHCl ₃	57	−0.220	Λ	45	−0.167	Λ	45	−0.170	Δ
30% DMSO/CHCl ₃	30	−0.129	Λ	22	−0.09	Λ	13	−0.053	Δ

^aDiastereoselectivity in %.^bH(4,7) in ppm.^cInduced configuration of the cation.^dNot measurable.

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Liquid Chromatographic Direct Resolution of β -Amino Acids on a Doubly Tethered Chiral Stationary Phase Containing N—H Amide Linkage Based on (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic Acid

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT A doubly tethered chiral stationary phase (CSP) prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to doubly tethered primary aminoalkyl silica gel was used for the resolution of various β -amino acids. All the β -amino acids tested were resolved quite well, the separation (α) and the resolution factors (R_S) being in the ranges 1.34–2.09 and 2.52–7.45, respectively, with a mobile phase of methanol–water (50:50, v/v) containing 10 mM acetic acid. The chiral recognition efficiency of the doubly-tethered CSP was found to be generally superior to that of the corresponding singly-tethered CSP in the resolution of β -amino acids. The chiral recognition behaviors for the resolution of β -amino acids on the doubly tethered CSP were examined by varying the type and content of organic and acidic modifiers in the aqueous mobile phase and the column temperature. *Chirality* 20:325–329, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: chiral stationary phase; chiral resolution; (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid; enantiomer separation; β -amino acid

INTRODUCTION

Direct liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) has been established as the most accurate and convenient means for the exact determination of the enantiomeric composition of chiral compounds and consequently various CSPs have been developed.^{1,2} CSPs based on chiral crown ethers are very effective for the resolution of racemic compounds containing a primary amino group.^{3,4} Among others, CSPs based on chiral crown ethers incorporating chiral binaphthyl^{5,6} or tartaric acid⁷ units and CSPs based on phenolic pseudo-chiral crown ethers^{8,9} have been most successful.

In our laboratory, we have developed CSP **1** (Fig. 1) by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid incorporating two tartaric acid unit to 3-aminopropylsilica gel.¹⁰ CSP **1** was quite successful in the resolution of various racemic primary amino compounds including α -amino acids,¹⁰ β -amino acids,^{11–13} racemic amines,¹⁴ racemic amino alcohols,¹⁴ and racemic fluoroquinolone antibacterials.¹⁵ The resolution of racemic primary amino compounds on CSP **1** has been usually performed under highly acidic conditions with an aqueous mobile phase containing sulfuric acid or acetic acid in order to convert the primary amino group into primary ammonium ions ($R-NH_3^+$), which are essential for chiral recognition by chiral crown ethers.^{3,4} In this instance, the CSP was not stable enough for long term use. In an effort to improve the stability of CSP **1**, we recently developed a doubly tethered CSP

(CSP **2**, Fig. 1) by attaching the second tethering group to silica gel through a carbon atom of the first tethering group of CSP **1**.¹⁶

CSP **2** was applied to the resolution of various α -amino acids, amines, and amino alcohols.¹⁶ As expected, the stability of CSP **2** was found to be greater than that of CSP **1**. In addition, CSP **2** was generally superior to CSP **1** in the resolution of α -amino acids, amines, and amino alcohols. However, CSP **2** has not yet been applied to the resolution of β -amino acids while CSP **1** has been found useful for the resolution of β -amino acids.^{11–13}

Optically active β -amino acids have attracted considerable attention due to their potent pharmacological activities and their usefulness as building blocks of many natural products.^{17,18} In this instance, analytical methods for the exact determination of the enantiomeric composition of β -amino acids are essential and liquid chromatographic chiral separations on CSPs might be the method of choice.

In this study, we wish to report the direct resolution of β -amino acids on CSP **2** in an effort to extend the uses of

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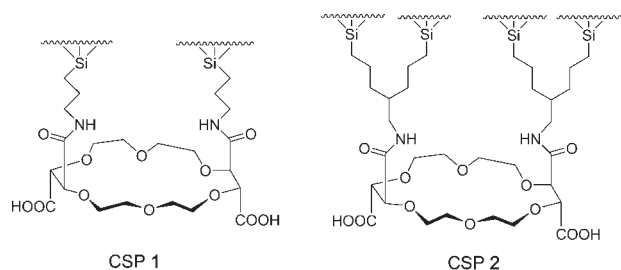


Fig. 1. Structures of CSP 1 and CSP 2.

CSP 2. Comparison of the chromatographic resolution results on CSP 2 with those on CSP 1 is expected to elucidate the characteristics of CSP 2 in the resolution of β -amino acids.

EXPERIMENTAL

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7725i injector with a 20 μ l sample loop, a Waters 2487 UV Dual λ Absorbance Detector operated at 210 nm UV, and a YongLin Autochro Data Module (software: YongLin Autochro-WIN 2.0 plus). The temperature of the chiral column was controlled using a JEIO TECH VTRC-620 Circulator (Seoul, Korea). Chiral column (150 mm $\times$ 4.6 mm i.d.) packed with CSP 2 was available from a prior study.¹⁶ Both enantiomers of the β -amino acids 3–11 shown in Figure 2 were purchased from Peptech (Cambridge, MA, USA). 3-Aminobutyric acid (12) and 3-aminoadipic acid (13) shown in Figure 2 were available as racemates from Aldrich. Injection samples were prepared by dissolving each analyte (racemic and 1:1 mixture of optically active analytes) in water at a concentration of 1 mg/ml. The elution orders of β -amino acids were determined by injecting configurationally known samples.

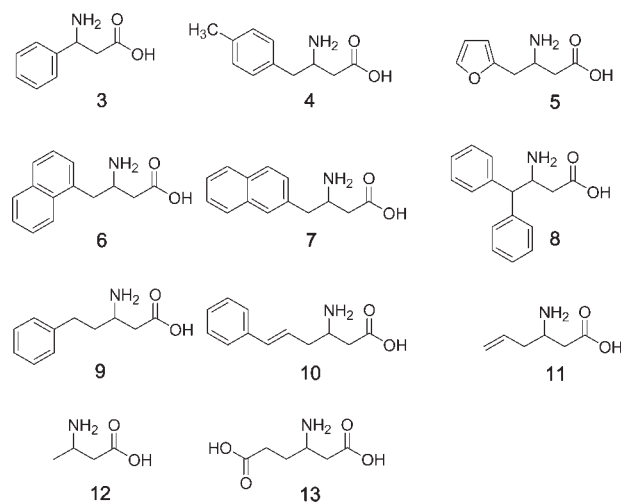


Fig. 2. The structures of β -amino acids used in this study. 3-Amino-3-phenylpropionic acid (3), 3-amino-4-(4-methylphenyl)butyric acid (4), 3-amino-4-(2-furyl)butyric acid (5), 3-amino-4-(1-naphthyl)butyric acid (6), 3-amino-4-(2-naphthyl)butyric acid (7), 3-amino-4,4-diphenylbutyric acid (8), 3-amino-5-phenylpentanoic acid (9), 3-amino-6-phenyl-5-hexenoic acid (10), 3-amino-5-xeenoic acid (11), 3-aminobutyric acid (12), and 3-aminoadipic acid (13).

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TABLE 1. Comparison of the resolution of β -amino acids 3–13 on CSP 1 and CSP 2 with a mobile phase of 50% methanol in water containing 10 mM acetic acid as acidic modifier^a

β -Amino acids	CSP 1			CSP 2		
	k_1	α	R_S	k_1	α	R_S
3	3.60 (R)	1.60	2.76	7.80 (R)	1.44	4.27
4	1.26 (S)	1.40	2.15	2.38 (S)	1.55	4.69
5	1.33 (S)	1.33	1.66	2.67 (S)	1.43	4.16
6	3.72 (S)	1.28	1.55	4.32 (S)	1.34	2.95
7	2.38 (S)	1.53	2.07	3.90 (S)	1.66	5.32
8	0.67 (S)	1.34	1.38	0.62 (S)	1.42	2.52
9	2.30 (S)	1.44	2.65	2.79 (S)	1.81	6.47
10	2.09 (S)	1.54	2.21	3.54 (S)	2.09	7.45
11	1.02 (S)	1.37	1.86	1.66 (S)	1.65	4.64
12	2.16	1.16	1.57	2.74	1.49	4.52
13	3.83	1.16	1.22	2.95	1.35	4.19

^aChromatographic data for CSP 1 are quoted from Ref. 12.

Flow rate, 0.5 ml/min; detection, 210 nm UV; temperature, 20°C; k_1 , retention factor of the first eluted enantiomer.

The absolute configuration of the first eluted enantiomer is presented in parenthesis.

α , separation factor; R_S , resolution factor.

RESULTS AND DISCUSSION

For the resolution of β -amino acids on CSP 1, 50% methanol in water containing 10 mM acetic acid has been successfully utilized as a mobile phase.^{11,12} For the purposes of comparison, the identical mobile phase was applied to the resolution of β -amino acids on CSP 2. The chromatographic results for the resolution of β -amino acids on CSP 2 are summarized and compared with those on CSP 1 in Table 1. As an example, a representative chromatogram for the simultaneous resolution of five β -amino acids on CSP 2 is presented in Figure 3. The elution orders shown in Table 1 were determined by injecting configurationally known samples. In the case of 3-aminobutyric acid (12) and 3-aminoadipic acid (13), configurationally known samples were not available and consequently the elution orders could not be determined.

As shown in Table 1 and Figure 3, the resolution of β -amino acids on CSP 2 is quite successful. Comparison of

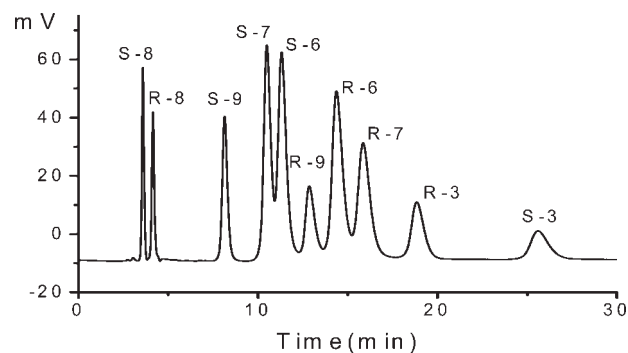


Fig. 3. Chromatogram for the resolution of the mixture of five β -amino acids (3, 6, 7, 8, and 9) on CSP 2. Mobile phase, 50% methanol in water + acetic acid (10 mM); flow rate, 0.5 ml/min; detection, 210 nm UV; temperature, 20°C.

TABLE 2. Resolution of 3-amino-3-phenylpropionic acid (**3**) and 3-amino-6-phenyl-5-hexenoic acid (**10**) on CSP **2** with the variation of the type and the content of organic and acidic modifiers in aqueous mobile phase and the column temperature^a

	Mobile phase	3			10		
		k_1	α	R_S	k_1	α	R_S
a	50% CH ₃ OH–10 mM H ₂ SO ₄ (20°C)	1.75 (S)	1.20	1.72	0.64 (S)	1.42	2.46
	50% CH ₃ CN–10 mM H ₂ SO ₄ (20°C)	0.84 (S)	1.15	1.11	0.28 (S)	1.18	0.74
	50% THF–10 mM H ₂ SO ₄ (20°C)	1.70 (S)	1.22	1.81	0.50 (S)	1.36	1.57
b	30% CH ₃ OH–10 mM H ₂ SO ₄ (20°C)	0.82 (S)	1.18	1.27	0.45 (S)	1.49	2.31
	50% CH ₃ OH–10 mM H ₂ SO ₄ (20°C)	1.75 (S)	1.20	1.72	0.64 (S)	1.42	2.46
	80% CH ₃ OH–10 mM H ₂ SO ₄ (20°C)	3.29 (S)	1.20	2.07	0.84 (S)	1.39	2.82
c	50% CH ₃ OH–10 mM H ₂ SO ₄ (20°C)	1.75 (S)	1.20	1.72	0.64 (S)	1.42	2.46
	50% CH ₃ OH–10 mM AcOH (20°C)	7.80 (R)	1.44	4.27	3.54 (S)	2.09	7.45
	50% CH ₃ OH–10 mM HClO ₄ (20°C)	1.70 (S)	1.19	1.45	0.74 (S)	1.36	2.17
d	50% CH ₃ OH–5 mM AcOH (20°C)	8.18 (R)	1.42	4.37	3.64 (S)	2.11	8.04
	50% CH ₃ OH–10 mM AcOH (20°C)	7.80 (R)	1.44	4.27	3.54 (S)	2.09	7.45
	50% CH ₃ OH–20 mM AcOH (20°C)	7.71 (R)	1.34	3.30	3.33 (S)	2.04	7.01
	50% CH ₃ OH–5 mM H ₂ SO ₄ (20°C)	2.10 (S)	1.20	1.81	0.77 (S)	1.48	2.86
	50% CH ₃ OH–10 mM H ₂ SO ₄ (20°C)	1.75 (S)	1.20	1.72	0.64 (S)	1.42	2.46
	50% CH ₃ OH–20 mM H ₂ SO ₄ (20°C)	1.42 (S)	1.18	1.56	0.50 (S)	1.38	1.98
e	50% CH ₃ OH–10 mM AcOH (20°C)	7.80 (R)	1.44	4.27	3.54 (S)	2.09	7.45
	50% CH ₃ OH–10 mM AcOH (10°C)	9.80 (R)	1.45	4.17	4.54 (S)	2.19	7.25
	50% CH ₃ OH–10 mM AcOH (5°C)	15.49 (R)	1.47	3.53	6.43 (S)	2.39	7.09
	50% CH ₃ OH–10 mM H ₂ SO ₄ (20°C)	1.75 (S)	1.20	1.72	0.64 (S)	1.42	2.46
	50% CH ₃ OH–10 mM H ₂ SO ₄ (10°C)	2.41 (S)	1.22	1.88	0.82 (S)	1.50	2.64
	50% CH ₃ OH–10 mM H ₂ SO ₄ (5°C)	3.04 (S)	1.24	1.93	1.02 (S)	1.51	2.88

^aFlow rate, 0.5 ml/min; detection, 210 nm UV; k_1 , retention factor of the first eluted enantiomer.

The absolute configuration of the first eluted enantiomer is presented in parenthesis.

 α , separation factor; R_S , resolution factor.

the resolution of β -amino acids **3–13** on CSP **1** and CSP **2** shown in Table 1 clearly demonstrates that CSP **2** is superior to CSP **1** in terms of both the separation (α) and the resolution factors (R_S) except for the resolution of 3-amino-3-phenylpropionic acid (**3**). Notably the resolution factors (R_S) on CSP **2** were always much greater than those on CSP **1**.

Retention factors (k_1) on CSP **2** are also greater than those on CSP **1** except for the resolution of 3-amino-4,4-diphenylbutyric acid (**8**) and 3-aminoadipic acid (**13**). These retention behaviors on CSP **1** and CSP **2** are somewhat different from those for the resolution of α -amino acids, amines, and amino alcohols.¹⁶ By adding the second tethering group, CSP **2** was expected to be more lipophilic than the corresponding singly tethered CSP (CSP **1**) especially under reverse phase conditions. In this instance, the retention factors (k_1) on CSP **2** were expected to be higher than those on CSP **1**.¹⁶ However, the retention factors for the resolution of α -amino acids, amines, and amino alcohols on CSP **2** have been reported to be smaller than those on CSP **1** except for highly lipophilic amines. These retention behaviors have been rationalized to arise from the quite low loading level of the chiral crown ether selector on CSP **2** (0.08 mmol) compared to that on CSP **1** (0.15 mmol) while the exceptional higher retention factors (k_1) for the resolution of highly lipophilic amines on CSP **2** have been proposed to arise from the greater lipophilicity of CSP **2** compared to CSP **1**.¹⁶ The β -amino acids shown in Figure 2 are quite lipophilic and consequently,

the greater retention factors (k_1) for the resolution of β -amino acids on CSP **2** might be also rationalized to be due to the greater lipophilicity of CSP **2**.

The elution order for the resolution of 3-amino-3-phenylpropionic acid (**3**) is different from that for the resolution of other β -amino acids as shown in Table 1. The inconsistent elution order for the resolution of 3-amino-3-phenylpropionic acid (**3**) has been rationalized to stem from the priority inversion of the substituents at the chiral center of the analyte according to the Cahn-Ingold-Prelog sequence rule.^{11,12}

In an effort to find whether the mobile phase condition (50% methanol in water containing 10 mM acetic acid) utilized for the resolution data in Table 1 is optimal and to elucidate the chromatographic characteristics for the resolution of β -amino acids on CSP **2**, two selected β -amino acids such as 3-amino-3-phenylpropionic acid (**3**) and 3-amino-6-phenyl-5-hexenoic acid (**10**) were resolved on CSP **2**, varying the type and the content of organic and acidic modifiers in aqueous mobile phase and the column temperature. The chromatographic resolution results are summarized in Table 2.

The influence of the organic modifiers on the resolution of β -amino acids on CSP **2** was observed with the use of methanol, acetonitrile, or tetrahydrofuran (THF) as organic modifiers in aqueous mobile phase containing 10 mM sulfuric acid as shown in panel a of Table 2. In the resolution of 3-amino-3-phenylpropionic acid (**3**), THF is slightly better than methanol, but in the resolution of

3-amino-6-phenyl-5-hexenoic acid (**10**), methanol is much better than tetrahydrofuran especially in terms of the resolution factor (R_S). Of the three organic modifiers, acetonitrile is worst in terms of both the separation (α) and the resolution factors (R_S). Overall, methanol turns out to be the best organic modifier.

The content of organic modifier in aqueous mobile phase also influences the resolution of β -amino acids on CSP **2**. The effect of content of organic modifier on the resolution of the β -amino acids on CSP **2** was examined with variation of the content of methanol in aqueous mobile phase containing 10 mM H_2SO_4 as shown in panel b of Table 2. An increase in the methanol content in the aqueous mobile phase increases the retention factors (k_1). These trends are exactly consistent with those for the resolution of β -amino acids on CSP **1**.¹¹ The resolution factors (R_S) always increase with increasing methanol content. However, the separation factor (α) for the resolution of 3-amino-3-phenylpropionic acid (**3**) shows almost no changes, while that for the resolution of 3-amino-6-phenyl-5-hexenoic acid (**10**) decreases slightly as the methanol content in aqueous mobile phase increases.

An acidic modifier added to aqueous mobile phase has been proposed to improve the formation of primary ammonium ions (R-NH_3^+) of analytes and enhance the complexation of primary ammonium ions (R-NH_3^+) inside the cavity of the chiral crown ether ring of CSP.^{3,4} The effect of the type of acidic modifiers on the resolution of β -amino acids on CSP **2** was tested with sulfuric acid, acetic acid, or perchloric acid in mobile phase consisting of 50% methanol in water. As shown in Panel c of Table 2, the use of acetic acid as an acidic modifier shows the best chiral recognition results in terms of both the separation (α) and the resolution factors (R_S). In addition, the retention factors (k_1) are also largest with acetic acid.

The elution orders are very interesting to note. As already mentioned, the notation of the absolute configuration of 3-amino-3-phenylpropionic acid (**3**) is different from those of other β -amino acids because of the priority inversion of the substituents at the chiral center.¹⁹ Consequently, the elution order for the resolution of 3-amino-3-phenylpropionic acid (**3**) on CSP **2** with the use of 50% methanol in water containing 10 mM acetic acid was different from others as shown in Table 1. However, the elution orders for the resolution of 3-amino-3-phenylpropionic acid (**3**) are inverted with the use of sulfuric acid or perchloric acid as an acidic modifier as shown in Table 2. At the present time, the exact reason for the inverted elution order is not yet clear. However, it seems that the chiral recognition mechanism may change with varying the type of acidic modifier from acetic acid to sulfuric acid or to perchloric acid.

The effect of the concentration of acidic modifier on the resolution of β -amino acids on CSP **2** was investigated with the use of acetic acid or sulfuric acid as shown in panel d of Table 2. As the concentration of acetic or sulfuric acid in aqueous mobile phase increases, the retention factors (k_1) decrease and these trends are exactly consistent with those of CSP **1**.¹¹ The increased ionic strength of the aqueous mobile phase with the increased concentration of acidic modifier has been proposed to be responsible

for the decrease in the retention factors.¹¹ The separation (α) and the resolution factors (R_S) also decrease slightly as the concentration of acidic modifier in aqueous mobile phase increases except that the separation factor (α) for the resolution of 3-amino-3-phenylpropionic acid (**3**) is greatest when the concentration of acetic acid is 10 mM. In general, 10 mM acetic acid seems to be the optimum concentration.

The effects of column temperature on the resolution of two selected analytes on CSP **2** are shown in panel e of Table 2. As the column temperature decreases, the retention and the separation factors increase while the resolution factors decrease. These chromatographic behaviors are exactly consistent with those on CSP **1**.¹¹ The formation of the two diastereomeric complexes with the two enantiomers of racemic β -amino acids inside the cavity of the crown ether of the CSP is expected to improve at lower temperature. In addition, the formation of the more stable diastereomeric complex is expected to be more favorable than that of the less stable diastereomeric complex at lower temperatures. Consequently, the retention (k_1) and the separation factors (α) should increase as the column temperature decreases. At lower temperatures, chromatographic peaks broaden because of the slow equilibrium for the formation of diastereomeric complexes and consequently, the resolution factors (R_S) decrease as the column temperature increases.

Overall the chromatographic characteristics for the resolution of β -amino acids on CSP **2** are very similar to those on CSP **1**. However, the chiral recognition mechanisms on the two CSPs seem to be slightly changed depending on the acidic modifier in aqueous mobile phase. The optimal mobile phase conditions for the resolution of β -amino acids are identical on both CSPs.

In conclusion, a doubly tethered CSP containing N-H amide linkage based on (+)-2,3,11,12-tetracarboxylic acid (CSP **2**) is superior to the corresponding singly tethered CSP (CSP **1**) for the resolution of various β -amino acids with a mobile phase of 50% methanol in water containing 10 mM acetic acid. The greater resolution factors (R_S) along with the greater separation (α) and the greater retention factors (k_1) on a relatively more stable CSP (CSP **2**) are expected to be quite attractive for the preparative scale resolution of β -amino acids even though both CSP **1** and CSP **2** are equally effective for the analytical scale resolution of β -amino acids.

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Review Article

Supramolecular Tilt Chirality in Crystals of Steroids and Alkaloids

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT The concept of supramolecular chirality has assumed increasing importance in association with the development of supramolecular chemistry over the last two decades. In chiral crystals, 2_1 helical molecular assemblies are frequently observed as key motifs. Helical handedness of the 2_1 assemblies, however, has not been determined from the mathematical or crystallographical viewpoints. In this context, we have proposed two new concepts, three-axial chirality and tilt chirality. On the basis of the concepts, we describe supramolecular chirality and determine the handedness of 2_1 assemblies that are composed of relatively complicated molecules with multiple stereogenic centers such as brucine, bile acids, and cinchona alkaloids as well as those of simple molecules. *Chirality* 20:330–336, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: crystallography; helical structure; molecular assembly; twofold screw axis; cholic acid; brucine; cinchona alkaloid

INTRODUCTION

Over the last two decades a number of sophisticated molecular self-assembly systems have been constructed in the field of supramolecular chemistry.¹ Simultaneously, the importance of the concept of supramolecular chirality has been increasing and, nowadays, it is one of the topics which chemists treating molecular assemblies must consider. Here, we focus on supramolecular chirality in 2_1 helical structures in the crystals of steroids and alkaloids.

Conventionally, point molecular chirality has been described with *R/S* nomenclature system according to the Cahn–Ingold–Prelog priority rules.^{2,3} On the other hand, even though a molecule does not have point chirality, it is possible for the molecule to exhibit chirality; namely, axial chirality and planar chirality described with *P/M*. One of the significant milestones in organic molecules having axial chirality must be 1,1'-bi-2-naphthol, which is often used as a ligand for transition-metal catalyzed asymmetric synthesis.⁴ Two achiral naphthols are staggered around covalent bond between 1 and 1' positions to give an asymmetric arrangement of naphthol moieties (Fig. 1a left). It is noteworthy that hypothetical removal of the covalent bond would give the appearance of supramolecular chirality (Fig. 1a right). In other words, supramolecular chirality is defined as the nonsymmetric arrangement of molecules in a noncovalent assembly.⁵ Can we observe such chirality in self-assembled supramolecular systems? If possible, in which system can we observe it?

SUPRAMOLECULAR CHIRALITY IN SIMPLE SYSTEMS

What is a fundamental of supramolecular chirality? To answer this initial question, we demonstrate here two general examples cited from literature. The first example is two solid models of the enantiomorphic polyhedrons (Fig. 1b) described in Gardner's book.⁶ The models composed of two identical components that are exactly alike in all geometrical properties. However, they are not obviously identical and are distinguishable. The second one is *La Coupe du Roi*, which is a parlor trick known among some families in France. It adequately describes relationships both in chiral assemblies and their achiral components, as well as in achiral assemblies and their chiral components. Mislow and coworkers⁷ described in their article in 1983 that finite geometric achiral objects are bisected into isomeric homochiral halves, with pointing to *La Coupe du Roi* as shown in the left part of Figure 1c. The chiral halves (A and B) can also be constructed from two quarters by coupling followed by twisting one quarter

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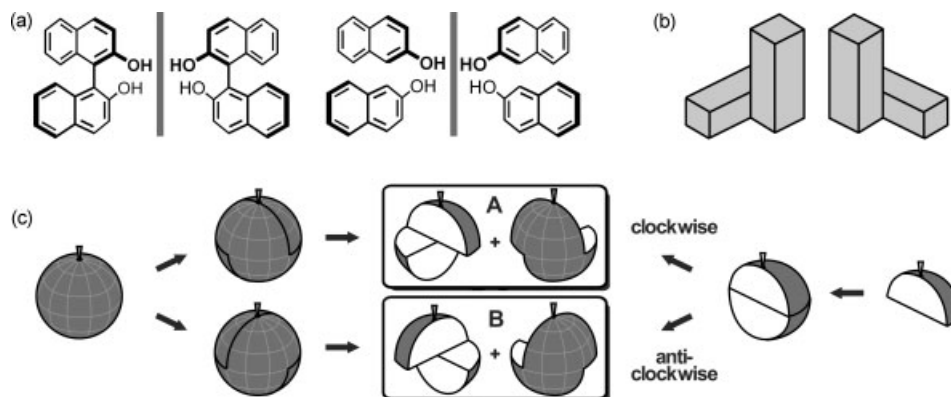


Fig. 1. Mirror image pairs of 1,1'-bi-naphthol and hypothetical asymmetric arrangement of two naphthol molecules (a). Chirality generated in assemblies composed of symmetrical objects. Mirror-pair image of an assembly composed of two cuboids (b), and La Coupe du Roi apples (c).

on the top in clockwise and anticlockwise directions, respectively, as shown in the right part of Figure 1c. In this way, it is classic that achiral two objects can form distinguishable enantiomorphic objects by tilting the components. This description suggests common appearances of supramolecular chirality.

Indeed, supramolecular chirality of bimolecules is often reported in discrete bimolecular systems having a twofold axis; one example is bis-porphyrinoid double-decker architectures.^{8,9} Bimolecules with twofold screw axis, which are hardly observed in solution also exhibit chirality; achiral banana-shaped molecules are reported to form 2_1 helical arrangements in liquid crystalline phases.^{10,11} In Figure 2, the schematic representation of bimolecular systems having twofold axis or twofold screw axis is presented. The model (a) shows assemblies in stacked face-to-face with the tilt, the model (b) is those in stacked edge-to-edge with the tilt. The only difference between C_2 and 2_1 assemblies is that the latter involves a translation operation. The hypothetical bimolecule composed of naphthol (Fig. 1a) belongs to the latter C_2 assembly.

Recently, we reported that the supramolecular chirality of benzene molecules is successfully observed in the 2_1 helical structure formed in the inclusion crystal of cholic acid.^{12,13} As shown in Figure 3, two kinds of benzene rings in the columns colored gray and white, respectively, are asymmetric arrangements. The supramolecular chirality observed is the same type as that in the 2_1 model in Figure 2b. We are convinced that this is one of the simplest and the most fundamental examples of supramolecular chirality.

CHIRALITY OF 2_1 HELICAL ASSEMBLIES

Molecular assemblies having twofold screw axes include chirality caused by molecular tilt. 2_1 Assemblies are quite familiar structures and are often observed in both organic and organometallic crystals, especially composed of asymmetric components, which is reasonable according to Kitaigorodskii.¹⁴ 2_1 Helical symmetry is exhibited in crystals with space groups such as $P2_12_12_1$ and $P2_1$, and according to Motherwell and coworkers¹⁵, about

10,000 organic crystal structures belonging to these space groups are registered in Cambridge Structural Database. These crystals have been investigated from the viewpoint of the spontaneous resolution of enantiomers and absolute asymmetric synthesis.^{16,17}

In connection with chirality of 2_1 symmetric structures, it is obvious that handedness of twofold screw axis operation cannot be determined from the mathematical or crystallographic viewpoint, because this includes 180° rotation as well as translation operation. Assemblies resulting from twofold screw axis operation of the object, on the other hand, may exhibit helical sense. For example, the stair with twofold screw axis shown in Figure 4a is a left-handed helix and its mirror image must be a right-handed helix (Fig. 4b), although the mathematical sense cannot be determined even in these cases. An important point to determine (or describe may be a better expression) the helical handedness of the 2_1 helical assemblies is, thus, to stipulate on what basis the handedness is determined. Similar examples must be observed in case of molecules

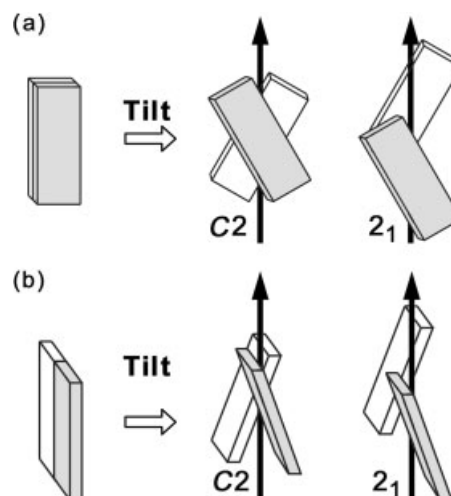


Fig. 2. Schematic representation of 2_1 and C_2 symmetrical dimers composed of planer molecules. Assemblies in face-to-face fashion (a) and in edge-to-edge fashion (b).

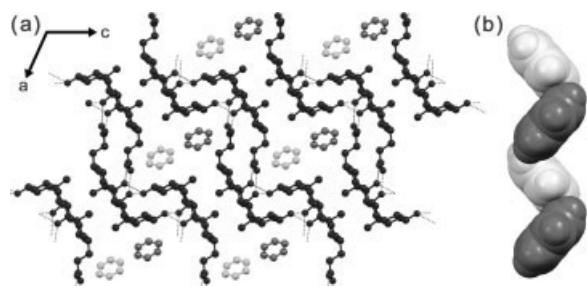


Fig. 3. (a) Packing diagram of the crystal of cholic acid including benzene. Hydrogen atoms are omitted for clarity. (b) 2_1 Helical assembly of benzene molecules in a cavity composed of cholic acid. Benzene molecules in front of and behind the twofold screw axis are colored gray and white, respectively.

in crystals. However, in the field of organic crystallography, it does not seem to have been either discussed or even described except in a few articles, in which handedness seems to be determined based on, for example, the sense of intermolecular interactions such as hydrogen bond networks or intuition from their appearance.^{18–20} This seems to be due to the practice of crystallography being dominated by mathematical treatments. Although absolute structures of chiral crystals have been determined by the use of the Flack parameter, schematic and systematic studies on the handedness of 2_1 helices have not been performed. Here, we emphasize the chirality of 2_1 helical assemblies and propose to determine the handedness of 2_1 helical assemblies on the basis of molecular tilt. As schematically shown in Figure 5, given the molecules in front of a 2_1 screw axis inclined to the right or left, the assemblies can be defined to be right- or left-handed, respectively. Thus, according to this method, the 2_1 assembly of benzene in Figure 3 is a right-handed helix.

2₁ HELICAL ASSEMBLIES COMPOSED OF ASYMMETRIC MOLECULES

So far, we have studied inclusion crystals of steroidal and alkaloidal asymmetric compounds such as brucine, bile acids, and cinchona alkaloids (Scheme 1). They fre-

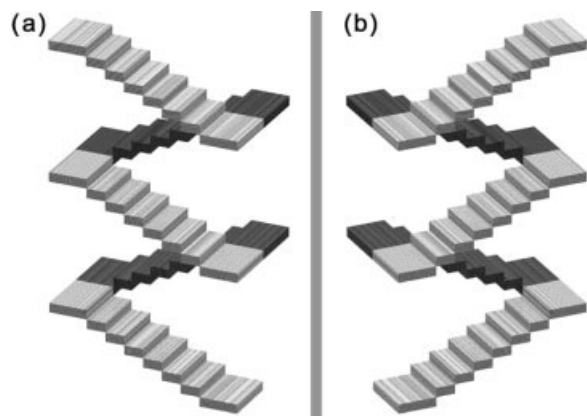


Fig. 4. 2_1 Helical stairs with left-handed (a) and right-handed (b) senses.

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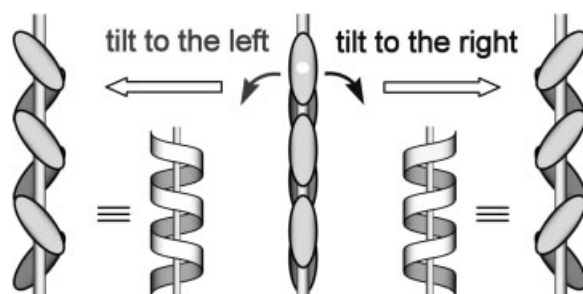
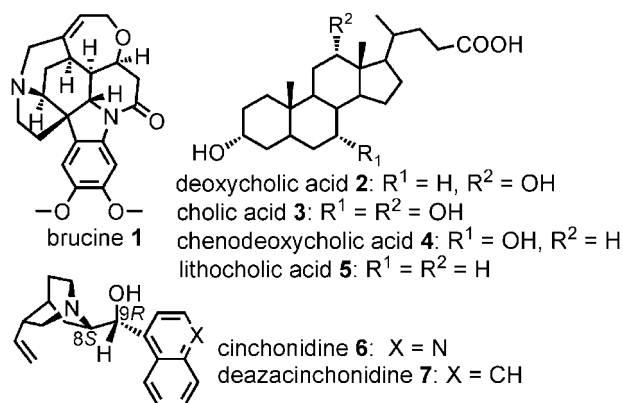


Fig. 5. Definition of left- or right-handedness of 2_1 helical assemblies on the basis of the tilt of objects against a twofold screw axis.

quently crystallized or cocrystallized with small molecules into crystals with twofold screw axes. They form intermolecular noncovalent interactions, for example, multihydrogen bonds and C—H/X (where X denotes O or N atom) weak interaction, to give inherent robust chiral frameworks. We obtained more than 300 types of inclusion crystals and revealed by X-ray diffraction analysis that these crystals take various types of host frameworks.²¹ To understand how the crystal structures are constructed from single molecules and how the guest molecules have an effect on the construction of the host frameworks, we developed a hierarchical interpretation process for the structures of inclusion crystals composed of bile acids, brucine, and cinchona alkaloids.^{22–24} In this process, we noticed that key structures with twofold screw axis are frequently observed. Thus, clarification of supramolecular chirality of 2_1 helical assemblies should lead to the understanding of whole asymmetric crystal structures. In this context, we introduced a new concept, three-axial chirality, and combined it with supramolecular tilt-chirality to describe the supramolecular chirality of 2_1 assemblies, which are composed of asymmetric molecules.

Three Axial Chirality

Conventionally, molecular asymmetry has been expressed in terms of point-, axis-, and plane-chirality as described earlier. In the case of the steroid and alkaloid molecules that have asymmetric, amphiphilic, and facial



Scheme 1.

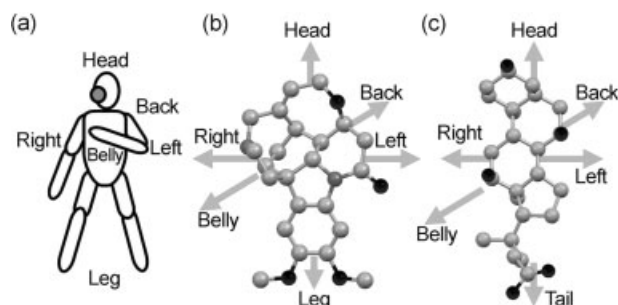


Fig. 6. Illustrations of a vertebrate animal (a), **1** (b), and **3** (c) with orthorhombic three-axes.

structures with multiple chiral carbon atoms, these terms are enough to express the local chirality of these molecules, but not suitable for the asymmetry of the whole molecules. Thus, to describe such three-dimensional anisotropic aspects of the molecules, we termed the aspects as three-axial chirality and proposed to depict it as follows. These characteristic highly anisotropic structures as described earlier connect the molecules with vertebrate animals, e.g., a human, whose bodies are expressed by the words; head and tail (or legs), right and left, as well as front and back, as shown in Figure 6a.^{25,26} Therefore, it may depict the whole three-dimensional anisotropic structures of the molecules on the basis of the orthorhombic three-axes, so-called, head–tail, belly–back, and right–left axes. Thus, we employ these words to define the three-axial chirality of the alkaloidal molecules such as **1** (Fig. 6b) and the steroidal ones such as **3** (Fig. 6c). At present, these directions are defined based on the relative molecular shapes. However, further detailed investigation may require other unified standards such as dipole moment to describe the three-directional chirality.

Combination of Three-Axial and Tilt Chirality of 2_1 Helix

The first step toward the molecular assemblies is logically the formation of dimers. Normally a simplified example is adequate for our purpose. When molecules with three-axial chirality (Fig. 7a) align in the same head-to-tail direction, they have the following three association modes; belly-to-belly, back-to-back as well as belly-to-back (Fig. 7b). The dimers with the former two modes can be expanded with 2_1 -symmetry operation to form the corresponding asymmetric 2_1 helical assemblies. Furthermore, each of the association modes of the bimolecules may have a right- or left-tilt. Thus, combination of the three-axial and tilt chirality enables us to define the helical chirality of the 2_1 helical assemblies. As shown in Figure 7c, dimers are classified by stacking modes depending on which side faces inside into two types, the belly-inside and back-inside modes, which are corresponding to the belly-to-belly and back-to-back assembly mode, respectively, followed by molecular tilt into totally four types; (a₁) right-handed with belly-inside, (b₁) left-handed with belly-inside, (c₁) right-handed with back-inside, and (d₁) left-handed with back-inside. They are extended to the corresponding

2_1 helical assemblies (a₂)–(d₂) and helical tapes (a₃)–(d₃), respectively.²⁶

We now describe concrete examples including 2_1 assembly systems of brucine, bile acids, and cinchona alkaloids.

In the Case of Brucine

Brucine (**1**), which is a natural host compound with six stereogenic centers, has been historically investigated as a separator of racemic mixtures by cocrystallization generally with organic molecules containing acidic functional groups.

Gould and Walkinshaw^{27,28} reported that **1** forms a characteristic corrugated monolayer sheet common to its many crystals, in spite of appearance of supramolecular isomeric brucinium frameworks. The sheet structures are stabilized by weak hydrogen bonds such as CH/O and CH/ π interactions. In our recent report describing inclusion crystals of **1** with various aliphatic alcohols, we revealed that a 2_1 helical structure, shown in Figure 8, is an important, inherent motif of the sheet and that supramolecular isomerism of the framework is derived by stacking the helix in a parallel or antiparallel fashion. According to our classification in Figure 7, the 2_1 helix belongs to the belly-inside right-handed helix.

Other 2_1 helices are also observed in crystals composed of brucinium 3,5-di-*tert*-butyl-4-hydroxybenzoate and benzoate hydrate. In the former, both two helical structures, the left-handed with front-inside and right-handed with back-inside, are achieved.²⁶ To our knowledge, coexistence of different supramolecular isomers in the same crystal is quite rare. The latter includes the back-inside left-handed helix. In this way, the supramolecular isomerism, induced by the addition of acidic components, observed in the 2_1 assemblies composed of brucine can be geometrically

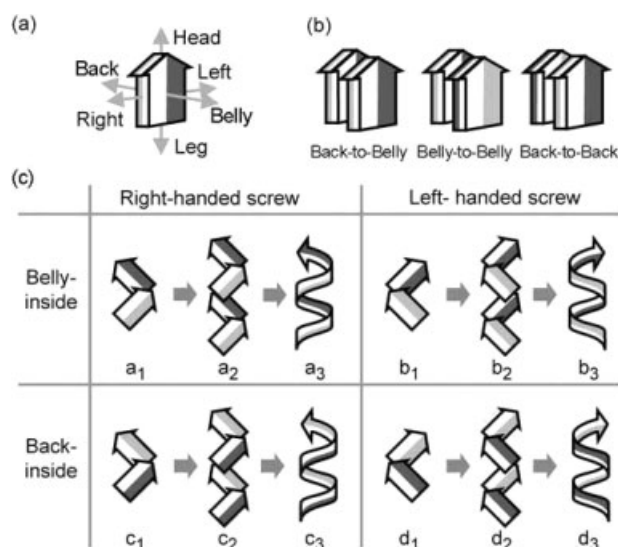


Fig. 7. Schematic representation of a chiral molecule with three-axes (a), three bimolecular association modes (b), and four kinds of bimolecular and 2_1 helical assemblies by combination of three-axial and tilt chirality (c).

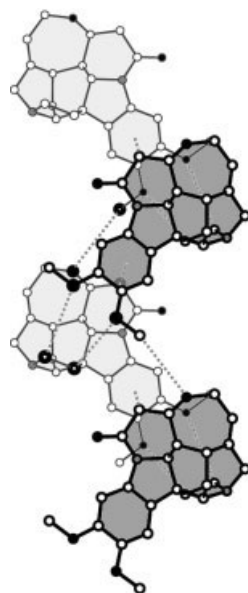


Fig. 8. Helical tape-like assembly as a motif common to the crystals of **1**. Dashed lines indicate weak hydrogen bonds such as CH/O and CH/ π interactions.

well understood by the bimolecular aggregation modes through the three-axial chirality.

In the Case of Bile Acids

Most of the inclusion crystals of cholic acid and their derivatives have crystallographic 2_1 or C_2 axes. The consideration of molecular arrangements around the axes led us to the finding of a notable relation between their molecular structures and the assembly modes.²⁹ Namely, the tilt and handedness of 2_1 helical assemblies of bile acids strongly depend on the substituted position of hydroxyl groups in steroidal skeleton. Figure 9 schematically illustrates the resulting helical tapes of the four bile acids; deoxycholic acid (**2**), cholic acid (**3**), chenodeoxycholic acid (**4**), and lithocholic acid (**5**). Bile acids **2**, **3**, and **5** form right-handed helical assemblies, whereas **4** forms left-handed assemblies. Deoxycholic acid **2** has the hydrogen bonding group (OH(12)) on the right side; as shown

in three-axis chirality expression (Fig. 6c). Since the two groups (OH(3) and OH(12)) face the 2_1 axis, the head slightly leans to the right and the tail greatly separates from the axis. The neighboring molecules adhere through intermolecular hydrogen bonds between OH(3) and OH(12) groups. The resulting assembly is a 2_1 right-handed helical tape, as shown in Figure 9a. Cholic acid **3** has one hydroxyl group on the right side (OH(12)) and the other on the left side (OH(7)). As shown in Figure 9b, there exists an additional hydrogen bond between OH(3) and OH(7) of the neighbored molecules, leading to the situation that cholic acid molecules lie in a perpendicular position to the axis, and that the tail greatly separates from the axis. However, since the distance between OH(3) and OH(7) is shorter than that between OH(3) and OH(12), the head slightly leans to the upper when compared with the perpendicular position. The resulting assembly is a 2_1 right-handed helical tape. Chenodeoxycholic acid **4** exhibits the opposite tendency when compared with **3**, as shown in Figure 9c. Since two hydroxyl groups (OH(3) and OH(7)) are on the left side, the head leans to the left and the tail separates from the axis. The resulting assembly is a 2_1 left-handed helical tape. In contrast, **5** has only one hydroxyl group (OH(3)), and the molecules lie in a perpendicular position to the 2_1 axis. Since the head slightly leans to the upper, the resulting assembly is a 2_1 right-handed helical tape (Fig. 9d). The 2_1 helices composed of **2**, **3**, and **5**, respectively, belong to the right-handed helices with belly-inside, whereas **4** forms the left-handed helix with belly-inside, according to the classification.

In the Case of Cinchona Alkaloids: More Complicated Systems

Brucine and bile acids have rigid facial structures with the long axis in the head-to-tail direction, making it easy to determine directions of the tilt. However, different organic molecules would change this situation. We selected cinchona alkaloids such as cinchonidine (**6**), which have a chiral carbon atom with hydroxyl, quinolinyl, and quinuclidinyl groups. Their fixed conformations are known to have facial structures with three-axial chirality. They form 2_1 helical assemblies through intermolecular hydrogen bonds between the hydroxyl groups and nitrogen atoms in the

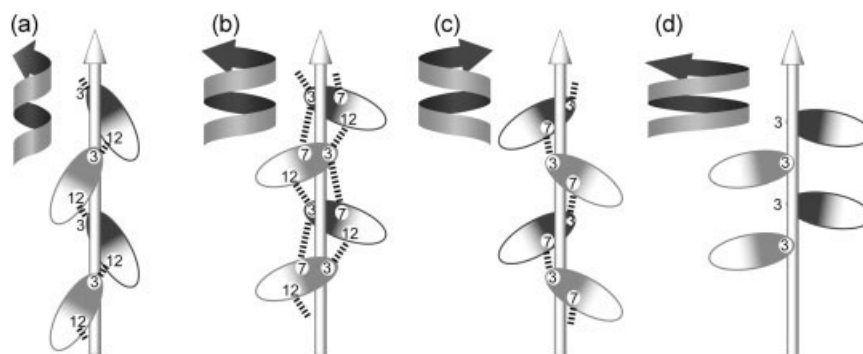


Fig. 9. Schematic representation of the helical tapes of four bile acids; (a) deoxycholic acid **2**, (b) cholic acid **3**, (c) chenodeoxycholic acid **4**, and (d) lithocholic acid **5**.

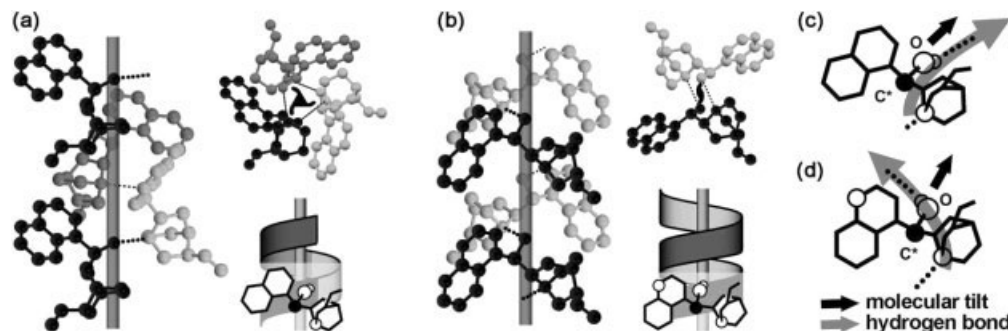


Fig. 10. 3_1 Helical structure of **7** (a) and 2_1 helical columnar assemblies of **6** (b), where side and top views of respective crystal structures and cartoon representations of the helices are described. Hydrogen atoms are omitted for clarity. Directions of the molecular tilts and hydrogen bond networks of **7** (c) and **6** (d) are illustrated in black and gray arrows, respectively.

quinuclidine rings. A problem encountered is how to take an axis for defining the tilt. We noticed that deazacinchonidine **7** forms a right-handed 3_1 helical assembly (Fig. 10a), indicating the right-tilt of the molecules.³⁰ Since the hydrogen bonds in both the systems are almost the same, between the OH group and the N atom in the quinuclidine ring, it can be considered that **6** forms a right-handed 2_1 helical assembly with the right-tilt (Fig. 10b) as in the case of **7** when the O—C* bond (C* notes chiral carbon atom at 9 position) is adopted as the axis for determination of the molecular tilt.³¹

Here, we have to recall that the determination of the handedness of 2_1 helical assemblies must show arbitrariness because of their mathematical aspects. Even in the clear, obvious cases of brucine and cholic acid, it is still possible to select another axis for depiction of molecular tilt, and when the new axis is perpendicular to the former, the handedness is completely opposite, shown in Figure 11a. Especially, setting the axis of cinchona alkaloids is difficult because of their molecular shapes. As described earlier, the handedness of their 2_1 assemblies is determined based on the molecular tilt of referenced 3_1 helical assembly. However, it is also possible to give opposite handedness on the basis of the sense of hydrogen bonded networks (Fig. 10d) in contrast with the assembly of **7**, in which both molecular tilt and hydrogen bonded network give the right-handed sense (Fig. 10c). The same fact is also observed in a peptide helix as shown in Figure 11b. As well known, peptides fold into a right-handed helix through hydrogen bonds of C=O...H—N. On the other

hand, when we focus on the chiral center C* and the adjacent four substituent groups, this is comparable with that of **6**. Thus, as in the case of **6**, the bond R—C* may be adapted to the axis for definition of the molecular tilt to give left-handed sense.

Finally, we again emphasize that an important point to determine the handedness of the 2_1 helical assemblies is to stipulate the basis of its determination. However, especially for the 2_1 assemblies without clearly directional interactions such as hydrogen bonds, molecular tilt must play a significant role in determining the helical handedness.

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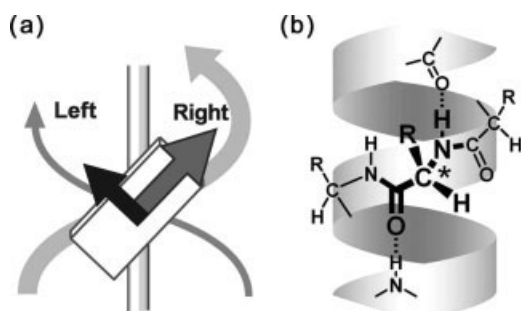


Fig. 11. (a) Schematic representation of two helices based on two perpendicular axes. (b) Helical structure of a peptide.

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A Study of Planar Chromophores in Dichromophoric Molecules by Circular Dichroism Spectroscopy

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT A synthesis of a series of enantiomerically pure polycyclic structures derived from (+)-(1*S*,5*S*)-bicyclo[3.3.1]nonane-2,6-dione was accomplished. Molecules containing single or two similar or different chromophores were obtained. Chiroptical properties of the synthesized chiral structures **2–5**, and **7** having carbonyl, 1,2-methylenedioxybenzene, and indole chromophores were studied. The sign of the cotton effect was related to sector rules and the orientation of the planes containing chromophores in dichromophoric molecules possessing 1,2-methylenedioxybenzene and carbonyl or indole chromophores. Interaction of the chromophores in dichromophoric molecules was examined by CD spectroscopy; however, no direct evidence was obtained. *Chirality* 20:337–343, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: absolute configuration; CD spectroscopy; chromophore interaction; enantiospecific synthesis

INTRODUCTION

Circular dichroism (CD) spectroscopy today is a well-established technique in stereochemistry studies.^{1,2} The most common application of CD relates spectra to the assignment of the absolute configuration via the sector and helicity rules, and different chromophores have been investigated extensively both experimentally and theoretically over the past decades.³ The goal is much more easily achieved when the chiral compound possesses a rigid skeleton or its geometry is known. However, semiempirical rules should be applied cautiously. Recently it was demonstrated that the application of the sector rule to the 1,2-methylenedioxybenzene chromophore to establish the absolute configuration of the polycyclic compound did not lead to an unequivocal conclusion, which is likely to be due to the interaction of the chromophores.⁴

Thus CD spectroscopy is not only a tool for studying the absolute configuration of organic molecules but also is a means to examine interactions between functional groups, i.e. chromophores. Most of synthetic and natural compounds are complex molecules containing several chromophores, which are sometimes introduced purposefully.^{5,6} In such cases the interchromophoric interactions usually provide the most significant contributions to the CD spectra. When two chromophores are located close in space in a chiral mutual orientation, the interactions between their transition dipoles are responsible for rotational strengths, often exceeding those associated with the perturbations on each chromophore exerted by the chiral nonchromophoric skeleton.⁷ The most important case arises when two chromophores with strong electric-dipole allowed transitions couple to each other (exciton coupling).^{8,9}

To study such phenomena, relevant dichromophoric molecular structures are required. Because of a proper molecular geometry bridged bicyclic compounds are suitable model structures for studying chiroptical properties and interaction between the nonconjugated chromophores.^{10–13} In this work we have synthesized several enantiomerically pure compounds of the bicyclo[3.3.1]nonane framework containing carbonyl and planar aromatic chromophores, namely 1,2-methylenedioxybenzene and indole, for an experimental study of chromophore properties and application of rules relating the sign of the Cotton effect (CE) to the absolute configuration.

MATERIALS AND METHODS

Instrumentation

Melting points were recorded with a Gallenkamp melting apparatus and are uncorrected. ¹H NMR and ¹³C NMR spectra were recorded on Bruker DRX400 spectrometer at 400 MHz for protons and 100.6 MHz for carbon and on a Varian Inova 300 spectrometer in CDCl₃, and are reported as chemical shifts in δ ppm relative to TMS, using the residual solvent peaks as internal standards. The CD spectra

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were recorded with a Jasco Model J-500A spectropolarimeter at 20°C in a 0.1–1.0 cm cell using 95% aqueous ethanol. The CD spectra were measured in millidegrees and normalized into $\Delta\epsilon_{\text{max}}$ ($\text{l mol}^{-1} \text{cm}^{-1}$)/ $\lambda(\text{nm})$ units. Optical rotations were measured with a Perkin-Elmer 141 polarimeter at the sodium D line and on a Polamat-A polarimeter (Carl Zeiss) at 546 nm and $[\alpha]^{20}$ values are given in $10^{-1} \text{deg cm}^{-1} \text{g}^{-1}$, and concentrations are given in units of $\text{g}/100 \text{cm}^3$. UV spectra were recorded on a Varian Cary 100Bio spectrophotometer using spectral grade ethanol.

IR spectra were recorded in KBr pellets on a PerkinElmer Spectrum BX spectrometer. Chromatography was performed using silica gel Merck Kieselgel 60 (0.040–0.063 mm) for flash chromatography and Merck Kieselgel 60 F₂₅₄ plates for TLC. Elemental analyses were performed by the analytical laboratory of the Chemistry Faculty, University of Vilnius. Semi-empirical and molecular mechanics calculations were performed using SPARTAN'06 for Windows Version 1.0.2 program package.¹⁴

Synthesis

(±)- and (+)-(1*S*,5*S*)-bicyclo[3.3.1]nonane-2,6-dione **1** were prepared according to the procedures described in literature,^{15,16} respectively.

(+)-(1'*S*,5'*S*)-Spiro[1,3-dioxolane-2,6'-bicyclo[3.3.1]nonan]-2'-one (6,6-ethylenedioxybicyclo[3.3.1]nonan-2-one) [(+)-3**].** Prepared according to the method described in the literature.¹⁹ Yield 0.34 g (88%). $[\alpha]_D^{20} = +49$ (c 0.083, EtOH). UV, λ_{max} (log ϵ): 289 (1.36), 194 (2.96). IR, ν_{max} : 1112, 1102 (C–O) cm^{-1} . ¹H NMR (400 MHz): 4.01–3.9 (m, 4H), 2.54–2.43 (m, 2H), 2.4–2.32 (m, 2H), 2.16–2.11 (dq, $J = 2.4$ and 13.4 Hz, 1H), 2.08–1.93 (m, 4H), 1.89–1.77 (m, 2H), 1.74–1.6 (m, 2H). ¹³C NMR: 216.2 (C2'), 110.2 (C6'), 64.5, 64.4 (C4 and C5), 43.7 (C1'), 37.4 (C3'), 35.4 (C5'), 30.2 (C9'), 29.2 (C7'), 27.2 (C4'), 22.4 (C8').

(+)-(1'*S*,5'*S*)-Spiro[1,3-benzodioxole-2,6'-bicyclo[3.3.1]nonan]-2'-one [(+)-2**].** Phosphorus pentoxide (0.7 g, 5 mmol) was added to a solution of (+)-(1*S*,5*S*)-dione **1** (300 mg, 1.97 mmol) in dry toluene (7 mL). Benzene-1,2-diol (217 mg, 1.97 mmol) was added in portions to the resulting mixture and heated under reflux until complete consumption of the starting diketone **1** (ca. 2 h; TLC, heptane-EtOAc 5:2), then cooled and the solution decanted from P₂O₅. The remaining solid residue was neutralized with water, the aqueous phase was extracted with benzene (3 × 5 mL), the combined solutions were washed with water, aqueous NaHCO₃, dried and evaporated. The residue was purified by column chromatography (heptane-EtOAc 8:2, R_f 0.35) to afford 380 mg (79%) of (+)-**2**, mp 149–151°C. $[\alpha]_D^{20} = +73$ (c 0.052, EtOH). IR ν_{max} : 1702, 1483, 1240, 1097, 737 cm^{-1} . UV, λ_{max} (log ϵ): 289 (sh, 3.55), 284 (3.68), 232 (3.47), 198 (4.74).

¹H NMR (400 MHz): 6.81–6.75 (m, 4H), 2.63–2.53 (m, 2H), 2.47–2.39 (m, 2H), 2.32–2.27 (dq, $J = 2.5$ and 13.7 Hz, 1H), 2.21–1.96 (m, 6H), 1.91–1.83 (dt, $J = 6.6$ and 13.2 Hz, 1H). ¹³C NMR: 215.2 (C-2'), 147.0, 147.0 (C-3a and C-

7a), 121.3, 121.2 (C-5a and C-6a), 118.9 (C-6' (C-2)), 108.7, 108.6 (C-4a and C-7a), 43.2 (C-1'), 37.1 (C-3'), 35.4 (C-5'), 30.2 (C-9'), 28.6 (C-7'), 26.4 (C-8'), 21.6 (C-4'). Anal Calcd for C₁₅H₁₆O₃: C 73.75; H 6.60. Found: C 74.03; H 6.64.

(+)-(1*S*,5*S*)-dispiro[benzo-1,3-dioxole-2,2',6'-bicyclo[3.3.1]nonane] [(+)-3**].** To a solution of (+)-(1*S*,5*S*)-dione **1** (50 mg, 0.3 mmol) and benzene-1,2-diol (350 mg, 3.17 mmol) in dry toluene (5 mL) phosphorus pentoxide (270 mg, 1.9 mmol) was added. The reaction mixture was heated under reflux for 3.5 h until the starting diketone **1** was consumed (TLC, heptane-EtOAc 5:2). The reaction mixture was cooled and the solution decanted from P₂O₅. The remaining solid was neutralized with water, the aqueous phase was extracted with benzene (3 × 5 mL), and the combined solutions washed with water, aqueous NaHCO₃, dried, and evaporated. The residue was purified by column chromatography (heptane-EtOAc 5:2, R_f 0.65) to afford 80 mg (73%) of (+)-**3**, mp 208–210 °C. $[\alpha]_D^{20} = +103$ (c 0.053, EtOH). UV, λ_{max} (log ϵ): 289 (sh, 3.89), 284 (3.99), 232 (3.79), 200 (4.97).

¹H NMR (400 MHz): 6.8–6.75 (m, 8H), 2.28–2.1 (m, 10H), 1.94–1.84 (m, 2H). ¹³C NMR: 147.2, 147.0 (C-3a and C-7a), 121.1, 121.0 (C-5 and C-6), 119.2 (C-2' and C-6'), 108.6, 108.5 (C-4 and C-7), 35.7 (C-3' and C-7'), 31.3 (C-1' and C-5'), 28.1 (C-9'), 22.3 (C-4' and C-8'). IR: 1482, 1235, 1069, 735 cm^{-1} . Anal Calcd for C₂₁H₂₀O₄: C 74.98; H 5.99. Found: C 75.11; H 6.23.

(+)-(6'*S*,10'*R*)-1'-H-spiro[1,3-benzodioxole-2,9'-[6',10']methanocyclooct[b]indole] [(+)-4**].** To a solution of ketone (+)-**2** (90 mg, 0.368 mmol) in ethanol (5 mL), phenyl hydrazine (50 mg, 0.46 mmol) was added. The reaction mixture was acidified with HCl (0.05 mL) and heated under reflux for 3.5 h until the starting ketone **3** was consumed (TLC, EtOAc-heptane 2:5). The cooled reaction mixture was evaporated to dryness and the resulting residue was purified by column chromatography (EtOAc-heptane 2:5, R_f 0.42) to afford 100 mg (86%) of (+)-**4**, mp 170–172°C. $[\alpha]_D^{20} = +74$ (c 0.05, EtOH). UV, λ_{max} (log ϵ): 289 (sh, 4.02), 283 (4.09), 228 (4.62), 199 (4.82).

¹H NMR (400 MHz): 7.67 (s, 1H, NH), 7.53–7.51 (m, 1H), 7.33–7.31 (m, 1H), 7.18–7.1 (m, 2H), 6.81–6.77 (m, 4H), 3.15–3.11 (d, ² $J = 17$ Hz, 1H, H_{endo}-11'), 3.11–3.08 (m, 1H, H-6'), 2.97–2.91 (dd, ² $J = 17$, ³ $J = 6.9$ Hz, H_{exo}-11'), 2.71–2.64 (m, 1H, H-10'), 2.39–2.34 (dt, ² $J = 12.8$, ³ $J = 3$ Hz, H_{exo}-8'), 2.18–2.08 (td, $J = 3.7$, 13 Hz, 1H), 2.07–2.02 (qd, $J = 2.9$, 12.8 Hz, 1H), 1.91–1.79 (m, 2H), 1.73–1.65 (dt, $J = 5$ and 13.5 Hz, 1H). ¹³C NMR: 147.3, 147.3 (C-3a and C-7a), 135.7, 135.7 (C-4'a and C-5'a), 127.4 (C-5'c), 121.3, 121.0 (C-5 and C-6), 121.0 (C-3'), 120.2 (C-1'), 119.3 (C-9'), 118.0 (C-2'), 110.6 (C-5'b), 108.9 (C-4'), 108.5, 108.5 (C-4 and C-7), 36.6 (C-6'), 29.9 (C-10'), 29.0 (C-12'), 28.9 (C-11'), 28.0 (C-8'), 21.9 (C-7'). IR: 3449, 1488, 1245, 1086, 737 cm^{-1} . Anal. Calcd for C₂₁H₁₉NO₂: C 79.47; H 6.03; N 4.41. Found: C 79.65; H 6.31; N 4.56.

(+)-(1'*S*,5'*S*)-Bis-spiro[1,3-dithiolane-1',3'-benzodioxole-2'',6''-bicyclo[3.3.1]nonane] [(+)-6**].** A mixture of ethanedithiol (0.06 mL, 0.72 mmol) and BF₃·Et₂O (0.055 mL) were added under continuous stirring to a solution of

ketoacetal **2** (100 mg, 0.4 mmol) in 3 mL of glacial acetic acid under a nitrogen atmosphere. The mixture was stirred for 18 h at room temperature. Then 3 g of ice was added, the mixture was washed with a 10% solution of NaOH, extracted with diethyl ether (5×10 mL), washed with 10% solution of Na_2CO_3 , and dried over anhydrous Na_2SO_4 . The solvent was evaporated in vacuo yielding 128 mg (98%) of (+)-**6**, m.p. 165–166°C, (heptane EtOAc 4:1 R_f 0.59). $[\alpha]_{546}^{20} = +59$ (c 1.4, CHCl_3). ^1H NMR (300 MHz): 6.75–6.8 (4H, m), 3.3–3.4 (4H, m), 2.4 (1H, m), 2.0–2.3 (8H, m). ^{13}C NMR: 147.6, 147.3 (C-3'a and C-7'a), 121.2, 121.3 (C-5' and C-6'), 119.8 (C-2''), 108.8, 108.7 (C-4 and C-7), 73.6 (C-6''), 41.4 (C-4), 39.4 (C-3''), 39.2, 39.1 (C-1' and C-5'), 36.0, 32.3, 31.5, 27.8, 25.0 (bicyclic skeleton carbon atoms). Anal. Calcd for $\text{C}_{17}\text{H}_{20}\text{O}_2\text{S}_2$: C 63.71; H 6.29; S 20.01. Found: C 63.65; H 6.36; S 20.07.

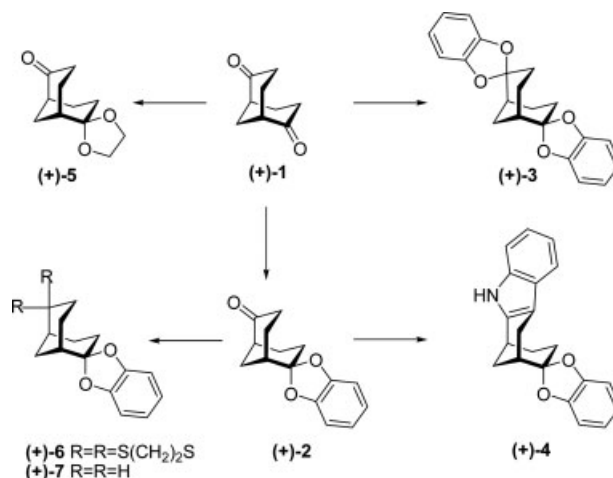
(+)-(1'S,5'S)-Spiro-1,3-benzodioxole-2,6'-bicyclo[3.3.1]nonane [(+)-7]. Freshly prepared Raney nickel (0.5 g) was added to a solution of monothioacetal **6** (120 mg, 0.37 mmol) in dry ethanol (12 mL) in a round-bottom flask equipped with a water condenser and magnetic stirrer. The reaction mixture was stirred under reflux for 6 h. The mixture was then, cooled to room temperature and hydrogenation was continued for additional 6 h at a pressure of 1 atm. The mixture was filtered through a 1-cm pad of CELITE[®], the solvent was evaporated, and the residue was purified by column chromatography (1% ethyl acetate in hexane) to afford 50 mg (60%) of (+)-**7**, m.p. 45–46°C. $[\alpha]_{546}^{20} = +40$ (c 1.2, CHCl_3). UV, λ_{max} (log ϵ): 293 (2.32), 286 (2.52), 233 (2.36), 208 (2.56). ^1H NMR (300 MHz): 6.8–6.85 (4H, m), 2.2–2.3 (2H, m), 1.45–2.1 (10H, m). ^{13}C NMR: 147.8, 147.5 (C-3a and C-7a), 121.1, 121.0 (C-5 and C-6), 108.7, 108.5 (C-4 and C-7), 37.3 (C-3'), 33.6, 31.9 (C-1' and C-5'), 31.3, 28.9, 26.7, 25.5, 20.7 (bicyclic skeleton carbon atoms). Anal. Calcd for $\text{C}_{15}\text{H}_{18}\text{O}_2$: C 78.23; H 7.88. Found: C 78.59; H 7.89.

RESULTS AND DISCUSSION

Enantiospecific Synthesis of Dichromophoric Compounds

An efficient resolution of enantiomers of bicyclo[3.3.1]nonane-2,6-dione **1** by baker's yeast employing a modified procedure reported by Hoffmann and Wiartalla^{17,18} after repeated fermentation afforded the (+)-(1*S*, 5*S*)-**1** enantiomer with 99% ee giving a crucial intermediate on the route to enantiomerically pure compounds containing different chromophores in a well defined spatial relationship. Thus, starting from (+)-**1** (>99% ee) enantiomerically pure derivatives **2–7**, having carbonyl, 1,2-methylenedioxybenzene and indole chromophores were obtained as shown in Scheme 1.

Monoprotected acetal derivative **5** and pyrocatechol acetals **2,3** were obtained by acetalization reactions. Reaction of (+)-**1** with an equimolar amount of benzene-1,2-diol (pyrocatechol) in toluene exclusively afforded monoacetal (+)-**2** in excellent yield. The introduction of the aromatic ring is confirmed by the ^1H and ^{13}C NMR spectra and the $\text{C}=\text{O}$ absorption band at 1702 cm^{-1} as well as the signal



Scheme 1.

at δ 215.0 ppm in the ^{13}C NMR spectrum confirming the presence of the carbonyl group. Treatment of (+)-**1** with an excess of benzene-1,2-diol afforded the bisacetal derivative (+)-**3**. Eleven distinct signals in the ^{13}C NMR spectrum are consistent with the C_2 molecular symmetry of **3**. The compound **7** with the 1,2-methylenedioxybenzene reference chromophore was synthesized via the protected monothioacetal derivative **6** obtained from **2** and subsequent reduction of the protective group with Raney nickel. It should be noted that the direct Wolff–Kishner reduction of carbonyl group in **2** gave a mixture of compounds including unsaturated derivative which could not be purified.

The reaction of the mono pyrocatechol acetal (+)-**2** with phenylhydrazine in the presence of a catalytic amount of hydrochloric acid under Fischer indolization reaction conditions afforded the indole derivative (+)-**4** in excellent yield.

The absolute configuration of the (+)-enantiomers of **2–5** and **7** is predetermined by the configuration of the starting (+)-(1*S*,5*S*)-diketone **1** since the acetalization and indolization reactions proceed without participation of the bridge atoms. As neither the configuration nor the priority in the sequence of the substituents of the bridged atoms changes, the absolute configuration is defined (1*S*,5*S*) for (+)-**2**, **3**, **5**, and **7**. However, the priority in the sequence of the substituents of the bridge atoms changes in the indole structure **4**. Therefore the absolute configuration of (+)-**4** is defined as (6'*S*,10'*R*).

Conformational Analysis of Compounds 2–5 and 7

The application of the CD spectral data to analyse the sign of the CE and relating it with the absolute configuration is basically achieved when the chiral compound possesses a rigid skeleton or is of predictable geometry. The necessity of conformational analysis becomes evident studying flexible molecules and conformational analysis should be undertaken in order to ascertain the most conformationally stable structures. We performed a lowest energy conformational search of **2–5** and **7** with Spartan'06 for Windows Version 1.0.2 using the Monte–Carlo method and MMFF94 force field. From two to four lowest

TABLE 1. Relative Energies of the Conformations of Compounds 2–5 and 7

Compound	ΔE (kcal/mol)				
	<i>b-c</i> ^a	<i>c-c</i>	<i>c-b</i>	<i>tb-tb</i>	<i>tb-tc</i>
2	1.49	0	5.54	9.52	–
3	6.27	0	–	14.13	–
4	–	0	–	–	9.75
5	1.63	0	5.42	9.54	–
7	6.41	0	4.12	–	–

^aConformation designation *c*, chair; *b*, boat; *tb*, twist boat; *tc*, twist chair; first symbol denotes the conformation of the ring bearing carbonyl group for **2,5** and acetal functionality for **4,7**.

energy conformers were located in each case.¹⁴ Relative energies of the conformers are presented in Table 1.

For all the investigated compounds **2–5** and **7**, the double chair (*c-c*) conformation was found to be the most stable and prevailed over other conformations. The fusion of the bicyclic framework with the indole ring notably reduces the conformational flexibility of the indole derivative **4**. Only two lowest energy conformers were obtained. The more stable was found to be the double chair conformation, the six membered ring bearing indole ring being considerably flattened. The next conformation, *i.e.* chair-boat, was calculated to be of much higher energy ($\Delta E = 9.75$ kcal mol^{−1}).

The conformational analysis revealed that for all the compounds investigated a single conformer, *i.e.* *c-c* is prevailing in the gas phase at room temperature. Consequently only the aforementioned conformers were taken for further consideration.

Analysis of the CD spectra

The CD spectra of compounds **2–5** and **7** were recorded and analyzed in order to study input of the chromophores into the CE and to relate the latter to the absolute configuration of the enantiomers (Table 2).

Compounds **5** and **7** are regarded as molecules having the reference chromophores, the carbonyl and the 1,2-methylenedioxybenzene, respectively. The carbonyl group $n \rightarrow \pi^*$ transition in **5** appears around 290 nm giving a positive CE and the intensity is typical for such a chromo-

phore (Fig. 1). The CD spectrum of enantiomer (+)-**7** in ethanol exhibited positive bands at around 280 nm and at about 250 nm. These bands of the 1,2-dioxophenylene chromophore are assigned to the α -band (ca. 285 nm) and the β -band (ca. 240 nm) (Fig. 1). The ¹L_b transition, the so-called α -band, of the 1,2-methylenedioxybenzene chromophore, which has C_{2v} symmetry is due to the interference of the $\pi \rightarrow \pi^*$ aromatic transitions and $n \rightarrow \pi^*$ transitions of the oxygen atoms.

The empirical rule, like the octant rule for the ¹L_b transition was proposed in the study of chiroptical properties of lycorine and related compounds.²⁰ This empirical rule served well for the determination of the absolute configuration of ketoacetal **8** (Fig. 4) and the corresponding diastereomeric *exo*- and *endo*-hydroxy derivatives obtained from the latter.²¹ To interpret the CD spectra of the acetal **7** we applied the sector rule developed for the 1,2-dioxophenylene chromophore. However, in this case the application of the sector rule placing the aromatic chromophore at the origin of coordinates (bold line on *x*-axis indicates the 1,2-dioxophenylene chromophore (Fig. 2) gives the opposite CE sign. This contravenes the previous application of the quadrant rule for this bicyclic molecule (*cf.* compound **8**) and thus a correlation between the absolute configuration of (+)-**7** and the sector rule could not be adapted.

The compounds **2–4** contain two chromophores. In this case a perturbation arises when two chromophores with nonvanishing electric dipole transition moments are nearby in the same molecule. This case, known as exciton coupling, gives rise to a definite chiroptical response. The rotational strength is dependent on the distance between the chromophores and, in particular, on the appropriate orientation of chromophores. Thus, we studied the CD spectra to rationale the CE signs in the dichromophoric compounds.

Dipyrrocatechol acetal (+)-**3** contains two 1,2-methylenedioxybenzene chromophores. The ¹L_b transition is either electrically or magnetically forbidden for this chromophore; however the rotational strength is still generated by a chiral environment. The UV absorption of **3** as well as the CD bands is analogous to compound **7** containing a single 1,2-methylenedioxybenzene chromophore (Fig. 1). The CD curves practically coincide and the intensity for dichromophoric compound **3** is nearly a sum of intensities. Thus no interaction is observed since the angle between ¹L_b transitions is nearly parallel, although the angle

TABLE 2. Specific Rotation and the CD Spectral Data for Compounds 2–5 and 7–9

Compound	$[\alpha]$, 10 ^{−1} deg cm ² g ^{−1}	CD, $\lambda_{\max}$, nm ($\Delta\epsilon$, dm ³ mol ^{−1} cm ^{−1})	Dihedral angle between chromophore planes, deg. (ca.)
(+)- 2	+73 (c 0.052, EtOH, 20°C)	222 (+0.51), 242 (−0.53), 291 (+0.96)	170
(+)- 3	+103 (c 0.053, EtOH, 20°C)	236 (+0.64), 280 (+0.79)	80
(+)- 4	+74 (c 0.05, EtOH, 20°C)	226 (+23), 267 (−2.0)	150
(+)- 7	+40 (c 0.012, CHCl ₃ , 20°C)	256 (+0.15), 283 (+0.35), 306 (+0.15)	–
(+)- 5	+49 (c 0.083, EtOH, 20°C)	290 (+0.39)	–
(+)- 8 ⁴	+40 (c 0.07, EtOH, 25°C)	225 (−0.80), 240 (+0.88)	70
(+)- 9 ²¹	+288 (c 0.125, EtOH, 16°C)	217 (+14.1), 290 (+9.5)	80

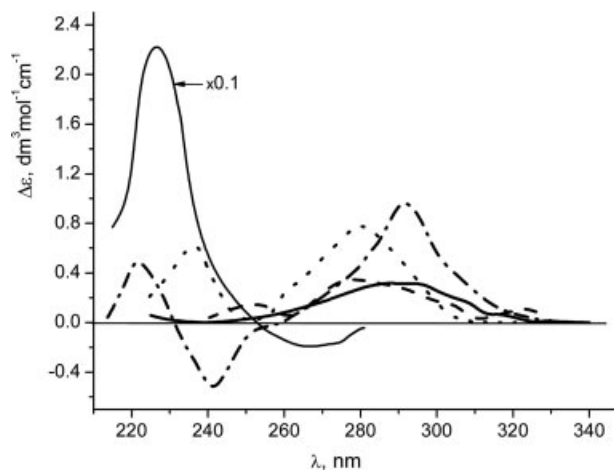


Fig. 1. CD spectra of ketone **5** (—), ketoacetal **2** (---), dipyrrocatechol acetal **3** (····), indole **4** (— · —), actual intensity $\times 10$, pyrocatechol **7** (---).

between planes containing two ^1L_b transition moments is about 80° (Fig. 3a). Location of any of chromophores in sectors gives the positive CE in contrary to compound **7** and the sector rule for 1,2-methylenedioxybenzene chromophore is applicable for this compound.

The carbonyl and 1,2-methylenedioxybenzene chromophores in (+)-**2** give rise to couplets of moderate intensity in a CD spectrum (Fig. 1). Two positive bands are observed at 222 (+0.51) and 291 nm (+0.96), and the negative CE is at about 240 nm. Since the ^1L_b transition is rather weak for aromatic chromophore and $n \rightarrow \pi^*$ electric transition for carbonyl chromophore is forbidden the observed CE is not due to the coupled interaction and likely there is no coupling mechanism in this case. The angle between the planes with chromophores is nearly parallel (Fig. 3b) thus the CE corresponds to separate carbonyl and 1,2-methylenedioxybenzene chromophores. Actually evaluation of the dihedral angle between the planes containing chromophores is not so straightforward in the minimized structures of the studied molecules. The band at about 290 nm corresponds to

$n \rightarrow \pi^*$ transition of carbonyl chromophore while the negative CE could be attributed to the p-band of the 1,2-methylenedioxybenzene chromophore. The sign of the CE at 290 nm is in accordance with the octant rule for the carbonyl chromophore, which predicts the positive CE of moderate intensity corresponding to $n \rightarrow \pi^*$ transition (Fig. 2).

A comparison of the CD spectra of **2** with compound **8** containing the same chromophores at different positions, namely **2** and **9**, in this bicyclic framework (Fig. 4) revealed that the band at 290 nm is of higher intensity in molecule (+)-**2**. The CE depends on the interchromophoric geometry and the rotational strength is dependent on the distance between the chromophores and in particular on the appropriate orientation of chromophores. An interesting observation relating the angle between planes of chromophores, which is about 170° (Fig. 3b) and the longer wavelength positive couplet in the CD spectrum of **2** may be accounted to positive chirality while an opposite couplet is observed at the lower wavelength (Fig. 1). The different intensity of the bands at 290 nm for (+)-**2** and **8** was accounted for by the geometric orientation of the chromophores in this structure. We attributed the low intensity of the ^1L_b transition in the CD spectrum to the orientation of the $n \rightarrow \pi^*$ transition with respect to the carbonyl group. The angle between the planes of these chromophores in the minimized structure of **8** was found to be about 70° (Fig. 4), thus the rotational strength should be rather weak. The interaction gives a weakly bi-signate CE, which leads to the similar or lower intensity of the band of the same sign compared to that of the isolated chromophores because of withdrawal of electrons from the carbonyl group by the aromatic chromophore.

The indole and 1,2-methylenedioxybenzene chromophores in compound **4** may give rise to an exciton coupling. The double chair conformation for **4** was found to be most stable, the six-membered ring bearing the indole ring being considerably flattened. The interpretation of the experimental CD spectrum and the assignment of observed CE's to particular electronic transitions are rather complicated due to the fact that both indole and 1,2-methylenedioxybenzene chromophores exhibit transitions

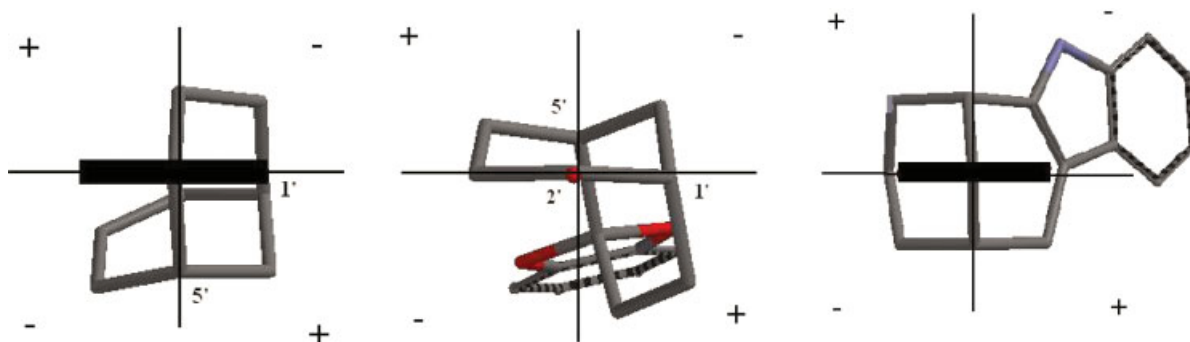


Fig. 2. Location of 1,2-dioxophenylene chromophore in acetal **7**, ketoacetal **2** and indole **4** (from the left to the right, respectively) into sectors placing the aromatic chromophore of **7** and **4** at the origin of the coordinates (bold line — on x -axis indicates the 1,2-dioxophenylene chromophore), and carbonyl chromophore of **2**. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

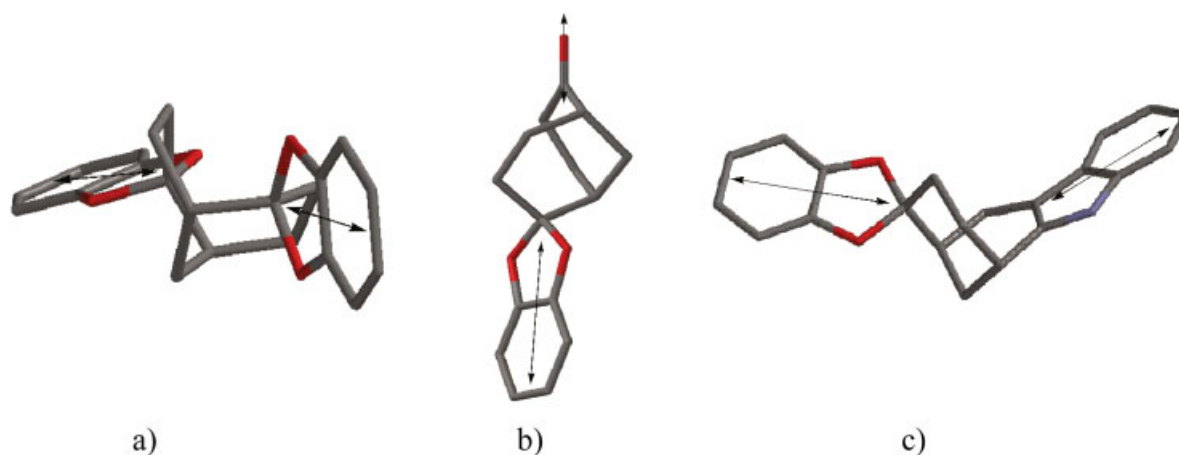


Fig. 3. Transition dipoles represented by arrows and dihedral angles between chromophore planes indicated in parenthesis: (a) dipyrocatechol acetal **3** (80°), (b) ketoacetal **2** (170°), and (c) indole **4** (150°). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

at the same wavelength. For the indole chromophore bands are observed at about 300 nm (^1L_b transition), about 290 nm (^1L_a), about 230 nm (^1B_b) and below 210 nm (^1B_a),^{22–24} whereas the 1,2-dioxophenylene chromophore exhibits bands at about 285 nm (α -band), about 240 nm (p-band) and about 220 nm.²⁰ The superimposition of the above mentioned bands of the same or opposite signs give resultant positions and magnitudes of the CEs in the CD spectrum of **4**. In agreement with this, the UV spectrum of **4** in ethanol shows three bands at 283 (shoulder at 289), 228 and 199 nm. The intensity of the longer wavelength band at about 270 nm is an order of the magnitude less due to the mix of transitions, namely ^1L_b and ^1L_a bands of the indole chromophore and the α -band of the 1,2-methylenedioxybenzene chromophore. The sector location of the latter chromophore gives the negative sign of the CE and the sector rule is valid (Fig. 2).

The CD spectrum of **9** containing the same chromophores but at different positions gives a positive CE in the CD spectrum due to the superimposition of the above mentioned bands of the same or opposite signs.⁴ The angle between the planes of these chromophores in the minimized structure of **9** was found to be about 80° (Fig. 4 cf. Fig. 3c). The analytical function of the coupling potential versus the dihedral angle has been first calculated by Mason²⁵ and it was later confirmed that coupling potential vanishes at $\theta \approx 110^\circ$. In accordance with the theoretical calculation and experimental observation for the compounds with dinaphthyl chromophores where a quantitative relationship between the angle and CD parameters was evidenced²⁶ the negative sign of the coupling band is observed at higher wavelength and when the dihedral angle is over 110° . Interestingly, this tendency despite without emerged coupling was observed for compounds **2** and **4** studied in this work.

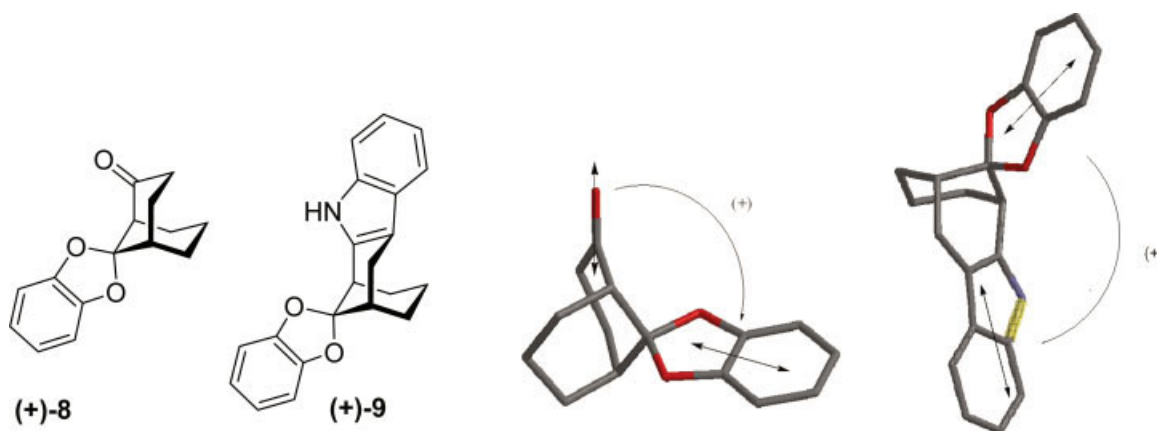


Fig. 4. Dichromophoric compounds spiro[benzo-1,3-dioxole-2,9'-bicyclo[3.3.1]nonan]-2'-one **8** and 1'*H*-spiro[1,3-benzodioxole-2,12'-[6',10'] methanocyclooct[b]indole] **9** containing the same chromophores as in molecules **2** and **4** though in different positions. Transition dipoles represented by arrows and the angles between planes containing chromophores in **8** and **9** are 70° and 80° , respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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Atropisomeric 3-Aryl-2-Oxo-4-Oxazolidinones and Some Thione Analogues—Enantiodifferentiation and Ligand Competition in Applying the Dirhodium Method

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT The atropisomeric 2-oxo-4-oxazolidinones **1Z** bind weakly to the rhodium atoms in the complex $\text{Rh}_2^{(\text{II})}[(R)-(+)\text{-MTPA}]_4$ (**Rh***, MTPA-H = methoxytrifluoromethylphenylacetic acid $\equiv$ Mosher's acid), presumably via the C-2 carbonyl oxygen atom. There are some ¹H and ¹³C NMR signals in these compounds which show small dispersion effects suitable for enantiodifferentiation. In contrast, the thiocarbonyl sulfur atoms in **2Z** and **3Z** bind strongly so that significant complexation shifts ($\Delta\delta$) and diastereomeric dispersion effects ($\Delta\nu$) can be observed, and chiral discrimination and the determination of enantiomeric ratios of these thiocarbonyl compounds is easy. So, it is shown that—as expected—C=S is a much better binding site when competing with C=O. In compounds of Series **2** a “syn-methyl effect” was discovered which describes the dependence of dispersion effects of syn-oriented methyl groups on the nature of the substituents Z. A mechanism of combined steric and electronic interaction influencing the conformational equilibria inside the adducts is proposed. Determination of absolute configurations by correlation fails, at least on the basis of the data available. *Chirality* 20:344–350, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: 3-aryl-2-oxo-4-oxazolidinone derivatives; rhodanines; dirhodium complexes; adduct formation; ¹H and ¹³C NMR; atropisomerism; axial chirality; chiral recognition

INTRODUCTION

The 3-*ortho*-aryl substituted 2-oxo-4-oxazolidinones, 2-thio-oxo-4-oxazolidinones and rhodanines are of manifold biological interest as they possess antibiotic activities. They are heterocyclic analogues of biphenyls and undergo similar thermal interconversion processes between the enantiomeric rotational isomers (axial chirality). Several studies have been done on these compounds regarding the proof of chirality,^{1–3} enantiodifferentiation in the presence of an optically active auxiliary,^{2,3} separation of enantiomers by enantioselective HPLC,^{2–4} the determination of barriers to rotation by temperature-dependent NMR³ or by thermal racemization of the resolved enantiomers^{2–4} and the determination of absolute conformations of some of the diastereomers⁵ and enantiomers^{2,3} of the series.

Although enantiodifferentiation by NMR has already been achieved,^{2,3} we were interested in applying our dirhodium method using a dirhodium tetracarboxylate complex.^{6,7} We have shown that the enantiomers of chiral soft-base ligands can easily be differentiated by adding an equimolar amount of the dirhodium complex $\text{Rh}_2^{(\text{II})}[(R)-(+)\text{-MTPA}]_4$ (**[Rh*]**, MTPA-H = methoxytrifluoromethylphenylacetic acid $\equiv$ Mosher's acid; see Scheme 1)⁸ to their CDCl₃ solution and monitoring the diastereomeric dispersion $\Delta\nu$ of their ¹H NMR

signals (or other nuclei) at room temperature (“dirhodium method” of chiral recognition).⁹ Very recently, we found that the dirhodium method may be of advantage even for hard-base ligands such as ethers¹⁰ and various carbonyl functionalities¹¹ because it shows remarkable selectivity if more than one such substituent exists in the ligand molecules.¹¹

To test this selectivity further, we studied the ligand behavior of a series of atropisomeric 3-aryl-2-oxo-4-oxazolidinones and some of their sulphur analogues (**1Z** to **3Z**; Scheme 1) which offer ligand sites of several categories defined by us recently.⁹ As mentioned before,^{10,11} the ligating atom or group can be identified by a deshielding of that atom or other nuclei close-by as compared with the respective chemical shifts of the free ligand (complexation shift $\Delta\delta > 0$), particularly for ¹³C nuclei.^{9–11}

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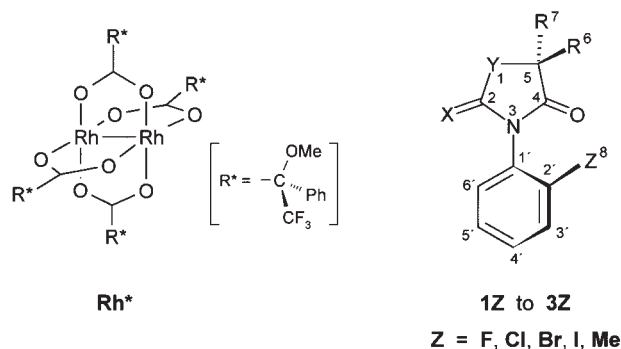
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Scheme 1. Structures of the dirhodium complex **Rh*** and the 3-aryl-2-oxo-4-oxazolidinone and their analogous thione derivatives **1Z** to **3Z** (**Me** = **CH₃**). All samples investigated were racemates or nonracemic mixtures; only the (*aR*)-enantiomer is depicted here.

EXPERIMENTAL

Syntheses

We investigated all samples in this study as racemates; some of them were prepared as pure enantiomers (see below). The 2-oxo-4-oxazolidinediones **1Z** have been synthesized by the reaction of ethyl α -hydroxyisobutyrate with *o*-arylisocyanates.³ The 2-thioxo-4-oxazolidinones **2Z** have been prepared from the reaction of ethyl α -hydroxyisobutyrate with *o*-arylisothiocyanates (Yilmaz and Doğan, Manuscript in preparation). The rhodamines **3Z** have been synthesized from either the reaction of *o*-aryldithiocarbamates with 2-bromoacetic acid or from ethyl thioglycolate with *o*-arylisothiocyanates¹ (Yilmaz and Doğan, Manuscript in preparation).

Separation of Enantiomers

Separation of the enantiomers of **2Cl**, **2Br**, **2I**, **2Me**, **3Cl**, **3I**, and **3Me** was performed by HPLC using a semipreparative Chiralpak AD-H column. Enantiomers coded by 'a' are eluted first and followed by 'b' enantiomers. The eluent was hexane:ethanol (95:5), and the column temperature was 7°C for **2Cl**, **2Br**, **2I**, **2Me**, and **3Me** and 20°C for **3Cl** and **3I**. For NMR measurements, nonracemic mixtures were prepared from enantiomerically pure samples, enantiomer **a** always being the major component (**a:b** = 2:1).

NMR Spectroscopy

¹H (400.1 MHz) and ¹³C (100.6 MHz) NMR spectra were performed on Bruker DPX-400 (9.4 Tesla). Solutions were about 0.07 molar in CDCl₃ with internal tetramethylsilane as standard (δ = 0 ppm) for both ¹H and ¹³C. Digital resolutions were 0.14 Hz/point in the ¹H and 0.24 Hz/point in the ¹³C. Bruker standard software has been used for recording DEPT, HMQC, HMBC, and ROESY spectra.

In the standard dirhodium experiment, **Rh*** and an equimolar amount of the ligands **1Z** to **2Z**, respectively, were dissolved in 0.7 ml CDCl₃. Quantities of 10–25 mg of **Rh*** (about 0.01–0.025 mmolar concentration) were employed. The dissolution process was accelerated by exposing the NMR sample tubes to an ultrasonic bath for a couple of minutes.

Note that $\Delta\nu$ -values are B_0 -dependent; in this work all dispersion values are given in Hz, as determined at B_0 = 9.4 Tesla corresponding to 400 MHz ¹H and 100.6 MHz ¹³C.

RESULTS AND DISCUSSION

NMR Signal Assignments

To obtain a reliable determination of complexation shifts ($\Delta\delta$) and diamagnetic dispersions ($\Delta\nu$), it is mandatory to have a safe NMR signal assignment of the free ligands themselves and in the presence of an equimolar amount of **Rh*** (standard dirhodium conditions). This was accomplished on the basis of one- and two-dimensional NMR experiments, such as DEPT, COSY, HMQC, HMBC, and ROESY. In the case of the fluorine-containing derivatives **1F**, **2F**, and **3F**, ¹⁹F-¹H- and ¹⁹F-¹³C-coupling constants were helpful as well. For the stereochemical assignments of the geminal methyl groups in compounds **2Z** see below. All pertinent data for the free ligands are collected in Table 1.

Complexation Shifts and Chiral Discrimination

All compounds studied here display significant complexation shifts, $\Delta\delta$, of their ¹H and ¹³C signals when the ligand molecules are mixed with an equimolar amount of **Rh***; positive values indicate deshielding. Moreover, many of those signals are split, i.e. dispersions $\Delta\nu$, indicating diastereomeric adducts. Note that all adducts have a short life-time relative to the NMR time-scale so that NMR signals are always averaged over those in the free ligand and the adduct(s).⁹ Furthermore, it can be taken for granted⁹ that none of the **Z** substituents—not even iodine¹²—is significantly involved in **Rh*** ligation in the presence of the C=O and/or C=S groups discussed here.

An inspection of the data in Tables 2–4 reveals the following trends:

- The 2-oxo-4-oxazolidinones **1Z** show only very small dispersion effects because of the weak binding of the carbonyl oxygens (category IV ligand)⁹ shown by their very low $\Delta\delta$ -values. Nevertheless, in all cases splitting of at least one signal occurs, suitable for enantiodifferentiation.
- The situation is totally different for the thioxo analogues **2Z** and **3Z** where the sulfur atoms act as quite strong binding sites (category II).⁹ Dispersion effects $\Delta\nu(^1\text{H})$ and $\Delta\nu(^{13}\text{C})$ (9.4 Tesla) of up to 28 and 13 Hz, respectively, can be observed, and enantiodifferentiation can be performed easily.
- The presence or absence of the geminal methyl groups has no significant effect on $\Delta\nu$ -magnitudes when compounds from Series **2** are compared with the corresponding ones of Series **3**; the change from Y=O in **2** to Y=S in **3** seems to have no effect either. This indicates that

TABLE 1. ^1H and ^{13}C chemical shifts of compounds **1Z** to **3Z**, in ppm relative to TMS; solvent: CDCl_3

Atom		2	4	5	6/7 (<i>syn/anti</i>)	8	1'	2'	3'	4'	5'	6'
1F^a	^1H	—	—	—	1.72	—	—	—	7.26	7.47	7.28	7.35
	^{13}C	152.5	174.3	84.4	23.8	—	118.5	157.4	116.9	131.6	124.9	129.1
1Cl	^1H	—	—	—	1.74/1.72	—	—	—	7.34	7.42	7.46	7.57
	^{13}C	152.5	174.4	84.5	24.1/23.4	—	132.6	128.3	129.9	128.0	131.4	130.6
1Br	^1H	—	—	—	1.76/1.72	—	—	—	7.34	7.38	7.47	7.74
	^{13}C	152.5	174.3	84.6	24.1/23.5	—	129.9	122.6	129.9	128.7	133.8	131.6
1I	^1H	—	—	—	1.79/1.72	—	—	—	7.97	7.21	7.50	7.30
	^{13}C	152.3	174.1	84.6	24.0/23.6	—	133.4	97.8	140.1	131.6	129.5	129.7
1Me	^1H	—	—	—	1.72	2.22	—	—	7.17	7.32	7.37	7.39
	^{13}C	153.3	175.1	84.0	24.1/23.5	17.47	135.8	129.4	131.4	130.1	127.2	127.9
2F^b	^1H	—	—	—	1.75/1.74	—	—	—	—	—	—	—
	^{13}C	187.7	174.8	87.1	23.8/23.3	—	120.3	157.3	116.3	132.0	124.9	130.0
2Cl	^1H	—	—	—	1.78/1.76	—	—	—	7.34	7.45	7.49	7.59
	^{13}C	187.6	174.7	87.2	23.9/23.2	—	130.1	132.6	130.4	128.1	131.6	130.6
2Br	^1H	—	—	—	1.76/1.79	—	—	—	7.75	7.41	7.50	7.33
	^{13}C	187.4	174.6	87.3	23.9/23.3	—	131.7	122.6	133.8	131.7	128.8	130.4
2I	^1H	—	—	—	1.83/1.75	—	—	—	7.98	7.23	7.52	7.29
	^{13}C	187.2	174.4	87.3	23.9/23.5	—	135.2	97.9	140.2	131.7	129.7	129.8
2Me	^1H	—	—	—	1.75/1.75	2.19	—	—	7.37	7.43	7.36	7.16
	^{13}C	188.3	175.4	86.7	23.9/23.4	17.3	131.3	135.9	127.3	130.4	131.4	128.3
3F^c	^1H	—	—	—	4.26/4.20 ^d	—	—	—	7.26	7.51	7.31	7.24
	^{13}C	199.9	172.5	36.4	—	—	122.4	157.6	117.0	131.9	124.9	130.4
3Cl	^1H	—	—	—	4.27/4.21 ^d	—	—	—	7.25	7.44	7.48	7.58
	^{13}C	199.6	172.5	36.4	—	—	132.7	132.8	130.6	128.1	131.4	130.6
3Br	^1H	—	—	—	4.27/4.21 ^d	—	—	—	7.75	7.39	7.49	7.24
	^{13}C	199.5	172.4	36.5	—	—	134.4	122.8	133.8	131.5	128.8	130.7
3I	^1H	—	—	—	4.27/4.20 ^d	—	—	—	7.97	7.22	7.52	7.22
	^{13}C	199.3	172.3	36.7	—	—	138.0	98.2	140.1	131.1	129.7	130.1
3Me	^1H	—	—	—	4.23/4.22 ^d	2.13	—	—	7.37	7.42	7.35	7.07
	^{13}C	200.3	173.1	36.4	—	17.4	134.0	136.2	131.4	130.2	127.4	128.5

^a ^{19}F , ^{13}C -values of **1F** in Hz; C-1': 13.0 (J_{FC}), C-2': 253.5 (J), C-3': 19.3 (J), C-4': 7.9 (J), C-5': 4.0 (J).

^b ^{19}F , ^{13}C -values of **2F** in Hz; C-1': 13.2 (J_{FC}), C-2': 253.6 (J), C-3': 19.2 (J), C-4': 8.0 (J), C-5': 4.0 (J).

^c ^{19}F , ^{13}C -values of **3F** in Hz; C-1': 13.0 (J_{FC}), C-2': 253.2 (J), C-3': 19.3 (J), C-4': 8.1 (J), C-5': 3.8 (J), C-6': 0.6 (J).

^dStereochemical differentiation of the two geminal protons at C-5 was not possible; ^1H signal assignments are arbitrary.

TABLE 2. ^1H and ^{13}C complexation shifts ($\Delta\delta$, in ppm; positive values indicate deshielding) and diastereomeric dispersions ($\Delta\nu$, in Hz) of compounds **1Z** in the presence of an equimolar amount of Rh^* ; in ppm relative to TMS; solvent: CDCl_3

Atom		2	4	5	6/7	8	1'	2'	3'	4'	5'	6'
1F	$\Delta\delta(^1\text{H})$	—	—	—	0	—	—	—	−0.01	−0.01	−0.02	0.01
	$\Delta\delta(^{13}\text{C})$	0.19	0.15	0.13	−0.03	—	−0.02	−0.01	−0.01	0	−0.01	−0.02
	$\Delta\nu(^1\text{H})$	—	—	—	1	—	—	—	1	0	0	0
	$\Delta\nu(^{13}\text{C})$	0	0	0	0	—	0	0	0	0	0	0
1Cl	$\Delta\delta(^1\text{H})$	—	—	—	0	—	—	—	0.01	−0.01	−0.01	−0.01
	$\Delta\delta(^{13}\text{C})$	0.19	0.16	0.13	−0.03/−0.04	—	−0.01	−0.02	−0.01	−0.01	0	−0.01
	$\Delta\nu(^1\text{H})$	—	—	—	0/1	—	—	—	0	2	1	1
	$\Delta\nu(^{13}\text{C})$	0	0	0	0	—	0	0	0	0	0	0
1Br	$\Delta\delta(^1\text{H})$	—	—	—	0	—	—	—	0.01	−0.02	−0.03	−0.02
	$\Delta\delta(^{13}\text{C})$	0.50	0.37	0.30	−0.70/0.52	—	−0.04	−0.05	0.12	−0.03	−0.03	−0.30
	$\Delta\nu(^1\text{H})$	—	—	—	1/2.5	—	—	—	n.d.	1	2	1.5
	$\Delta\nu(^{13}\text{C})$	0	0	0.4	0	—	0	0	0	0	0	0
1I	$\Delta\delta(^1\text{H})$	—	—	—	0.01/0.01	—	—	—	0.02	0	0.01	0.05
	$\Delta\delta(^{13}\text{C})$	0.54	0.39	0.34	−0.07/−0.10	—	−0.07	−0.10	−0.02	0.01	−0.01	−0.01
	$\Delta\nu(^1\text{H})$	—	—	—	1/3	—	—	—	2	2	3	2
	$\Delta\nu(^{13}\text{C})$	0	0	0	0	—	—	0	0	1	0	1
1Me	$\Delta\delta(^1\text{H})$	—	—	—	0.01/0.01	0	—	—	0.02	−0.01	−0.01	−0.01
	$\Delta\delta(^{13}\text{C})$	0.23	0.20	0.16	−0.04/−0.04	0	0.02	−0.02	−0.01	0	−0.02	−0.01
	$\Delta\nu(^1\text{H})$	—	—	—	0/3	—	—	—	n.d. ^a	2	2	2
	$\Delta\nu(^{13}\text{C})$	0	2	0	0	0	0	0	0	0	0	0

The $\Delta\nu$ -values correspond to 9.4 Tesla, i.e. 400 MHz ^1H and 100.6 MHz ^{13}C .

“n.d.” stands for “not detectable.”

TABLE 3. Complexation shifts ($\Delta\delta$, in ppm; positive values indicate deshielding) and diastereomeric dispersions ($\Delta\nu$, in Hz) of compounds **2Z** in the presence of an equimolar amount of Rh^* ; in ppm relative to TMS; solvent: CDCl_3

Atom		2	4	5	6 (<i>syn</i>)	7 (<i>anti</i>)	8	1'	2'	3'	4'	5'	6'
2F	$\Delta\delta(^1\text{H})$				−0.19	−0.18				+0.10	+0.10	+0.10	+0.24
	$\Delta\delta(^{13}\text{C})$	+5.75	−0.78	+2.27	−0.62	−0.66		−0.83	+0.03	+0.35	+0.70	+0.41	0
	$\Delta\nu(^1\text{H})$				28	6				n.d. ^a	2	4	8
	$\Delta\nu(^{13}\text{C})$	0	6	5	11	13		0	0	3	0	2	1
2Cl	$\Delta\delta(^1\text{H})$				−0.17	−0.18				+0.21	+0.09	+0.09	+0.09
	$\Delta\delta(^{13}\text{C})$	+5.53	−0.71	+2.18	−0.64	−0.58		−0.86	+0.07	+0.04	+0.35	+0.61	+0.37
	$\Delta\nu(^1\text{H})$				−18	+10				5 ^b	5 ^b	−2	2
	$\Delta\nu(^{13}\text{C})$	0	−5	−3	−9	+7		0	+1	−1	−2	0	+2
2Br	$\Delta\delta(^1\text{H})$				−0.15	−0.20				+0.09	+0.14	+0.10	+0.17
	$\Delta\delta(^{13}\text{C})$	+5.60	−0.70	+2.23	−0.65	−0.56		−0.89	−0.03	+0.39	−1.25	+0.34	+1.90
	$\Delta\nu(^1\text{H})$				−11	+12				n.d. ^a	+8	6	−5
	$\Delta\nu(^{13}\text{C})$	0	+4	0	+7	−7		0	+2	+2	0	−1	0
2I	$\Delta\delta(^1\text{H})$				−0.18	−0.15				+0.09	+0.09	+0.10	+0.25
	$\Delta\delta(^{13}\text{C})$	+5.49	−1.0	+2.0	−0.99	−0.88		−1.30	−0.56	+0.13	+0.29	+0.02	−0.21
	$\Delta\nu(^1\text{H})$				+1	+14				+1	+4	+8	+13
	$\Delta\nu(^{13}\text{C})$	4	−2	+3	+7	−4		+3	+4	+1	0	0	0
2Me	$\Delta\delta(^1\text{H})$				−0.20	−0.14	+0.11			+0.09	+0.09	+0.10	+0.23
	$\Delta\delta(^{13}\text{C})$	+5.54	−0.68	+2.42	−0.70	−0.56	−0.09	−0.88	+0.13	+0.36	+1.43	−0.40	+0.05
	$\Delta\nu(^1\text{H})$				−6	+9	−9			n.d. ^a	n.d. ^a	n.d. ^a	8
	$\Delta\nu(^{13}\text{C})$	0	−2	−2	+7	−9	+2	0	+1	−2	+3	0	−3

The $\Delta\nu$ -values correspond to 9.4 Tesla, i.e. 400 MHz ^1H and 100.6 MHz ^{13}C . Dispersion values ($\Delta\nu$) with signs (+ or −) refer to nonracemic mixtures as defined in the Experimental Part (**a:b** = 2:1).

^a“n.d.” stands for not detectable.

^bSign could not be determined unambiguously due to signal overlap.

the conformational equilibria within the adducts are similar for each substituent **Z** in both series.

- d. Assigning the stereochemical position of the geminal methyl groups in the **2Z** compounds was an intriguing problem which could be solved unambiguously only for the methyl derivative **2Me** in the presence of an equimolar amount of Rh^* . The existence of ROESY peaks (Fig. 1) proved that the low-frequency methyl group

signals correspond to the *syn*-position (vertical) with respect to the tolyl-methyl group (horizontal); these are the (pro-*S*)-methyl groups in the (*aR*)-enantiomer (*M*-enantiomer) as shown in Scheme 1 and Figure 1. HMQC and HMBC spectroscopy (Fig. 2) proved which of the *syn*- (s) and *anti*-methyl (a) belongs to the each of the two enantiomers, arbitrarily designated **A** and **B**. First, the ^{13}C nuclei directly attached to the ^1H nuclei

TABLE 4. Complexation shifts ($\Delta\delta$, in ppm; positive values indicate deshielding) and diastereomeric dispersions ($\Delta\nu$, in Hz) of **3Z** in the presence of an equimolar amount of Rh^* ; in ppm relative to TMS; solvent: CDCl_3

Atom		2	4	5	6 ^a (<i>syn</i>)	7 ^a (<i>anti</i>)	8	1'	2'	3'	4'	5'	6'
3F	$\Delta\delta(^1\text{H})$				−0.18	−0.16				+0.01	+0.07	+0.10	+0.09
	$\Delta\nu(^1\text{H})$				20	15				n.d. ^b	1	n.d. ^b	n.d. ^b
3Cl	$\Delta\delta(^1\text{H})$				−0.15	−0.14				+0.17	+0.05	+0.06	+0.05
	$\Delta\delta(^{13}\text{C})$	+7.59	−0.01	−0.33				−0.06	−0.61	−0.10	+0.39	+0.69	+0.31
	$\Delta\nu(^1\text{H})$				−13	−15				−10	−4	−2	0
	$\Delta\nu(^{13}\text{C})$	+2	+5	−4				−5	−4	+3	0	+3	+2
3Br	$\Delta\delta(^1\text{H})$				−0.16	−0.14				+0.06	+0.08	+0.07	+0.20
	$\Delta\nu(^1\text{H})$				9	18				0	3	4	12
3I	$\Delta\delta(^1\text{H})$				−0.02	+0.05				+0.01	+0.01	+0.01	+0.03
	$\Delta\delta(^{13}\text{C})$	+7.15	−0.25	−0.29				−0.46	−0.48	+0.33	+0.56	+0.42	−0.04
	$\Delta\nu(^1\text{H})$				−6	+18				+2	+3	+5	+13
	$\Delta\nu(^{13}\text{C})$	+4	−6	+7				+6	+11	−3	−1	n.d. ^b	n.d. ^b
3Me	$\Delta\delta(^1\text{H})$				−0.13	−0.14	+0.06			+0.04	+0.07	+0.06	+0.20
	$\Delta\delta(^{13}\text{C})$	+7.23	+0.11	−0.14			−0.28	−0.46	−0.01	+0.24	+0.59	+0.31	−0.07
	$\Delta\nu(^1\text{H})$				−7	−5	+12			n.d. ^b	0	n.d. ^b	n.d. ^b
	$\Delta\nu(^{13}\text{C})$	−3	+7	−7			−7	−3	−3	0	+2	+4	+5

The $\Delta\nu$ -values correspond to 9.4 Tesla, i.e. 400 MHz ^1H . Dispersion values ($\Delta\nu$) with signs (+ or −) refer to nonracemic mixtures as defined in the Experimental Part (**a:b** = 2:1).

^aStereochemical differentiation of the two geminal protons at C-5 was not possible; ^1H signal assignments are arbitrary.

^b“n.d.” stands for “not detectable.”

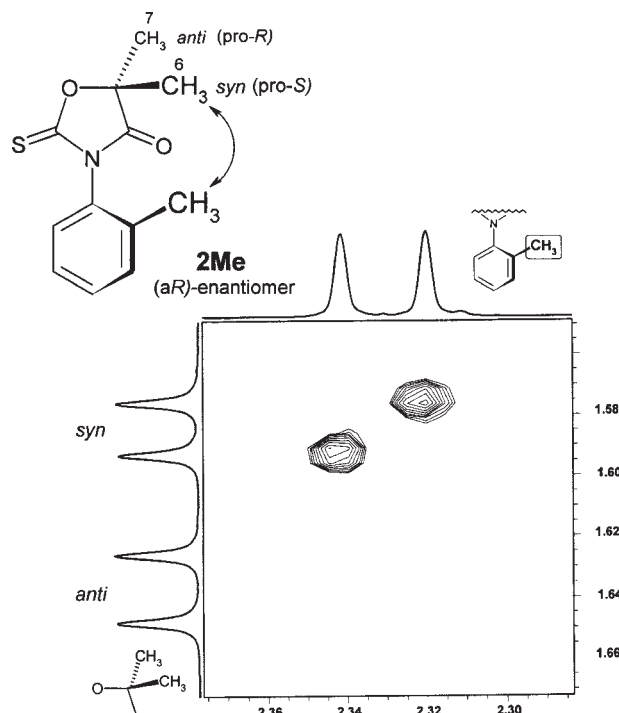


Fig. 1. Section of the ROESY spectrum of racemic **2Me** in the presence of an equimolar amount of **Rh***, indicating the stereochemical assignment of the geminal methyl groups by spatial contact of the *syn*-methyl of the *ortho*-tolyl group (double arrow) in both diastereomeric **Rh***–**2Me** adducts; the structure formula depicts only the (*aR*)-enantiomer (see text).

were identified by HMQC (Fig. 2, left). Second, the HMBC cross-peaks (Fig. 2, right) prove that the two outer methyl ^1H signals belong to one enantiomer (**A**) and, analogously the inner ones to the other (**B**).

Analogous experiments were conducted with the other compounds of the Series **2**. Here, NOE contacts are not available so that the stereodifferentiation of the diastereotopic methyl groups was tentatively performed on the basis of the observation that in all cases of **2Z**, the sequence of their carbon atoms and their ^{13}C chemical shifts is remarkably uniform (Table 3 and Fig. 3, vertical dimensions) so that we assigned all *anti* and *syn* positions in analogy to the methyl derivative **2Me**. The same argument allowed us to state tentatively that in this series of compounds all enantiomers **A** should belong to the same absolute configuration and *vice versa* for **B**.

Once this assignment of the geminal methyl ^{13}C signals and the relative configurations of the compounds in the series **2Z** is accepted, we are able to assign all signals of the attached ^1H atoms as well. Figure 3 shows this for the halogenated derivatives in this series **2**; note that here the original spectra have been replaced by schematic representations for sake of clarity. It is immediately evident that a similar signal sequence, as found for the ^{13}C nuclei, does not exist for the protons. In contrast, it can be seen that for the *syn*- CH_3 groups—which are close to the substituent **Z**—the absolute values of the dispersion effects $\Delta\nu$ are strongly dependent on the nature of the halogen atoms; **2F**: $\Delta\nu = 28$ Hz, **2Cl**: $\Delta\nu = 18$ Hz, **2Br**: $\Delta\nu = 11$ Hz, **2I**: $\Delta\nu = 1$ Hz, and **2Me**: $\Delta\nu = 6$ Hz (*syn*-effect), whereas

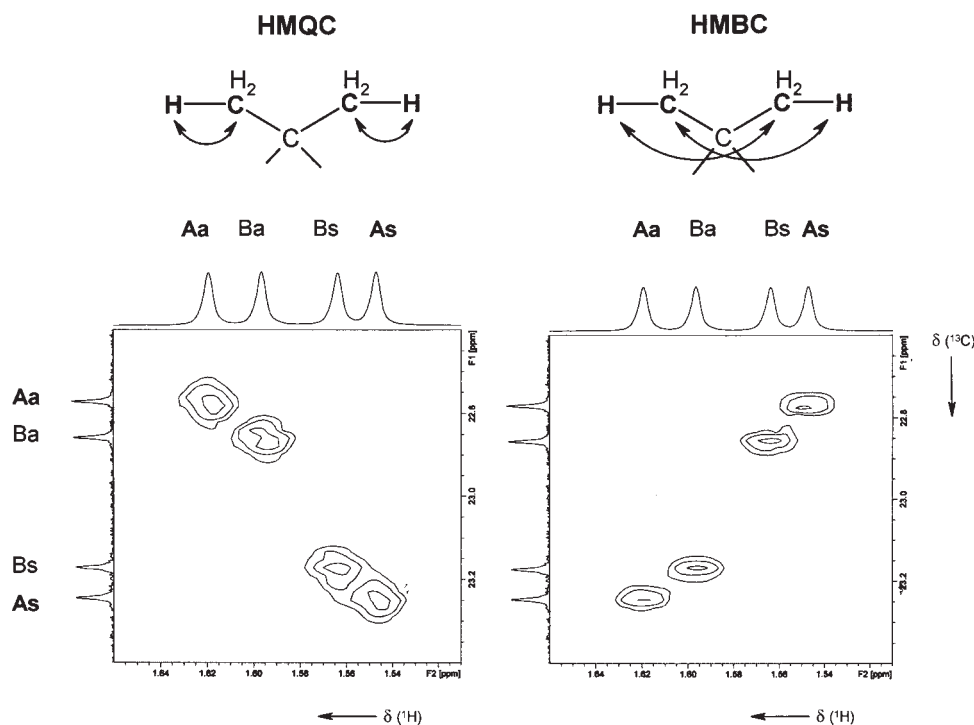


Fig. 2. Mutual assignments of the hydrogen and carbon atoms within the *syn*- and *anti*-methyl groups in the adducts of **Rh*** and the enantiomers **A** and **B** of **2Me** by a combination of HMQC (one-bond ^1H , ^{13}C correlations; left) and HMBC (three-bond ^1H , ^{13}C correlations; right) experiments. For further explanation see text.

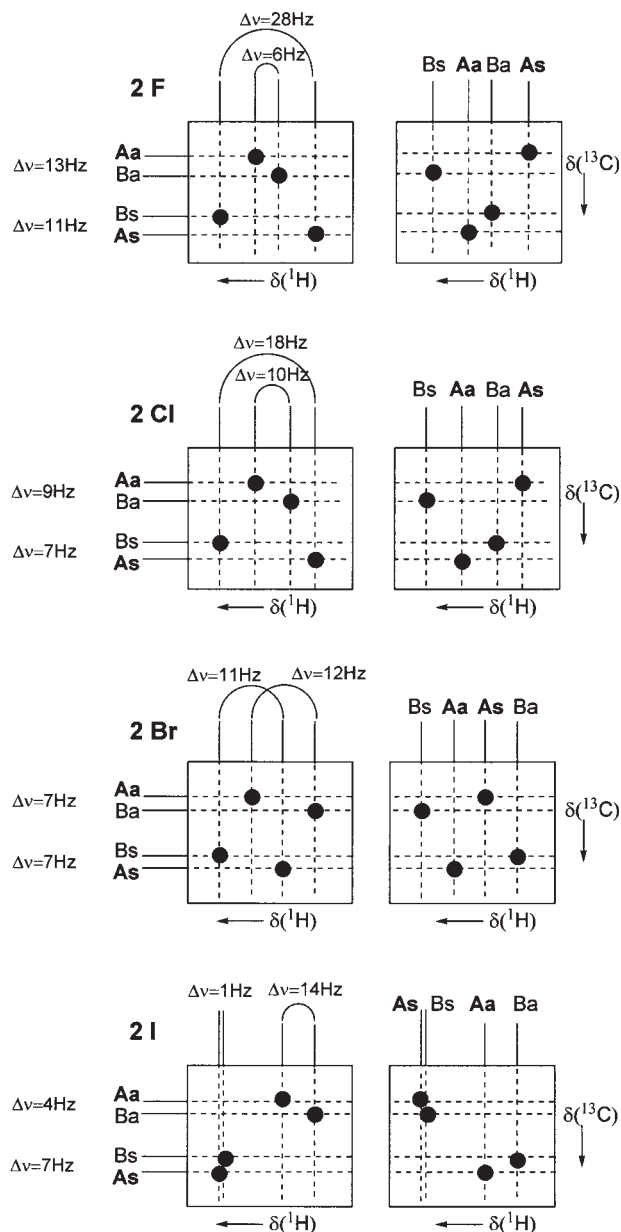


Fig. 3. Schematic representations of the HMQC and HMBC experiments (in analogy to Fig. 2) of the halogenated derivatives of compounds series **2**, including signal assignments.

such sequence does not exist for the *anti*-CH₃ groups situated on the back-side relative to Z; **2F**: $\Delta v = 6$ Hz, **2Cl**: $\Delta v = 10$ Hz, **2Br**: $\Delta v = 12$ Hz, **2I**: $\Delta v = 14$ Hz, and **2Me**: $\Delta v = 9$ Hz. We present the following rationalization for this surprising observation: the substituent Z influences the conformational equilibria inside the **Rh*** $\cdots$ **2Z** adducts so that the nearby *syn*-methyl protons, which are on the same side of the molecule as substituent Z, experience more efficient chiral discrimination in the diastereomeric complexes exerted by the Mosher phenyl groups than the *anti*-methyl protons which are further away. Steric effects alone, however, do not explain the Δv -value variation. It is well-known for several decades that Vögtle's *n*-values rep-

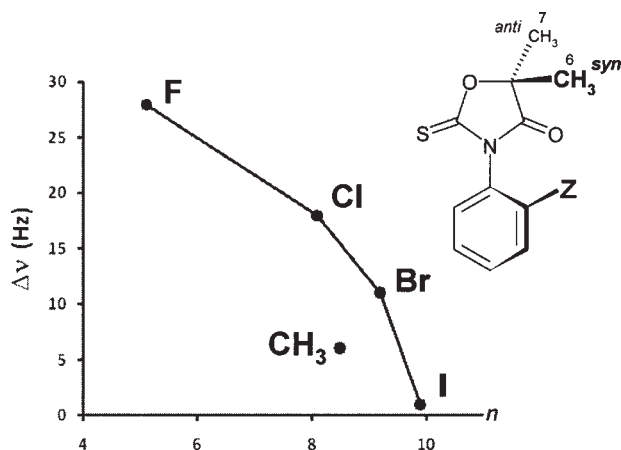


Fig. 4. Comparison of the *syn*-methyl effects in the dispersions Δv (H-6) of **2Z** with Vögtle's steric *n* parameters of the substituents Z.

resent excellently the steric demand and the relative volumes of a great variety of spherical substituents.¹³ Plotting our Δv (*syn*-methyl)-values of the **2Z** series against those *n*-values, however, shows that the value for Z = methyl should be two to three times larger if steric interaction alone controlled the *syn*-methyl effects (Fig. 4). Therefore, we expect that dipole interaction produced by the halogens contributes as well.

The same procedure for assigning the geminal dimethyl signals failed with the compounds of series **1Z**, presumably because the binding is much weaker than in **2Z** and all $\Delta\delta$ and Δv effects are much less pronounced. Finally, we refrained from detailed stereochemical assignments of the ¹H signals of the geminal protons at C-5 in series **3** compounds; they are often very close (AB-quartets) so that no significant information would emerge from such assignments anyway. Moreover, ROESY experiments to identify H-5 $\cdots$ CH₃ contacts failed. Figure 5 displays an example.

Ligand Competition

All data extracted from the ¹H and ¹³C NMR spectra of **1Z** to **3Z** in the presence of an equimolar amount of **Rh*** are collected in Tables 2, 3, and 4, respectively.

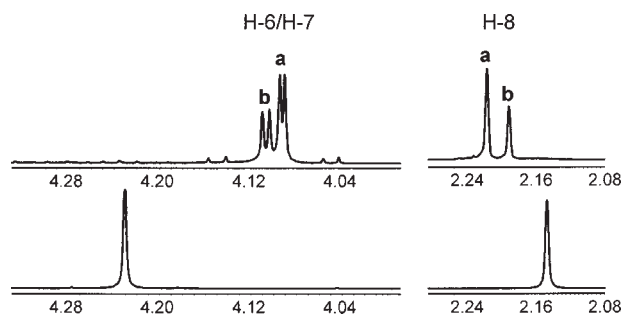


Fig. 5. Signals of H-6/H-7 and H-8 of nonracemic **3Me**; normal ¹H NMR spectrum of the free ligand (bottom) and in the presence of an equimolar amount of **Rh*** (top). The letters "a" and "b" refer to the first (a) and second (b) enantiomer eluted (see Experimental Part). For the dispersion effects Δv (in Hz) see Table 4.

An inspection of the complexation shifts $\Delta\delta(^{13}\text{C})$ of the **1Z** derivatives show that significant values exist only for the carbons in the 2-oxo-4-oxazolidinone ring so that there is no indication that any halogen atom contributes to adduct formation, even not iodine.¹² From our previous experience with amides and lactones,¹¹ we expect that both carbonyl oxygens can be binding sites. However, $\Delta\delta$ -values for C-2 as well C-4 are very low indicating weak complexation, i.e. the equilibrium of the two adduct components **Rh*** and **1Z** together with the adduct **Rh*...1Z** is apparently strongly directed to the component side (category IV). Moreover, slight differences in the $\Delta\delta$ -values seem to indicate that any binding—although weak—should favor the C-2 carbonyl oxygen atom because access to the C-4 carbonyl is sterically hindered by the nearby two methyl groups.

This is completely different for the 2-thioxo derivative **2Z** which show significant deshielding of thiocarbonyl C-2 ($\Delta\delta = 5.5$ – 5.8 ppm) by adduct formation whereas the carbonyl C-4 ($\Delta\delta = -1.0$ to -0.8 ppm) is practically unaffected. This behavior is not surprising since sulfur is a binding atom belonging to category II,⁹ i.e. to the class of ligands where the adduct formation equilibrium is shifted towards the adduct side.

Finally, the **Rh* ... S=C** binding in **3I** seems to be reinforced as compared with **2I** ($\Delta\delta = 7.2$ ppm vs. 5.5 ppm, respectively). A significant competitive binding of the endocyclic S-1 can be excluded because one would expect a much stronger deshielding of C-5 if S-1 were the dominating binding site. In order to explain why the $\Delta\delta(\text{C}=\text{S})$ -value is markedly larger in **3Z** than in all Series **2** compounds, it is plausible to assume that the sterically demanding methyl groups 6 and 7 in **2Z** may produce some congestion when the ligand approaches the complex **Rh***, and this effect is missing in **3I**. So, the absence of those groups in **3I** leads to a further shift of the adduct formation equilibrium towards the adduct so that the time-averaged signal shift ($\Delta\delta$) becomes larger. Again, the carbonyl group (C-4) does not play a substantial role in the adduct formation ($\Delta\delta = -0.2$ ppm).

Determining Absolute Configurations?

In previous papers on the application of the dirhodium method we have shown that absolute configurations can be determined by correlation if all compounds in a series are structurally closely related and the absolute configuration of at least one compound is known. This is possible because of a significant uniformity in the signs of dispersion effects ($\Delta\nu$) and has been exemplified before for chalcogenuranes^{14,15} and phospholenes.¹⁶

We reexamined a selection of nonracemic mixtures (**2Cl**, **2Br**, **2I**, **2Me**, **3Cl**, **3I**, and **3Me**; the fluorine-containing derivatives are configurationally unstable). These samples were prepared by mixing the respective pure enantiomers **a** and **b** in a ratio of 2:1; the separations were performed by HPLC (see Experimental Part and Tables 3 and 4) and enantiomer **a** is the one eluted first in each case. It should be noted, however, that the absolute configurations of none of the enantiomers was known and we assumed, as a working hypothesis, that all samples **a** belong to the same absolute configuration and all **b** to the other.

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Inspecting the $\Delta\nu$ -values of the compounds (Tables 3 and 4), we found rather erratic behavior of $\Delta\nu$ -signs. There are only a few protons or carbons displaying the same sign. Even if one takes into account that our working hypothesis is wrong, there should be some regularity in changing signs but this is not the case either.

Therefore, we have to state that—on the basis of the data available—it is unlikely that correlation of $\Delta\nu$ -values for absolute configuration determination works in the case of the thio-derivatives of oxazolidinones (**2Z** and **3Z**). This failure may originate in the existence of a variety of functionalities available for adduct formation.

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The Origin of an Unusually Large ^{19}F Chemical Shift Difference Between the Diastereomeric α -Cyano- α -Fluoro-*p*-Tolylacetic Acid (CFTA) Esters of 3',4',5,7-Tetra-*O*-Methylepicatechin

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT During a study on the relationship between the ^{19}F chemical shift difference for the diastereomeric α -cyano- α -fluoro-*p*-tolylacetic acid (CFTA) esters of chiral secondary alcohols and the absolute configurations of the alcohols, an unusually large ^{19}F chemical shift difference has been observed for the CFTA esters of 3',4',5,7-tetra-*O*-methylepicatechin. This large chemical shift difference has been reproduced by ab initio calculations of molecular geometry and GIAO calculations of ^{19}F chemical shifts on the stable conformations of the diastereomeric α -cyano- α -fluorophenylacetic acid (CFPA) esters of *cis*-flavan-3-ol as model systems. The origin of the large chemical shift difference has been further investigated using NBO analysis. This revealed that the interaction between $\pi(\text{C}=\text{C})$ of the aryl group in the chiral auxiliary and $\sigma^*(\text{C}-\text{F})$ is a very important determinant of the ^{19}F chemical shifts and this interaction depends on the torsion angle between the plane of aryl group and the $\text{C}-\text{F}$ bond. *Chirality* 20:351–356, 2008. © 2007 Wiley-Liss, Inc.

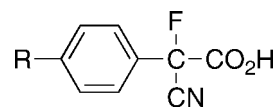
KEY WORDS: ab initio calculation; molecular geometry; GIAO calculation; α -cyano- α -fluorophenylacetic acid; *cis*-flavan-3-ol; NBO analysis; $\pi(\text{C}=\text{C})-\sigma^*(\text{C}-\text{F})$ interaction

INTRODUCTION

Diastereomer formation with chiral derivatizing agents (CDAs) followed by ^1H NMR analysis has proved to be a powerful tool to assess the relevant absolute stereochemistry of chiral molecules.^{1–3} Recently, Takeuchi et al. introduced enantiopure α -cyano- α -fluorophenylacetic acid (CFPA, **1**)^{4,5} and α -cyano- α -fluoro-*p*-tolylacetic acid (CFTA, **2**),⁶ as versatile CDAs that have shown impressive ^1H chemical shift differences ($\Delta\delta_{\text{H}}$) between their diastereomeric pairs. Thus, a very reliable determination of the stereochemistry of chiral alcohols and amines is allowed through simple ^1H NMR spectroscopic analyses.^{7–9}

In addition, a good correlation was observed between the ^{19}F NMR chemical shift differences ($\Delta\delta_{\text{F}}$) and absolute configurations of substrate moieties for a series of diastereomeric CFTA esters⁷ of chiral secondary alcohols and of CFTA amides¹⁰ of α -amino esters. Although the correlation between $\Delta\delta_{\text{F}}$ and absolute configurations of alcohol and amine moieties in diastereomeric esters and amides has been examined for some fluorine-containing CDAs,^{11,12} the relation observed between $\Delta\delta_{\text{F}}$ and absolute configurations was not simple, and a convincing explanation for the origin of $\Delta\delta_{\text{F}}$ has not been reported so far. However, in order to use the ^{19}F NMR chemical shift difference for determining absolute configuration, accumulation of data as well

as a better understanding of the mechanism that gives the algebraic sign of $\Delta\delta_{\text{F}}$ is necessary.



R = H: CFPA (**1**)
R = CH₃: CFTA (**2**)

EXPERIMENTS AND CALCULATIONS

General Procedures

Melting points were measured with a Yanaco micro melting point apparatus and are uncorrected. Spectroscopic measurements were carried out with the following instruments: optical rotations, JASCO DIP-1000 digital polarimeter; IR spectra, JASCO FT/IR-460 Plus; mass

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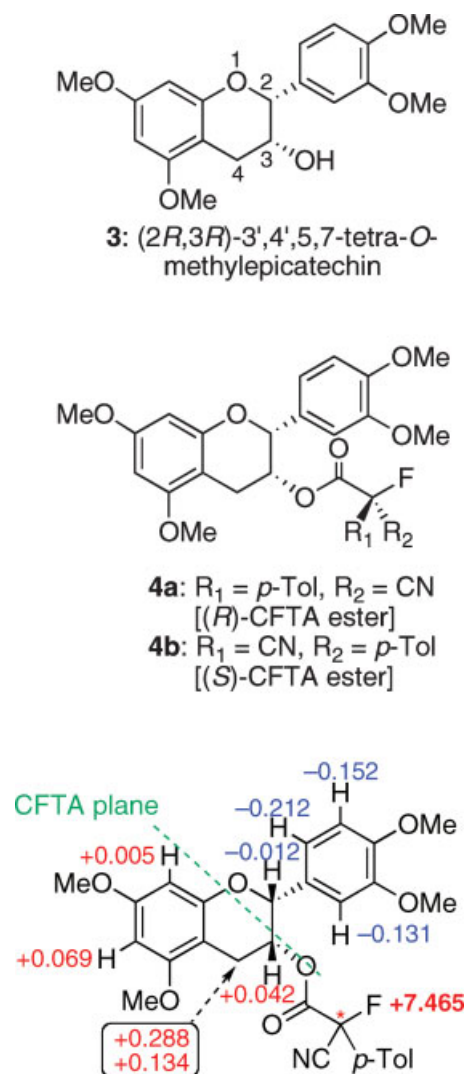


Fig. 1. $\Delta\delta_{\text{H}}$ and $\Delta\delta_{\text{F}}$ values for the diastereomeric CFTA esters, **4a,b**. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

spectra (MS), JEOL JMS-GCmate; high resolution mass spectra (HRMS), JEOL JMS-AX 505 HAD; ^1H NMR spectra, Varian Unity 500 (500 MHz) in CDCl_3 with TMS ($\delta = 0$ ppm) as an internal standard; ^{19}F NMR spectra, Varian Unity 500 (470 MHz) in CDCl_3 with CFCl_3 ($\delta = 0$ ppm) as an internal standard. Flash column chromatography and thin layer chromatography were performed on silica gel [Merck silica gel 60 (0.040–0.063 mm) and Merck 5715, respectively]. Further purification was carried out using recycling HPLC on silica gel column (Kusano Si-10). (–)-3',4',5,7-Tetra-*O*-methylepicatechin (**3**) was prepared from (–)-(2*R*,3*R*)-epicatechin (Aldrich) according to literature.¹³

Preparation of (*R*)-CFTA Ester of (–)-3',4',5,7-Tetra-*O*-Methylepicatechin (**4a**)

(*R*)-CFTA ester of (1*S*,3*R*,4*R*,6*R*)-3,7,7-trimethylbicyclo[4.1.0]heptane-3,4-diol (101 mg, 0.29 mmol) was dissolved in 1.5 ml of THF/ H_2O (1:1). To this solution was

added 1 M lithium hydroxide aqueous solution (0.3 ml, 0.3 mmol) and the mixture was stirred at room temperature for 10 min. After evaporation of THF under reduced pressure, the unreacted ester was removed by ether extraction. The aqueous layer was acidified (pH 1) with 10% aqueous HCl at 0°C. The solution was saturated with NaCl and extracted with AcOEt. The organic layer was washed with brine and dried over MgSO_4 . After removal of the solvent, the residual water was azeotropically removed with benzene to give crude (*R*)-CFTA (62 mg) as a pale yellow oil. This was subsequently dissolved in dried CH_2Cl_2 (2.5 ml) containing a catalytic amount of DMF (3.5 μl , 0.04 mmol) under a nitrogen atmosphere. To the solution was added oxalyl chloride (51 μl , 0.58 mmol) at 0°C and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated to give the crude acid chloride as a yellow oil. This was dissolved in 2.0 ml of dried CH_2Cl_2 under a nitrogen atmosphere. To this solution were added pyridine (0.12 ml, 1.46 mmol) and (–)-3',4',5,7-tetra-*O*-methylepicatechin (**3**) (91 mg, 0.26 mmol) and the mixture was stirred at room temperature for 15 h. The solvent was evaporated and the residue was dissolved in AcOEt. The solution was washed with water and brine. The organic layer was dried over MgSO_4 and concentrated. Purification by flash column chromatography (eluent: hexane/AcOEt = 3:1) and recycling HPLC (eluent: hexane/ CHCl_3 /AcOEt = 4:4:1) gave **4a** (47 mg, 31% from the starting CFTA ester) as a pale red solid (A small amount of unidentified impurity was present): mp 47–49°C; $[\alpha]_{\text{D}}^{26} -58.9$ (*c* 0.5, CHCl_3); IR (KBr) 2255 (vw), 1774, 1753 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 2.32 (s, 3H), 2.69 (dd, 1H, *J* = 18.0, 1.6 Hz), 2.84 (dd, 1H, *J* = 18.0, 4.3 Hz), 3.68 (s, 3H), 3.82 (s, 3H), 3.90 (s, 3H), 3.92 (s, 3H), 5.06 (s, 1H), 5.44 (m, 1H), 6.05 (d, 1H, *J* = 2.2 Hz), 6.20 (d, 1H, *J* = 2.2 Hz), 6.87 (d, 1H, *J* = 8.1 Hz), 7.00 (d, 2H, *J* = 8.1 Hz), 7.01 (dd, 1H, *J* = 8.1, 1.9 Hz), 7.07 (d, 1H, *J* = 1.9 Hz), 7.16 (d, 2H, *J* = 8.1 Hz); ^{19}F NMR (470 MHz, CDCl_3) δ -150.13 (s); MS *m/z* 521 (M^+), 355, 328, 179, 167, 151; HRMS calcd for $\text{C}_{29}\text{H}_{28}\text{FNO}_7$ 521.1850, found 521.1858.

Preparation of (*S*)-CFTA Ester of (–)-3',4',5,7-Tetra-*O*-Methylepicatechin (**4b**)

In a similar manner for preparation of **4a**, (*S*)-CFTA ester **4b** (64 mg, 41%) was obtained as a pale red solid from (*S*)-

TABLE 1. $\Delta\delta_{\text{F}}$ values for the diastereomeric CFTA esters of some chiral alcohols

Alcohol	$\Delta\delta_{\text{F}}/\text{ppm}^a$
2-Octanol ^b	+0.32
1-Phenylethanol ^b	+0.87
Borneol ^b	+0.74
Menthol ^b	+0.59
3	+7.45

^a $\Delta\delta_{\text{F}} = \delta_{\text{F}}^{\text{SR}} - \delta_{\text{F}}^{\text{RR}}$ or $\delta_{\text{F}}^{\text{RS}} - \delta_{\text{F}}^{\text{SS}}$. The esters of (*S*)-acids with (*R*)-alcohols are designated *SR* and those of (*R*)-acids with (*R*)-alcohols are designated *RR*.

^bRef. 7.

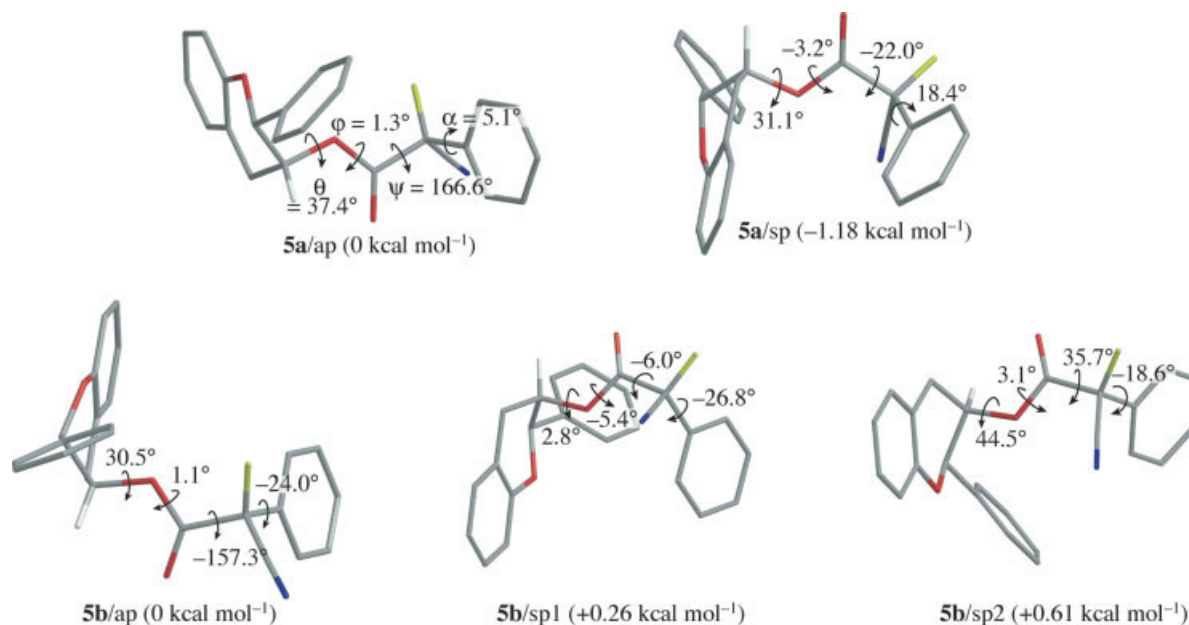


Fig. 2. Geometries and relative energies of (*R*)- and (*S*)-CFPA esters, **5a,b**. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CFTA ester of (1*S*,3*R*,4*R*,6*R*)-3,7,7-trimethylbicyclo[4.1.0]heptane-3,4-diol: mp 46–49°C; $[\alpha]_D^{28}$ –28.5 (*c* 1.0, CHCl_3); IR (KBr) 2254 (vw), 1761 cm^{-1} ; ^1H NMR (270 MHz, CDCl_3) δ 2.36 (s, 3H), 2.98 (dd, 1H, $J = 18.0, 3.0$ Hz), 2.98 (dd, 1H, $J = 18.0, 3.8$ Hz), 3.76 (s, 3H), 3.80 (s, 3H), 3.82 (s, 3H), 3.88 (s, 3H), 5.05 (s, 1H), 5.48 (ddd, 1H, $J = 3.8, 3.0, 1.1$ Hz), 6.12 (d, 1H, $J = 2.2$ Hz), 6.21 (d, 1H, $J = 2.2$ Hz), 6.72 (d, 1H, $J = 8.4$ Hz), 6.80 (dd, 1H, $J = 8.4, 1.9$ Hz), 6.93 (d, 1H, $J = 1.9$ Hz), 7.06 (d, 2H, $J = 8.2$ Hz), 7.17 (d, 2H, $J = 8.2$ Hz); ^{19}F NMR (470 MHz, CDCl_3) δ –142.67 (s); MS m/z 521 (M^+), 355, 328, 179, 167, 151; HRMS calcd for $\text{C}_{29}\text{H}_{28}\text{FNO}_7$ 521.1850, found 521.1850.

Calculations

A conformer search for the structures of (2*R*,3*R*)-flavan-3-ol (*R*)- and (*S*)-CFTA esters, **5a** and **5b**, were conducted systematically using the AM1 level of theory. The structures were further refined at HF/STO-3G level. The full geometry optimizations were performed at the B3LYP/6-31G** level. Nuclear magnetic shielding constants of ^{19}F nuclei in **5a**, **5b**, and CFCl_3 were calculated using the GIAO method at the HF/6-311+G(2d,p) level. Natural bond orbital (NBO) analysis for the structures **5a** and **5b** was conducted at the same level. All calculations except for the initial conformer search were carried out by using the Gaussian 03 suite of the program.¹⁴

RESULTS AND DISCUSSION

^1H and ^{19}F NMR Chemical Shifts of Diastereomeric CFTA Esters of (2*R*,3*R*)-3',4',5,7-Tetra-*O*-Methylepicatechin

We examined the relation between $\Delta\delta_{\text{F}}$ and the absolute configuration of a typical flavonoid alcohol, (2*R*,3*R*)-

3',4',5,7-tetra-*O*-methylepicatechin (**3**). The $\Delta\delta_{\text{F}}$ values, i.e., ^{19}F chemical shift differences ($\Delta\delta_{\text{F}} = \delta_{\text{F}}^{\text{SR}} - \delta_{\text{F}}^{\text{RR}}$, the ester of (*S*)-acid with (*R*)-alcohol is designated *SR* and that of (*R*)-acid with (*R*)-alcohol is designated *RR*)⁷ between the diastereomeric (*R*)- and (*S*)-CFTA esters of epicatechin, **4a** and **4b**, is shown in Figure 1 together with the $\Delta\delta_{\text{H}}$ values ($\Delta\delta_{\text{H}} = \delta_{\text{H}}^{\text{S}} - \delta_{\text{H}}^{\text{R}}$). The $\Delta\delta_{\text{H}}$ values for the protons on the left side of the CFTA plane^{7,8} are positive and those on the right side of the plane are negative. This result correctly indicates that the absolute configuration at C_3 is *R* and strongly suggests that the conformation with an all synperiplanar (sp) $\text{F}-\text{C}\alpha-\text{C}(=\text{O})-\text{O}-\text{C}-\text{H}$ arrangement is preferred for both **4a** and **4b**.

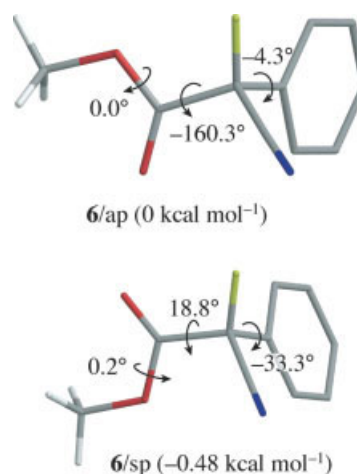


Fig. 3. Geometries and relative energies of (*S*)-CFPA methyl ester **6**. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 2. Calculated relative energies and ^{19}F chemical shifts^a for **5a**, **b** and **6**

	5a		5b			6	
	ap	sp	ap	sp1	sp2	ap	sp
$\Delta E/\text{kcal mol}^{-1}$	0	-1.17	0	+0.27	+0.62	0	-0.48
$\delta_{\text{F}}^{\text{calcd}}/\text{ppm}$	-153.92	-151.41	-150.82	-145.67	-148.70	-155.62	-143.38
$\delta_{\text{F}}^{\text{obs}}/\text{ppm}^{\text{b}}$		-150.12 ^c		-142.67 ^d			-144.43 ^e

^aRelative shifts to CFCl_3 .^bCFTA esters.^c**4a**.^d**4b**.^eCFTA methyl ester (**7**).

In ^{19}F NMR, we found that the signs of $\Delta\delta_{\text{F}}$ values obtained from **4a** and **4b** are positive, as anticipated.⁷ However, the magnitude of $\Delta\delta_{\text{F}}$ (+7.45 ppm) was surprisingly greater than the usual values previously obtained (< 0.9 ppm), as shown in Table 1.

Molecular Geometry Calculations, GIAO Calculations of ^{19}F Chemical Shifts, and NBO Analyses on Model Systems

To explain this unusual phenomenon, we conducted ab initio molecular geometry calculations, GIAO calculations of ^{19}F chemical shifts and NBO analyses on the diastereomeric CFPA esters of (2*R*,3*R*)-*cis*-flavan-3-ol, **5a** and **5b**, as the simplified model structures for **4a** and **4b**, respectively.

Regarding the conformation with respect to the rotation axis of $\text{C}(\text{F})\text{—C}(\text{=O})$ bond, there were two predominant conformers, i.e. the sp and ap (antiperiplanar) conformers, which have been found for the α -fluoro carbonyl structure.^{15–17} As for the conformation of the 2,3-*cis*-disubstituted 3,4-dihydropyran ring, the half-chair conformation with 2-equatorial/3-axial substitution was found to be more stable than that with 2-axial/3-equatorial substitution, for both ap and sp conformers. This result is consistent with the experimental data, with no large *J*'s were observed between H_3 's and H_4 's in **4a** and **4b**. The stable conformations having 3-axial substitution and the relative energies between the ap and sp conformers, for each diastereomer, **5a** and **5b**, are shown in Figure 2.

Geometry and energies of the conformers of the CFPA methyl ester (**6**) were also calculated for comparison (Fig. 3). As is seen in Table 2, the calculated δ_{F} value of the sp conformer of **6** is lower than that of the ap conformer by 12.2 ppm. Similar differences in ^{19}F chemical shifts were obtained for (*S*)- and (*R*)-CFPA menthyl esters⁷ (16.7 and 13.4 ppm for (*S,R*) and (*R,R*) esters, respectively) in our previous calculation. On the other hand, the δ_{F} value of the conformer of **5a**/sp (-151.41 ppm) is close to that of the corresponding **5a**/ap (-153.92 ppm). The δ_{F} value of **5a**/sp is considerably higher than that of **6**/sp (-143.38 ppm), whereas the δ_{F} of **5a**/ap is similar to that of **6**/ap. The δ_{F} 's of all conformers of **5b** are relatively close and lower than those of the conformers in **5a**. Since the δ_{F} 's of the conformers of **5a** as well as those of **5b** are similar, the δ_{F} of each diastereomer in the fast conformational equilibrium should not be greatly dependent on the

population of the conformers. Since the preferred conformation of **4a** and **4b** has been experimentally known to be synperiplanar from the signs of $\Delta\delta_{\text{H}}$, the δ_{F} values are roughly estimated to be $\sim$ -151 ppm for **5a** and $\sim$ -146 ppm for **5b**. Thus the positive sign and the large magnitude of the $\Delta\delta_{\text{F}}$ (+ 7.45 ppm) observed for CFTA esters **4a** and **4b** can be reproduced from the theoretical calculations on the model system of **5**.

To reveal the origin of the unusually high and low δ_{F} values calculated for the **5a**/sp and **5b**/ap, respectively, we employed the NBO analysis¹⁸ on the electronic interactions in **5a,b**, and those in **6** for comparison. It is now known that electron donation to the local environment of a fluorine nucleus in many alkyl fluoride systems decreases shielding and causes a downfield shift instead of the upfield shift that is expected from simple shift theory.^{19–22} Adcock conducted an NBO analysis of the electronic interactions in a series of structurally similar 7-fluoronorbornyl systems and demonstrated that the delocalization of elec-

TABLE 3. NBO $E^{(2)}$ energies (kcal mol^{-1}) of interactions of $\sigma^*(\text{C—F})$ as the acceptor in **5a,b** and **6**^a

Donor	5a		5b			6	
	ap	sp	ap	sp1	sp2	ap	sp
$\sigma(\text{C}^{\text{a}}\text{—C}^{\text{b}})$	–	0.69	–	0.80	0.69	–	0.76
$\sigma(\text{C}^{\text{b}}\text{—O}^{\text{h}})$	–	0.86	–	0.90	0.63	–	0.86
$\pi(\text{C}^{\text{b}}=\text{O}^{\text{i}})$	–	–	–	–	–	0.66	–
$\pi(\text{C}^{\text{b}}=\text{O}^{\text{j}})$	–	–	0.74	–	0.77	–	–
$\pi(\text{C}^{\text{c}}\equiv\text{N})$	6.94	6.80	7.02	6.83	7.01	7.02	6.97
$\sigma(\text{C}^{\text{d}}=\text{C}^{\text{e}})$	0.67 ^b	0.62 ^b	0.64 ^c	0.55 ^b	0.65 ^b	0.66 ^b	1.17 ^c
$\pi(\text{C}^{\text{d}}=\text{C}^{\text{e}})$	– ^b	0.84 ^b	– ^c	1.82 ^b	0.88 ^b	– ^b	3.17 ^c
$\sigma(\text{C}^{\text{d}}\text{—C}^{\text{f}})$	1.64	1.51	1.63	1.31	1.50	1.65	–
$n(\text{C}^{\text{a}})$	2.54	2.39	2.59	2.37	2.42	2.55	2.40
$n(\text{O}^{\text{h}})$	–	0.64	–	0.78	–	–	0.63
$n(\text{O}^{\text{i}})$	1.13	–	1.03	–	–	1.06	–
$\pi^*(\text{C}^{\text{b}}=\text{O}^{\text{j}})$	–	–	0.88	–	–	0.69	–
$\pi^*(\text{C}^{\text{d}}=\text{C}^{\text{e}})$	–	0.54	–	1.09	1.04	–	–
$\pi^*(\text{C}^{\text{d}}=\text{C}^{\text{f}})$	–	–	–	–	0.57	–	1.38

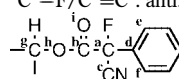
^a $E^{(2)}$ energies larger than 0.5 kcal mol^{-1} are shown.^b $\text{C}^{\text{a}}\text{—F}/\text{C}^{\text{d}}=\text{C}^{\text{e}}$: syn.^c $\text{C}^{\text{a}}\text{—F}/\text{C}^{\text{d}}=\text{C}^{\text{e}}$: anti.

TABLE 4. Calculated ^{19}F chemical shifts, $E^{(2)}$ energies^a and dihedral angles α for **5a**, **b** and **6**

	6 /sp ^b	5b /sp1 ^b	5b /sp2 ^b	5b /ap ^c	5a /sp ^b	5a /ap ^b	6 /ap ^c
$\delta_{\text{calc}}/\text{ppm}$	-143.38	-145.67	-148.70	-150.82	-151.41	-153.92	-155.62
$E^{(2)}/\text{kcal mol}^{-1}$	3.17	1.82	0.88	—	0.84	—	—
$ \alpha $	33.3	26.8	18.6	18.4	18.4	5.1	4.3

^a $E^{(2)}$ energies larger than 0.5 kcal mol⁻¹ are shown.^bC—F/ $\pi(\text{C}=\text{C})_{\text{CFPA-Ph}}$: syn.^cC—F/ $\pi(\text{C}=\text{C})_{\text{CFPA-Ph}}$: anti.

trons into the σ^* orbital of the C—F bond ($\sigma^*(\text{C—F})$) is a major factor governing this “inverse behavior.”²³ This report clearly showed the usefulness of an NBO analysis for the ^{19}F chemical shift problem.

Among the NBO occupancies relevant to F atoms of these compounds, those of $\sigma(\text{C—F})$ and lone pairs were practically the same. Different occupancies were found only for $\sigma^*(\text{C—F})$, especially for **6**/ap (0.02765) and **6**/sp (0.03141). Although the differences were small, the higher occupancy of $\sigma^*(\text{C—F})$ in the **6**/sp conforms to the lower δ_{F} of the fluorine in the sp conformer than that of the fluorine in the ap one. Thus it has become quite certain that the $\sigma^*(\text{C—F})$ plays an important role in determining δ_{F} in the CFPA esters.

Table 3 shows the energies of second-order perturbation of Fock matrix elements, $E^{(2)}$, which indicate the magnitude of interaction between the donors and the $\sigma^*(\text{C—F})$ as the acceptor. The most noticeable difference between the $E^{(2)}$ of the sp and the ap in **6** can be seen in $\pi(\text{C}=\text{C})-\sigma^*(\text{C—F})$. The interaction, $\pi^*(\text{C}=\text{C})-\sigma^*(\text{C—F})$, is also different but the energy is smaller. The decrease of the $E^{(2)}$ in the **5a**/sp compared to that in the **6**/sp corresponds to the decrease of deshielding which gives higher δ_{F} in the former than in the latter. All the conformers of **5b** showed slightly larger $E^{(2)}$ values compared to **5a**/sp, and this can explain the experimentally found lower δ_{F} in **4b** in comparison with the δ_{F} in **4a**.

Table 4 summarizes δ_{calc} , the $E^{(2)}$, and the magnitude of the torsion angles ($|\alpha|$) of F—C—C=C (CFPA-Ph ring) for the conformers of **5a**, **5b**, and **6**. As is seen in the table, the larger $E^{(2)}$ corresponds to the lower δ_{calc} with the only exception being **5b**/ap. A fairly good correlation can be also seen between the $E^{(2)}$ and the $|\alpha|$. In the conformers of **5a**, **b** and **6**, where the π bonds of Ph in CFPA interacting with $\sigma^*(\text{C—F})$ are syn to C—F, the order of the magnitude of $E^{(2)}$, **5b**/sp1 (1.82 kcal mol⁻¹) > **5b**/sp2 (0.88 kcal mol⁻¹) $\approx$ **5a**/sp (0.84 kcal mol⁻¹) > **5a**/ap (< 0.5 kcal mol⁻¹) $\approx$ **6**/ap (< 0.5 kcal mol⁻¹), corresponds to the order of the $|\alpha|$, **5b**/sp1 (27°) > **5b**/sp2 (19°) $\approx$ **5a**/sp (18°) > **5a**/ap (5°) $\approx$ **6**/ap (4°). This result can be understood by the angular dependence of the $\pi(\text{C}=\text{C})-\sigma^*(\text{C—F})$ interaction, which is considered to be a maximum at $|\alpha| = 90^\circ$ and a minimum at 0° . The magnitude of the torsion angle $|\alpha|$ (33°) in **6**/sp is the largest of those in all conformers of **5a**, **b** and **6** shown in Figures 2 and 3. Although, unlike others, the donor $\pi(\text{C}=\text{C})_{\text{CFPA-Ph}}$ in **6**/sp is anti to the C—F bond, the magnitude of the $E^{(2)}$ energy (3.17 kcal mol⁻¹) is the largest of those in all conformers of **5a**, **b** and **6**. The

angular dependence of $E^{(2)}$ energy in **6**/sp was confirmed by the calculations of the energies at $|\alpha| = 0^\circ$, 10° , and 20° . The calculations afforded the $E^{(2)}$ energy values < 0.5, < 0.5, and 1.0 kcal mol⁻¹, respectively. It must be noted that the donor $\pi(\text{C}=\text{C})_{\text{CFPA-Ph}}$ is syn to C—F bond at $|\alpha| = 20^\circ$, while the donor changes to anti at $|\alpha| = 33^\circ$. Although the origin of the different α is not clear at present, it is likely that the $\pi-\pi$ interaction between phenyl group in the CFPA moiety and the aromatic rings of flavan moiety causes a slight change of the $|\alpha|$.

In conclusion, we have found an unusually large ^{19}F chemical shift difference for the CFTA esters **4a** and **4b**. This large difference was reproduced in ab initio calculations of molecular geometry, GIAO calculations of ^{19}F chemical shifts on the model systems. The interaction between $\pi(\text{C}=\text{C})$ of the aryl group in the chiral auxiliary and $\sigma^*(\text{C—F})$ is a very important factor determining the ^{19}F chemical shifts.

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The Effects of Conformation and Solvation on Optical Rotation: Substituted Epoxides

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT The vapor-phase optical rotation (or circular birefringence) of (*S*)-1,2-epoxybutane, (*S*)-epichlorohydrin, and (*S*)-epifluorohydrin has been measured at the nonresonant excitation wavelengths of 355 nm and 633 nm by means of Cavity Ring-Down Polarimetry (CRDP). Complementary solution-phase studies were performed in a wide variety of dilute solvent media to highlight the pronounced influence of solute–solvent interactions. Density functional theory calculations of optical activity have been enlisted to unravel the structural and electronic provenance of experimental observations. Three stable, low-lying conformers have been identified and characterized for each of the targeted chiral species, with thermal (relative population weighted) averaging of their antagonistic chiroptical properties allowing specific rotation values to be predicted under both isolated and solvated conditions. For (*S*)-epichlorohydrin and (*S*)-epifluorohydrin, a self-consistent isodensity polarizable continuum model (SCI-PCM) has been exploited to gain further insight into the underlying nature of solvation effects. *Chirality* 20:357–369, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: intrinsic optical activity; conformational effects; solvation processes; nonresonant chiroptical response; density functional theory; vapor-phase optical activity; cavity ring-down polarimetry; polarizable continuum model

INTRODUCTION

The determination of solution-phase optical activity with sodium *D*-line (589.3 nm) excitation long has been used to establish the enantiomeric purity (or percent enantiomeric excess, %ee) of organic compounds¹; however, practicing chemists largely have ignored the important roles that conformational flexibility and solvent effects can play in these routine analyses. While a variety of empirical schemes (e.g., Brewster's rules^{2–4}) have been proposed to predict and interpret chiroptical behavior, their quantitative application has proven to be limited, at best. Our basic understanding of such nonresonant phenomena has been enhanced greatly by two recent developments. The advent of reliable quantum chemical calculations for optical activity based upon the efficient formalism of linear response theory has provided a powerful tool for unraveling the structural and electronic provenance of chiral molecule-field interactions.^{5–10} Likewise, the ability to measure specific polarization rotation (or circular birefringence) parameters for isolated (solvent-free) species has afforded a viable means for critically assessing computational predictions and elaborating the influence of environmental perturbations.^{11–14} The present work has exploited both of these advancements to examine the nonresonant optical activity for three nonrigid members of the epoxide family, (*S*)-1, 2-epoxybutane, (*S*)-epichlorohydrin, and (*S*)-epifluorohydrin, with complementary studies being performed in vapor and solution phases.

Previous work in our laboratory has exploited the technique of Cavity Ring-Down Polarimetry (CRDP) to interrogate the nonresonant chiroptical response evoked from isolated molecules in their gaseous state.^{11–14} These efforts have shown that the optical rotatory dispersion (ORD) curves for most rigid compounds, such as α -pinene, β -pinene, and *cis*-pinane, do not manifest *gross* solvent-induced modifications. Nevertheless, the direct comparison of analogous solution-phase and vapor-phase results did reveal a number of intriguing effects. For example, most chemists would select an innocuous solvent for routine optical activity measurements so as to minimize solute–solvent perturbations, with cyclohexane often being the medium of choice since it is aprotic (*sans* hydrogen bonding), essentially nonpolar (weak multipolar interactions), and exhibits a low dielectric constant ($\epsilon = 2.02$). In contrast to conventional wisdom, polarimetric studies performed on the afore-

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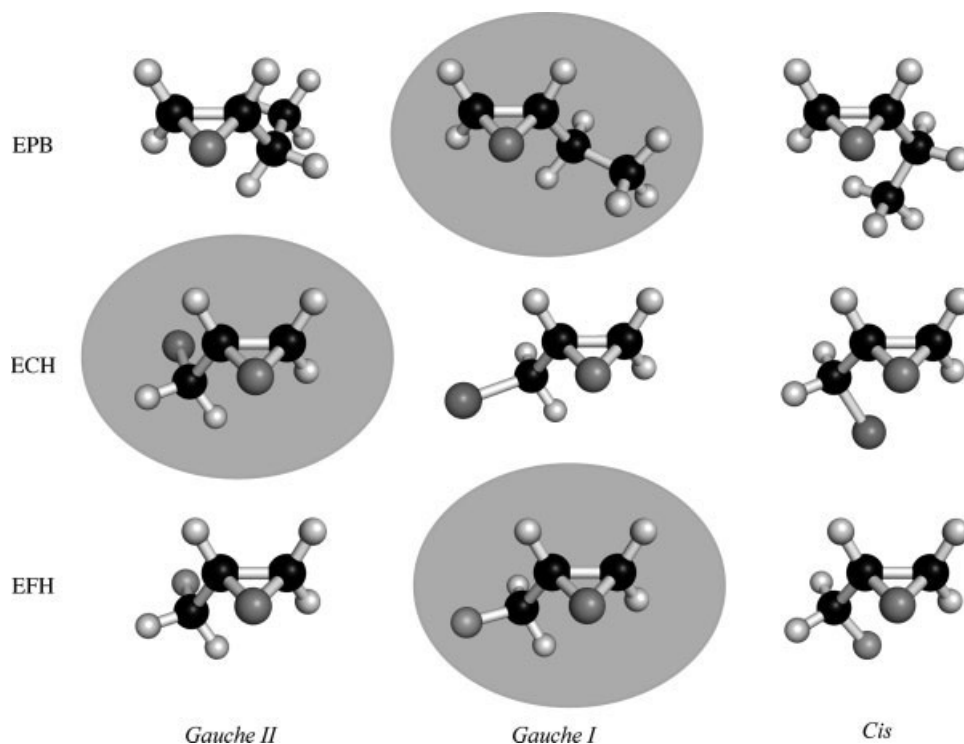


Fig. 1. Conformational structures of substituted epoxides. The three stable conformers of (*S*)-1,2-epoxybutane (EPB), (*S*)-epichlorohydrin (ECH), and (*S*)-epifluorohydrin (EFH) as predicted by the MP2(full)/6-311+G* (for EPB) or MP2/6-311+G* (for ECH and EFH) level of theory are shown, with the equilibrium (minimum-energy) form of the isolated (gas-phase) species being distinguished by superimposing a shaded envelope. In each case, molecular structures have been designated systematically by identifying the *Gauche II*, *Gauche I*, and *Cis* conformations.

mentioned terpene derivatives have found that strongly-polar acetonitrile ($\epsilon = 36.6$) affords the best mimic for isolated-molecule behavior. This counterintuitive assertion has been corroborated for a variety of other conformationally rigid species, including the small (*S*)-methyloxirane system. The present investigation focuses on related members of the epoxide family that are flexible enough to support several distinct, low-lying minima within the ground electronic potential energy surface. Owing to their antagonistic contribution to overall optical activity and their differential stabilization in various solvent media, these conformational structures can be expected to influence profoundly the role that the environment plays as a mediator of chiroptical response.

EXPERIMENTAL

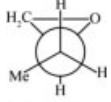
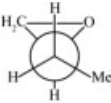
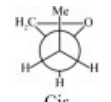
The present measurements of gas-phase optical activity have exploited the same basic CRDP instrumentation and alignment procedures used for previous studies,^{11–14} as well as the accompanying data collection and analysis protocols. To minimize potential sources of contamination, each sample of chiral target molecules was subjected to at least three freeze–pump–thaw cycles before being introduced into the ring-down apparatus. The resulting specific rotation parameters for isolated (solvent-free) species were compared with analogous solution-phase values obtained from a commercial polarimeter (Perkin Elmer 341; 1 dm sample pathlength) operating at discrete excitation wavelengths filtered from Hg I and Na I atomic emission lamps.

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The solvents employed for such work were of spectrophotometric grade (Sigma-Aldrich), except for ethanol (ACS grade, 200 proof) and carbon tetrachloride (ACS grade, 99.9%). Computational predictions for the relaxed geometries, relative energies, and chiroptical properties of individual conformers were based upon the theoretical methods found in the Gaussian 03 ab initio package,¹⁵ as were the implicit solvation models used to further elucidate the influence of environmental perturbations.

Since (*S*)-1, 2-epoxybutane and (*S*)-epichlorohydrin samples of superior chemical and enantiomeric quality were available from commercial sources (Sigma-Aldrich), these materials were procured and used without further purification. Racemic epifluorohydrin of high chemical purity was obtained commercially (Sigma-Aldrich) and kinetically resolved by exploiting the catalytic method of Jacobsen and coworkers.¹⁶ More specifically, 199 mg (0.005 equiv) of (*R,R*)-(-)-*N,N'*-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexane-diaminocobalt(II) (Acros Organics) was dissolved in 5 mL of toluene, reacted with 200 μ L of acetic acid, and allowed to stir at room temperature for $\sim$ 30 min. Following removal of the solvent, the prepared catalyst was dissolved in 5.0 g of racemic epifluorohydrin, with the resulting solution being cooled to 0°C before 0.6 mL (0.55 equiv) of water was added. The mixture was allowed to warm to room temperature and stirred for $\sim$ 17 h, at which point 1.24 g (49.6% yield) of (*S*)-epifluorohydrin was isolated by vacuum transfer to a cold trap maintained at -78°C . The specific rotation of the final material was measured to be $[\alpha]_D^{25^\circ\text{C}} = -3.33 \text{ deg}^{-1} \text{ dm}^{-1} (\text{g/mL})^{-1}$

TABLE 1. Calculated properties for the conformers of (S)-1, 2-epoxybutane

Epoxybutane conformer	O—C—C—Me torsion angle	H—C—C—Me torsion angle	Electric dipole moment μ (D)	Vapor-phase ($\epsilon = 1.0$) energies and populations			
				Relative energy (kcal/mol)	Fractional conformer population	Relative Gibbs free energy (kcal/mol)	Fractional conformer population
 Gauche II	163.2	-59.7	2.50	0.27	0.339	0.14	0.385
 Gauche I	-73.6	61.8	2.40	0.00	0.534	0.00	0.487
 Cis	45.3	-179.6	2.39	0.85	0.127	0.79	0.128

The fully-optimized MP2(full)/6-311+G* geometries predicted for the stable conformers of (S)-1, 2-epoxybutane are illustrated by means of Newman projections and defined in terms of their key torsion angles. The relative energies (ΔE_r) from MP2(full)/6-311+G* calculations and the free energies (ΔG_r) from G3 analyses also are tabulated, as are the corresponding Boltzmann-weighted populations (at 25°C) for the isolated Gauche I, Gauche II, and Cis forms. Since the computed magnitude of the permanent electric dipole moment, μ , for each species is similar, the changes in dielectric constant ($\epsilon > 1$) of the surrounding medium that accompany solvation are not expected to alter the gas-phase ($\epsilon = 1$) energy-ordering of conformational structures.

($c = 1.95$ g/100 mL in CHCl_3), which corresponds to 80.4% ee as determined by comparison to the known solution-phase $[\alpha]_D^{25^\circ\text{C}}$ value.¹⁶

RESULTS AND DISCUSSION

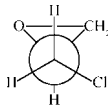
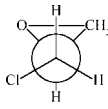
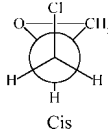
The three molecules of interest for the present study, (S)-1,2-epoxybutane (EPB), (S)-epichlorohydrin (ECH), and (S)-epichlorofluorohydrin (EFH), are depicted schematically in Figure 1. These results, as well as the accompanying structural parameters compiled in Tables 1–3, follow from MP2/6-311+G* [MP2(full)/6-311+G* in the case of EPB] geometry optimization procedures that identified three stable, low-lying conformers for each species. All of the targeted epoxides have been designated as (S)-enantiomers; however, the spatial arrangement of EPB clearly is different from that of ECH and EFH. This fact can be attributed to changes in the substituent sequence used to assign absolute stereochemical configuration by means of the Cahn–Ingold–Prelog (CIP) protocol (or *R*, *S* convention).^{17,18} More specifically, the chloromethyl and fluoromethyl groups attached to the lone stereogenic center of ECH and EFH, respectively, take precedence over all other substituents, while the lower-ranked ethyl moiety of EPB does not. At least three prior investigations of conformer-mediated chiroptical properties have been reported for ECH^{19–22}; however, to the best of our knowledge, the ensuing work represents the first detailed exploration of such phenomena for the related EPB and EFH systems.

The shaded elliptical rings in Figure 1 highlight the minimum-energy structures predicted at the MP2/6-311+G* level of theory for each of the targeted epoxides. The equilibrium configuration for EPB, designated as the Gauche I

form, has an O—C—C—Me dihedral angle of $\tau = -73.6^\circ$. Rotation about this internal coordinate leads to the remaining two minima on the ground state potential energy surface: the Gauche II conformer at $\tau = 163.2^\circ$ (0.27 kcal/mol) and the Cis conformer at $\tau = 45.3^\circ$ (0.85 kcal/mol), where values in parentheses denote the corresponding relative MP2(full) energies. In contrast, electrostatic repulsion between the oxygen and chlorine atoms of ECH causes the Gauche II isomer, possessing an O—C—C—Cl dihedral angle of $\tau = -163.2^\circ$, to become the equilibrium geometry, with the Gauche I form at $\tau = 80.5^\circ$ and the Cis form at $\tau = -45.7^\circ$ having relative MP2 energies of 0.55 and 1.58 kcal/mol, respectively. Similar conclusions hold true in the case of EFH; however, the reduction in steric hindrance obtained by replacing the bulky chlorine atom by a smaller fluorine atom (relative van der Waals radii of 1.75 and 1.47 Å)²³ significantly changes the degree of destabilization experienced by the individual conformers. While the Gauche I geometry with an O—C—C—F dihedral angle of $\tau = 85.0^\circ$ represents the minimum energy configuration of EFH, the Gauche II ($\tau = -160.5^\circ$) and Cis ($\tau = -54.5^\circ$) forms are estimated to reside only 0.07 and 0.31 kcal/mol higher in energy. As shown by our previous studies of 2-substituted butanes²⁴ and 3-substituted-1-butenes,²⁵ thermal distribution of population among such distinct, low-lying conformers can exert a profound influence upon both the magnitude and the sign of observed chiroptical response.

Figure 2 presents ORD data for (S)-1,2-epoxybutane and (S)-epichlorohydrin obtained by monitoring nonresonant optical activity (or circular birefringence) as a function of excitation wavelength, the latter being far removed from known electronic absorption bands of the targeted

TABLE 2. Calculated properties for the conformers of (*S*)-epichlorohydrin

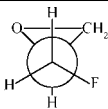
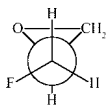
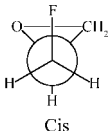
Epichlorohydrin conformer	O—C—C—Cl torsion angle	H—C—C—Cl torsion angle	Electric dipole moment μ (D)	Vapor-phase ($\epsilon = 1.0$) energies and populations			
				Relative energy (kcal/mol)	Fractional conformer population	Relative Gibbs free energy (kcal/mol)	Fractional conformer population
 Gauche II	−163.2	59.7	0.634	0.00	0.683	0.00	0.680
 Gauche I	80.5	−57.1	3.432	0.55	0.270	0.60	0.247
 Cis	−45.7	176.1	2.486	1.58	0.047	1.32	0.073

The fully-optimized MP2/6-311+G* geometries predicted for the stable conformers of (*S*)-epichlorohydrin are illustrated by means of Newman projections and defined in terms of their key torsion angles. The relative energies (ΔE_n) from MP2/6-311+G* calculations and the free energies (ΔG_n) from G3 analyses also are tabulated, as are the corresponding Boltzmann-weighted populations (at 25°C) for the isolated Gauche I, Gauche II, and Cis forms. Since the computed magnitude of the permanent electric dipole moment, μ for each species differ markedly, the changes in dielectric constant ($\epsilon > 1$) of the surrounding medium that accompany solvation are expected to alter the gas-phase ($\epsilon = 1$) energy-ordering of conformational structures.

species. The specific optical rotation, $[\alpha]_{\lambda}^T$, expressing the angle of linear polarization rotation (deg) observed at temperature T (25°C) and wavelength λ (nm) per unit path-length (dm) and per unit concentration (g/mL) has been

plotted on the ordinate scale. The dotted curves follow from the interpolation of canonical solution-phase polarimetric measurements performed at the discrete excitation frequencies (ν) denoted by individual opened symbols,

TABLE 3. Calculated properties for the conformers of (*S*)-epifluorohydrin

Epifluorohydrin conformer	O—C—C—F torsion angle	H—C—C—F torsion angle	Electric dipole moment μ (D)	Vapor-Phase ($\epsilon = 1.0$) energies and populations			
				Relative energy (kcal/mol)	Fractional conformer population	Relative Gibbs free energy (kcal/mol)	Fractional conformer population
 Gauche II	−160.5	62.9	0.687	0.07	0.358	0.09	0.389
 Gauche I	85.0	−52.6	3.932	0.00	0.402	0.00	0.452
 Cis	−54.5	169.2	3.191	0.31	0.240	0.62	0.159

The fully-optimized MP2/6-311+G* geometries predicted for the stable conformers of (*S*)-epifluorohydrin are illustrated by means of Newman projections and defined in terms of their key torsion angles. The relative energies (ΔE_n) from MP2/6-311+G* calculations and the free energies (ΔG_n) from G3 analyses also are tabulated, as are the corresponding Boltzmann-weighted populations (at 25°C) for the isolated Gauche I, Gauche II, and Cis forms. Since the computed magnitude of the permanent electric dipole moment, μ , for each species differ markedly, the changes in dielectric constant ($\epsilon > 1$) of the surrounding medium that accompany solvation are expected to alter the gas-phase ($\epsilon = 1$) energy-ordering of conformational structures.

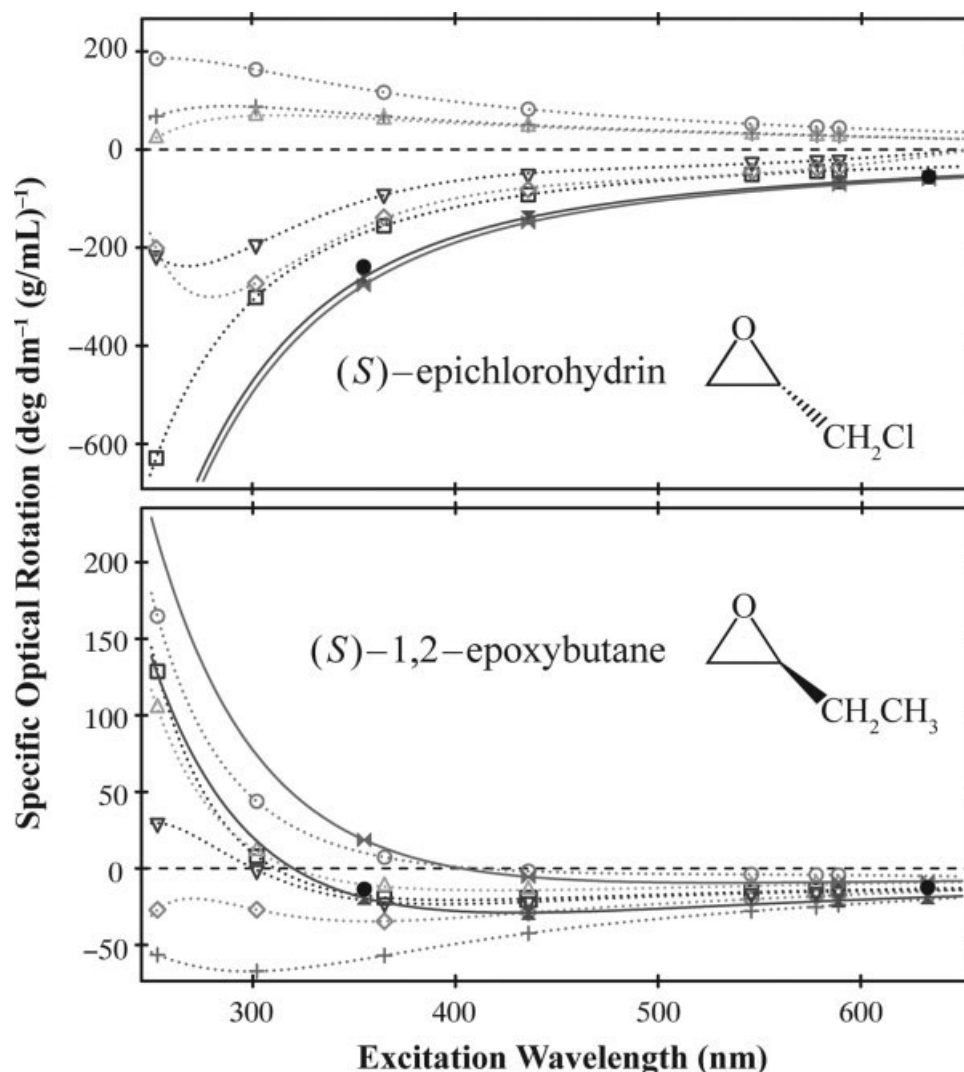


Fig. 2. Nonresonant optical activity in model epoxide systems. Optical Rotary Dispersion (ORD) curves, showing the dependence of specific optical rotation on excitation wavelength, are plotted for (S)-1,2-epoxybutane (top panel) and (S)-epichlorohydrin (bottom panel). The depicted experimental results follow from canonical liquid-phase polarimetric studies performed on a variety of dilute solutions [dotted curves and opened symbols: cyclohexane ($\square$); carbon tetrachloride ($\diamond$); di-*n*-butylether (∇); ethanol ($\triangle$); benzene (+); and acetonitrile ($\circ$)], as well as from analogous CRDP measurements of vapor-phase behavior ($\bullet$). The corresponding theoretical predictions (solid curves) stem from linear response calculations of optical activity (B3LYP/aug-cc-pVDZ) for the three stable conformers of each species, with requisite Boltzmann averaging (25°C) being performed through use of both relative energies ($\boxtimes$) and relative free energies ($\boxplus$) as deduced from G3 treatments. The first electronic absorption bands for the targeted epoxides appear well below 200 nm, highlighting the nonresonant nature of the observed chiroptical response.

with concentrations being kept low enough so as to minimize solute–solute interactions. The selected solvent media (cf., figure caption) span a wide range of chemical and physical characteristics, including distinct values of permanent electric dipole moment μ , static dielectric constant ϵ , and frequency-dependent refractive index $n(\nu)$. Even a cursory inspection of Figure 2 reveals dramatic differences among the various solution-phase results, highlighting the substantial role of solvation and showing that the measured chiroptical response does not reflect an intrinsic property of the isolated solute molecule.

The filled circular symbols in Figure 2 follow from CRDP measurements of optical activity performed on ambient, bulk-gas samples of (S)-1,2-epoxybutane and (S)-epichlorohydrin at the nonresonant excitation wavelengths

of 355 and 633 nm. For each of these data points, the linear polarization rotation per pathlength was examined as a function of sample pressure and the desired value of $[\alpha]_{\lambda}^T$ was extracted from the slope of the resulting linear plot.¹⁴ Owing to the removal of strong environmental perturbations, these vapor-phase specific rotation parameters should reflect the intrinsic properties of the isolated target molecules. In the case of rigid chiral species, previous work from our laboratory has found that solvents of high dielectric constant such as acetonitrile consistently provide the best mimic for gas-phase chiroptical response.¹⁴ Figure 2 shows this no longer to be true for the conformationally flexible epoxide systems, with cyclohexane reproducing the observed gas-phase results most uniformly, in keeping with the expectations drawn from chemical intuition.

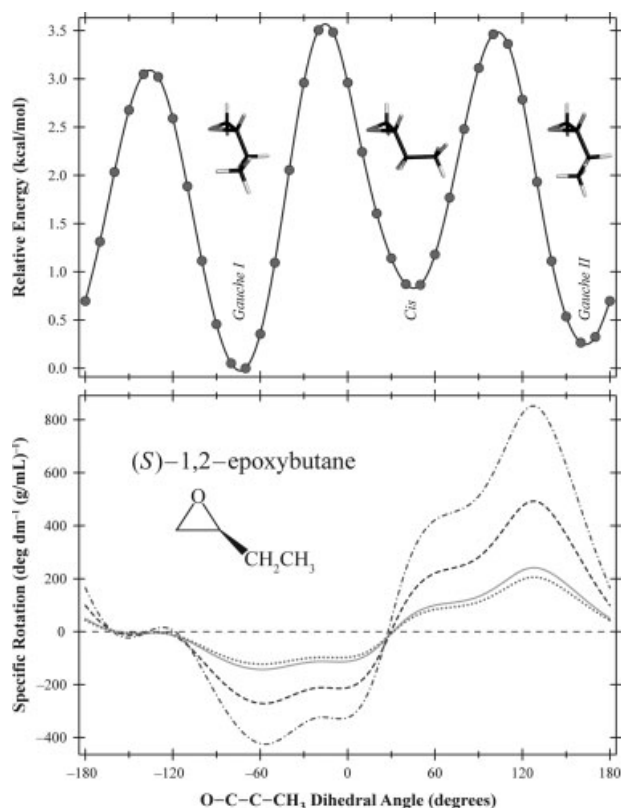


Fig. 3. Dependence of potential energy and optical activity on dihedral-angle. The top panel shows the relative potential energy for (S)-1,2-epoxybutane as a function of the O—C—C—Me dihedral angle, τ , highlighting the three distinct minima that correspond to the Gauche I, Cis, and Gauche II forms. These results follow from constrained MP2(full)/6-311+G* geometry optimizations performed at the values of τ indicated by circular symbols, with the superimposed line being drawn for clarity. The bottom panel illustrates the τ -dependence of optical rotation calculated by means of B3LYP/aug-cc-pVDZ linear response theory for several different excitation wavelengths: 633 nm (dotted curve), 589 nm (solid curve), 436 nm (dashed curve), and 355 nm (dashed-dotted curve). While the overall magnitude of the predicted chiroptical response increases with decreasing wavelength, the Gauche II structure always exhibits a polarity opposite to that of the Gauche I and Cis conformers.

An MP2/6-311+G* potential energy scan was performed as a function of the O—C—C—X (X = Me, Cl, F) dihedral angle, τ , for each of the targeted epoxides, with the structures emerging as energy minima subsequently undergoing full geometry optimization. The top panel of Figure 3 provides a representative example of this analysis in the case of EPB. The nonresonant optical activity for every stable conformer identified in this manner was calculated by exploiting the linear response formalism^{26–29} as implemented within the framework of density functional theory.^{5–7,10,30–33} This treatment employed the B3LYP correlation-exchange functional in combination with the aug-cc-pVDZ basis set, an approach that has been shown to provide a reasonable compromise between computational cost and accuracy.^{6,7,14,32} The values of $[\alpha]_{\lambda}^T$ predicted at four distinct excitation wavelengths (355, 436, 589, and 633 nm) have been compiled in Tables 4–6 for the three low-lying structural isomers of EPB, ECH, and EFH, respectively. The pronounced dependence of specific optical rotation on internal coordinate τ is illustrated graphically by the bottom panel of Figure 3, which highlights the

varying magnitude and sign of chiroptical response expected for the individual conformers of EPB.

Given the antagonistic chiroptical properties exhibited by the individual conformers of EPB, ECH, and EFH (cf., Tables 4–6), reliable prediction of the overall response evoked from an equilibrated ensemble of such molecules demands careful consideration. Rigorously speaking, this analysis should require the determination of multidimensional vibrational eigenvalues, E_v , and eigenfunctions, $\psi_v(\mathbf{Q})$, the latter spanning pertinent minima within the ground electronic potential energy surface. When combined with a coordinate-dependent optical activity function of comparable complexity, $[\alpha]_{\lambda}(\mathbf{Q})$, these quantities will permit the evaluation of expectation values, $\langle [\alpha]_{\lambda}(\mathbf{Q}) \rangle_v$, that subsequently would be thermally averaged to account for the incoherent occupation of discrete quantum states. While such arduous procedures can be simplified by restricting attention to a relevant subset of vibrational degrees of freedom (e.g., see the $[\alpha]_{\lambda}(\tau)$ function in the bottom panel of Fig. 3), valuable insight can be gleaned from the population weighted average of the specific rotation contributed by each of the stable conformers: $[\alpha]_{\lambda,\eta}$ where η denotes Gauche I, Gauche II, or Cis for the epoxide systems of interest. Provided that vibrationally mediated isomerization and tunneling processes can be excluded, this rudimentary approximation enables the predicted nonresonant chiroptical behavior at a given wavelength and temperature to be cast as a straightforward linear combination:

$$[\alpha]_{\lambda}^T = \sum_{\eta}^{\text{all conformers}} c_{\eta}(T) [\alpha]_{\lambda,\eta} \quad (1)$$

where the temperature dependent fractional population coefficients, $c_{\eta}(T)$, must be properly normalized so that they sum to unity:

$$\sum_{\eta}^{\text{all conformers}} c_{\eta}(T) = 1 \quad (2)$$

The requisite $[\alpha]_{\lambda,\eta}$ parameters for eq. 1 have been estimated through application of B3LYP/aug-cc-pVDZ linear response theory, with the resulting values being compiled in Tables 4–6 for EPB, ECH, and EFH, respectively. For each of the selected excitation wavelengths, such calculation show that the predicted chiroptical behavior for the Gauche I conformer always will be of opposite polarity to that for either the Gauche II or Cis form. The corresponding fractional population coefficients, $c_{\eta}(T)$, can be formulated most readily in terms of the relative conformer energies, ΔE_{η} , emerging from ab initio (MP2) geometry optimization procedures. More specifically, these quantities follow from the canonical Boltzmann equilibrium distribution at temperature T :

$$c_{\eta}(T) = \frac{e^{-\Delta E_{\eta}/k_B T}}{q} \quad (3)$$

where k_B is the Boltzmann constant and the conformer partition function q ensures that the restriction of eq. 2 is satisfied:

TABLE 4. Optical rotation parameters for isolated (S)-1, 2-epoxybutane

Excitation wavelength (nm)	Calculated specific optical rotation for individual conformers			Predicted specific optical rotation (Boltzmann-averaged at 25°C)		Measured vapor-phase specific optical rotation
	Gauche II	Gauche I	Cis	Based on energy ΔE	Based on Gibbs free energy ΔG	
355	414.9	−371.1	310.0	−18.2	+18.6	−13.56 ± 0.95
436	242.9	−244.1	155.7	−28.3	−5.5	−
589	120.1	−130.9	67.3	−20.7	−8.9	−
633	102.5	−113.0	56.2	−18.5	−8.4	−12.33 ± 0.35

B3LYP/aug-cc-pVDZ linear response theory has been employed to calculate specific optical rotation parameters (in deg dm^{−1} (g/mL)^{−1}) at a series of nonresonant excitation wavelengths for each of the three structural isomers identified in (S)-1,2-epoxybutane. These results have been thermally-averaged (25°C) by exploiting Boltzmann weights deduced independently from relative energies (ΔE_η ; MP2(full)/6-311+G* level of theory) and relative free energies (ΔG_η ; G3 composite level of theory), with the former found to be in better agreement with experimental measurements performed in the vapor phase.

$$q = \sum_{\eta}^{\text{all conformers}} e^{-\Delta E_\eta/k_B T}. \quad (4)$$

Tables 4–6 compare the overall specific optical rotation values predicted at 25°C through combined use of eqs. 1 and 3 with the analogous quantities determined for isolated EPB, ECH, and EFH molecules by means of vapor-phase CRDP measurements. Reasonable accord is obtained between computation and experiment, as corroborated by the theoretical ORD curves found in both panels of Figure 2 (viz., upright hourglass symbols and superimposed solid lines).

Since the thermodynamic specification of equilibrium relies upon both enthalpic and entropic criteria, it would seem more appropriate to base the above analyses of overall optical activity upon the relative Gibbs free energy of conformers, ΔG_η , rather than their corresponding relative MP2 energies, ΔE_η . To make this possible, a series of Gaussian-3 (G3) calculations³⁴ was performed to predict the requisite values of ΔG_η for each of the three structural isomers identified in EPB, ECH, and EFH. These results have been compiled in Tables 1–3 along with the corresponding fractional population coefficients, $c_\eta(T)$, deduced from eqs. 3 and 4 by substituting ΔG_η for ΔE_η . As expected only minor changes ($\leq 10\%$) in the room temperature (25°C) occupation of individual conformers is realized by employing relative Gibbs free energies instead of rela-

tive MP2 energies. The only notable exceptions to this assertion can be found for the Cis forms of ECH (+55.3%) and EFH (−34.2%) where the quantities in parentheses denote the percentage changes in $c_\eta(T)$ obtained by switching from ΔE_η to ΔG_η .

As highlighted by the numerical results presented in Tables 4–6 and reinforced by the theoretical ORD curves in Figure 2, the seemingly small modifications in conformer population introduced by the use of relative Gibbs free energies (rather than relative MP2 energies) can lead to surprisingly large differences in overall chiroptical response. For ECH and EFH, the values of $[\alpha]_\lambda^{25^\circ\text{C}}$ determined on the basis of ΔE_η and ΔG_η are quite comparable to one another and are in reasonable accord with CRDP measurements conducted on isolated molecules. In contrast, the optical activity for EPB changes dramatically when the Boltzmann weighted factors are cast in terms of the Gibbs free energy, with the computed specific rotation at 355 nm having a sign opposite to that estimated from the analogous ΔE_η calculations. This anomaly is exhibited clearly in the bottom panel of Figure 2, where the vertical and horizontal hourglass symbols (as well as interpolating solid lines) distinguish the predictions from energies and free energies, respectively. The ΔE_η results reproduce the vapor-phase experimental findings for EPB considerably better than their ΔG_η counterparts; however, the well-established thermodynamic criteria for equilibrium and

TABLE 5. Optical rotation parameters for isolated (S)-epichlorohydrin

Excitation wavelength (nm)	Calculated specific optical rotation for individual conformers			Predicted specific optical rotation (Boltzmann-averaged at 25°C)		Measured vapor-phase specific optical rotation
	Gauche II	Gauche I	Cis	Based on energy ΔE	Based on Gibbs free energy ΔG	
355	−565.6	505.3	−234.4	−260.8	−276.8	−238.7 ± 2.3
436	−327.9	335.9	−117.3	−138.7	−148.5	−
589	−160.8	166.9	−50.4	−67.1	−71.8	−
633	−137.0	143.4	−42.0	−56.8	−60.8	−55.0 ± 1.7

B3LYP/aug-cc-pVDZ linear response theory has been employed to calculate specific optical rotation parameters (in deg dm^{−1} (g/mL)^{−1}) at a series of nonresonant excitation wavelengths for each of the three structural isomers identified in (S)-epichlorohydrin. These results have been thermally-averaged (25°C) by exploiting Boltzmann weights deduced independently from relative energies (ΔE_η ; MP2/6-311+G* level of theory) and relative free energies (ΔG_η ; G3 composite level of theory), with both found to well reproduce experimental measurements performed in the vapor phase.

TABLE 6. Optical rotation parameters for isolated (S)-epifluorohydrin

Excitation wavelength (nm)	Calculated specific optical rotation for individual conformers			Predicted specific optical rotation (Boltzmann-averaged at 25°C)		Measured vapor-phase specific optical rotation
	Gauche II	Gauche I	Cis	Based on energy ΔE	Based on Gibbs free energy ΔG	
355	−91.6	63.3	−348.6	−90.9	−62.3	−79.6 ± 4.4
436	−53.1	59.3	−186.6	−39.9	−23.5	—
589	−26.3	38.2	−85.5	−14.5	−6.5	—
633	−22.4	33.7	−71.9	−11.7	−4.9	−21.5 ± 2.7

B3LYP/aug-cc-pVDZ linear response theory has been employed to calculate specific optical rotation parameters (in deg dm^{−1} (g/mL)^{−1}) at a series of nonresonant excitation wavelengths for each of the three structural isomers identified in (S)-epichlorohydrin. These results have been thermally-averaged (25°C) by exploiting Boltzmann weights deduced independently from relative energies (ΔE_η ; MP2/6-311+G* level of theory) and relative free energies (ΔG_η ; G3 composite level of theory), with both found to well reproduce experimental measurements performed in the vapor phase.

the inherent reliability of G3 theory³⁴ (vide infra) provide strong arguments in favor of the ΔG_η approach. This would tend to place doubts on the analyses of optical activity contributions in EPB, suggesting that the conformer specific $[\alpha]_{\lambda,\eta}$ parameters are in error or that complete exclusion of vibrational dynamics is an oversimplification.^{25,35–39} The disparate behavior seen for the three epoxide systems targeted by the present study might also reflect the greater degrees of conformational freedom available to the ethyl group of EPB as opposed to the less flexible halomethyl substituents of ECH and EFH.

The incongruities uncovered during G3 analysis of EPB prompted further efforts based upon the potent Complete Basis Set – Quadratic Configuration Interaction/Atomic Pair Natural Orbital (CBS-QCI/APNO) scheme of Petersson and coworkers.^{40,41} While the Gaussian-3 paradigm is believed to reproduce chemical energy differences (including enthalpies of formation, ionization potentials, and electron/proton affinities) to better than ±1 kcal/mol,³⁴ the more demanding CBS-QCI/APNO approach has been shown to reduce mean deviations between experiment and theory to ±0.5 kcal/mol.^{40,41} Application of the CBS-QCI/APNO procedure to the conformers of EPB gave Gibbs free energies of $\Delta G_{\text{Gauche II}} = 0.21$ kcal/mol and $\Delta G_{\text{Cis}} = 0.82$ kcal/mol, as defined relative to the lowest-lying Gauche I form ($\Delta G_{\text{Gauche I}} = 0$). The attendant fractional population coefficients at 25°C were found to be $c_{\text{Gauche II}} = 0.359$ (−6.5%), $c_{\text{Gauche I}} = 0.512$ (5.2%), and $c_{\text{Cis}} = 0.128$ (−0.02%), representing only modest changes (as indicated in parenthesis) from their G3 counterparts in Table 1. When combined with the intrinsic optical activities computed for the structural isomers of EPB (cf., Table 4), these quantities lead to ensemble-averaged specific rotations [in deg dm^{−1} (g/mL)^{−1}] of −1.2, −17.8, −15.3, and −13.8 at the selected excitation wavelengths of 355, 436, 589, and 633 nm, respectively. In contrast to the G3 studies summarized in Table 4, the CBS-QCI/APNO treatment does predict the proper sign of polarization rotation at 355 nm; however, the corresponding magnitude still deviates significantly from the vapor-phase result of -13.56 ± 0.95 . Much better agreement is realized at 633 nm, where the CBS-QCI/APNO estimate for $[\alpha]_{633\text{nm}}^{25^\circ\text{C}}$ differs from the CRDP measurement of -12.33 ± 0.35 by less than 12%. As a final

point of comparison, harmonic frequency calculations performed at the MP2(full)/6-311+G* level of theory gave free energies of $\Delta G_{\text{Gauche II}} = 0.22$ kcal/mol and $\Delta G_{\text{Cis}} = 1.03$ kcal/mol (i.e., relative to $\Delta G_{\text{Gauche I}} = 0$). The values of $[\alpha]_{\lambda}^{25^\circ\text{C}}$ deduced from such force-field analyses (viz., −16.3, −26.4, −19.4, and −17.4 at 355, 436, 589, and 633 nm, respectively) closely parallel those obtained from MP2(full)/6-311+G* predictions of ΔE_η (cf., Table 4) and are in reasonable accord with experimental findings.

The discrepancies obtained by applying various quantum chemical methods to the EPB system underscore the important roles that conformational degrees of freedom play in governing overall chiroptical behavior, with the quantitative interpretation of bulk behavior demanding that high-level estimates of optical activity be coupled with sophisticated treatments of thermodynamic parameters. As part of this endeavor, it is important to recognize how uncertainties associated with the relative (free) energies and the specific rotations of individual conformers propagate into the final calculated value of $[\alpha]_{\lambda}^T$. By assuming $\sigma(\Delta G_\eta)$ and $\sigma([\alpha]_{\lambda,\eta})$ to represent uncorrelated estimates for the root-mean-square deviations (or standard deviations) in ΔG_η and $[\alpha]_{\lambda,\eta}$, respectively, a straightforward, albeit tedious, analysis shows that the variance (or mean-square deviation) for the ensemble-averaged response at wavelength λ and temperature T will be given by⁴²:

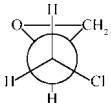
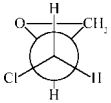
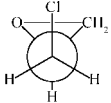
$$\sigma^2([\alpha]_{\lambda}^T) = \sum_{\eta}^{\text{all conformers}} \left[\left\{ c_{\eta}(T) \sigma([\alpha]_{\lambda,\eta}) \right\}^2 + \left\{ \frac{c_{\eta}(T)}{k_B T} ([\alpha]_{\lambda}^T - [\alpha]_{\lambda,\eta}) \sigma(\Delta G_{\eta}) \right\}^2 \right] \quad (5)$$

where the temperature uncertainty has been presumed to be negligibly small (i.e., $\sigma(T) \approx 0$). The corresponding one standard deviation error in $[\alpha]_{\lambda}^T$ follows from the square root of eq. 5:

$$\sigma([\alpha]_{\lambda}^T) = \sqrt{\sigma^2([\alpha]_{\lambda}^T)}, \quad (6)$$

thereby leading to the canonical 68.3% confidence interval of $[\alpha]_{\lambda}^T \pm \sigma([\alpha]_{\lambda}^T)$. Even if uncertainties in the conformer-

TABLE 7. Computed solvation parameters for conformers of (*S*)-epichlorohydrin

Epichlorohydrin conformer	Acetonitrile solution phase ($\epsilon = 36.6$)		Cyclohexane solution phase ($\epsilon = 2.02$)		<i>n</i> -Butyl ether solution phase ($\epsilon = 3.06$)		Vapor-Phase ($\epsilon = 1.0$)	
	Relative Gibbs free energy (kcal/mol)	Fractional conformer population	Relative Gibbs free energy (kcal/mol)	Fractional conformer population	Relative Gibbs free energy (kcal/mol)	Fractional conformer population	Relative Gibbs free energy (kcal/mol)	Fractional conformer population
 Gauche II	0.42	0.298	0.00	0.582	0.00	0.512	0.00	0.680
 Gauche I	0.00	0.606	0.33	0.333	0.15	0.398	0.60	0.247
 Cis	1.09	0.096	1.14	0.085	1.03	0.090	1.32	0.073

A self-consistent implicit polarizable continuum model (SCI-PCM) for solvation has been used to predict the relative free energies and the ambient (25°C) populations for the three stable conformers of (*S*)-epichlorohydrin in diverse solvent environments: acetonitrile ($\epsilon = 36.6$), cyclohexane ($\epsilon = 2.02$), and *n*-butylether ($\epsilon = 3.06$), where values in parentheses denote the corresponding dielectric constants. Also tabulated for purposes of comparison are the analogous quantities deduced for the isolated (gas-phase) molecule. The differential stabilization that the Gauche I, Gauche II, and Cis forms exhibit in the selected solvent media can be ascribed to the distinct charge distributions supported by each species, as evinced by their corresponding permanent electric dipole moments.

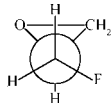
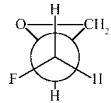
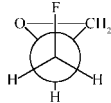
specific contributions to optical activity are ignored by setting $\sigma([\alpha]_{\lambda,\eta}) \approx 0$, eqs. 5 and 6 suggest that the relative (free) energies for the individual conformers of EPB must be predicted with substantially better than 10% accuracy (viz., $\sigma(\Delta G_{\eta})/\Delta G_{\eta} < 0.1$) for the values of $\sigma([\alpha]_{\lambda}^T)$ to be on the order of $1 \text{ deg dm}^{-1} (\text{g/mL})^{-1}$. This requirement would appear to be well below the ± 1 and ± 0.5 kcal/mol limits imposed upon chemical energy differences by the G3 and CBS-QCI/APNO paradigms; however, such tolerances reflect a broad spectrum of thermochemical calculations performed on a diverse family of test molecules. Since the present investigations of nonrigid chiral species rely on the relative (free) energies for stable structural isomers that have essentially identical bonding motifs, *ab initio* estimates for ΔE_{η} and ΔG_{η} might be expected to exhibit errors more commensurate with those required for fruitful application of the rudimentary ensemble-averaging scheme embodied in eq. 1. Nevertheless, the intrinsic reliability of different quantum chemical approaches for computing Boltzmann-weighted conformer populations (as required for the determination of nonresonant optical activity) is a topic of active research in our laboratory.

An extensive literature exists for the prediction of molecular properties in the presence of solvent^{43–50}; however, theoretical descriptions of chiral solute–solvent perturbations are surprisingly limited, often being relegated to the venerable (albeit now largely discredited)^{7,32,51} Lorentz local field correction.^{52–54} As illustrated by the ORD curves in Figure 2, solvation long has been known to markedly influence both the magnitude and the sign of

chiroptical response.^{52,54} In the case of flexible solutes, these observations commonly have been attributed to geometrical relaxation of the nuclear framework that results in the differential stabilization of individual conformers, each of which contributes uniquely to the ensemble-averaged optical activity (cf., Tables 4–6). Nevertheless, previous work in our laboratories has shown that even nominally rigid chiral species undergo pronounced changes,¹⁴ which ultimately reflect the static and dynamic redistribution of electron density incurred by complex solvation processes. Since a detailed understanding of such phenomena is essential for the modeling of behavior in condensed media, a series of quantum chemical calculations have been performed to unravel the solvent dependence of specific rotation in the substituted epoxides targeted by the present study.

The ensuing analyses of solution-phase chiroptical response have exploited a self-consistent isodensity polarizable continuum model⁵⁵ (SCI-PCM) that treats the solvent as an isotropic and homogeneous medium characterized by the static dielectric constant ϵ . This implicit solvation framework builds upon the well-established precepts of the Onsager reaction field,⁵⁶ where the charge distribution of a potentially polarizable solute molecule, imbedded in a continuum dielectric solvent, induces reflection moments (in the solvent) that, in turn, act to electrostatically stabilize the solute. Despite their exclusion of explicit first solvation-shell effects in the cybotactic region, such approaches accurately portray the strong, long-range forces (of primary electrostatic origins) that often domi-

TABLE 8. Computed solvation parameters for conformers of (*S*)-epifluorohydrin

Epifluorohydrin conformer	Acetonitrile solution phase ($\epsilon = 36.6$)		Cyclohexane solution phase ($\epsilon = 2.02$)		<i>n</i> -Butyl ether solution phase ($\epsilon = 3.06$)		Vapor-phase ($\epsilon = 1.0$)	
	Relative Gibbs free energy (kcal/mol)	Fractional conformer population	Relative Gibbs free energy (kcal/mol)	Fractional conformer population	Relative Gibbs free energy (kcal/mol)	Fractional conformer population	Relative Gibbs free energy (kcal/mol)	Fractional conformer population
 Gauche II	1.15	0.109	0.40	0.286	0.58	0.232	0.09	0.390
 Gauche I	0.00	0.761	0.00	0.564	0.00	0.621	0.00	0.452
 Cis	1.05	0.130	0.78	0.150	0.85	0.147	0.62	0.158

A self-consistent implicit polarizable continuum model (SCI-PCM) for solvation has been used to predict the relative free energies and the ambient (25°C) populations for the three stable conformers of (*S*)-epifluorohydrin in diverse solvent environments: acetonitrile ($\epsilon = 36.6$), cyclohexane ($\epsilon = 2.02$), and *n*-butylether ($\epsilon = 3.06$), where values in parentheses denote the corresponding dielectric constants. Also tabulated for purposes of comparison are the analogous quantities deduced for the isolated (gas-phase) molecule. The differential stabilization that the Gauche I, Gauche II, and Cis forms exhibit in the selected solvent media can be ascribed to the distinct charge distributions supported by each species, as evinced by their corresponding permanent electric dipole moments.

nate solvation processes.^{43,44} As such, they have been found to provide reasonable descriptions for aprotic and nonaromatic solvents that do not engage in specific solute–solvent interactions.^{57–60} The particular PCM implementation selected for the present work incorporates electron correlation effects at the B3LYP/6-311+G* level of density functional theory and constructs the solute cavity in a self-consistent manner based upon the corresponding electron isodensity surface⁵⁵ (isodensity value of 4×10^{-4}). The relative Gibbs free energies for individual conformers, ΔG_{η} , thus can be calculated taking into account the additional stabilization afforded by the surrounding dielectric environment.

Since the continuum dielectric model emphasizes the role of electrostatic interactions, our SCI-PCM treatment can be expected to show pronounced solvation effects only when changes in molecular conformation lead to substantial modifications in the electronic charge distribution. Tables 1–3 contain the magnitude of the permanent electric dipole moment, μ_{η} , computed at the MP2/6-311+G* level of theory for all optimized geometries of the targeted epoxides. While the three identified structural isomers of EPB support nearly equal values of this lowest-order multipole moment ($\mu_{\eta} = 2.4 - 2.5$ D), the inherent polarity of the C–X bond in ECH and EFH produces large variations in this quantity among conformers, with $\mu_{\text{Gauche I}} > \mu_{\text{Cis}} \gg \mu_{\text{Gauche II}}$. The measured solution-phase ORD curves for the halogen containing epoxides are in keeping with this implicit prediction for the preferential stabilization of individual conformers, displaying much larger solvent-depend-

ent variations that their EPB counterparts. Consequently, the SCI-PCM calculations of relative Gibbs free energy in the presence of a continuum-dielectric solvent have been restricted to the ECH and EFH systems.

The key parameters extracted from SCI-PCM calculations of conformationally flexible ECH and EFH molecules have been compiled in Tables 7 and 8, respectively. In particular, values of relative Gibbs free energy, ΔG_{η} , and fractional conformer population, $c_{\eta}(T)$, have been computed for implicit solvation models of acetonitrile ($\epsilon = 36.6$), cyclohexane ($\epsilon = 2.02$), and *n*-butylether ($\epsilon = 3.06$), where parentheses denote the corresponding static dielectric constant. While solvents of low polarity (viz., cyclohexane and *n*-butylether) yield results comparable to those predicted for the vapor-phase, strongly-polar media (viz., acetonitrile) are found to lower substantially the energy of structural isomers that possess large permanent electric dipole moments. For ECH, this differential stabilization is sufficient to alter the energy ordering of conformers from the isolated-molecule ranking of Gauche II < Gauche I < Cis to Gauche I < Gauche II < Cis. Such reordering does not occur in EFH; however, the near degeneracy exhibited by the Gauche I and Gauche II forms in the gas-phase is lifted, with the free energy of the former dropping substantially below that of the latter in a polar solvent.

Taking into account the distinct optical activity expected for the stable conformations of ECH and EFH, the modifications in $c_{\eta}(T)$ parameters emerging from SCI-PCM calculations should lead to dramatic changes in chiroptical properties. As highlighted in Table 9, the overall specific

TABLE 9. Solution-phase optical rotation of (S)-epichlorohydrin

Excitation wavelength (nm)	Acetonitrile solution		Cyclohexane solution		<i>n</i> -Butyl ether solution	
	Predicted	Measured	Predicted	Measured	Predicted	Measured
355	114.8	123.3	−180.5	−167.7	−109.9	−104.6
436	94.4	82.1	−88.7	−91.9	−44.9	−50.6
589	48.3	44.5	−42.2	−42.5	−20.5	−21.6
633	42.0	37.8	−35.5	−30.4	−16.9	−7.4

The B3LYP/aug-cc-pVDZ linear response calculations of optical activity for individual (solvent-free) conformers of (S)-epichlorohydrin have been combined with the Boltzmann weights (25°C) suggested by polarizable continuum models of implicit solvation (SCI-PCM) to predict nonresonant specific rotation parameters (in deg dm^{−1} (g/mL)^{−1}) for three model solvents. These ensemble-averaged results are in good agreement with experimental findings, in particular, reproducing the changes in sign observed between acetonitrile solution-phase and isolated gas-phase polarimetric measurements.

rotation computed for ECH in acetonitrile, cyclohexane, and *n*-butyl ether on the basis of eq. 1 are in near-quantitative accord with laboratory measurements performed at four discrete excitation wavelengths spanning the ultraviolet and visible portions of the spectrum. Indeed, our analysis reproduces the change in sign of $[\alpha]_{\lambda}^{25^{\circ}\text{C}}$ that takes place when ECH is dissolved in acetonitrile, reflecting the preferential stabilization of the *Gauche* I structure that makes $[\alpha]_{\lambda,\eta}$ contributions of opposite polarity to those of the *Gauche* II and *Cis* forms. The quality of agreement achieved between theory and experiment is especially remarkable given the rudimentary nature of the present treatment, which has cobbled together solvation energy estimates for localized structural isomers with vapor-phase predictions of their attendant chiroptical response. In contrast, analogous calculations by Tam and Crawford²² were not able to reproduce successfully the nonresonant optical activity exhibited by ECH in dilute CH₂Cl₂, CHCl₃, and CCl₄ media. This discrepancy in computational results can be attributed, in part, to known deficiencies inherent to PCM descriptions of such chlorinated solvents.⁵¹

The SCI-PCM estimates of optical activity for solvated EFH have been compiled in Table 10. Although not displaying the same near-quantitative agreement between theory and experiment as found for ECH, these computational results do reproduce the gross trends uncovered by laboratory measurements. For all three of the solvent systems under investigation, the ORD curve is predicted to undergo a sign change (or exhibit a “zero-crossing”) in the blue/ultraviolet region of the spectrum; however, polarimetric studies show this to be true only for EFH sol-

vated by acetonitrile. Nevertheless, in contrast to prior investigations of rigid chiral species,¹⁴ the SCI-PCM analyses of ECH and EFH suggest that cyclohexane should provide a better mimic for isolated molecules behavior than either acetonitrile or *n*-butylether. This assertion can be rationalized through the small degree of differential stabilization that the weakly-polar cyclohexane medium affords to individual conformers, thereby implying that their antagonistic contributions to the overall chiroptical response remain essentially unchanged from those of the vapor phase.

SUMMARY AND CONCLUSIONS

The nonresonant optical activity (or circular birefringence) for three conformationally flexible members of the epoxide family has been studied under solvated and isolated conditions by employing conventional (solution-phase) polarimetric techniques and ultrasensitive CRDP, respectively. The resulting specific rotation parameters for (S)-1,2-epoxybutane, (S)-epichlorohydrin, and (S)-epifluorohydrin were compared to linear response predictions obtained from high levels of density functional theory, with a self-consistent isodensity polarizable continuum model (SCI-PCM) being enlisted to unravel the role of solvation. For all of the targeted species, environmental perturbations were found to affect chiroptical properties in a dramatic fashion, causing solvent-dependent changes to appear in both the magnitude and the sign of ORD curves. These observations could be rationalized in terms of the

TABLE 10. Solution-phase optical rotation of (S)-epifluorohydrin

Excitation wavelength (nm)	Acetonitrile solution		Cyclohexane solution		<i>n</i> -Butyl ether solution	
	Predicted	Measured	Predicted	Measured	Predicted	Measured
355	−7.0	−28.4	−42.9	−88.4	−33.2	−54.5
436	15.1	−3.5	−9.8	−38.3	−3.0	−20.2
589	15.1	3.7	1.2	−13.5	5.0	−5.1
633	13.9	5.0	1.8	−9.3	5.1	−2.6

The B3LYP/aug-cc-pVDZ linear response calculations of optical activity for individual (solvent-free) conformers of (S)-epifluorohydrin have been combined with the Boltzmann weights (25°C) suggested by polarizable continuum models of implicit solvation (SCI-PCM) to predict nonresonant specific rotation parameters (in deg dm^{−1} (g/mL)^{−1}) for three model solvents. These ensemble-averaged results are in reasonable accord with experimental findings, reproducing the basic trends observed in solution-phase polarimetric measurements.

differential stabilization experienced by three stable, low-lying conformers, which, in turn, make distinct and, often-times, antagonistic contributions to the overall (ensemble-averaged) optical activity.

For the flexible epoxides probed by the current work, direct comparison of solution-phase and vapor-phase values of specific rotation has revealed that a weakly-polar liquid medium (e.g., cyclohexane) provides the best mimic for isolated-molecule behavior. This assertion would appear to corroborate long-established tenets of chemical intuition; however, prior investigations of nominally-rigid chiral compounds surprisingly have found that solvents of high dielectric constant (e.g., acetonitrile) most favorably reproduce the chiroptical response measured under rarefied (gas-phase) conditions.¹⁴ The obvious disparity between these conclusions can be attributed to the conformational dynamics that characterize the targeted epoxide systems, as elucidated by combining calculations of the intrinsic optical rotation for each structural isomer with Boltzmann-weighted estimates for their relative populations. In the case of nonspecific solvation, strong electrostatic coupling to the environment has been implicated as a primary driving force for the preferential stabilization of conformers and, consequently, for the pronounced solvent dependence observed in the attendant chiroptical response.

The present analyses have proven to be moderately successful at reproducing laboratory measurements; however, several aspects of the highlighted computational approach demand additional consideration. Underlying all of these efforts are the use of particular quantum chemical methods and basis sets, with a more systematic exploration of these choices being needed to assess the quality of resulting equilibrium structures, relative (free) energies, and optical rotation parameters. While the restriction of calculations to localized conformer geometries greatly facilitates evaluation of ensemble-averaged properties, this approximation has excluded vibrational degrees of freedom and their potentially important influence upon chiroptical effects.^{25,35–39,61,62} Likewise, our theoretical description of solvation has been limited to an implicit (continuum dielectric) model that neglects the detailed nature of solute–solvent interactions in the cybotactic region. This treatment has enabled solvent-mediated changes in conformer structures and populations to be estimated, but attendant modifications in chiroptical behavior caused by the surrounding medium (as reflected in the dynamical response of the solvent to oscillating electric and magnetic fields)⁵¹ have not been taken into account. All of these issues and their ramifications are the subject of ongoing investigations. Nevertheless, it is hoped that the concerted experimental and theoretical work reported in this paper will motivate more extensive studies of chiral solvation phenomena and foster further development of reliable ab initio paradigms for the prediction of nonresonant optical activity.

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Chiral NMR Discrimination of Amines: Analysis of Secondary, Tertiary, and Prochiral Amines Using (18-Crown-6)-2,3,11,12-tetracarboxylic Acid

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT Enantiomeric discrimination is observed in the ^1H and ^{13}C NMR spectra of secondary and tertiary amines in the presence of (–)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (**1**). Nonequivalence of the resonances of prochiral nuclei in primary and secondary amines is also observed when they associate with **1**. The amines are added in their neutral form and are protonated by the carboxylic acid groups of **1** to produce the corresponding ammonium and carboxylate ions. Secondary amines associate with **1** through two hydrogen bonds and an ion pair interaction. Tertiary amines can only form one hydrogen bond to accompany the ion pairing. Chiral discrimination in the ^1H and ^{13}C NMR spectra of a series of aryl-containing secondary amines is of sufficient magnitude to determine enantiomeric purities. The discrimination in the spectra of tertiary amines with **1** is smaller, but ^{13}C NMR spectra provided enough distinction for the determination of enantiomeric purity. *Chirality* 20:370–378, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: chiral discrimination; NMR; crown ethers; secondary amines; tertiary amines; prochiral amines

INTRODUCTION

NMR spectroscopy is one of the most common techniques used for the analysis of chiral compounds. Chiral derivatizing agents, solvating agents, metal complexes, or liquid crystals are employed in NMR applications.^{1–6} Chiral derivatizing agents react with the substrate to form a covalently bonded compound. Provided no kinetic resolution or racemization occurs in the derivatization reaction, the distinction in the NMR spectrum of the resulting diastereomers can often be used to determine ee or assign absolute configuration. Chiral solvating agents and metal complexes are much simpler to use than derivatizing agents as they are typically added to the substrate in the NMR tube and associate through non-covalent interactions. Kinetic resolution and racemization are not a concern with chiral solvating agents, and the chemical shift anisotropy of the resulting diastereomeric complexes can be used to determine ee and to sometimes assign absolute configuration. Chiral liquid crystals adopt preferred alignments in a magnetic field. Pairs of enantiomers often exhibit different packing orders in the liquid crystal leading to differences in dipolar or quadrupolar coupling constants. Liquid crystals are applicable to many families of compounds, although assigning resonances in the NMR spectra of compounds dissolved in liquid crystals can be challenging because of the different coupling constants that occur.⁷

Cavity compounds such as cyclodextrins and crown ethers have been widely utilized as chiral NMR solvating

agents. By altering the substituent groups on the cyclodextrin, derivatives that associate with a wide variety of substrate compounds in both organic and aqueous solvents have been developed.⁸ Crown ethers, the most common of which have an 18-crown-6 unit, are primarily known for their ability to form host–guest complexes with and distinguish chiral primary amines.^{9–10} The primary amine is analyzed as its protonated ammonium ion. The NH_3^+ unit forms three hydrogen bonds to oxygen atoms of the 18-crown-6 unit as shown in Figure 1a. Many chiral crown ethers have been studied as liquid chromatographic stationary phases and several of these are commercially available. Among those studied as chiral NMR solvating agents, (18-crown-6)-2,3,11,12-tetracarboxylic acid (**1**) is noteworthy. The enantiomeric discrimination in the NMR spectra of primary amines with **1** is generally larger than that with other chiral crown ethers.^{11–15} Furthermore, **1** is the only commercially available chiral crown ether for use as a chiral NMR solvating agent.

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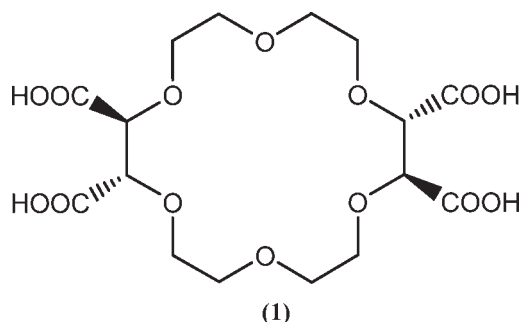
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Secondary amines generally bind poorly to 18-crown-6 ethers because the protonated ammonium ion can only form two hydrogen bonds with oxygen atoms of the crown and steric effects about secondary amines hinder association with the crown. The use of a much larger pseudo-24-crown-8 ring provided a cavity that was large enough to accommodate secondary amines, but this reagent is not commercially available and its synthesis is rather involved.^{16–17} Alternatively, a specialized receptor compound for secondary amines has been reported, but this compound is not commercially available.^{18,19} We recently showed that **1** is an effective chiral NMR solvating agent for secondary amines, especially pyrrolidines, piperidines, and piperazines.^{20–22}

In this report we extend the application of **1** to a series of secondary amines with aryl substituent groups. We further show that **1** can be used to distinguish prochiral nuclei in primary and secondary amines. Finally, we demonstrate that **1** binds with tertiary amines to produce a small amount of enantiomeric discrimination in the resulting NMR spectrum. Analyses are performed by recording the ¹H and/or ¹³C NMR spectra.

EXPERIMENTAL

Reagents

All substrates and deuterated NMR solvents were obtained from commercial suppliers and used as received. The (–)-isomer of **1** was obtained from Sigma-Aldrich.

Instrumentation

All ¹H (16 scans, sweep width of 20.7 ppm, digital resolution of 18, pulse angle of 30°, delay time of 1 sec) and ¹³C spectra (4096 scans, sweep width of 238.3 ppm, digital resolution of 17, pulse angle of 30°, delay time of 1 sec) were collected on a 400 MHz NMR spectrometer. Spectra were run in methanol-*d*₄ at ambient probe temperature and calibrated using tetramethylsilane as an internal reference. When necessary, assignments were confirmed using 2D-COSY spectra.

Procedures for Chiral Discrimination Studies

Solutions of the chiral substrates (10 mM) in methanol-*d*₄ with 0.05% TMS were prepared, and enriched with one of the enantiomers when available. Increments of **1** were

added to the sample by appropriately sized aliquots of a 0.3 M stock solution in methanol-*d*₄.

RESULTS AND DISCUSSION

The substrates examined herein are mixed with **1** as neutral amines. An acid-base neutralization reaction occurs between the substrate and **1** to produce the corresponding ammonium and carboxylate ions. Large shifts to higher frequencies of the resonances of hydrogen atoms α to the nitrogen atom in all of the secondary and tertiary amines examined herein provide evidence that a neutralization reaction occurs. For secondary amines, the neutralization reaction provides a situation in which the substrate can interact with **1** through three points of association, as shown in Figure 1b. Two of these involve hydrogen bonds between NH groups and oxygen atoms of the crown. The third is an ion pair between the ammonium and carboxylate group. The ion pairing interaction is important in facilitating association of secondary protonated amines and **1** and causing the large enantiomeric discrimination in the NMR spectrum. The spectra in Figure 2 and data in Table 1 for mixtures of neutral ephedrine (**2**) (Fig. 3) and the ephedrinium ion (as ephedrine hydrochloride) with **1** demonstrate the importance of the ion pairing interaction. The term ΔΔδ is used to denote the difference in chemical shift between the resonances of the two enantiomers. The large shifts to higher frequency that occur on mixing **2** with **1** are seen in Figure 2b and provide evidence that a neutralization reaction occurs. More important is the substantial enantiomeric discrimination of the C-methyl, *N*-methyl, and methine resonances (Figs. 2b–2d). The reso-

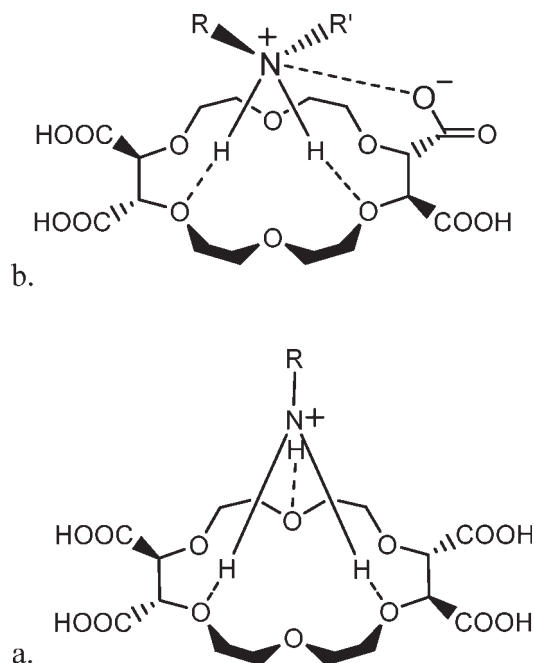


Fig. 1. Association geometries of the ammonium salts of (a) protonated primary amines with neutral **1** and (b) protonated secondary amines with the carboxylate anion of **1**.

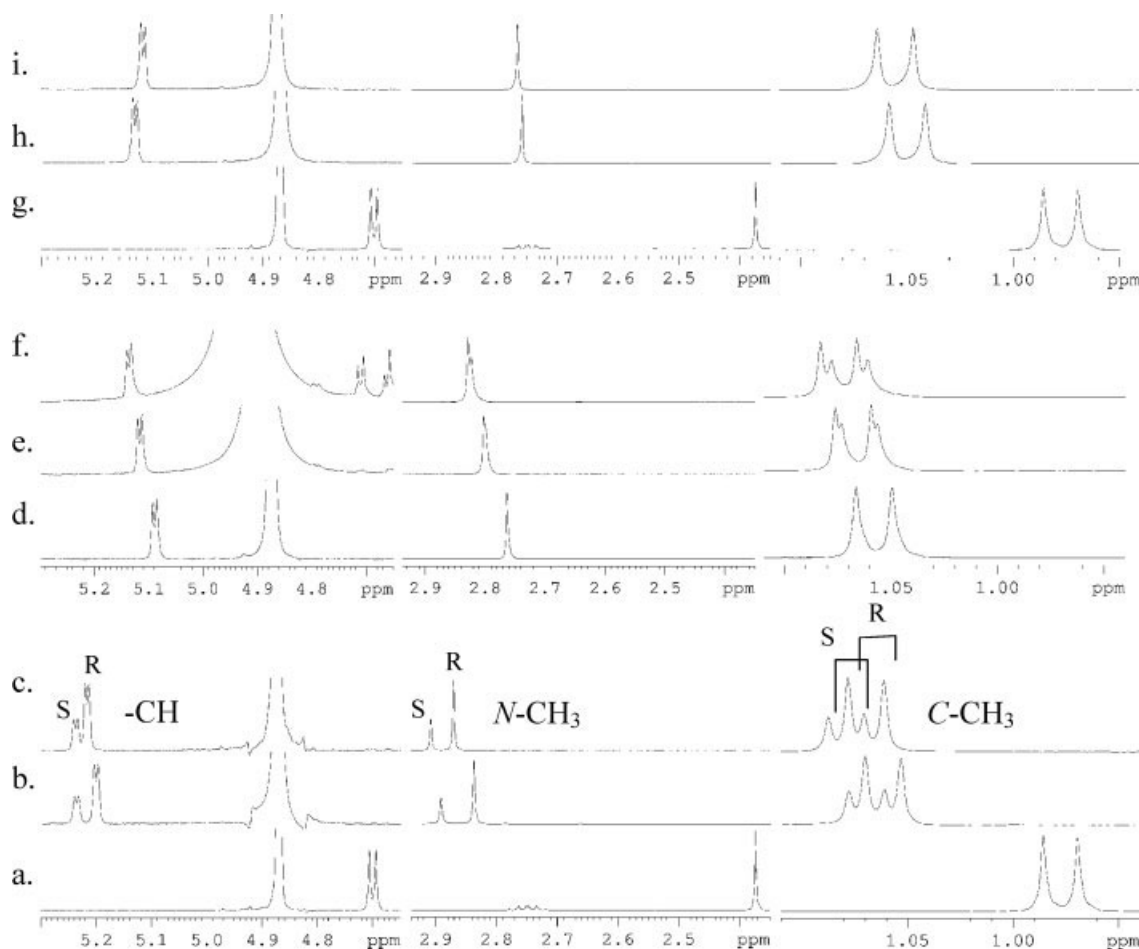


Fig. 2. The ^1H NMR (400 MHz) of the C-methyl, N-methyl, and CHOH resonances of (a) ephedrine with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM; and (e) ephedrine-HCl with **1** at (f) 30 mM, and (g) 60 mM; and (h) ephedrine with tartaric acid at (i) 10 mM, and (j) 20 mM.

nances in the spectrum of ephedrine hydrochloride are already shifted to higher frequency as seen in Figure 2e. The ephedrinium ion cannot undergo ion pairing with neutral **1** and the resonances show only modest enantiomeric discrimination in the resulting NMR spectrum (Figs. 2f and 2g), and require much higher concentrations of **1** to achieve any distinction.

Mixing tartaric acid, the chiral component on which **1** is based with secondary amines leads to substantial shifts to higher frequency in the NMR spectrum because of an acid-base neutralization reaction as seen in the spectrum of **2** in Figure 2h. The spectrum of **2** with tartaric acid does not show any enantiomerically nonequivalent resonances (Figs. 2i and 2j), indicating that the crown moiety of **1** is necessary for chiral NMR discrimination.

For tertiary amines, association with **1** can only involve one hydrogen bond and the ion pairing interaction. Steric or electrostatic effects between the substituent groups of the tertiary amine and **1** provide the potential for a third point of interaction required for chiral recognition.²³ Relatively modest enantiomeric discrimination is observed in the ^1H and/or ^{13}C NMR spectra of most tertiary amines we examined in the presence of **1**. Therefore tertiary amines associate with **1** and the substituent groups of the

two enantiomers must interact differently with the crown ether.

When using **1** as a chiral NMR solvating agent, we have found that it is best to record a series of spectra in which the concentration of **1** is gradually increased relative to the concentration of substrate. Using a substrate concentration of 10 mM, spectra with concentrations of **1** at 5, 10, and 20 mM are typically recorded. In many cases, the spectrum with 5 mM of **1** exhibits substantial broadening, which is indicative of an intermediate rate of crown-substrate exchange. The spectrum in Figure 4b of two of the alkene resonances of *N*-allyl- α -methylbenzylamine (**3**) with **1** shows a representative example of the broadening that occurs. The broadening usually goes away when the concentration of **1** is raised to 10 mM or higher, indicating that the system is now under conditions of fast exchange. The spectra in Figures 4c and 4d for **3** demonstrate the reduction in broadening that is observed. Enantiomeric discrimination is often greater at higher concentrations of **1**, although this is not always the case as seen by comparing the spectra for **3** in Figures 4c and 4d.

The series of spectra for **2** with **1** (Figs. 2a–2d) further demonstrate the value of obtaining a series of spectra at different crown-substrate ratios. In this example, excellent

TABLE 1. Enantiomeric discrimination ($\Delta\Delta\delta$ —ppm) in the ^1H NMR spectra (400 MHz) of secondary benzyl amines (10 mM) with **1** in methanol- d_4

Substrate	Proton	$\Delta\Delta\delta$	1:Substrate
Ephedrine (2)	CH—OH	0.036	0.5:1
	C—CH ₃	0.017	1:1
	N—CH ₃	0.057	0.5:1
Ephedrine HCl (2)	C—CH ₃	0.004	4:1
	N—CH ₃	0.004	4:1
<i>N</i> -Allyl- α -methylbenzylamine (3)	<i>ortho</i>	0.019	1:1
	CH ₃ CH	0.027	1:1
	CH ₃	0.013	2:1
	H ₅	0.113	2:1
	H ₆	0.028	1:2
α,α -Di(2-naphthyl)pyrrolidine methanol (4)	H ₄	0.068	2:1
	H ₇	0.041	2:1
	H ₉	0.025	2:1
	H ₁₁	0.026	2:1
α,α -Diphenylpyrrolidinemethanol (5)	H ₇	0.005	2:1
	H ₁₁	0.026	2:1
2-(Diphenylmethyl)pyrrolidine (6)	H ₅	0.137	2:1
	H ₇	0.019	2:1
	H ₁₄	0.019	2:1
<i>cis</i> -2-Benzylaminocyclohexanemethanol (7)	H ₂	0.033	2:2
	<i>ortho</i>	0.077	0.5:1
2- <i>tert</i> -Butylamino-1-phenylethanol (8)	CH—OH	0.008	1:1
	<i>t</i> -butyl	0.003	0.5:1
	<i>ortho</i>	0.016	0.5:1
Dimethylbenzylamine (9)	C—CH ₃	0.026	2:1
	N—CH ₃	0.043	2:1
	CH—CH ₃	0.045	2:1
<i>N</i> -Methyl 1-(1-naphthyl)ethylamine (10)	C—CH ₃	0.021	0.5:1
	N—CH ₃	0.065	0.5:1
	CH—CH ₃	0.008	2:1
	H ₂	0.073	1:1
	H ₈	0.012	0.5:1
<i>N</i> -Benzyl- α -methylbenzylamine (11)	CH ₃	0.013	2:1
	H ₄	0.020	2:1
	H ₄ '	0.016	2:1
	N—CH ₃	0.025	0.5:1
α -Methylaminomethylbenzyl alcohol (12)	CH—OH	0.035	1:1
	H ₃	0.064	2:1
2-Benzylamino-1-phenylethanol (13)	H ₃ '	0.113	2:1
	H ₄	0.138	1:1
	H ₄ '	0.007	1:1

enantiomeric discrimination with minimal broadening is observed for a crown-substrate ratio of 0.5. Increasing the concentration of **1** relative to the substrate causes a diminishment in the nonequivalence of the methine and *N*-methyl resonances, while causing an increase in the nonequivalence of the *C*-methyl resonance. Enantiomeric recognition with chiral solvating agents can involve two possible mechanisms. One involves the diastereomeric nature of the two crown-substrate complexes and the fact that the diastereomers can have different chemical shifts. The second is that the two enantiomers often have different association constants with the chiral solvating agent. One enantiomer has a higher proportion in the bound form than the other, such that different time-averaged solvation environments occur. A distinction caused by different association constants is likely to be more pronounced

when the two substrate enantiomers compete for binding to the solvation agent. Unfortunately, we are not able to measure association constants of the substrates with **1** using common methods, because of the presence of neutralization and association processes in this system.²¹

The diminishment in enantiomeric discrimination observed for the alkene resonances of **3** (Fig. 4) and *N*-methyl and methine resonances of **2** (Fig. 2) at higher crown-substrate ratios suggests that the chiral recognition with these nuclei is dominated by differences in association constants of the two enantiomers. Raising the concentration of **1** then reduces the importance of this effect by reducing competition for the crown ether. The reverse behavior of the *C*-methyl resonance of **2** as a function of crown-substrate ratio suggests that chiral recognition of this nucleus is dominated by different chemical shifts of

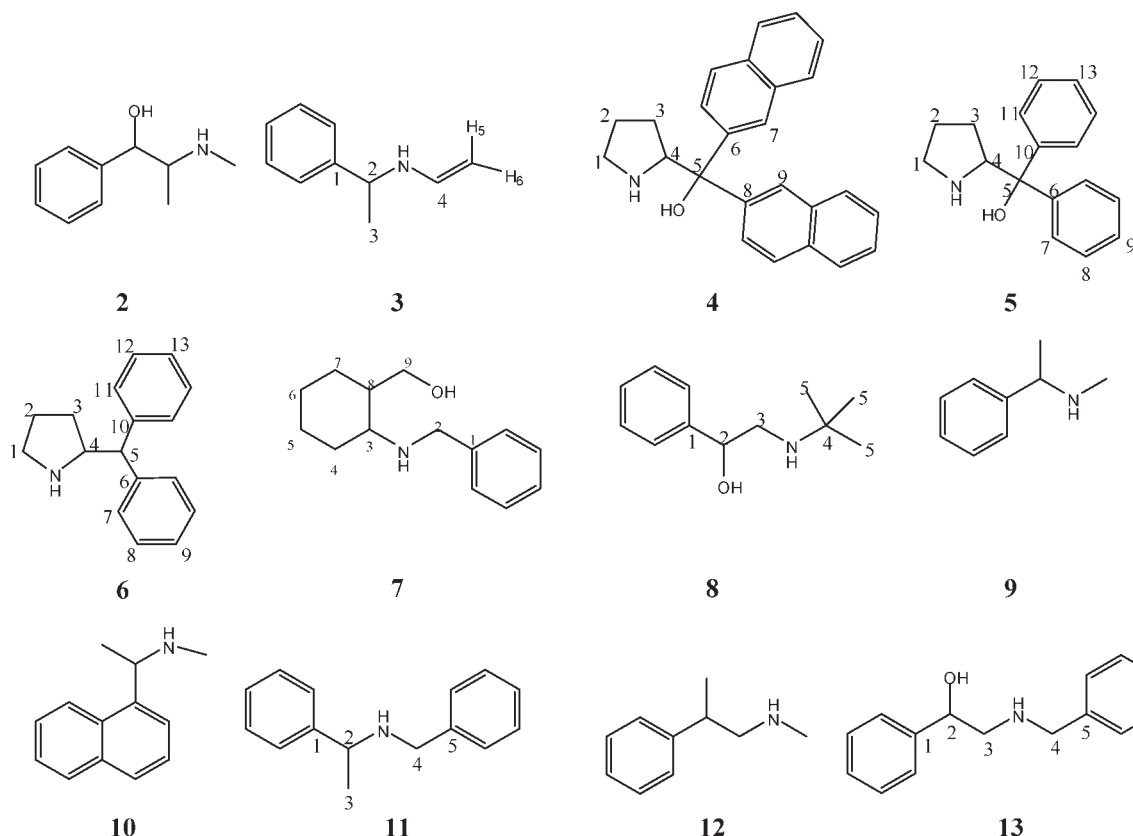


Fig. 3. Structures of the secondary aryl-containing amines.

the two diastereomeric complexes. Nonequivalence values herein are reported for the optimal separation of resonances and the crown–substrate ratios are indicated in the tables.

Analysis of Aryl-Containing Secondary Amines

We have previously shown the utility of **1** as a chiral NMR solvating agent for pyrrolidines,²¹ piperidines, and piperazines.²² Only in the case of a sterically hindered sub-

strate such as 2,2,5,5-tetramethyl-3-pyrrolidine carboxylate, which has four substituent groups α to the nitrogen atom, was the enantiomeric discrimination negligibly small (0.018 ppm at a 2:1 crown–substrate ratio).²¹ All other substrates had substantial enantiomeric nonequivalence in the ^1H and/or ^{13}C NMR spectra. Herein we report ^1H and ^{13}C NMR data for a series of aryl-containing secondary amines with **1** (Fig. 3 and Table 1). Certain resonances of compounds **4**, **5**, and **6** exhibit substantial enantiomeric discrimination in their NMR spectra and indicate the extent

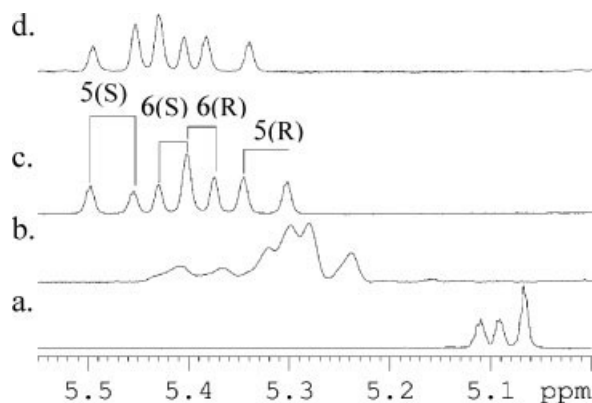


Fig. 4. ^1H NMR spectra (400 MHz) of the allyl group of **3** with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM.

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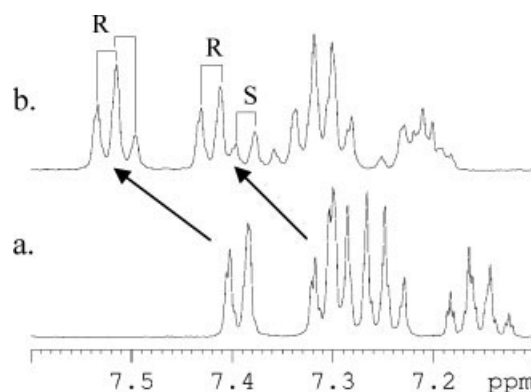


Fig. 5. (a) The ^1H NMR (400 MHz) of the *ortho*-hydrogens of **6** with **1** at (b) 20 mM.

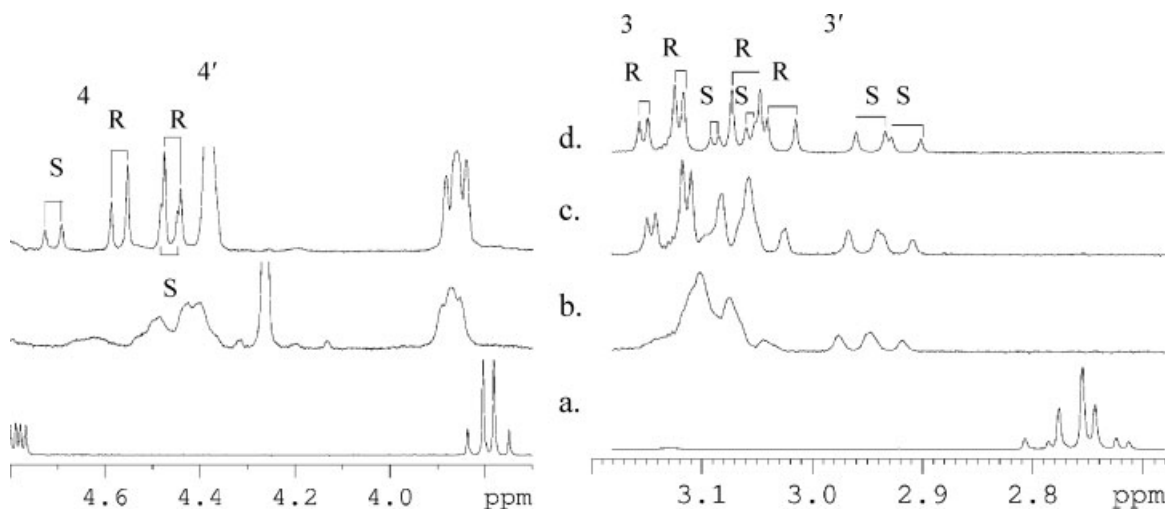


Fig. 6. (a) The ^1H NMR (400 MHz) resonances of the diastereotopic protons of **13** (enriched in the (*R*)-enantiomer) with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM.

to which potential steric hindrance from the phenyl and naphthyl rings does not inhibit association with **1**. Compounds **7** and **8** have bulky cyclohexyl and *tert*-butyl groups at the α -position, whereas **2**, **3**, **9**, **10**, and **11** have methyl or hydroxyl groups at the α -position. These substrates also associate with **1** as evidenced by the presence of enantiomerically distinct resonances in their ^1H and/or ^{13}C NMR spectra. The enantiomeric discrimination in the spectrum of **8** in the presence of **1** is small for the *tert*-butyl and methylene resonances, but the large nonequivalence of the *ortho*-hydrogen resonances of the aromatic ring is suitable for determining ee.

The aromatic rings in **4**, **5**, and **6** are diastereotopic and the H_f resonances of all three exhibit both diastereotopic resolution and enantiomeric discrimination in the presence of **1**. The spectra in Figure 5 provide an excellent example of the nonequivalence observed for a set of these aromatic resonances in the presence of **1**. The aromatic hydrogen nuclei that are enantiomerically discriminated in **4**, **5**, and **6** are five or more bonds removed from the secondary amine site, demonstrating that chiral recognition can occur for relative remote nuclei of the substrate when using **1** as a chiral solvating agent.

Compounds **2**, **3**, **9**, **10**, **11**, and **12** all have *C*- and/or *N*-methyl resonances that are close to the amine group or chiral center and are either singlets or doublets. All of these resonances show rather substantial enantiomeric nonequivalence in the presence of **1** and several are useful for the analysis of ee. The spectra in Figure 6 for **13** provide an example of the enantiomeric nonequivalence that occurs with diastereotopic methylene groups α to the secondary nitrogen atom. Of particular interest are the relative shifts of the 3,3' and 4,4' resonances. For the 3,3' pair of diastereotopic nuclei, the signal for the (*R*)-enantiomer of both is at higher frequency than that of the (*S*)-enantiomer. For the 4,4' pair, the signal of the (*S*)-enantiomer of both is at higher frequency than that of the (*R*)-enantiomer. A reversal of shift order for nuclei of the same sub-

strate implies that the diastereomeric nature of the crown-substrate complexes is more significant than differences in association constants in determining the relative shift order. A similar observation occurs with **3** (Fig. 4) as the shift order of the enantiomers for the methyl and alkene resonances is reversed.

We recorded the ^{13}C NMR spectra of several aryl-containing secondary amines in the presence of **1** and in every case observed rather substantial enantiomeric discrimination for several resonances (Table 2). Nonequivalence on the order of 0.1 ppm or greater is common, and these baseline-resolved ^{13}C resonances are amenable for the analysis of ee. Since analogous ^{13}C nuclei of the two enantiomers may have different relaxation times and NOEs in the presence of a chiral solvating agent, an inverted gated acquisition mode is required when using chiral solvating agents for determining optical purity.

Analysis of Tertiary Amines

We previously examined enantiomeric discrimination in the ^1H NMR spectrum of the dibasic substrate 2-phenyl-4-methylpiperazine, which contains both secondary and tertiary amines, in the presence of **1**.²² A notable observation was the pronounced enantiomeric nonequivalence in the *N*-methyl resonance (0.116 ppm at a crown-substrate ratio of 2). Since the *N*-methyl group is relatively remote from the secondary amine, which was the presumed site of association, the large nonequivalence of the *N*-methyl resonance suggested that the substrate might associate with **1** at both the secondary and tertiary nitrogen atoms. We therefore examined a range of tertiary amines (Fig. 7) to determine whether these did associate with **1** and, if so, the extent to which enantiomeric discrimination is observed in the ^1H and ^{13}C NMR spectra.

The series of spectra for the *N*-methyl resonance of **14** with increasing amounts of **1** is shown in Figure 8. Substantial shifts to higher frequency occur for several resonances of **14** on adding **1**, indicating that protonation of the nitro-

TABLE 2. Enantiomeric discrimination ($\Delta\Delta\delta$, ppm) in the ^{13}C NMR spectra (100 MHz) of secondary benzyl amines (10 mM) with **1 (20 mM) in methanol- d_4**

Substrate	Carbon	$\Delta\Delta\delta$
<i>N</i> -Allyl- α -methylbenzylamine (3)	C1	0.131
	C2	0.174
α,α -Di-(2-naphthyl) pyrrolidine methanol (4)	C2	0.494
	C3	0.238
	C4	0.806
	C5	0.205
α,α -Diphenylpyrrolidine methanol (5)	C2	0.510
	C3	0.230
	C4	0.730
	C5	0.210
	C7	0.160
2-(Diphenylmethyl)pyrrolidine (6)	C8	0.141
	C9	0.145
	C12	0.097
	C1	0.432
	C2	0.082
	C3	0.377
	C4	0.313
	C5	0.143
	C6	0.172
	C7	0.065
	C8	0.042
	C9	0.107
	C10	0.265
<i>cis</i> -2-Benzylaminocyclohexanemethanol (7)	C11	0.136
	C12	0.247
	C13	0.141
	<i>para</i>	0.044
	C1	0.055
<i>N</i> -Benzyl- α -methylbenzylamine (11)	C2	0.110
	C4	0.034
	C7	0.148
	C1	0.085
	C2	0.244
2-Benzylamino-1-phenylethanol (13)	C4	0.100
	C5	0.078
	C1	0.077
	C2	0.279
	C3	0.471
	C4	0.329
	C5	0.063

gen atom occurs through a neutralization reaction. Broadening of the resonances is observed at 1:2 and 1:1 crown-substrate ratios (Figs. 8b and 8c), and diminishes considerably at a 2:1 crown-substrate ratio. The nonequivalence of the methyl singlet of the two enantiomers is apparent in the spectrum in Figure 8d. The enantiomeric discrimination in the *N*-methyl resonance of **15** is substantially less than that of the *N*-methyl resonance of **14**. One possibility is that steric hindrance of the substituent group in **15** limits binding with **1**, although it is also possible that interaction of the substituent group with the carboxylic acid groups of **1** is important in determining chiral recognition.

Using routine one-dimensional ^1H and ^{13}C NMR spectra, any resonances for which observable nonequivalence

occurs are reported in Tables 3 and 4. Only the *N*-methyl resonance of **14** and *C*-methyl resonance of **16** exhibit enough enantiomeric discrimination in the ^1H NMR spectrum such that the nonequivalence is suitable to determine ee. Most of the other tertiary amine substrates have one or more ^1H resonances that exhibit enantiomeric discrimination in the presence of **1**, although the nonequivalence is only a few thousandths of a ppm. This degree of nonequivalence is sufficient to assess whether a sample is optically pure or not, but is not useful for determining ee. Nonequivalence in certain ^{13}C NMR resonances of many of the tertiary amines with **1** is usually on the order of several hundredths of a ppm, which is sufficient to accurately determine ee.

Substrates **16–21** have *N,N*-dimethyl groups that all appeared as one singlet in the ^1H spectrum of the substrate, even though these methyl groups are diastereotopic. In the case of **16**, **18**, **19**, and **20**, addition of **1** to enantiomerically enriched mixtures split the *N*-methyl signal into two singlets of equal area, one for each of the diastereotopic methyl groups. No evidence for enantiomeric discrimination of the *N*-methyl signals of **16**, **18**, **19**, and **20** is observed in the presence of **1**. For enantiomerically enriched mixtures of **17** and **21** in the presence of **1**, the *N*-methyl signal split into two resonances whose area correlated with the degree of enrichment. The values of the enantiomeric discrimination for the *N*-methyl resonances of **17** and **21** are reported in Table 3.

Analysis of Substrates with Prochiral Nuclei

Compounds **22–26** (Fig. 9) are achiral but have nuclei that are prochiral. Prochiral nuclei are formally nonequivalent when the substrate is bound to an optically pure chiral solvating agent. The ability of chiral solvating agents to render prochiral nuclei nonequivalent in the resulting NMR spectrum has been noted with many other systems.²⁴

2-Aminopropane (**22**) and 2-aminopentane (**23**) provide an interesting comparison. The methyl resonances in the ^1H NMR spectrum of **22** (10 mM) do not split at concentrations of **1** from 5 to 20 mM. By contrast, the methyl resonances in the ^1H NMR spectrum of **23** do split as seen in Figure 10 and Table 5. The methyl groups of **23** extend further from the amine than those in **22** and likely undergo greater interaction with the chiral portion of **1**, thereby accounting for the enhanced nonequivalence. The prochiral methyl groups of **22** are distinguished in the ^{13}C NMR spectrum of **22** (10 mM) in the presence of **1** (20 mM) (Table 5).

The methylene hydrogen atoms of ethylmethylamine (**24**) and methyl groups of *cis*-2,6-dimethylpiperidine (**25**) and *cis*-2,6-dimethylpiperazine (**26**) are prochiral. Addition of **1** to **24**, **25**, and **26** caused a nonequivalence of the resonances of these prochiral groups (Table 5). The series of spectra of **26** with **1** are shown in Figure 11. In this example, the resonances show continuous shifts to higher frequency with increasing concentrations of **1**. This behavior is quite different from the observations with substrates that contain only a single nitrogen atom, and likely indicates that both nitrogen atoms of **26** eventually become

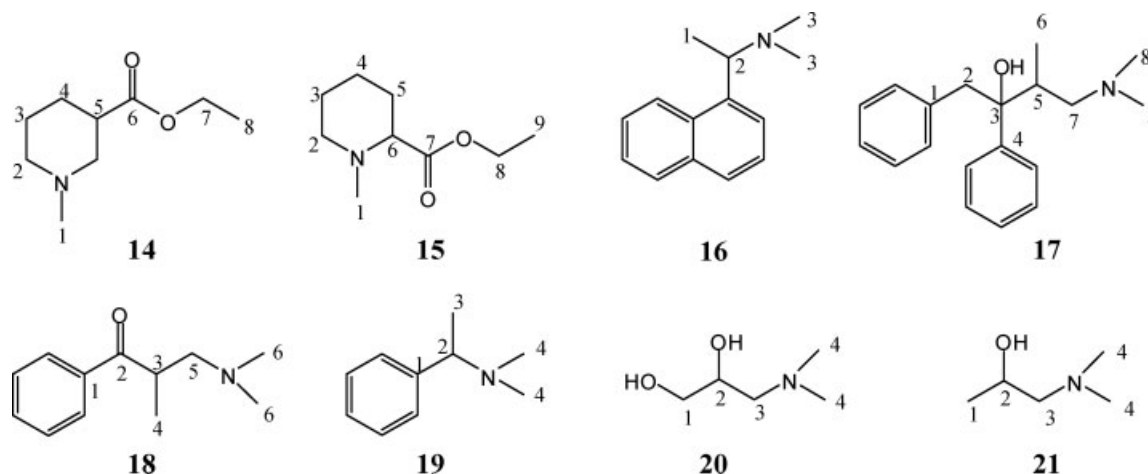
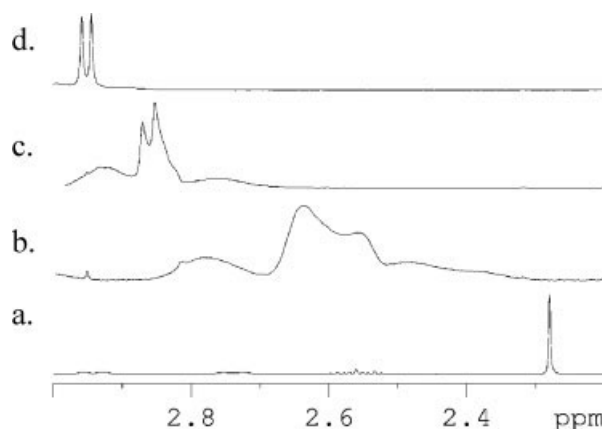


Fig. 7. Structures of the tertiary amines.

Fig. 8. (a) The ^1H NMR (400 MHz) spectra of the *N*-methyl resonance of **14** with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM.TABLE 3. Enantiomeric discrimination ($\Delta\Delta\delta$, ppm) in the ^1H NMR (400 MHz) spectra of the tertiary amines (10 mM) with **1** in methanol- d_4

Substrate	Proton	$\Delta\Delta\delta$	1:Substrate
Ethyl 1-methyl-3-piperidinecarboxylate (14)	NCH_3	0.028	2:1
	H_7	0.004	2:1
	H_8	0.002	2:1
Ethyl 1-methylpipecolate (15)	NCH_3	0.003	2:1
	H_8	0.005	2:1
<i>N,N</i> -Dimethyl-1-(1-naphthyl) ethylamine (16)	CCH_3	0.017	1:1
4-(Dimethylamino)-3-methyl-1,2-diphenyl-2-butanol (17)	CCH_3	0.010	0.5:1
	NCH_3	0.004	2:1
3-Dimethylamino-2-methylpropionophenone (18)	H_5	0.003	2:1
	H_5'	0.009	2:1
1-(Dimethylamino)-2-propanol (21)	H_3	0.004	2:1
	H_3'	0.008	1:1
	NCH_3	0.007	2:1

TABLE 4. Enantiomeric discrimination ($\Delta\Delta\delta$, ppm) in the ^{13}C NMR spectra (100 MHz) of tertiary amines (10 mM) with **1** (20 mM) in methanol- d_4

Substrate	Carbon	$\Delta\Delta\delta$
<i>N,N</i> -Dimethylnaphthylethylamine (16)	C_3	0.078
	arom	0.046
	arom	0.066
	arom	0.036
4-(Dimethylamino)-3-methyl-1,2-diphenyl-2-butanol (17)	C_1	0.022
	C_3	0.029
1-(Dimethylamino)-2-propanol (21)	C_3	0.129
	C_4	0.077
3-(Dimethylamino)-1,2-propanediol (20)	C_1	0.028
	C_2	0.020
	C_3	0.119

protonated at high enough concentrations of **1**. The spectra of these prochiral substrates indicate that **1** is a useful chiral NMR solvating reagent for the analysis of prochiral nuclei in primary and secondary amines.

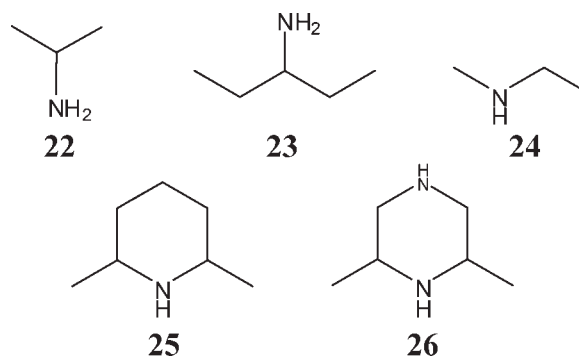


Fig. 9. Structures of the prochiral substrates.

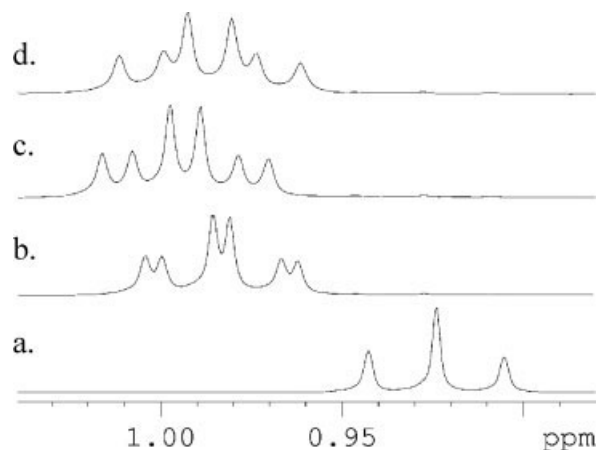


Fig. 10. The ^1H NMR (400 MHz) of the methyl group of **23** with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM.

TABLE 5. Nonequivalence ($\Delta\Delta\delta$, ppm) in the ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra of prochiral amines (10 mM) with **1** in methanol- d_4

Substrate	Proton/Carbon	$\Delta\Delta\delta$	1:Substrate
Isopropyl amine (22)	CH_3 (^{13}C)	0.158	2:1
1-Ethylpropylamine (23)	CH_3 (^1H)	0.012	2:1
<i>N</i> -Ethylmethylamine (24)	CH_2 (^1H)	0.008	3:1
<i>cis</i> -2,6-Dimethylpiperidine (25)	CH_3 (^1H)	0.006	0.5:1
<i>cis</i> -2,6-Dimethylpiperazine (26)	CH_3 (^1H)	0.012	2:1

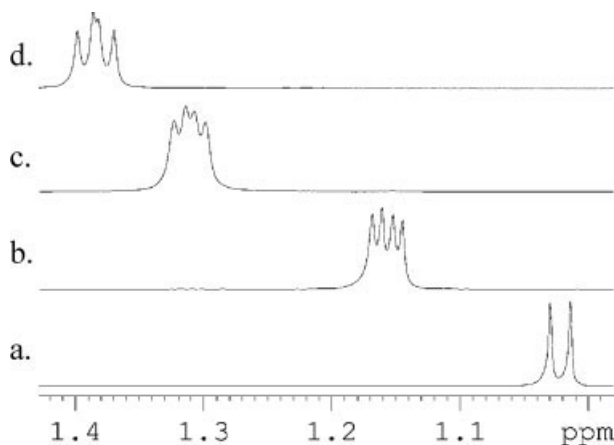


Fig. 11. The ^1H NMR (400 MHz) of the methyl groups of **26** with **1** at (b) 5 mM, (c) 10 mM, and (d) 20 mM.

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Circular Dichroism of Diglycosyl Dichalcogenides in Solution and Solid State

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT Solution and solid-state CD spectra of nine peracetylated and deacetylated diglycosyl disulfides were measured to study the relationship between the low-energy CD transitions ($n_1 \rightarrow \sigma_{S-S}^*$ and $n_2 \rightarrow \sigma_{S-S}^*$) and helicity of the inherently chiral disulfide chromophore as perturbed by chiral carbohydrate moieties. The solid-state CD spectra were directly correlated with the reported X-ray structures of Ac₄GlcSSGlcAc₄ and Ac₄GlcSSGalAc₄, and the CD data revealed that all the disulfides have *M* helicity with C1–S–S–C1' dihedral angles $-90^\circ < \varphi < 0^\circ$ both in solution and in the solid state. A TDDFT CD study was carried out on dimethyl diselenide which confirmed that the same quadrant rule is relevant between the signs of the low-energy CD transitions and the diselenide torsional angle as formulated previously for the disulfide chromophore. The CD spectra of Ac₄GlcSeSeGlcAc₄ measured in solution and in the solid state were correlated with its X-ray structure and reproduced well by TDDFT CD calculations performed on its tetra-*O*-acetyl derivative. *Chirality* 20:379–385, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: disulfide; diselenide; solid-state CD; TDDFT CD calculation

INTRODUCTION

The disulfide linkage is an important structural motif to define and maintain the tertiary structures of proteins and oligopeptides; it was, however, virtually nonexistent within carbohydrates of either synthetic or natural origin. This type of binding gained importance in carbohydrate chemistry with the synthesis of novel types of neoglycoconjugates via attachment of glycosyl units to proteins through S–S linkages.^{1–3}

The disulfide bond may, on the other hand, be regarded as a representative of a special connection between two sugar units via a *three-bond interglycosidic linkage* incorporating two heteroatoms.⁴ The importance of such unusual type of glycosidic linkage was emphasized by the example of the calicheamicin–esperamicin family of antibiotics incorporating a hydroxylamine bond as an interglycosidic connection in the oligosaccharide part of the molecules. These molecules are endowed with potent antitumor activity which is due to specific cleavage of double-stranded DNA.⁵ Tight binding of the drug in the minor groove of the DNA is ensured by the carbohydrate part due to a specific, preferred conformation of the N–O interglycosidic linkage.⁶

Symmetric carbohydrate disulfides^{7,8} formed through oxidation of 1-thioaldoses have been known for a long time and symmetric diglycosyl diselenides can be also obtained similarly.⁹ Unsymmetrical diglycosyl disulfides, containing two different monosaccharide units connected by a disulfide linkage, were unknown until recently. Two

of us reported the first syntheses of derivatives wherein two glycopyranosyl units are connected through a disulfide bridge via their anomeric carbons.¹⁰ Pinto and co-workers recently described disaccharides with disulfide bonds between anomeric and nonanomeric carbons.¹¹ These derivatives are of significant interest as novel carbohydrate scaffolds with potential biological activity.

The disulfide or diselenide chromophore in R–Z–Z–R structures (with Z = S, Se, or Te, named generally as dichalcogenides) is inherently chiral due to the preferred torsional angle φ of the R–Z–Z–R bonds (*P* or *M* helicity, Fig. 1a) which tends to assume values near to $\pm 90^\circ$.

If R is an achiral group, the two forms are enantiomeric and the compounds R–Z–Z–R are optically inactive in solution, since the *P* and *M* forms are isoenergetic and in general fast-exchanging, and hence their ratio is 1:1 at the equilibrium.¹² However, Shimizu and coworkers were able to isolate the *P*-helical enantiomers of diphenyl dichalcogenides (R = Ph, Z = S, Se, Te) from their optically inactive solutions by preferential crystallization.¹³ If R is a chiral

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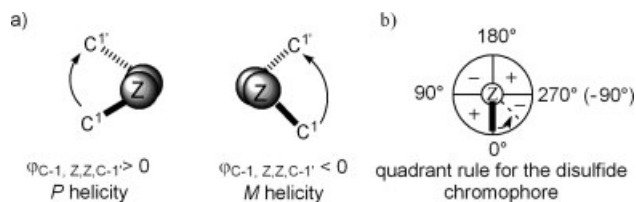


Fig. 1. (a) Definition of *P* and *M* helicity by the C1–Z–Z–C1' dichalcogenide torsional angle. (b) Quadrant rule between the sign of the lowest energy $n \rightarrow \sigma^*_{S-S}$ CD transition and the C1–S–S–C1' torsional angle.

substituent, as in diglycosyl dichalcogenides, the *P* and *M* forms are diastereomers, and hence their solution is optically active, since their ratio deviates from 1:1 at the equilibrium. With crystallization, the equilibrium can be shifted toward the diastereomer that crystallizes faster (in a crystallization-induced asymmetric transformation fashion), which enables its isolation and structural study by X-ray diffraction and solid-state CD.

The relation between the geometry of the disulfide linkage and the appearance of CD spectra of chiral disulfides in the low-energy region is well established.^{12,14} In the 220–300 nm range, two Cotton effects of opposite sign are observed, appearing as a couplet-like feature, allied to the $n_1 \rightarrow \sigma^*_{S-S}$ and $n_2 \rightarrow \sigma^*_{S-S}$ transitions of the inherently chiral disulfide chromophore. The n_1 and n_2 molecular orbitals represent the anti-phase and in-phase combinations of the two *p*-type sulfur lone-pair atomic orbitals.¹⁵ The relative energy of the two MO's, and consequentially the transition energies of the “couplet” components and the absolute sign of the latter, depend on the value assumed by the C–S–S–C dihedral angle φ . For acute dihedrals ($-90^\circ < \varphi < 90^\circ$), the anti-phase A-symmetric MO n_1 is the HOMO, thus the first Cotton effect at longer wavelength is allied to $n_1 \rightarrow \sigma^*_{S-S}$ transition; its sign is positive for *P* helicity ($0 < \varphi < 90^\circ$) and negative for *M* helicity ($-90^\circ < \varphi < 0^\circ$) of the disulfide torsion. For obtuse dihedrals ($\varphi < -90^\circ$ or $\varphi > 90^\circ$), the in-phase B-symmetric MO n_2 becomes the HOMO so the couplet sign is reversed. At $\varphi \approx 90^\circ$ (where n_1 and n_2 are degenerate), the overall CD shape should depend on higher-order mechanisms of optical activity (perturbations due to surrounding chirotopic elements) rather than to the intrinsic chirality of the disulfide chromophore. This picture has been consistently derived at various levels of theory by Linderberg and Michl,¹⁶ Woody,¹⁷ and Snatzke,¹⁵ and nicely fits experimental observations;^{14,18,19} it may also be expressed in terms of the quadrant rule first formulated by Linderberg and Michl,¹⁶ which correlates the sign of the first, longest-wavelength CD transition with the disulfide helicity (Fig. 1b). The second transition is instead more sensitive to perturbation by other chromophores or solvents and was thus not recommended for diagnostic purpose.¹⁶

In contrast, no calculation had been available for a long time on the diselenide chromophore, but the correlations obtained with chiral disulfides were supposed to be applicable to diselenides as well.^{13,20–22} Only recently has a systematic study appeared describing the chiroptical properties of several dichalcogenides (Ch = S, Se, Te) on a firm theoretical background, based on the symmetry adapted

cluster–configuration interaction (SAC-CI) theory.²³ We are rather interested in applying Time-Dependent Density Functional Theory (TDDFT) method to the prediction of CD of diselenides, especially since TDDFT studies of this class of compounds are scarce in the literature.²⁴

To examine the effect of chiral saccharide moieties on the helical preference in diglycosyl dichalcogenides and the correlation between the characteristic CD bands and the helicity of the inherently chiral dichalcogenide chromophore, the CD spectra of symmetrical and unsymmetrical disulfides and a symmetrical diselenide derivative were studied both in solution and solid state. The solid-state results were checked against X-ray structures when available and, in the case of the diselenide, supported with TDDFT calculations on an input structure derived from the solid-state geometry, in analogy with the solid-state TDDFT CD method.^{25–28}

MATERIALS AND METHODS

The CD spectra were recorded on a J-810 spectropolarimeter. For solid-state CD protocol, see Refs. 25–28. The effect of the rotation-dependent macroscopic anisotropy of the KCl disc was checked by sample rotation; CD spectra were recorded with 0° , 90° and 180° , 270° rotations about the propagation of light (*Z* axis) which resulted in near identical spectra. The compounds studied were prepared by literature procedures.^{10,29,30}

Computational Section

DFT and TDDFT calculations were run with Gaussian'03W, Revision B.05, (Gaussian, Pittsburgh PA, 2003). The functionals employed were the pure GGA functional BP86 and the hybrid functionals B3LYP and BH&HLYP with 20 and 50% HF exchange character, respectively.[†] The basis sets employed were: (1) for DFT geometry optimizations, the native 6-31G(d) and 6-311+G(d,p) basis sets; (2) for TDDFT calculations, the native TZVP, 6-311+G(d,p) and cc-pVTZ basis sets,[†] and the “aug-TZVP” obtained by augmenting TZVP with diffuse functions on C and Se atoms taken from the aug-cc-pVTZ set. The input geometry of compound **6'** was obtained from the X-ray structure of compound **6** (see text) upon replacement of 3, 3', 4 and 4' ester groups by hydrogens and reoptimization, with B3LYP/6-31G(d), of all hydrogen atoms. CD spectra were generated using rotational strengths computed with dipole-length gauge formulation, in the view of their faster convergence towards the basis set limit observed for dimethyl diselenide, with respect to dipole-velocity values. A Gaussian band-shape was applied to each rotational strength with 3300 cm^{-1} half-height width (corresponding to 22 nm at 330 nm).

RESULTS AND DISCUSSION

Structures of the compounds investigated are collected in Chart 1. For the dichalcogenides Ac₄GlcSSGlcAc₄ (**1**),³¹

[†]See Gaussian'03 documentation at http://www.gaussian.com/g_ur/g03mantop.htm for details on basis sets and DFT functionals.



	short name	conf. of B	Z-Z	R ₁	R ₂	R ₃	R ₄	R ₅
1	Ac ₄ GlcSSGlcAc ₄	β-D-glucO	S-S	Ac	OAc	H	H	OAc
1a	GlcSSGlc	β-D-glucO	S-S	H	OH	H	H	OH
2	Ac ₄ GlcSSManAc ₄	β-D-manno	S-S	Ac	H	OAc	H	OAc
2a	GlcSSMan	β-D-manno	S-S	H	H	OH	H	OH
3	Ac ₄ GlcSSGalAc ₄	β-D-galacto	S-S	Ac	OAc	H	OAc	H
3a	GlcSSGal	β-D-galacto	S-S	H	OH	H	OH	H
4	Ac ₄ GlcSSGlcNAc	β-D-glucNAc	S-S	Ac	NAc	H	H	OAc
5	Ac ₄ GlcSManAc ₄	β-D-manno	S	Ac	H	OAc	H	OAc
6	Ac ₄ GlcSeSeGlcAc ₄	β-D-glucO	Se-Se	Ac	OAc	H	H	OAc
7	Ac ₄ GlcSeGlcAc ₄	β-D-glucO	Se	Ac	OAc	H	H	OAc

R = Ac: Ac₄GalSSGalAc₄ (**8**)
 R = H: GalSSGal (**8a**)

Models used in the calculations:

6'

9

Chart 1.

Ac₄GlcSSGalAc₄ (**3**),²⁹ and Ac₄GlcSeSeGlcAc₄ (**6**),³¹ X-ray structures of the single crystals were reported, which revealed that the C1–Z–Z–C1' torsional angles are –82.5°, –83.4°, and –81.8°, respectively, in the crystalized conformer.

The X-ray torsional angles of the disulfide chromophore imply that **1** and **3** have *M* helicity (left-handed helix), for which the disulfide empirical rule,¹⁹ MO-based arguments,¹⁵ and direct calculations^{16,17} all predict a negative long-wavelength $n_1 \rightarrow \sigma_{S-S}^*$ Cotton effect. The solution CD spectra of **1** and **3** showed indeed a negative Cotton effect (CE) around 240 nm in acetonitrile as a wide shoulder of the intense negative 182 nm CD transition (Table 1 and Fig. 2a).

Their solid-state CD measurements (as KCl discs) allowed the direct correlation of the characteristic $n_1 \rightarrow \sigma_{S-S}^*$ CD transition with the torsional angles obtained from the X-ray analysis; this also helps clarifying the solution CDs where the *P*- and *M*-helicity forms are in equilibrium. The solid-state CD spectra of Ac₄GlcSSGlcAc₄ (**1**) and Ac₄GlcSSGalAc₄ (**3**) showed similar negative CEs for the long-wavelength CD transition (Table 1) in full accordance with the *M*-helicity deduced by the X-ray analysis. The unsymmetrical disulfides Ac₄GlcSSManAc₄ (**2**) and Ac₄GlcSSGlcNAc (**4**) also have negative $n_1 \rightarrow \sigma_{S-S}^*$ CE around 240 nm (Table 1 and Fig. 2a), which shows that the change of the configuration at C-2 or the change of the acetoxy group to acetamido at C-2 does not invert the helicity of the disulfide chromophore. The solution and solid-state CD spectra of the symmetrical disulfide Ac₄GalSSGalAc₄ (**8**) also showed the same pattern as those of **1–4** indicating the same helicity of these disulfides. It is noteworthy that the corresponding transitions have the same signs both in the solution and solid-state CD spectra of the disulfides **1–4** and **8**, and except for some wavelength shift ($n_1 \rightarrow \sigma^*$ of Ac₄GlcSSManAc₄ at 239 nm in solution and 224 nm in the solid-state CD), their solution and solid-state CD spectra are nearly identical. This demonstrates that the solid-state conformers of Ac₄GlcSSGlcAc₄ (**1**) and

Ac₄GlcSSGalAc₄ (**3**), obtained by crystallization and characterized by X-ray analysis, are also the dominant conformers in solution, which is also true for Ac₄GlcSSManAc₄ (**2**), Ac₄GlcSSGlcNAc (**4**), and Ac₄GalSSGalAc₄ (**8**). Ac₄GlcSManAc₄ (**5**) contains only one sulfur atom in the

TABLE 1. Solution and solid-state CD data of the studied compounds

Compound	λ [nm] ($\Delta\epsilon$) for solution and λ [nm] (ϕ) ^a for solid-state CD measurements (KCl disc)
1	MeCN: 243 (–2.53), 183 (–72.40)
1a	KCl: 246 (–10.45), negative down to 187 nm MeOH: 262 (–0.43), 229 (0.76), 203 (–1.74) H ₂ O: 257 (–0.48), 227 (0.28), 185 (–4.64)
2	KCl: 279 (–3.66), 235 (2.29), 210 (–6.08) MeCN: 239 (–1.71), 183 (–51.14)
2a	KCl: 224 (–10.92), 188 (–95.36) ^b
3	H ₂ O: 251 (–0.65), 225 (0.18), 194 (–4.27) MeCN: 247 (–2.18), 182 (–69.56)
3a	KCl: 250 (–2.16), 185 (–48.46) ^b MeOH: 261 (–0.83), 230 (0.79), 198 (–7.33) H ₂ O: 256 (–0.76), 227 (0.27), 186 (–11.11)
4	KCl: 272 (–6.41), 232 (13.43), 202 (–35.94) MeCN: 245 (–2.56), 200sh (–19.17), negative down to 185 nm KCl: 246 (–10.45), 193 (–89.33) ^b
5	MeCN: 207 (–1.22)
6	MeCN: 314 (–0.66), 274 (0.30), 191 (–65.81)
7	KCl: 321 (–11.31), 274 (8.98), 195 (–62.1) ^b
8	MeCN: 247 (1.11), 218 (–1.27)
8a	MeCN: 250 (–1.13), 183 (–30.61) KCl: 253 (–7.00), 187 (–43) ^b MeOH: 261 (–0.59), 230 (0.53), 198 (–5.03) H ₂ O: 258 (–0.69), 227 (0.32), 188 (–10.32) KCl: 276 (–6.29), 233 (16.46), 206 (–28.38)

^aValues of ellipticity [ϕ (mdeg)] are not corrected for the concentrations.

^bFor the solid-state measurement of the high-energy CD transition below 200 nm, KCl disc of lower sample concentration was used.

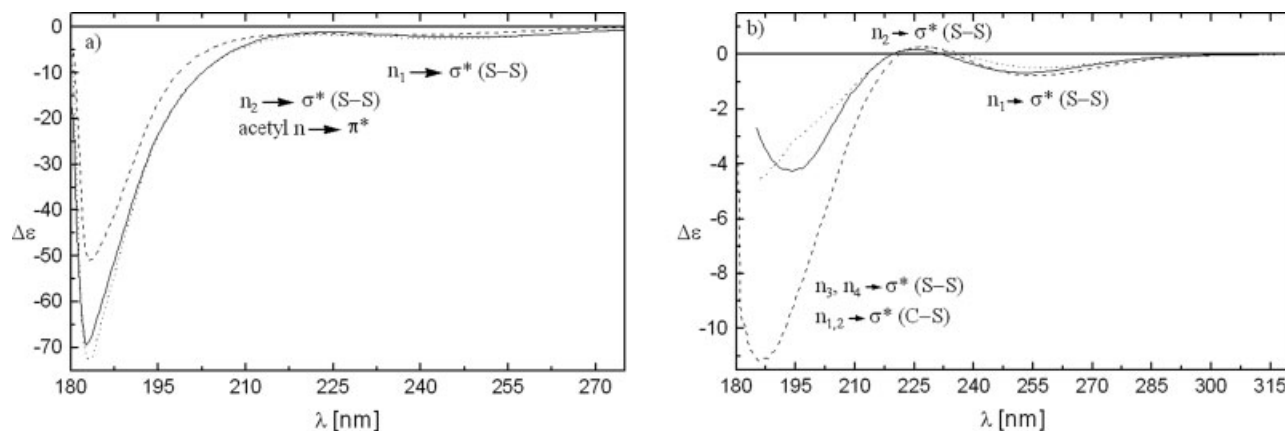


Fig. 2. (a) CD spectra $\text{Ac}_4\text{GlcSSGlcAc}_4$ (**1**, dotted curve), $\text{Ac}_4\text{GlcSSManAc}_4$ (**2**, dashed curve), and $\text{Ac}_4\text{GlcSSGalAc}_4$ (**3**, solid curve) in acetonitrile. (b) CD spectra GlcSSGlc (**1a**, dotted curve), GlcSSMan (**2a**, solid curve), and GlcSSGal (**3a**, dashed curve) in water.

interglycosidic linkage and its longest wavelength transition is at 207 nm.

In the disulfides **1–4** and **8**, the CD bands allied to the transitions of the eight acetate groups are also present in the short-wavelength region of the CD spectra overlapping with high-lying transitions of the disulfide chromophore. To gain information on the pure transitions of the disulfide chromophore, the CD spectra of the deacetylated derivatives GlcSSGlc (**1a**), GlcSSMan (**2a**), GlcSSGal (**3a**), and GalSSGal (**8a**) were studied in solution and solid-state (Table 1, Figs. 2b and 3a,b).

This revealed the presence, besides the negative $n_1 \rightarrow \sigma^*_{\text{S-S}}$ disulfide transition, of the additional positive CE at 225–230 nm that derives from the $n_2 \rightarrow \sigma^*_{\text{S-S}}$ disulfide transition (see Introduction), and that is obscured by the acetate $n \rightarrow \pi^*$ transition in the octa-*O*-acetyl derivatives **1**, **2**, **3**, **4**, and **8**. The solution CD spectra of the deacetylated derivatives GlcSSGlc (**1a**), GlcSSMan (**2a**), GlcSSGal (**3a**), and GalSSGal (**8a**) showed the same CD pattern; a negative $n_1 \rightarrow \sigma^*$ CE, a positive $n_2 \rightarrow \sigma^*$ CE and a more

intense negative CE below 200 nm in methanol and water. These CD transitions of the disulfide chromophore, especially the lowest energy one, confirmed that the *M* helicity form is dominant in solution. Moreover, the disulfide transitions are not disturbed by other chromophores in the deacetylated derivatives and hence the pure transitions can be studied. One of the consequences of the $n_{1,2} \rightarrow \sigma^*_{\text{S-S}}$ origin of the CD of disulfides above 220 nm is the pronounced band wavelength dependence on the C–S–S–C dihedral angle, as it was expected and found.¹⁷ According to Woody's results, the lowest energy transition appears at 250 nm for $\varphi = 90^\circ$, when the $n_1 \rightarrow \sigma^*_{\text{S-S}}$ and $n_2 \rightarrow \sigma^*_{\text{S-S}}$ transitions become degenerate, and it is shifted to red as the dihedral angle is increasing or decreasing.¹⁷ The wavelength of the lowest energy transition of **1a–4a** is in the range of 251–258 nm in water, 261–262 nm in methanol, and 272–279 nm in KCl. The 251–258 nm range in water suggests C–S–S–C dihedral angles close to the 90° which is in keeping with the values assumed in the crystals of the peracetylated derivatives **1**

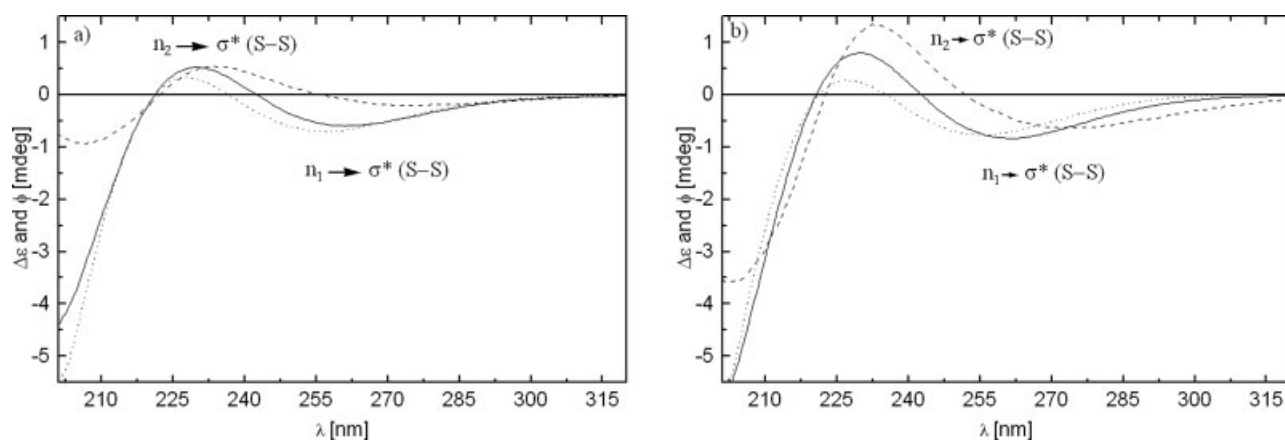


Fig. 3. (a) CD spectra of GalSSGal (**8a**) in water (dotted curve), methanol (solid curve), and as a KCl disc (dashed curve). The solid-state CD spectra is shown as ellipticity [mdeg] and multiplied by 3.3×10^{-2} for comparison with the solution spectra. (b) CD spectra of GlcSSGlc (**3a**) in water (dotted curve), methanol (solid curve), and as a KCl disc (dashed curve). The solid-state CD spectra is shown as ellipticity [mdeg] and multiplied by 0.1 for comparison with the solution spectra.

TABLE 2. TDDFT-calculated CD spectrum for dimethyl diselenide (9**) with $\phi = +90^\circ$ with various functionals and basis sets**

		Band I		Band II	
		λ/nm	R^a	λ/nm	R^a
B3LYP	TZVP	333.5	−15.3	331.4	16.4
	6-311+G(d,p)	337.0	−17.3	335.0	19.0
	aug-TZVP	337.9	−16.1	336.1	17.4
	cc-pVTZ	330.0	−13.9	328.2	14.1
BP86	TZVP	347.6	−14.2	345.0	14.2
BH&HLYP	TZVP	309.8	−14.7	308.5	17.9

^aRotational strengths in 10^{-40} cgs, computed with dipole-length gauge.

(-82.5°) and **3** (-83.4°).^{29,31} However, these minima are shifted to the red in methanol which suggests that the C—S—S—C dihedral angle may increase in this solvent, although other kinds of solvent effects are also possible. For GlcSSGlc (**1a**), GlcSSGal (**3a**), and GalSSGal (**8a**), the solid-state CD spectra measured as KCl disc showed the same CD pattern as the solution spectra and thus it confirmed that a conformer of *M* helicity crystallizes in the solid state as well, which also determines the solution CD spectra.

As anticipated in the Introduction, we considered it of some interest to apply TDDFT theory to the prediction of CD of $\text{Ac}_4\text{GlcSeSeGlcAc}_4$ (**6**), the diselenide analogue of the disulfide derivative **1**. Therefore we tested several DFT functionals and basis sets on the model compound dimethyl diselenide $\text{CH}_3\text{—Se—Se—CH}_3$ (**9**). The geometry of this model compound was optimized with DFT at B3LYP/6-311+G(d,p) level; the absolute minimum had Se—Se distance = 2.35 Å, C—Se—Se angle = 101.2° , and C—Se—Se—C dihedral angle $\phi = 82^\circ$, in good agreement with other calculation results,^{23,32} and with experimental values in the gas phase³³ and in the solid state (93 entries for diselenides found in the Cambridge Crystallographic Database).³⁴ The DFT-computed barriers for the C—Se—Se—C torsion of **9** are 8.7 kcal/mol at $\phi = 0^\circ$ and 5.3 kcal/mol at $\phi = 180^\circ$, again in keeping with other calculations.³⁵

TDDFT calculations were then run on **9** in the structure with $\phi = +90^\circ$, employing various functionals (BP86, B3LYP, and BH&HLYP) and basis sets (TZVP, 6-311+G(d,p), aug-TZVP, cc-pVTZ). All calculations predicted a similar CD spectrum in the long wavelength region (see Table 2), consisting of a negative couplet-like feature arising from the two quasi-degenerate $n_1 \rightarrow \sigma^*_{\text{Se—Se}}$ and $n_2 \rightarrow \sigma^*_{\text{Se—Se}}$ transitions. Four other transitions follow, starting from 232 nm for B3LYP/TZVP, of the $n_{1,2} \rightarrow \sigma^*_{\text{C—Se}_{1,2}}$ type.

We are especially interested in the first two transitions, however, for several reasons: (1) the determination of absolute configurations through sector rules, in analogy with disulfides; (2) their red-shifted position where overlap with other transitions, e.g., allied with acetate groups in compound **6**, does not occur; (3) inherent higher accuracy of TDDFT method in the prediction of low-lying states.³⁶

The substantial independence of TDDFT results on the functional and basis employed, in terms of rotational strengths sign and intensities (Table 2), demonstrates the robustness of the application of TDDFT method to chiral diselenides; B3LYP/TZVP was chosen for the following calculations.

Again compound (*P*)-**9** was taken as a model, and its CD spectrum calculated as a function of the dihedral ϕ varied in the $60\text{--}120^\circ$ range (see Fig. 4).

The calculations nicely reproduce the same trend observed for disulfides^{16,17} and recently confirmed for diselenides,²³ the plots in Figure 4 are in accordance with recent TDDFT calculations on dimethyl diselenide employing a larger basis set.²⁴ The two $n_{1,2} \rightarrow \sigma^*_{\text{Se—Se}}$ transitions have predicted energies increasing (n_1) and decreasing (n_2) with ϕ , such that $n_1 \rightarrow \sigma^*_{\text{Se—Se}}$ (B symmetry, positive CD) is the first (longest wavelength) transition for $0 < \phi < 90^\circ$, while $n_2 \rightarrow \sigma^*_{\text{Se—Se}}$ (A symmetry, negative CD) is the first transition for $90 < \phi < 180^\circ$; they are almost degenerate at $\phi = 90^\circ$. As a consequence, for *P* helicity, the intrinsic dissymmetry of the diselenide chromophore is responsible for a positive couplet-like feature for $\phi < 90^\circ$, and a negative one for $\phi > 90^\circ$; at $\phi = 90^\circ$, the overall shape should depend on higher-order mechanisms of optical activity, as envisaged for disulfides.^{16,17} This confirms that the same quadrant rule is relevant for diselenides as used for the disulfide chromophore (Fig. 1b).

X-ray analysis of $\text{Ac}_4\text{GlcSeSeGlcAc}_4$ (**6**)³⁰ reported a $\phi = -81.8^\circ$ C1—Se—Se—C1' torsional angle for its single

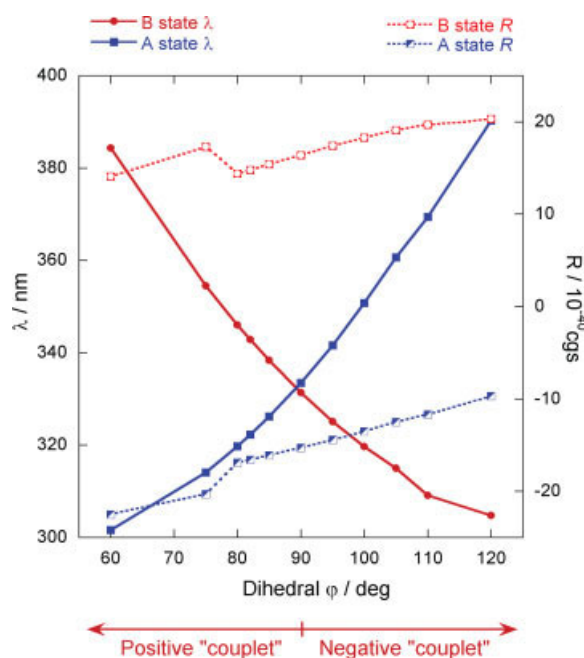


Fig. 4. Wavelength and rotational strengths calculated with TDB3LYP/TZVP for the first two transitions $n_1 \rightarrow \sigma^*_{\text{Se—Se}}$ (B symmetry) and $n_2 \rightarrow \sigma^*_{\text{Se—Se}}$ (A symmetry) of dimethyl diselenide (**9**) with *P* helicity as a function of C—Se—Se—C dihedral angle. *R*: rotatory strength. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

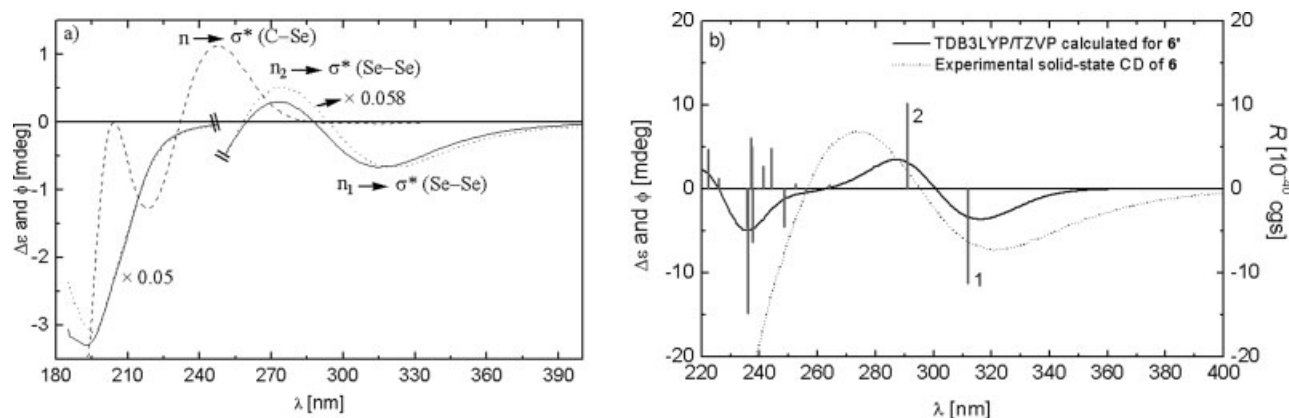


Fig. 5. (a) CD spectra of $Ac_4GlcSeSeGlcAc_4$ (**6**) measured in acetonitrile (solid curve) and as KCl disc (dotted curve), and of $Ac_4GlcSeGlcAc_4$ (**7**) in acetonitrile (dashed curve). The curves of $Ac_4GlcSeSeGlcAc_4$ (**6**) were multiplied by 5×10^{-2} below 250 nm and the curve of the solid-state curve was multiplied by 5.8×10^{-2} for comparison with the solution curve. (b) TDB3LYP/TZVP calculated CD spectrum for tetra-*O*-acetyl model compound **6'** (solid curve), compared with the experimental solid-state spectrum of **6** (dotted curve). Vertical bars represent rotational strengths R in 10^{-40} cgs.

crystal of $P2_12_12_1$ space group, obtained from methanol at ambient temperature. In the CD spectra of **6** in acetonitrile (Fig. 5a), a negative CE at 314 nm ($n_1 \rightarrow \sigma^*_{Se-Se}$), a positive one at 274 nm ($n_2 \rightarrow \sigma^*_{Se-Se}$), and a negative one at 191 nm were observed as also published by Snatzke and co-workers.²¹

The $n_1 \rightarrow \sigma^*_{Se-Se}$ and the $n_2 \rightarrow \sigma^*_{Se-Se}$ transitions were clearly missing from the CD spectrum of $Ac_4GlcSeGlcAc_4$ (**7**), confirming their origin (Fig. 5a). In solution, the *P* and *M* helicity forms of $Ac_4GlcSeSeGlcAc_4$ (**6**) are in equilibrium with each other and the solid-state structure is not necessarily the dominant one in solution. Thus the microcrystalline solid-state CD spectrum of **6** crystallized from methanol at ambient temperature was measured as KCl disc which truly reflected the C1–Se–Se–C1' torsional angle of the X-ray structure. The solid-state CD spectrum of **6** (Fig. 5a) showed the same CD pattern as the solution CD spectra with CEs at 321, 274, and 191 nm. Thus the long-wavelength negative CE at 321 nm and the positive one at 274 derive from the inherently chiral biselenide chromophore of *M* helicity with -81.8° C1–Se–Se–C1' torsional angle.

Potrzebowski et al.³¹ also reported a different X-ray structure of $Ac_4GlcSeSeGlcAc_4$ (**6**), whose single crystal was grown from methanol at -10°C and which has $P2_12_12$ space group and $\phi = -109.2^\circ$ C1–Se–Se–C1' torsional angle. This torsional angle should give a positive couplet-like CD for the low energy transition, i.e., opposite to the CEs of that crystallized at ambient temperature. Unfortunately, our attempts to reproduce the crystallization of Potrzebowski et al. at -10°C were unsuccessful. The solid-state CD of the crystals grown from methanol at -10°C was identical with those grown at ambient temperature, which suggested that the same conformer crystallized at -10°C as at ambient temperature.

Compound **6** was then considered for TDDFT calculations using its solid-state structure reported by Michalska et al.³⁰ with $\phi = -81.8^\circ$ as input geometry. Since its absolute configuration is known, the main purpose here is to

test our solid-state TDDFT CD approach^{25–28} on a compound containing the diselenide moiety, to which applications of TDDFT calculations are scarce (TDDFT calculations on selenium-containing compounds: refs 37, 38).

Regrettably, only the long wavelength region of the CD spectra could be considered for two reasons. First, the whole molecular systems of **6** is quite large and complicated; to save computational time, the “reduced” model **6'** (see Chart 1) was taken into consideration, obtained from the X-ray structure of **6** upon replacing ester groups attached at 3 and 4 positions (the more remote from the dicalchogenide bridge) by hydrogens. Second, the first two CD bands for **6** are allied to low-lying transitions, well separated from the following ones, which are expected to be accurately predicted by TDDFT as demonstrated above for dimethyl diselenide. The next CD bands, instead, result from the superimposition of several transitions, including ester-centered ones (which need to be overlooked for models **6'**); most of them also involve unbound virtual orbitals for which TDDFT method is less accurate.³⁶ Figure 5b shows the solid-state CD spectrum of **6** compared with TDB3LYP/TZVP calculated spectrum for **6'**. The position, sign, and relative intensity of the two $n_{1,2} \rightarrow \sigma^*_{Se-Se}$ transitions is well reproduced; although TDDFT calculations may easily lead to sizeable errors in individual rotational strengths, the global shape of the calculated CD spectra are often accurate, like in the current case. The CD curve in the low energy region resembles that calculated for the lowest energy structure of $CH_3-Se-Se-CH_3$ (**9**) with $\phi = -82^\circ$. In fact, the rotational strengths and the energy difference for the $n_{1,2} \rightarrow \sigma^*_{Se-Se}$ transitions (no. 1–2 in Fig. 5b) are very similar ($R_{1,2} = -11.3/+10.2 \times 10^{-40}$ cgs for **6**, $R_{1,2} = -14.8/+16.6$ for **9**; $\Delta\nu_{1,2} = 2300\text{ cm}^{-1}$ for **6**, $\Delta\nu_{1,2} = 1800\text{ cm}^{-1}$ for **9**), and only a blue-shift of 30 nm is estimated for **6**. This finding demonstrates that the CD of **6** above 250 nm may be almost entirely ascribed to the inherent chirality of the diselenide chromophore, and is poorly influenced by neighboring groups, including acetal oxygens and ester

chromophores. In fact, the n_1 , n_2 , and σ^* Kohn–Sham orbitals, for **6'**, are almost confined to selenium atoms and the participation of vicinal oxygens and carbons is small.

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Synthesis and Chiroptical Properties of a Helical Poly(phenylacetylene) Bearing Optically Active Pyrene Pendants

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT A novel poly(phenylacetylene) derivative bearing optically active pyrene moieties as the pendant groups (poly-(*R*)-**1**) was prepared by the polymerization of the corresponding monomer (*R*)-**1** in the presence of a rhodium catalyst, and its chiroptical property was investigated. Poly-(*R*)-**1** exhibited an induced circular dichroism (ICD) in the polymer backbone region due to the predominantly one-handed helical conformation. The ICD pattern dramatically changed and was accompanied by inversion of the Cotton effect sign in response to a change in the temperature and solvent, indicating that poly-(*R*)-**1** underwent a helix–helix transition in response to the external stimuli. *Chirality* 20:386–392, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: helix; chirality; circular dichroism; fluorescence; polyacetylenes

INTRODUCTION

Biological macromolecules such as DNA and proteins adopt a right-handed double helix and an α -helix, respectively, because of the homochirality of their components. Their one-handed helical structures play important roles in their sophisticated and fundamental functions in nature. Since the discovery of the helical structures in these biopolymers, significant attention has been paid to developing synthetic helical polymers with a predominant one-handedness. Such studies are interesting and valuable not only due to their biomimicking features, but also their variety of applications, such as chiral recognition, liquid crystals, and asymmetric catalysis. To date, several synthetic helical polymers showing optical activity only due to their one-handed helicity, being stable in solution, have been prepared.^{1–7} Some of them are known to exhibit a helix–helix transition in response to various external stimuli such as a change in temperature and solvent. For example, the right-handed helices of DNA^{8,9} and polypeptides^{10,11} invert into opposite left-handed helices regulated by the addition of salts and temperature, respectively. Inspired by the discovery of such a helicity inversion in biopolymers, chemists have been interested in developing artificial macromolecules showing an inversion of the helicity. Up to now, several synthetic helical polymers undergoing a transition from one helix to another induced by external stimuli have been reported.^{1–7,12–29} These switchable helical polymers have potential applications in sensors, data storage, and chiral separation.

We now report that a poly(phenylacetylene) derivative bearing optically active pyrene moieties as the pendant groups (poly-(*R*)-**1**) (Scheme 1) forms a preferred-handed

helical conformation and undergoes a helix–helix transition in response to external stimuli such as temperature and solvent. The helical array of chromophores, such as a pyrene group along the polymer backbone, is of particular interest because such a system may produce a possible molecular wire through which excitation energy and charge may be one-dimensionally transported. To this end, the pyrene group has often been introduced at the side chains of helical RNA,³⁰ DNA,^{31,32} peptides,^{33,34} and artificial polymers,^{35–38} and their changes in the fluorescence spectra due to a helical array of the pyrenes have been investigated. However, a pyrene-bound helical polymer, which shows an inversion of the helicity, has not been reported.

EXPERIMENTAL PROCEDURES

Materials

Triethylamine (Et₃N) was dried over KOH pellets and distilled onto KOH under nitrogen. Et₃N was redistilled from KOH under high vacuum just before polymerization. Chloroform (CHCl₃) was dried over calcium hydride, distilled, and stored under nitrogen. Bis[(Norbornadiene)rhodium(I) chloride] ([Rh(nbd)Cl]₂) and 1-hydroxybenzotriazole monohydrate (HBT) were purchased from

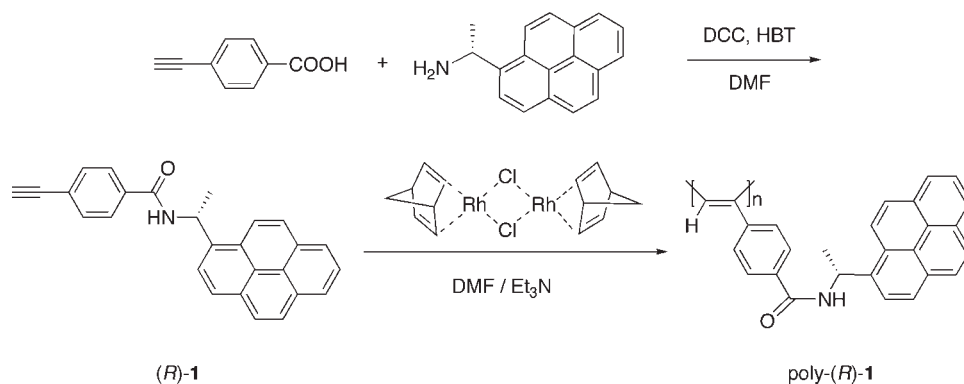
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Scheme 1

Aldrich. Anhydrous *N,N*-dimethylacetamide (DMA), *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), and methanol (MeOH) (water content < 0.005%) were purchased from Wako (Osaka, Japan). Anhydrous lithium chloride (LiCl) and *N,N'*-dicyclohexylcarbodiimide (DCC) were obtained from Kishida (Osaka, Japan). *(R)*-(1-Pyrenyl)ethylamine³⁴ and 4-ethynylbenzoic acid^{39,40} were prepared according to the reported methods.

Synthesis of *(R)*-4-((1-(1-Pyrenyl)ethyl)carbamoyl)phenylacetylene (*(R)*-1)

4-Ethynylbenzoic acid (50 mg, 0.34 mmol) and HBT (53 mg, 0.34 mmol) were dissolved in DMF (2.5 ml), and to this was added DCC (71 mg, 0.34 mmol) at 0°C. After the reaction mixture was stirred at 0°C for 1 h and then at room temperature for 1 h, *(R)*-1-(1-pyrenyl)ethylamine (100 mg, 0.41 mmol) was added, and the reaction mixture was stirred at room temperature for 24 h. After evaporating the solvent, the crude product was purified by silica gel chromatography with CHCl₃ as the eluent, and then recrystallized from *n*-hexane–ethyl acetate (1/1, v/v) to give 90 mg of *(R)*-1 as a pale yellow crystal in 70% yield.

Mp 219.1–219.7°C; IR (KBr): ν = 3301 (—CH), 1635 (amide I), 1542 cm⁻¹ (amide II). ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.73 (d, CH₃, *J* = 6.6 Hz, 3H), 4.39 (s, —CH, 1H), 6.21–6.31 (m, CH, 1H), 7.56–8.55 (m, aromatic, 13H), 9.28 (d, NH, *J* = 7.8 Hz, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 22.2, 45.4, 82.9, 83.0, 122.8, 123.4, 124.0, 124.1, 124.5, 125.0, 125.1, 125.3, 126.3, 127.0, 127.2, 127.4, 127.6, 127.8, 129.8, 130.3, 130.9, 131.6, 134.5, 138.7, 164.8. Anal. Calcd. for C₂₇H₁₉NO: C, 86.84; H, 5.13; N, 3.75. Found: C, 86.61; H, 5.33; N, 3.65. $[\alpha]_D^{25}$ = +460° (*c* = 0.5, CHCl₃).

Polymerization

Polymerization was carried out in a dry glass ampoule under a dry nitrogen atmosphere using [Rh(nbd)Cl]₂ as a catalyst. A typical polymerization procedure is described below (Scheme 1).

(R)-1 monomer (50 mg, 0.134 mmol) was placed in a dry glass ampoule, which was then evacuated on a vacuum line and flushed with dry nitrogen. After this evacuation-flush procedure was repeated three times, a three-way

stopcock was attached to the ampoule and dry DMF (0.47 ml) and Et₃N (0.018 ml) were added with a syringe. To this was added a solution of [Rh(nbd)Cl]₂ (0.005 M) in DMF at 30°C. The concentrations of the monomer and the rhodium complex were 0.2 and 0.002 M, respectively. The polymerization proceeded rapidly, and a yellow polymer was precipitated within a few seconds. After 2 h, the resulting polymer was precipitated into a large amount of MeOH, collected by centrifugation, and dried in vacuo overnight to give 50 mg of poly-*(R)*-1 quantitatively. The number average molecular weight (*M*_n) and its distribution (*M*_w/*M*_n) were 2.4 × 10⁵ and 1.8, respectively, as determined by size exclusion chromatography (SEC) using poly(ethylene oxide) and poly(ethylene glycol) standards in DMF containing 10 mM LiCl as the eluent.

IR (KBr): ν = 1638 (amide I), 1525 cm⁻¹ (amide II). ¹H NMR (300 MHz, DMA-*d*₉ containing 10 mM LiCl, 100°C): δ 1.64 (d, CH₃, 3H), 5.85 (s, =CH, 1H), 6.25 (s, CH, 1H), 6.72 (s, aromatic, 2H), 7.56–8.35 (m, aromatic, 11H), 8.94 (s, NH, 1H). Anal. Calcd for C₂₇H₁₉NO·H₂O: C, 82.84; H, 5.41; N, 3.58. Found: C, 83.2; H, 5.16; N, 3.12. $[\alpha]_D^{25}$ = +468° (*c* = 0.5, DMA containing 10 mM LiCl).

Instruments

Melting points were measured on a Yanako micro melting point apparatus and are uncorrected. IR spectra were recorded using a Jasco Fourier transform IR-620 spectrophotometer (Hachioji, Japan). Optical rotation was measured in a 5-cm quartz cell on a Jasco P-1030 polarimeter. NMR spectra were taken on a Varian Mercury 300 spectrometer (300 MHz for ¹H) with TMS (for DMA-*d*₉) or a solvent residual peak (for DMSO-*d*₆) as the internal standard. The absorption and circular dichroism (CD) spectra were measured in a 1.0-mm quartz cell unless otherwise noted using a Jasco V-570 spectrophotometer and a Jasco J-725 spectropolarimeter, respectively. The temperature was controlled with a Jasco PTC-348 WI apparatus. Fluorescence spectra were measured in a 10-mm quartz cell on a Jasco FP 6500 spectrofluorometer. SEC measurements were performed with a Jasco PU-980 liquid chromatograph equipped with an RI (Jasco RI-930) detector and a column oven (Jasco CO-965) at 40°C using Tosoh TSK-GEL α -3000 (30 cm) and α -5000 (30 cm) SEC columns in series,

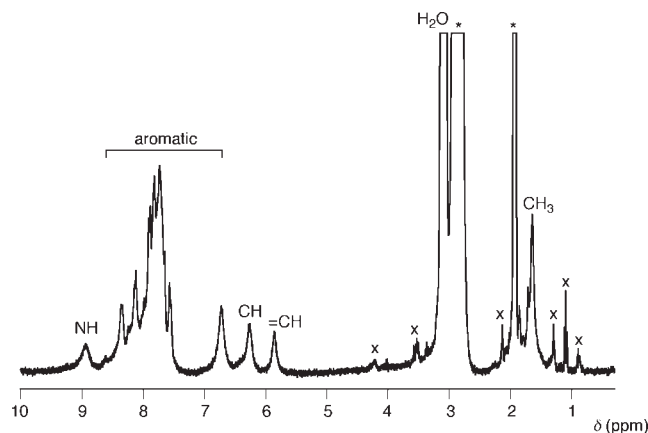


Fig. 1. ^1H NMR spectrum of poly-(*R*)-1 in $\text{DMA-}d_9$ containing LiCl (10 mM) at 100°C . The asterisks and x denote protons from $\text{DMA-}d_9$ and impurities contained in the solvent, respectively.

and DMF containing 10 mM LiCl was used as the eluent at a flow rate of 0.5 ml/min. The molecular weight calibration curve was obtained with poly(ethylene oxide) and poly(ethylene glycol) standards (Tosoh).

CD Measurements: Effect of Solvent on ICD of Poly-(*R*)-1

A typical experimental procedure is described below. A stock solution of poly-(*R*)-1 (5 mg/ml) in DMA containing 10 mM LiCl (DMA-LiCl) was prepared in a 2-ml flask equipped with a stopcock. A 100- μl aliquot of the poly-(*R*)-1 solution was transferred to four 1-ml flasks equipped with stopcocks. To each flask was added DMA-LiCl (900, 700, 600, and 400 μl) using a micropipette (Nichiryo Nichipet EX, Japan), and the solutions were diluted with THF so as to keep the poly-(*R*)-1 concentration at 0.5 mg/ml. The absorption and CD spectra were then taken for each flask at different temperatures (0 – 55°C). In a similar manner, effects of other poor solvents (MeOH and CHCl_3) on induced circular dichroism (ICD) of poly-(*R*)-1 were investigated.

Concentration Effect of Poly-(*R*)-1 on ICD

A stock solution of poly-(*R*)-1 (1 mg/ml) in DMA-LiCl was prepared in a 2-ml flask equipped with a stopcock, and the initial CD and absorption spectra at 0, 25, and 55°C were recorded with a 1.0-mm quartz cell. A 200- μl aliquot of the polymer solution was transferred to a 2-ml flask with a micropipette, and the solution was diluted with DMA-LiCl, giving a 0.1-mg/ml solution of poly-(*R*)-1. The CD and absorption spectra of this solution were taken at different temperatures. The same dilution procedure was repeated to prepare a 0.01-mg/ml solution of poly-(*R*)-1 and the CD and absorption spectra were measured. In the same way, the concentration effect of poly-(*R*)-1 on ICD in the mixtures of DMA-LiCl and the poor solvents (THF, MeOH, and CHCl_3) were also investigated.

Fluorescence Measurements

A stock solution of poly-(*R*)-1 (0.01 mg/ml) in DMA-LiCl was prepared in a 5-ml flask equipped with a stopcock and stored under shielded light. A 200- μl aliquot of the

poly-(*R*)-1 solution was transferred to a 2-ml flask equipped with a stopcock. To this was added DMA-LiCl (0.8 ml) using a micropipette, and the solution was diluted with THF so as to keep the poly-(*R*)-1 concentration at 0.001 mg/ml. Fluorescence spectrum was then measured. Fluorescence measurements of poly-(*R*)-1 in DMA-LiCl/ CHCl_3 (7/3, v/v) and DMA-LiCl/MeOH (96/4, v/v) were performed in the same way.

RESULTS AND DISCUSSION

Synthesis and Structural Characteristics of Poly-(*R*)-1

Poly-(*R*)-1 was prepared by the polymerization of (*R*)-1 using $[\text{Rh}(\text{nbd})\text{Cl}]_2$ as a catalyst in DMF in the presence of Et_3N at 30°C . The polymerization rapidly proceeded and a polymer precipitated within a few seconds. After 2 h, a high molecular weight poly-(*R*)-1 was quantitatively obtained as a MeOH-insoluble part. The obtained polymer was partially and completely soluble in DMA and DMA containing 10 mM LiCl (DMA-LiCl), respectively, but insoluble in the common organic solvents such as THF, CHCl_3 , toluene, pyridine, DMF, and DMSO. The M_n and M_w/M_n values were 2.4×10^5 and 1.8, respectively, as determined by SEC using poly(ethylene oxide) and poly(ethylene glycol) standards in DMF containing 10 mM LiCl as the eluent. The stereoregularity of poly-(*R*)-1 was investigated by ^1H NMR spectroscopy. The ^1H NMR spectrum of poly-(*R*)-1 in $\text{DMA-}d_9$ containing 10 mM LiCl showed a relatively sharp singlet centered at 5.85 ppm due to the main chain protons, which can be assigned to the cis-transoidal main chain's olefinic protons (see Fig. 1).^{41–46} This result demonstrates that poly-(*R*)-1 possesses a highly cis-transoidal stereoregular structure.

Chiroptical Property of Poly-(*R*)-1 and Helix-Helix Transition

Figure 2A displays the CD and absorption spectra of poly-(*R*)-1 and (*R*)-1 in DMA-LiCl. The enantiomerically pure (*R*)-1 gave rise to the typical absorption and CD signals assigned to the optically active pyrenylethylcarbamoyl residues in the region between 300 and 360 nm. The poly-(*R*)-1 exhibited similar absorption and CD spectra in the pendant chromophore region, whereas the polymer showed a very weak ICD in the long wavelength regions due to the π -conjugated polymer backbone (370–500 nm) in DMA-LiCl at 25°C ($\Delta\epsilon_{371} = 0.49 \text{ M}^{-1} \text{ cm}^{-1}$), although the polymer is considered to adopt a predominantly one-handed helical conformation induced by the optically active bulky pendant groups. However, the ICD intensity and spectral pattern significantly changed with temperature; the ICD intensity increased with the increasing temperature and almost reached a plateau value at 55°C ($\Delta\epsilon_{371} = 3.86 \text{ M}^{-1} \text{ cm}^{-1}$) (Figs. 2A and 2B and Table 1). (*R*)-1 showed no CD and absorption spectra at wavelengths greater than 365 nm, indicating that poly-(*R*)-1 has a preferred-handed helical conformation at high temperature and the helical sense excess is rather small at 25°C . Interestingly, the ICD pattern of poly-(*R*)-1 was dramatically changed at low temperature, and the Cotton effect sign inverted to give an almost mirror image at 0 – 15°C in the

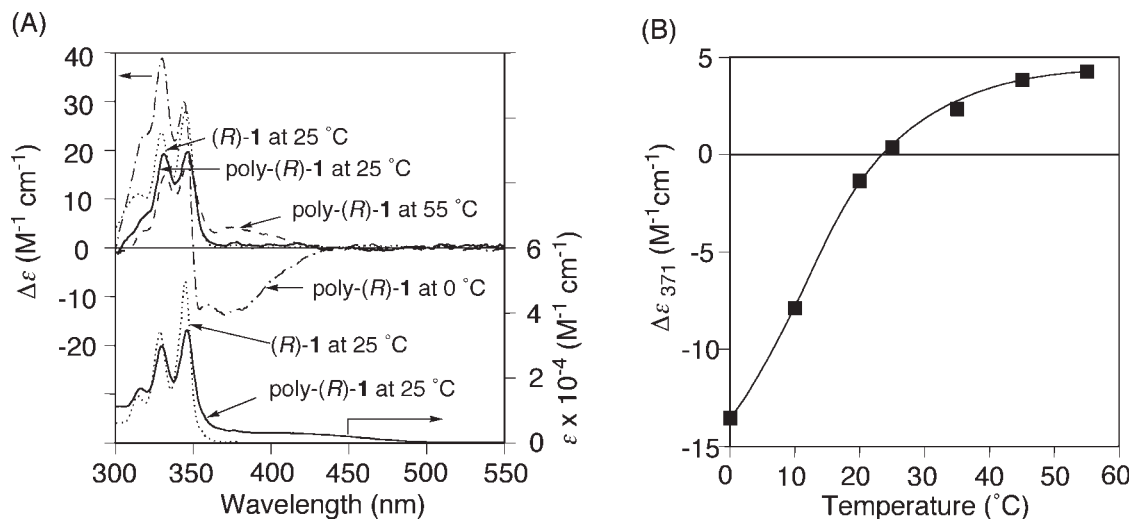


Fig. 2. (A) CD and absorption spectra of poly-(*R*)-1 and (*R*)-1 at various temperatures in DMA containing 10 mM LiCl. The concentration of poly-(*R*)-1 and (*R*)-1 was 0.1 mg/ml. (B) Plots of the CD intensity at 371 nm ($\Delta\epsilon_{371}$) for poly-(*R*)-1 versus temperature.

long absorption region of the polymer backbone ($\Delta\epsilon_{371} = -13.6 \text{ M}^{-1} \text{ cm}^{-1}$). These CD spectral changes were completely reversible, suggesting inversion of the helicity of poly-(*R*)-1 which undergoes a transition from one helix to another in response to the temperature. A similar inversion of the macromolecular helicity has also been observed for other helical polyacetylenes, polyisocyanates, and polysilanes in response to solvent, temperature, and chirality.^{1–7,12–29} The right- and left-handed helices of poly-(*R*)-1 are not exactly enantiomers but diastereomers, due to the presence of the optically active pyrenylethylcarbamoyl residues of the polymer, and therefore, their CD spectra differ from one another. The CD and absorption spectra of poly-(*R*)-1 did not change after the samples had been allowed to stand at room temperature for 1 day.

Another possible explanation for the helix inversion of poly-(*R*)-1 may be due to aggregations of the polymer chains depending on the temperature. It is well known that aggregations are highly sensitive to the polymer concentration. However, the CD and absorption spectral changes are independent of the poly-(*R*)-1 concentration (0.01–1.0 mg/ml), indicating that the formation of aggregates depending on the temperature could be excluded.

Besides temperature, the helix-sense of poly-(*R*)-1 could also be controlled by changing the solvent composition.

Figure 3 shows the CD and absorption spectra of poly-(*R*)-1 in DMA–LiCl in the presence of poor solvents such as THF, CHCl_3 , and MeOH at 25 °C. The addition of the poor solvents to a solution of poly-(*R*)-1 in DMA–LiCl at 25 °C caused a change in its ICD pattern; poly-(*R*)-1 exhibited intense ICDs with positive Cotton effects at 371 nm in DMA–LiCl/THF (5/5, v/v) and DMA–LiCl/ CHCl_3 (7/3, v/v), while the ICD pattern of poly-(*R*)-1 was inverted in DMA–LiCl/MeOH (96/4, v/v) (see also Table 1). The changes in the ICDs were accompanied by a negligible change in their absorption spectra. These results suggest that the predominant helix-sense of poly-(*R*)-1 in DMA–LiCl/MeOH is opposite to those in DMA–LiCl/THF and DMA–LiCl/ CHCl_3 . Polar solvents can interact with the polar amide groups, and this solvation effect may cause such a helicity inversion of the polymer depending on the difference in the solvent polarity. The magnitudes of the ICDs of poly-(*R*)-1 in these solvent mixtures hardly changed over the concentration range of poly-(*R*)-1 from 0.5 to 0.01 mg/ml, indicating that the formation of aggregates could also be excluded.

The helical structure of poly-(*R*)-1 is sensitive to temperature as well as to solvent as already mentioned. We then investigated the temperature dependent ICD intensity changes of poly-(*R*)-1 in various solvent mixtures (see Fig. 4). As expected, the ICD intensities at 25 °C increased

TABLE 1. CD, absorption, and fluorescence spectral data of poly-(*R*)-1 in various solvents^a

Entry	Solvent	Temperature (°C)	$\Delta\epsilon_{371} (\text{M}^{-1} \text{ cm}^{-1})$	$\epsilon_{400} (\text{M}^{-1} \text{ cm}^{-1})$	$\text{FL}_{462}/\text{FL}_{381}^b$
1	DMA–LiCl	0	–13.6	2970	0.403
2	DMA–LiCl	25	0.49	3050	0.635
3	DMA–LiCl	55	3.86	2990	0.672
4	DMA–LiCl/THF (5/5, v/v)	25	10.1	2990	0.384
5	DMA–LiCl/MeOH (96/4, v/v)	25	–5.03	3100	0.446
6	DMA–LiCl/ CHCl_3 (7/3, v/v)	25	6.22	3020	0.476

^aCD and absorption spectra were measured in a 1.0-mm quartz cell with a poly-(*R*)-1 concentration of 0.5 mg/ml. Fluorescence spectra were taken in a 10-mm rectangular quartz cell with a poly-(*R*)-1 concentration of 0.001 mg/ml.

^bThe fluorescence intensity ratio at 462 and 381 nm.

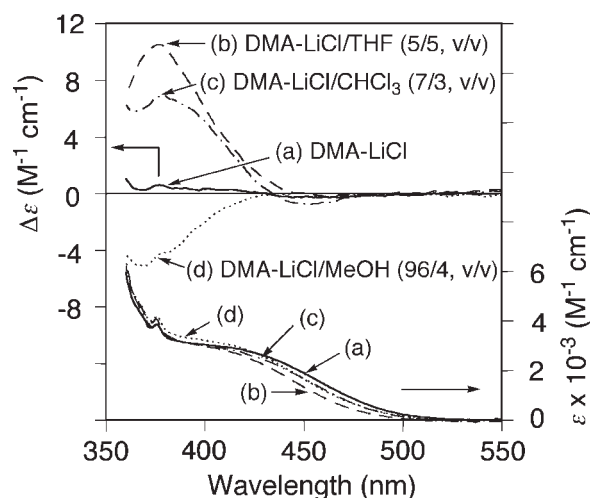


Fig. 3. CD and absorption spectra of poly-(*R*)-**1** (0.5 mg/ml) in DMA containing 10 mM LiCl (a), DMA-LiCl/THF (5/5, v/v) (b), DMA-LiCl/CHCl₃ (7/3, v/v) (c), and DMA-LiCl/MeOH (96/4, v/v) (d) at 25°C.

in the positive or negative direction with increasing volumes of THF (A) and CHCl₃ (B) or MeOH (C), respectively. The ICD intensities of the polymer in specific solvent mixtures further changed with temperature and the signs inverted through a transition temperature (T_m), where the $\Delta\epsilon_{371}$ values became almost zero. The T_m values tended to decrease with an increasing amount of THF and CHCl₃ (Figs. 4A and 4B), but they increased with increasing amounts of MeOH (Fig. 4C). These results indicate that the preferred-handed helical conformation of poly-(*R*)-**1** is likely determined by a delicate balance of the interactions between the polymer chain and solvents. As a consequence, we could control the T_m values by tuning the solvent compositions.

Fluorescence Properties

Next we investigated the fluorescence property of poly-(*R*)-**1**. The monomer (*R*)-**1** exhibited a characteristic monomer emission with peaks of 381 and 395 nm due to

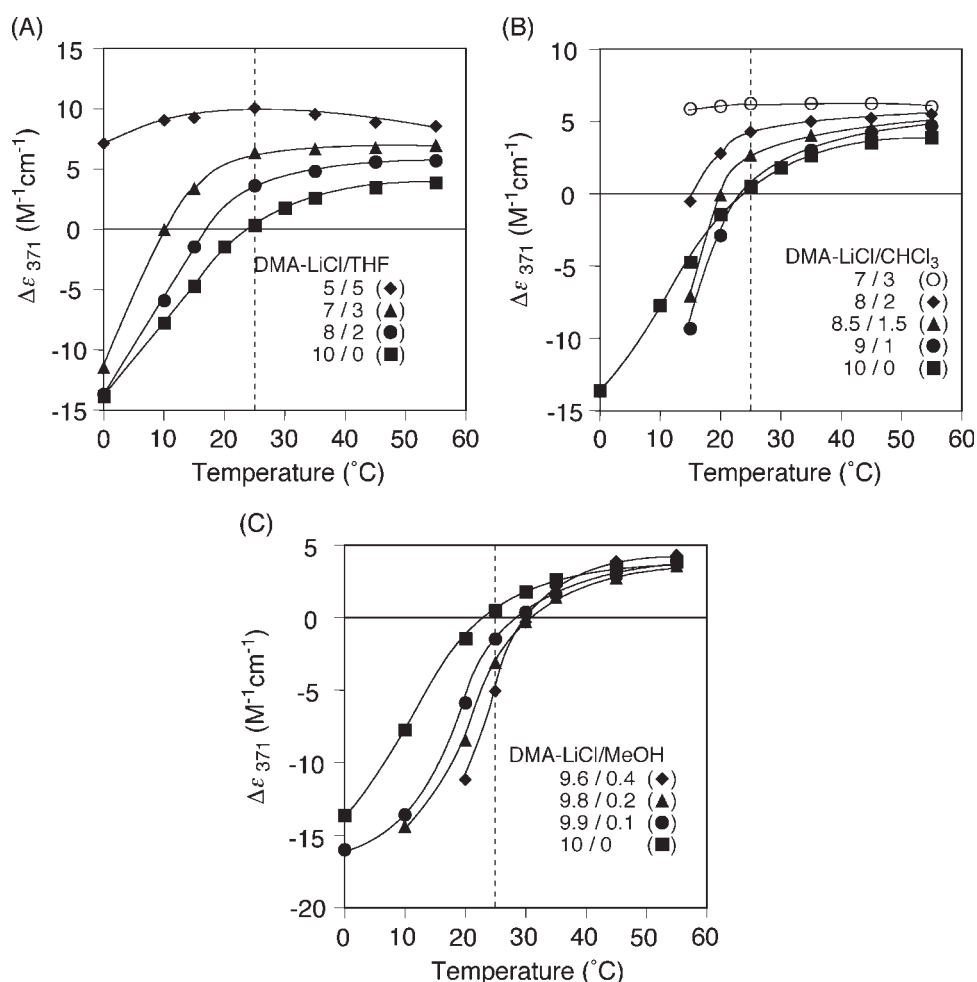


Fig. 4. Plots of the CD intensities at 371 nm ($\Delta\epsilon_{371}$) for poly-(*R*)-**1** versus temperature in DMA-LiCl/THF (A), DMA-LiCl/CHCl₃ (B), and DMA-LiCl/MeOH (C).

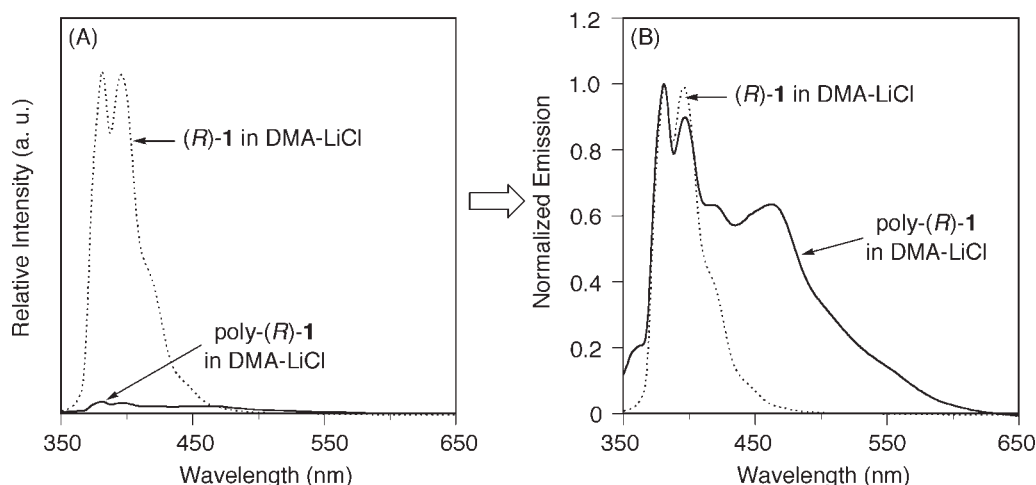


Fig. 5. (A) Fluorescence spectra of poly-(*R*)-1 and (*R*)-1 in DMA containing 10 mM LiCl at 25°C. The concentration of poly-(*R*)-1 and (*R*)-1 was 0.001 mg/ml. $\lambda_{\text{ex}} = 340$ nm. (B) The emission spectra of poly-(*R*)-1 and (*R*)-1 normalized at 381 nm.

the pyrene unit upon excitation at 340 nm in DMA-LiCl (Fig. 5A). In sharp contrast, poly-(*R*)-1 showed a very weak monomer emission as well as a weak, but apparent broad excimer emission from the pyrene pendants at around 462 nm under identical conditions (Figs. 5A and 5B). The main chain of the poly-(*R*)-1 consists of π -conjugated double bonds, so that this fluorescence quenching phenomenon may indicate a possible intramolecular energy transfer or electron transfer from the pyrene pendant to the polymer backbone. A similar fluorescence quenching was also observed for analogous helical poly(phenylacetylene)s bearing a chiral ruthenium (Ru) complex as a bulky pendant through the amide linkage or a C—C triple bond, and the latter showed negligibly weak emissions.⁴⁷ On the other hand, Masuda and coworkers reported a large excimer-based fluorescence of the pyrene-functionalized helical poly(*N*-propargylamide)s compared to the corresponding monomers, where the pyrene pendants were linked to the polymer backbones through nonconjugated aliphatic linkers.^{35–38} Further studies including laser flash photolysis and time-resolved emission spectral measurements are necessary to determine the origin of the fluorescence quenching observed in the poly-(*R*)-1.

In addition, the facts that poly-(*R*)-1 showed a broad excimer emission whose intensity was independent of the poly-(*R*)-1 concentration (0.01 to 0.0001 mg/ml) suggest that the excimer is formed between the adjacent pyrene pendants along the poly-(*R*)-1 backbone. It is well known that the excimer formation between two pyrene rings is sensitive to their distance, orientation, and the surroundings. Therefore, the intensity of the excimer emission of the pyrene pendants along the polymer chain may be influenced by changes in the secondary structures of poly-(*R*)-1 in response to temperature and solvent. As expected, the emission intensity ratio of the excimer to the monomeric state (FL_{462}/FL_{381}) changed in response to the temperature and solvent composition (Table 1). However, no significant relationship between the CD intensities of poly-(*R*)-1 and the FL_{462}/FL_{381} values was observed.

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The Prediction of the Circular Dichroism of the Benzene Chromophore: TDDFT Calculations and Sector Rules

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT The CD spectra of the series PhCH(Me)R, with R = Et (**1**), ⁿPr (**2**), ⁱPr (**3**), and ^tBu (**4**), are reported (**1–3** for the first time) at room temperature in the 185–280 nm range and at 183 K. These purely hydrocarbon compounds represent the simplest chiral systems containing the phenyl chromophore and exhibit Cotton effects exclusively allied with the benzene transitions. The bands in ¹L_a and ¹L_b regions were checked against the available sector rules, with discordant outcomes. Time-dependent density-functional theory calculations, with various functionals and basis sets tested, correctly reproduced the prominent CD bands observed for **1–4**. *Chirality* 20:393–399, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: benzene chromophore; circular dichroism; TDDFT calculations; sector rules

INTRODUCTION

In recent years, time-dependent density-functional theory (TDDFT) has emerged as the most convenient method for the calculation of electronic spectra in view of its very favorable accuracy/cost compromise.^{1,2} Among other properties, TDDFT lends itself to the prediction of electronic circular dichroism (CD) spectra.^{3,4} As TDDFT CD calculations were made routinely available with commercial software, the comparison between experimental and TDDFT-computed CD spectra has become a popular tool for assigning absolute configurations.^{5–10}

Aromatic rings constitute the most common and important chromophores among organic molecules. The parent benzene chromophore, in particular, represents a crucial benchmark for the evaluation of the performance of computational techniques in the prediction of excitation energies of organic molecules. Several TDDFT calculations on benzene and polycyclic aromatic hydrocarbons have been reported in the past.^{11–14} While the results are often superior to other techniques with a similar computational cost,¹⁴ it has been found that aromatic hydrocarbons often pose a question of functional choice, because a different amount of exact Hartree-Fock exchange may be required for an optimal description of various excited states (¹B_u, ¹L_a, and ¹L_b in Platt's nomenclature),¹⁵ on account of their diverse characters (ionic vs. covalent).^{11,12} A further problem is represented by the vibrational progression encompassing the low-energy ¹L_a and (especially) ¹L_b bands, whose prediction would require additional calculations of the vibronic structure.^{16–19}

The obvious interest in chiroptical properties of aromatic molecules—as a source of structural information—has led in the past to the development of various semi-

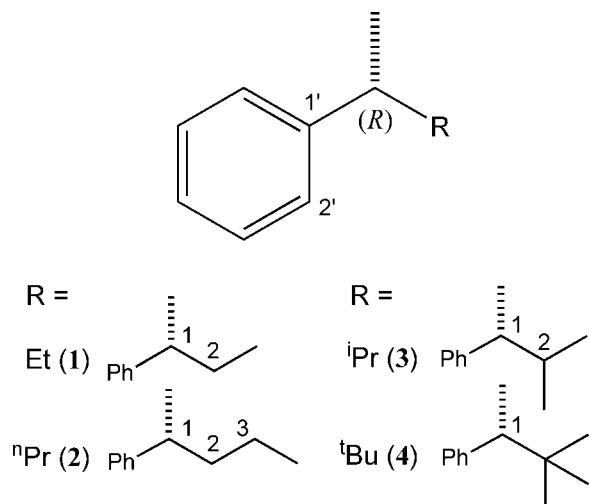
empirical rules, relating the sign of ¹L_a and chiefly ¹L_b Cotton effects to the absolute configuration of chiral molecules containing a phenyl or substituted benzene chromophore.^{20–23} These rules have mostly been applied to important natural products belonging to the families of phenylcarbinamines, phenylcarbinols, benzylcarbinamines, phenylalanines, mandelic acids, and so on. In a different approach, we deemed it interesting to take into consideration chiral hydrocarbons, devoid of any heteroatom and/or any other chromophoric group, and to investigate the Cotton effects associated with the “pure” benzene chromophore. It is noteworthy that, to the best of our knowledge, the CD spectrum of (*S*)-2,2-dimethyl-3-phenylbutane (**4**, see Chart) reported by one of us in 1972 still remains the only published CD spectrum belonging to the above category.²⁴ Here we report the CD spectra of the smaller homologues²⁵ (*R*)-**1–3** (see Chart) based on a similar chiral pattern, i.e., the α-phenylethyl with an ethyl (**1**), *n*-propyl (**2**), isopropyl (**3**), and *tert*-butyl (**4**) group attached at the chiral center; we then check the consistency of ¹L_a and ¹L_b Cotton effects measured for **1–3** with the relative sector rules. Second, we test the performance of TDDFT method (without including vibronic structure calculations)^{26,27} in the prediction of CD spectra of compounds **1–4**.

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EXPERIMENTAL SECTION

Compounds (*R*)-**1–3** were available in our laboratory with optical purities >95%,²⁵ which were checked again prior to use. CD spectra were measured on a Jasco J-710 Spectropolarimeter equipped with a low-temperature apparatus, with the following conditions: scan speed 20 nm/min, time constant 1–2 sec, band-width 1 nm, 16–64 scans. For each compound, a solution 2–5 mM in hexane (**1,2**) or heptane (**3**) was prepared and CD spectra were measured using cells with different path-lengths: 0.01 cm (185–200 nm), 0.02–0.05 cm (200–230 nm), and 1 cm (240–280 nm). CD spectra for (*S*)-**4** in heptane were taken from Ref. 24 and inverted.

COMPUTATIONAL SECTION

Conformational searches were run employing Molecular Merck force field (MMFF), with standard parameters and convergence criteria, implemented in Spartan'06, Wavefunction, Irvine, CA. All minima thus found were optimized with DFT, B3LYP/6-31G(d) level,²⁸ using Gaussian'03W, Revision B.05, Gaussian, Pittsburgh, PA. TDDFT calculations were run with G03W on all minima within 2 kcal/mol, employing various functionals (BP86, B3LYP, BH&HLYP) and basis sets (TZVP, ADZP,²⁹ aug-cc-pVTZ).²⁸ CD spectra were generated by applying a Gaussian band-shape with 2500 cm⁻¹ half-height width (corresponding to 11 nm at 210 nm) to rotational strengths computed with the dipole-length gauge formulation.

RESULTS AND DISCUSSION

Experimental CD Spectra of **1–4**

Figures 1a–1d report CD spectra of compounds **1–4** in the 185–280 nm range. They consist of three regions allied to the ¹B_b (below 200 nm), ¹L_a (205–225 nm), and ¹L_b (240–275 nm) transitions of the benzene chromophore.¹⁵ The overall spectral intensity increases when passing from **1** to **4**, i.e., with the bulkiness of substituent R at the chiral center.

This is intuitive because in our compounds of formula PhCH(Me)R, the chirality arises from the difference between R and Me groups, thus the bulkier and the more polarizable is R, the larger is the dissymmetry of the perturbation exerted on the phenyl ring. Because of unfavorable $\Delta\epsilon/\epsilon$ ratio, the 185–200 nm region could be entirely covered only for compounds **3** and **4**; however, its low-energy side clearly suggests that, for all compounds with (*R*) configuration, the dominant CD band allied to ¹B_b transition is negative. A weak CD band, endowed with an apparent vibrational progression with 900–1000 cm⁻¹ spacing, is measured in the ¹L_a region, again with negative sign for all compounds with (*R*) configuration. Low-temperature CD spectra were also recorded, shown with dashed lines in Figures 1a–1d: at the low temperature limit (183 K), ¹L_a Cotton effects for **1–3** feature a twofold intensity increase while preserving the same sign and shape. In the long-wavelength region allied to ¹L_b transition, as a consequence of its inherent weakness due to basically dipole-forbidden character, the corresponding Cotton effects are substantially dominated by the vibrational contribution,³⁰ and appear as a sequence of pronounced minima and maxima (compounds **3** and **4**) or of maxima of alternating sign (**1** and **2**). This spectral shape is due to two distinct vibrational progressions with opposite signs, both associated with a totally symmetric vibration (ring breathing) with frequency $\approx$ 920–1000 cm⁻¹: an allowed one, starting in correspondence with the 0–0 absorption band at 267–268 nm; and a “forbidden” one, starting from a 0–*v* band, where *v* $\approx$ 420 cm⁻¹ is associated with a nontotally symmetric vibration.^{31,32} The overall ¹L_b-band integral has the same sign of the allowed progression, which is distinctly negative for **3** and **4** and only weakly positive for **1** and **2**. The situation changes dramatically at low temperature, where the dominant sign becomes clearly positive (the same of the allowed progression) for **1–3**, while the CD of **4** is unchanged. Similarly marked changes upon cooling have been observed for ¹L_b bands of other chiral benzene derivatives.³¹

Geometry Optimizations

To predict the chiroptical properties of **1–4** with either sector rules or TDDFT computations, their conformation was studied by means of MMFF conformational searches and DFT calculations at B3LYP/6-31G(d) level, the results of which are summarized in Table 1. While **4** has the rotation around the C1'–C1 bond (see Chart for numbering) as its only degree of freedom, **1** and **3** have one additional C1–C2 rotation, and **2** a further C2–C3 rotation allowed. In **4**, the phenyl–C1 torsional mode has a single energy minimum with dihedral C2'–C1'–C1–H1 around 15° (Table 1); this is in keeping with a CD spectrum unaffected by temperature changes (Fig. 1d).²⁴ For **1** and **3**, three minima with DFT energy within 2 kcal/mol were found (Table 1), the most stable one having C2'–C1'–C1–H1 dihedral close to 0° (H1 eclipsed with the ring). For **2**, five minima were found, the most stable one again with C2'–C1'–C1–H1 dihedral close to 0° (Table 1). In each case, the absolute minimum optimized with either B3LYP/6-31G(d) or MP2/6-31G(d) coin-

TABLE 1. B3LYP/6-31G(d) optimized structures^a and relative energies (in kcal/mol) for compounds 1–4

(<i>R</i>)-1  Abs. min. +0.64 +1.55			(<i>R</i>)-3  Abs. min. +0.34 +0.70		
(<i>R</i>)-2  Abs. min. +0.62 +0.80			(<i>R</i>)-4  +1.44 +1.56 Abs. min.		

^aEnergy minima within 2 kcal/mol from the lowest energy are shown.

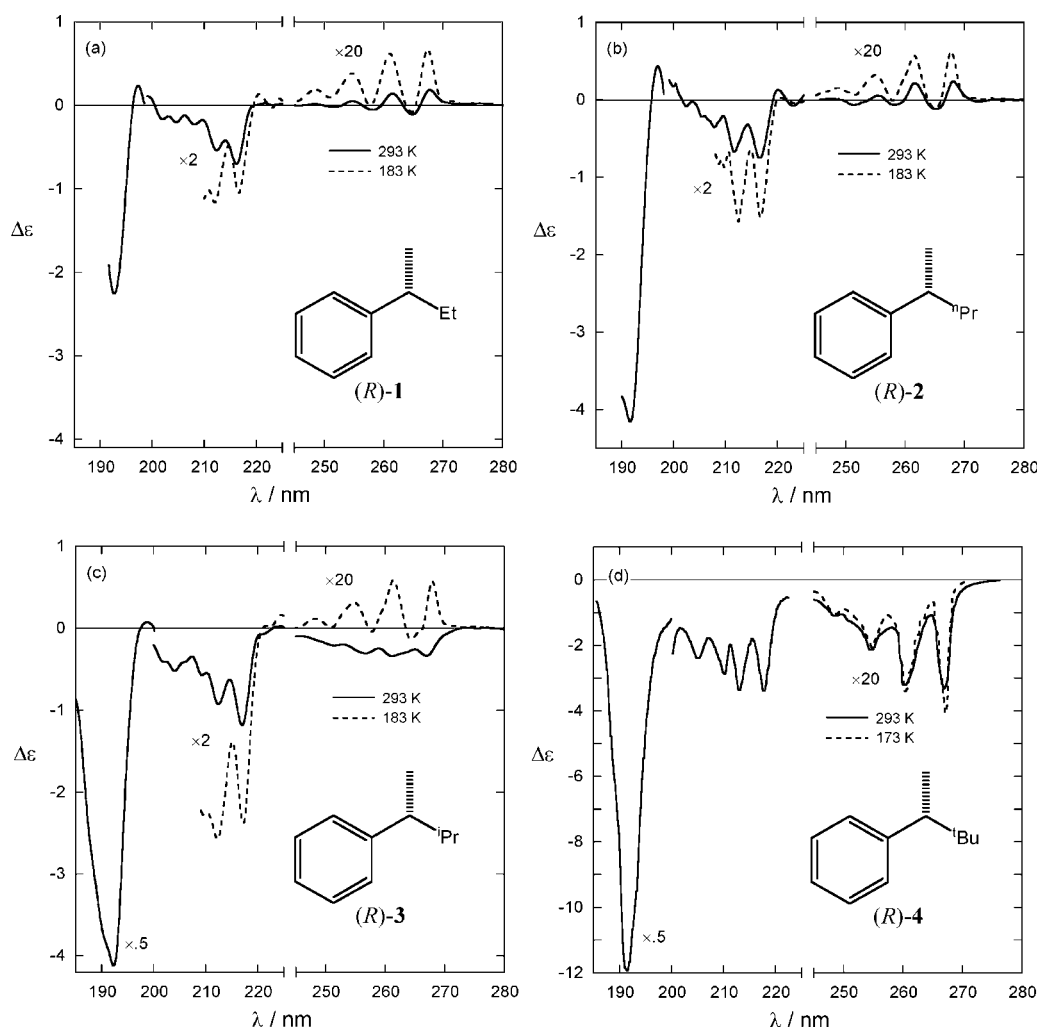
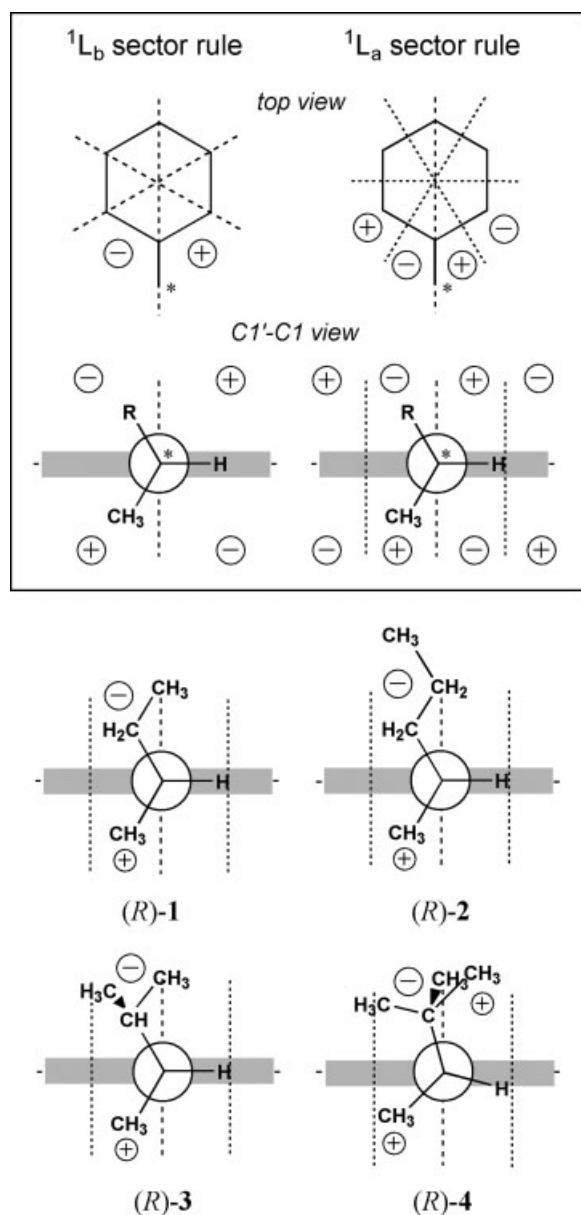


Fig. 1. Experimental CD spectra of compounds (*R*)-1–4 at room temperature (solid lines) and at 173–183 K (dashed lines), in hexane or heptane. Sample concentrations 2–5 mM, cell path length 0.01 cm (185–200 nm), 0.02–0.05 cm (200–230 nm), and 1 cm (240–280 nm).



Scheme 1. Top: benzene sector rules for ^1L_a and ^1L_b Cotton effects;^{20,21} each substituent to the chiral center is expected to give a contribution to the CD with sign corresponding to that of the sector it lies in, and magnitude proportional to its polarizability ($R > CH_3 > H$). Bottom: schematic structures for the absolute minima computed with B3LYP/6-31G(d) for compounds **1–4**, with sector contributions expected for the various groups.

cided.³³ Interestingly enough, the lowest-energy structures computed for **1–3** (see Table 1 and Scheme 1) are very similar, which is in agreement with the fact that at 183 K, where the absolute minima are more fully populated, the CD spectra of **1–3** converge into the same feature.

Application of Sector Rules

Only for **4** has, so far, the sign of CD spectrum been checked against the well-known sector rule for the phenyl Chirality DOI 10.1002/chir

^1L_b Cotton effect: the negative CD band for (*R*) absolute configuration was found in agreement with the general trend.²⁰ We must consider, however, this agreement fortuitous, because the conformation adopted by **4** (Scheme 1 and Table 1) is quite far from that with H1 eclipsed with the ring, prescribed by the rule;²⁰ analogous misinterpretations based on wrong conformational assumptions have been demonstrated for other benzene derivatives.³⁴ On the contrary, for the absolute minima of compounds **1–3**, which do have the prescribed conformation, the dominant sign of the ^1L_b Cotton effect (that of the allowed progression) is positive for (*R*) absolute configuration (see spectra at 183 K in Fig. 1), thus establishing three relevant exceptions to the rule (Scheme 1).

A different result is found when employing the relatively less popular sector rule for the ^1L_a Cotton effect.^{21,22} We prefer to focus particularly on this band because it has the same sign for all compounds **1–4**, irrespective of temperature, and it is less affected by the vibronic coupling (for consistency with TDDFT calculations where it is not considered). Here, for compounds **1–3** in the lowest energy conformation, a negative ^1L_a Cotton effect is expected and consistently found (Fig. 1 and Scheme 1). The situation for **4** is ambiguous because some groups cannot be safely assigned to a defined sector.

TDDFT CD Calculations

As a benchmark for testing TDDFT calculations we chose compound **4** in view of its defined conformation and most intense CD spectrum. We employed the pure GGA functional BP86 and the hybrid functionals B3LYP and BH&HLYP with increasing HF exchange character (20 and 50%, respectively).²⁸ As basis sets, we used the triple- ζ basis set with polarization functions TZVP, the double- ζ basis set with polarization and diffuse functions ADZP,²⁹ and the correlation consistent triple- ζ basis set with polarization and diffuse functions aug-cc-pVTZ.²⁸ As anticipated above, the vibronic coupling^{26,27} was not considered. All the above functionals were able to reproduce the CD spectrum of **4** in terms of sign and relative intensity of bands (Fig. 2), although none of them did it perfectly. In general, BP86 predicted the energies better, while hybrid functionals provided more accurate rotational strengths; B3LYP offered the best compromise. The basis sets including diffuse functions performed apparently worse than TZVP, because of the collapse of higher energy bands onto the ^1L_a one. This is especially surprising because with TZVP, but not with ADZP and aug-cc-pVTZ, all transitions beyond the second one involve unbound virtual orbitals and have energies above the computed ionization potential (between 6–7 eV, depending on the functional); therefore, the underestimation of ^1B_u transition energy with the augmented basis sets is in principle less expected,³⁵ though not unprecedented.¹⁴ As a consequence, the B3LYP/TZVP method was chosen for the following calculations, also in consideration that the difference between rotational strengths computed with the dipole-length and the dipole-velocity gauge (which is an indication of basis set incompleteness)^{3,4,36} is acceptable

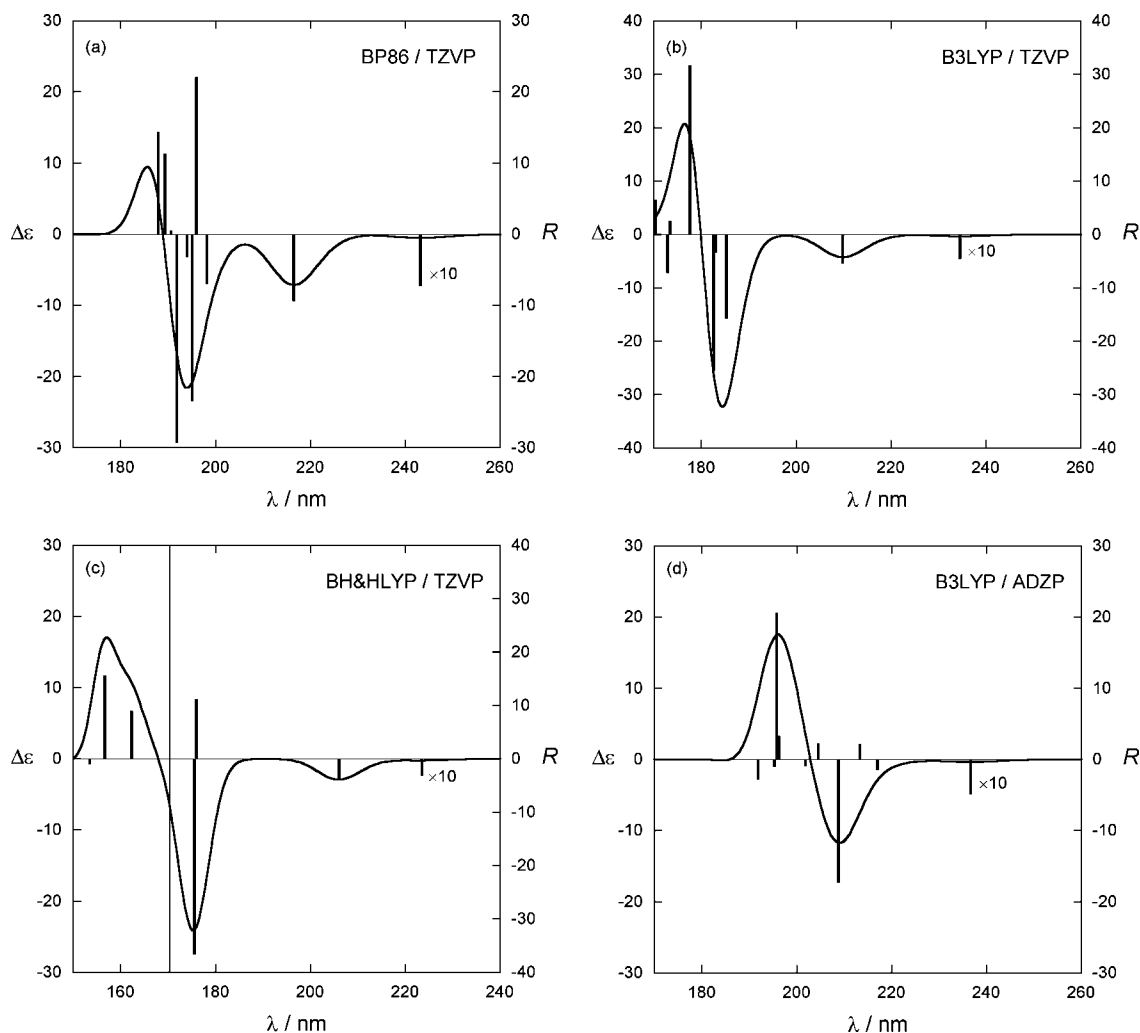


Fig. 2. TDDFT-computed CD spectra for compound (*R*)-**4** on B3LYP/6-31G(d) optimized structure (Table 1) with various functionals and basis sets. Vertical bars represent rotational strengths *R* in 10^{-40} cgs.

with TZVP, i.e., below 15% for the main transitions for **4**. CD spectra were computed for each structure found above for compounds **1–3**, then weighted according to Boltzmann's distributions at 293 and 183 K; Figures 3a–3c report the two average spectra as well as that calculated for each absolute minimum (see Table 1). Comparative inspection of Figures 1 and 3 reveals that the sign and relative intensity of ^1B_b and ^1L_a Cotton effects is well reproduced for all compounds, while the absolute intensities are overestimated especially for **1** and **2** by a factor 10. For each compound, the two average spectra are almost coincident in the ^1L_a region, which is at odds with experimental results at different temperatures; most probably the effective conformational ensemble populated at room temperature is not adequately represented by the 3–5 minima considered in the calculations. On the other hand, calculated averages of ^1L_b bands are strongly affected by temperature; in this region, however, appropriate inclusion of vibronic effects^{26,27} would be obviously needed for a correct prediction.

In conclusion, we have reported the CD spectra at different temperatures of three simple hydrocarbon compounds **1–3** exhibiting Cotton effects obviously allied to the phenyl chromophore transitions; they represent the smaller homologues of compound **4** already described. Despite their apparent structural simplicity, prediction of the CD spectra of this series is not a trivial task. The well-known phenyl sector rule failed to predict the sign of ^1L_b Cotton effects; *ab initio* calculations of this band must necessarily take into account the pronounced vibronic structure. The situation is less problematical in the high energy region, where compounds **1–3** obey the sector rule available for ^1L_a Cotton effect, and TDDFT calculations reproduce the observed spectra in terms of signs and relative intensity. It must be stressed that, in the most common application of TDDFT CD calculations for assigning absolute configurations,^{5–10} one would logically focus on the most intense region of the spectrum (185–230 nm); in the current case, TDDFT results would unambiguously provide the correct answer for all compounds **1–4** considered.

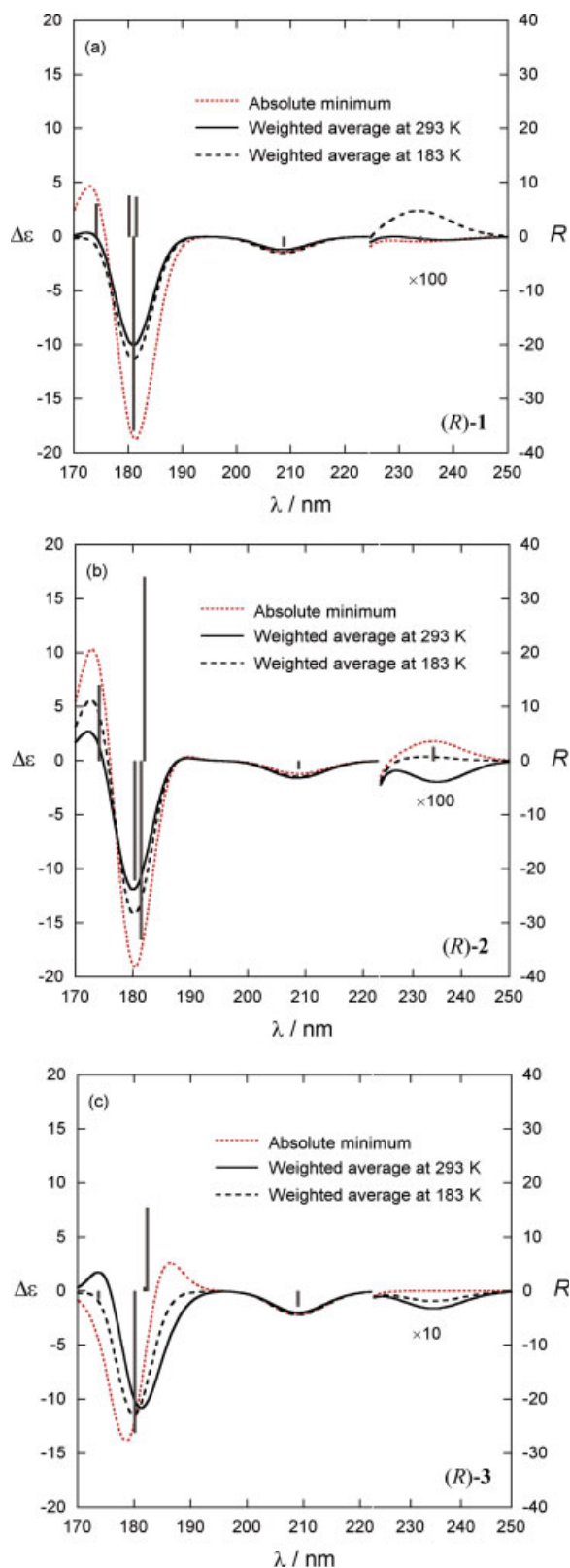


Fig. 3. TDB3LYP/TZVP computed CD spectra for compounds (R)-1-3 in the lowest energy B3LYP/6-31G(d) conformations (vertical bars represent rotational strengths R in 10^{-40} cgs) and Boltzmann-weighted averages at 293 and 183 K for all conformations within 2 kcal/mol (see structures in Table 1). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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Molecular Mechanics (MM3(π)) Conformational Analysis of Molecules Containing Conjugated π -Electron Fragments: Leucomycin-V

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT The conformations of the 16-membered macrolide antibiotic leucomycin-V (**1**) were studied with molecular mechanics. Leucomycin-V contains a conjugated π -electron fragment and necessitates special treatment with the MM3(π) modeling protocol. Comparison was made with results from the standard MM3 scheme. The CONFLEX conformational search procedure was used for finding low-energy conformations. The computed data are indicative for the existence of mainly one conformation of the macro-ring of **1** and minor participation of several others. Intramolecular hydrogen bonds play important roles for the preferred geometry of the macro-ring and the conformations of the side chains. The most probable macro-ring conformation of **1** is very similar to the preferred conformation of another 16-ring macrolide antibiotic, tylosin. The same order of conformational preference for **1** was estimated with the MM3 and the MM3(π) methods. Surprisingly, when changing the chirality of the C(9) macro-ring atom of **1**, the two methods produced different order of conformational preferences for the 9-epi form (**2**), as well as enhanced population of several clusters of conformations. *Chirality* 20:400–410, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: leucomycin-V; conformational analysis; molecular mechanics; MM3(π); CONFLEX search

INTRODUCTION

The macrolide antibiotics have a broad spectrum of antimicrobial effect, and they are studied extensively.^{1–15} They play therapeutically an important role, particularly with the emergence of new pathogens¹² and are considered as the preferred therapeutic agents for the treatment of upper and lower respiratory tract infections with proven safety and efficacy. Macrolides exert their antibacterial effect by selectively binding to the 50S subunit of the bacterial ribosome and thus inhibiting protein synthesis.¹⁴ As a large family of both natural and semisynthetic antibiotics, macrolides are classified according to the size of the lactone ring consisting of 12–16 atoms. Other differences are related to the number and nature (neutral or basic) of the sugars.

Erythromycins belong to the superfamily of complex polyketides that are of constant interest due to their pharmacological activity and useful agricultural properties.¹⁶ It is thought that the biological activity of complex polyketides is dependent on conformational preorganization, which directs functional groups in space and reduces the entropic penalty involved in binding.¹⁷ Some complex macrocyclic polyketides are promising neuroregenerative agents.¹⁶ Erythromycin A and its derivatives, such as clarithromycin, which has a 14-membered lactone ring, are among the most commonly prescribed macrolides. As a result of extensive usage, however, erythromycin-resistant

pathogens have become increasingly prevalent. Accordingly, the problem of bacterial resistance had been addressed by the development of a new generation of 14-membered macrolides known as ketolides, such as telithromycin and cethromycin.¹⁴

The 16-membered ring macrolide antibiotics (e.g., leucomycins and tylosin) constitute an important clinically useful series of naturally occurring compounds within the macrolide class of antibiotics, as they show some advantages over 14-membered erythromycin A derivatives such as gastrointestinal tolerance and activity against resistance-expressing strains. After cleavage of the mycarose sugar of tylosin and subsequent modification of the 4'-hydroxyl group, novel 4'-substituted 16-membered ring macrolides were recently synthesized.¹⁴ One of them exhibited much improved activities against some key

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erythromycin-resistant pathogens compared with tylosin and erythromycin A. Thus promising opportunities appeared for the development of new macrolide antibiotics to overcome bacterial resistance. Moreover, high-resolution X-ray cocrystal structures of the bacterial ribosome with the macrolides have given an insight into the detail of these interactions at the atomic level and provided further means for understanding the mechanism of action of the macrolides and for improving their activity.¹³

Recently, we reported results from a molecular mechanics study of the preferred conformations of tylosin.^{18,19} Two different approaches were examined: (i) a Monte-Carlo conformational search technique in BatchMin^{20–22} with the AMBER* force field as a model, and the GB/SA method²³ for modeling the solvent, and (ii) a thorough search for the lowest energy conformations based on the CONFLEX^{24–26} procedure as a searcher and the MM3(92)^{27–30} force field as a molecular mechanics model. A specific characteristic and an advantage of the Allinger's MM3 force field is that an extension of the force field, MM3(π),^{31,32} has been devised to model molecules with conjugated π -electron fragments. We now apply the same methodology in the conformational analysis of another system with many degrees of freedom for intramolecular rotations, leucomycin-V (**1**), as well as on its 9-epi analogue (**2**). Leucomycin-V is among the first macrolide antibiotics for which experimental conformational studies were carried out with spectral methods.⁴ X-ray crystal data of leucomycin-A5 are also available.¹⁰

Our study on tylosin¹⁹ posed several questions that need further elucidation:

1. What is the dependence of the macro-ring conformation from the side chain sugar moieties? The computed global-minimum conformation of tylosin resulted from hydrogen bonding through the macro-ring between the two side chain sugar fragments. This conformation was rejected however from consideration as being of low probability.¹⁹ Now we consider the case with a mycarosyl mycaminoside disaccharide as the only big substituent to the macro-ring.
2. Hydrogen bonding of the OH group at C(2') on mycaminoside with the nitrogen atom of the dimethyl amino group is observed in the IR spectrum of leucomycin-V.⁴ We could not find such a conformation among the computed lowest energy forms of tylosin. Is the presence of the mycinose sugar responsible for that computed result?
3. Tylosin required treatment with the π -version of the MM3 method.¹⁹ Is this necessary also for **1**? Now we have a diene instead of a dienone unsaturated fragment.

As an overall result of methodological significance from the present study are the differences between two very similar molecules **1** and **2** (they differ only by the chirality of the C(9) macro-ring carbon atom) when comparative studies are carried out using the MM3 and MM3(π) molecular mechanics modeling protocols.

COMPUTATIONAL DETAILS

The version 3.6 of CONFLEX^{24–26} was used. The starting conformation for **1** was derived by molecular graphics

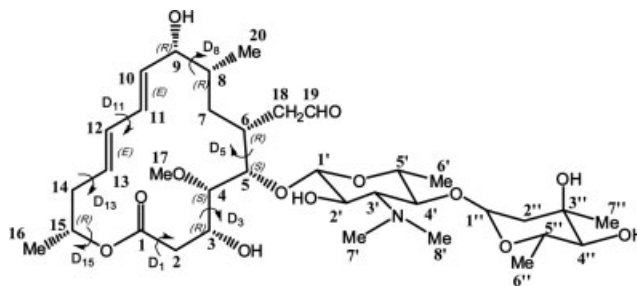


Fig. 1. Numbering of atoms in leucomycin-V (**1**).

from optimized coordinates of tylosin. Portions of the lactone ring were perturbed by bond flip and corner flap to generate new 16-ring conformations, while stepwise rotations were applied for side-chain fragments to generate new rotamers during the conformational search. Thirteen stepwise rotation dihedral angles were selected (D_1 – D_{16}) designate the endocyclic dihedral angles of the aglycone ring; the numbering of the atoms of **1** is displayed in Fig. 1): $D_{17} = C(4)–C(5)–O–C(1')$, $D_{18} = C(5)–O–C(1')–O$, $D_{19} = C(2')–C(3')–N–Me$, $D_{20} = C(5)–C(6)–C(18)–C(19)$, $D_{21} = C(3)–C(4)–O–Me$, $D_{22} = C(3')–C(4')–O–C(1')$, $D_{23} = C(4')–O–C(1')–O$, $D_{24} = C(6)–C(18)–C(19)=O$, $D_{25} = C(2)–C(3)–O–H$, $D_{26} = C(8)–C(9)–O–H$, $D_{27} = C(1')–C(2')–O–H$, $D_{28} = C(2'')–C(3'')–O–H$, $D_{29} = C(3'')–C(4'')–O–H$. The same dihedral angles were used for describing the conformations of 9-epi-leucomycin (**2**). For ease of comparison and to avoid confusion, we followed the same designation of dihedral angles (non-IUPAC nomenclature) we used in our earlier study on tylosin,¹⁹ and also utilized by others in experimental studies we cite in this work. Five steps of 60° were generated for the rotation about the $C(18)–C(19)$ bond. Two rotations of $\pm 120^\circ$ were accomplished in all other cases. The six-membered rings were not subjected to any perturbations. The chirality of the asymmetric carbon atoms of the 16-membered ring was kept during the search. Restrictions were imposed to keep the starting *10-trans* and *12-trans* configurations about the double bonds. Thus, 29 torsional angles were considered to vary when searching for low-energy conformations of **1** and **2**. A gradual increase to a search limit of 5.0 kcal mol^{–1} determined the energy range from which the initial structures for the search were selected.^{24–26} The torsional angles used for conformation comparison when eliminating identical conformers comprised the 16 endocyclic dihedrals of the macro-ring plus the 13 dihedral angles of stepwise rotations. The default values of the options were used when the steric energies and the dihedral angles were compared in the redundancy test. The search for **1** and **2** ended with, respectively, 4978 and 4996 conformations stored with relative energies less than 7.0 kcal mol^{–1}. The whole sets of conformations were subjected to a cluster analysis^{33,34} and the resulted structures were sorted into 50 clusters for **1** and 86 clusters for **2**. Fourteen clusters of **1** and 21 clusters of **2** contain more than 50 conformations each (Tables I-S and III-S in Supplemen-

tary Material). The analysis of the dihedral angles revealed that most of them appear as pairs of identical conformations, which differ by inversion of the dimethylamino group on mycaminose. Unique conformations were processed with the π -version (MM3(π)) of the MM3(92) program.^{30–32} This approach is more economical than the direct search with the computational scheme for modeling conjugated π -electron segments in the molecule. The block-diagonal Newton–Raphson minimization procedure was used.³⁰ Electrostatic interactions were estimated with the value 2.0 of the dielectric parameter.

RESULTS AND DISCUSSION

Computed conformational data for **1** are summarized in Tables 1 and 2. These are average steric energies and populations of clusters (Table 1) and the relative energies of the low-energy conformations within each cluster obtained with MM3 and MM3(π) (Table 2), and a letter-code representation of the important dihedral angles. The macro-ring dihedral angles of the most probable conformations (clusters **A** and **C**) of leucomycin and tylosin are compared in Table 3. A comparison was also made between computed dihedral angles of **1** with estimates from X-ray analysis for leucomycin-A5¹⁰ (Table 4). For the sake of compactness of the presentation we used a letter-code designations of dihedral angles as given in Figure 2.¹⁸ A letter **G** designates a dihedral angle in the range from 20° to 100°, **G'**, from 100° to 150°; **A**, from 150° to –150°, etc. The conformations of **1** are sorted into 50 groups (clusters) designated with capital letters from **A** to **Z**, **A1** to **X1**, etc. They differ by the conformation of the lactone ring. The conformations within each cluster are listed in the order of increasing relative energies, together with the different dihedral angles for side-chain rotations (common dihedral angles within each cluster are presented separately only once). Table 5 summarizes the information for the patterns of the computed intramolecular hydrogen bonded networks. The optimized geometries of the lowest energy conformations for each one of the first six clusters and the patterns of hydrogen bonding for **1** are displayed in Figure 3. Table 6 presents

computed data for **2**. Low-energy conformations of **2** are presented in Figure 4.

Leucomycin-V

We emphasized in our previous work¹⁹ the importance of using at the final stage of the minimization the version of MM3 devised to model molecules with conjugated π -electron fragments. Such kind of treatment required the dienone portion of tylosin. For **1**, that has a smaller conjugated π -electron system, we obtained the same order of relative energies with both methods, MM3 and MM3(π): **A.1** < **B.1** $\approx$ **C.1** < **D.1** < **E.1**. It is not however the case with its 9-epi analogue **2**.

Five clusters of conformations represent more than 96% of the population of all conformations found for **1** after the CONFLEX search (Table 1). Cluster **A** has the highest computed population (74%), and comprise about one-third of all local minima found. Compared with clusters **B** and **C**, cluster **A** has eight conformations (**A.1**–**A.8**) with energies lower than **B.1** and **C.1**. The average relative steric energies of clusters **B**, **C**, and **D** differ by less than 0.5 kcal mol^{–1} and possess the total of about 20% population.

The following general features characterize the leucomycin-V conformations:

1. Preference for the *s-trans* conformation of the diene portion (D_{10} , D_{11} , D_{12}).
2. C=O/O–C- eclipsed conformation at the lactone moiety (D_{16}).
3. Favored extended conformation for the rotation about the C(3)–C(4) bond.
4. Prevalence of folded conformations of the endocyclic dihedrals D_5 , D_8 , D_{14} , and D_{15} .
5. The global minima conformations for the most populated clusters (**A.1**, **B.1**, **C.1**, **D.1**, **F.1**) acquire the same or very similar conformation of the disaccharide portion (dihedrals D_{17} , D_{18} , D_{19} , D_{22} , D_{23} , D_{27} , D_{28} , D_{29}), as well as for the hydroxyl group at C(9) (D_{26}), and the methoxy group at C(4) (D_{21}) (Table 2).
6. Two five-ring hydrogen bonds, C(2')O–H...O<C(5) (distance 2.45 ÷ 2.53 Å; in tylosin 2.36 ÷ 2.66 Å¹⁹),

TABLE 1. Cluster analysis of low-energy conformations of leucomycin-V (**1**)^a

	Dihedral angles of macro-ring clusters																Number of conform.	ΔE_o^b	ΔE_{av}^c	Population ^d
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	O–1				
A	G	A	A	G'	G'	A	G'	G'	G	A	A	A	G	G'	G	A	1725	0.0	1.4	0.740
B	A	G'	A	A	G	G	A	G	G	A	A	A	G	G'	G	A	461	1.3	2.3	0.106
C	G'	A	A	G'	G'	A	G'	G'	G	A	A	A	G	G'	G'	A	545	1.2	2.5	0.061
D	G	A	A	G'	G'	A	G'	G'	G	A	A	A	G	G'	A	A	231	1.7	2.8	0.021
E	A	G'	G	A	G	G	G'	G'	G	A	A	A	G	G'	G	A	351	2.6	3.3	0.028
F	A	A	A	G'	G'	A	A	G	G	A	A	A	G	G'	G	A	292	2.5	3.6	0.013
G	A	G'	A	A	G	G	A	G	G'	A	E	A	G	G'	G	A	97	3.0	3.9	0.004
H	A	A	G	G	A	A	G'	G'	G	A	A	A	G	G'	G	A	92	3.1	4.0	0.003
I	G	G'	A	A	G	G	A	G	G'	A	A	A	G'	G'	G	A	85	3.1	4.0	0.003

^aUnits are kcal mol^{–1}. Table I-S in the Supplementary Material contains the data for all clusters obtained from the analysis.

^bRelative steric energy of the lowest-energy conformation of the cluster.

^cAverage steric energy of the cluster.

^dAt 300 K.

TABLE 2. Computed conformational data for the dihedral angles of side-chain rotations of leucomycin-V (1)^a

									ΔE^a	
Conformation	D ₁₇	D ₁₈	D ₂₀	D ₂₁	D ₂₂	D ₂₄	D ₂₅	D ₂₆	MM3	MM3(π)
Conformation A (D ₁₉ , D ₂₃ , D ₂₇ , D ₂₈ , D ₂₉) $\equiv$ (G'G'GGG) ^b										
A.1	G'	G'	G'	G	G	E	G'	A	0.0	0.0
A.2	G'	G'	G'	G	G	G'	G'	A	0.6	0.6
A.3	G'	G'	G'	G	G	G	G'	A	0.7	0.7
A.4	G'	G'	G'	G	G	E	G'	A	0.8	0.8
A.5	G'	G'	G'	G	G	E	G'	G'	0.9	0.9
A.6	G'	G'	G'	G	G	E	G'	A	1.1	1.0
A.7	G'	G'	G'	G	G	G	G'	A	1.1	1.1
A.8	G'	G'	G'	G	G	E	G'	A	1.1	1.1
A.9	G'	G'	G'	G	G	G'	G'	G'	1.2	1.2
A.10	G'	G'	G'	G	G	E	G'	A	1.3	1.4
A.11	G'	G'	G	G	G	G	G'	A	1.3	1.4
A.12	G'	G'	G'	G	G	G'	G'	A	1.3	1.3
A.13	G'	G'	G'	G	G	G'	G'	G'	1.4	1.4
A.14	G'	G'	A	G	G	E	G'	G'	1.4	1.5
A.15	G'	G'	G'	G	G	G	G'	G'	1.4	1.4
A.16	G'	G'	G'	G	G	G	G'	A	1.5	1.5
A.17	G'	G'	G'	G	G	G	G'	A	1.5	1.5
A.18	G'	G'	A	G	G	G'	G'	A	1.6	1.6
A.19	G'	G'	G'	G	G	E	G'	G'	1.7	1.7
A.20	G'	G'	G	G	G	G'	G'	A	1.7	1.7
A.21	G'	G'	G	G	G	G	G'	G'	1.7	1.7
A.22	G'	G'	G	G	G	E	G'	A	1.7	1.7
A.23	G'	G'	G'	G	G	G	G'	A	1.7	1.7
A.24	G'	G'	G'	G	G	E	G'	A	1.9	1.8
A.25	G'	G'	G'	G	G	G	G'	G'	1.9	1.8
A.26	G'	G'	G'	G	G	E	G'	A	1.9	1.8
A.27	G'	G'	G'	G'	G	E	G'	A	1.9	1.9
A.28	G'	G'	G'	G	G	E	G'	A	1.9	1.9
A.29	G'	G'	G'	G	G	G	G'	A	1.9	1.9
A.30	G'	G'	G'	G	G	E	G'	A	1.9	1.9
A.31	G'	G'	G'	G	G	E	G'	G'	1.9	1.9
A.32	G'	G'	G'	G	G	E	G'	A	1.9	1.9
A.33	G'	G'	G'	G	G	G'	G'	G'	2.0	2.0
Conformation B (D ₁₇ , D ₁₈ , D ₁₉ , D ₂₁ , D ₂₂ , D ₂₃ , D ₂₇ , D ₂₈ , D ₂₉) $\equiv$ (G'G'G'GGG'GGG) ^b										
B.1			A			E	G	A	1.3	1.4
B.2			A			E	G	A	1.5	1.6
B.3			A			E	G	G'	1.6	1.7
B.4			A			E	G	A	1.6	1.7
B.5			A			E	G	G'	1.6	1.8
B.6			A			G'	G	A	1.7	1.8
B.7			A			G'	G	A	1.7	1.9
B.8			A			E	G	A	1.9	2.0
B.9			G'			G	G	A	1.9	2.1
B.10			A			E	G	G'	2.0	2.0
B.11			A			G'	G	A	2.0	2.1
Conformation C (D ₁₇ , D ₁₈ , D ₁₉ , D ₂₀ , D ₂₁ , D ₂₂ , D ₂₃ , D ₂₆ , D ₂₇ , D ₂₈ , D ₂₉) $\equiv$ (G'G'G'G'GGG'AGGG) ^b										
C.1						E	G'		1.2	1.4
C.2						G'	G'		1.9	2.1
C.3						E	G'		2.0	2.2
C.4						E	G'		2.1	2.3
C.5						G	G'		2.3	2.5
C.6						E	A		2.4	2.6
C.7						G'	G'		2.6	2.7
C.8						G'	G'		2.6	2.8
C.9						E	G'		2.6	2.8
C.10						G'	G'		2.6	2.8
C.11						E	G'		2.9	3.1
C.12						E	G		3.0	3.1

TABLE 2. Continued

Conformation	D ₁₇	D ₁₈	D ₂₀	D ₂₁	D ₂₂	D ₂₄	D ₂₅	D ₂₆	ΔE^a	
									MM3	MM3(π)
C.13						E	G'		3.0	3.2
C.14						G	G'		3.0	3.2
Conformation D (D ₁₉ , D ₂₀ , D ₂₁ , D ₂₃ , D ₂₄ , D ₂₇ , D ₂₈ , D ₂₉) $\equiv$ (G'G'GG'EGGG) ^b										
D.1	G'	G'			G		A	A	1.7	1.9
D.2	G'	G'			G		A	A	2.5	2.7
D.3	G'	G'			G		A	A	2.6	2.8
D.4	G'	G'			G		A	G'	2.6	2.9
D.5	G'	G'			G		G	A	2.8	3.0
D.6	G'	G'			G		A	A	3.0	3.1
Conformation E (D ₁₇ , D ₁₈ , D ₁₉ , D ₂₀ , D ₂₂ , D ₂₃ , D ₂₇ , D ₂₈ , D ₂₉) $\equiv$ (AG'G'AGG'GGG) ^b										
E.1				G		E	G'	G'	2.6	2.7
E.2				G		E	G'	A	2.7	2.7
E.3				G		E	G'	G'	2.7	2.7
E.4				G		E	G'	A	2.7	2.7
E.5				G		E	G'	G'	2.7	2.7
E.6				G		E	A	G'	2.8	2.9
E.7				G		E	G'	G'	2.8	2.8
E.8				G		E	G'	A	2.8	2.9
E.9				G		E	G'	G'	2.8	2.8
E.10				G		E	G'	A	2.9	2.9
E.11				G		G'	G'	A	2.9	2.9
E.12				G		G'	G'	A	2.9	2.9
E.13				G		E	A	A	2.9	2.9
E.14				G		E	A	A	2.9	3.0
E.15				G		G'	G'	A	2.9	2.9
E.16				G		G'	A	A	3.0	3.1
Conformation F (D ₁₇ , D ₁₈ , D ₁₉ , D ₂₀ , D ₂₁ , D ₂₂ , D ₂₃ , D ₂₅ , D ₂₇ , D ₂₈ , D ₂₉) $\equiv$ (G'G'G'G'GGG'G'GGG) ^b										
F.1						E		A	2.5	2.6
F.2						E		G'	2.9	2.9
F.3						G		A	3.0	2.9

^a Units are kcal mol⁻¹. Table II-S in the Supplementary Material contains the complete sets of data for all clusters obtained from the analysis.

^b These dihedral angles have the same values in all conformations of a cluster of conformations.

C(4'')O—H...OH—C(3'') (distance 2.20 Å; in tylosin from 2.14 to 2.42 Å), and one six-ring hydrogen bond C(3'')O—H...O<C(4') (distance 1.86 Å; in tylosin from 1.86 to 1.89 Å), are present in all cases (Table 5).

Conformation A. This is the largest cluster (1725 local minima) and has 116 conformations with relative steric energies less than 3.0 kcal mol⁻¹. The hydrogen bond pattern of this conformation resembles the configuration of hydrogen bonds in clusters **A**, **E**, **G**, and **H** of tylosin (six-membered C(3)—OH...O=C(1) hydrogen bonded ring; distance 2.13 Å)¹⁹ (Fig. 3). The same hydrogen bond appears also in conformation **F** of **1**. The four hydrogen bonds present in the lowest energy conformations of cluster **A**, namely C(3)—OH...O=C(1), C(2')—OH...O<C(5)

(2.51 Å), C(3'')—OH...O<C(4') (1.86 Å), and C(4'')—OH...OHC(3'') (2.20 Å), are observed in the IR spectra.⁴ According to the spectral study of **1**, the hydroxyl group at C(2') participates in two hydrogen bonds in equilibrium with the C(5) oxygen and with the nitrogen from the dimethylamino group. As was the case with tylosin,¹⁹ we did not find a hydrogen bond with the dimethylamino group. The relative shifts in O—H stretching vibrations are in a qualitative correlation with the computed energy contributions for hydrogen bonding: C(3)O—H...O=C(1) (3514 cm⁻¹/–1.5 kcal mol⁻¹), C(3'')O—H...O<C(4') (3513 cm⁻¹/–1.5 kcal mol⁻¹), C(4'')O—H...OHC(3'') (3530 cm⁻¹/–0.6 kcal mol⁻¹), C(2')O—H...O<C(5) (3606 cm⁻¹/–0.2 kcal mol⁻¹). The hydroxyl group at C(9) position is free from participation in intramolecular hydrogen bonding in all low energy clus-

TABLE 3. Comparison between the macro-ring dihedral angles of the most probable conformations of leucomycin-V (cluster A) and tylosin (cluster C¹⁹)

	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇	D ₈	D ₉	D ₁₀	D ₁₁	D ₁₂	D ₁₃	D ₁₄	D ₁₅	D ₁₆
Leu	G	A	A	G'	G'	A	G'	G'	G	A	A	A	G	G'	G	A
Tyl	A	A	A	G'	G'	A	G'	G'	A	A	A	A	G	G'	G	A

TABLE 4. Comparison between computed geometry for leucomycin-V (cluster A) and crystallographic data for leucomycin-A5¹⁰ (angles in degrees)

	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇	D ₈	D ₉	D ₁₀	D ₁₁	D ₁₂	D ₁₃	D ₁₄	D ₁₅	D ₁₆	D ₁₇	D ^a	D ₂₂	D ^b
Leu-V _{CONFLEX/MM3}	145	-173	-176	-58	-62	178	-60	-59	137	-177	180	-174	111	-57	92	-171	-98	174	138	156
Leu-A5 _{X-Ray}	134	180	-171	-60	-54	175	-60	-63	136	-179	-179	-171	112	-61	108	-174	-103	169	147	180

^aDihedral angle C(5)-O-C(1')-C(2').^bDihedral angle C(4')-O-C(1')-C(2').

ters. This is not however the case with 9-epi-leucomycin-V (vide infra). The aglycone ring geometry of cluster A is practically identical with the most probable conformation of tylosin (Table 3).¹⁹ Data for important dihedral angles from crystallographic studies of leucomycin-A5¹⁰ nicely correlate with our computed values (Table 4). Leucomycin-A5 differs from leucomycin-V only by the hydroxyl group at C(4'') of the mycarose part which is esterified with butyric acid in the former case. This may be the cause of the most significant difference (24°) for one of the dihedral angles (Table 4). The good correlation is gratifying between the values for the dihedral angles determining the orientation of the sugar side chain. Except for B, a preference for antiperiplanar disposition for the rotation about bond C(6)–C(7) characterizes the conformations belonging to the lowest-energy clusters. Besides, four computed short contacts between protons in A, namely H(3)/H(6) (2.12 Å), H(10)/H(20) (2.22 Å), H(11)/H(13) (2.40 Å), and H(5)/H(1') (2.50 Å) resemble the NOE data for tylosin.⁹

Hydrophilic groups like the formylmethyl, the lactone carbonyl and the two hydroxyl groups at C(3) and C(9) positions in the lactone ring are all situated on one side of an average plane passing through the 16-ring. This is an analogous arrangement of the functional groups on the aglycone part of other leucomycins and tylosin,¹⁹ and it was suggested to have an important role in their biological activity.⁴ The mechanism of antibiotic action of macrolides (tylosin) is related to the formation of a molecular complex with ribosomes and the consequent inhibition of bacterial protein synthesis.⁷ The one-side-hydrophilic layered structure of the aglycone ring conformation A may eventually present the minimum structural unit required for bioactivity.

Conformation B. This cluster is represented by 461 conformations. 39 of these conformations have relative steric energies less than 3.0 kcal mol⁻¹. The hydroxyl groups in C(2') and C(3) together with the C(4) methoxy group and the bridging oxygen between the macro-ring and mycaminoose constitute a net of hydrogen bonds (two

pairs of bifurcated hydrogen bonds) that lower the energy of B.1 by about 3.0 kcal mol⁻¹ (Fig. 3). The macro-ring conformation of B differs from A mostly in the portion C(4) to C(9) (Table 1).

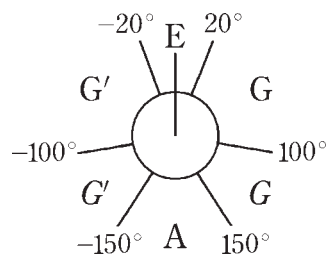
Conformation C. The relative energy of the global minimum conformation of this cluster is practically the same as the energy of the corresponding conformation of cluster B, but the computed population is about the half of the population estimated for cluster B. Another new six-ring hydrogen bond with the participation of C(3) hydroxyl group appears in this cluster (Fig. 3). The proton acceptor is the lactone oxygen (O<C(1)) in this case. The geometry of the macro-ring in cluster C differs from cluster A only by two dihedrals in the lactone moiety, namely D₁ and D₁₅. Two short contacts of the proton of the same hydroxyl group with the π -electron system are of minor significance (both -0.5 kcal mol⁻¹). Only 14 local minima among 545 conformations found for this cluster have relative steric energies less than 3.0 kcal mol⁻¹.

Conformation D. Again the differences between cluster D and clusters A and C are the values for dihedral

TABLE 5. Computed hydrogen bonding contacts D–H...A (r_{ij} ; units are Å) and energies of individual interactions (E_{ij} ; kcal mol⁻¹) of leucomycin-V (1)

D–H...A	Conformation									
	A, F		B		C		D		E	
	r_{ij}	E_{ij}	r_{ij}	E_{ij}	r_{ij}	E_{ij}	r_{ij}	E_{ij}	r_{ij}	E_{ij}
C(3)O–H										
O=C(1)	2.1	-1.5								
O=C(19)							2.2	-2.0		
O<C(1)					2.0	-1.0				
O<C(4)			2.2	-0.6						
O<C(5)			2.2	-0.5						
π C(12)					3.4	-0.2				
π C(13)					3.1	-0.3				
C(2')O–H										
O=C(1)									2.1	-1.5
OH–C(3)			1.9	-1.6						
O<C(4)	1.8 ^a	-1.8								
O<C(5)	2.5	-0.2	2.5	-0.3	2.5	-0.2	2.5	-0.2	2.5	-0.3
C(3'')O–H										
O<C(4')	1.9	-1.5	1.9	-1.5	1.9	-1.5	1.9	-1.5	1.9	-1.5
C(4'')O–H										
OH–C(3'')	2.2	-0.6	2.2	-0.6	2.2	-0.6	2.2	-0.6	2.2	-0.6

^aThis hydrogen bond is present only in some conformations of A with relative steric energies higher than 1.1 kcal mol⁻¹.

**Fig. 2.** Designation of dihedral angles.

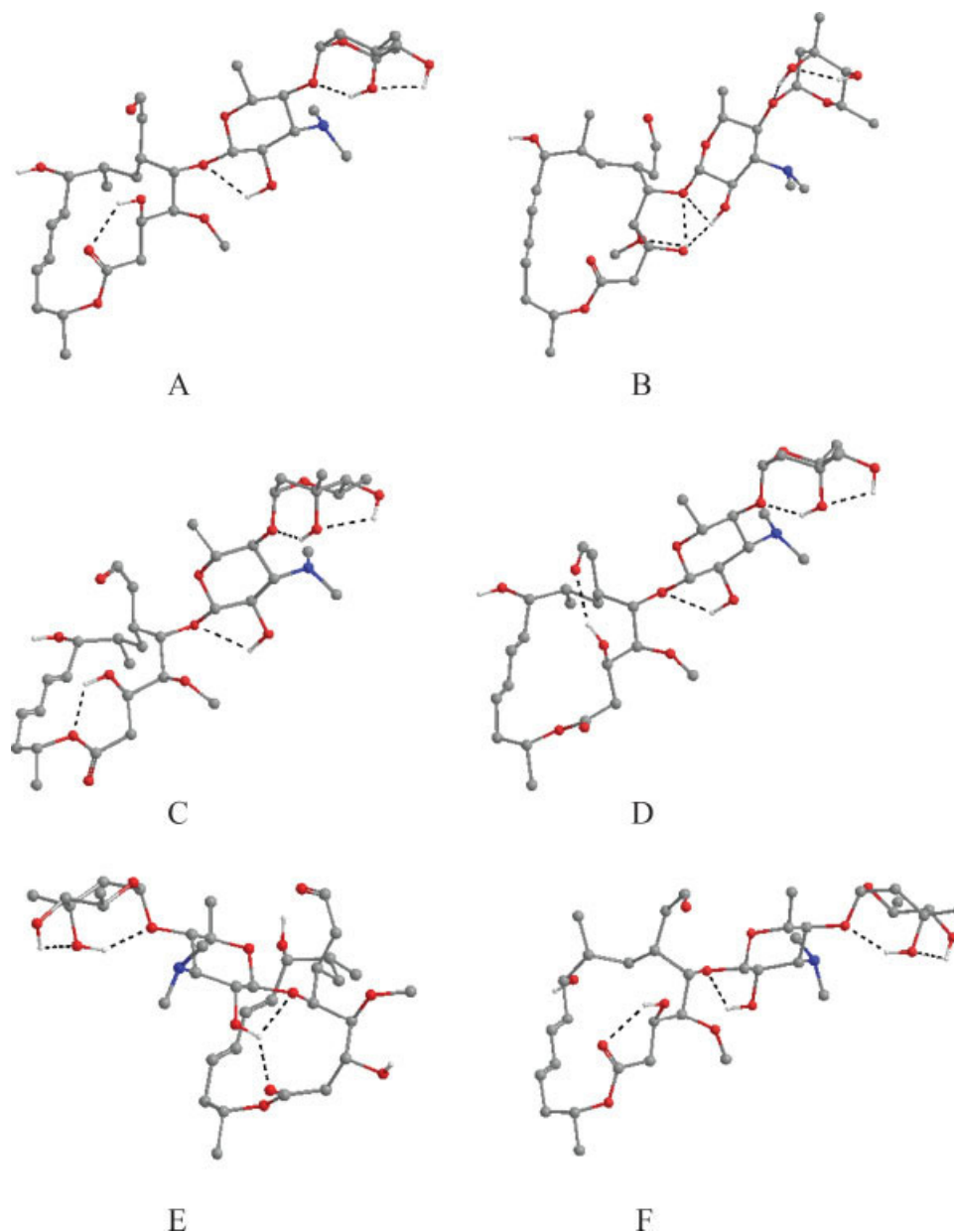


Fig. 3. Optimized geometries of low-energy conformations of leucomycin-V (1). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

angles D_1 and D_{15} , with a gauche conformation for D_1 and antiperiplanar conformation for D_{15} . This arrangement allows the formation of a nine member hydrogen bond ring with C(3)OH as a proton donor to the oxygen atom of the formylmethyl group, C(3)—OH $\cdots$ O=C(19) (2.17 Å) (Fig. 3). This is the strongest hydrogen bond found in the present analysis and lowered the energy of conformation **D** with 2.0 kcal mol $^{-1}$. Two hundred thirty-one local minima represent this conformation, six of which have relative steric energies less than 3.0 kcal mol $^{-1}$.

Conformation E. The C(2') hydroxyl group participates in a bifurcated hydrogen bond with the proton

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acceptors O=C(1) (2.13 Å; this hydrogen bond is not present in the low-energy forms of tylosin¹⁹) and the bridging oxygen between the macro-ring and mycaminoside (2.48 Å), thus forming an eight member hydrogen-bonded ring in a distorted boat-chair conformation. The macro-ring has a broader shape on the C(2)—C(3)—C(4) side, whereas C(5) and C(6) point inwards. The disaccharide moiety is attached almost perpendicularly to the average plane of the macro-ring and is slightly bent on to it.

Conformation F. About 300 conformations were determined from the cluster analysis in this case, but only three local minima have relative steric energies less than

TABLE 6. Cluster analysis of low-energy conformations of 9-epi-leucomycin-V (2)^a

Dihedral angles of macro-ring clusters																	Number of conform.	ΔE_o^b	ΔE_{av}^c	Population ^d
1—2—3—4—5—6—7—8—9—10—11—12—13—14—15—O—1																				
a	A	G'	A	A	G	G	A	G	G	A	A	A	G	G'	G	A	267	0.0	1.0	0.168
b	A	G'	A	A	G	G	A	G	G'	A	E	A	G	G'	G	A	236	0.0	1.0	0.126
c	G	A	A	G'	G'	A	G'	G'	G	A	A	A	G	G'	G	A	734	0.0	1.2	0.266
d	G	G'	A	A	G	G	A	G	G'	A	A	A	G'	G'	G	A	231	0.4	1.4	0.084
e	A	A	G	G	A	A	G'	G	G'	A	A	A	G'	G	G	A	383	0.3	1.5	0.112
f	G	A	A	G'	G'	A	G'	G	G'	A	A	A	G'	G	G	A	538	0.5	1.9	0.091
g	G	A	A	G	A	A	G'	G	G'	A	A	A	G'	G	G	A	294	1.2	2.3	0.040
h	A	A	G'	G'	G'	A	G'	G	G'	A	A	A	G'	G	G'	A	38	1.8	2.5	0.003
i	A	A	A	G'	G'	A	G'	G	G	A	A	A	G	G'	G	A	265	1.6	2.6	0.021
j	G	G'	A	A	G	G	A	G	G'	A	A	A	G'	G	G	A	62	2.0	2.8	0.005

^aUnits are kcal mol⁻¹. Table III-S in the Supplementary Material contains the data for all clusters obtained from the analysis.

^bRelative steric energy of the lowest-energy conformation of the cluster.

^cAverage steric energy of the cluster.

^dAt 300 K.

3.0 kcal mol⁻¹. Conformation **F** has the same pattern of hydrogen bonding as cluster **A** and differs from the latter mainly in the deformations of the macro-ring portion between the formylmethyl group and the hydroxyl group at C(9) (dihedral angles D₇ and D₈).

The geometry of the preferred conformations of the macrolide antibiotics plays an important role for their biological activity. It is natural to ask what consequences the variation of the configuration about C(9) (R → S) have for the preferred conformations (the C(9) position displays the main difference in the macro-rings of tylosin and leucomycin). Thus we supplemented our results with the examination of the conformational preferences in 9-epi-leucomycin-V (**2**). Surprisingly this minor change of the chirality of only one macro-ring carbon atom leads to a completely different conformational distribution. To avoid confusion we will use small letters for designating clusters and conformations of **2**.

We emphasized in our previous work¹⁹ the importance of using at the final stage of the minimization the version of MM3 devised to model molecules with conjugated π -electron fragments. This treatment required the dienone portion of tylosin. A different order of relative energies was obtained with the MM3(π) method also for **2** (Table 6): **b.1** < **c.1** (ΔE = 1.1 kcal mol⁻¹) $\approx$ **a.1** (1.2) < **d.1** (1.5) = **e.1** (1.5) < **f.1** (1.8).

Six clusters of conformations represent about 85% of the population of all the conformations found after the CONFLEX search. Cluster **c** has the highest computed population (27%), as well as one of the lowest-energy local minima. Clusters **a** (population 17%) and **b** (population 13%) have lower average steric energies compared with cluster **c**. They also possess local minima of the lowest energy. The total number of conformations in clusters **a** and **b** is 503, i.e., less than the number of conformations in cluster **c**.

The next three clusters, **d**, **e**, and **f** contain local minima with relative steric energies less than 0.5 kcal mol⁻¹ and they represent about 30% of the population estimated for the total of 1152 local minima. Clusters **g** and **h** have average relative steric energies higher than 2.0 kcal mol⁻¹.

The same general features pointed out for **1** characterize also the macro-ring conformations of **2**. The only exception is cluster **b** that displays preference for the *s-cis* conformation of the diene portion (dihedrals D₁₀, D₁₁, D₁₂).

Conformation a. Conformation **a** of **2** is identical to conformation **B** of **1**. The unsaturated carbons are in a *11,12-s-trans* conformation. The hydroxyl groups in C(2') and C(3) together with the C(4) methoxy group and the bridging oxygen between the macro-ring and mycaminose constitute a net of hydrogen bonds (two bifurcated hydrogen bonds; Fig. 4). These four interactions decrease the energy of **a** by about 3 kcal mol⁻¹. Conformation **b** is very similar to **a** except for the *11,12-s-cis* configuration of the diene system, and matches conformation **G** of **1**. Cluster **d** has minor differences in the macro-ring conformation and, accordingly, the same pattern of hydrogen bonding as **a** and **b**.

Conformation c. The macro-ring conformation **c** of **2** is identical to conformation **A** of **1**. This is the largest cluster (734 local minima) and has 112 conformations with relative energies less than 3.0 kcal mol⁻¹. The hydrogen bond pattern of this conformation resembles the configuration of hydrogen bonds in clusters **A**, **E**, **G**, and **H** of tylosin (C(3)—OH...O=C(1) hydrogen bonding).¹⁹ Two hydrogen bonds in **c** are not present in conformations **a** and **b** (Fig. 4): a new hydrogen bond, not present in tylosin, of the hydroxyl group on C(9) and the formylmethyl carbonyl (distance 2.20 Å) encircles the hydrogen bonded nine-ring; a six-ring is also formed by C(3)O—H...O=C(1) (distance 2.12 Å). These two hydrogen bonds lower the energy of **c.1** by 3.2 kcal mol⁻¹. Except for the hydrogen bond with the participation of the formylmethyl carbonyl, the same hydrogen bond pattern characterizes also conformations **e**, **f**, and **h**. Cluster **c** has practically identical geometry of the aglycone ring with the most probable conformation of tylosin.¹⁹

Conformation g. This cluster has three hydrogen bonds not present in any of the other low-energy forms:

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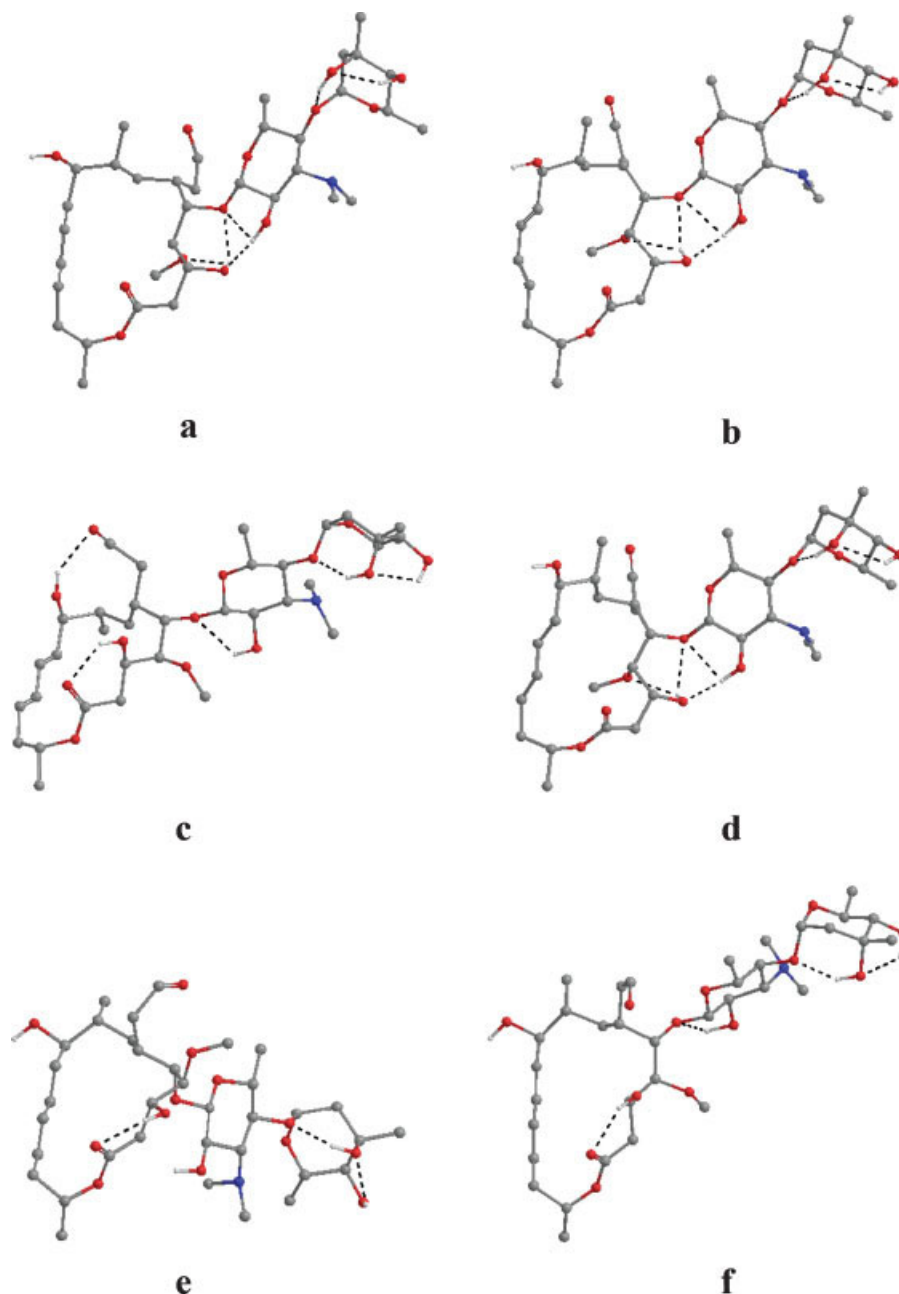


Fig. 4. Optimized geometries of low-energy conformations of 9-epi-leucomycin-V (**2**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

hydrogen bonding between the hydroxyl group at C(2') and the diene conjugated π -system (distances from 3.1 to 3.8 Å and 0.8 kcal mol⁻¹ lowering of the energy from this interaction), C(3)—OH $\cdots$ O=C(19) (nine-ring, distance 2.26 Å, 1.6 kcal mol⁻¹ lowering of the energy; similar to conformation **C** of tylosin) and a weak bifurcated hydrogen bonding C(2')O—H $\cdots$ O<C(1) (11-ring).

A careful examination of the results from the cluster analyses of **1** and **2** (Tables I–S III–S in Supplementary Material) reveals that each cluster of **1** has its counterpart among the clusters of **2** even for the higher energy regions. Not in all cases the values of all macro-ring dihe-

dral angles are the same, but the deviations are not so significant to deserve special discussion. Besides, pairs of analogous conformations (e.g., **B.1** and **a.1**) have in some cases the same contributions from different energy terms, whereas in other instances (e.g., **A.1** and **c.1**) different balances of interactions produce the same macro-ring conformation. Conformation **A.1** is favored by angle bending and torsional energy contributions, whereas the energy of conformation **c.1** is lowered from the electrostatic interactions.

In summary, the analysis of the results for **2** suggests the highest probability for occurrence of the conforma-

tions in cluster **c** with participation in equilibrium of the other *11,12-s-trans* low-energy clusters **a**, **d-f**. The most probable macro-ring conformation of **2** (cluster **c**) is very similar to the preferred form (cluster **C**) of tylosin.¹⁹ Computed low-energy conformations with hydrogen bonding of the OH group at C(2') on mycaminose with the nitrogen atom of the dimethyl amino group were not obtained also in this case. The order of conformational preference is different using MM3 and the MM3(π) methods, i.e., 9-epi-leucomycin-V (**2**) also requires treatment with the π -version of the MM3 method. From a methodological point of view this result presents an interesting case—the two molecules, **1** and **2**, differ only in the configuration of one macro-ring carbon atom but display completely different behavior under the treatment of two different modeling protocols.

According to literature data, 9-epi-leucomycin-A5 had been synthesized from leucomycin-A5. The only difference between leucomycin-A5 and leucomycin-V is a butyrate instead of a hydroxyl group at C(4''). Practically identical antimicrobial activities were observed with some tests strains for both epimers, but 9-epi-leucomycin-A5 appeared reduced in some bacteria such as *Staphylococcus epidermidis* sp-al-1 and *Streptococcus pyogenes* N.Y.5.³⁵ This fact may be due to the lower population of the bioactive conformation in the 9-epi form.

In summary, these results suggests a strong preference for the conformations of cluster **A** of leucomycin-V (**1**) (confirmed also with the prevalence of cluster **c** from the computational experiment with the 9-epi form (**2**)), and minor participation in equilibrium of the other low-energy clusters **B-F**. The answers to the questions formulated at the beginning can be summarized as follows:

1. The most probable macro-ring conformation of **1** (cluster **A**) is very similar to the preferred form (cluster **C**) of tylosin.¹⁹ Other, different probable macro-ring geometries were also obtained.
2. Computed low-energy conformations with hydrogen bonding of the OH group at C(2') on mycaminose with the nitrogen atom of the dimethyl amino group were not obtained in this case.
3. The same order of conformational preference for **1** was estimated with the MM3 and the MM3(π) methods (Table 2). The change of the chirality of the C(9) macro-ring atom however produced different order of conformational preference for the 9-epi form **2**, estimated with the two methods, as well as enhanced population of several clusters of conformations.

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Review Article

Chiral Memory: Induction, Amplification, and Switching in Porphyrin Assemblies

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT The interaction between the tetra-anionic porphyrin H₂TPPS and its copper derivative, CuTPPS, with the tetra-cationic porphyrin H₂T4 and its copper derivative, CuT4, leads, in aqueous solution, to the formation of remarkably stable and kinetically inert heteroaggregates. The aggregation process is under hierarchic control and, in the presence of a suitable chiral mold, leads to the formation of chiral porphyrin heteroassemblies as stable and inert as the achiral ones. Because of these properties, the chirality of the porphyrin “imprinted” heteroaggregates not only survives the disruption of the template, but also to its complete removal from the solution. Notably, the template-free chiral porphyrin system is an excellent mold for its own self-replication. The relevant characteristics of these chiral heteroaggregates together with the knowledge of the forces that guide the aggregation processes permitted us to design a new but similar system. This system not only is able to store chiral information, but also is capable to release and restore it reversibly, in a cyclic manner. This has been achieved by modulating the charges carried by one of the two coupled porphyrins through protonation under various pH conditions. The role of the central metal ion and the template-free chiral structure of the CuT4-H₂TPPS heteroaggregate, determined through EDXD analysis, are also presented. *Chirality* 20:411–419, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: supramolecular chiral information transfer; porphyrin heteroaggregates; circular dichroism; modulation

INTRODUCTION

There is great interest in the processes concerning the transfer and storing of chiral information from a chiral mold to achiral molecules and supramolecular systems, especially when this is achieved through noncovalent interactions. These processes not only play a key role in nature, but are also candidates for technological application. The full knowledge of the forces that guide these noncovalent interactions and chiral information transfer processes is essential in order both to understand the transfer of information in biologically relevant phenomena and aggregation processes (i.e. prion-mediated disease, diabetes, cataract, etc.) and to be able to design supramolecular systems with predictable properties and behavior. Also, with this aim in mind, hierarchic control represents a powerful and promising tool to synthesize rationally non-covalent complex species with designed properties.

Chirality generates optical activity which is commonly related to molecular dissymmetry. Symmetric molecules, however, may also present supramolecular chirality (i) by forming intrinsically chiral assemblies,^{1–8} or (ii) by aggregating on to chiral polymeric templates (extrinsic chirality).^{9–15} The latter case leads to induced circular dichroism

(ICD) signals which, having a conformational origin⁹ (i.e. arising from self-organization of the achiral guest by chiral templates), modify or disappear^{9,10,12} following matrix conformational transition. Supramolecular chirality can derive from the complex nonsymmetric arrangement of various molecular components in a noncovalent ensemble.

Owing to the intrinsic “weakness” of noncovalent interactions, the addressability of this approach in terms of sequence and stoichiometry of the final species is poorer than that achieved from covalent syntheses and might lead to a mixture of species deriving from the coexistence of different chemical equilibria. The approach mostly used to by-pass these limitations exploits a network of noncovalent interactions whose cooperativity increases both the

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thermodynamic and kinetic stability of the designed non-covalent species. Most of times the structure (i.e. the sequence, stoichiometry, chirality, etc.) of the molecules designed and synthesized in this way is under hierarchical control, that is, the structure of the final product is controlled by a time-dependent specific sequence of chemical events (addition of reagents, pH changes, time evolution of the species)¹⁶ that lead to one major or unique final product. If, during one of these synthetic steps, a chiral reagent is introduced, then the final complex can show chirality at a supramolecular level. In some cases, the kinetic inertia of the chiral supramolecular complex can lead to the so-called "memory" phenomenon.^{13–15,17–27}

The chiral memory phenomenon is of particular interest because, in principle, using this synthetic approach one can choose the desired molecular (monomeric) pieces having specific functions (binding, catalytic, or others) and (with the aid of the appropriate chiral mold) noncovalently assemble them in a permanent chiral fashion. The resulting supramolecular complex can exert predetermined functions accompanied by chiral discrimination.

Porphyrins, in particular, are excellent building blocks to assemble supramolecular architectures. Their remarkable and tunable spectroscopic properties have been, in fact, exploited to design and realize a paramount number of porphyrin arrays potentially useful as sensors, optoelectronic devices, antenna systems, models of metalloenzymes, etc.^{14,16,28–51}

We have already presented in a review⁵² some results we achieved in the field of chiral memory. Here, after summarizing past results for sake of clarity, we focus on some quite new and important results concerning: (i) the role played by noncovalent interactions different from the electrostatic ones, such as the effect of the central metal ion, (ii) the determination of the template-free chiral H₂TPPS-CuT4 heteroaggregate structure by the energy dispersive X-ray diffraction (EDXD) analysis and (iii) the noncovalent synthesis of a designed supramolecular system with which it is possible to perform a complete cycle of imprinting, storing, releasing, and restoring of the memorized chirality.

MATERIALS AND METHODS

Poly-L-glutamate and poly-D-glutamate sodium salts (mean degree of polymerization 95), L-phenylalanine (L-Phe), and D-phenylalanine (D-Phe) were purchased by Sigma-Aldrich. *meso*-Tetrakis(4-sulfonatophenyl)porphine (sodium salt), H₂TPPS (Fig. 1), *meso*-tetrakis(4-*N*-methylpyridyl)porphine (chloride salt), H₂T4 (Fig. 1), and *meso*-tetrakis(4-pyridyl)porphine, H₂TPyP (Fig. 1), were obtained from mid-century. Porphyrin metallation (with copper oxide) was performed by literature methods. The formation of the porphyrin assemblies is obtained by addition of equimolar amounts of the two macrocycles, because the stoichiometry of the aggregates has been already determined to be 1:1 (cationic:anionic porphyrin).³ Amino acids were removed by ultrafiltration, using Centricon filters (cut-off = 10 kDa). Noteworthy, about

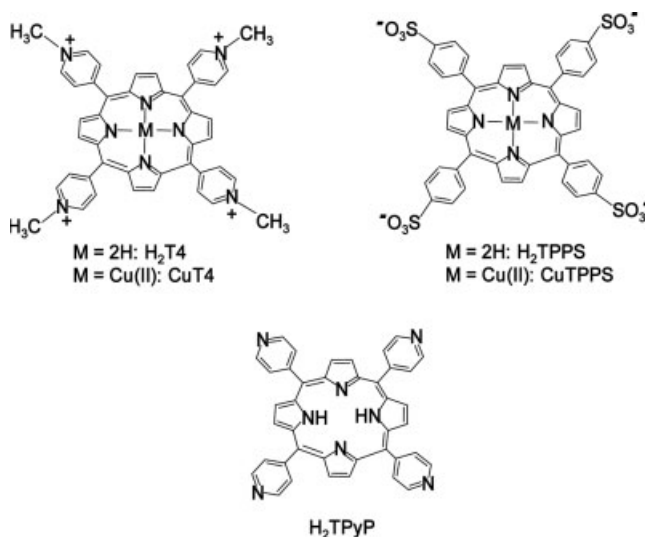


Fig. 1. Schematic structure of H₂T4, CuT4, H₂TPPS, CuTPPS, H₂TPyP.

95% of the amino acid is already removed after the first filtration. Residual amino acid concentration after ultrafiltration was checked both in the aggregate solution and in the filtrate by fluorescence: the lower detection limit is $\approx 1 \times 10^{-8}$ M. Absorption measurements were recorded on a Varian Cary 500, circular dichroism measurements on a Jasco J-810. All the experiments were carried out using 1 cm optical path quartz cuvettes and ultra-pure water (Milli Q-RG system) was used throughout. No buffer was used; the pH solution was adjusted with HCl or H₂SO₄ and NaOH.

RESULTS AND DISCUSSION

Chiral Memory I: Porphyrin Hetero-Self-Assemblies in the Presence of Poly-Glutamate

Our interest on chiral memory processes in porphyrin aggregates began with the synthesis of the chiral ternary heteroassembly formed by the achiral tetra-cationic CuT4 (Fig. 1) and tetra-anionic H₂TPPS (Fig. 1) porphyrins self-aggregated onto the helical anionic poly-L- or D-glutamate.^{12,13,15} This complex is the first example of a ternary porphyrin assembly where two components (one of the porphyrins and the template) bears the same charge. Its formation is made possible by the shielding effect of the cationic unit, CuT4 (that is added to the poly-glutamate solution before the anionic H₂TPPS), which minimizes the electrostatic repulsion between the two anionic components. We have shown that these ternary supramolecular species are remarkably (thermodynamically and kinetically) stable, which allows them to "memorize" the chiral information imprinted by the template and to retain it even if the matrix chirality is "disrupted" by pH-induced conformational transitions. In fact, polyglutamic acid adopts a helical conformation when the side carboxylic groups are partially protonated⁵³ and undergoes a helix to coil transition following deprotonation of the carboxylic moieties.

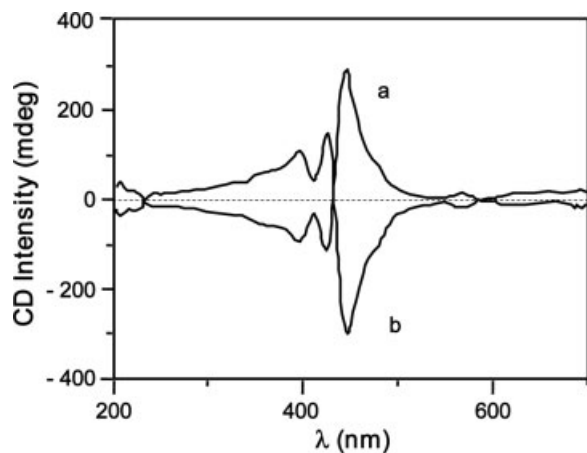


Fig. 2. CD spectra of an aqueous solution of CuT4 (4 μ M) and H₂TPPS (4 μ M) aggregated in the presence of (a) poly- α -glutamate (200 μ M) and (b) poly- β -glutamate (200 μ M) at pH 3.6.

The formation of the ternary chiral complex not only is accompanied by a strong hypochromic effect (about 90%) of the porphyrin Soret band and an almost complete quenching of H₂TPPS fluorescence (CuT4 does not emit), but also induces a quite intense ICD signal in the porphyrin Soret region of the spectrum (Fig. 2).

Notably, heteroaggregation of the title porphyrins in ultrapure water, in the absence of poly-glutamate, leads to achiral 1:1 complexes. This aggregation process is also accompanied by a hypochromic absorbance effect and fluorescence quenching similar to that observed in the presence of poly-glutamate, but no CD signal is observed (Fig. 3, curve a). More interestingly, no dichroic signal is also observed in the Soret region if poly-glutamate is added after porphyrin self-aggregation (Fig. 3, curve b). This behavior already testifies to the relevant kinetic inertia of these porphyrin supramolecular species.

Another proof of this notable inertia is given by the persistence of the ICD after the poly-glutamate pH-induced helix to coil conformational transition. In fact, since the chirality of the supramolecular complex is transferred from poly-glutamate to porphyrins, the pH-induced helix to coil transition (from pH 4 to 12) should cause the disappearance of the ICD of the ternary species. Surprisingly,

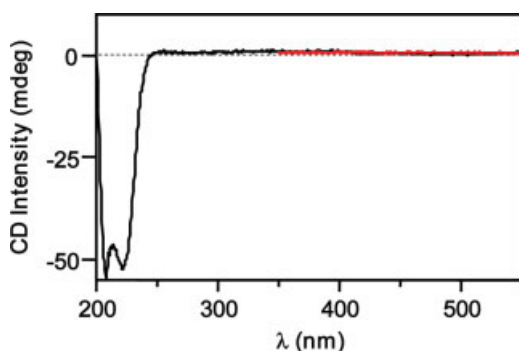


Fig. 3. CD spectra of CuT4 (4 μ M) and H₂TPPS (4 μ M) aggregated in water at pH 3.6 (red curve) and of the same solution after the addition of poly- α -glutamate (200 μ M).

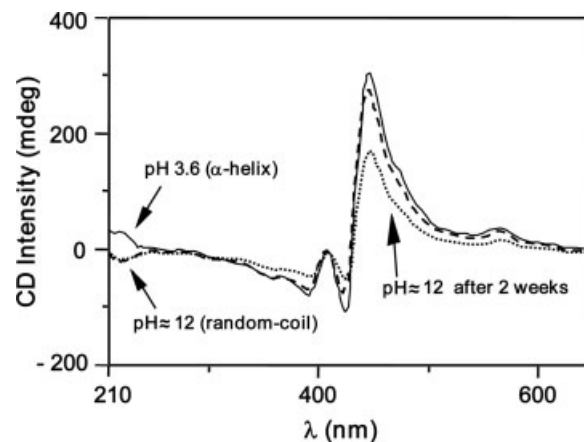


Fig. 4. CD spectra of an aqueous solution of CuT4 (4 μ M) and H₂TPPS (4 μ M) aggregated in the presence of poly- α -glutamate (200 μ M) (full line) at pH 3.6 and of the same solution at pH 12.0 (dashed line) after few minutes and (dotted line) after 2 wk.

increasing the pH to 12 does not perturb the ICD, strongly indicating that these porphyrin assemblies retain their “original” chirality even when the matrix loses it (Fig. 4). We have also checked the time stability under such critical experimental conditions (pH 12). It turns out that these complexes remain stable for several days, as indicated by the CD intensity in the Soret region which decreases only by 30% over about 2 wk¹⁵ (Fig. 4).

We have also tested the behavior of other couples of porphyrins bearing opposite charges: H₂T4-H₂TPPS, H₂T4-CuTPPS, CuT4-CuTPPS (Fig. 1).²² We chose to use only the free-base and copper derivatives (Fig. 1) because penta- or hexa-coordinated porphyrins or planar metallo-derivatives bearing a positive charge in the inner core (as the gold(III) derivatives) lead to the formation of supramolecular species for which the chiral memory phenomenon does not occur.⁵⁴ All the three porphyrin systems mentioned earlier follow the same trend presented by the CuT4-H₂TPPS couple.

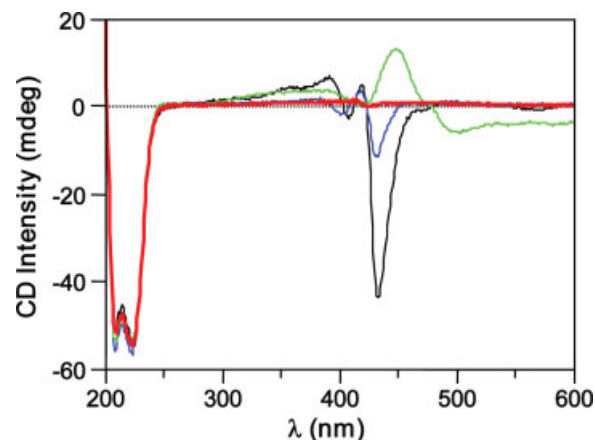


Fig. 5. CD spectra of the couples CuT4-H₂TPPS (black curve), H₂T4-H₂TPPS (green curve), H₂T4-CuTPPS (blue curve), and CuT4-CuTPPS (red curve) (4 μ M each) aggregated in the presence of poly-L-glutamate (200 μ M) at pH 4.0.

The only significantly different behavior was found out for the CuT4-CuTPPS system. This species exhibits the remarkable inertness and the chiral memory phenomenon presented by the other porphyrin aggregates, but its CD signal is considerably smaller than those exhibited by the other porphyrin couples (H₂T4-H₂TPPS, CuT4-H₂TPPS, H₂T4-CuTPPS) (Fig. 5). These findings suggested us that the title aggregate is smaller than the others, hypothesis that was also supported by the RLS signals (data not shown).

The study of the aggregation behavior of the reported systems confirms that net electrostatic interactions (i) constitute the driving force of the self-assembly process and (ii) lead, due to their large number and strength, to the formation of inert ensembles. Kinetic inertia, then, turn out to be crucial in determining the memory phenomenon. Finally, the aggregation process is affected by other non-covalent interactions as well. In particular, the central copper ion does not play a trivial role; in fact, it is able to affect the assembly dimension. This finding may turn out to be useful for modulating the aggregate size, whose control is crucial for the possible applications of these systems.

Chiral Memory II: Porphyrin Hetero-Self-Assemblies in the Presence of Phenylalanine

If we have, now, a deeper insight into the previous described systems and reconsider the memory phenomenon observed for all of them, it is possible that the presence of the porphyrin cationic *N*-methyl groups close to the side carboxylate groups of poly-glutamate could shield electrostatic repulsions, preserving small regions of the polypeptides from the helix to coil transition, (partially) explaining the persistence of the CD signal of the porphyrin aggregate after the pH jump experiment. A definitive and unambiguous demonstration of the memory phenomenon needs the survival of the ICD in the Soret region after removal of the chiral mold (e.g. after ultrafiltration).

The latter experiment is not feasible when the mold is a (covalent) polymer, because it can remain trapped by the

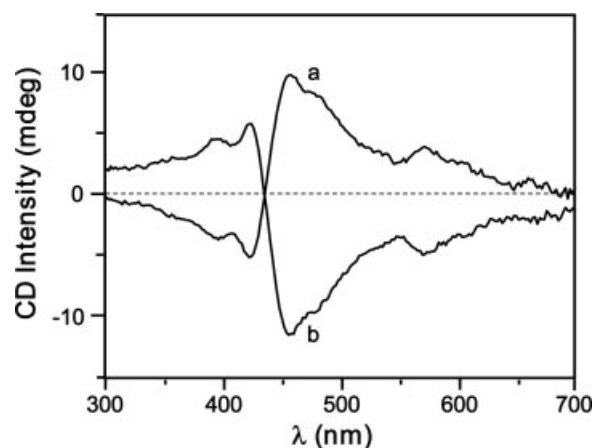


Fig. 6. CD spectra of an aqueous solution of CuT4 (2 μ M) and H₂TPPS (2 μ M) aggregated in the presence of (a) L-phenylalanine (8 mM) and (b) D-phenylalanine (8 mM).

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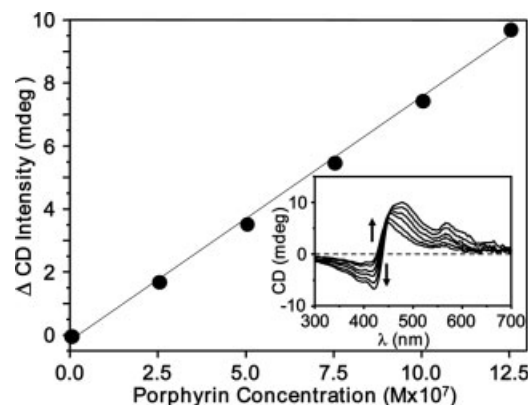


Fig. 7. CD variations versus porphyrin added concentration. In the inset the CD spectra relative to various titration points are reported. The experiment was conducted using a CuT4-H₂TPPS L-Phe-free imprinted aggregate solution to which equimolar amounts of the title porphyrins were individually added.

quite big porphyrin aggregates (heteroaggregate solutions are cloudy, as supported by elastic light scattering data).

We choose, then, as first candidate, “monomeric” Phenylalanine (Phe). In fact, it has a good fluorescence yield in a region where porphyrins only slightly absorb, and its removal from the solution can be easily checked. Moreover, considering that ICD signals, the fingerprint of (extrinsic) chirality, have conformational origin, Phe, due to the hydrophobic character of a large portion of the molecule, ensures greater aggregation than other amino acids.

Indeed, addition of the cationic CuT4 and anionic H₂TPPS to an aqueous solution of L-Phe or D-Phe leads to an ICD in the Soret region (Fig. 6). The threshold concentration of Phe to induce the ICD is about 1×10^{-3} M. Therefore, in contrast to all the previous examples reported in the literature, in this case the preferential conformation of the porphyrin aggregates is borrowed by chiral noncovalent polymers.¹⁴

According to our previous results,¹³ the title porphyrin aggregates are inert enough to memorize the chirality of polymeric helical templates even after helix disruption. A similar result is expected also here upon Phe removal. Ultrafiltration of the solution leaves, in fact, the CD signal almost unaltered (Fig. 6a) showing that the imprinted aggregates are now intrinsically chiral. The residual concentration of Phe has been checked by fluorescence, whose remarkable sensitivity allows detecting up to 1×10^{-8} M of Phe. This concentration is far below the concentration threshold necessary to transfer chirality to the porphyrin aggregates (about 10^{-3} M). Dynamic light scattering measurements¹⁴ have shown that, in these conditions, the concentration of aggregated Phe is very small, being about 10^{-7} M. Therefore, as soon as most of the monomeric Phe is removed the equilibrium $n \text{ Phe} \leftrightarrow (\text{Phe})_n$ can be considered completely shifted toward the monomeric form.

The same results were obtained in the presence of D-Phe.

A direct consequence of the “memory” phenomenon is that the title aggregates are inherently chiral and thus, in

principle, excellent templates for their self-replication. Figure 7 shows that when equimolar amounts of CuT4 and H₂TPPS are individually added to solutions containing about 6×10^{-13} M of imprinted-assemblies (each of them formed by about 2×10^6 porphyrin molecules, see Ref. 14) the ICD of the imprinted-aggregates increases indeed does doubles with doubling porphyrins concentration (inset of Fig. 7). The linear increase of the CD with porphyrin concentration shows that the chiral growth process is substantially enantiospecific.

Recently, the solid state structure of the template-free chiral CuT4-H₂TPPS heteroaggregate has been determined through EDXD analysis.⁵⁵ It represents the first structural determination of a porphyrin (chiral) heteroaggregate. Also, a comparison with the amorphous heteroaggregate structure that the same porphyrins form in the absence of the chiral template is reported. Morphology and structure of the porphyrin aggregates are responsible of the porphyrin physical and chemical-physical properties. This relationship is particularly true for the properties of the chiral assemblies. In this perspective, the determination of the supramolecular system structure becomes an essential point to clarify the structure-functions relationship in order to increase our ability to design systems having the desired properties.

The synthesis of the CuT4-H₂TPPS chiral heteroaggregate samples for EDXD analysis was carried out following a purely noncovalent approach: equimolar amounts of each porphyrin (the addition order of the two porphyrins has no relevance) were added to a 200 ml aqueous solution of L-Phe 8×10^{-3} M (extrapure Milli-Q water was used throughout the procedure). Each addition resulted in a porphyrin concentration of 4 μ M. After three additions of the cationic and the anionic porphyrin (the final concentration in solution of each porphyrin is 12 μ M), the amino acid was removed from the solution by mean of various centrifugation steps, using Centricon filters (cut-off = 10 KDa). The concentration of phenylalanine was checked both in the aggregate solution and in the filtrate by fluorescence (λ_{ex} = 218 nm, λ_{em} = 284 nm). Here is worth noting that the lowest detectable concentration of Phe is of order of 0.01 μ M, while the minimum Phe concentration necessary to induce a chiral structure in the porphyrin heteroaggregate is of the order of 100 μ M. Three further "cleaning" centrifugation steps were performed even after no Phe was detected in the sample.

After the amino acid removal, the aggregates were suspended again in 200 ml aqueous solution and various equimolar addition of CuT4 and H₂TPPS were performed until the desired amount of aggregate was assembled (in particular, three couples of additions of 4 μ M in each porphyrin were still done, while all the following additions were of the order of 10 μ M).

When all the necessary growing steps were performed, the aggregate solution was maintained to 45–50°C until complete water evaporation. The powder sample was then recovered for EDXD characterization.

The same procedure were followed for the assembly of the CuT4-H₂TPPS chiral heteroaggregate in the presence of D-Phe, for the amino acid removal and, then, for the "as-

sembly amplification" in order to have a sufficient amount of compound for the EDXD analysis (~200 mg).

Finally, the CuT4-H₂TPPS achiral heteroaggregate was prepared according with the following procedure: equimolar amounts of each porphyrin (the addition order of the two porphyrins has no relevance) were added to 200 ml aqueous solution (extrapure Milli-Q water was used throughout the procedure) until the desired amount of aggregate was assembled. In particular, six additions of 4 μ M in each porphyrin were first done, while all the following additions were of 10 μ M of each porphyrin.

When all the necessary growing steps were performed, the aggregate solution was maintained to 45–50°C until complete water evaporation. The powder sample was, then, recovered for EDXD characterization.

Several parameters influence the porphyrin assembly. The most important ones are surely the attractive forces between opposite net charges and the intermolecular interactions of the extended aromatic systems. Also, the types of metal, the peripheral substituents, the molecular packing in solid state play a role.

The structural study of L-Phe-imprinted heteroaggregates of CuT₄-H₂TPPS compared to the equivalent nonimprinted sample in solid state, performed with EDXD, has evidenced that the template imprints an α -helix superstructure responsible for the CD properties. The lack of template during the aggregation process leads to a randomly arranged structure and a lack of CD signal in the region of absorbance of the porphyrins. The supramolecular chiral heteroassembly α -helix arrangement is, more precisely, characterized by a pitch length of 62.17 ± 0.20 Å, a radius of 5.50 ± 0.30 Å and an interplanar distance of 3.90 ± 0.10 Å. On the contrary, the arrangement of the nontemplate-imprinted sample is completely disordered, amorphous, and hence achiral.

Chiral Memory III: Switching On and Off the Chiral, Template-Free, Porphyrin Hetero-Self-Assemblies "ICD" Signal

As said earlier, the relevant properties of the title chiral heteroporphyrin complexes arise mainly from the extended network of electrostatic interactions between the tetra-cationic and tetra-anionic porphyrins. Such interactions are the driving force of the assembly process and give rise to the remarkable inertness and stability characteristic of these systems. The latter property then affects the ability of the aggregates to act as a template for the enantiospecific propagation of their own chiral structure.

On the other hand, as a consequence of the inertness and stability of such interactions, the stiffness of these systems is unwelcome when dynamic and reversible behavior is required.

Recently we have overcome this problem by "switching on and off" the network of electrostatic interactions that hold up the noncovalent supramolecular structure.⁵⁶ With this strategy it is possible to reversibly release and restore the chiral information, i.e. the CD signal, previously induced (and stably transferred) from a chiral mold to the porphyrin heteroassemblies (by mean of the described imprinting process).

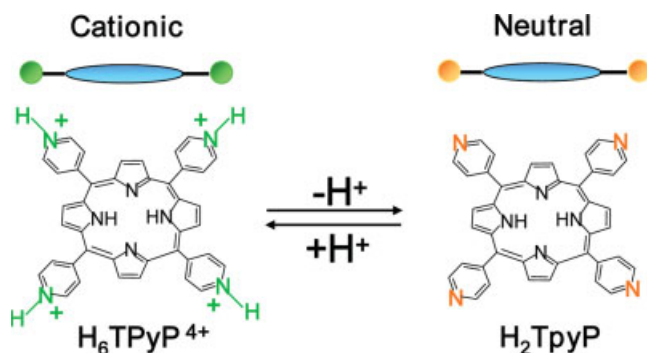


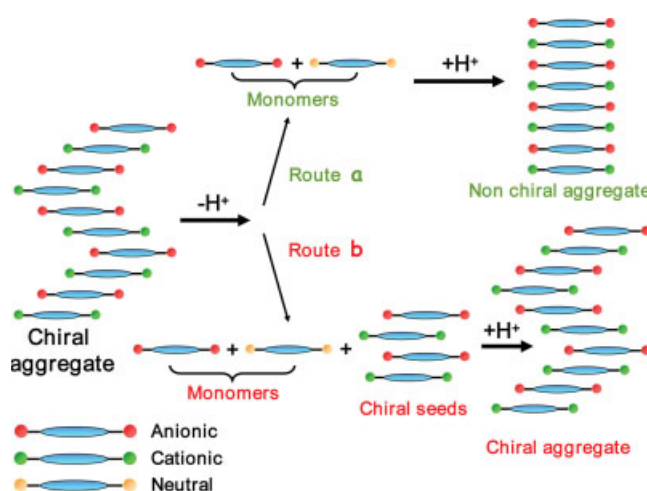
Fig. 8. H_2TPyP deprotonation equilibrium with the consequent change from the charged (cationic) state to the neutral one.

In theory, the switching of the electrostatic interactions can be achieved by replacing the four permanently cationic groups in the meso position of the porphine ring with four *ionisable*, cationic (acid) groups.

In particular, we have substituted the cationic CuT4 with the free base of the nonmethylated analogous: H_2TPyP (Figs. 1 and 8). This porphyrin is uncharged in neutral-basic aqueous solution, but carries four positive charges following protonation in acidic conditions ($pK_a \approx 4$). Finally, we used CuTPPS as anionic counterpart, maintaining L-Phe and D-Phe as chiral templates.

In our design, deprotonation of the four pyridines causes the loss of the positive charges (Fig. 8) and consequently the disassembly of the supramolecular complex. However, it is the reassembly of the chiral supramolecular structure after the chiral template removal, the crucial and most tricky step of the whole “erase and rewrite” process.

In fact, apparently, “switching on” the electrostatic interactions by reprotonating the four meso basic groups (Fig. 8) should lead to the *achiral* complex between the two porphyrins because the chiral information could be lost after the erase reaction (Scheme 1, route a). Yet, reminding



Scheme 1. Picture of the pH-induced disassembly of the chiral porphyrin aggregate and of the two possible reassembling paths. Route a: the chiral aggregate is completely disassembled after the pH-jump. Route b: some undetectable chiral seeds survive the pH-jump.

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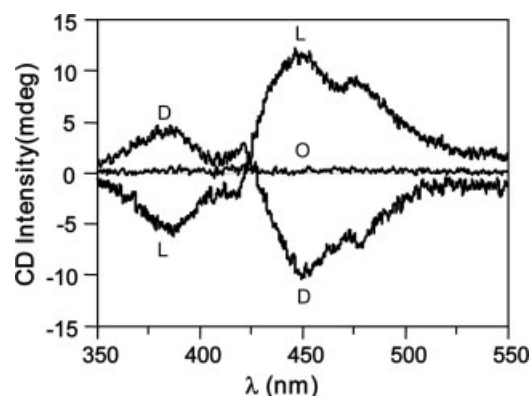


Fig. 9. CD spectra of an aqueous solution of H_2TPyP (4 μM) and CuTPPS (4 μM) aggregated (L) in the presence of L-phenylalanine (8 mM), (D) in the presence of D-phenylalanine (8 mM), and (O) in simple water at pH 2.3.

that the chirality storing complexes are (a) kinetically inert and (b) excellent chiral templates for their self-propagation, it is conceivable to expect that: (a) deprotonation of the four acid groups does not cause the complete destruction of the chiral complexes but leaves a small amount of chiral seeds (Scheme 1, route b), and (b) this lasting amount of chiral seeds is enough to promote the formation of the *chiral* supramolecular complex (Scheme 1, route b). Therefore, route b should prevail over route a (Scheme 1).

The first and preliminary step of our experiment was to check that L- or D-Phe are effectively able to imprint a chiral structure in the porphyrin heteroassembly and to verify the reversible appearance and disappearance of the induced CD signal cycling between basic and acidic condition in the presence of the amino acid.

In fact, addition of an equimolar amount of H_6TPyP^{4+} and CuTPPS (Fig. 1) (at pH 2.3 by HCl) to a solution of L- or D-Phe leads to an induced CD signal in the Soret region (Fig. 9). On the contrary, no CD signal is observed when the two porphyrins are mixed in pure water (pH 2.3), i.e. in the absence of L- or D-Phe (Fig. 9). Both in the absence and in the presence of phenylalanine, however, porphyrin interaction leads to about 90% of hypochromicity of the Soret band testifying aggregation (Fig. 10). Then, if the

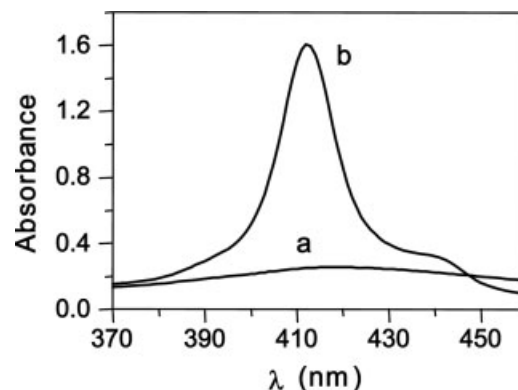


Fig. 10. Absorbance spectra of the couple H_2TPyP -CuTPPS in the aggregated state at pH 2.3 (curve a) and in the disassembled state at pH 9.0 (curve b).

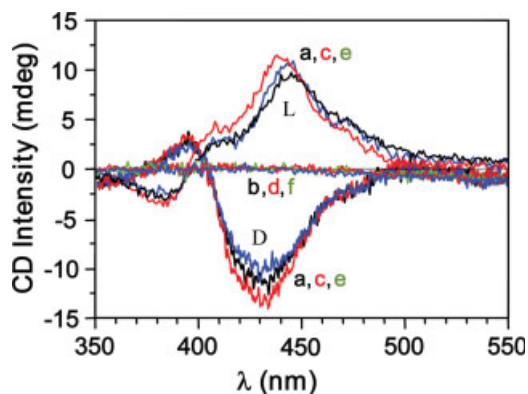


Fig. 11. CD spectra of the Phe-free H_2TPyP - $CuTPPS$ L-imprinted and D-imprinted aggregates at pH 2.3 (curves a, c, e) and at pH 9.0 (curves b, d, f) subjected to various disassembling–reassembling cycles.

solution pH is increased from 2.3 to 9.0, it is observed that the CD of the chiral porphyrin aggregates disappears and the absorption spectra experience quite a strong hyperchromicity, indicating that most of the porphyrins are in a monomeric state. Reducing again the pH to 2.3 the CD signal of the porphyrin aggregate is restored (after about 10 min). So, the system responds quite well to the external stimuli and, in the presence of phenylalanine, is able to “indefinitely” switch off and on the supramolecular chiral information.

At this point, the more delicate stages of our work have to be performed: (i) removal of the amino acid from the imprinted porphyrin heteroaggregate solution (in acidic condition) and (ii) cycling between acidic and basic conditions to “erase and rewrite” the CD signal (i.e. the chiral information).

Indeed, elimination of phenylalanine, by ultrafiltration of the solution, does not affect the chirality of the aggregates, as shown by the persistence of the CD signal in the Soret region. Also, addition of the two porphyrins to the solution containing the supramolecular chiral assembly leads to the growth of the chiral architectures confirming the remarkable ability of these chiral aggregates to template their own enantiospecific self-propagation.

Finally, the “erase and rewrite” experiment was performed using solutions of the amino acid-free chiral porphyrin assemblies.

In particular, the experiment shown in Figure 11 demonstrates that, indeed, the ICD of the phenylalanine-free chiral aggregate can be cyclically switched “on and off.” The CD for both the L- and D-imprinted aggregates disappears by increasing the pH from 2.3 to 9.0 (Figs. 11b, 11d, and 11f), and it is restored by lowering back the pH at 2.3 (Figs. 11a, 11c, and 11e). No ICD is observed for the achiral aggregates when cycling between pH 2.3 and 9.0.

Again, CD changes are parallel to the remarkable absorption variations. Protonation at pH 2.3 of the H_2TPyP peripheral nitrogens leads to aggregation and to about 90% of hypochromicity (Fig. 10, spectrum a), whereas deprotonation (at pH 9.0) restores the Soret intensity (Fig. 10, spectrum b).

Apparently, the remarkable kinetic inertia of the imprinted aggregates allows for the persistence of a spectroscopically undetectable amount of chiral seeds which drives the reassembly of the chiral structure (Scheme 1, route b). We have performed up to 10 consecutive cycles, but there are no apparent limitations to the number of cycles that can be performed. The reassembly process is time dependent. The CD signal reappears 5 min after the pH change (from 9.0 to 2.3) aggregation and increases with time. The spectra reported in Figure 11 have been recorded 10 min after the pH variation.

To demonstrate that the chiral porphyrin reassembly is due to the presence of the (spectroscopically silent) chiral seeds of the porphyrin aggregate, we have kept various solutions of the chiral aggregates at pH 9.0 (that is in the disassembled state) for different time intervals before reassembling them (lowering the pH at 2.3). If the chiral reassembly is driven by the presence of inert chiral seeds, then (at pH 9.0) there must be a time interval after which the chiral seeds will disassemble. Then, chirality would not be reversible anymore and the system will reassemble in a nonchiral fashion (see route a) in Scheme 1. Indeed, after about 24 h at pH 9, the CD at pH 2.3 is not restored anymore because also the chiral seeds disassembled.

The results reported here permit to outline the system general characteristics necessary to get an erasable-rewritable (chiral) memory device: (i) a network of electrostatic interactions because their strength (quite higher than other noncovalent interactions) ensures a quite deep local energy minimum (that is a high value of E_A , at the basis of the kinetic inertia), (ii) the possibility of switching between the imprinted and erased memory by changing the “nature” of the system component(s) through photo-, redox-, or pH-driven reactions without affecting the thermodynamic energy state of the chiral heteroaggregates. The last point is very important because erasing the memory by increasing the temperature could cause the destruction of the chiral seed hampering the back reaction to the chiral assemblies.

CONCLUSIONS

Our data present evidence for a correlated sequence of induction, memory, and amplification of chirality in mesoscopic assemblies. They also show that the role of the chiral template is crucial only in the very first step of the formation of these ternary chiral species. Once formed, these porphyrin heteroaggregates have an “existence” independent from the template and not only retain “memory” of the shape of the mold but are also able to self-propagate. This peculiar characteristic permitted us to design a totally noncovalent memory system able to cycle between “written” and “erased” states. We think that this example opens a route for a general approach and new pathways to design systems able to memorize imprinted chiral information and successively cycle between erased and rewritten states.

Also, the structure determination of the $CuT4$ - H_2TPPS chiral and achiral heteroaggregates, for the first time,

allows the association of the CD signal of the chiral system to its α -helical structure, while the achiral heteroaggregate does not present any CD signal because of its amorphous nature.

The simplicity of this “mix and shake” synthetic approach, the robustness, and the remarkable ability to build exact duplicates of themselves candidate this imprinted noncovalent aggregates (obtained with low-price and commercially available chemicals) for a wide range of possible technological applications as enantioselective catalyst, resolution agents, remarkably sensitive amplifiers of chirality, and versatile chiral supramolecular reversible information storing systems.

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Chirality Sensing of Fullerenes Using Cyclic Hosts Having a Chiral *N*-Substituted Porphyrin: A Remote Substituent Effect

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT Cyclic host molecules **1_{2H}** and **2_{2H}**, having a chiral *N*-methylporphyrin unit, include C₇₆ in their asymmetrically distorted π -electronic cavity and are capable of spectral discrimination of the enantiomers of C₇₆ by means of ¹H NMR. Although the only structural difference between **1_{2H}** and **2_{2H}** is in the para-substituents R on their meso-phenyl groups, the association constant of C₇₆ with **1_{2H}** is more than twice as large as that with **2_{2H}**. The ΔH values for the association of C₇₆ with these hosts are hardly different from one another, but a rather big difference exists in their ΔS values (**1_{2H}**, -17.7 ± 0.7 J mol⁻¹; **2_{2H}**, -26.3 ± 1.0 J mol⁻¹). The difference in ΔS is most likely due to a steric effect of the substituents on the conformational freedom of the hexamethylene linkers. Although the R groups are topologically remote from the binding site, they also affect the resolution of the diastereoisomerically split *N*-Me signals in the ¹H NMR spectra of the hosts upon inclusion of C₇₆. *Chirality* 20:420–424, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: host–guest chemistry; porphyrin; *N*-alkylporphyrin; fullerene; chirality sensing

INTRODUCTION

Some higher fullerenes such as C₇₆ (Fig. 1), C₇₈, and C₈₄ are chiral, having asymmetrically curved π -electronic surfaces.^{1–3} Sensing of the asymmetric structures of these carbon clusters is a challenging issue in chiral chemistry, because they are devoid of any appropriate functionalities for three-point recognition, as generally required for chiral recognition of asymmetric compounds. We have found that cyclic dimers of metalloporphyrins can accommodate fullerenes in their cavities.^{4–13} We expected that their chiral versions may have the potential for sensing chiral fullerenes. In a previous paper, we have reported that heterocyclic porphyrin dimer **1_{Rh}** (Fig. 1) containing a chiral *N*-methylporphyrin^{14,15} is indeed capable of sensing chiral fullerene C₇₆ by means of ¹H NMR.⁴ *N*-Alkylporphyrins are nonplanar because of a steric effect of the *N*-alkyl groups on the porphyrin core. Thus, host **1_{Rh}** possesses an asymmetrically distorted π -electronic surface. In the present paper, we report inclusion complexation of chiral free-base porphyrins **1_{2H}** and **2_{2H}** (Fig. 1) with C₇₆, and highlight an interesting substituent effect on their affinity and sensing capability.

MATERIALS AND METHODS

General Methods

Electronic absorption spectra were recorded on a JASCO model U-best V-560 or V-570 spectrometer. Circular dichroism (CD) spectra were recorded on a JASCO

model J-720 spectropolarimeter. ¹H NMR spectra were recorded on a JEOL Type GSX-500 spectrometer, using a residual nondeuterated solvent as an internal reference. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on an Applied Biosystems BioSpectrometry Workstation™ model Voyager-DE™ STR spectrometer using dithranol as a matrix. Preparative high-performance liquid chromatography (HPLC) was performed at 20°C using a $\phi 20 \times 250$ mm CHIRALPAK IA (Daicel Chemical) or 5PBB (Nakarai Tesque) column on a Japan Analytical Industry model LC-918 recycling preparative HPLC, equipped with JASCO Type 875-UV and CD-1595 variable-wavelength detectors. Square wave voltammetry was performed on a BAS model ALS-619B voltammetry controller using Pt working and counter electrodes along with an Ag pseudo-reference electrode. The ferrocene/ferrocenium redox couple was used as an internal standard.

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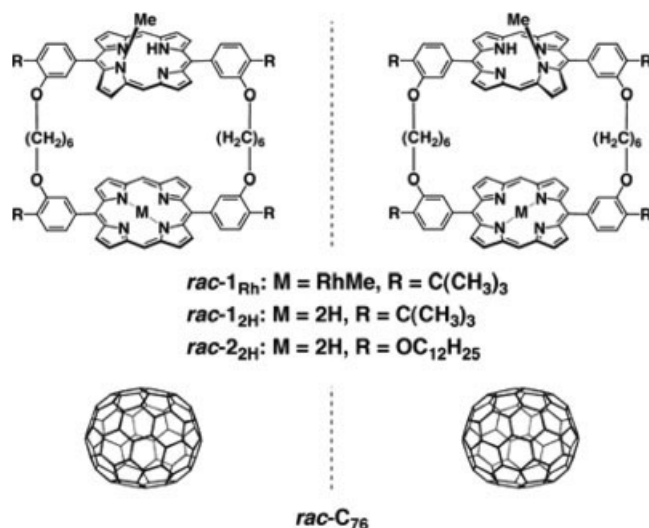


Fig. 1. Schematic structures of the hosts and C₇₆.

Evaluation of Association Constants by Spectroscopic Titrations

Association constants K_{assoc} were evaluated from the following equation by applying a nonlinear curve-fitting method to the observed changes in absorbance (ΔAbs) upon titration of hosts **1_{2H}** and **2_{2H}** with C₇₆:

$$\Delta\text{Abs} = (L(1 + K_{\text{assoc}}X + K_{\text{assoc}}A) - (L^2(K_{\text{assoc}}X + K_{\text{assoc}}A + 1)^2 = 4K_{\text{assoc}}^2AXL^2)^{0.5})/2K_{\text{assoc}}A$$

where X and A represent $[\text{Guest}]_{\text{total}}$ and $[\text{Host}]_{\text{total}}$, respectively; L denotes ΔAbs at 100% complexation; L and K_{assoc} are parameters.

Preparations of the Materials

Preparation and optical resolution of **1_{2H}** and C₇₆ were reported in Ref. 4.

Synthesis of rac-2_{2H} (Scheme 1). **3** was synthesized according to a literature method.¹² A mixture of **3** (30 mg, 16 μmol), MeI (1.0 mL, 16 mmol), and AcOH (0.50 mL) in CHCl₃ (5.0 mL) was stirred for 2 days at 55°C and evaporated to dryness. The residue was dissolved in CHCl₃ and washed successively with aqueous NH₃ (28%) and water, dried over Na₂SO₄, and evaporated to dryness. The residue was chromatographed on silica gel with CHCl₃/Et₂NH (100/0.1) as an eluent, where the second fraction was collected and evaporated to dryness. The residue was recrystallized from CH₂Cl₂/MeCN to give *rac*-**2_{2H}** as purple powder (10 mg, 30%). Because of the presence of conformational isomers, *rac*-**2_{2H}** showed a complicated ¹H NMR spectrum, which however was simplified upon inclusion of C₆₀.

¹H NMR (*rac*-**2_{2H}**⊂C₆₀, 500 MHz, toluene-*d*₈, 20°C) δ 9.87 (s, 1H, meso-H), 9.80 (s, 1H, meso-H), 9.66 (s, 1H, meso-H), 9.52 (s, 1H, meso-H), 9.16–9.13 (m, 3H, pyrrole- β -H), 9.07 (d, 1H, pyrrole- β -H), 9.04–9.02 (m, 3H, pyrrole- β -H), 8.99–8.96 (m, 3H, pyrrole- β -H), 8.91–8.88 (m, 3H,

pyrrole- β -H), 8.83 (d, 1H, pyrrole- β -H), 8.09 (m, 1H, C₆H₃), 7.95–7.88 (m, 4H, pyrrole- β -H and C₆H₃), 7.72 (d, 1H, pyrrole- β -H), 7.45–7.42 (m, 4H, C₆H₃), 7.21–7.15 (m, 4H, C₆H₃), 4.16–4.12 (m, 8H, OCH₂C₁₁H₂₃), 3.86–3.33 (m, 8H, OCH₂(CH₂)₄CH₂O), 2.21–0.86 (m, 108H, OCH₂(CH₂)₄CH₂O and OCH₂C₁₁H₂₃), –2.70 (s, 1H, NH of *N*-methylporphyrin), –2.93 (s, 2H, NH of porphyrin), –4.46 (s, 3H, NCH₃); MALDI-TOF-MS m/z calcd. for C₁₂₅H₁₆₂N₈O₈ (MH⁺) 1903.25, found 1903.34; UV-vis (toluene): λ_{max} (log ϵ) 414 (5.68), 508 (4.44), 553 (4.24), 637 (3.57), 663 (3.55) nm.

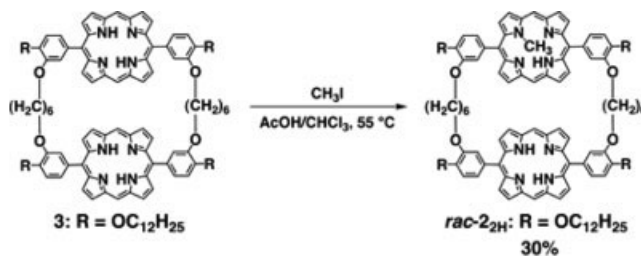
RESULTS AND DISCUSSION

Optical Resolution and Redox Properties of **1_{2H}** and **2_{2H}**

As reported previously,⁴ optical resolution of **1_{2H}** was achieved by preparative HPLC on CHIRLAPAK IA with hexane/CH₂Cl₂/Et₂NH (87.5/12.5/0.1, v/v/v) as an eluent. The fractions corresponding to the enantiomers of **1_{2H}** were separated well and displayed CD spectra that were mirror images of one another (Fig. 2a). Since the first and second fractions showed intense negative and positive CD bands at 410 nm, respectively, they are denoted as (–)-**1_{2H}** and (+)-**1_{2H}** here. Since the solubility of **1_{2H}** was not satisfactory in common organic solvents, we newly synthesized **2_{2H}**, which bears dodecyloxy groups in place of the *tert*-butyl groups at the meso-phenyl rings attached to the linker parts. Similar to the case of **1_{2H}**, the enantiomers of **2_{2H}** were successfully separated with hexane/CH₂Cl₂/Et₂NH (75.0/25.0/0.1, v/v/v) as an eluent. By analogy to the enantiomers of **1_{2H}**, those obtained as the first and second fractions are denoted as (–)-**2_{2H}** and (+)-**2_{2H}**, respectively (Fig. 2b). The absorption spectra of **1_{2H}** and **2_{2H}** were substantially the sum of those of the corresponding *N*-substituted and unsubstituted porphyrin precursors. Square wave voltammetry of a CH₂Cl₂ solution of **1_{2H}** (0.1 mM) containing 0.1 M Bu₄NPF₆ as a supporting electrolyte showed two peaks at 0.43 and 0.56 V vs Fc/Fc⁺, which are assignable to the oxidation of the *N*-substituted and unsubstituted porphyrin parts, respectively. It is of interest that the corresponding oxidation potentials of **2_{2H}** were more negative (0.36 and 0.52 V).

Inclusion Complexation of C₇₆ with Host Molecules

Both **1_{2H}** and **2_{2H}** included C₇₆ in their cavity without any detectable enantioselectivity. In ¹H NMR spectro-



Scheme 1

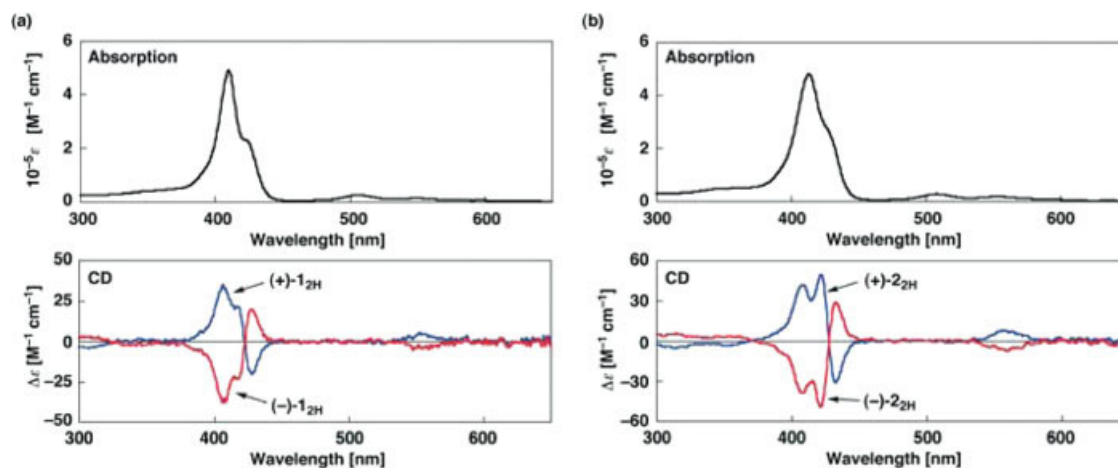


Fig. 2. Absorption and CD spectra of the enantiomers of (a) **1**_{2H} ((-)-**1**_{2H} and (+)-**1**_{2H}) and (b) **2**_{2H} ((-)-**2**_{2H} and (+)-**2**_{2H}) in toluene at 20°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

copy, *rac*-**1**_{2H} alone in toluene-*d*₈ at 30°C showed multiple *N*-Me signals in a range from $\delta = -4.3$ to -4.9 ppm, due to the presence of some conformational isomers (Fig. 3a). However, upon mixing *rac*-**1**_{2H} with an equimolar amount of *rac*-C₇₆, the spectrum became simplified, displaying a single *N*-Me signal at $\delta = -4.56$ ppm. Molecular models, optimized with a molecular mechanics calculation program CACheTM, predicted that better fitting of the π -electronic cavity of **1**_{2H} to C₇₆ requires unfavorable conformational isomers of **1**_{2H} to rotate the *N*-methylporphyrin unit to point their *N*-Me group outward. The same was true for *rac*-**2**_{2H}, where the multiple *N*-Me signals ranging from $\delta = -4.2$ to -4.9 ppm were merged, upon inclusion of *rac*-C₇₆, to give a single signal at $\delta = -4.49$ ppm (Fig. 3b). Titration of *rac*-**1**_{2H} with *rac*-C₇₆ in toluene at 30°C resulted in a bathochromic spectral change at the Soret absorption region (Fig. 4a), where the absorbance change at 410 nm allowed for the evaluation of K_{assoc} as $2.2 \times 10^6 \text{ M}^{-1}$. Host *rac*-**2**_{2H}, upon titration with *rac*-C₇₆ under identical conditions, displayed an absorption spectral change similar to that observed for **1**_{2H}C₇₆ (Fig. 4b). Interestingly, the K_{assoc} value, thus evaluated for **2**_{2H}C₇₆ ($8.9 \times 10^5 \text{ M}^{-1}$), was less than a half of that obtained for **1**_{2H}C₇₆.

Thermodynamic Parameters for the Inclusion Complexation

We have reported that a porphyrin-to-fullerene charge transfer (CT) interaction operates in the inclusion complexation of metalloporphyrin cyclic hosts with fullerenes.^{5,6,11} Since the first oxidation potentials of the porphyrin units in **2**_{2H} are a little lower than those in **1**_{2H} (vide ante), we expected that the CT interaction in **2**_{2H}C₇₆ might be more pronounced than that in **1**_{2H}C₇₆. However, as described in the above section, the K_{assoc} value for **2**_{2H}C₇₆ is obviously smaller than that for **1**_{2H}C₇₆. As shown in Fig. 5, plots of $\ln K_{\text{assoc}}$ versus T^{-1} gave a linear correlation, from which ΔH and ΔS were obtained. While the ΔH values for the formation of **1**_{2H}C₇₆ (-42.2

$\pm 0.2 \text{ kJ mol}^{-1}$) and **2**_{2H}C₇₆ ($-42.5 \pm 0.3 \text{ kJ mol}^{-1}$) were hardly different from one another, the ΔS value for the former complexation ($-17.7 \pm 0.7 \text{ J mol}^{-1}$) was much less negative than that for the latter ($-26.3 \pm 1.0 \text{ J mol}^{-1}$). As already discussed, the host molecules lose their conformational freedom upon guest inclusion (Fig. 3).⁵ Therefore, the negative ΔS values are quite reasonable. Then why is ΔS for **1**_{2H} less negative than the other? We have noticed that the linker parts of the cyclic hosts play an important role in the inclusion complexation.^{5,12} According to CPK model studies, the hexamethylene linkers in **1**_{2H} appear to be conformationally constrained because of the presence of the sterically demanding *tert*-butyl groups. In relation to this indication, host **1**_{2H} at -20°C most likely adopts two dominant conformations, judging from the ¹H NMR spectral pattern of the *N*-Me signals in Figure 6a ($\delta = -4.64$ and -4.99 ppm; 84%). In contrast, host **2**_{2H} under identical conditions obviously displayed some minor *N*-Me signals ($\delta = -4.31$, -4.76 , and -4.86 ppm; 24%) along with two dominant signals ($\delta = -4.49$ and -4.92 ppm, 76%; Fig. 6b). Thus, the major conformational isomer of **1**_{2H} is more populated than that of **2**_{2H}. This may account for the smaller entropy loss of the system of **1**_{2H} than that of **2**_{2H} for the complexation with C₇₆.

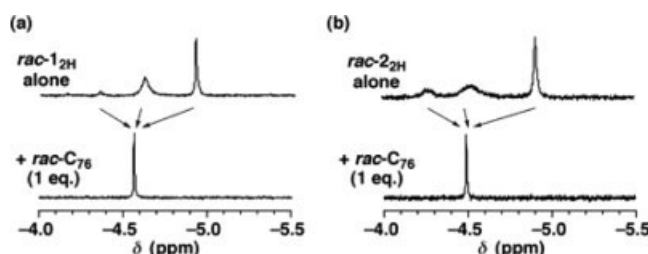


Fig. 3. ¹H NMR (500 MHz) spectra (*N*-Me resonance region) of (a) *rac*-**1**_{2H} and (b) *rac*-**2**_{2H} in the absence and presence of an equimolar amount of *rac*-C₇₆ in toluene-*d*₈ at 30°C. [**1**_{2H}] = [**2**_{2H}] = $1.5 \times 10^{-4} \text{ M}$.

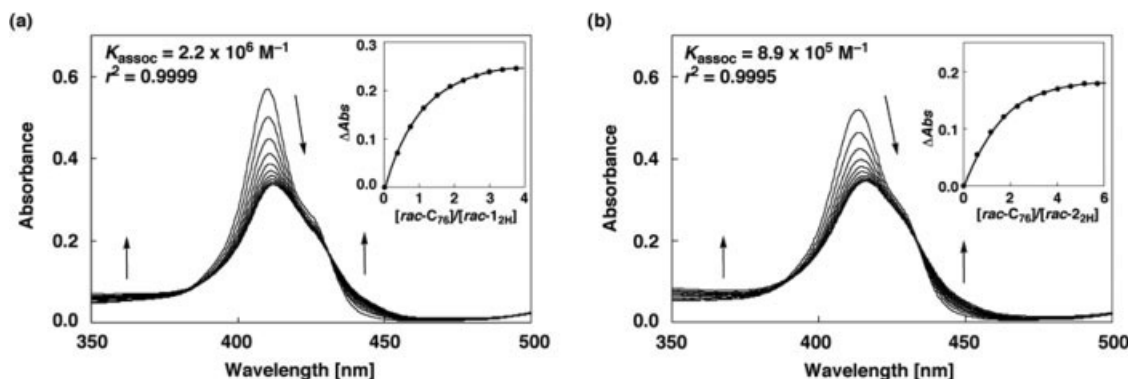


Fig. 4. Spectral changes of (a) *rac*-**12H** and (b) *rac*-**22H** upon titration with *rac*-C₇₆ in toluene at 30°C. Insets: changes in ΔAbs at (a) 410 and (b) 414 nm for the evaluation of K_{assoc} . [**12H**] = 1.2×10^{-6} M; [*rac*-C₇₆]/[**12H**] = 0–3.7, [**22H**] = 1.1×10^{-6} M; [*rac*-C₇₆]/[**22H**] = 0–5.7. The spectra were obtained by subtracting the contribution of *rac*-C₇₆.

Chirality Sensing of C₇₆

In the ¹H NMR spectra of a toluene-*d*₈ solution of *rac*-**12H**⊃*rac*-C₇₆, several signals due to **12H** underwent splitting upon lowering the temperature to –20°C. The splitting of the *N*-Me signal (Fig. 7a) was noteworthy, with a $\Delta\delta$ value at –20°C as large as 5.4 Hz. Obviously, the split signals are due to the diastereoisomers of *rac*-**12H**⊃*rac*-C₇₆, since neither of the two signals was observed when (+)-C₇₆ was allowed to complex with (–)-**12H** or (+)-**12H** (Fig. 7a). By reference to the ¹H NMR spectra of these diastereoisomerically pure samples, the *N*-Me signals at δ = –4.61 and –4.62 ppm were assigned to (+)-**12H**⊃(+)-C₇₆/ (–)-**12H**⊃(–)-C₇₆ and (–)-**12H**⊃(+)-C₇₆/ (+)-**12H**⊃(–)-C₇₆, respectively. Inclusion complex *rac*-**22H**⊃*rac*-C₇₆, upon lowering the temperature to –20°C, showed an analogous ¹H NMR spectral change profile. However, signal separation was inferior to that of *rac*-**12H**⊃*rac*-C₇₆ (Fig. 7b), indicating that **12H** is a better chirality sensor than **22H** for C₇₆. As discussed in our previous paper,⁴ the diastereoisomeric signal splitting is affected by the host/guest complexation dynamics. We investigated here the dynamics of the complexation of **12H** with C₇₆ at a low temperature such as –20°C. By ¹H NMR line-shape analy-

sis with a program DNMR5,^{4,8,12} the guest-exchange rate constant between (–)-**12H**⊃(+)-C₇₆ and (–)-**12H**⊃(–)-C₇₆ was 1.1 s^{-1} , as evaluated in regard to the *N*-Me resonance in an equimolar mixture of (–)-**12H** and *rac*-C₇₆. As expected, (–)-**22H**⊃*rac*-C₇₆ showed an obviously larger guest-exchange rate constant (1.7 s^{-1}). These results are consistent with our previous conclusion⁴ that a cyclic host molecule having a larger K_{assoc} value with C₇₆ shows a lower guest-exchange activity.

CONCLUSION

We have demonstrated here that cyclic host molecules **12H** and **22H**, having a chiral *N*-methylporphyrin unit, can include C₇₆ in their asymmetrically distorted π -electronic cavity and are capable of spectral discrimination of the enantiomers of C₇₆ by means of ¹H NMR. The only structural difference between these two hosts exists only in the para-substituents on the meso-phenyl groups. While these are topologically remote from the guest-binding site, **12H** exhibits a larger affinity and a better sensing capability for C₇₆ than **22H**. These features are most likely due to a steric effect of the bulky *tert*-butyl groups in **12H** that more or less limit the conformation of the hexamethylene linkers. Neither **12H** and **22H** is enantioselective in the inclusion of C₇₆, and they are suitable for determining the enantiomeric purity of chiral fullerenes.

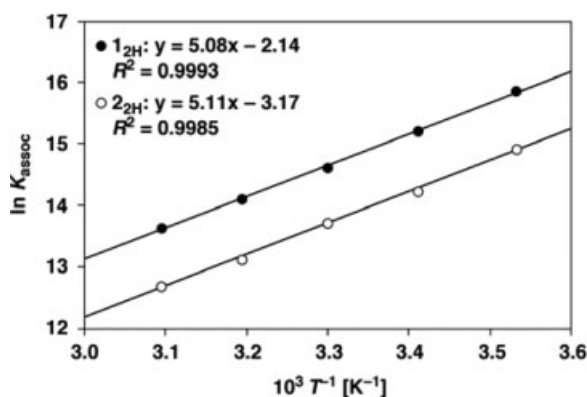


Fig. 5. van't Hoff plots for the complexation of hosts **12H** and **22H** with C₇₆ in toluene: (●) **12H**; and (○) **22H**.

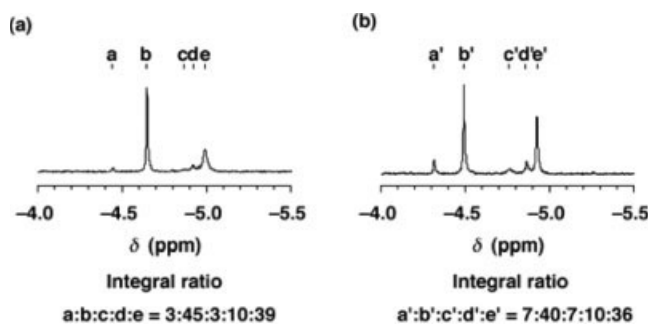


Fig. 6. ¹H NMR (500 MHz) spectra (*N*-Me resonance region) of *rac*-host in toluene-*d*₈ at –20°C. Host = (a) **12H** and (b) **22H**. [**12H**] = [**22H**] = 1.5×10^{-4} M.

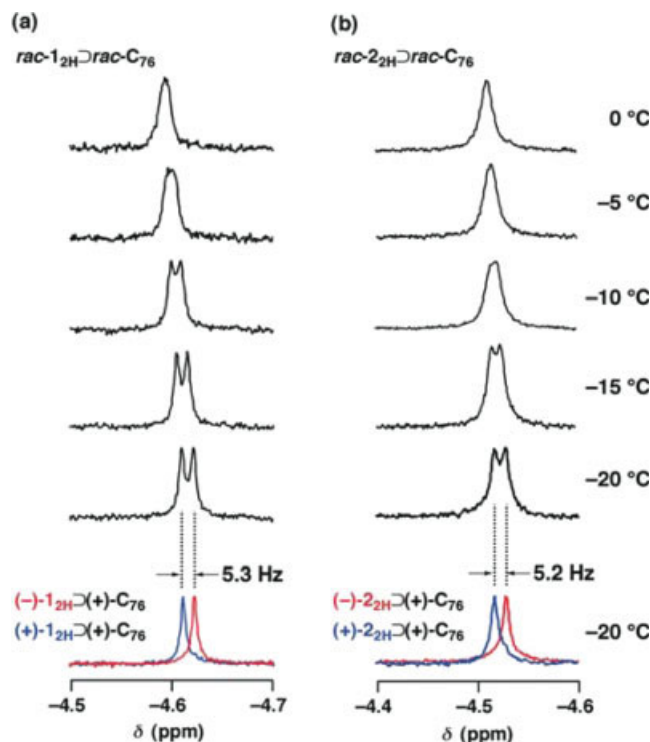


Fig. 7. VT ^1H NMR (500 MHz) spectra (N -Me resonance region) of equimolar mixtures of *rac*-host/*rac*- C_{76} (black curves), (+)-host/(+)- C_{76} (blue curves), and (-)-host/(+)- C_{76} (red curves) in toluene- d_8 . Host = (a) $1_{2\text{H}}$ and (b) $2_{2\text{H}}$, $[(+)\text{-}\text{C}_{76}] = [\text{rac}\text{-}\text{C}_{76}]/2 = 7.7 \times 10^{-5}$ M. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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Chiral Ligand Optimization in the Asymmetric Zirconium–Zinc Transmetalation Aldehyde Addition Reaction

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT The in situ hydrozirconation–transmetalation–aldehyde addition process is a convenient method for the generation of allylic alcohols. Ongoing research has focused on enhancing the enantioselectivity and substrate scope of this process. A chiral β -amino thiol scaffold was evaluated in the addition reaction. Amino thiols tend to provide the highest *ee*'s, in part due to the higher affinity of sulfur for zinc over zirconium. A class of valine-based thiol ligands was identified to be effective for the formation of enantiomerically enriched allylic alcohols in terms of low ligand loading and high % *ee*. *Chirality* 20:425–430, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: chiral allylic alcohols; β -amino thiol; dimethyl zinc; transmetalation; enantioselectivity; catalysis; alkenylzinc addition

INTRODUCTION

Allylic alcohols are not only found as a common motif in natural products but also represent convenient building blocks for a variety of synthetic applications. These include [3,3]-sigmatropic rearrangements,¹ enantio- and diastereoselective hydroxy-directed addition to alkenes,² S_N2' -displacements with cuprates,³ palladium catalyzed π -allyl substitution,⁴ and cationic cyclizations.⁵ Because of this versatility, efficient methods for the formation of enantioenriched allylic alcohols are highly desirable.

Transition metal-mediated vinylations, which rely on the nucleophilic addition of an alkenyl metal reagent to a carbonyl group, represent a popular route for the generation of allylic alcohols. Asymmetric variants include the Nozaki–Hiyama–Kishi reaction employing a chiral sulfonamide ligand pioneered by Kishi et al.,⁶ Ni-catalyzed reactions using a chiral phosphine ligand introduced by Jamison et al.⁷ and an *N*-heterocyclic carbene ligand by Montgomery et al.,⁸ and an iridium-catalyzed hydrogenative coupling approach utilizing a chiral phosphoric acid derived from BINOL developed by Krische and Komanduri.⁹ Alkenylzinc reagents can also be utilized to gain access to this functionality. Oppolzer has demonstrated the effectiveness of hydroboration–transmetalation of an alkyne followed by addition to a carbonyl compound in the presence of (+)-DAIB to afford the enantioenriched allylic alcohols.¹⁰ This original protocol has been investigated with a variety of diverse ligand scaffolds, including isoborneol-based amino alcohol (–)-MIB by Walsh et al.,¹¹ paracyclophane based ketimines by Bräse and Dahmen,¹² aminonaphthols by Chan et al.,¹³ and β -amino alcohols by Yang and Tseng.¹⁴ The in situ hydrozirconation–transmetalation is an alternative protocol developed in our laboratory and is a convenient method for the generation of

allylic alcohols.^{15,16} In this method, hydrozirconation of the alkyne is followed by transmetalation to dimethyl zinc at -65°C ; in the presence of an aldehyde the corresponding allylic alcohol product is generated in a one-pot reaction in high yields (Scheme 1).¹⁷

Previously, we have reported on the development of an asymmetric version of this protocol, which has resulted in the discovery of a class of thiol benzylamine ligands pioneered by van Koten et al.,¹⁸ which showed high enantioselectivity in the alkenylzirconium/zinc addition (Scheme 2).¹⁹ The thiol benzylamine **3** provided excellent enantioselectivities (83–99% *ee*) in the addition of terminal and internal alkynes to aromatic aldehydes, but a decrease in enantioselectivity (63–74% *ee*) was observed with aliphatic and aromatic aldehydes containing electron-donating groups in the *para*-positions. Several more highly substituted analogues of this scaffold were investigated but yielded no further improvement in enantioselectivity. It was also observed that incorporation of a sulfur atom was essential for high enantioselectivity. In general, sulfur-containing ligands have shown superior enantioselectivities when compared with their alcohol counterparts in this addition process.²⁰ The enhanced performance can be attributed to the properties of sulfur. Thiols proved to have a higher affinity towards zinc than zirconium.²¹ This selec-

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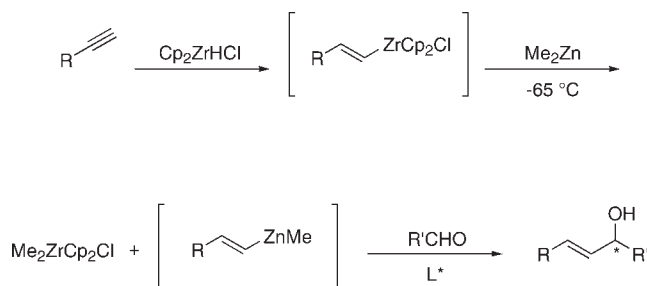
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Scheme 1. The in situ hydrozirconation-transmetalation addition protocol.

tivity is quite significant since in the in situ method both zinc salts and zirconium complexes are present in the reaction medium, and a higher affinity of the ligand for zinc is desirable. Thiolates also have less of a tendency to lower the Lewis acidity of zinc. This feature is important since the Lewis acidity of the metal activates the carbonyl group towards addition.

In ongoing research, we are focused on the development of thiol ligands with alternative scaffolds that demonstrate high asymmetric induction with a wide variety of aldehydes and alkynes. In addition, we are also interested in identifying a ligand complex that allows for lower loadings without a significant drop in enantiomeric excess.

EXPERIMENTAL SECTION

General: Me_2Zn was purchased from the Aldrich Chemical and Cp_2ZrHCl was prepared according to a modification of a literature protocol.²² Analytical thin layer chromatography was performed on precoated silica gel 60 F-254 plates (particle size 0.040 mm–0.055 mm, 230–400 mesh). Flash chromatography on SiO_2 was used to separate and purify the crude reaction mixtures. NMR spectra were recorded at 300 MHz/75 MHz (^1H NMR/ ^{13}C NMR) at 21 °C in CDCl_3 unless otherwise noted. Chemical shifts (δ) are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sx = sextet, sp = septet, o = octet, dt = doublet of triplet, dq = doublet of quartet, m = multiplet, b = broad), integration, and coupling constants. Mass spectra were obtained on a double focusing instrument. Optical rotations were measured on a Perkin–Elmer 241 polarimeter at $T = 25^\circ\text{C}$. Chiral HPLC analysis was performed on a Dynamax SD-200 delivery system in conjunction with a Dynamax UV-1 absorbance detector. A Chiralcel OD (0.46 cm $\times$ 25 cm) or AD-H (0.46 cm $\times$ 25 cm) column was used for determination of enantiomeric excess.

(S)-3-Methyl-2-pyrrolidin-1-yl-butan-1-ol (6a)

To a solution of 2.00 g (19.4 mmol) of (L)-valinol in 80 ml of CH_3CN were added 5.36 g (38.8 mmol) of K_2CO_3 and 2.54 ml of 1,4-dibromobutane. The reaction mixture was heated at reflux for 18 h, cooled, filtered, and concentrated in vacuo. The resulting oil was diluted with H_2O , extracted with EtOAc, dried (MgSO_4), and concentrated in vacuo to yield 2.63 g (86%) of **6a** as a colorless oil: $[\alpha]_{\text{D}}^{25} + 39.2$ (c 1.5, CHCl_3); ^1H NMR δ 3.63 (dd, 1H, $J = 4.8$, 10.5 Hz), 3.41 (dd, 1H, $J = 6.9$, 10.5 Hz), 2.73 (bs, 4H), 2.34 (q, 1H, $J = 6.3$ Hz), 1.92 (o, 1H, $J = 6.6$ Hz), 1.88–1.70 (m, 4H), 0.99 (d, 3H, $J = 6.9$ Hz), 0.91 (d, 3H, $J = 6.6$ Hz); ^{13}C NMR δ 66.97, 59.65, 49.43, 28.63, 23.66, 21.67, 18.73; MS (EI) m/z (relative intensity): 126 ($[\text{M}-\text{CH}_2\text{OH}]^+$, 100), 114 (98), 96 (19), 84 (40), 70 (53), 55 (32); HRMS (EI) Calcd for $\text{C}_9\text{H}_{19}\text{NO}$ 158.1545, found 158.1540.

10.5 Hz), 3.41 (dd, 1H, $J = 6.9$, 10.5 Hz), 2.73 (bs, 4H), 2.34 (q, 1H, $J = 6.3$ Hz), 1.92 (o, 1H, $J = 6.6$ Hz), 1.88–1.70 (m, 4H), 0.99 (d, 3H, $J = 6.9$ Hz), 0.91 (d, 3H, $J = 6.6$ Hz); ^{13}C NMR δ 66.97, 59.65, 49.43, 28.63, 23.66, 21.67, 18.73; MS (EI) m/z (relative intensity): 126 ($[\text{M}-\text{CH}_2\text{OH}]^+$, 100), 114 (98), 96 (19), 84 (40), 70 (53), 55 (32); HRMS (EI) Calcd for $\text{C}_9\text{H}_{19}\text{NO}$ 158.1545, found 158.1540.

Thioacetic acid (S)-(3-methyl-2-pyrrolidin-1-yl-butyl) ester (7a)

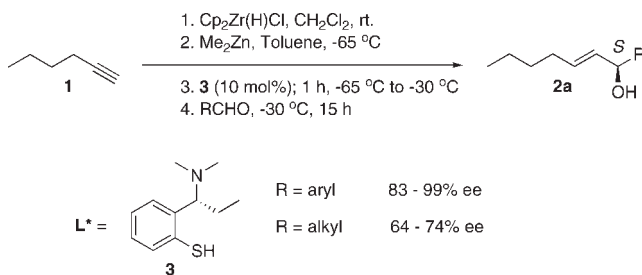
To a well-stirred solution of 2.68 g (10.2 mmol) of PPh_3 in 75 ml of THF at 0 °C was added 2.00 ml (10.2 mmol) of diisopropylazodicarboxylate. The resulting solution was stirred for 30 min, followed by simultaneous addition of 0.73 ml (10.2 mmol) of thioacetic acid and 800 mg (5.10 mmol) of **6a**. The reaction mixture was stirred for 1 h at 0 °C, 1 h at rt, and concentrated in vacuo. The resulting crude residue was purified by chromatography on SiO_2 (2% EtOAc/pet. Et₂O) to yield 725 mg (66%) of **7a** as an orange oil: $[\alpha]_{\text{D}}^{25} + 52.6$ (c 0.5, CHCl_3); ^1H NMR δ 3.17 (A of ABX, 1H, $J = 4.5$, 13.8 Hz), 3.00 (B of ABX, 1H, $J = 5.4$, 13.8 Hz), 2.68–2.55 (m, 4H), 2.32 (s, 3H), 2.04–1.88 (m, 1H), 1.82–1.69 (m, 4H), 1.34–1.24 (m, 1H), 0.96 (d, 3H, $J = 7.2$ Hz), 0.93 (d, 3H, $J = 6.9$ Hz); ^{13}C NMR δ 195.97, 67.61, 50.72, 30.79, 30.46, 28.34, 23.49, 20.35, 17.81; HRMS (EI) Calcd for $\text{C}_{11}\text{H}_{21}\text{NOS}$ 216.1422, found 216.1409.

(S)-3-Methyl-2-pyrrolidin-1-yl-butane-1-thiol (8a)

To a suspension of 42 mg (1.1 mmol) of LiAlH_4 in 4 ml of Et₂O at 0 °C was added 60 mg (0.28 mmol) of **7a** in 2 ml of Et₂O. The resulting slurry was stirred for 45 min at rt, cooled to 0 °C, and quenched with 1 ml of H_2O , 1 ml of 15% NaOH, 3 ml of H_2O , and filtered through Celite. The resulting filtrate was concentrated in vacuo to yield 35 mg (72%) of **8a** as a colorless oil: $[\alpha]_{\text{D}}^{25} + 31.8$ (c 0.25, CHCl_3); ^1H NMR δ 2.80–2.55 (m, 4H), 2.33 (q, 1H, $J = 5.4$ Hz), 1.96 (o, 1H, $J = 6.0$ Hz), 1.82–1.68 (m, 4H), 1.61 (bs, 1H), 1.26 (s, 1H), 0.96 (d, 3H, $J = 6.3$ Hz), 0.94 (d, 3H, $J = 6.3$ Hz); ^{13}C NMR δ 69.79, 50.14, 30.33, 24.01, 23.68, 20.64, 18.51.

(S)-3-Methyl-2-piperidin-1-yl-butan-1-ol (6b)²³

According to the preparation of **6a**, **6b** (76%) was obtained as a colorless oil: $[\alpha]_{\text{D}}^{25} + 23.3$ (c 0.25, CHCl_3); ^1H NMR δ 3.98 (s, 1H), 3.56–3.45 (dd, 1H, $J = 5.4$, 10.2 Hz), 3.16 (t, 1H, $J = 10.2$ Hz), 2.91–2.78 (m, 2H), 2.63–2.47 (m, 2H), 2.35–2.21 (m, 1H), 1.92–1.78 (m, 1H), 1.68–1.42



Scheme 2. Enantioselectivity observed with thiol benzylamine **3**.

(m, 6H), 1.03 (d, 3H, J = 6.6 Hz), 0.81 (d, 3H, J = 6.6 Hz); MS (EI) m/z (relative intensity) 154 ($[M-OH]^+$, 13), 140 (60), 128 (20), 98 (20), 91 (61), 84 (82), 69 (74), 63 (100).

Thioacetic acid (S)-(3-methyl-2-piperidin-1-yl-butyl) ester (7b)²³

According to the preparation of **7a**, **7b** (56%) was obtained as an orange oil: $[\alpha]_D^{25} +42.4$ (c 1.0, $CHCl_3$); ^1H NMR δ 3.07 (d, 2H, J = 5.5 Hz), 2.62–2.53 (m, 2H), 2.53–2.40 (m, 2H), 2.32 (s, 3H), 2.34–2.22 (m, 1H), 1.83 (o, 1H, J = 6.8 Hz), 1.53–1.46 (m, 4H), 1.46–1.36 (m, 2H), 0.95 (d, 3H, J = 6.7 Hz), 0.92 (d, 3H, J = 6.7 Hz); MS (EI) m/z (relative intensity) 229 (M^+ , 31), 210 (80), 186 (93), 144 (76), 140 (100), 110 (49), 103 (70).

(S)-3-Methyl-2-pyrrolidin-1-yl-butane-1-thiol (8b)²³

According to the preparation of **8a**, **8b** (63%) was obtained as an orange oil: $[\alpha]_D^{25} +72.2$ (c 0.6, $CHCl_3$); ^1H NMR δ 3.06 (A of ABX, 1H, J = 7.2, 12.6 Hz), 3.04 (B of ABX, 1H, J = 4.8, 12.9 Hz), 2.65–2.53 (m, 2H), 2.53–2.44 (m, 2H), 1.82 (o, 1H, J = 6.6 Hz), 1.63–1.48 (m, 4H), 1.48–1.35 (m, 2H), 0.95 (d, 3H, J = 6.6 Hz), 0.93 (d, 3H, J = 6.3 Hz); ^{13}C NMR δ 70.68, 50.69, 39.32, 30.23, 26.71, 25.09, 21.17, 20.57; MS (EI) m/z (relative intensity) 188 (M^+ , 7), 186 (10), 140 (100), 111 (16), 98 (6), 84 (18), 69 (7), 55 (13); HRMS (EI) Calcd for $C_{10}H_{21}NS$ 187.1395, found 187.1397.

(S)-3,3-Dimethyl-2-piperidin-1-yl-butan-1-ol (6c)

According to the preparation of **6a** except starting from (S)-*t*-leucinol, **6c** (75%) was obtained as a white flaky solid: $[\alpha]_D^{25} +31.4$ (c 0.5, $CHCl_3$); ^1H NMR δ 3.58–3.40 (m, 2H), 3.05–2.95 (m, 2H), 2.75–2.60 (m, 2H), 2.42 (dd, 1H, J = 1.4, 10.8 Hz), 1.52–1.43 (m, 6H), 0.97 (s, 9H); ^{13}C NMR δ 74.82, 57.31, 51.59, 36.84, 29.08, 27.81, 24.92; MS (EI) m/z (relative intensity) 185 (M^+ , 23), 170 (70), 154 (72), 128 (100), 98 (60), 83 (57), 68 (48); HRMS (EI) Calcd for $C_{11}H_{23}NO$ 185.1779, found 185.1780.

Thioacetic acid (S)-(3,3-dimethyl-2-piperidin-1-yl-butyl) ester (7c)

According to the preparation of **7a**, **7c** (63%) was obtained as an orange solid: $[\alpha]_D^{25} +114$ (c 0.1, $CHCl_3$); ^1H NMR δ 3.34 (dd, 1H, J = 3.2, 13.6 Hz), 2.96 (dd, 1H, J = 11.5, 13.6 Hz), 2.85–2.73 (m, 2H), 2.63–2.52 (m, 2H), 2.33 (s, 3H), 2.28 (dd, 1H, J = 3.1, 11.6 Hz), 1.57–1.40 (m, 6H), 0.94 (s, 9H); ^{13}C NMR δ 196.19, 73.04, 52.88; 37.95, 30.58, 27.71, 27.60, 27.13, 25.01.

(S)-(3,3)-Dimethyl-2-piperidin-1-yl-butane-1-thiol (8c)

According to the preparation of **8a**, **8c** (73%) was obtained as a colorless oil: $[\alpha]_D^{25} +64.6$ (c 0.85, $CHCl_3$); ^1H NMR δ 2.91–2.78 (m, 4H), 2.77 (d, 1H, J = 5.4 Hz), 2.69–2.56 (m, 1H), 2.29 (dd, 1H, J = 3.0, 11.1 Hz), 1.95 (bs, 1H), 1.56–1.39 (m, 6H), 0.91 (s, 9H); ^{13}C NMR δ 76.83, 52.39, 38.28, 28.08, 27.35, 25.12, 22.96.

General Protocol for formation of allylic alcohols. (R)-1-Phenyl-hept-2-en-1-ol (2b) using 10 mol % of ligand 11

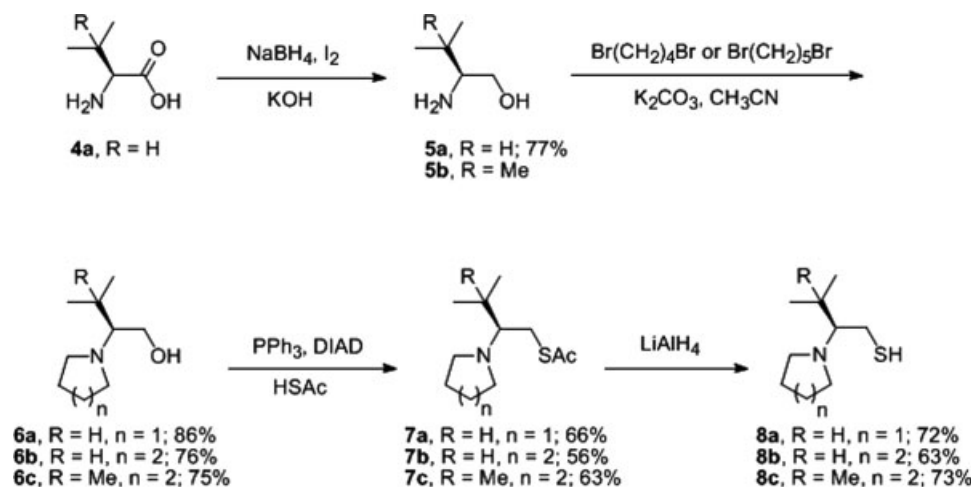
To a suspension of 500 mg (1.93 mmol) of zirconocene hydrochloride in 4 ml of CH_2Cl_2 under N_2 was added 175 μ l (1.52 mmol) of 1-hexyne at room temperature. After 5 min, an additional 80 μ l (0.70 mmol) of 1-hexyne was added. The reaction mixture was stirred for a further 10 min, and the solution was concentrated in vacuo. To a solution of the resulting orange oil in 4 ml of toluene at $-65^\circ C$ was added 650 μ l (1.29 mmol) of Me_2Zn (2.0 M solution in toluene). After 10 min, 28 mg (0.129 mmol) of ligand **11** was added. The reaction mixture was then warmed to $-30^\circ C$ over a period of 1 h, and 134 μ l (1.29 mmol) of freshly distilled benzaldehyde was added. The solution was stirred for 15 h at $-30^\circ C$, quenched by addition of $NaHCO_3$ solution, filtered through a plug of Florisil and extracted with EtOAc. The organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting residue was purified by chromatography on SiO_2 (10% EtOAc/Hexanes) to yield 198 mg (81%) of **2b** as a colorless oil. The enantiomeric excess was determined using chiral HPLC (Chiralcel OD), using 1% *i*-PrOH/hexanes at a flow rate of 1 ml/min (R_t major = 13.1 min, R_t minor = 20.5 min) with a Dynamax UV-1 absorbance detector: ^1H NMR δ 7.37–7.22 (m, 5H), 5.70 (dt, 1H, J = 6.2, 15.3 Hz), 5.61 (dd, 1H, J = 6.5, 15.4 Hz), 5.13 (d, 1H, J = 6.3 Hz), 2.10–2.00 (m, 2H), 1.83 (bs, 1H), 1.41–1.24 (m, 4H), 0.85 (t, 3H, J = 6.8 Hz).

RESULTS AND DISCUSSION

In an effort to investigate alternate ligand scaffolds, conformationally relatively restricted compounds containing the amino and thiol moieties in a 1,2-relationship were considered. The desired analogues were synthesized starting from (L)-valine and (S)-*t*-leucinol (Scheme 3). (L)-Valine was reduced using Meyer's reduction protocol to afford (L)-valinol **5a** in 77% yield.²⁴ Dialkylation of the amine employing 1,4-dibromobutane and 1,5-dibromopentane in the presence of K_2CO_3 resulted in the formation of **6a** and **6b** in 86 and 76% yield, respectively. Conversion of the hydroxy moiety to the thioacetate utilizing Mitsunobu reaction conditions followed by $LiAlH_4$ reduction afforded the desired ligands **8a** and **8b**.²⁵ Analogously, ligand **8c** was synthesized starting from (S)-*t*-leucinol in the manner described earlier.

The β -amino thiol ligands **8a**, **8b**, and **8c** were screened in the alkenyl zirconium/zinc addition. Initial results showed good yields (80–81%) with moderate enantioselectivities (71–77%) in the presence of ligands **8b** and **8c** at 15 mol % ligand loading (Table 1). Thiol **8a** was rather ineffective (22% *ee*). Not unexpectedly, the corresponding β -amino alcohol ligands also showed a dramatic decrease in enantioselectivity (2–3%). The latter effect can be attributed to the characteristics of sulfur atoms and the presence of at least two metal complexes as previously mentioned.

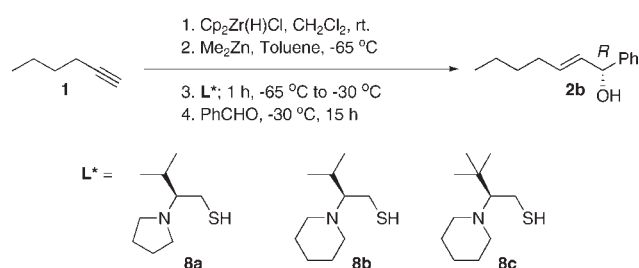
Our preliminary results with these rather simple β -amino thiol ligands prompted a further exploration of this β -amino alcohol scaffold. In the hope of increasing the

Scheme 3. Synthesis of β -amino alcohol and thiol ligands.

enantioselectivity to appreciable levels, steric bulk was introduced at the α -carbon next to the thiol. Ligands with this specific substitution pattern have previously been reported by Yang et al. for the alkenylboron/zinc addition to aldehydes.¹⁴ Yang observed high enantioselectivity when employing β -amino thiol **11**. This ligand was synthesized and examined in the alkenylzirconocene/zinc addition protocol. In our standard reaction, β -amino thiol **11** showed the highest enantioselectivity at 94% *ee* at a ligand loading of 10 mol % (Table 2, entry 6). A high enantioselectivity for the formation of **2b** could be retained even at 2.5 mol % loading. In comparison to the corresponding amino alcohol ligand, the amino thioacetate and amino thiol

ligands both showed higher enantioselectivities (entries 1,2,6).

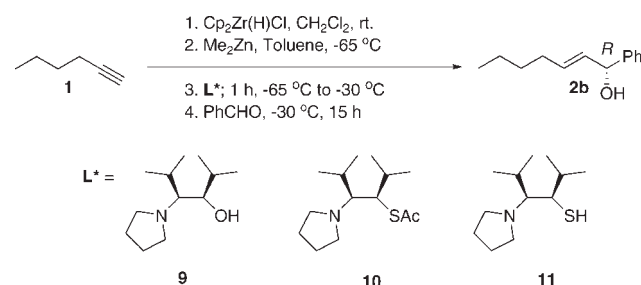
It is important to note that while the alkenylzirconocene/zinc addition protocol offers reagents with a greater reactivity and faster carbonyl addition rate as well as improved tolerance for functional groups on the alkyne precursor,^{15,16,26} the Lewis-acidic zirconocene byproduct of the transmetalation step enhances the background reaction leading to racemic product. Accordingly, the asymmetric ligand accelerated process has to perform at a much more efficient level than what is required for the alkenylboron/zinc addition reaction, where the borane

TABLE 1. Asymmetric addition of **1** to benzaldehyde in the presence of ligands **8a–8c**

Entry	Ligand	Loading (mol %)	Yield (%)	<i>ee</i> ^a (%) 2b
1	8a	10	69	22
2	8b	10	75	64
3	8b	15	82	77
4	8b	20	79	76
5	8c	10	75	61
6	8c	15	81	71

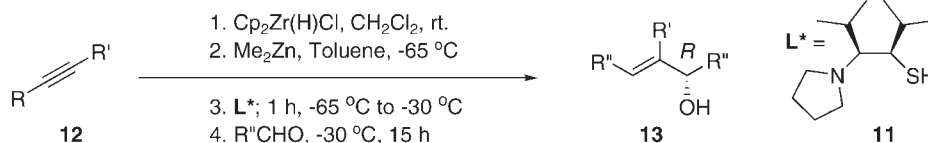
^a*ee*'s were determined by chiral HPLC analysis [Chiralcel OD, flow rate 1 ml/min using *i*-PrOH/hexanes (1:99)].

Chirality DOI 10.1002/chir

TABLE 2. Asymmetric additions of **1** to benzaldehyde in the presence of ligands **9–11**

Entry	Ligand	Loading (mol %)	Yield (%)	<i>ee</i> ^a (%) 2b
1	9	10	81	7
2	10	10	77	84
3	11	1	75	66
4	11	2.5	82	90
5	11	5	79	93
6	11	10	81	94
7	11	15	76	94

^a*ee*'s were determined by chiral HPLC analysis [Chiralcel OD, flow rate 1 ml/min using *i*-PrOH/hexanes (1:99)].

TABLE 3. Asymmetric addition in the presence of ligand **11**

Entry	Alkyne (R, R')	Aldehyde (R'')	Loading (mol %)	Yield (%)	<i>ee</i> ^a (%) 13
1	$^nC_4H_9$, H	Ph CH_2CH_2	5	78	79
2	$^nC_4H_9$, H	(<i>p</i> -OMe) C_6H_4	5	72	36
3	$^nC_4H_9$, H	(<i>p</i> -OMe) C_6H_4	10	65	42
4 ^b	$^nC_4H_9$, H	(<i>p</i> -OMe) C_6H_4	10	60	14
5 ^c	$^nC_4H_9$, H	(<i>p</i> -OMe) C_6H_4	10	62	5
6 ^d	$^nC_4H_9$, H	(<i>p</i> -OMe) C_6H_4	10	15	10
7	$^nC_4H_9$, H	C_6H_{11}	5	65	72 ^e
8	$^nC_4H_9$, H	C_6H_{11}	10	70	73 ^e
9 ^f	$^nC_4H_9$, H	C_6H_{11}	10	23	24 ^e
10	CH_3CH_2 , CH_3CH_2	Ph	10	89	90 ^g
11	CH_3CH_2 , CH_3CH_2	Ph CH_2CH_2	10	65	53 ^g
12	CH_3CH_2 , CH_3CH_2	(<i>p</i> -OMe) C_6H_4	10	63	87 ^g
13	TIPSOC(O) CH_2CH_2 , H	Ph	10	72	71 ^h

^a*ee*'s were determined by chiral HPLC analysis [Chiralcel OD, flow rate 1 ml/min using *i*-PrOH/hexanes (1:99)].

^bNo premixing of ligand.

^cReaction mixture was stirred at $-50\text{ }^\circ C$ for 15 h.

^dReaction mixture was stirred for 1 h.

^e*ee*'s were determined by ^1H NMR analysis of the derivatized product using Mosher's ester.

^fLigand was premixed at $0\text{ }^\circ C$ and stirred at $0\text{ }^\circ C$ for 15 h.

^g*ee*'s were determined by chiral HPLC analysis [Chiralcel AD-H, flow rate 1 ml/min using *i*-PrOH/hexanes (1:99)].

^hThe corresponding allylic alcohol was treated with $LiAlH_4$ followed by *ee* determination of the resulting diol by chiral HPLC analysis [Chiralcel AD-H, flow rate 1 ml/min using *i*-PrOH/hexanes (5:95)].

side product does not appear to compete in the aldehyde addition. For example, 1 mol % of ligand **11** provided the benzaldehyde addition product **2b** in only 66% *ee* in the alkenylzirconocene/zinc addition process, whereas the analogous product was formed in 99% *ee* from the alkenylboron species.¹⁴

The scope of the alkenylzirconocen/zinc addition process in the presence of the β -aminothiol ligand **11** is further illustrated in Table 3. It was observed that amino thiol **11** catalyzed reactions resulted in moderate enantioselectivity for aliphatic aldehydes (73–79% *ee*, entries 1 and 8). Interestingly, this level of enantioselectivity is slightly improved over the corresponding alkenylboron/zinc addition to aliphatic aldehydes with ligand **11**.¹⁴ A decrease in enantioselectivity was found for the electron rich *p*-anisaldehyde (entry 3). Attempts at optimizing this reaction resulted in no appreciable increase in enantioselectivity (entries 4–6). The reaction scope was further investigated using internal alkynes. Promising results were obtained in the reaction of 3-hexyne with benzaldehyde, which resulted in an 89% yield and 90% *ee* for the corresponding allylic alcohol (entry 10). The analogous reaction with *p*-anisaldehyde resulted in 87% *ee*, although at a lower yield of 63% (entry 12). Utilization of a silyl ester functionalized alkyne provided the substituted allylic alcohol in 72% yield with an enantioselectivity of 71% (entry 13).

CONCLUSIONS

Ligand **11** represents a new β -amino thiol scaffold that was found to be effective in the alkenylzirconium/zinc addition process. In comparison to thiol benzylamine **3**, β -amino thiol **11** shows comparable enantioselectivity at a lower ligand loading of 5 mol %. β -Amino thiol **11** can potentially increase the asymmetric induction in substrates where ligand **3** has been shown to be mediocre. Further research in this area will be focused on new synthetic manipulations of this β -amino thiol scaffold as well as the exploration of novel functional group arrays for the alkenylzirconium/zinc addition process.

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Measured and Calculated CD Spectra of G-Quartets Stacked with the Same or Opposite Polarities

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT Circular dichroism (CD) spectroscopy is widely used to characterize the structures of DNA G-quadruplexes. CD bands at 200–300 nm have been empirically related to G-quadruplexes having parallel or antiparallel sugar-phosphate backbones. We propose that a more fundamental interpretation of the origin of the CD bands is in the stacking interactions of neighboring G-quartets, which can have the same or opposing polarities of hydrogen bond acceptors and donors. From an empirical summation of CD spectra of the d(G)₅ G-quadruplex and of the thrombin binding aptamer that have neighboring G-quartets with the same and opposite polarities, respectively, the spectra of aptamers selected by the Ff gene 5 protein (g5p) appear to arise from a combination of the two types of polarities of neighboring G-quartets. The aptamer CD spectra resemble the spectrum of d(G₃T₄G₃), in which two adjacent quartets have the same and two have opposite polarities. Quantum-chemical spectral calculations were performed using a matrix method, based on guanine chromophores oriented as in d(G₃T₄G₃). The calculations show that the two types of G-quartet stacks have CD spectra with features resembling experimental spectra of the corresponding types of G-quadruplexes. *Chirality* 20:431–440, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: circular dichroism; matrix method calculations; DNA aptamers; Ff gene 5 protein; G-quadruplexes

INTRODUCTION

Guanine-rich nucleic acid motifs that can form G-quadruplexes in vitro are found in naturally occurring sequences such as telomeres,^{1,2} IgG switch regions,³ fragile X syndrome nucleotide repeats,⁴ and the control region of the *c-myc* oncogene.⁵ Recent reviews have shown the diversity of G-quadruplex structures and the ability of G-quartets to self-assemble into complexes with enantiomeric selectivity.^{6,7} The importance of G-quadruplex structures in biological systems is supported by the number of proteins that interact with G-quadruplexes in vitro. These proteins fall into four classes: (a) Molecular chaperones. At least four proteins have been shown to promote G-quadruplex formation and thus act as molecular chaperones. They are the telomere binding proteins in *Oxytricha* (β subunit)⁸ and in yeast (RAP1),⁹ yeast DNA-binding protein Hop1,¹⁰ and human DNA topoisomerase I.¹¹ (b) Nucleases. The eukaryotic topoisomerase II causes a sequence-specific cleavage on the 5' side of parallel G-quadruplexes,¹² while other proteins show a G-quadruplex-dependent nuclease activity.^{12–14} (c) Helicases. The yeast Sgs1 protein,¹⁵ human Werner syndrome helicase (WRN)¹⁶ and Bloom's syndrome helicase (BLM)¹⁷ are three closely related helicases that unwind G-quadruplexes more efficiently than they unwind double helices.

In addition, a resolvase from human placental tissue unwinds G-quadruplexes but not DNA duplexes.¹⁸ (d) G-quadruplex binding proteins. Some proteins have been shown to have high affinity for binding to G-quadruplexes but may not have enzymatic activities.^{19–21} The formation of G-quadruplex structures may play a positive role in some cellular functions, but could hinder other functions.²² Possible examples of the latter include the possibility that formation of quadruplex DNA could result in errors during recombination, unless a helicase is present

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to unwind the structure,^{17,22} and disease-associated CGG triplet expansion, which could result from DNA replication inhibition by the G-quadruplex structure.²³ The thrombin-binding DNA aptamer d(GGTTGGTGTGGTTGG) was originally identified as the result of a selection for DNA sequences that bind to thrombin and inhibit its enzymatic activity in the chain of reactions that lead to blood clotting.²⁴

Ff Gene 5 Protein Binds G-Quadruplexes

We and others have shown that the gene 5 protein (g5p) of the Ff (F-pilus-specific filamentous) bacterial viruses, which binds to and saturates the nascent viral single-stranded genomic DNA to form a precursor for virion assembly,^{25,26} also binds with high affinity to G-quadruplexes of RNA or DNA.^{27,28} The g5p has been shown to repress translation of the Ff gene 2 mRNA by binding to a 16-mer leader sequence (GU₅G₄CU₄C) in the 5' untranslated region of the mRNA.²⁹ Kneale and colleagues showed that the DNA analogue of this leader sequence forms a four-stranded G-quadruplex structure through association of the central G₄ blocks from each strand, and that g5p preferentially binds to the structured form.^{27,30} We used Systematic Evolution of Ligands by EXponential enrichment (SELEX)³¹ to identify a 58-mer ssDNA sequence (named I-3) to which g5p binds preferentially under physiologically relevant conditions of 200 mM NaCl, pH 7.4, and 37°C.²⁸ The central 26 nucleotides of I-3 (named I-3c26) consist of the sequence d(GGGGTCAGGCTGGGGTTGTGCAGGTC) which has a CD spectrum in 200 mM NaCl that is considered to be diagnostic of a G-quadruplex structure. Other experiments showed that the quadruplex is an intramolecular structure and that g5p preferentially binds to the G-quadruplex structure.^{32,33} CD and chemical modification experiments led to a proposed structure for I-3c26 that involves three G-quartets, two of which are noncanonical, containing either one adenine or one thymine base. Another 26-nucleotide sequence (named G-8c26) that was SELEX-selected by g5p binding has a CD spectrum quite similar to that of I-3c26; it seems likely that G-8c26 forms a G-quadruplex with three canonical G-quartets having all guanine bases.^{28,32} The structure proposed for these intramolecular G-quadruplexes, which have antiparallel strands, has two stacked G-quartets that have the same polarity, while a third G-quartet in the stack has a polarity opposite to that of the first two quartets, as in the structure of d(G₃T₄G₃).^{34–36}

CD Spectroscopy of Quadruplexes

CD spectroscopy has been widely used to diagnose the existence of G-quadruplexes and to study the effects of different cations and solution conditions.^{7,37–40} However, the usefulness of CD spectra in analyzing G-quadruplex structures has been limited by the fact that interpretations have relied on empirical relationships with spectra of known or inferred G-quadruplex structures.

The empirical relationship between structure and CD spectra is illustrated by the following examples. The structure of a parallel quadruplex (with all strands having the same 5' to 3' orientation) consisting of four strands of

d(TG₄T) is a right-handed helix with a four-fold symmetry and with all guanines having an *anti* conformation of the glycosyl bonds.⁴¹ The CD spectra of similar G-quadruplexes have a positive band at ~260–265 nm and a small negative band at ~240–245 nm, typified by the spectrum of a d(G)₅ quadruplex.^{30,42,43} In contrast, the structure of an antiparallel quadruplex formed by two folded-back strands of d(G₄T₄G₄) is also a right-handed helix but the guanines have alternating *syn* and *anti* glycosyl conformations along each strand.^{44,45} The CD spectrum of this sequence exhibits a positive band at ~295 nm, a negative band at 260–265 nm, and a small positive band at ~245 nm.^{46,47} Interestingly, these two types of CD spectra seem to be inverted with respect to each other above 230 nm, except for the 295-nm band. CD spectra of other sequences expected to form G-quadruplexes between parallel or antiparallel strands mostly follow similar patterns.^{7,44–48} The dogma has been that these two types of CD spectra are dependent on whether the G-quartets form between DNA strands that are all parallel or between DNA strands that are a mixture of parallel and antiparallel strands. However, as pointed out by Burge et al.⁷ in a review of telomeric sequences: “As more complex quadruplex-forming sequences are examined, the reliability of assigning topology based on the comparison of a spectrum with the CD signature of known parallel or antiparallel telomeric quadruplexes cannot always be assumed . . .” A better understanding of the relationship of the CD signatures of G-quadruplexes to their structural attributes would be invaluable in assessing how G-quadruplex structures in solution vary with DNA sequence and solution conditions.

Wen and Gray³² hypothesized that the existence of positive bands at both 255–260 and 290–295 nm in the CD spectra of G-quadruplexes such as for the inferred quadruplex structures of I-3c26 and G-8c26 (see below) might be related to the stacking orientation of G-quartets. They specifically proposed that a band near 260 nm arises from the stacking of quartets of the same polarity while a band at longer wavelength arises from the stacking of quartets with alternating polarities. That is, when the glycosyl bonds of the guanines alternate in *syn* and *anti* conformations along each strand for quadruplexes having antiparallel strands, the G-quartet polarity also alternates (i.e., the direction of proton donors and acceptors around adjacent quartets alternates),^{35,44,45} while quadruplexes with parallel strands and all *anti* glycosyl bonds have nonalternating G-quartet polarity.⁴¹ Since CD is very sensitive to base-stacking geometry, quadruplexes with alternating vs. nonalternating G-quartet polarity should have different CD spectra. The polarity of stacked quartets, not the relative orientations of the backbone strands, may be the determining factor for G-quadruplex CD spectra. Some quadruplexes with antiparallel strands, such as d(G₃T₄G₃)₂^{34–36} and the proposed structures for I-3c26 and G-8c26, have mixed polarities of stacked G-quartets because the glycosyl bonds do not always alternate along each strand. These quadruplexes would have CD spectra that are composites of contributions of the two types of G-quartet stacks. Connecting loops, normally present only in the folded-back antiparallel structures, may also affect the CD spec-

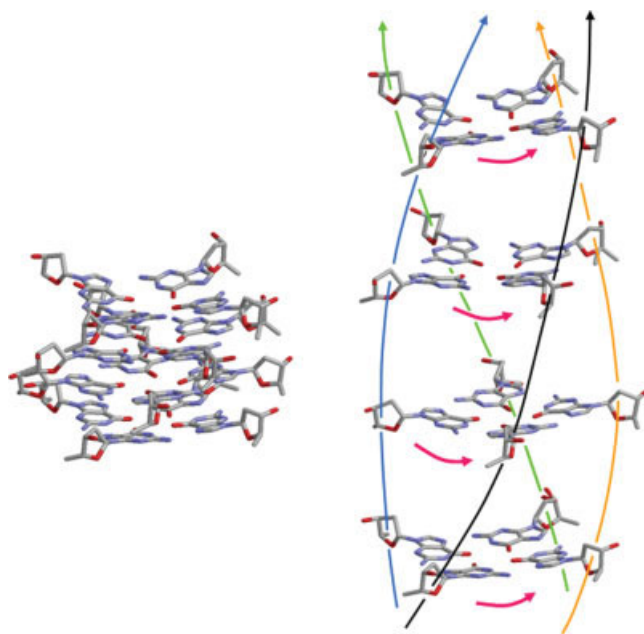


Fig. 1. Arrangement of stacked guanines between four paired strands of d(TG₄T) from Protein Data Bank (PDB) crystallographic structure 352d.⁴¹ The left panel shows the guanosine nucleosides, stacked as in the PDB structure. In the right panel, the G-quartets are shown separated for easier viewing. The glycosyl bonds are all *anti*, and the four sugar-phosphate backbones that connect neighboring guanines are all parallel in their 5'-to-3' orientations. The guanine quartets all have the same polarity (orientation of hydrogen bond donors relative to acceptors), as indicated by the small curved arrows. Figures 1–3 were generated using the RasMol modeling program, Windows version 2.7.3.1. The structures were rotated 30° around the crystallographic x-axis, to more clearly show the G-quartets, in Figures 1 and 2.

trum,^{7,36,49} although the effects of substituting different bases in connecting loops in telomere-like sequences do not seem to obscure the presence of a ~295 nm peak in the CD spectra in quadruplexes having antiparallel strands.^{7,38,40}

In the present article, we show by comparing experimental spectra and by quantum-chemical calculations using a matrix method⁵⁰ that the relative polarities of stacked G-quartets in G-quadruplexes are indeed related to the two canonical types of G-quadruplex CD bands and that the CD spectra of I-3c26 and G-8c26 are consistent with our proposed structure for these aptamers.

MATERIALS AND METHODS

DNA Sequences

The d(G)₅ sequence, the G-8c26 sequence 5'-d(TAGGG CAGGGGTCGTCGGGTAGGGC)-3', the thrombin binding aptamer (TBA) sequence 5'-d(GGTTGGTGTGGTTGG)-3', and 5'-d(G₃T₄G₃)-3' were obtained from Midland Certified Reagent Company (Midland, TX), all purified by gel filtration. The I-3c26 sequence 5'-d(GGGGTCAGGCTGGG GTTGTGCAGGTC)-3' was purchased from Oligos Etc. (Wilsonville, OR) in HPLC purified form. The I-3c26 and G-8c26 sequences were named for the clones from which they were derived following SELEX selection as aptamers for the Ff gene 5 protein.²⁸

CD and UV Measurements

CD spectra were measured using a Jasco Model J715 spectropolarimeter (Jasco, Inc., Easton, MD) as in previous work.⁵¹ CD spectra were measured with the d(G)₅ at 20°C in 20 mM Na⁺ (phosphate), pH 7.0, the TBA at 20°C in 30 mM KCl, 10 mM Tris-HCl, pH 7.7, the d(G₃T₄G₃) at 20°C at either 0 or 100 mM KCl in 10 mM Tris-HCl, pH 7.5, and the I-3c26 and G-8c26 sequences at 37°C in 200 mM NaCl, 10 mM Tris-HCl, pH 7.4. Extinction coefficients used were all per mole of nucleotide as follows: $\epsilon_{256 \text{ nm}} = 9900$ for d(G₃T₄G₃)³⁴; $\epsilon_{260 \text{ nm}} = 9530 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ for d(G)₅ in the quadruplex form,⁴² and 9860, 9800, and 10,098 $\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ for TBA, I-3c26, and G-8c26, respectively, in their single-stranded forms. The latter were obtained by nearest-neighbor calculations as in Gray et al.⁵² Experimental CD spectra are plotted in units of molar CD, $\epsilon_L - \epsilon_R$ ($\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$), per mole of nucleotide, except as noted for Figure 9B.

CALCULATION OF SPECTRA

Structure and Orientation of Adjacent G-Quartets

For reader orientation, we show in Figure 1 the crystal structure for d(TG₄T)₄, a G-quadruplex in which the four supporting phosphodiester backbone strands all run in the

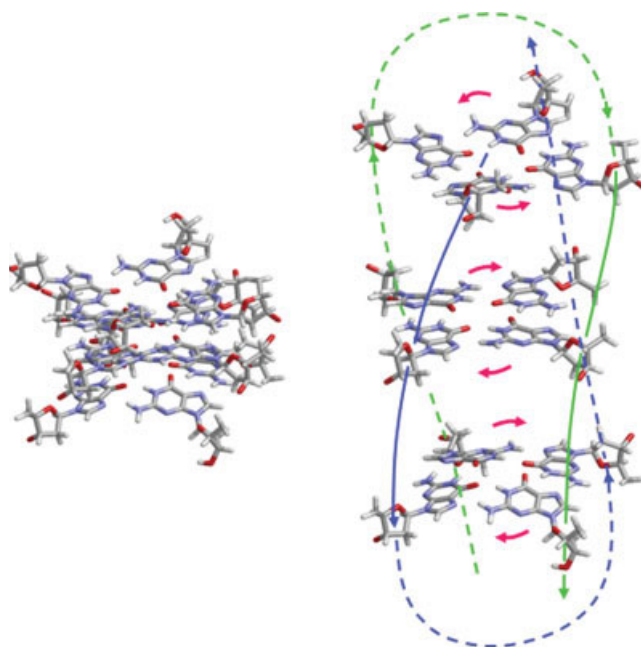


Fig. 2. Arrangement of stacked guanines between two-folded, paired strands of d(G₃T₄G₃) from PDB structure 1FQP.³⁶ One of five NMR structures is shown. The left panel shows the guanosine nucleosides, stacked as in the PDB structure. In the right panel, the G-quartets are shown separated for easier viewing. The glycosyl bonds are *syn*, *anti*, *anti* (top to bottom, i.e. 5' to 3') in the two strands nearer the viewer and they are *syn*, *syn*, *anti* (bottom to top, i.e. 5' to 3') in the two strands further from the viewer (dashed lines). The nearer strands are parallel to each other but are antiparallel with respect to the more distant strands. In this structure the guanine quartets have the same hydrogen bond polarities in the bottom two G-quartets but have the opposite hydrogen bond polarities in the top two G-quartets, as indicated by the small curved arrows.

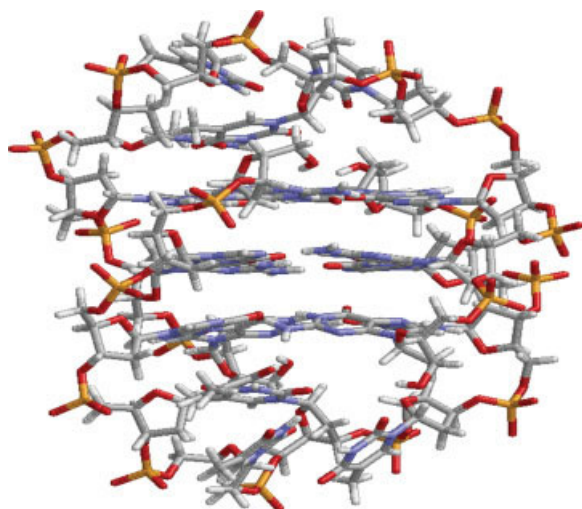


Fig. 3. The dimeric $d(G_3T_4G_3)_2$ structure (PDB 1FQP, showing all atoms.³⁶ Thymine is in the loops at the ends and the three G-quartets are in the center of the structure.

same 5' to 3' direction (parallel strands) and in which all of the glycosyl bonds are *anti*. It can be seen from the expanded view in the right panel that the orientation of hydrogen bonds is the same in each of the stacked G-quartets, so that in every instance neighboring G-quartets have hydrogen bonds of the same donor-to-acceptor polarity. We propose that the CD spectral characteristics of such a classical parallel-stranded G-quadruplex are a consequence of the stacking of G-quartets in such a way that adjacent quartets have the same directional orientation of hydrogen-bond polarities. We define this as stacking of adjacent G-quartets with the same polarity. This stacking orientation results from the parallel orientation of the backbone strands.

The oligomer $d(G_3T_4G_3)$ has been shown by NMR studies^{34–36} to form a quadruplex in the presence of Na^+ ions consisting of three stacked quartets between two folded (antiparallel) oligomer strands. Within a given quartet, the guanines bonded to antiparallel segments of the same strand differ in that one has a *syn* and the other has an *anti* glycosyl bond. The G-quartets of one of the five NMR structures are shown in Figure 2. The expanded view in the right panel of Figure 2 shows that hydrogen bonds in two of the adjacent G-quartets (the lower two quartets) have the same polarity, while hydrogen bonds in the other two adjacent G-quartets (the upper two quartets) have opposite polarities with respect to the directional orientation of proton donors and acceptors around the quartets. We define this as stacking of adjacent G-quartets with opposite polarities. This stacking arrangement is due to the fact that the conformations of glycosyl bonds along a given strand are not all the same, being either *syn*, *syn*, *anti* or *syn*, *anti*, *anti* (in the 5' to 3' direction; see Ref. 35). The CD spectrum of this oligomer has characteristics that are consistent with the existence of G-quartet stacks having both the same and opposite orientations of hydrogen bond polarities, as is discussed below.

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The complete dimeric structure of $d(G_3T_4G_3)_2$ with the T_4 loops is presented in Figure 3.

Figure 4A gives a view parallel to the planes of the three guanine quartets isolated from the $d(G_3T_4G_3)$ structure (PDB structure 1FQP³⁶) for the purpose of theoretical calculations, Figure 4B shows a view perpendicular to the plane of a single quartet, and Figures 4C and 4D show the neighboring quartets that have the same (C) or opposite (D) polarity. CD spectra were calculated for each of the isolated structures shown in Figures 4A, 4C, and 4D. For calculations of CD spectra, only the guanine molecules were considered as chromophore groups. The influence of the sugar-phosphate backbone was neglected, since its transitions are at shorter wavelengths than the wavelengths of the measured CD spectra. Moreover, the influence of the nucleic acid bases other than the guanine quartets on the CD spectrum was also neglected, since their distances from the quartets are relatively large in comparison with the distances between the neighboring guanine molecules within the quartets.

Computational Methods

To understand at least qualitatively the differences between the CD spectra of the three different G-quadruplexes (Figs. 4A, 4C, and 4D) we performed calculations with the quantum-chemical matrix method of Bayley et al.⁵⁰ using the program MATMAC⁵³ where the rotational strengths have been calculated with the origin-independent

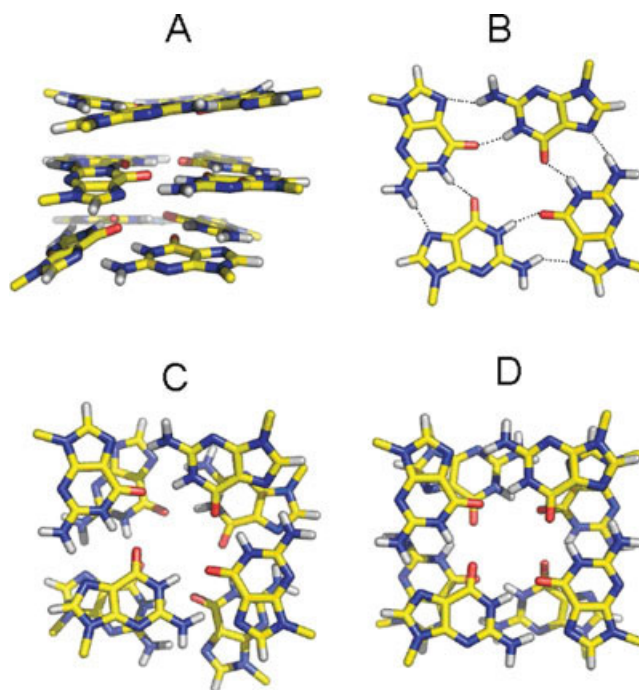


Fig. 4. (A) The three guanine quartets isolated from the structure of Figure 3. These are the chromophore groups used for theoretical calculations. (B) View perpendicular to the plane of a single guanine quartet. (C) Axial view of two neighboring guanine quartets that have the same polarity. (D) Axial view of two neighboring guanine quartets that have opposite polarities.

TABLE 1. Experimental and calculated excitation energies (ΔE , eV) and wavelengths (λ , nm), oscillator strengths (f), absolute values of the electric transition moments ($|\mu|$, Debye) and transition moment directions (Θ , degrees) for the two energetically lowest electronic transitions guanine ($\pi \rightarrow \pi^*$)

		Exp. ^a	TDDFT ^b	INDO/S
1.	ΔE	4.4–4.5	5.0	4.0
	λ	279	248	311
	f	0.16	0.13	0.35
	$ \mu $	3.08	2.63	4.82
	Θ	–4	–58	49.6
2.	ΔE	4.9–5.0	5.3	4.6
	λ	250	234	271
	f	0.25	0.25	0.39
	$ \mu $	3.65	3.52	4.77
	Θ	–75	–103	–42

For the definition of Θ see Figure 5.

^aFrom Ref. 55.

^bTDDFT/B3LYP/TZVP.

velocity formalism.⁵⁴ As mentioned above, the only chromophoric group (in the sense of Tinoco's CD theory for macromolecules) in these calculations is the guanine moiety. The structure of the G-quartets was extracted from the solution NMR structure of the Na⁺ form of the dimeric guanine quadruplex [d(G₃T₄G₃)] determined by Keniry et al.³⁶ Five types of calculations were performed:

1. In the first calculation (Method 1), we employed the dipole–dipole approximation and used experimental transition energies and electric transition moments⁵⁵ (see Table 1 and Figure 5) of the two energetically lowest $\pi \rightarrow \pi^*$ transitions of an isolated guanine molecule. Higher transitions were neglected since they lie outside the spectral range of interest in this article. Moreover, the underlying experimental data, such as transition moment directions, may be uncertain. The center of the positive charge of the heavy atoms is chosen as the origin of the dipole. The geometry of the guanine molecule was optimized under constraint of C_s symmetry at the B3LYP/TZVP^{56–59} level of density functional theory as implemented in the GAUSSIAN03 suite of quantum-chemical routines.⁶⁰ Since no experimental values for the magnetic transition moment were available, they were omitted in these calculations. Moreover, the transition moment between the excited states and also the three permanent electric moments (the ground state and the two excited states) were neglected.
2. To test for the influence of magnetic transition moments, we performed a second series of calculations where all experimental data used so far were replaced by the results from TDDFT⁶¹ calculations with the same functional and basis set as in the geometry optimization (see Table 1). The calculations were performed omitting (Method 2a) and including (Method 2b) the two magnetic transition moments.
3. Finally, we evaluated the influence of the inclusion of the hitherto neglected transition charge density

between the two excited states and also the other permanent charges. For that purpose we performed MAT-MAC calculations using the monopole approximation. Calculations were performed without (Method 3a) and with (Method 3b) transition monopoles between the two excited states. The permanent charges were retained in both calculations. The monopoles of the guanine molecule were obtained by the method of Kramer as described in the paper of Kurapkat et al.⁶² employing an INDO/S^{63,64} CI wave function including only $\pi \rightarrow \pi^*$ transitions. The DZDO/MCD3SP program package⁶⁵ was used for the INDO/S calculations.

The calculated CD curves were obtained as a sum of Gaussians, centered at the wavelengths of the corresponding transitions, multiplied by the rotational strength which was divided by the number of guanine molecules in the corresponding structure. The Gaussians were generated using the empirical formula⁶⁶ $\Gamma = k \cdot \lambda^{1.5}$ for the half bandwidth Γ at $\Delta\varepsilon/e$. The parameter k was set to 0.00375 (Dr. RW Woody, Colorado State University, personal communication) which yields half bandwidths from 14.8 to 69.5 between 250 and 700 nm.

RESULTS AND DISCUSSION

CD Spectra of G-Quartets Oriented with the Same or Opposite Polarities

The oligomer d(G)₅ was first characterized in the Gray laboratory^{42,67} as being able to form a structure with the CD spectral signature that is now documented to be that of a parallel-stranded G-quadruplex, in which adjacent stacked G-quartets have the same polarity.^{7,38,41,46,68,69} Formation of the d(G)₅ quadruplex occurs upon slow freezing of the oligomer at –20°C.^{42,67} The solid line in Figure 6A is the spectrum of thawed d(G)₅ in 20 mM Na⁺ (phosphate) at 20°C. Spectra of the oligomer at 80°C

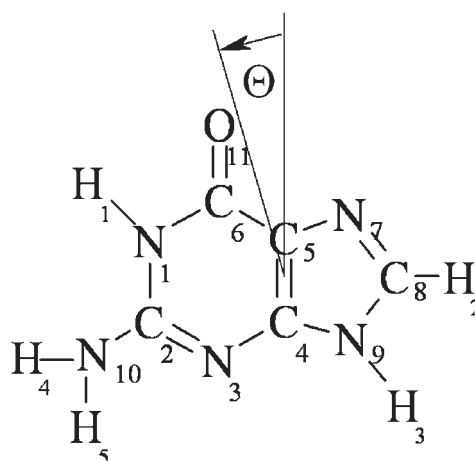


Fig. 5. Geometry of the guanine base. Θ defines the angle of transition moment directions (Table 1). The angle Θ is measured in the plane of the molecule relative to the C₄–C₅ bond.

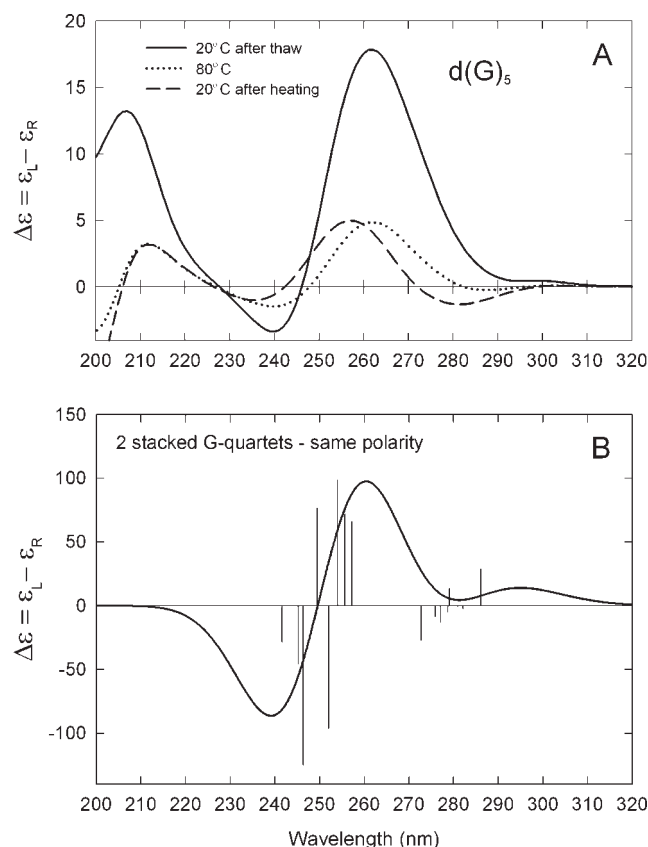


Fig. 6. (A) Experimental CD spectrum of d(G)₅ after freezing to form the quadruplexes⁴² and then warming to 20°C (solid line); measured at 80°C after heating the quadruplexes (dotted line); and measured at 20°C after heating and cooling (dashed line). Spectra were measured in 20 mM Na⁺ (phosphate), pH 7.0. $\epsilon_L - \epsilon_R$ is given per mole of nucleotide; each quadruplex is assumed to contain four d(G)₅ strands and five G-quartets. (B) Theoretical CD spectrum calculated using the matrix method (Method 1) for two G-quartets of the same polarity (Fig. 4C) isolated from the structure of d(G₃T₄G₃) (see Fig. 3). The vertical bars (same dimensions as $\Delta\epsilon$) in Figures 6B, 7B, 8, and 10B are proportional to the rotational strengths of the corresponding transitions and have lengths ($\Delta\epsilon_{\max}$) calculated from the rotational strength (R in DBM) and the transition wavelength (λ_0 in nm) using the formula $\Delta\epsilon_{\max} = 607.57 \cdot \lambda_0^{-1/2} \cdot R$.

(dotted line), and at 20°C after heating (dashed line), demonstrate the loss of the structured form upon heating. Figure 6B shows the calculated CD spectrum for the pair of same-polarity adjacent G-quartets within d(G₃T₄G₃), from a calculation using structural parameters from the structure of d(G₃T₄G₃) shown in Figure 4C. The spectrum calculated by Method 1 shows a positive CD band at 260 nm and a negative band at 239 nm. Unlike the experimental spectrum (Fig. 6A) where the intensity of the positive band is much higher than that of the negative one, the calculated positive and negative bands have approximately the same intensity. In spite of this discrepancy, we assign the bands calculated at 260 and 239 nm to those observed at about 260 and 240 nm. In addition, a very weak positive CD band observed around 300 nm is computationally reproduced at 294 nm. Thus, the general shape of the spectrum calculated by Method 1 closely resembles that of the experimental one.

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The thrombin-binding aptamer d(GGTGGGTGGTGGTGG) has been shown by NMR and crystallographic studies^{70–74} to be a G-quadruplex containing two G quartets whose residues alternate *anti-syn-anti-syn* around each quartet and whose adjacent stacked G-quartets have opposite polarities. The CD spectrum of the thrombin aptamer, measured at 20°C in 30 mM KCl, is typical of the CD spectra of quadruplexes whose adjacent G-quartets have opposite polarities (Fig. 7A). Figure 7B shows the CD spectrum calculated for the pair of opposite-polarity adjacent G-quartets isolated from the structure of d(G₃T₄G₃) shown in Figure 4D. Again the general shape of the calculated CD curve corresponds to that of the experimental one. Thus, like the experimental spectrum the calculated spectrum has positive Cotton effects at 291 and 237 nm and a negative one at 264 nm. However, it must be noted that the calculated intensity at 237 nm is much too low. The reason for this appears to be the neglect of higher states which come into play above about 230 nm (Fig. 7A).

Figure 8 compares the calculated CD spectra for the two G-quadruplexes isolated from d(G₃T₄G₃) in which adjacent G-quartets have the same (solid line) or opposite (dashed line) polarities. As in the experimental spectra,

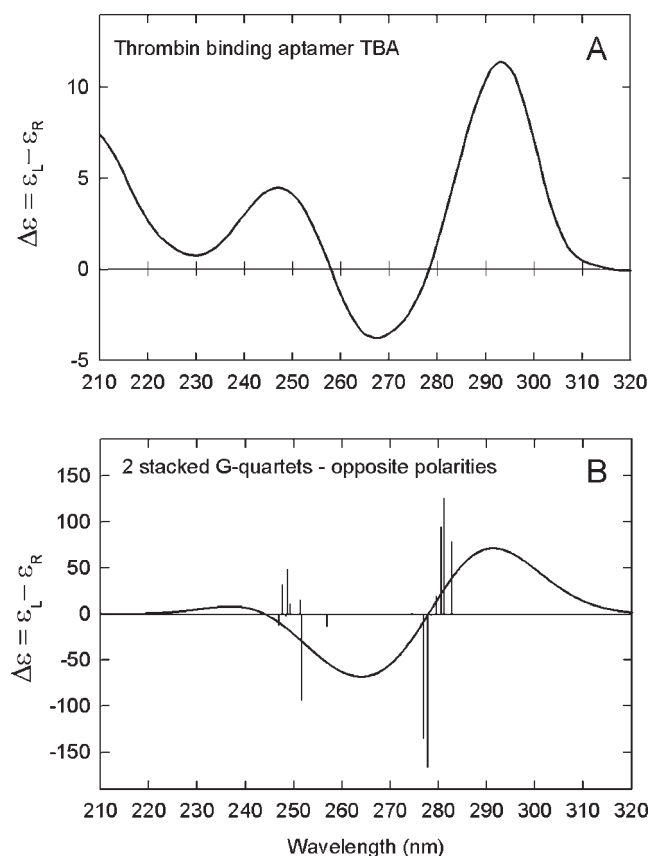


Fig. 7. (A) Experimental CD spectrum of the thrombin binding aptamer (TBA) at 20°C in 30 mM KCl, 10 mM Tris-HCl, pH 7.7. $\epsilon_L - \epsilon_R$ is given per mole of nucleotide; TBA forms a quadruplex containing a single folded strand and two stacked G-quartets.^{70–74} (B) Theoretical CD spectrum calculated using the matrix method (Method 1) for two G-quartets of the opposite polarity (Fig. 4D) isolated from the structure of d(G₃T₄G₃) (see Fig. 3).

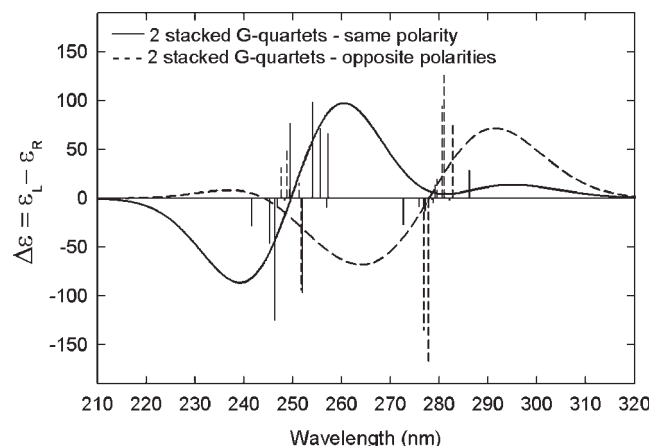


Fig. 8. Superimposed calculated CD spectra of two G-quartets stacked with the same (solid line) or opposite (dashed line) polarities.

the Cotton effects at wavelengths below ~ 280 nm have opposite signs.

CD Spectra of Quadruplexes with G-Quartet Neighbors of Mixed Relative Polarities

Experimental CD spectra are shown in Figure 9A for two G-quadruplex aptamers, I-3c26 and G-8c26, selected by preferential binding to the Ff gene 5 protein. We previously presented biochemical data to support an intramolecular quadruplex model for I-3c26 in 200 mM NaCl, and we infer that the G-8c26 aptamer forms a similar intramolecular quadruplex.³² These spectra are given per mole of nucleotide. In Figure 9B are shown spectra of $d(G)_5$ (quadruplex in which G-quartets have the same polarity; long dashes), and of the thrombin binding aptamer TBA (quadruplex in which neighboring stacked G-quartets have opposite polarities; short dashes). These spectra are also given per mole of nucleotide, except that the TBA spectrum has been scaled up by a factor of 15/8, reflecting the fact that only eight of the 15 nucleotides are guanines in stacked G-quartets. This adjustment in magnitude was made to approximate the spectral contribution per stacked guanine base, assuming that the G-quartets dominate the spectrum. The spectrum shown as a solid line is the average of the $d(G)_5$ and scaled TBA spectra. The averaged spectrum indicates the relative magnitudes that the positive bands might be expected to have in a quadruplex with neighboring G-quartets of mixed relative polarity. The averaged spectrum closely resembles the shape of the spectra of the g5p-binding aptamers shown in Figure 9A, supporting our proposal³² that these aptamers are quadruplexes in which one pair of neighboring G-quartets (in a stack of three quartets) have the same polarity while G-quartets in the other neighboring pair have opposite polarities. Moreover, the averaged spectrum (solid line) has positive bands that are similar to those in the spectrum of I-3c26 scaled by a factor of 26/10 and shown in Figure 7B as a dotted line, making the rough approximation that the 10 stacked guanines in G-quartets dominate the spectrum. The experimental CD spectrum of $d(G_3T_4G_3)$, which has stacked G-quartets of the same and opposite polar-

ities,³⁴ is plotted in Figure 10A. This spectrum is also similar to the spectra of I-3c26 and G-8c26 in having two positive bands above 240 nm, although in the case of $d(G_3T_4G_3)$ the longer-wavelength band at ~ 290 nm has about 2.5 times the magnitude of the band at 250–260 nm in the presence of 100 mM K^+ or 125 mM Na^+ .³⁴

Figure 10B shows the CD spectrum calculated by Method 1 for the three isolated stacked quartets of the $d(G_3T_4G_3)$ structure containing two adjacent G-quartets of the same polarity and two adjacent G-quartets of opposite polarities as shown in Figure 4A. The general features of the experimental CD spectrum for $d(G_3T_4G_3)$ in Figure 10A are all predicted well, including two positive bands of which the one at 290 nm is larger in magnitude, a small

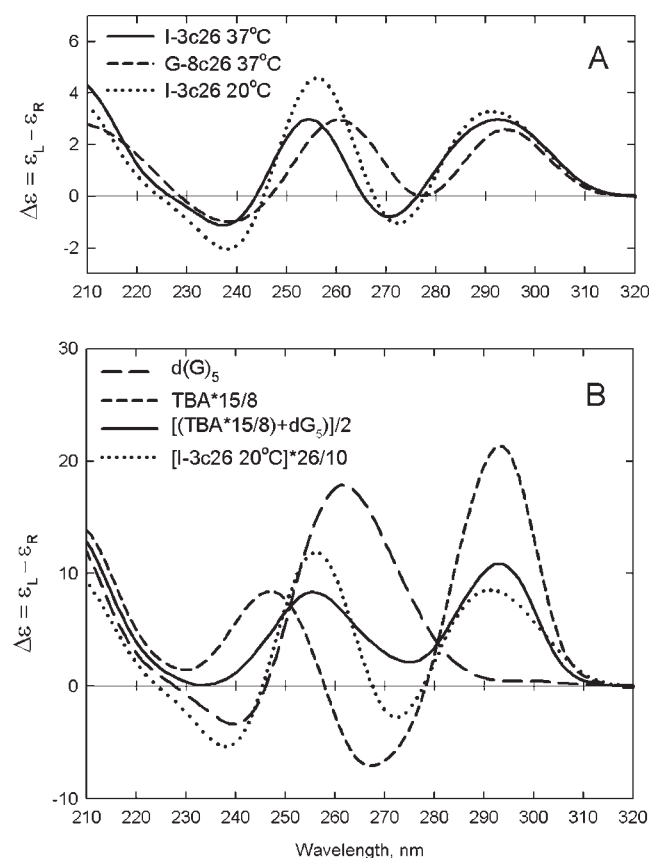


Fig. 9. (A) CD spectra of I-3c26 (solid line) and G-8c26 (dashed line) at 37°C, and I-3c26 at 20°C (dotted line), in 200 mM NaCl, 10 mM Tris-HCl, pH 7.4. $\epsilon_L - \epsilon_R$ is given per mole of nucleotide for both sequences. Based on biochemical data, these oligomers are proposed to form intrastand quadruplexes with three G-quartets of which two neighboring quartets have the same hydrogen bond donor to acceptor polarities and the other two neighboring quartets have opposite hydrogen bond donor to acceptor polarities.³² (B) CD spectra of $d(G)_5$ at 20°C in 20 mM Na^+ (phosphate), pH 7.0 (long dashes), and the thrombin binding aptamer (TBA) at 20°C in 30 mM KCl, 10 mM Tris-HCl, pH 7.7 (short dashes). $\epsilon_L - \epsilon_R$ is plotted per mole of nucleotide, except that the TBA spectrum is scaled up by a factor of 15/8 to reflect the fact that only 8 of the 15 nucleotides are guanines in stacked G-quartets (see text). The solid line is the sum of the spectra of $d(G)_5$ and the TBA spectrum $\times 15/8$, divided by 2, to give an average, estimated CD spectrum for quadruplexes having stacked G quartets of both the same [$d(G)_5$] and alternating [TBA] polarities. The dotted line shows the I-3c26 spectrum at 20°C, scaled up by 26/10 as if only the 10 guanines in three putative G-quartets contributed to the spectrum.

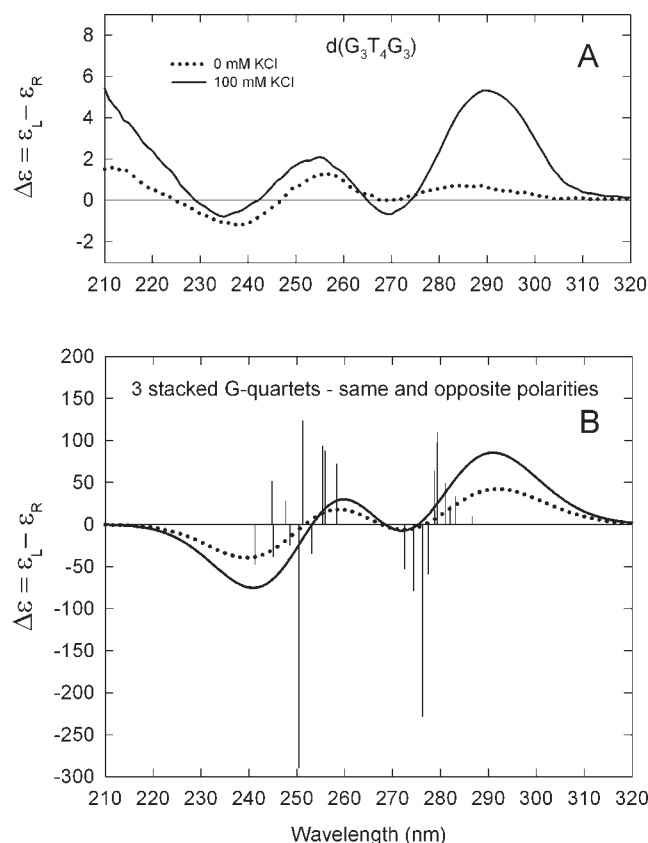


Fig. 10. (A) Experimental CD spectrum of $d(G_3T_4G_3)$ at 20°C in 0 mM KCl or 100 mM KCl, 10 mM Tris-HCl, pH 7.5. $\epsilon_L - \epsilon_R$ is given per mole of nucleotide; $d(G_3T_4G_3)$ forms a quadruplex containing neighboring G-quartets of which two have the same and two have opposite hydrogen bond donor to acceptor polarities^{34–36} (Figs. 2 and 3). (B) Theoretical CD spectrum (solid line) calculated using the matrix method (Method 1) for the three G-quartets (Fig. 4A) isolated from the structure of $d(G_3T_4G_3)$ (see Fig. 3). The dotted line shows the average of the calculated spectra shown in Fig. 8, that is, the average of spectra calculated for a stack of two G-quartets in $d(G_3T_4G_3)$ having the same polarity and for a stack of two G-quartets having opposite polarities.

negative band at 272 nm, and a negative Cotton effect below 250 nm. The main difference between the calculated and measured spectral shapes for $d(G_3T_4G_3)$ is in the positions of the short wavelength bands; for example, the positive band is calculated to be at about 260 nm whereas it appears closer to 255 nm in the measured spectrum. In contrast to the experimental spectra in Figure 9A, the intensity of the longer-wavelength positive calculated CD band is substantially greater than that of the shorter-wavelength positive band. The calculated CD spectrum shows a negative Cotton effect at about 240 nm as in the spectra of I-3c26 and G-8c26, although the calculated negative band at 272 nm appears only in the spectrum of I-3c26. The CD bands of I-3c26 are increased in magnitude as the temperature is decreased from 37 to 20°C (Fig. 9A). Given the effects of temperature and possible CD contributions of bases that are not paired in the 26-mer aptamers, the overall agreement between the spectrum calculated for three stacked quartets in $d(G_3T_4G_3)$ and the measured spectra of $d(G_3T_4G_3)$ and the g5p aptamers is quite good. One

possible inference from the relatively larger 290 nm band in the measured and calculated spectra of $d(G_3T_4G_3)$ as opposed to the spectra of the g5p aptamers is that the G-quartets with opposing polarity are less stacked or more flexible in the latter oligomers.

The average of the spectra calculated for a pair of stacked quartets of same or opposite polarity (calculated spectra from Fig. 8; their average is shown as a dotted line in Fig. 10B) has the same spectral shape as the calculated spectrum for a stack of three quartets of alternating polarities, further supporting the hypothesis that CD spectra of G-quadruplexes have shapes dominated by the stacking polarities of nearest-neighboring G-quartets.

Calculations using method 2 and method 3. The most satisfactory calculations were those performed using Method 1. Our calculations employing transition energies and transition moments from TDDFT calculations (Method 2a,b) gave spectra which, in comparison with the experimental CD curves, are strongly shifted to the blue (18–36 nm). The signs of at least the first two observed Cotton effects are correctly reproduced. Inclusion of magnetic moments does not improve the computational results significantly, and the spectra obtained without (Method 2a) and with (Method 2b) magnetic moments are very similar and almost visually indistinguishable.

Also, the spectra calculated using the monopole method (Method 3a,b) reproduce the general shape of the experimental CD curves. However, while spectra calculated using Method 3a for the G-quartets with opposite polarity and for the structure containing adjacent G-quartets of opposite and same polarity are shifted to the blue, the spectrum obtained for the quartets with same polarity is shifted to the red. Including the transition density between the first and the second excited state (Method 3b) slightly reduces these shifts by up to 8 nm. Moreover, inclusion of this density also affects the relative intensity of the CD bands and this effect is the strongest for the G-quartets with same polarity. The spectra obtained with Methods 2 and 3 are not included in this paper but, along with structural parameters for guanine, are part of the supplementary material (Figs. S1–S12 and Table S1).

CONCLUSIONS

In summary, first, CD spectra generated using the matrix method in the dipole–dipole approximation with experimental transition energies and transition moments reproduce the general features of the experimental spectra of quadruplexes in which neighboring G-quartets are of the same or opposite polarity. Our rather qualitative calculations do not reproduce the measured intensities very well. In most cases the measured values are about 1/5 of the calculated values; the reason for this is still unclear. Our calculations, on the whole, do reproduce the spectral shapes of experimental CD spectra in each case. While no substantial red or blue shift is observed, the relative intensities of the CD bands are not well reproduced. The spectrum calculated for the set of three stacked G-quartets in $d(G_3T_4G_3)$ exhibits two positive peaks at ~260 and ~290

nm resembling those seen at ~255 and ~290 nm in the experimental CD spectrum of d(G₃T₄G₃) (Fig. 10A). In the case of d(G₃T₄G₃) we calculate a negative band at 272 nm. This band is experimentally found for I-3c26 but not for G-8c26, while the positions of the calculated positive bands are more closely matched by those of G-8c26. However, the experimental spectra include contributions from a majority of bases that are not involved in G-quartets.

Second, the matrix method calculations show that the polarity of stacked G-quartets can indeed affect CD spectra, with a substantial long wavelength band appearing in the spectra of G-quartets stacked with opposite polarities. This band also appears in spectra of stacked G-quartets having the same polarity, but at a much lower intensity (see Figs. 6A and 6B). An additional calculation where the guanine molecule that is not coplanar with the other three molecules was adjusted to co-planarity showed no significant changes in comparison to the results obtained for the original structure as shown in Figure 4A.

Finally, the results of both our matrix method calculations and our empirical summation of experimental CD spectra indicate that the presence of two positive CD bands above 240 nm in the spectra of the Ff gene 5 protein aptamers is consistent with the presence of G-quartets stacked in such a way that the hydrogen bond donor to acceptor polarities are in the same direction in one neighboring pair of quartets but are in the opposite direction in another neighboring pair of quartets.

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An Improved Synthesis of 10,11-Didehydro *Cinchona* Alkaloids

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT A revised procedure for the conversion of the four major *Cinchona* alkaloids (quinine, quinidine, cinchonidine, and cinchonine) into their respective 10,11-didehydro derivatives is described. The reported protocol offers several advantages over a recently published synthetic route. These include (i) enhanced robustness (ii) ready scalability (iii) reduced operational complexity and number of steps (iv) chromatography-free work-up. In addition, toxic solvents were replaced by environmentally less problematic alternatives. *Chirality* 20:441–445, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: 10,11-Didehydro *Cinchona* alkaloids; *Cinchona* alkaloids; quinine; elimination; alkynes

INTRODUCTION

Cinchona alkaloids and their derivatives occupy a privileged position as asymmetric organocatalysts,^{1–3} chiral recognition materials,^{4–6} and resolving agents.⁷ For many applications, the stereodiscriminating catalytic and recognition properties of parent *Cinchona* alkaloids can be enhanced by dedicated chemical modifications.^{1–3} In this context, strategies allowing noninvasive immobilization of functionalized *Cinchona* derivatives onto supporting carriers have received special attention.^{1–3,8–11} Because of its remote position from the active 1,2-aminoalcohol domain the 10,11-vinyl group of *Cinchona* alkaloids appears to be ideally suited for immobilization purposes. However, few mild procedures for the activation of this functionality are known (for most frequently used radical thiol addition to the *Cinchona* vinyl group see ref. 1–3 and 11).^{12–17} Its transformation into the corresponding 10,11-alkyne derivatives (to yield didehydro *Cinchona* alkaloids **4a–d**, Scheme 1) appears to be especially attractive, opening the vast synthetic opportunities associated with terminal acetylenes,^{18,19} including the recently discovered Cu(I)-triggered alkyne/azide-type *click chemistry*.²⁰

Didehydro *Cinchona* alkaloids were described by Christensen as early as in 1904,²¹ but have not received much attention for almost a century. In 2000, Hoffmann reinvestigated the synthesis of didehydro *Cinchona* alkaloids, and reported their use for various metal-catalyzed cross coupling reactions, such as Sonogashira-, Heck-, and homocoupling protocols.^{22,23} In recent years, these derivatives are receiving increasing attention from the asymmetric synthesis community. Thus, 10,11-Didehydrocinchonidine (**4c**) was utilized by Soós and coworkers to create fluorine-tagged *Cinchona* reagents.²⁴ Quaternary salts derived from **4c** were evaluated by Cahard and coworkers for enantioselective imine alkylation,²⁵ and by Castle's group for enantioselective aldol reaction²⁶ (Scheme 1). Our group has established an efficient strategy for the prepara-

tion of chiral stationary phases by using the immobilization of 10,11-didehydro *Cinchona* alkaloids and their derivatives to azido-modified silica gels employing 1,3-dipolar Huisgen cycloaddition (*click chemistry*).²⁷ An overview of the employed 10,11-didehydro *Cinchona* derivatives is summarized in Scheme 1.

In course of these studies, we were challenged to prepare multigram quantities of 10,11-didehydro *Cinchona* alkaloids. To access these compounds, we attempted to reproduce the three-step procedure recently published by Hoffmann's group.²² This strategy involved the bromination of the *Cinchona* vinyl group to form a diastereoisomeric mixture of dibromides **2a–d**, followed by a triethylamine-mediated elimination to the corresponding bromovinyl compounds **3a–d**. Consecutive treatment of the chromatographically purified **3a–d** with KOH in presence of a phase transfer catalyst was claimed to give, after an additional chromatographic purification step, the corresponding didehydro alkaloids **4a–d** in overall yields up to 74%. In our hands and in those of others [difficulties of reproducing Hoffmann's synthesis of *Cinchona* alkynes were also encountered by Castle's and Soós's groups (personal communication)], however, the reported procedure gave disappointingly low yields (typically less than 40%). Moreover, following strictly Hoffmann's procedure, we obtained products being heavily contaminated (up to 40%) with the rather difficult-to-separate vinyl bromides **3a–d**. We also found this protocol being short of important experimental details, a feature further complicating our efforts of reproduction.

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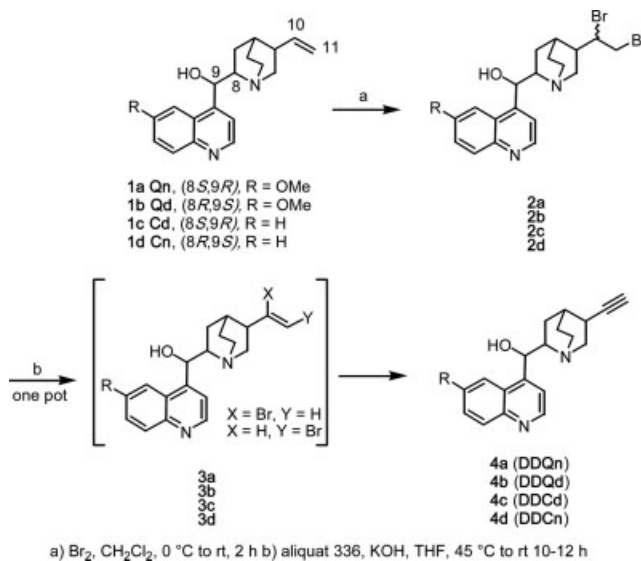
6.37 g (66%). Optionally, the crude material could be purified by flash chromatography (silica gel, AcOEt followed by AcOEt-MeOH 8:1 v/v as eluent), furnishing **4a** in a somewhat improved yield (6.66 g, 69%).

10,11-Didehydroquinidine 4b, **10,11-Didehydrocinchonidine 4c** and **10,11-Didehydrocinchonine 4d**: Crude didehydro *Cinchona* alkaloids **4b-d** were dissolved in the minimum volume of AcOEt by heating. The resultant solutions were allowed to crystallize at ambient temperature and then for further 12 h at 4 °C. The formed crystals were isolated by filtration, washed with pre-cooled AcOEt-petroleum ether (25 ml, 1:20) and dried on air. Yields: 5.70 g (59%) **4b**; 4.85 g (59%) **4a** and 4.38 g (50%) **4d**. In the case of crude **4b**, employing flash chromatography (silica gel, AcOEt followed by AcOEt:MeOH 8:1 v/v) as an alternative purification methodology gave a slightly improved yield (6.27 g, 65%).

10,11-Didehydroquinidine 4a 5.78 g, 60%; off-white solid; mp. 186–190 °C; $[\alpha]_D^{20}$ –218.3 (c 1.0, MeOH); IR (KBr): 3305, 2936, 2832, 2107, 1622, 1591, 1508, 1470, 1452, 1430, 1372, 1323, 1241, 1138, 1106, 1030, 920, 883, 856 cm^{–1}; ¹H NMR (CDCl₃): δ = 8.65 (d, J = 4.5 Hz, 1H), 7.99 (d, J = 9.3 Hz, 1H), 7.49 (d, J = 4.6 Hz, 1H), 7.34 (dd, J = 9.1, 2.8 Hz, 1H), 7.26 (d, J = 2.8 Hz, 1H), 5.53 (d, J = 4.8 Hz, 1H), 3.91 (s, 3H), 3.43–3.36 (m, 2H), 3.19 (dd, J = 13.4, 10.1 Hz, 1H), 2.90 (dt, J = 13.4, 6.3, 3.5 Hz, 1H), 2.65 (m, 1H), 2.52 (m, 1H), 2.04 (m, 1H), 1.96 (d, J = 2.5 Hz, 1H), 1.92 (m, 1H), 1.73–1.67 (m, 2H), 1.44 (m, 1H); ¹³C-NMR (CDCl₃) δ = 158.14, 147.98, 147.71, 144.71, 131.97, 127.13, 121.98, 118.88, 101.76, 88.10, 72.43, 69.11, 59.87, 58.31, 56.08, 43.07, 28.00, 27.49, 26.48, 22.82; Anal. Calc. for C₂₀H₂₂N₂O₂ (322.17) C, 74.51; H, 6.88; N, 8.69; Found C, 73.95; H, 6.84; N, 8.28.

10,11-Didehydroquinidine 4b 59%; white crystals; mp. 196–198 °C; $[\alpha]_D^{20}$ +182.3 (c 1.0, MeOH); IR (KBr): 3214, 3071, 2999, 2878, 2833, 1621, 1591, 1567, 1512, 1461, 1450, 1420, 1385, 1337, 1323, 1264, 1187, 1174, 1153, 1111, 1093, 1076, 1040, 996 cm^{–1}; ¹H NMR (CDCl₃) δ = 8.74 (d, J = 4.6 Hz, 1H), 8.02 (d, J = 9.1 Hz, 1H), 7.53 (d, J = 4.5 Hz, 1H), 7.37 (dd, J = 2.8, 9.1 Hz, 1H), 7.31 (d, J = 2.5 Hz, 1H), 5.64 (d, J = 5.6 Hz, 1H), 3.92 (s, 3H), 3.40 (dd, J = 6.1, 12.6 Hz, 1H), 3.22 (q, J = 9.1, 14.7 Hz, 1H), 3.09 (dd, J = 9.6, 12.6 Hz, 1H), 2.89 (m, 1H), 2.74 (m, 1H), 2.52 (m, 1H), 2.30 (m, 1H), 2.16 (d, J = 2.3 Hz, 1H), 2.04 (m, 1H), 1.60–1.41 (m, 2H); ¹³C-NMR (CDCl₃) δ = 158.14, 148.12, 147.38, 144.93, 132.21, 127.20, 121.95, 118.95, 101.73, 72.65, 69.62, 60.37, 56.04, 50.85, 49.98, 28.44, 28.34, 25.54, 23.11; Anal. Calc. for C₂₀H₂₂N₂O₂ (322.17) C, 74.51; H, 6.88; N, 8.69; Found C, 73.88; H, 6.96; N, 8.35.

10,11-Didehydrocinchonidine 4c 4.85 g, 59%; white crystals; mp. 188–195 °C; $[\alpha]_D^{20}$ –172.6 (c 1.0, MeOH); IR (KBr): 3176, 3076, 3032, 2941, 2924, 2879, 1592, 1570, 1508, 1461, 1421, 1381, 1351, 1236, 1172, 1159, 1130, 1112, 1099, 1020, 998, 950, 883, 821, 800, 770, 754 cm^{–1}; ¹H NMR (CDCl₃) δ = 8.91 (d, J = 4.5 Hz, 1H), 8.15 (dd, J = 0.8, 8.6 Hz, 1H), 8.09 (d, J = 8.6 Hz, 1H), 7.74 (m, 1H), 7.59 (m, 2H), 5.66 (d, J = 4.8 Hz, 1H), 3.42 (m, 2H), 3.21 (dd, J = 10.1, 9.9 Hz, 1H), 2.93 (m, 1H), 2.66 (m, 1H), 2.54 (m, 1H), 2.04 (m, 1H), 1.97 (d, J = 2.5 Hz, 1H), 1.95–1.88 (m, 1H), 1.78–1.62 (m, 3H), 1.46 (m, 1H); ¹³C NMR



Scheme 2

(CDCl₃) δ = 150.65, 149.00, 147.3, 130.86, 129.49, 127.12, 123.46, 118.55, 87.53, 72.44, 69.10, 60.25, 58.33, 43.09, 28.04, 27.55, 26.52, 22.93; Anal. Calc. for C₁₉H₂₀N₂O (292.16) C, 78.05; H, 6.89; N, 9.58; Found C, 77.55; H, 6.90; N 9.42.

10,11-Didehydrocinchonine 4d 50%; white crystals; mp. 205–209 °C; $[\alpha]_D^{20}$ +166.0 (c 1.0, MeOH); IR (KBr): 3295, 3067, 2938, 2880, 2110, 1591, 1568, 1507, 1455, 1340, 1322, 1206, 1170, 1158, 1128, 995, 949, 888, 829, 801, 785 cm^{–1}; ¹H NMR (CDCl₃) δ = 8.92 (d, J = 4.6 Hz, 1H), 8.16 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 8.6 Hz, 1H), 7.75 (dt, J = 1.3, 6.8 Hz, 1H), 7.60 (m, 2H), 5.71 (d, J = 5.8 Hz, 1H), 3.39 (dd, J = 2.5, 7.1 Hz, 1H), 3.22 (m, 1H), 3.10 (dd, J = 1.5, 10.4 Hz, 1H), 2.90 (m, 2H), 2.74 (m, 1H), 2.54 (m, 1H), 2.29 (m, 1H), 2.18 (d, J = 2.5 Hz, 1H), 2.04 (m, 1H), 1.75 (m, 1H), 1.51 (m, 1H); ¹³C NMR (CDCl₃) δ = 150.2, 149.9, 148.2, 130.1, 129.6, 127.2, 126.1, 123.4, 119.0, 87.3, 71.7, 69.6, 60.3, 50.5, 49.7, 28.4, 28.3, 25.3, 22.0.

RESULTS AND DISCUSSION

Synthetic Route is Shown in Scheme 2

In order to reduce the complexity of the original procedure, we envisioned an alternative one-pot elimination scheme, obviating the need of isolation of the unstable bromovinyl intermediates **3a-d**. Considerable efforts to establish a double dehydrobromination procedure were also described by Hoffmann's group, who screened different types of bases in a range of solvents and at various temperatures.²² However, the yields of the chromatographically isolated didehydro *Cinchona* alkaloids did generally not exceed 50%, with increasing temperature and extended reaction times further compromising product recovery because of decomposition. From these observations, we concluded that successful double dehydrobromination demands a set of carefully matched reaction condi-

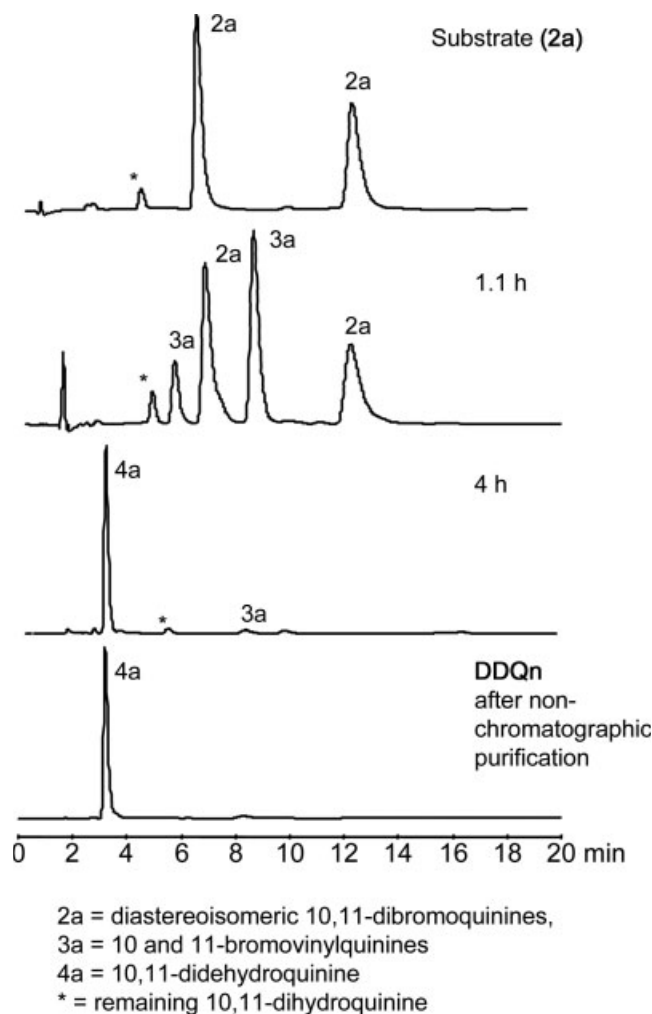


Fig. 1. HPLC monitoring of synthesis of **3a** (DDQn), for chromatographic condition see experimental part.

tions, including relatively mild bases and low temperatures.

To guide our efforts to identify optimal reactions conditions we developed a dedicated RP-HPLC assay, allowing the convenient monitoring of all starting materials, intermediates, as well as the final products. With this analytical tool in hand, we first explored the most appealing all-in-one-pot option for the formation of **4a**, namely the bromination of **1a** in THF and the subsequent *in situ* KOH-mediated double dehydrobromination. However, several HPLC-detectable byproducts were formed along with a diastereomeric mixture of dibromides **2a**, despite of the fact the bromination was carried out at 0°C with stoichiometric amount of bromine. Moreover, at ambient temperature the mixture of bromination products was found to be rather resistant towards elimination both with triethylamine and/or KOH/aliquat 366. Elevated temperature (>40°C) in combination with a large excess of KOH (8 equiv) was required to promote elimination, which proceeded sluggishly to give after 24 h **4a** in a disappointingly low yield (<40%) along with additional byproducts.

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Next we decided to investigate the one-pot double dehydrobromination^{28–30} utilizing preformed dibromide **2a**. Considering the toxicity of carbon tetrachloride and of chloroform employed in the original procedure, these solvents were replaced by the environmentally and toxicologically less problematic dichloromethane. We were pleased to find that in this solvent the reported excess of bromine (3 equiv) can be reduced (2 equiv) without affecting the level of conversion. The resultant dibromides **2a–d** was obtained in high purity and essentially quantitative yield.

Treatment of a solution of dried **2a** in THF (0.13 M) with excess of pre-dried, finely powdered KOH (12 equiv) in presence of a transfer catalyst (Aliquat 336, 10 mol %) at 45°C resulted in a clean and relatively fast alkyne formation. Under these conditions, more than 90% of **2a** was converted to **4a** in 4 h. Chromatograms representing the progress of the elimination reaction are depicted in Figure 1. Complete conversion was observed after 12 h stirring at room temperature. This elimination protocol proved robust and scalable, producing for multiple 30 mmol-scale runs **4a** in yields of 65–69% (established by RP-HPLC). We were pleased to find that this protocol, without any additional modifications, was also applicable to the synthesis of the quinidine, cinchonidine, and cinchonine congeners, providing the corresponding didehydro *Cinchona* alkaloids **4b**, **4c**, and **4d** in 65, 64, and 50% yields, respectively.

The isolation of the didehydro *Cinchona* alkaloids could be achieved by removal of the formed salts, concentration of the filtrates, and filtration through a short pad of silica, which eliminated polar colored impurities. For further purification, a recrystallization from ethyl acetate proved to be efficient for **4b**, **4c**, and **4d**, providing the corresponding alkynes in 50–59% yield. Crystallization of **4a** was found to be difficult because of a pronounced tendency to organogel formation in many solvents (AcOEt, dioxane, and toluene). However, stirring the pre-purified material with a mixture of AcOEt-petrolether (1:4) eliminated essentially all impurities, leaving behind crystalline **4a** in 60% yield. The so obtained products **4a–c** showed a chemical purity >95% as established by RP-HPLC. The overall yields can further be raised by subjecting the remaining mother liquors to chromatographic purification on silica gel.

CONCLUSION

In summary, the represented two-step procedure allows straightforward access to multigram amounts of the didehydro *Cinchona* alkaloids in satisfactory yields. As compared with the reported strategy,²² the described procedure offers the advantages of robustness, scalability, operational simplicity, use of as-received solvents, and chromatography-free work-up. We are confident that with the now ready access of didehydro *Cinchona* alkaloids, these versatile scaffolds will see frequent future use in the fields of asymmetric synthesis and chiral recognition.

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Observation and Characterization of a Specific Vibrational Circular Dichroism Band in Phenyl Glycosides

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT Application of vibrational circular dichroism (VCD) spectroscopy to structural analysis of carbohydrates has recently progressed. However, few studies on glycoconjugates VCD have thus far been reported, despite the fact that naturally occurring carbohydrates exist as various glycoconjugates. To further explore the application of the VCD technique, we have measured a series of aromatic glycosides and found that axial aromatic glycosides exhibited a negative band at around 1230 cm^{-1} while equatorial ones showed flat features in this region. This is the first structure–spectra relationship on glycoconjugate VCD that distinguishes the stereochemistry of the sugar anomers. Several model compounds were prepared and their vibrational properties calculated by using the density functional theory (DFT) method, which assigned the vibrational mode of this band based on the stretching motion of the glycosidic oxygen and aromatic carbon. This concept that aglycan parts can reflect stereochemical information of sugar moieties may encourage further VCD studies on glycoconjugates to realize practical structural analysis of carbohydrates. *Chirality* 20:446–453, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: vibrational circular dichroism; density functional theory; carbohydrate; glycoconjugate; aromatic glycoside

INTRODUCTION

Vibrational circular dichroism (VCD) spectroscopy, characterized by circular dichroic measurement in the infrared region, was first observed in the 1970s^{1–3} and is becoming one of the most convenient and reliable chiral analysis tools.^{4–6} Because of the advantages of high theoretical predictive reliability and the accessibility of a broad range of vibrational transitions, VCD has been applied to the determination of absolute configuration and conformation of chiral molecules. In particular, its application to the study of polypeptides has been extensive, unveiling that their secondary structure interrelates with VCD patterns in the amide regions.⁷ These empirical structure–spectra relationships have been theoretically supported by density functional theory (DFT) calculations.^{7–9} VCD studies on nucleic acids have been developed in a similar manner.^{10,11} On the other hand, although carbohydrate VCD was first studied a few decades ago,^{12–14} characterization of carbohydrate structure–VCD spectra relationships and theoretical calculation of carbohydrate spectra only have been achieved recently.^{15,16} Furthermore, naturally occurring carbohydrates have frequently been found as various forms of glycoconjugates, including glycopeptides, glycoproteins, glycolipids, and glycosylated natural products, but there have been no VCD studies on glycoconjugates except for a few reports on glycopeptides and glycoproteins.^{16–18}

Carbohydrates can be regarded as chiral carbon ensembles, but their structure has been analyzed primarily based

on achiral techniques such as NMR and MS. With the aim to establish a novel method for the analysis of carbohydrate structures, we have applied VCD spectroscopy to a variety of carbohydrates. Currently, a few structure–spectra relationships on carbohydrate VCD are being established through comprehensive analysis of monosaccharides and disaccharides.^{15,16,19–21} Moreover, we have recently extended VCD spectroscopy to glycopeptides and have found that vibration involving the sugar anomeric carbon affords information on the sugar structure while amide stretching of the polypeptide informs about the polypeptide secondary structures.¹⁶ This result demonstrated the effectiveness of VCD as a structural analysis tool for molecules ranging from simple saccharides to complicated glycoconjugates. For future practical use of VCD spectroscopy, further investigations on glycoconjugates, not only glycoproteins but also glycolipids and glycosylated natural products, are required.

Among the various glycosylated natural products, aromatic glycosides such as antibiotics (e.g. the vancomycin

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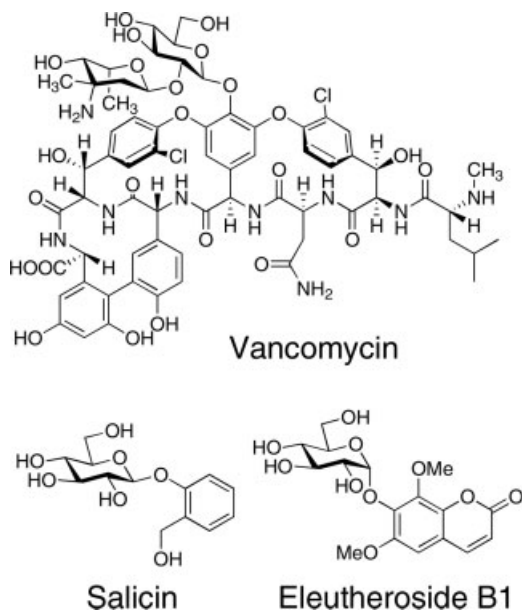


Fig. 1. Examples of naturally occurring glycosylated aromatic compounds.

family) and glycosylated phenols of plant origin (e.g. Salicin and eleutheroside B1, Fig. 1) are one of the most extensively studied groups because of their attractive pharmacological properties.^{22,23} These are currently a broad range of research targets for a new synthetic methodology^{24,25} and physicochemical properties,^{26–28} in addition to dietary and pharmaceutical study. To explore the applicability of the VCD technique to aromatic glycoconjugate analysis, we have, for the first time, measured VCD spectra of a series of aromatic glycosides. Herein, we describe a new structure–spectra relationship that distinguishes the anomeric configuration of aromatic glycosides. DFT calculations have also been conducted using model compounds to analyze the vibrational mode of this band. The complementary approach from systematic measurements and theoretical calculations revealed that vibrational motion on the aglycan part, not on the sugar part, gives rise to this VCD signal that is informative for the sugar structure. This insight will likely promote further VCD application to glycoconjugates, which have been less studied compared to other biomolecules like polypeptides and nucleic acids.

MATERIALS AND METHODS

Instrumentation

¹H NMR (600 MHz) and ¹³C NMR (150 MHz) spectra were recorded on a Bruker Avance spectrometer with TMS as an internal standard. EI-MS were measured on a JEOL JMS-FABmate at the Center of Instrumental Analysis, Hokkaido University. Optical rotations were obtained on a Perkin-Elmer 343 polarimeter using a 1 cm optical cell at ambient temperature. Phenyl glycosides **1–4** and *p*-nitrophenyl glycosides **5–11** were purchased and measured without further purification (Tokyo Chemical Industry for **1**, **2**, and **5**; Sigma for **3**, **4**, **6–11**). VCD and IR

spectra were measured on a Bomem/BioTools Chiralir spectrometer with or without the second photoelastic modulator. All spectra were recorded for 2 or 3 h at a resolution of 8 cm^{−1} at ambient temperature. DMSO-*d*₆ was selected as a solvent for the measurements of **1–11** for its solubility and weak absorption in the region of interest. For **12–14**, CDCl₃ was used for reliable comparison between the observed and calculated spectra. DMSO-*d*₆ and CDCl₃ were purchased from Cambridge Isotope Laboratories. The VCD and IR spectra were corrected with a solvent spectrum obtained under the same experimental conditions. Other conditions such as concentrations and type of cell are described in each figure caption.

Computational Calculation

Theoretical VCD/IR calculations for **12–14** started from MMFF94 systematic conformational searches using the Spartan02 package,²⁹ followed by further optimization at the B3LYP/6-311G(d,p) level of theory without considering solvent effect using the Gaussian03 package.³⁰ Conformer(s) within 2 kcal/mol from the most stable one have been taken into account for VCD/IR calculations at the same level of theory. VCD and IR spectra were simulated with Lorentzian bandshapes of 6 or 8 cm^{−1} half-width. Boltzmann populations were calculated using ΔG values at 298 K. Final spectra were obtained based on Boltzmann population-average of each spectrum with their wavenumbers scaled by 0.98.

Synthesis

Compounds **12–14** were prepared using a similar procedure as previously described.¹⁶ A catalytic amount of triphenylphosphine hydrobromide (60 mg, 0.17 mmol) was added to an anhydrous dichloromethane (7 mL) solution of 3,4-di-*O*-acetyl-D-xylal (**15**, 766 mg, 3.83 mmol) and phenol (1.25 g, 13.3 mmol), giving phenyl 3,4-di-*O*-acetyl-2-deoxy- α -D-threo-pentopyranoside (**16**, 8 mg) and phenyl 3,4-di-*O*-acetyl-2-deoxy- β -D-threo-pentopyranoside (**17**, 108 mg). Deacetylation of **16** (6.8 mg) and **17** (92 mg) using a catalytic sodium methoxide gave **12** (4.6 mg) and **13** (59 mg), respectively. To a stirred solution of **13** (31 mg) and tetrabutylammonium bromide (14 mg) in 1,2-dichloroethane was added 20% NaOH aqueous solution, and the mixture was then stirred at 84°C with refluxing for 3 days, yielding **14** (20 mg). These conditions were not optimized.

Phenyl 3,4-di-*O*-acetyl-2-deoxy- α -D-threo-pentopyranoside (16**).** ¹H NMR (CDCl₃) δ 7.29 (2H, dd, J = 7.5, 8.2 Hz, *m*-H), 7.06 (2H, d, J = 8.2 Hz, *o*-H), 7.02 (1H, t, J = 7.5 Hz, *p*-H), 5.58 (1H, broad d, J = 2.6 Hz, H-1), 5.47 (1H, ddd, J = 5.1, 9.0, 10.0 Hz, H-3), 4.97 (1H, ddd, J = 5.2, 8.9, 9.1 Hz, H-4), 3.84 (1H, dd, J = 5.2, 11.3 Hz, H-5eq), 3.74 (1H, dd, J = 9.7, 11.0 Hz, H-5ax), 2.43 (1H, ddd, J = 2.5, 5.0, 13.2 Hz, H-2eq), 2.08 (3H, s, —OAc), 2.05 (3H, s, —OAc), 1.94 (1H, ddd, J = 3.2, 10.4, 13.2 Hz, H-2ax); ¹³C NMR (CDCl₃) δ 170.1 (—COCH₃), 170.0 (—COCH₃), 156.2 (*i*), 129.4 (*m*), 122.2 (*p*), 116.3 (*o*), 95.2 (C-1), 69.3 (C-4), 68.2 (C-3), 60.2 (C-5), 34.5 (C-2), 20.9 (—COCH₃), 20.7 (—COCH₃); [α]_D +98.6° (CHCl₃, *c* 1.0).

Phenyl 3,4-di-*O*-acetyl-2-deoxy- β -D-threo-pentopyranoside (17**).** ¹H NMR (CDCl₃) δ 7.29 (2H, dd, J = 7.3,

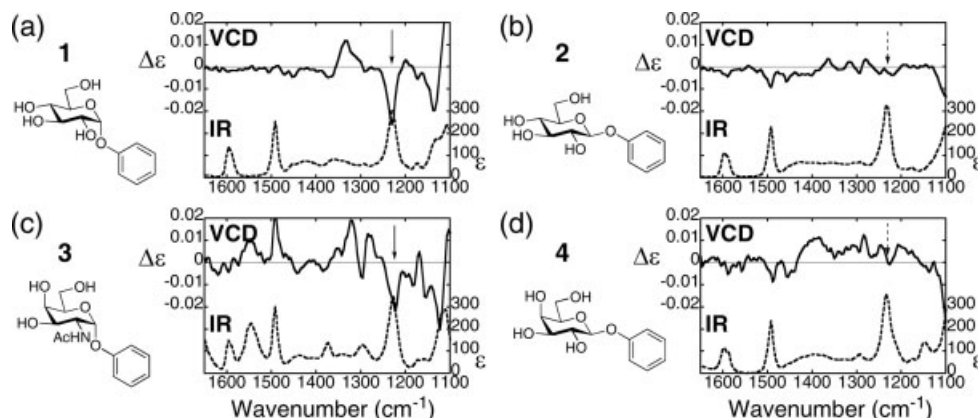


Fig. 2. VCD (solid lines) and IR (dotted lines) spectra of phenyl glycosides in DMSO- d_6 ($c = 0.16$ M for **1** and **2**, $c = 0.08$ M for **3** and **4**, $l = 72$ μ m, BaF₂ cell). (a) phenyl α -D-glucopyranoside, (b) phenyl β -D-glucopyranoside, (c) phenyl 2-acetamide-2-deoxy- α -D-galactopyranoside, and (d) phenyl β -D-galactopyranoside. The solid arrows indicate the characteristic VCD feature, while the dotted arrows indicate the absence of this band in the corresponding region.

8.7 Hz, m -H), 7.03–7.00 (3H, m, o -H x 2, p -H), 5.44 (1H, dd, $J = 4.0, 4.1$ Hz, H-1), 5.02 (1H, ddd, $J = 4.7, 4.7, 4.7$ Hz, H-3), 4.88 (1H, ddd, $J = 3.3, 5.0, 5.0$ Hz, H-4), 4.24 (1H, dd, $J = 3.0, 12.6$ Hz, H-5a), 3.58 (1H, dd, $J = 4.7, 12.6$ Hz, H-5b), 2.37 (1H, ddd, $J = 4.0, 4.0, 14.3$ Hz, H-2a), 2.13 (4H, m, H-2, $-\text{OAc}$), 2.11 (3H, s, $-\text{OAc}$); ^{13}C NMR (CDCl₃) δ 170.1 ($-\text{COCH}_3$), 170.0 ($-\text{COCH}_3$), 156.8 (i), 129.5 (m), 122.3 (p), 116.5 (o), 95.3 (C-1), 68.3 (C-4), 67.2

(C-3), 60.2 (C-5), 31.5 (C-2), 21.1 ($-\text{COCH}_3$), 21.0 ($-\text{COCH}_3$); HRMS (EI) m/z for C₁₅H₁₈O₆ (M^+), calcd 294.1103, found 294.1108; $[\alpha]_D -140^\circ$ (CHCl₃, c 1.0).

Phenyl 2-deoxy- α -D-threo-pentopyranoside (12). ^1H NMR (CDCl₃) δ 7.29 (2H, dd, $J = 7.5, 8.4$ Hz, m -H), 7.04–7.00 (3H, m, o -H x 2, p -H), 5.62 (1H, broad s, H-1, $W_{1/2} = 7.5$ Hz), 4.14 (1H, ddd, $J = 5.0, 8.4, 11.2$ Hz, H-3), 3.76

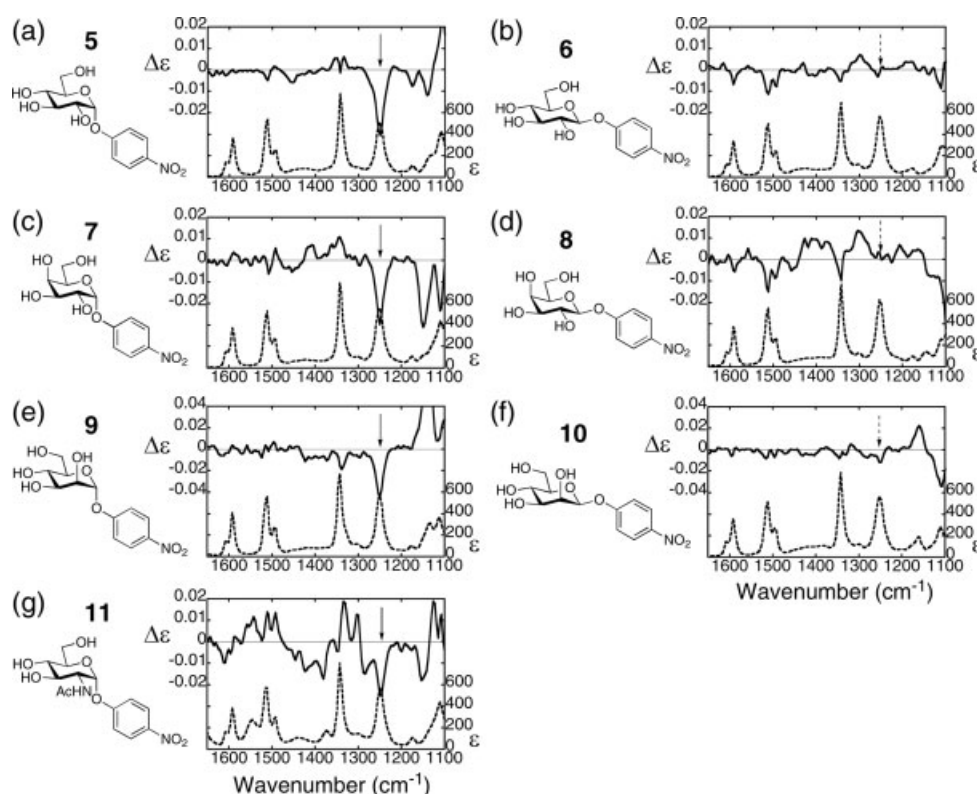
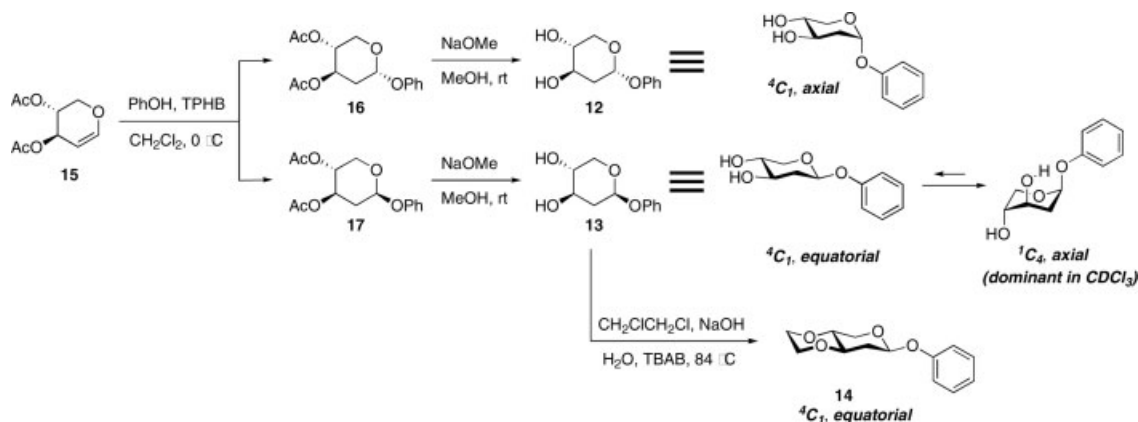


Fig. 3. VCD (solid lines) and IR (dotted lines) spectra of p -nitrophenyl glycosides in DMSO- d_6 ($c = 0.08$ M for **5**, $c = 0.16$ M for the others, $l = 72$ μ m, BaF₂ cell). (a) p -nitrophenyl α -D-glucopyranoside, (b) p -nitrophenyl β -D-glucopyranoside, (c) p -nitrophenyl α -D-galactopyranoside, (d) p -nitrophenyl β -D-galactopyranoside, (e) p -nitrophenyl α -D-mannopyranoside, (f) p -nitrophenyl β -D-galactopyranoside, and (g) p -nitrophenyl 2-acetamide-2-deoxy- α -D-glucopyranoside. The solid arrows indicate the characteristic VCD feature, while the dotted arrows indicate the absence of this band in the corresponding region.



Scheme 1. Preparation of model compounds **12–14** and their conformation.

(1H, dd, $J = 4.6, 10.4$ Hz, H-5eq), 3.66 (1H, ddd, $J = 4.7, 10.1, 10.1$ Hz, H-4), 3.60 (1H, dd, $J = 10.3, 10.3$ Hz, H-5ax), 2.35 (1H, ddd, $J = 1.5, 4.9, 13.1$ Hz, H-2eq), 1.82 (1H, ddd, $J = 3.4, 11.3, 13.3$ Hz, H-2ax); ^{13}C NMR (CDCl_3) δ 156.5 (*i*), 129.5 (*m*), 122.1 (*p*), 116.4 (*o*), 96.1 (C-1), 72.0 (C-4), 69.5 (C-3), 62.8 (C-5), 37.2 (C-2); HRMS (EI) m/z for $\text{C}_{11}\text{H}_{14}\text{O}_4$ (M^+), calcd 210.0892, found 210.0887; $[\alpha]_{\text{D}} +63.3^\circ$ (MeOH, c 1.0).

Phenyl 2-deoxy- β -D-threo-pentopyranoside (13). ^1H NMR (CDCl_3) δ 7.30 (2H, dd, $J = 7.0, 7.7$ Hz, *m*-H), 7.04 (3H, m, *o*-H x 2, *p*-H), 5.49 (1H, broad s, H-1, $W_{1/2} = 8.8$ Hz), 4.25 (1H, dd, $J = 1.6, 12.5$ Hz, H-5a), 3.87 (1H, broad d, $J = 3.5$ Hz, H-3), 3.73 (1H, broad s, H-4), 3.52 (1H, dd, $J = 4.2, 12.5$ Hz, H-5b), 2.42 (1H, broad dd, $J = 3.2, 11.0$ Hz, H-2a), 2.02 (1H, ddd, $J = 4.6, 4.6, 10.6$ Hz, H-2b); ^{13}C NMR (CDCl_3) δ 156.3 (*i*), 129.6 (*m*), 122.7 (*p*), 116.6 (*o*), 97.0 (C-1), 69.3 (C-4), 68.4 (C-3), 62.0 (C-5), 33.2 (C-2); HRMS (EI) m/z for $\text{C}_{11}\text{H}_{14}\text{O}_4$ (M^+), calcd 210.0892, found 210.0887; $[\alpha]_{\text{D}} -65.9^\circ$ (MeOH, c 1.0).

Phenyl 2-deoxy-3,4-*O*-ethylene- β -D-threo-pentopyranoside (14). ^1H NMR (600 MHz, CDCl_3) δ 7.28 (2H, dd, $J = 7.5, 8.5$ Hz, *m*-H), 7.02 (3H, m, *o*-H x 2, *p*-H), 5.18 (1H, dd, $J = 2.1, 9.5$ Hz, H-1), 4.01 (1H, dd, $J = 4.4, 11.0$ Hz, H-5eq), (4H, m, $-\text{OCH}_2\text{CH}_2\text{O}-$), 3.48–3.42 (2H, m, H-3, H-4), 3.37 (1H, dd, $J = 10.0, 10.9$ Hz, H-5ax), 2.31 (1H, ddd, $J = 2.2, 3.9, 12.4$ Hz, H-2eq), 1.94 (1H, ddd, $J = 9.7, 11.9, 11.9$ Hz, H-2ax); ^{13}C NMR (150 MHz, CDCl_3) δ 156.8 (*i*), 129.4 (*m*), 122.6 (*p*), 116.5 (*o*), 98.5 (C-1), 75.8 (C-4), 75.8 (C-3), 67.3, 67.1 ($-\text{OCH}_2\text{CH}_2\text{O}-$), 64.3 (C-5), 36.1 (C-2); HRMS (EI) m/z for $\text{C}_{13}\text{H}_{16}\text{O}_4$ (M^+), calcd 236.1048, found 236.1045; $[\alpha]_{\text{D}} -27.0^\circ$ (CHCl_3 , c 1.0).

RESULTS AND DISCUSSION

To gain fundamental insight into aromatic glycoside VCD, we started with the measurement of four glycosides **1–4** whose aglycan moiety is the simplest aromatic system (**1**, phenyl α -D-glucopyranoside; **2**, phenyl β -D-glucopyranoside; **3**, phenyl 2-acetamide-2-deoxy- α -D-galactopyranoside; **4**, phenyl β -D-galactopyranoside). The VCD and IR spectra of $\text{DMSO}-d_6$ solutions of **1–4** are shown in Figure 2. Their

vibrational spectra exhibit characteristic absorption bands at around 1600, 1500, and 1230 cm^{-1} . These bands could be ascribed to the phenyl aglycan part since they were not recognized in previously studied aliphatic glycosides.^{16,19} Interestingly, the IR spectra of axial glucopyranoside **1** and equatorial glucopyranoside **2** are very similar, but their VCD spectra are strikingly different. In particular, the VCD feature corresponding to the 1230 cm^{-1} IR band showed a strong negative peak for **1** but was almost flat for **2**. This dramatic difference should be the outcome of a subtle structural change at the anomeric carbon. This negative band is also observable in axial glycoside **3**, in the presence of additional absorption bands arising from the acetamide function. On the other hand, equatorial glycoside **4** did not show a negative band at around 1230 cm^{-1} .

The generality of this negative band has been examined by systematic VCD studies on a set of *p*-nitrophenyl glycosides **5–11**, which are frequently used as substrates for enzyme reaction monitoring by recording the fluorescence of the cleaved product. As shown in Figure 3, these molecules exhibit additional IR peaks at around 1500 and 1350 cm^{-1} caused by the NO_2 group. The negative VCD peak was commonly recognized in all axial glycosides (α -anomers) at around 1250 cm^{-1} , a slightly higher wave-number than that of simple phenyl glycosides. On the other hand, their equatorial counterparts (β -anomers) did not show such characteristic signal in this region. These results suggested this feature be a new carbohydrate structure–VCD spectra relationship that distinguishes the anomeric configuration of aromatic glycosides.* To gain further understanding on the vibrational properties of aromatic glycosides and to examine the theoretical predictability of the VCD spectra of glycoconjugates, we conducted DFT calculation of phenyl glycosides **12–14**.

*This characteristic band is different from our recently discovered “glycoside band,” a sharp and intense VCD band near 1145 cm^{-1} exhibited by sugars with axial glycosidic linkage (Refs. 16 and 19). The glycoside band is attributed to C1–H1 deformation coupled with C1–O1 stretching vibration, while the present band comes from a completely different vibrational mode, as clarified later. Moreover, we have observed the disappearance of the glycoside band from those connecting with carbonyl and aromatics, due to alteration of the strength of the C1–O1 linkage.

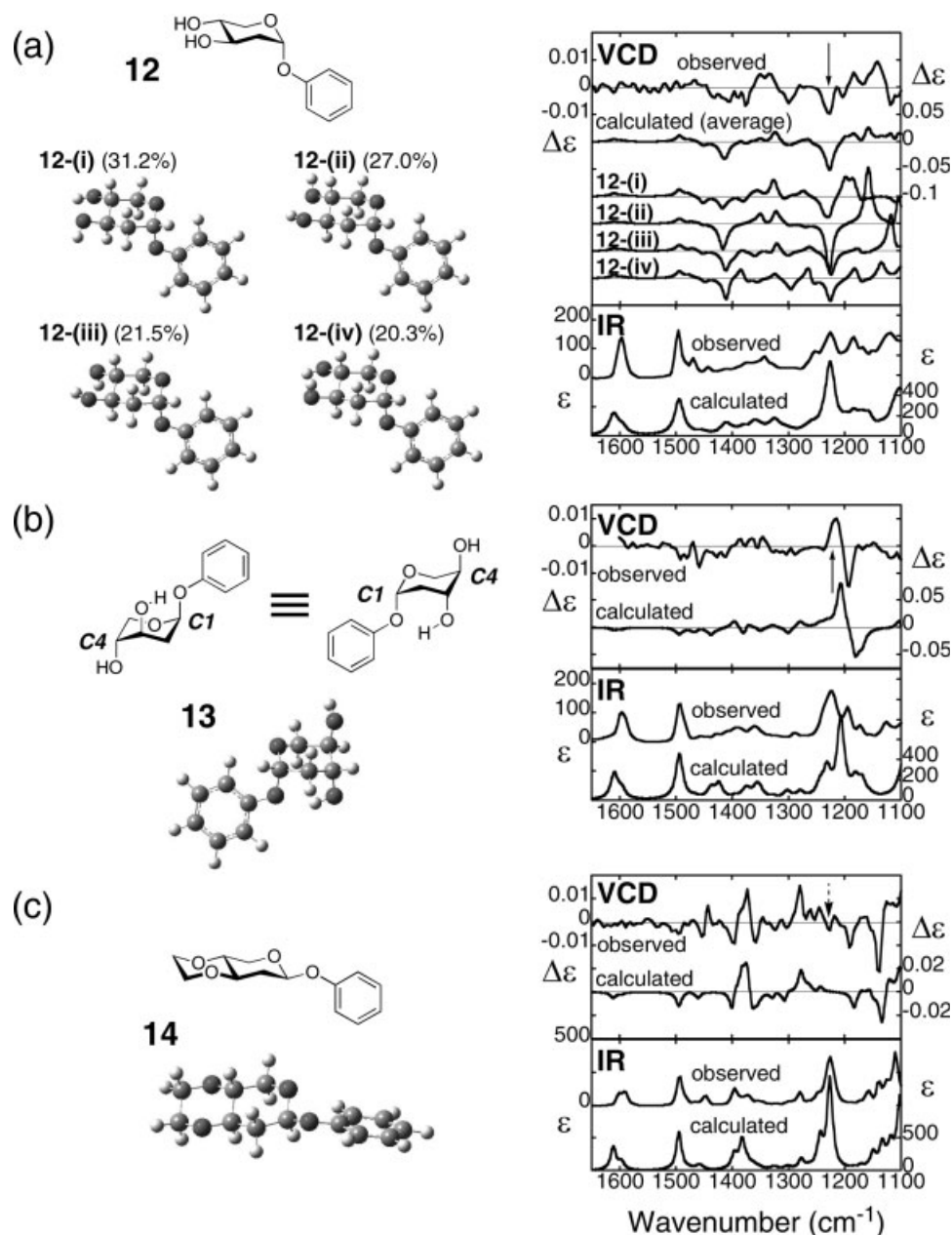


Fig. 4. Calculated stable conformers (left) and the comparison of observed and calculated VCD/IR spectra (right) of (a) **12**, (b) **13**, and (c) **14**. The Boltzmann populations of conformers of **12** are shown in parentheses. Experimental spectra were obtained in CDCl_3 ($c = 0.10 \text{ M}$, $l = 100 \mu\text{m}$, CaF_2 cell). **13** is reoriented to bring C1 to the front and C4 to the back.

A combination of DFT calculation and experimental VCD spectroscopy has been a powerful approach in determining the molecular conformation and the absolute stereochemistry.^{31–34} However, DFT prediction of carbohydrate VCD has not been straightforward because of the flexibility of the sugar structure and solvation effects. Recently, we have introduced calculation-compatible model compounds that possess simple and rigid structures and are soluble in non-hydrogen bonding solvent, such as CDCl_3 and CCl_4 , thus enabling reliable carbohydrate VCD theoretical study. The molecules **12–14** were designed based on a similar concept to have rigidity and solubility

in such solvents, with reflecting axial and equatorial aromatic glycoside structures. Although these structures are simple, the insight obtained from their theoretical calculation would be useful in expanding VCD application to more complicated aromatic glycoconjugates.

Compounds **12–14** were synthesized from 3,4-di-*O*-acetyl-D-xylal according to Scheme 1 (see Materials and Methods). Their VCD and IR spectra were measured in CDCl_3 and compared with the theoretical ones (Fig. 4). Theoretical VCD calculations were conducted starting from conformation analysis, which led to four conformers for **12**, and one conformer for **13** and **14**. Although **13** was prepared

		12	13	14
Observed	Wavenumber [cm^{-1}]	1227	~1220	1223
	IR intensity (ϵ [$\text{L mol}^{-1} \text{cm}^{-1}$])	156	176 (at 1223 cm^{-1})	318
	VCD intensity ($\Delta\epsilon \times 10^2$ [$\text{L mol}^{-1} \text{cm}^{-1}$])	-1.0	+1.0 (at 1219 cm^{-1})	+0.0
Calculated	Wavenumber [cm^{-1}]	1228	1208	1227
	Dipole strength [$10^{-40} \text{esu}^2 \text{cm}^2$]	149	194	260
	Rotational strength [$10^{-44} \text{esu}^2 \text{cm}^2$]	-62.0	+175.2	+0.5

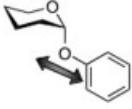
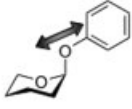
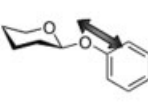
Type of Conformation			
	${}^4C_1$, axial	${}^1C_4$, axial	${}^4C_1$, equatorial
Typical VCD feature			
	negative	positive	flat

Fig. 5. Vibrational properties at the aromatic ether stretching mode of **12–14** and the relationship between the conformation of aromatic glycosides and the sign of the VCD signal. Two-headed arrows represent the stretching motion of the glycosidic oxygen and aromatic carbon in the aglycan part.

with the aim of obtaining an equatorial model structure, DFT calculation in a vacuum condition concluded that the pyranose ring of **13** is flipped from the ordinary ${}^4C_1$ conformation to the ${}^1C_4$ one as illustrated in Scheme 1. This calculation result was supported by the smallness of the ${}^1\text{H}$ NMR coupling constants between the anomeric proton and the vicinal protons (**13**: H1, broad s, $W_{1/2} = 8.8$ Hz). While, the ring conformation of **14** is fixed to ${}^4C_1$ with its phenyl aglycan oriented toward equatorial fixed by the *trans*-decalin framework, which is in agreement with the ${}^1\text{H}$ NMR result (**14**: H1, dd, $J = 2.1, 9.5$ Hz). For the DFT-optimized geometries, VCD and IR spectra were calculated at DFT/B3LYP/6-311G(d, p) level of theory, affording the final spectra.

Both the observed and the calculated IR spectra exhibit peaks at around 1600, 1500, and 1230 cm^{-1} , as observed in phenyl glycosides **1–4**. At the 1230 cm^{-1} region, the experimental VCD spectrum of **12** displays the negative band, while **14** does not present such characteristic features, in agreement with other axial and equatorial aromatic glycosides. These observed VCD trends were well reproduced in the theoretical VCD spectra, namely, a negative band for **12**, a positive band for **13**,[†] and an almost flat trace for **14**.

[†]The model compound **13** also shows an intense negative VCD band at around 1190 cm^{-1} . The analysis of vibrational mode of **13** suggested that a negative VCD peak at 1182 cm^{-1} was mainly attributed to coupled deformation movement of C2–H2, C3–H3, C4–H4, and O4–H4, and not attributed to the anomeric nor aglycan parts. Because this negative tendency at 1190 cm^{-1} is related to the very sugar structure of **13**, this would not be a part of the structures–structure relationship described so far. A small VCD shoulder at 1170 cm^{-1} recognized in calculated **13** was contributed by C–H deformations of the benzene ring.

This result showed that structure–spectra relationships in glycoconjugate VCD are predictable from theoretical calculation, as with polypeptides and nucleic acids.^{7–9,11}

The analysis of the vibrational mode of **12** revealed that the stretching motion of the glycosidic oxygen and the aromatic carbon contributes to the vibration at this negative band. The assignment of the 1230 cm^{-1} band as an aromatic C–O stretch is consistent with the other model compounds **13** and **14**, although their VCD patterns are completely different (Fig. 5). This assignment is in accord with previous IR studies in which aromatic ethers show an absorption band at around 1250 cm^{-1} because of resonance effects.^{35–37} Further analysis of vibrational modes identified the vibrations at around 1600 and 1500 cm^{-1} as aromatic C=C stretching, while additional peaks at 1500 cm^{-1} and 1350 cm^{-1} displayed in *p*-nitrophenyl glycosides arise from antisymmetric and symmetric NO_2 stretch, respectively. The dominant contribution of the aglycan moiety to this 1230 cm^{-1} band can explain the independence of this band from the type of sugars (see Figs. 2 and 3). The other atoms in the aromatic aglycan did not seem to have significant effects on this vibrational mode, and in fact the *p*-substitution by a nitro group did not alter this structure–spectra relationship. This result makes it interesting to apply VCD spectroscopy to more complicated aromatic glycosylated natural products.

It is noteworthy that the stretching motion of an aglycan moiety can provide information on the sugar structure. This situation is reminiscent of our recent findings on the vibrational chirality probe phenomenon where a specific IR chromophore that is not a sugar itself, for example a simple methyl group, can extract stereochemical informa-

tion on nearby chiral carbons.^{15,20,38} Currently, most VCD studies on carbohydrates have been limited to sugar samples without aglycans, but this work indicates the importance of further studies on glycoconjugates, which would be useful in the structural analysis of aglycan as well as sugar parts.

In conclusion, as a first step of glycoconjugate VCD studies, we have measured a series of aromatic glycosides and found a characteristic VCD peak that distinguishes the sugar anomeric structure. This band was assigned as the stretching motion of the glycosidic oxygen and the adjacent carbon in the aglycan moiety by means of DFT calculation of model compounds. This is the first example of theoretical calculation of the structure–spectra relationship on glycoconjugate VCD. Furthermore, aglycan parts have been found to have a potential to provide stereochemical information on sugar moieties.

VCD studies on carbohydrates have lagged behind polypeptides and nucleic acids and there have been few insights on glycoconjugate VCD. However, the complementary approach of using systematic measurements (empirical) and theoretical calculation (semi- or nonempirical) employed here will afford further understanding of the spectra of glycoconjugates.

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Determination of the Absolute Configurations of Natural Products via Density Functional Theory Calculations of Vibrational Circular Dichroism, Electronic Circular Dichroism, and Optical Rotation: The Iso-Schizozygane Alkaloids Isoschizogaline and Isoschizogamine

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

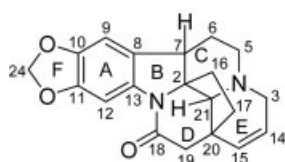
ABSTRACT The development of density functional theory (DFT) methods for the calculation of vibrational circular dichroism (VCD), electronic circular dichroism (ECD), and transparent spectral region optical rotation (OR) has revolutionized the determination of the absolute configurations (ACs) of chiral molecules using these chiroptical properties. We report the concerted application of DFT calculations of VCD, ECD, and OR to the determination of the ACs of the isoschizozygane alkaloid natural products, isoschizogaline, and isoschizogamine, whose ACs have not previously been determined. The ACs of naturally occurring (–)-isoschizogaline and (–)-isoschizogamine, are both determined definitively to be 2*R*, 7*R*, 20*S*, 21*S*. *Chirality* 20:454–470, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: natural product; absolute configuration; density functional theory; vibrational circular dichroism; electronic circular dichroism; optical rotation

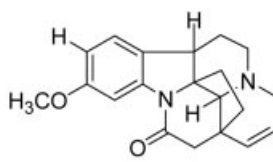
INTRODUCTION

The alkaloids schizozygine (**1**), schizogaline (**2**), schizogamine (**3**), isoschizogaline (**4**), and isoschizogamine (**5**) were isolated by Renner and coworkers^{1–4} from the East African plant *Schizozygia caffaeoides* (Boj.) Baill., belonging to the *Apocynaceae* family, a plant used in tradi-

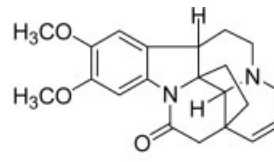
tional medicine in Kenya for the treatment of skin diseases. These alkaloids were structurally characterized via their chemical reactions and spectroscopic properties. For schizozygine (**1**), schizogaline (**2**), and schizogamine (**3**), the structures obtained were



Schizozygine, **1**



Schizogaline, **2**



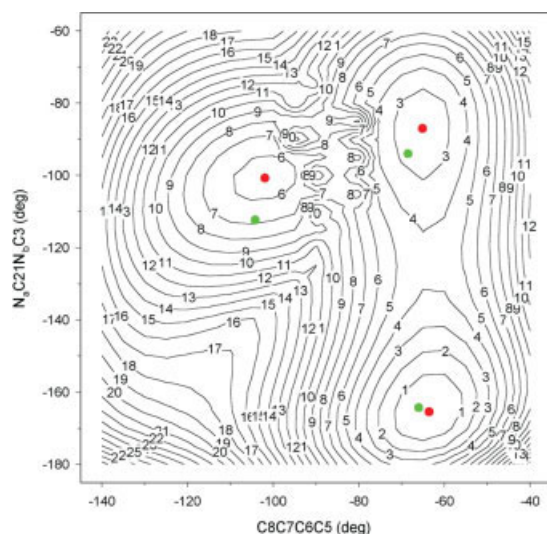
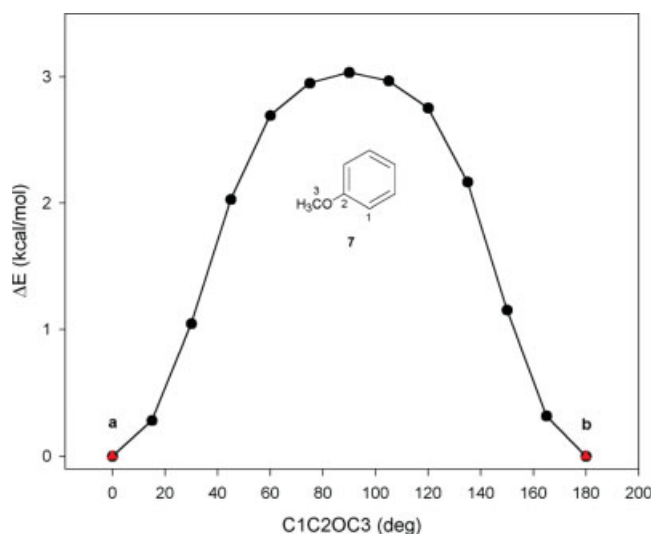
Schizogamine, **3**

This article contains supplementary material available via the Internet at <http://www.interscience.wiley.com/jpages/0899-0042/suppmat>.
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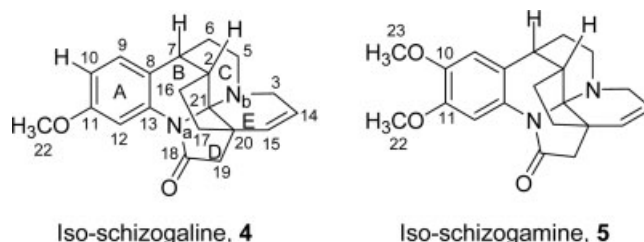
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TABLE 1. The relative energies (ΔE)^a and dihedral angles,^b D1–D4, of the conformations of **6**

Conformer	MMFF94	B3LYP/6-31G*	MMFF94 dihedral angles				B3LYP/6-31G* dihedral angles			
	ΔE	ΔE	D1	D2	D3	D4	D1	D2	D3	D4
I	0.00	0.00	34.62	43.86	40.05	16.60	35.74	48.35	40.12	16.12
II	9.65	2.38	34.85	50.94	−49.73	10.11	40.13	52.85	−54.55	10.43
III	10.54	5.64	39.56	−46.37	−36.59	14.99	46.73	−53.41	−48.44	14.94

^aIn kcal/mol.^bIn degrees. D1: C13N_aC21C2; D2: C21N_bC5C6; D3: C14C3N_bC21; D4: N_aC18C19C20.**Fig. 1.** B3LYP/6-31G* PES scan of **6**. The dihedral angles C8C7C6C5 and NaC21NbC3 were varied in 5° steps. Contours are shown at 1.0 kcal/mol intervals. The red circles are B3LYP/6-31G* optimized minima and the green circles are MMFF94 minima.**Fig. 2.** B3LYP/6-31G* PES scan of **7**. The dihedral angle C1C2OC3 was varied in 15° steps. The red triangles are the B3LYP/6-31G* optimized minima.

and have continued to be the accepted structures. However, in the cases of isoschizogaline (**4**) and isoschizogamine (**5**), recent NMR studies have modified the structures originally proposed^{5,6} and their structures are now known to be



In addition, total synthesis of racemic **5**⁷ has further confirmed the structure of **5**. **4** and **5** thus represent the most characterized members of the isoschizozigane alkaloid group.

All of the naturally occurring alkaloids **1–5** exhibit non-zero optical rotations,¹ consistent with the chirality of their structures. Until very recently, none of the absolute configurations (ACs) of these natural products had been determined. Recently, we have reported the determination of the AC of **1**, via comparison of density functional theory (DFT) calculations of the vibrational circular dichroism (VCD), electronic circular dichroism (ECD), and optical rotation (OR) to experimental VCD, ECD, and OR data.⁸ The naturally occurring (+)-schizozigine was shown to have the AC 2*R*, 7*S*, 20*S*, 21*S*. The natural products **2** and **3** were consequently assigned the same ACs,⁸ given the very small differences in their structures from that of **1** and assuming a common biosynthetic pathway for all three alkaloids.

In this article, we extend our work on the AC of **1** to the determination of the ACs of the differently structured alkaloids **4** and **5**, again using their VCD, ECD, and OR.

METHODS

Isolation of Isoschizogaline, **4** and Isoschizogamine, **5**

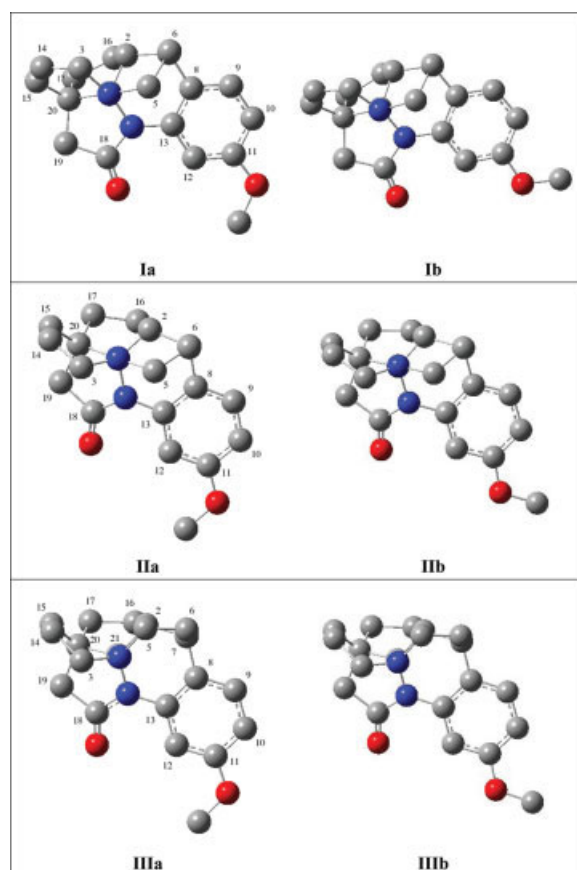
Leaves of *Schizozigia caffaeoides* (Boj.) Baill. were collected in July 1996 in Kenya, on the seashore of the Mom-

TABLE 2. The relative energies (ΔE)^a and free energies (ΔG)^a of the conformations of **4**

Conformer	MMFF94	B3LYP/6-31G*			B3LYP/TZ2P			B3PW91/TZ2P		
	ΔE	ΔE	ΔG	P (%) ^b	ΔE	ΔG	P (%) ^b	ΔE	ΔG	P (%) ^b
Ia	0.00	0.00	0.00	77.96	0.00	0.00	79.95	0.00	0.00	81.84
Ib	1.85	1.15	0.89	17.34	1.13	0.99	16.99	1.10	0.96	16.17
IIa	9.73	2.50	1.66	4.70	2.68	2.29	3.06	2.91	2.20	1.99
IIb	11.38	3.62	2.68	0.00	3.77	3.31	0.00	3.98	3.06	0.00
IIIa	10.47	5.74	5.30	0.00	5.60			5.72		
IIIb	12.27	6.87	6.45	0.00	6.71			6.81		

^aIn kcal/mol.^bPopulation percentages based on ΔG , assuming Boltzmann statistics at $T = 298.15$ K.**TABLE 3.** The dihedral angles, ^a D1–D5, of the conformations of **4**

Conformer	B3LYP/6-31G*					B3PW91/TZ2P				
	D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
Ia	35.99	48.21	39.71	15.97	0.43	36.28	48.68	40.63	16.13	0.27
Ib	35.95	48.43	40.00	16.07	−179.70	36.18	48.96	41.29	16.22	−179.80
IIa	40.14	52.58	−54.61	9.10	1.39	40.70	53.16	−55.57	9.70	1.15
IIb	40.04	52.80	−54.74	10.42	179.91	40.65	53.32	−55.55	11.41	179.73
IIIa	46.54	−52.98	−48.16	13.83	0.77	46.82	−53.65	−47.99	14.43	0.60
IIIb	47.03	−53.13	−48.41	14.45	−179.65	47.21	−53.72	−48.23	15.18	−179.80

^aIn degrees. D1: C13N_aC21C2; D2: C21N_bC5C6; D3: C14C3N_bC21; D4: N_aC18C19C20; D5: C12C11OC22.**Fig. 3.** B3LYP/6-31G* conformations of (2*R*,7*R*,20*S*,21*S*)-**4**. The structures are viewed along the C6–C7 and N_b–C21 bonds with C6 and N_b pointing towards the viewer.

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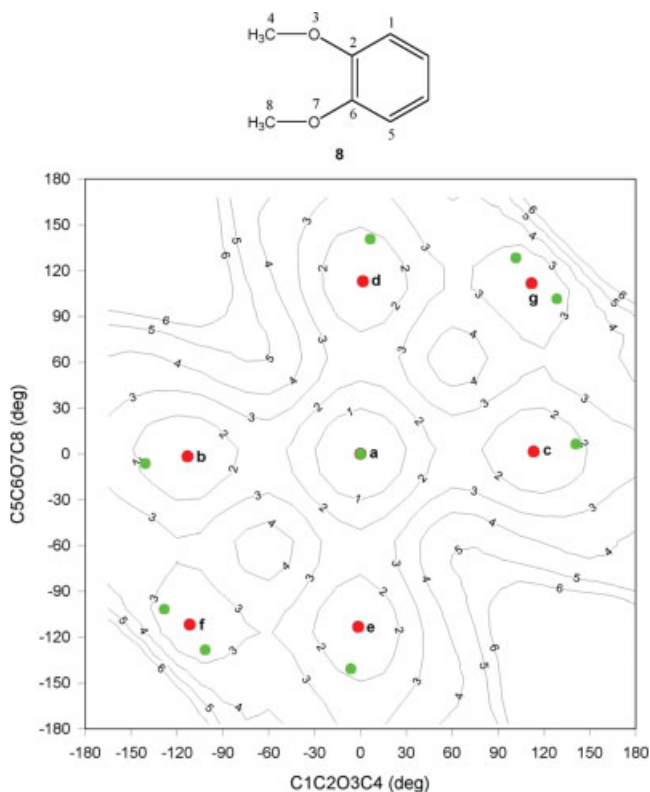
**Fig. 4.** B3LYP/6-31G* PES scan of **8**. The dihedral angles C1C2O3C4 and C5C6O7C8 were varied in 15° steps. Contours are shown at 1.0 kcal/mol intervals. The red circles are B3LYP/6-31G* optimized minima and the green circles are MMFF94 minima.

TABLE 4. The relative energies (ΔE)^a and dihedral angles of the conformations of **8**

Conformer	B3LYP/6-31G*			MMFF94		
	ΔE	C5C6O7C8	C1C2O3C4	ΔE	C5C6O7C8	C1C2O3C4
a	0.00	−0.02	0.01	1.10	0.00	0.00
b	1.09	−1.55	−113.17	0.35	−6.38	−140.73
c	1.09	1.58	113.18	0.35	6.38	140.73
d	1.09	113.17	1.55	0.35	140.73	6.38
e	1.09	−113.12	−1.69	0.35	−140.73	−6.38
f	2.07	−111.69	−111.69	0.00	−128.33	−101.56
g	2.07	111.82	111.80	0.00	−101.56	−128.33
				0.00	101.56	128.33
				0.00	128.33	101.56

^aIn kcal/mol.

basa region, by Sedaherb S.A. (Saint Léger sur Dheume, France). Dried material was grained and macerated repeatedly with methanol. The macerate was concentrated and subjected to acido–basic treatment, providing a mixture of alkaloids. Individual alkaloids were obtained by

chromatography, first on alumina and then on silica gel columns. Crude (–)-isoschizogaline was recrystallized from ether/hexane, mp 108–110.5°C, $[\alpha]_D^{23}$ −260.1 (c 0.99; CHCl₃), (lit.^{1,2} m.p. 110–112°C, $[\alpha]_D^{25}$ −262.3 (c 1; CHCl₃)). Crude (–)-isoschizogamine was recrystallized

TABLE 5. The relative energies (ΔE),^a free energies (ΔG),^a equilibrium populations (P)^b and dihedral angles,^c D1–D6, of the conformations of **5**

Conformer ^d	MMFF94	B3LYP/6-31G*			B3LYP/TZ2P			B3PW91/TZ2P			B3LYP/6-31G* dihedral angles					
	ΔE	ΔE	ΔG	P (%)	ΔE	ΔG	P (%)	ΔE	ΔG	P (%)	D1	D2	D3	D4	D5	D6
Ia	1.44	0.00	0.00	29.69	0.00	0.00	38.68	0.00	0.00	53.33	35.88	48.36	40.14	16.09	0.51	−0.41
Ib	0.00	0.53	0.02	28.49	0.74	0.22	26.52	1.18	0.62	18.74	35.62	48.30	40.07	16.04	−0.82	−114.49
Ic	0.00	0.67	0.03	28.28	0.91	0.21	27.32	1.38	0.53	21.64	35.78	48.32	40.20	16.08	1.78	114.97
Id	2.19	2.18	1.37	2.91	2.36	1.44	3.40	2.81	1.75	2.76	36.05	48.42	40.06	16.93	109.60	1.01
Id'	2.30	2.18														
Ie	2.26	2.22	1.56	2.12	2.45	1.76	1.99	2.87	2.15	1.41	35.74	48.44	40.27	16.02	−111.62	−2.06
Ie'	2.33	2.22														
Ila	11.06	2.42	1.59	2.02	2.59	1.73	2.09	2.82	1.91	2.12	40.22	52.68	−54.59	9.41	1.05	0.09
If		2.58	1.71	1.65	3.11			3.90			35.57	48.38	40.15	15.98	−109.87	−112.21
Ig		2.65	1.52	2.27	3.17			3.97			36.20	48.27	40.04	16.52	108.38	111.53
Ilb	9.76	2.95	1.90	1.20	3.35			4.03			40.11	52.63	−54.34	9.19	0.02	−114.78
Ilc	9.79	3.07	1.82	1.37	3.49			4.17			39.98	52.52	−54.43	10.01	2.42	114.58
IId	11.59	4.32	3.16	0.00	4.61			5.25			40.34	52.79	−54.51	12.32	108.76	1.23
IId'	11.70	4.32														
Ile	11.90	4.60	3.20	0.00	4.99			5.64			39.97	52.97	−54.69	10.64	−111.98	−2.31
Ilg		4.90	3.06	0.00	5.53			6.56			40.52	52.64	−54.83	11.14	107.09	111.63
IIf	10.55	4.97	3.35	0.00	5.68			6.71			40.04	52.83	−54.56	9.77	−110.36	−112.64
IIla	11.79	5.65									46.25	−52.98	−48.06	14.42	1.03	0.11
IIlb	10.44	6.21									46.15	−52.88	−48.13	14.28	−0.39	−114.02
IIlc	10.37	6.22									46.42	−52.86	−48.16	14.19	2.04	114.98
IIId	12.48	7.67									46.73	−53.09	−48.13	15.40	108.87	1.25
IIId'	12.59	7.67														
IIle	12.57	7.82									46.55	−53.29	−48.17	14.78	−111.95	−2.29
IIle'	12.62	7.82														
IIlg		8.19									46.80	−53.30	−48.30	15.00	107.59	112.17
IIIf		8.22									46.29	−53.01	−48.03	14.65	−110.51	−111.94

^aIn kcal/mol.^bPopulation percentages based on ΔG , assuming Boltzmann statistics at $T = 298.15$ K.^cIn degrees. D1: C13N₆C21C2; D2: C21N₆C5C6; D3: C14C3N₆C21; D4: N₆C18C19C20; D5: C12C11OC22; D6: C9C10OC23.^dOptimized **Id'**, **Ie'**, **IId'**, **IIId'**, and **IIle'** are the same as **Id**, **Ie**, **IId**, **IIId**, and **IIle**.

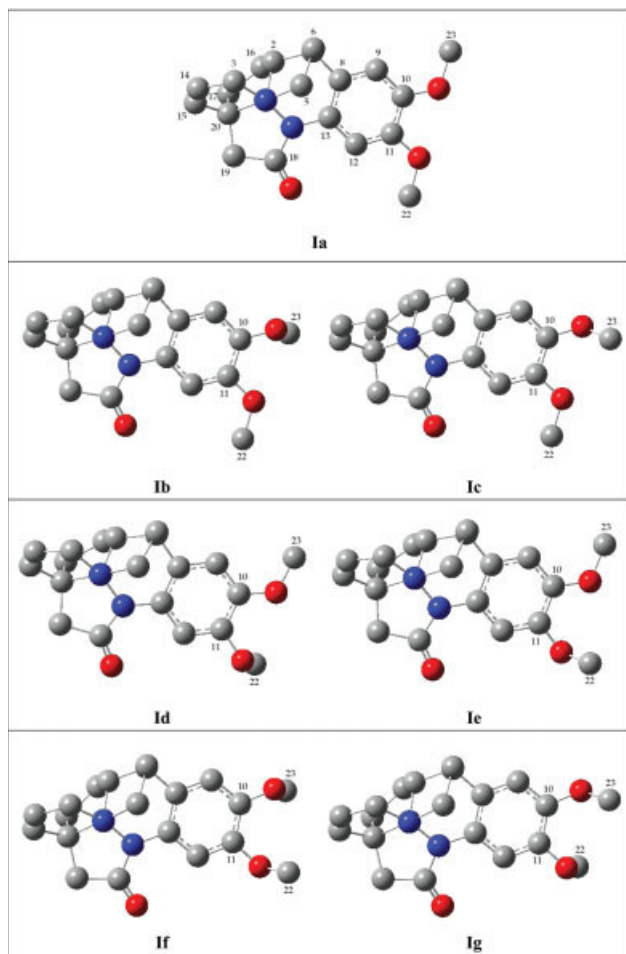


Fig. 5. B3LYP/6-31G* conformations of (2*R*,7*R*,20*S*,21*S*)-**5**. The structures are viewed in the same way as those in Figure 3.

from ethyl acetate, mp 181.5–184.5°C, $[\alpha]_D^{24}$ –239.8 (c 0.54; CHCl₃), (lit.^{1,2} m.p. 184–185°C, $[\alpha]_D^{25}$ –239 (c 1; CHCl₃)).

Calculations

Calculations have been carried out on (2*R*,7*R*,20*S*,21*S*)-**4** and (2*R*,7*R*,20*S*,21*S*)-**5**. Initial conformational analyses were carried out using Monte-Carlo searching together with the MMFF94 molecular mechanics force field via the SPARTAN 02 program.⁹ Geometry optimizations of the MMFF94 conformations obtained were then carried out using DFT via the GAUSSIAN 03 program,¹⁰ followed by calculations of their harmonic vibrational frequencies to verify their stability and to calculate conformational free energies. Simultaneously with vibrational frequency calculations, harmonic vibrational dipole strengths, and rotational strengths were calculated and IR and VCD spectra obtained thence, using *Chirality* DOI 10.1002/chir

Lorentzian band shapes.^{11,12} The DFT calculation of vibrational rotational strengths in GAUSSIAN 03 uses the equation of Stephens¹³ and its DFT implementation by Cheeseman et al. using gauge-invariant atomic orbitals (GIAOs), which guarantee origin-independent rotational strengths.¹⁴

Experimental vibrational frequencies, dipole strengths, and rotational strengths were obtained from experimental IR and VCD spectra by Lorentzian fitting^{11,12} using the PeakFit program.¹⁵

Electronic excitation energies, oscillator strengths and both length- and velocity-representation rotational strengths of the DFT conformations of **4** and **5** have been calculated using TDDFT via GAUSSIAN 03 and ECD spectra obtained thence using Gaussian band shapes.¹⁶

Optical rotations of the DFT conformations of **4** and **5** have been calculated using TDDFT and GIAOs via GAUSSIAN 03.

As in prior work,^{16–40} DFT calculations have used the functionals B3LYP and B3PW91 and the basis set TZ2P for IR and VCD calculations^{17–32} and B3LYP and aug-cc-pVDZ for ECD and OR calculations.^{16,33–40}

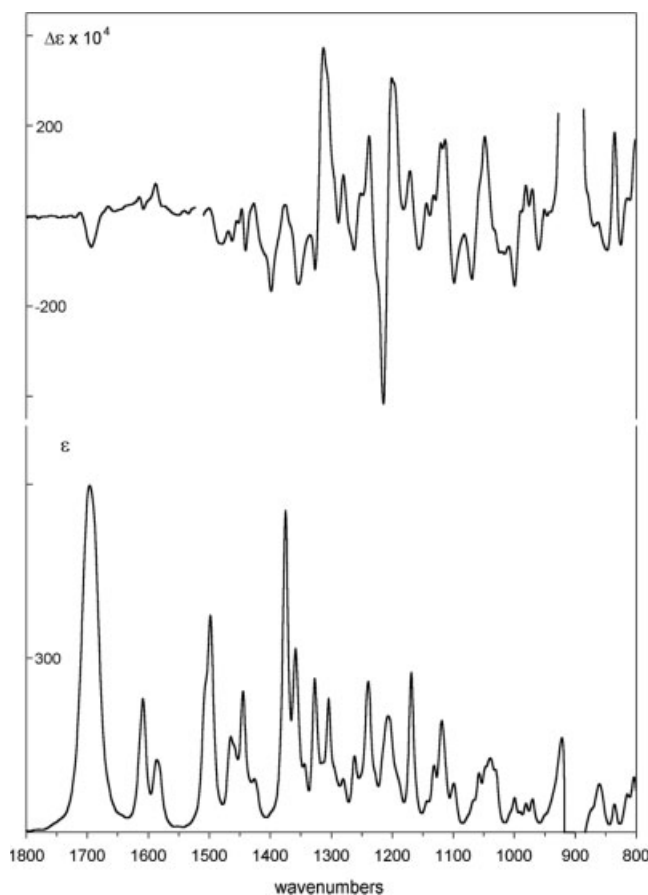


Fig. 6. Experimental IR and VCD spectra of (–)-**4** in CDCl₃; concentration 0.255 M; pathlength 50 μm.

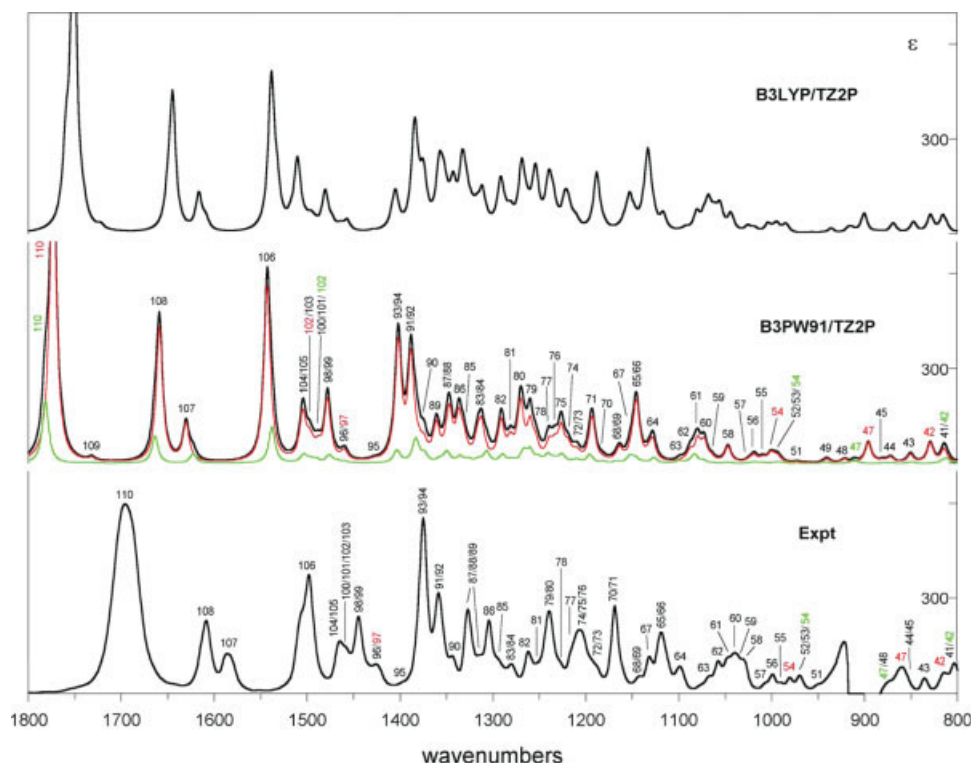


Fig. 7. Comparison of the experimental and B3LYP/TZ2P and B3PW91/TZ2P IR spectra of **4** for the range 800–1800 cm^{-1} . The black calculated spectra are the conformationally-averaged spectra of conformations **Ia**, **Ib**, and **IIa**. The red and green calculated spectra are the population-weighted IR spectra of conformations **Ia** and **Ib** of **4** respectively. The numbers define the fundamentals contributing to resolved bands. Red and green numbers refer to fundamentals of **Ia** and **Ib** respectively. Black numbers are used when the bands of **Ia** and **Ib** are not resolved. Bandshapes of the calculated spectra are Lorentzian ($\gamma = 4.0 \text{ cm}^{-1}$). The assignment of the experimental spectrum is based on the B3PW91/TZ2P calculated spectrum.

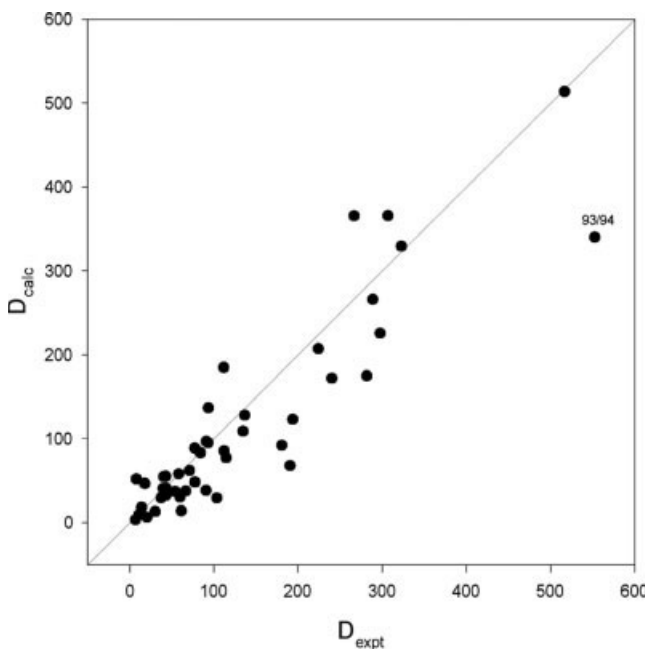


Fig. 8. Comparison of experimental and B3PW91/TZ2P dipole strengths of **4**. Calculated dipole strengths are population-weighted averages of conformations **Ia**, **Ib**, and **IIa**. The line is of slope +1. Dipole strengths are in $10^{-40} \text{ esu}^2 \text{ cm}^2$.

Spectroscopy

The IR and VCD spectra of CDCl_3 solutions of **4** and **5** were measured using a Bruker IFS-66/S spectrometer equipped with a PMA 37 VCD/IRRAS module containing a BaF_2 polarizer, a ZnSe photoelastic modulator, a MCT detector and a filter restricting frequencies to $<1800 \text{ cm}^{-1}$, as described previously.⁴¹ For all measurements an A145 (Bruker) cuvette with KBr windows and a $50 \mu\text{m}$ pathlength was used. All spectra were measured at 4 cm^{-1} resolution, averaging 8 blocks, each containing 2240 interferometric scans with zero filling factor of 4. ECD and UV-vis spectra of CHCl_3 solutions of **4** and **5** were recorded simultaneously using a Jasco J-810 spectropolarimeter and a $100 \mu\text{m}$ quartz cuvette, with a 1 nm resolution, a time constant of 1 sec , a scan speed of 100 nm/sec and 10 accumulations. Optical rotations of CHCl_3 solutions of **4** and **5** were measured using a Rudolph AUTOPOL IV polarimeter, at 365, 405, 436, 546, 589, and 633 nm and 20°C .

RESULTS

Conformational Analysis: Isoschizogaline, **4**

In analyzing the conformations of (2*R*,7*R*,20*S*,21*S*)-**4** we first examine the conformations of its hexa-cyclic core, **6**:

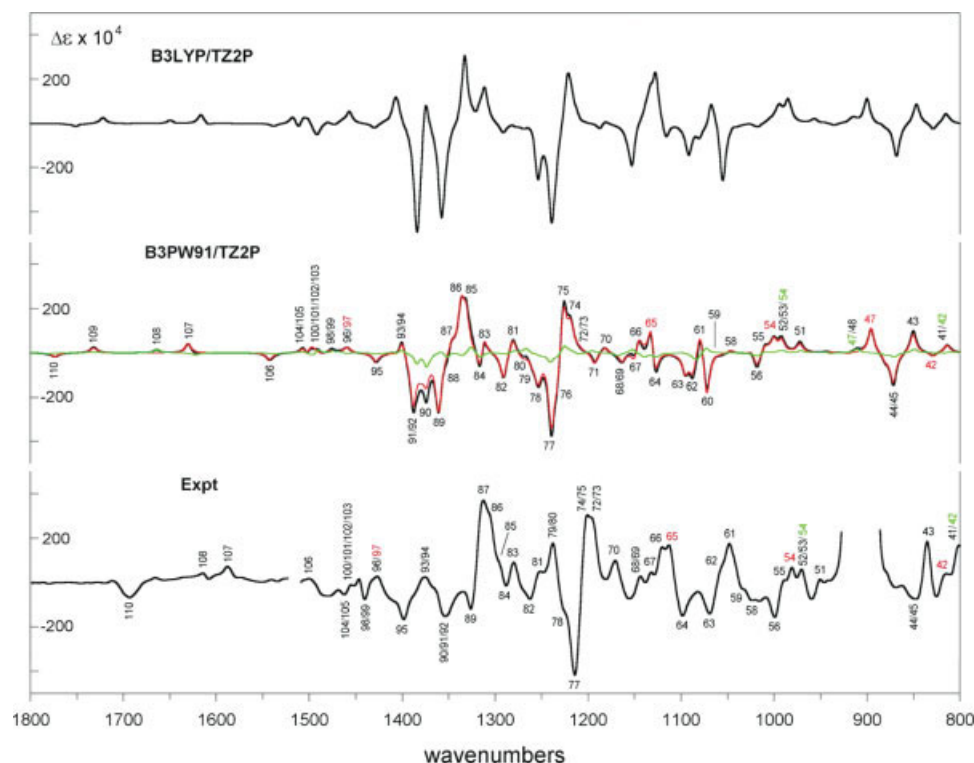
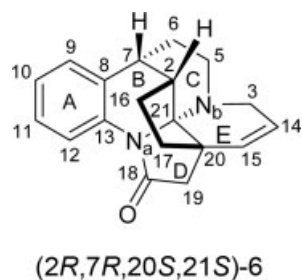


Fig. 9. Comparison of the experimental and B3LYP/TZ2P and B3PW91/TZ2P VCD spectra of **4** for the range 800–1800 cm^{-1} . The black calculated spectra are the conformationally-averaged spectra of conformations **Ia**, **Ib**, and **IIa**. The red and green calculated spectra are the population-weighted IR spectra of conformations **Ia** and **Ib** of **4** respectively. The numbers define the fundamentals contributing to resolved bands. Red and green numbers refer to fundamentals of **Ia** and **Ib** respectively. Black numbers are used when the bands of **Ia** and **Ib** are not resolved. Bandshapes of the calculated spectra are Lorentzian ($\gamma = 4.0 \text{ cm}^{-1}$). The assignment of the experimental spectrum is based on the B3PW91/TZ2P calculated spectrum.



Monte Carlo searching using the MMFF94 molecular mechanics force field found three conformations of **6** within a 20 kcal/mol window. The relative energies of these conformations, **I**, **II**, and **III** are given in Table 1. Dihedral angles in rings B, C, D, and E of these conformations, listed in Table 1, show that the three conformations result from changes in the conformations of rings C and E; the conformations of rings B and D do not change greatly. Reoptimization of the geometries of **I–III** using DFT at the B3LYP/6-31G* level leads to the relative energies and dihedral angles also given in Table 1. The DFT relative energies of **II** and **III** are very different from the MMFF94 relative energies. In contrast, the dihedral angles D1–D4 are similar, all changes being $<12^\circ$. To fur-

ther evaluate the reliability of the conformational analysis of **6**, we have carried out a 2D Potential Energy Surface (PES) scan at the B3LYP/6-31G* level, varying the dihedral angles C8C7C6C5 and N_aC21N_bC3. The results, shown in Figure 1, support the conclusion that only three stable conformations of **6** exist.

In Figure 2 we report the B3LYP/6-31G* PES of methoxy-benzene (anisole), **7**, as a function of the C1C2OC3 dihedral angle. As the CH₃ group rotates around the O–C2 bond, there are two stable conformations, each with the C3O group coplanar with the phenyl ring. B3LYP/6-31G* optimization of these structures shows that they are of identical energy and have C1C2OC3 angles of 0° and 180° respectively.

We anticipate on the basis of these results for **6** and **7** that six stable conformations of **4** will exist with three core conformations similar to those of **6** and two methoxy conformations similar to those of **7**. Monte-Carlo searching using MMFF94 finds six conformations, as expected, with the relative energies given in Table 2. B3LYP/6-31G* optimization of these conformations leads to the relative energies and dihedral angles D1–D5 also given in Tables 2 and 3. Comparison of the D1–D5 values to those for **6** and **7** identifies the core conformations, **I–III**, and the methoxy conformations **a** and **b** of the six conformations of **4**. The relative energies of **Ia**, **IIa**, and **IIIa** are similar

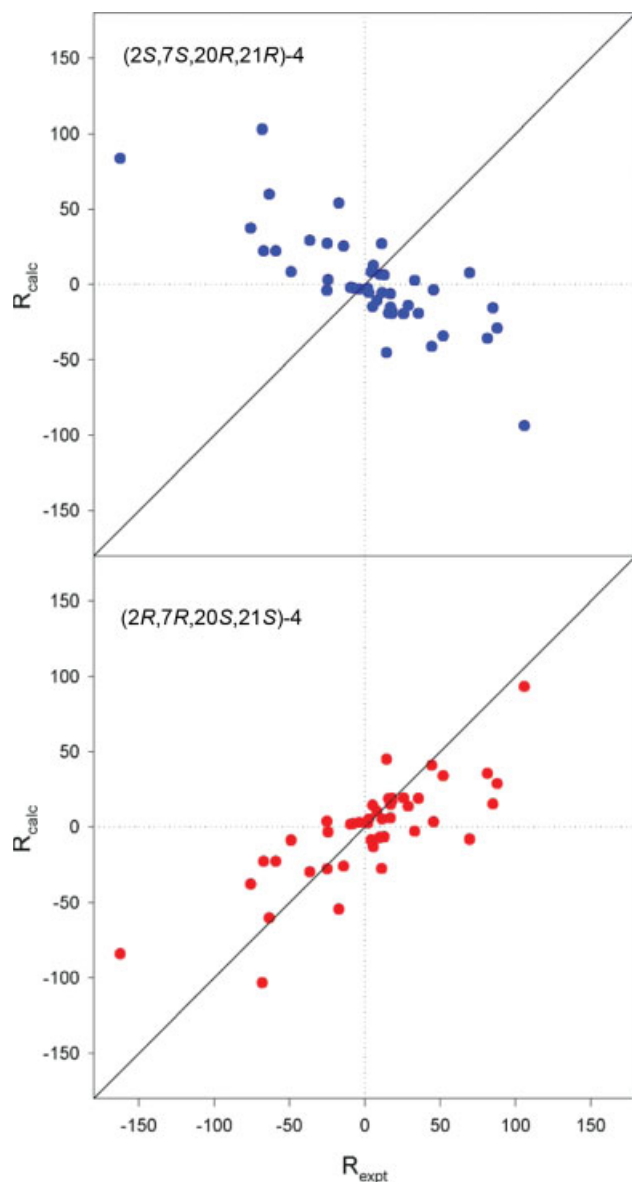


Fig. 10. Comparison of experimental and B3PW91/TZ2P rotational strengths of **4**. Experimental rotational strengths are for (–)-**4**. Calculated rotational strengths are for (2*R*,7*R*,20*S*,21*S*)- and (2*S*,7*S*,20*R*,21*R*)-**4**. Calculated rotational strengths are population-weighted averages of conformations **Ia**, **Ib**, and **IIa**. The lines are of slope +1. Rotational strengths are in $10^{-44} \text{ esu}^2 \text{ cm}^2$.

to those of conformations **I**, **II**, and **III** of **6**, as are those of **Ib**, **IIb**, and **IIIb**. However, for each core conformation, the two methoxy conformations **a** and **b** differ in energy by ~ 1 kcal/mol, reflecting the impact of rings B–E on the symmetry of the methoxy-benzene moiety. The B3LYP/6-31G* structures of the six conformations of **4** are shown in Figure 3.

The stability of the six conformations of **4** is confirmed by B3LYP/6-31G* harmonic vibrational frequency calculations, which also lead to the room-temperature relative

free energies and equilibrium populations given in Table 2. Only three conformations, **Ia**, **Ib**, and **IIa**, are predicted to be significantly populated, **Ia** being the dominant conformation. Further optimization of the six conformations of **4** at the B3LYP/TZ2P and B3PW91/TZ2P levels leads to the relative energies and free energies, equilibrium populations and dihedral angles given in Tables 2 and 3; all TZ2P properties are similar to the 6-31G* values.

Conformational Analysis: Isoschizogamine, **5**

In **5**, the methoxy-benzene moiety of **4** is replaced by an ortho-dimethoxybenzene (veratrole) moiety. The conformations to be expected for this moiety in **5** are examined by a 2D B3LYP/6-31G* PES scan of ortho-dimethoxybenzene, **8**. The results, shown in Figure 4, demonstrate the existence of seven stable conformations, **a–g**. Optimization leads to the relative energies and structures detailed in Table 4. MMFF94 Monte-Carlo searching gives nine conformations, five with dihedral angles close to those of B3LYP/6-31G* conformations **a–e**, two with dihedral angles close to conformation **f**, and two close to conformation **g**. B3LYP/6-31G* optimization of the MMFF94 structures gives the same seven conformations as obtained via the B3LYP/6-31G* PES scan.

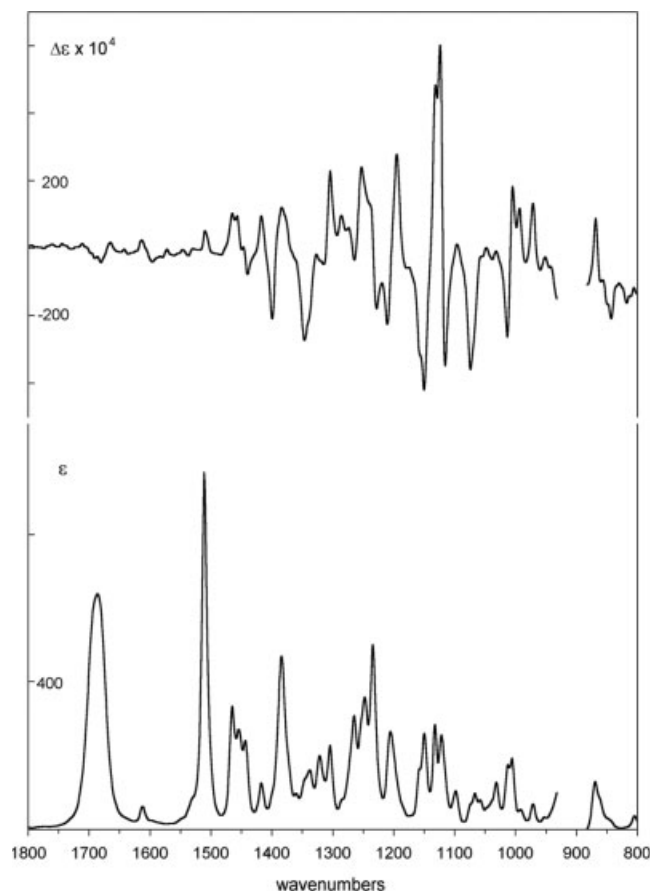


Fig. 11. Experimental IR and VCD spectra of (–)-**5** in CDCl_3 ; concentration 0.225 M; pathlength 50 μm .

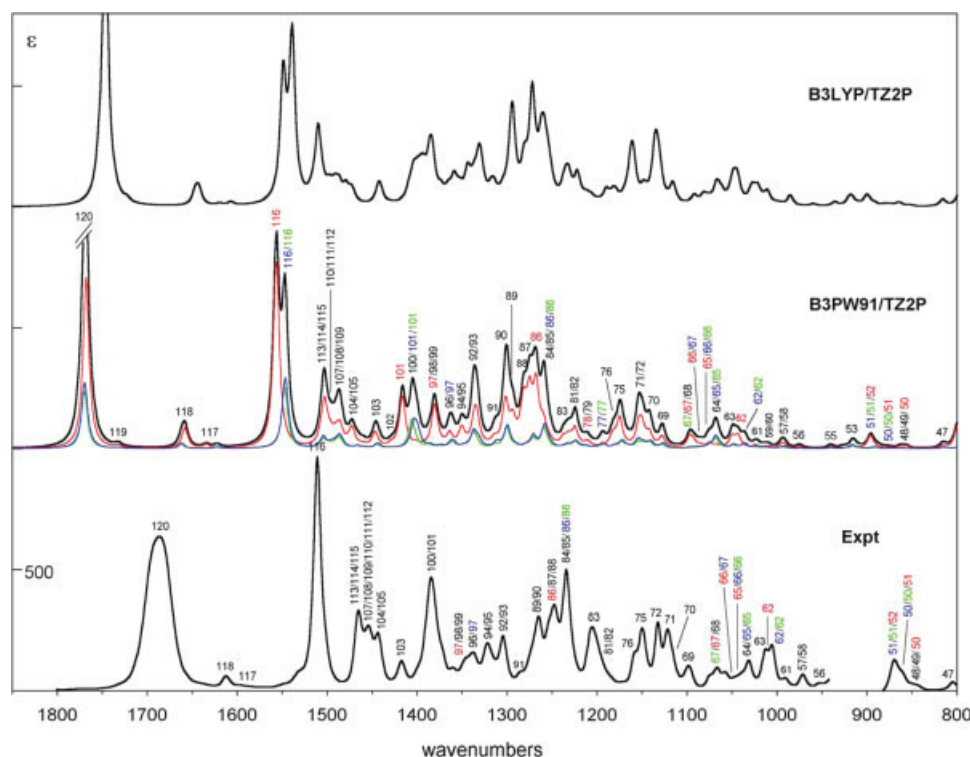


Fig. 12. Comparison of the experimental and B3LYP/TZ2P and B3PW91/TZ2P IR spectra of **5** for the range 800–1850 cm^{-1} . The black calculated spectra are the conformationally-averaged spectra of conformations **Ia–Ie** and **IIa**. The red, green, and blue calculated spectra are the population-weighted IR spectra of conformations **Ia**, **Ib**, and **Ic** of **5** respectively. The numbers define the fundamentals contributing to resolved bands. Red, green, and blue numbers refer to fundamentals of **Ia**, **Ib**, and **Ic** respectively. Black numbers are used when the bands of **Ia**, **Ib**, and **Ic** are not resolved. Bandshapes of the calculated spectra are Lorentzian ($\gamma = 4.0 \text{ cm}^{-1}$). The assignment of the experimental spectrum is based on the B3PW91/TZ2P calculated spectrum.

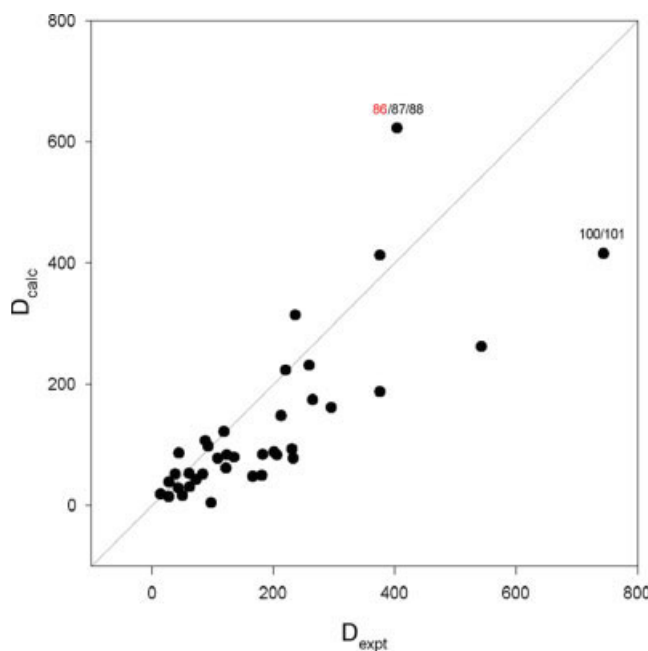


Fig. 13. Comparison of experimental and B3PW91/TZ2P dipole strengths of **5**. Calculated dipole strengths are population-weighted averages of conformations **Ia–Ie** and **IIa**. The line is of slope +1. Dipole strengths are in $10^{-40} \text{ esu}^2 \text{ cm}^2$.

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On the basis of these results, we anticipate there to be 21 conformations of **5**, with three core conformations **I–III** and seven dimethoxybenzene conformations **a–g**. A Monte-Carlo MMFF94 search for **5** does lead to 21 conformations, with the relative energies given in Table 5.

However, B3LYP/6-31G* optimizations of these structures lead to only 16 inequivalent conformations; their relative energies and dihedral angles D1–D6 are given in Table 5. Comparison of the dihedral angles to those of **6** and **8** shows that conformations **Ia–Ie**, **IIa–IIg**, and **IIIa–IIIg** have been obtained; consequently, **If**, **Ig**, **IIg**, **IIIg**, and **IIIg** are missing. We have therefore built and optimized the five missing conformations with the results given in Table 5. The B3LYP/6-31G* structures of conformations **Ia–Ig** of **5** are shown in Figure 5.

For the 14 conformations with relative energies within 5 kcal/mol we have calculated the room temperature free energy differences and equilibrium populations, with the results given in Table 5. Conformations **Ia**, **Ib**, and **Ic** are dominant, constituting nearly 90% of the equilibrium mixture. We have further reoptimized the 14 B3LYP/6-31G* conformations at the B3LYP/TZ2P and B3PW91/TZ2P levels and calculated the relative free energies and equilibrium populations, with the results given in Table 5. At these levels, **Ia**, **Ib**, and **Ic** constitute >90% of the equilibrium conformational mixture.

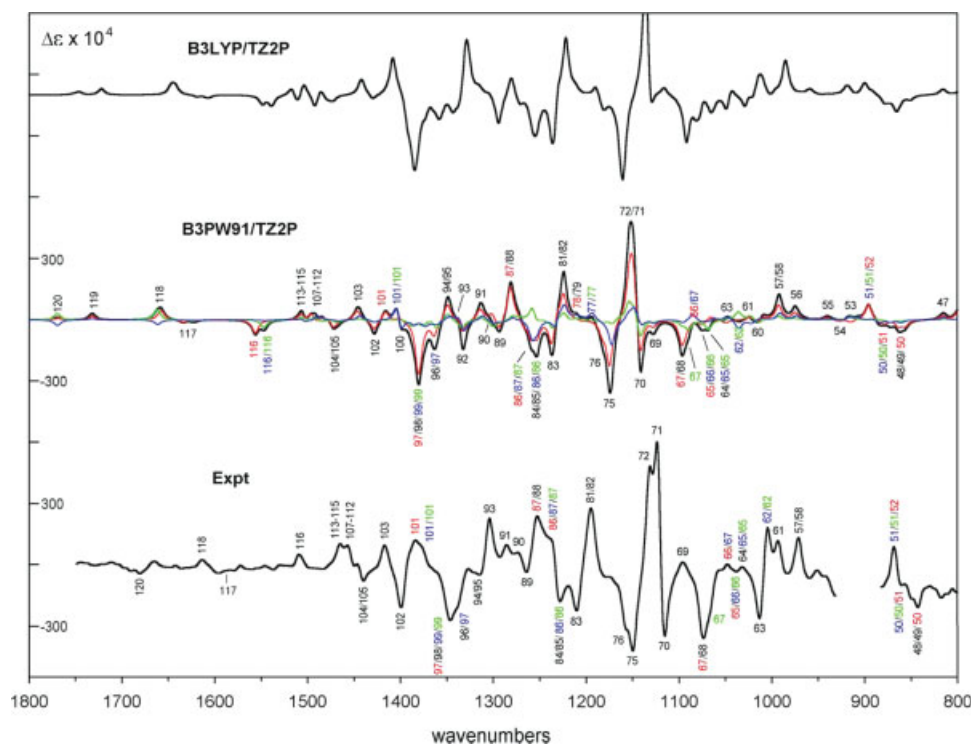


Fig. 14. Comparison of the experimental and B3LYP/TZ2P and B3PW91/TZ2P VCD spectra of **5** for the range 800–1800 cm^{-1} . The black calculated spectra are conformationally-averaged spectra of conformations **Ia–Ie** and **IIa**. The red, green, and blue calculated spectra are the population-weighted VCD spectra of conformations **Ia**, **Ib**, and **Ic** of **5** respectively. The numbers define the fundamentals contributing to resolved bands. Red, green, and blue numbers refer to fundamentals of **Ia**, **Ib**, and **Ic** respectively. Black numbers are used when the bands of **Ia**, **Ib**, and **Ic** are not resolved. Band-shapes of the calculated spectra are Lorentzian ($\gamma = 4.0 \text{ cm}^{-1}$). The assignment of the experimental spectrum is based on the B3PW91/TZ2P calculated spectrum.

IR and VCD Spectra of **4**

The IR and VCD spectra of the naturally-occurring (–)-**4** in a 0.255 M CDCl_3 solution in the mid-IR spectral region are shown in Figure 6. The harmonic frequencies, dipole strengths and rotational strengths of conformations **Ia**, **Ib**, and **IIa** have been calculated using DFT at the B3LYP/TZ2P and B3PW91/TZ2P levels, with the results given in Table 1 of the supplementary material (SM). The IR spectra of the three conformations are obtained thence using Lorentzian band shapes, weighted by their equilibrium populations, and summed to give the conformationally-averaged IR spectrum. The conformationally-averaged B3LYP/TZ2P and B3PW91/TZ2P IR spectra of **4** are compared to the experimental IR spectrum in Figure 7. The B3PW91/TZ2P spectrum is in closer agreement with the experimental spectrum than is the B3LYP/TZ2P spectrum; the assignment of the experimental spectrum is consequently based on the B3PW91/TZ2P spectrum, as detailed in Figure 7 and Table 1 of the SM.

Experimental frequencies and dipole strengths have been obtained by Lorentzian fitting, with the results given in Table 1 of the SM. Experimental and B3PW91/TZ2P dipole strengths are compared in Figure 8. Their good agreement supports the assignment of the IR spectrum of **4** and the reliability of the B3PW91/TZ2P conformational geometries and populations and their predicted IR spectra.

The conformationally-averaged B3LYP/TZ2P and B3PW91/TZ2P VCD spectra of (2*R*,7*R*,20*S*,21*S*)-**4** are compared to the experimental VCD spectrum of (–)-**4** in Figure 9. The assignment of the experimental spectrum follows from the assignment of the experimental IR spectrum (since each vibrational mode contributes to the IR and VCD spectra at the same frequency), and is detailed in Figure 9. Comparison of the calculated and experimental spectra again shows that the B3PW91/TZ2P spectrum is in better agreement with the experimental spectrum than is the B3LYP/TZ2P spectrum. On the basis of the good agreement of the B3PW91/TZ2P VCD spectrum of (2*R*,7*R*,20*S*,21*S*)-**4** and the experimental VCD spectrum of (–)-**4**, it follows that the AC of **4** is (2*R*,7*R*,20*S*,21*S*)-(–).

Experimental rotational strengths have been obtained by Lorentzian fitting, with the results given in Table 1 of the SM. Experimental rotational strengths for (–)-**4** and B3PW91/TZ2P rotational strengths for both enantiomers of **4** are compared in Figure 10. The agreement for (2*R*,7*R*,20*S*,21*S*)-**4** is good, while for (2*S*,7*S*,20*R*,21*R*)-**4** it is extremely bad, confirming unambiguously that the AC of **4** is (2*R*,7*R*,20*S*,21*S*)-(–).

IR and VCD Spectra of **5**

The IR and VCD spectra of the naturally-occurring (–)-**5** in a 0.225 M CDCl_3 solution in the mid-IR spectral

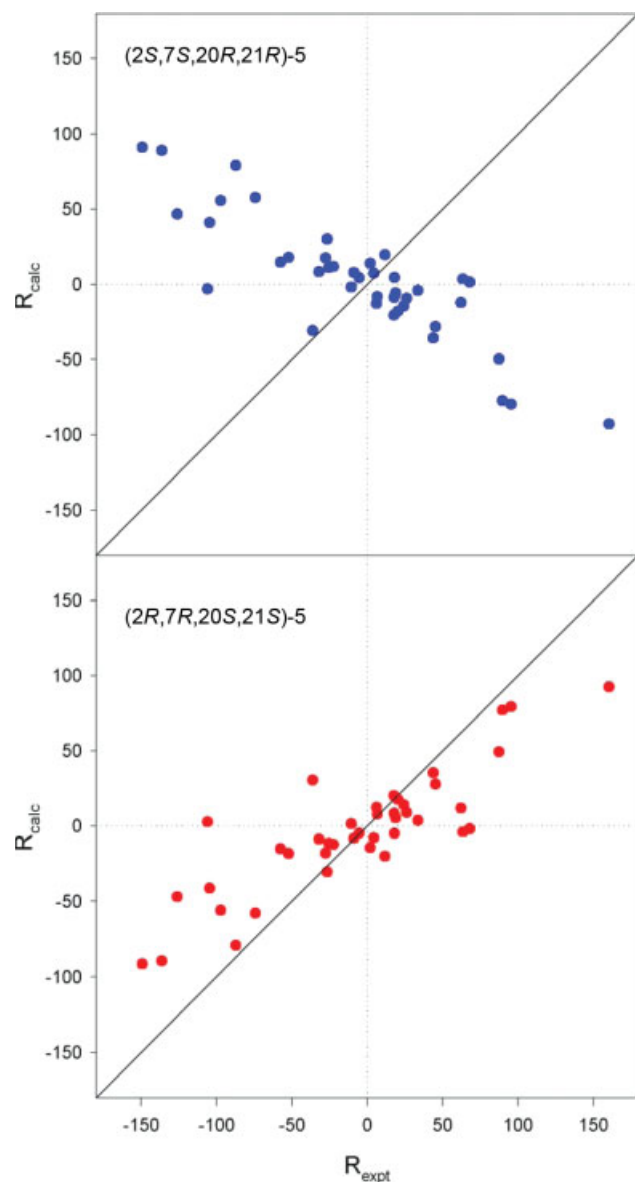


Fig. 15. Comparison of experimental and B3PW91/TZ2P rotational strengths of **5**. Experimental rotational strengths are for (–)-**5**. Calculated rotational strengths are for (2*R*,7*R*,20*S*,21*S*)- and (2*S*,7*S*,20*R*,21*R*)-**5**. Calculated rotational strengths are population-weighted averages of conformations **Ia–Ie**, and **Ila**. The lines are of slope +1. Rotational strengths are in 10^{-44} esu² cm².

region are shown in Figure 11. The harmonic frequencies, dipole strengths and rotational strengths of conformations **Ia–Ie** and **Ila** have been calculated using DFT at the B3LYP/TZ2P and B3PW91/TZ2P levels, with the results (for the most populated conformations **Ia–Ic**) given in Table 2 of the SM. The IR spectra of the six conformations are obtained thence using Lorentzian band shapes, weighted by their equilibrium populations, and summed to give the conformationally-averaged IR spectrum. The conformationally-averaged B3LYP/TZ2P and B3PW91/TZ2P *Chirality* DOI 10.1002/chir

IR spectra of **5** are compared to the experimental IR spectrum in Figure 12. The B3PW91/TZ2P spectrum is in closer agreement with the experimental spectrum than is the B3LYP/TZ2P spectrum; the assignment of the experimental spectrum is consequently based on the B3PW91/TZ2P spectrum, as detailed in Figure 12 and Table 2 of the SM.

Experimental frequencies and dipole strengths have been obtained by Lorentzian fitting, with the results given in Table 2 of the SM. Experimental and B3PW91/TZ2P dipole strengths are compared in Figure 13. Their good agreement supports the assignment of the IR spectrum of **5** and the reliability of the B3PW91/TZ2P conformational geometries and populations and their predicted IR spectra.

The conformationally-averaged B3LYP/TZ2P and B3PW91/TZ2P VCD spectra of (2*R*,7*R*,20*S*,21*S*)-**5** are compared to the experimental VCD spectrum of (–)-**5** in Figure 14. The assignment of the experimental spectrum follows from the assignment of the experimental IR spectrum and is detailed in Figure 14. Comparison of the calculated and experimental spectra again shows that the

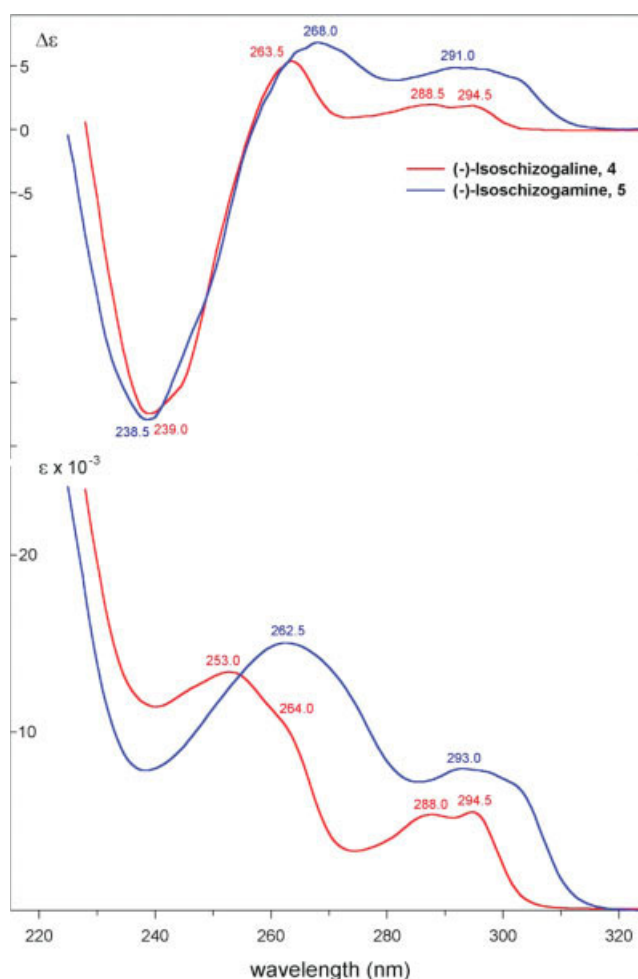


Fig. 16. Experimental UV and ECD spectra of (–)-**4** and (–)-**5** in CHCl₃; concentrations **4**: 0.0125 M; and **5**: 0.012 M; pathlengths for both: 100 μm.

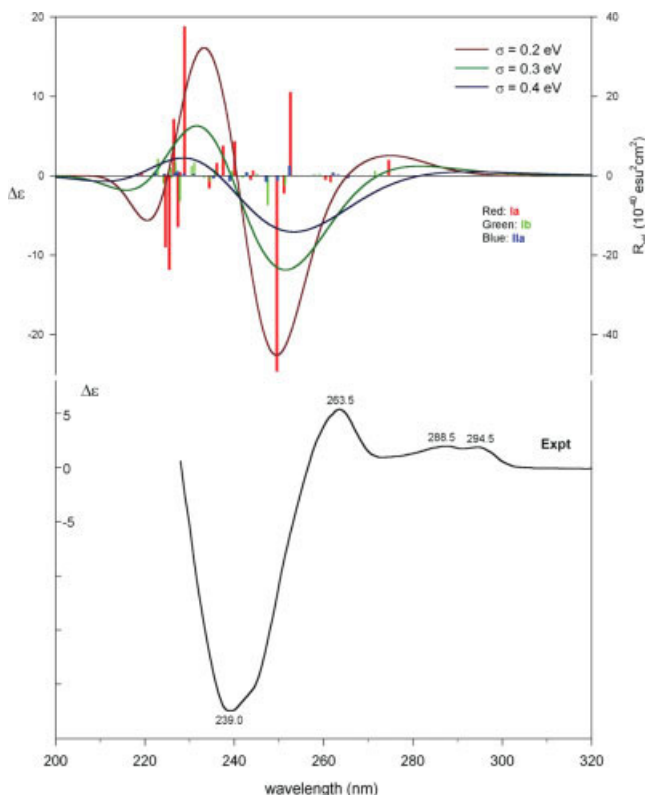


Fig. 17. Comparison of the B3LYP/aug-cc-pVDZ//B3LYP/6-31G* population-weighted velocity rotational strengths of the conformations of (2*R*,7*R*,20*S*,21*S*)-**4** and the conformationally-averaged ECD spectrum, simulated using Gaussian band shapes ($\sigma = 0.2, 0.3$, and 0.4 eV), to the experimental ECD spectrum of (–)-**4**.

B3PW91/TZ2P spectrum is in much better agreement with the experimental spectrum than is the B3LYP/TZ2P spectrum. On the basis of the excellent agreement of the B3PW91/TZ2P VCD spectrum of (2*R*,7*R*,20*S*,21*S*)-**5** and the experimental VCD spectrum of (–)-**5**, it follows that the AC of **5** is (2*R*,7*R*,20*S*,21*S*)-(–).

Experimental rotational strengths have been obtained by Lorentzian fitting, with the results given in Table 2 of the SM. Experimental rotational strengths for (–)-**5** and B3PW91/TZ2P rotational strengths for both enantiomers of **5** are compared in Figure 15. The agreement for (2*R*,7*R*,20*S*,21*S*)-**5** is good, while for (2*S*,7*S*,20*R*,21*R*)-**5** it is extremely bad, confirming unambiguously that the AC of **5** is (2*R*,7*R*,20*S*,21*S*)-(–).

The ECD Spectra of **4** and **5**

The experimental UV and ECD spectra of (–)-**4** and (–)-**5** are shown in Figure 16. Both UV and ECD spectra of **4** and **5** are qualitatively similar. The most intense features in the ECD spectra are the negative ECD at 239 nm.

The electronic excitation energies, oscillator strengths, velocity rotational strengths, and length rotational strengths of the 20 lowest excitations of (2*R*,7*R*,20*S*,21*S*)-**4** have been calculated at the B3LYP/aug-cc-pVDZ//B3LYP/6-31G* level, with the results given in Table 3 of the SM. In Figure 17, the equilibrium population-weighted

origin-independent velocity rotational strengths are plotted, together with the conformationally-averaged ECD spectra, obtained using Gaussian bandshapes with $\sigma = 0.2, 0.3$, and 0.4 eV. In Figure 18, the predicted ECD spectra for both enantiomers of **4** are compared to the experimental spectrum of (–)-**4**. At wavelengths > 240 nm, the calculated spectrum of (2*R*,7*R*,20*S*,21*S*)-**4** exhibits (+) and (–) features at ~ 270 and 250 nm, the latter being substantially greater in intensity, in qualitative agreement with the experimental spectrum. At wavelengths > 240 nm the calculated spectrum of (2*S*,7*S*,20*R*,21*R*)-**4** is inconsistent with the experimental ECD. The comparison of calculated and experimental ECD spectra thus leads to the conclusion that the AC of **4** is (2*R*,7*R*,20*S*,21*S*)-(–).

The electronic excitation energies, oscillator strengths, velocity rotational strengths, and length rotational strengths of the 20 lowest excitations of (2*R*,7*R*,20*S*,21*S*)-**5** have been calculated at the B3LYP/aug-cc-pVDZ//B3LYP/6-31G* level, with the results given in Table 4 of the SM. In Figure 19, the equilibrium population-weighted velocity rotational strengths are plotted, together with the conformationally-averaged ECD spectra, obtained using Gaussian bandshapes with $\sigma = 0.2, 0.3$, and 0.4 eV. In

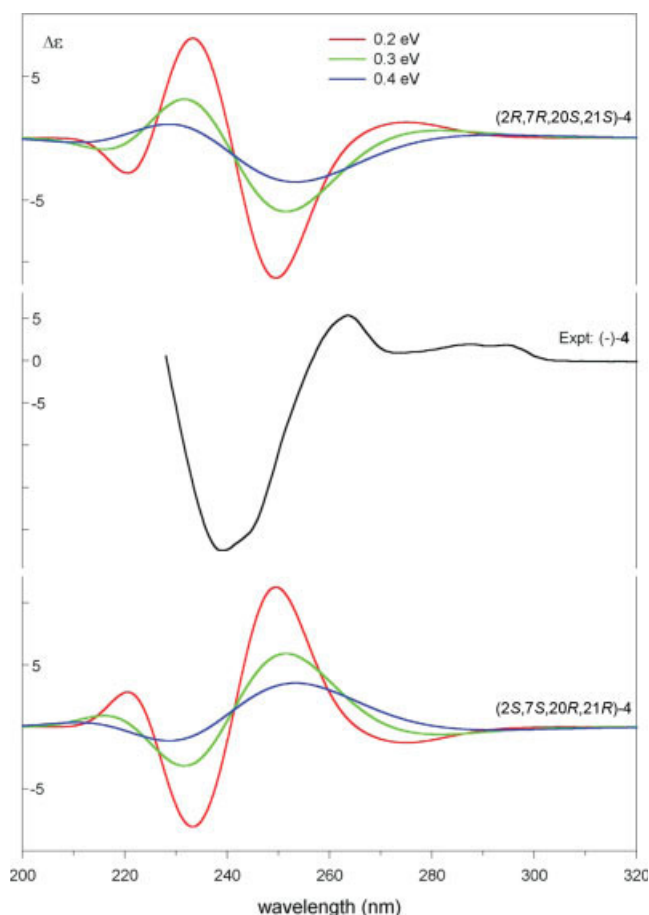


Fig. 18. Comparison of the experimental ECD spectrum of (–)-**4** and the B3LYP/aug-cc-pVDZ//B3LYP/6-31G* ECD spectra of the two enantiomers of **4**.

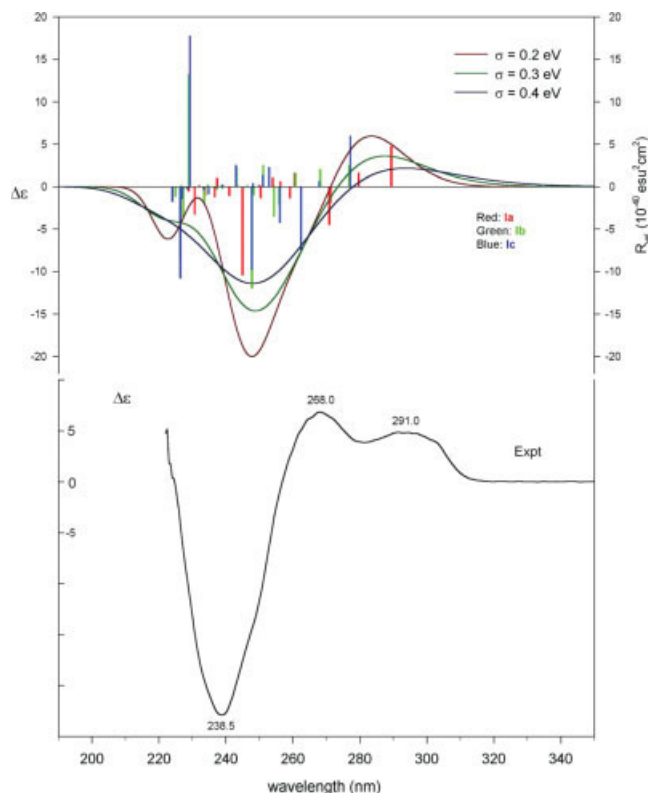


Fig. 19. Comparison of the B3LYP/aug-cc-pVDZ//B3LYP/6-31G* population-weighted velocity rotational strengths of the conformations of (2*R*,7*R*,20*S*,21*S*)-**5** and the conformationally-averaged ECD spectrum, simulated using Gaussian band shapes ($\sigma = 0.2, 0.3$, and 0.4 eV), to the experimental ECD spectrum of (–)-**5**.

Figure 20, the predicted ECD spectra for both enantiomers of **5** are compared to the experimental spectrum of (–)-**5**. At wavelengths > 230 nm, the calculated spectrum of (2*R*,7*R*,20*S*,21*S*)-**5** exhibits (+) and (–) features at ~ 280 and 250 nm, the latter being substantially greater in intensity, in qualitative agreement with the experimental spectrum. At wavelengths > 230 nm the calculated spectrum of (2*S*,7*S*,20*R*,21*R*)-**5** is inconsistent with the experimental ECD. The comparison of calculated and experimental ECD spectra thus leads to the conclusion that the AC of **5** is (2*R*,7*R*,20*S*,21*S*)-(–).

The Optical Rotations of **4** and **5**

Both natural products **4** and **5** exhibit negative specific rotations over the transparent wavelength range 633–365 nm, increasing in magnitude with decreasing wavelength. At all wavelengths, the rotations of **4** are greater than those of **5**.

The specific rotations of conformations **Ia**, **Ib**, and **Ila** of (2*R*,7*R*,20*S*,21*S*)-**4** and conformations **Ia–Ie** and **Ila** of (2*R*,7*R*,20*S*,21*S*)-**5** have been calculated at the experimental wavelengths using TDDFT at the B3LYP/aug-cc-pVDZ//B3LYP/6-31G* level, with the results given in Tables 6 and 7, together with the conformationally-averaged rotations, which are plotted in Figure 21 for both enantiomers of **4** and **5**. For the AC 2*R*, 7*R*, 20*S*, 21*S* the *Chirality* DOI 10.1002/chir

predicted rotations of both **4** and **5** are in perfect qualitative agreement and good quantitative agreement with the experimental rotations, leading unambiguously to the AC (2*R*,7*R*,20*S*,21*S*)-(–) for **4** and **5**. It is further noteworthy that the predicted rotations of **4** are greater in magnitude than those of **5**, as observed experimentally.

DISCUSSION

Comparisons of DFT calculations of the VCD, ECD, and OR of **4** and **5** to experimental VCD, ECD, and OR data of the natural products (–)-**4** and (–)-**5** yield the ACs:

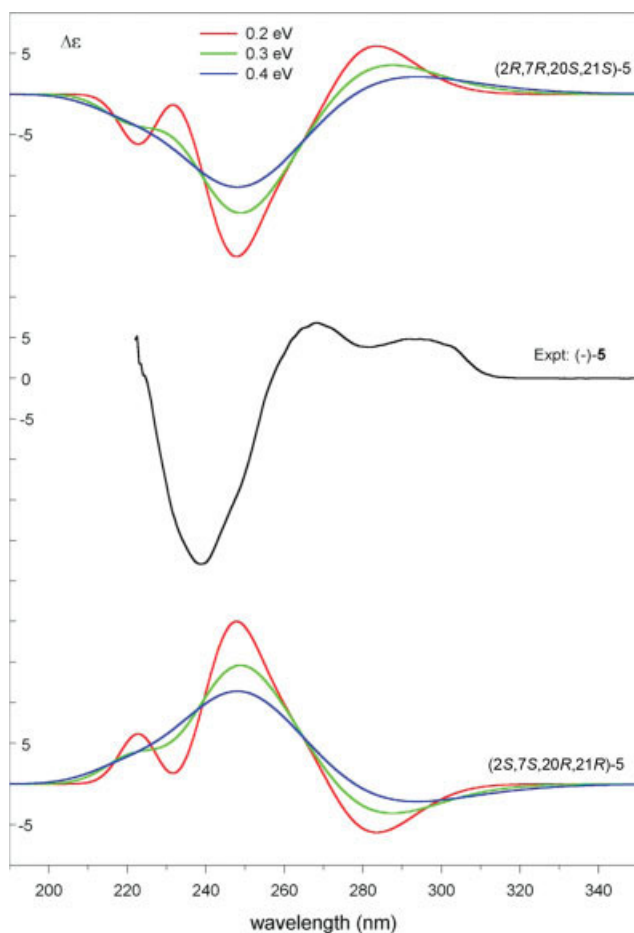
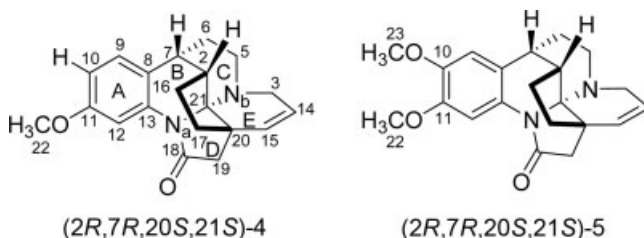


Fig. 20. Comparison of the experimental ECD spectrum of (–)-**5** and the B3LYP/aug-cc-pVDZ//B3LYP/6-31G* ECD spectra of the two enantiomers of **5**.

TABLE 6. The calculated and experimental specific rotations^a of **4**

Conformer	$[\alpha]_{633}$	$[\alpha]_D$	$[\alpha]_{546}$	$[\alpha]_{436}$	$[\alpha]_{405}$	$[\alpha]_{365}$
1a	−273.06	−322.17	−386.03	−683.12	−838.44	−1145.04
1b	−276.34	−326.16	−391.01	−693.39	−851.85	−1165.18
11a	−205.54	−240.07	−283.85	−470.45	−556.31	−700.12
Avg ^b	−270.46	−319.00	−382.09	−674.91	−827.51	−1127.62
Expt ^c	−210.40	−247.30	−298.80	−520.70	−634.10	−853.60

^aSpecific rotations in degrees[dm/g/cm³]^{−1}. The specific rotations are calculated at the B3LYP/aug-cc-pVDZ//B3LYP/6-31G* level for (2*R*,7*R*,20*S*,21*S*)-**4**.

^bPopulation-weighted average.

^cCHCl₃ (c 0.275) [Ref. 1: $[\alpha]_D$ −262.3 in CHCl₃ (c 1.00)].

TABLE 7. Comparison of the experimental and calculated specific rotations^a of **5**

Conformer	$[\alpha]_{633}$	$[\alpha]_D$	$[\alpha]_{546}$	$[\alpha]_{436}$	$[\alpha]_{405}$	$[\alpha]_{365}$
5-1a	−266.19	−313.07	−373.48	−643.75	−775.20	−1001.44
5-1b	−272.46	−321.32	−384.73	−676.75	−826.45	−1112.14
5-1c	−241.02	−283.52	−338.38	−586.96	−711.77	−943.70
5-1d	−191.39	−224.71	−267.54	−458.45	−551.93	−718.72
5-1e	−326.02	−384.55	−460.49	−808.71	−985.05	−1312.16
5-11a	−214.07	−248.79	−292.05	−461.60	−525.41	−582.98
Avg ^b	−258.40	−304.13	−363.22	−630.68	−764.06	−1006.93
Expt ^c	−187.00	−218.00	−260.00	−440.00	−521.00	−643.00

^aSpecific rotations in degrees[dm/g/cm³]^{−1}. The specific rotations are calculated at the B3LYP/aug-cc-pVDZ//B3LYP/6-31G* level for (2*R*,7*R*,20*S*,21*S*)-**5**.

^bPopulation-weighted average.

^cCHCl₃ (c 0.219) [Ref. 1: $[\alpha]_D$ −239.0 in CHCl₃ (c 1.00)].

The consistency of the ACs resulting from the three chiroptical properties for each molecule supports the conclusion that the ACs are definitive. At the same time, it must be noted that the agreement of calculated and experimental data is far superior for the VCD and ORD than for the ECD. As a result, the ACs of **4** and **5** are most definitively determined by their VCD and ORD. The lower utility of ECD spectra has been previously observed in our recently published studies of the natural products schizozigine,⁸ plumericin and isoplumericin.⁴² Indeed, in the cases of the latter two molecules, TDDFT ECD spectra did not permit the determination of their ACs at all. This situation can be attributed to the substantial density of low-energy electronic excited states in these molecules giving rise to electronic excitations in the near-UV, preventing the resolution and assignment of individual transitions. As a result, the predicted ECD spectra are sensitive to the band-shapes and -widths of the electronic transitions, which are neither exactly Gaussian nor of constant width, as assumed in the simulation of the ECD spectra. It follows that the use of ECD for determining ACs is most effective when the low-energy electronic transitions are limited in number and density, and hence well-resolved experimentally. Such a situation was observed in our study of the ECD of the natural product quadron,⁴³ where the carbonyl group $n \rightarrow \pi^*$ transition was well-resolved.

Given the close similarity in structure of **4** and **5**, one might expect their chiroptical properties to be similar, and that the AC of **5** could be determined empirically simply

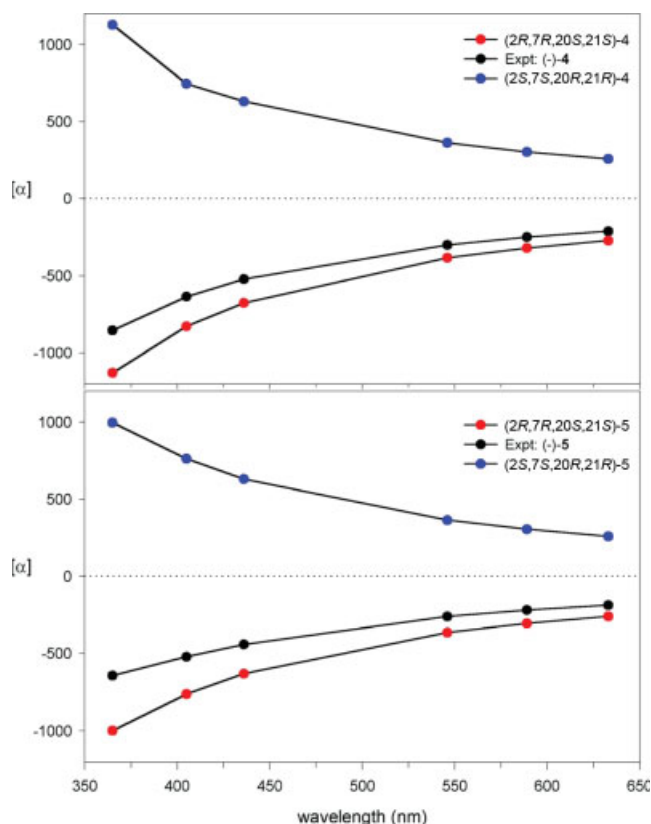


Fig. 21. Comparison of experimental and calculated specific rotations of **4** and **5**.

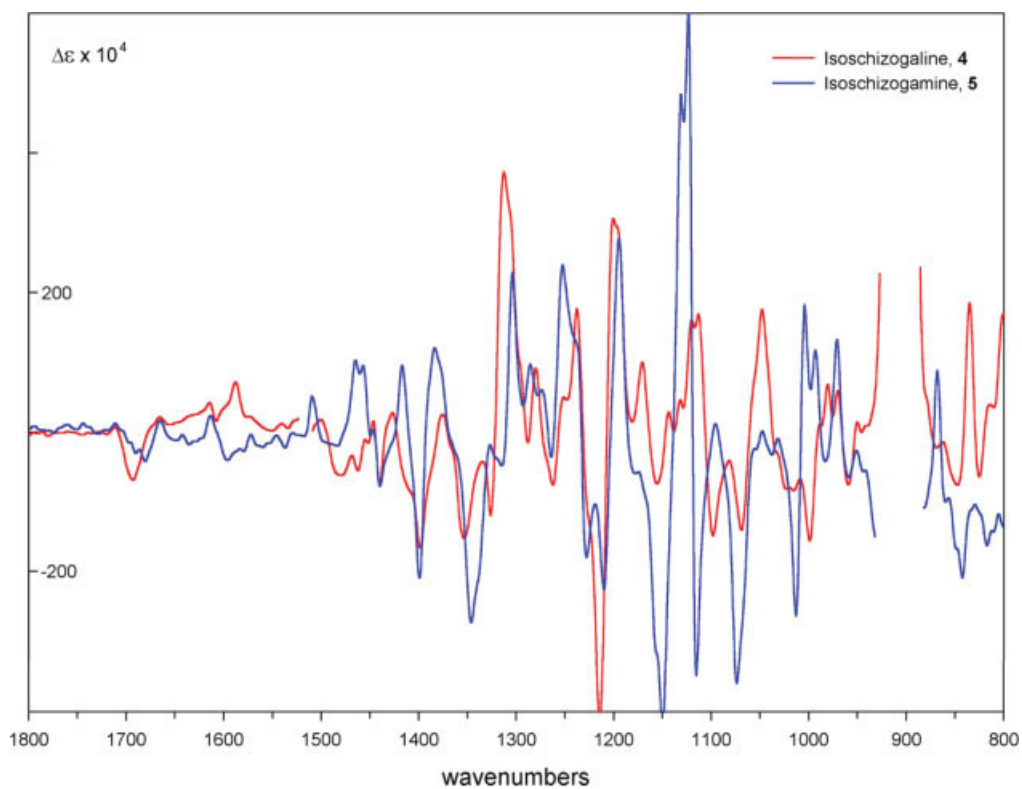


Fig. 22. Superposition of the experimental VCD spectra of (–)-4 and (–)-5.

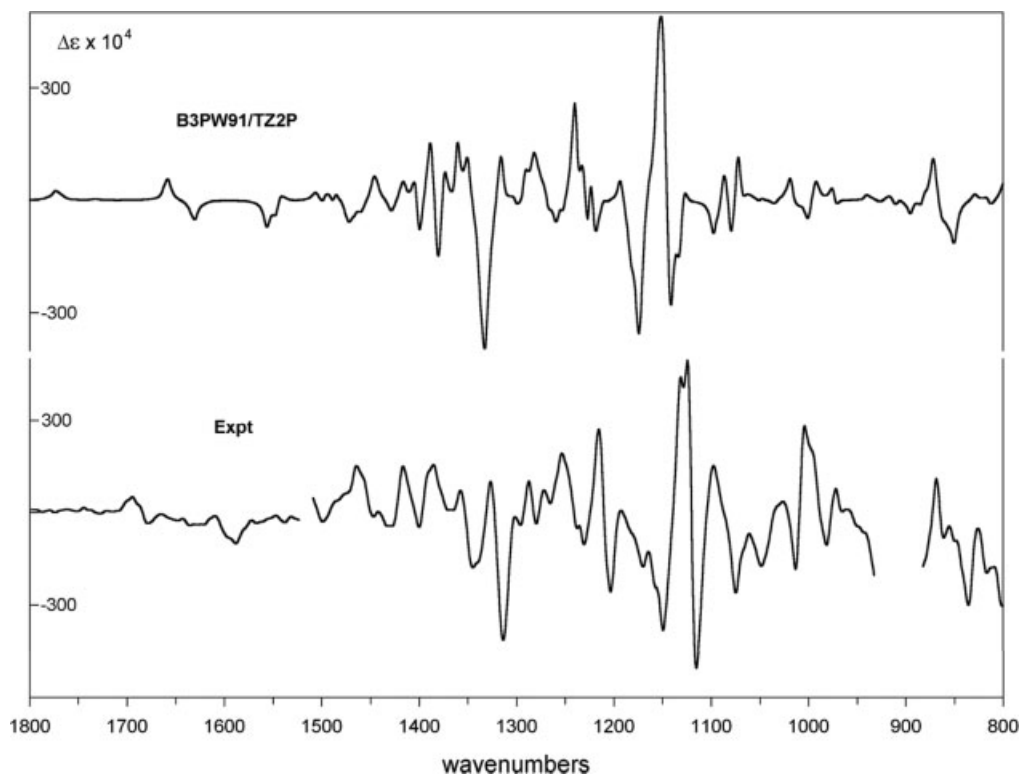
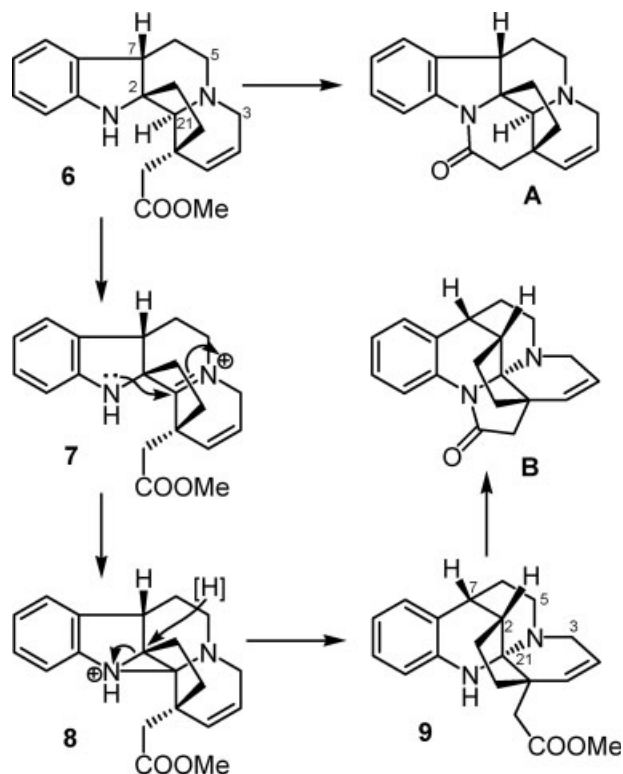


Fig. 23. Comparison of the B3PW91/TZ2P difference VCD spectrum, $\Delta\epsilon(4) - \Delta\epsilon(5)$, of (2*R*,7*R*,20*S*,21*S*)-4 and (2*R*,7*R*,20*S*,21*S*)-5 to the experimental difference VCD spectrum $\Delta\epsilon((-)-4) - \Delta\epsilon((-)-5)$.



Scheme 1. Biosynthesis proposal for schizozygane alkaloids (A) and isoschizozygane alkaloids (B).

by comparison of its chiroptical properties to those of **4**, or vice versa. Interestingly, while the experimental ECD spectra and ORD of (–)-**4** and (–)-**5** are indeed very similar, their VCD spectra are significantly different, as shown in Figure 22, demonstrating that vibrational spectra are much more sensitive to molecular structure than are electronic spectra. To provide further support for the reliability of the B3PW91/TZ2P VCD calculations for **4** and **5** we have predicted the difference VCD spectrum and compared to the experimental difference spectrum, as shown in Figure 23. The agreement of calculated and experimental difference spectra is excellent, further confirming the excellent reliability of the VCD calculations and the ACS of **4** and **5** derived thence.

In 1998⁵ we postulated that both schizozygane (A) and isoschizozygane alkaloids (B) could originate biosynthetically from a common intermediate **9**, as shown in Scheme 1. The ACS of (–)-isoschizogaline (**4**) and (–)-isoschizogamine (**5**) and the recently determined ACS of the schizozygane alkaloids schizozygine (**1**), schizogaline (**2**), and schizogamine (**3**) together provide further support for this biosynthesis proposal.

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Review Article

The Use of Circular Dichroism Spectroscopy for Studying the Chiral Molecular Self-Assembly: An Overview

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT Self-assembly plays an important role in the formation of many chiral biological structures and in the preparation of chiral functional materials. Therefore the control of chirality in synthetic or biological self-assembled systems is important either for the comprehension of recognition phenomena or to obtain materials with predictable and controllable properties. Circular dichroism was developed to study molecular chirality, however, because of its outstanding sensitivity to chiral perturbations of the system under investigation; it has been extended more recently to supramolecular chemistry. In particular, self-assembly processes leading to the formation of chiral supramolecular architectures (and eventually to gels or liquid crystal phases) can be monitored by CD. Furthermore, CD spectroscopy often allows one to obtain structural information on the assembled structures. This review deals with representative contributions to the study of supramolecular chirality by means of circular dichroism. *Chirality* 20:471–485, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: circular dichroism; self-assembly; chirality; supramolecular chemistry; liquid crystal

INTRODUCTION

Chiroptical spectroscopies, optical rotatory dispersion (ORD), and circular dichroism (CD)¹ were developed in the 1960s to study mainly steroidal ketones^{2,3} and octahedral metal complexes⁴ whose electronic transitions, with favourable anisotropic factors ($g = \Delta\epsilon/\epsilon$), were accessible to the instrumentation available.

Subsequently, several different chromophores were investigated by CD and interpreted using different theoretical analyses⁵; the exciton approach, in particular, has been very successful for studying molecular systems containing two or more absorbing chromophores, for example, biaryls^{5,6} and proteins.⁷ At the present time, the outstanding sensitivity of CD is employed mainly to study biological macromolecules and their perturbations by external factors. In the field of supramolecular chemistry, CD has several interesting applications. In particular, assembly processes leading to the formation of helical superstructures can be investigated.⁸ In those cases where gels are formed by chiral monomers through self-assembly processes, it seems natural to use CD to monitor the progress of the process and, hopefully, to obtain information on the assembled structures supporting those obtained with other techniques.

The basic principles of CD spectroscopy can be found in several specialized publications.^{1,5,6,9} CD is measured either as ellipticity Θ (in mdeg) or as differential absorption ΔA (in absorbance units), the two values being correlated through the following relation $\Delta A = \Theta/32,980$.

Literature data are usually reported as molar ellipticity ($[\Theta] = \Theta/(10 c_M l)$, where c_M is the molar concentration and l the path length in cm) or molar CD [$\Delta\epsilon = \Delta A/(c_M l)$]. In a few cases the anisotropy factor g is used; it is independent of concentration and linearly related to the enantiomeric excess.

The CD technique is typically suitable for samples measured in isotropic solution (or more rarely in the gas phase). Factors such as sample turbidity, birefringence, and linear dichroism (LD) (caused for example by macroscopic anisotropies) may seriously affect the data.¹⁰ Solid sampling may be difficult or may call for dedicated accessories/special hardware.¹¹ The limitations are many. The main one originates in the optical properties of the photoelastic modulator whose residual static birefringence may distort data. Furthermore, any optical imperfection of the various components (for example, the photomultiplier

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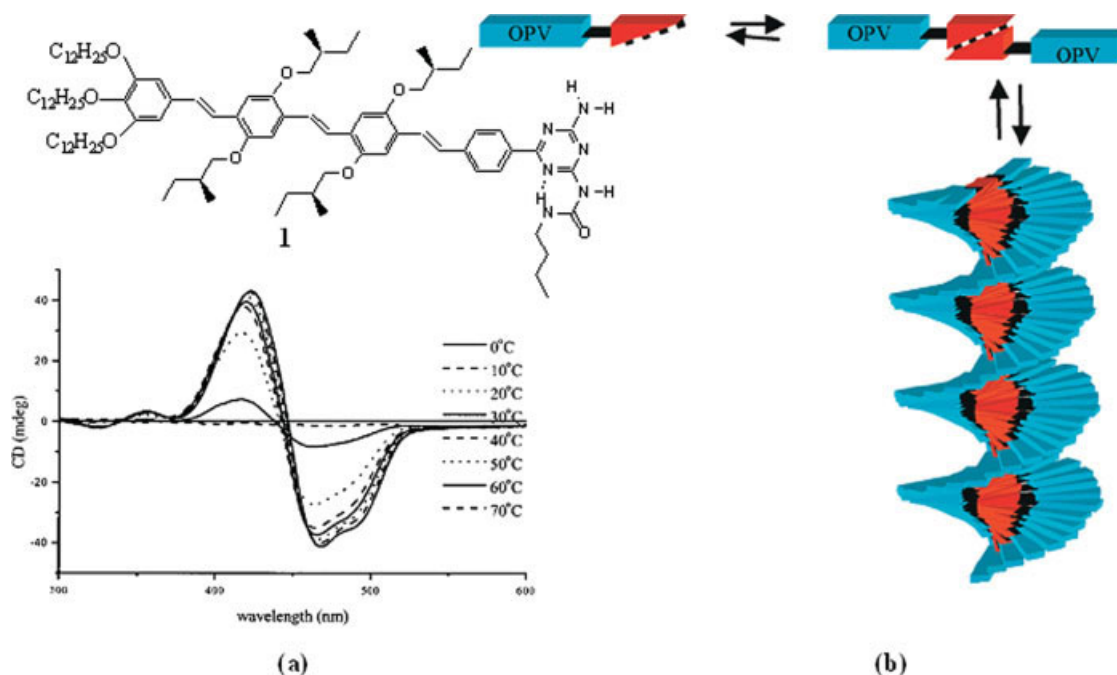


Fig. 1. Temperature-dependent CD spectrum of monofunctional OPV **1** in dodecane (a) and the schematic representation of the hierarchical organization (b). (Reprinted with permission from Reference 19. Schenning APHJ, et al. *Journal of the American Chemical Society* 2001;123:409–416. Copyright 2001 American Chemical Society.)

tube surface) may cause artifacts when working with weakly dichroic samples.¹² The lock-in detection technique, which measures the signal only at the modulation frequency of the photoelastic modulator crystal, is also prone to errors. Signals at double frequency (like those generated by sample LD) are impossible to remove completely and may distort the final data. For these and other reasons, the observed CD spectra may be different from the real ones, and ways to compensate for or correct the spectra are not easy. Thus, several data in the literature are known to be questionable or even incorrect, since no care has been taken to prevent or correct for artifacts.¹³

As a general rule, simultaneous measurement of the LD spectra may give clear warnings that the CD spectra are not correct. Although relatively little attention has been paid to these effects in the past, more recent literature provides a deeper understanding of these effects and the use of experimental methods to control them.¹⁴

Samples which scatter light will significantly reduce the number of photons reaching the detector; in such cases, the usual approach is to place the sample as close as possible to the photomultiplier tube surface to increase solid angle collection; more sophisticated approaches, such as employing a fluoroscat cell,¹⁵ have been proposed, but rarely used.

In dealing with problematic samples, a rule of thumb is to run the spectra of the samples obtained from both enantiomers, see for example Refs. 16, 17.

THE EXCITON CD AND THE SELF-ASSEMBLY PROCESSES

In general, for solutions, there is a direct correlation between the regions of absorption and CD. In the case of *Chirality* DOI 10.1002/chir

noncoupled chromophores, the shapes of the two spectra are similar, although the vibrational fine structure can be different. If two or more strongly absorbing chromophores are oriented chirally with respect to each other, an exciton spectrum is observed characterized by the presence of two bands with opposite signs, where λ_{max} in absorption corresponds, or nearly corresponds, to zero CD intensity.^{1,5,6} In general, the two bands of an exciton spectrum do not necessarily have the same intensity: a conservative exciton CD spectrum (equal intensity of the two bands) occurs only if the coupled chromophores have an allowed transition well isolated from other transitions. Nondegenerate couplings with other transitions of the same chromophore may alter the relative intensity of the two bands.^{5,6,18}

The exciton case is rather common during the formation of helical aggregates by self-assembly, which is also a common basis of gel formation by chiral gelators. The appearance of an exciton spectrum during the self-assembly of molecules, which show noncoupled spectra in the isolated state, is observed in these cases. This indicates that in the aggregate, the chromophores are arranged in a chiral array, often helicoidal. However, to obtain more precise information on the structure of the assembled species from an exciton spectrum, it is necessary to know the direction of the transition moments of the chromophores involved. The coupling of these moments in a possible model can be calculated, and comparison between the theoretical results and the experimental data can refine the hypotheses. In general, it is not easy to obtain structural information from CD spectra alone, and other complementary techniques should also be employed.

An instructive example is the work of Meijer and co-workers on *p*-oligo(phenylene vinylene)s (OPV) functional-

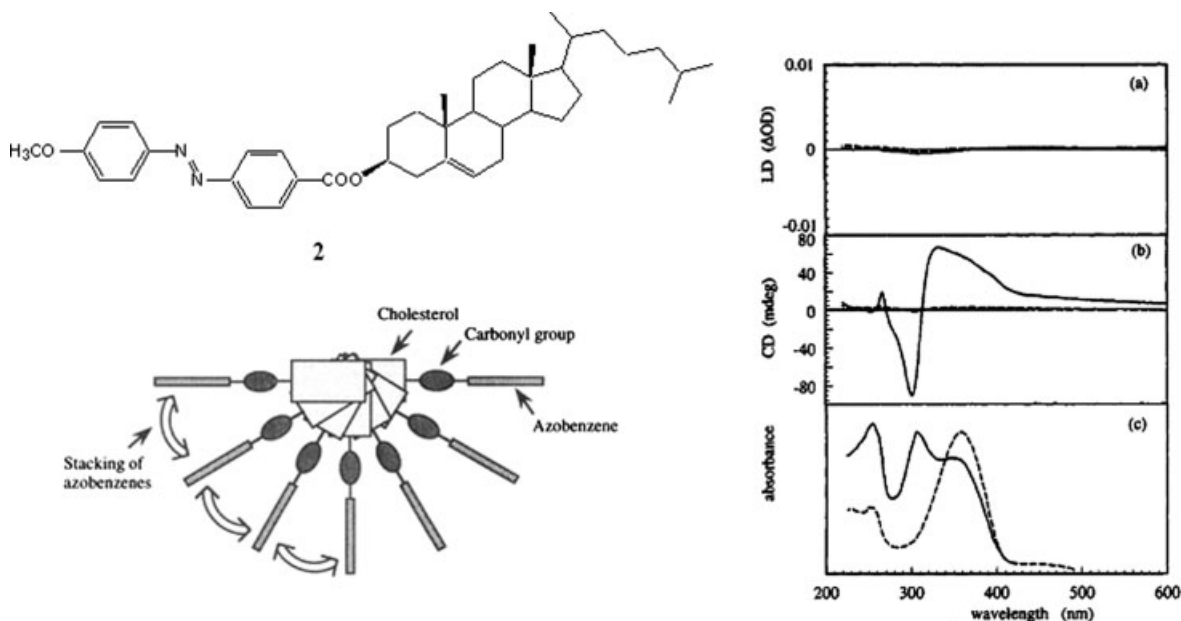


Fig. 2. LD, CD, and absorption spectra of **2** in 1-butanol in the gel (solid line), and the solution state (dash line), and the schematic representation of the aggregation model. (Reprinted with permission from Reference 21. Murata K, et al. *Journal of the American Chemical Society* 1994;116:6664–6676. Copyright 1994 American Chemical Society.)

ized with ureido-S-triazine units.¹⁹ Monofunctional OPV **1** displays an allowed, well isolated $\pi\pi^*$ transition at ca. 440 nm. OPV **1** dimerizes in chloroform without showing significant CD, however, in dodecane, a strong conservative exciton spectrum is observed indicating that the dimers aggregate in a chiral supramolecular stack (Fig. 1). Temperature dependence of the CD allows the detection of the melting temperature of the helical aggregate. More recently, the Meijer group monitored by CD a nucleation process in the self-assembly of OPVs into helical supramolecular fibrillar structures.²⁰

Shinkai and coworkers²¹ report an interesting example of the appearance of an exciton pattern in the CD spectrum of a cholesterol derivative with appended *p*-alkoxyazobenzene groups **2**, during gelation of several solvents (Fig. 2). Before gelation (in the sol state), the spectra in the region of the azo chromophore absorptions are flat; after gelation, an exciton pattern appears.

From the temperature dependence of the CD at a single wavelength, the T_g of the gel was estimated. It agrees well with that obtained on the basis of a mechanical test. To propose a model for the self-assembled species, the authors needed also the help of scanning electron microscopy: fibrils with a left-handed helical structure give a negative exciton couplet and those with a right-handed structure a positive one. In their final model (see Fig. 2), the cholesterol moieties are piled up to give a chiral columnar structure with the chromophoric azobenzene groups at the periphery.

A similar study was described in Ref. 22 on a similar derivative **3** with a stilbene chromophore, hanging at position 3 of cholesterol instead of the azobenzene. The model reported in Figure 3 was formulated with the help of CD spectra and STM microscopy.

Oligo- and poly-thiophenes tend to aggregate to give complex supramolecular structures; an interesting study of this self-assembly was carried out by Meijer and his group²³ on a series of polythiophenes with chiral chains attached at the β positions (e.g., **4**). In the assembled state the CD of these compounds show a complex, nonconservative exciton pattern with vibrational fine structure in the region between 500 and 650 nm corresponding to the polythiophene chromophore (Fig. 4). Furthermore, an odd-even effect was observed when the stereogenic centre of the side-chain is shifted along the chain.²⁴ The CD is attributed to the helical packing of predominantly planar chains to give a superstructure similar to a cholesteric phase.

The interactions between porphyrins can easily be detected and elucidated following the modifications occurring in the UV and CD spectra on passing from isolated molecules to assembled species. To this end, the study of model systems with fixed geometry is a useful starting point.²⁵ In aqueous solutions, porphyrins can assemble into different aggregates according to their structures and experimental conditions. At high concentrations, colloidal solutions and gel-like phases have been observed.

These assembly processes are particularly interesting as they are very sensitive to chiral induction.

Homoassociation of achiral diprotonated *meso*-tetraphenylsulphonato porphyrins **5** (Fig. 5), gives rise to hedge-to-hedge (J) or face-to-face (H) assembled species at low and high concentrations, respectively. Surprisingly, J aggregation of the trisulphonato derivative is chirally sensitive to stirring (Fig. 6)^{26–28}; in particular, Ribò group has carried out a systematic study of the relationship between the handedness of the stirring and the CD observed for J aggregates. The CD spectra seem free from LD and other

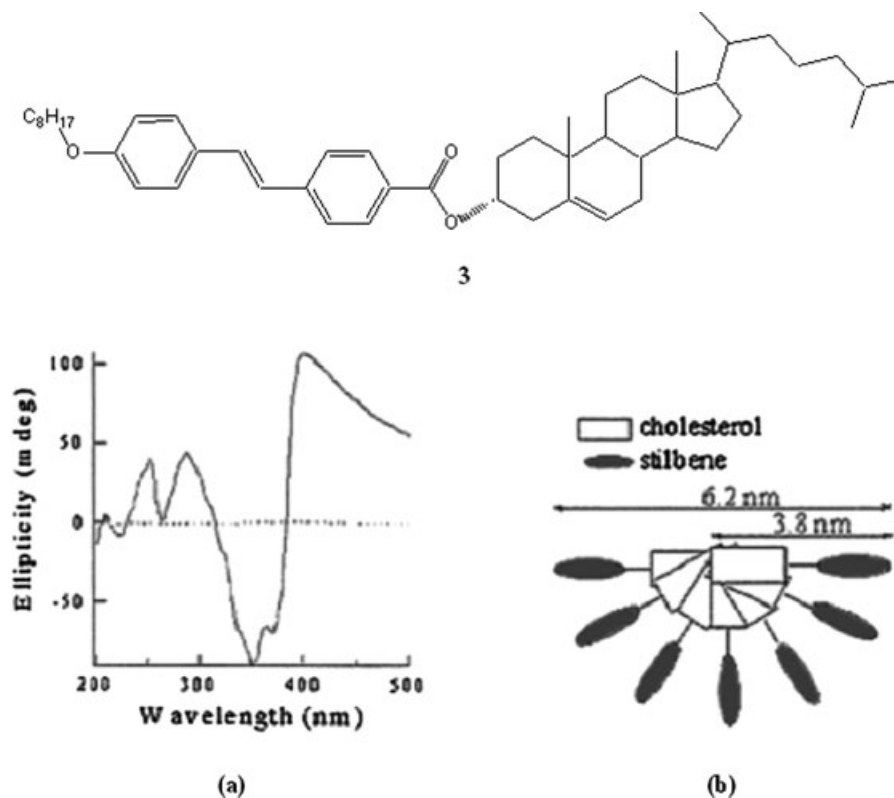


Fig. 3. Circular dichroism spectra (a) of sol (dash line) and gel (solid line) for **3** in octanol, and the possible helical stacking model of the seven molecules in a unit fiber (b). (Reprinted with permission from Reference 22. Wang R, et al. *Journal of the American Chemical Society* 2000;122:2399–2400. Copyright 2000 American Chemical Society.)

artefacts and are very neat in the Soret region. A statistically convincing correlation is observed between the sign of the CD exciton at 480 nm and the stirring handedness: clockwise vortex generate right handed chirality of the J aggregate and viceversa for left-handed vortex.

In the same field, Purrello and his group²⁹ have studied the self-assembly of equimolecular mixtures of achiral cationic and anionic porphyrins in the presence of D- or L-phenylalanine: one observes opposite exciton CD in the Soret region. The chiral aggregates display a memory effect as

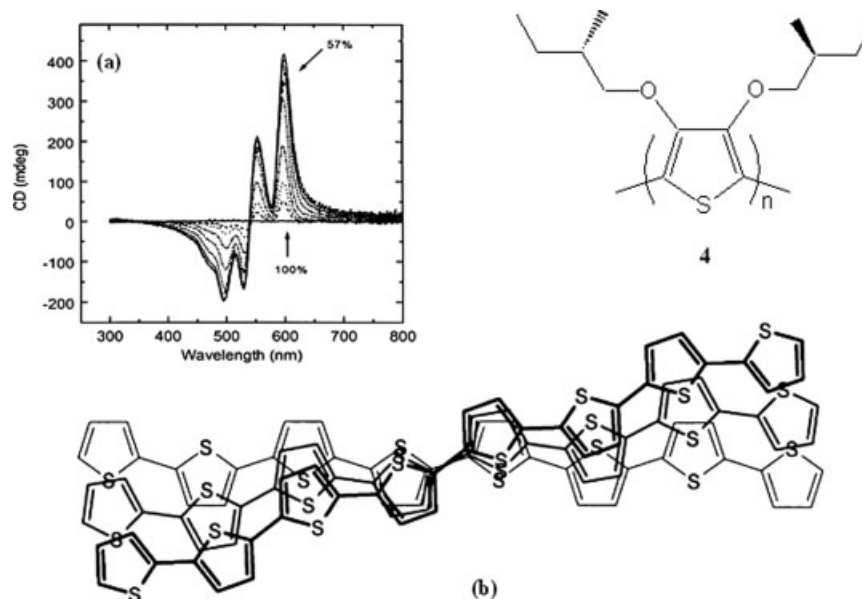


Fig. 4. CD spectra of **4** in various CHCl₃/MeOH mixtures at room temperature (a) and the helical packing of predominantly planar chains of polythiophenes (b). (Reprinted from Reference 23. Langeveld-Voss BMW, et al. *Journal of Molecular Structure* 2000;521:285–301. Reproduced with permission of Elsevier.)

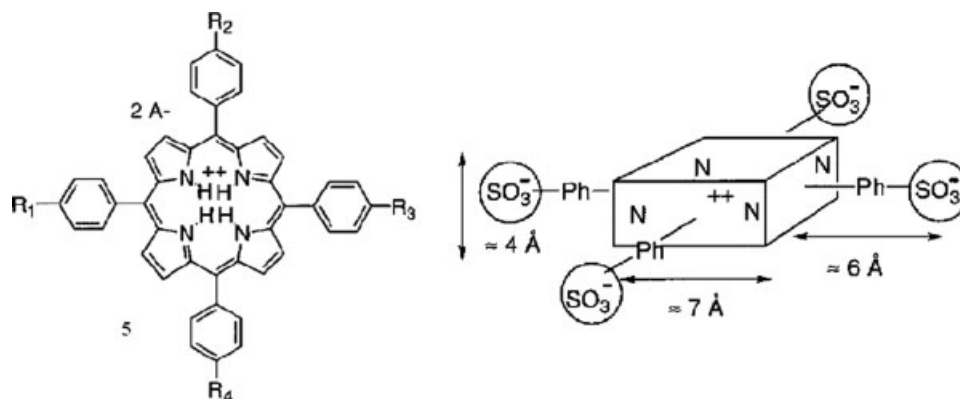


Fig. 5. *Meso*-sulfonatophenyl porphyrins **5** and their schematic structure. (Reprinted from Reference 26. Rubires R, et al. *Chem Eur J* 2001;7:436–446. By permission of Wiley-VCH.)

after removal of Phe by ultrafiltration, the CD signal is almost unaltered.

In another paper,³⁰ the influence of [60]fullerene on the gelling properties of zinc–porphyrin-appended cholesterol derivatives **6** was studied. In particular, CD spectra corresponding to the porphyrin Soret band show a clear exciton pattern in the gel phase obtained only in the presence of fullerene, which seems to impose a left-handed arrangement of the porphyrins through the formation of sandwich-like complexes (Fig. 7). This structure was deduced from CD spectra using the known direction of the effective transition moment of the substituted porphyrin chromophore in the framework of the exciton theory. The authors were very careful during the CD measurements in the gel phase and recorded also the LD spectra. In fact, as mentioned earlier, it is rather dangerous to attribute CD spectra from anisotropic phases without assessing the importance of the LD (that may be much stronger than the CD).^{10,31} If the CD spectrometer cannot measure LD, it

has been recommended to repeat the measurements several times after successive rotation of the cell. If drastic changes of the spectra are caused by rotation, the measurements are meaningless with regard to CD because they are strongly influenced by LD and LB (linear birefringence).^{13,32}

CD AND TUNABLE CHIROPTICAL PROPERTIES

Amplification of chirality occurs when a small initial amount of chiral bias induces a high diastereo- or enantiomeric excess and consequently a strong chiroptical activity. This is commonly referred to as “sergeants and soldiers” principle. After the seminal studies by Green and coworkers in covalent polymeric systems,^{33,34} this phenomenon has been studied in noncovalent supramolecular polymers and in discrete supramolecular assemblies.

Reinhoudt and coworkers investigated the amplification of chirality in double rosette assemblies. (For a general

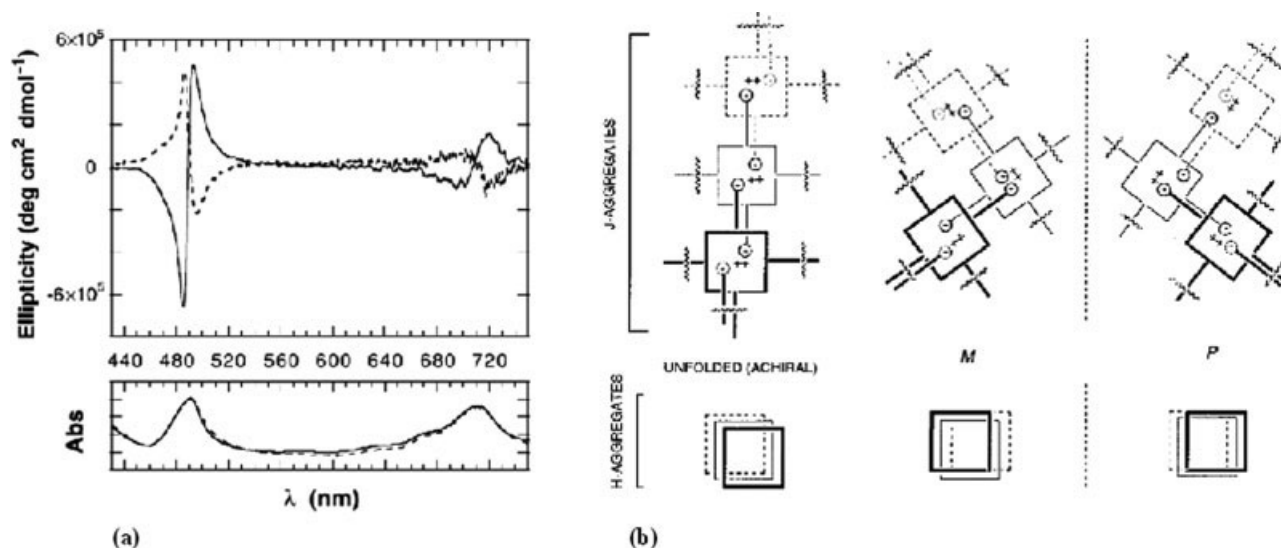


Fig. 6. CD and UV/vis spectra (a) of two solutions (3 μ M) of homoassociates of **5** corresponding to two characteristic experiments: the vortex direction during the rotary evaporation was opposite for the two samples (solid and dashed lines). Schematic arrangement of **5** in the different homoassociates (b). (Reprinted from Reference 26. Rubires R, et al. *Chem Eur J* 2001;7:436–446. By permission of Wiley-VCH and from Reference 27. Ribo JM, et al. *Science* 2001;292:2063–2066. Reprinted with permission from AAAS.)

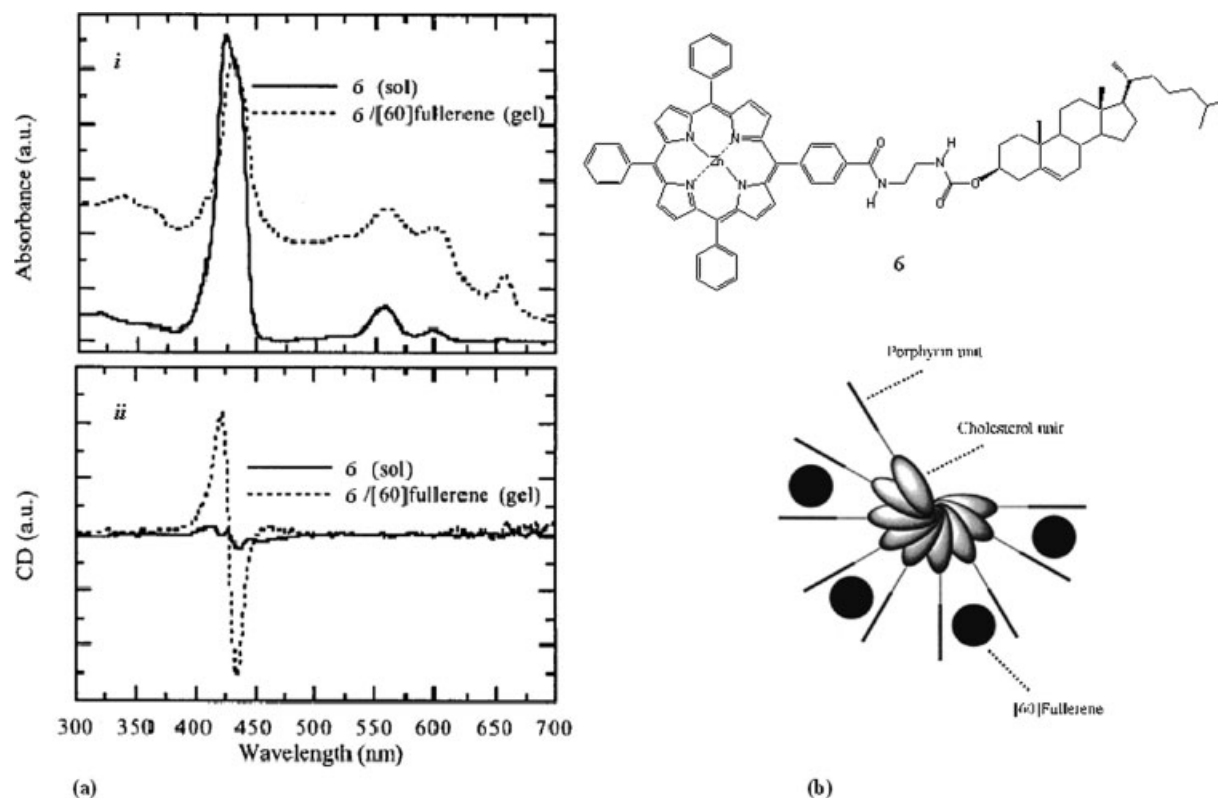


Fig. 7. Absorption and CD spectra (a) of **6** and **6**/[60]fullerene complex (2:1 molar ratio) in toluene (sol phase for **6** and gel phase for **6**/[60]fullerene complex) and the aggregate models for **6** in the presence of [60]fullerene in the gel phase (b). (Reprinted with permission from Reference 30. Ishi-T, et al. *Langmuir* 2001;17:5825–5833. Copyright 2001 American Chemical Society.)

overview on the control over chirality for self-assembled system in solution, see Ref. 35). Solutions of chiral and achiral assemblies in benzene were mixed in ratios varying between 9:1 and 1:9. The CD intensities increase as a result of the formation of the heteromeric assemblies: the plot of the relative CD against the mole fraction of the chiral component shows the typical nonlinear behaviour of the “sergeants and soldiers” regime (Fig. 8) (Scheme 1).

Meijer and coworkers investigated the association of C_3 symmetrical disc-like molecules **9a** and **9b**.^{36,37} These molecules may originate in chiral columnar stacks through noncovalent interactions when dissolved in apolar solvents and possibly gels or LC phases. The chiral enantiopure compound **9a** shows a very strong Cotton effect around 380 nm. Interestingly, the achiral derivative **9b** shows in the optically active solvent (*R*)-2,6-dimethyloctane a CD spectrum almost identical to the spectrum of **9a**. The effect of this chiral solvation (The conformational change in poly(isocyanate)s driven by chiral solvents has been investigated with CD by Green and coworkers. See, for example Refs. 38 and 39) indicate a strong cooperative effect and the tendency of the stacks to take on a helical arrangement (Fig. 9). This is confirmed by the following remarkable observation: as expected, **9b** does not show any Cotton effect in hexane; however addition of 2.5% of chiral enantiopure **9a** to achiral **9b** generates a strong Cotton effect similar, in magnitude, to pure **9a**. The “ser-

geants and soldiers” effect is operative even in dynamic supramolecular polymer.

Taking inspiration from the spontaneous self-assembly of naturally occurring guanylic acids and related molecules (vide infra), Fenniri and coworkers investigated in details the properties of a derivative of heterobicyclic base G·C.⁴⁰ Because of the distribution of the H-bonding array around the periphery of this moiety, derivative **10** undergoes, in appropriate conditions, a hierarchical self-assembly process to form a six-membered macrocycle and finally a tubular architecture (see Fig. 10). Under these conditions, the addition of a chiral amino acid, e.g., *L*-Ala that binds to the crown ether moiety of **10** promotes the transition from racemic rosette nanotubes to chiral nanotubes with predefined helicity as monitored by the induced CD corresponding to the achiral chromophoric molecule **10**. The CD titration of **10** with *L*-Ala has been employed to reveal that the molecular chirality is expressed by the supramolecular organisation collectively only when all the crown ether sites are occupied with an amino acid. This all-or-none response is at variance with the sergeant and soldier’s mechanism described earlier, in which a small chiral subpopulation determines the collective chiroptical destiny of the entire pool.

The self-assembly of complementary diaminopyridine (DAP) **11** and flavine derivatives **12** has been widely investigated by Rotello and coworkers by means of CD.⁴¹

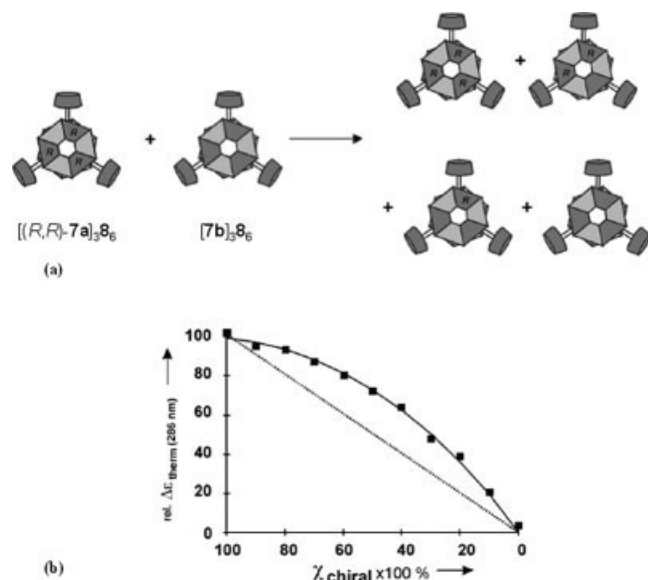


Fig. 8. Schematic representation of the formation of the heteromeric assemblies of **7** and **8** (a) and the plot of the relative CD-intensities at the thermodynamic equilibrium for different mole fractions of the chiral component (b). The dotted line represents the values in absence of chiral amplification. (Reprinted from Reference 35. Mateos-Timoneda M, et al. *Chemical Society Reviews* 2004;33:363–372. Reproduced by permission of The Royal Society of Chemistry.)

No significant Cotton effect was observed in halomethanes (where DAP-flavine hydrogen bonding still occurs) when achiral flavine **12** is mixed with chiral enantiopure **11S** or **11R**: this lack of induced CD indicates that formation of the DAP-flavine dyad does not result in efficient chiral stacking. In contrast, strong CD was observed in less polar hydrocarbons, such as hexane, indicating the formation of helical assembled structures (see Fig. 11). Given that the direction of the transition moments are established, the coupling in the flavine visible region allows the determination of the chirality of stacking.

The presence of a stereogenic centre also in the flavin unit shows cooperative and chiral amplification effects. The non linear behaviour of the chiroptical response as a function of the molar fraction of **12S** (in the mixture **11S-12** and **11S-12S**) indicate that flavin **12S** can direct achiral flavine **12** to follow the preorganized helical structure in a “sergeants and soldiers” fashion. A dyad with 80% of chiral building block **12S** provides essentially the same chiroptical response of a dyad prepared with 100% of chiral **12S**.

THE CD OF THE CHIRAL LIQUID CRYSTAL PHASES

Scattering effects or formation of liquid-crystalline phases (cholesteric or chiral smectic C) can give rise to CD signals outside the chromophoric absorption region. Typically, a cholesteric phase has a total reflection CD band that is unrelated to chromophoric absorption, which can appear at wavelengths far from the chromophore absorption region. The theory of the origin of this reflection band can be found in specialized texts.^{42,43} The wavelength of the reflection band is related to the pitch p of the

cholesteric helix by the relation, $\lambda = np$, where n is the average refractive index of the mesophase.

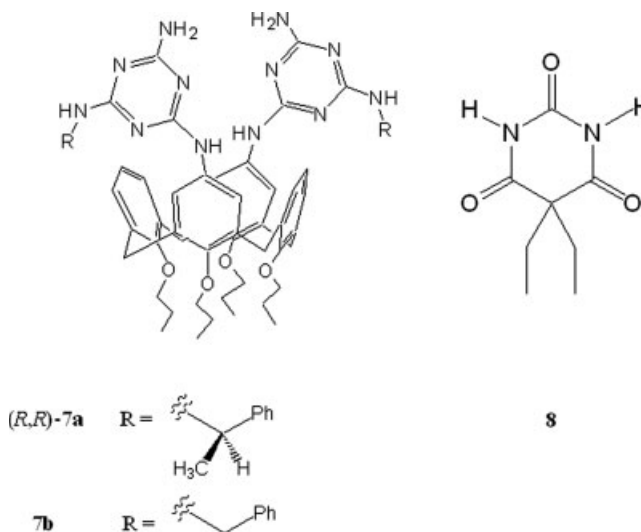
Cholesteric phases show CD bands that correspond to molecular absorptions, as well. It should be emphasized that these CD bands are not generated by exciton couplings, but by the LD of the individual molecules composing the phase.⁴⁴ For these reasons, special care must be taken when recording CD spectra of cholesteric phases. The sample must have a planar texture (i.e., the cholesteric helix axis should be perpendicular to the cell walls) and the light beam must also be normal to the cell walls (i.e., parallel to the cholesteric helix axis).⁴⁵

Enantiomerically pure 12-hydroxyoctadecanoic acid in CCl_4 and benzene is a classic example of formation of chiral liquid-crystalline gels.¹⁷ In the solid state, this molecule is assembled in helical fibrillar structures with the handedness determined by the enantiomer considered. The gel in CCl_4 displays a CD spectrum characterized by a broad band at ca. 350 nm, in a wavelength range far from the chromophoric absorption, ca. 230 nm (Fig. 12). This band is interpreted as a reflection band of a chiral mesophase, tentatively a smectic one. In this example, CD does not provide information about the helical fibrils whose formation depends on the nature of the solvent.

An example of intense CD bands that correspond to the chromophore absorption exhibited by cholesteric phases is shown later in the section dedicated to the guanine chromophore.

THE SELF-ASSEMBLY OF GUANOSINE DERIVATIVES

Guanine is an important heterocyclic base present in DNA and RNA. Telomeres are particularly rich in guanine, which is responsible for their unusual structure. Telomeres, the terminal part of eukaryotic chromosomes, play a fundamental role for the stability of these structures and are connected to aging and possibly to cell proliferation in cancer.⁴⁶ Furthermore, guanosine derivatives are known to undergo extensive self-assembly processes,⁴⁷ which



Scheme 1

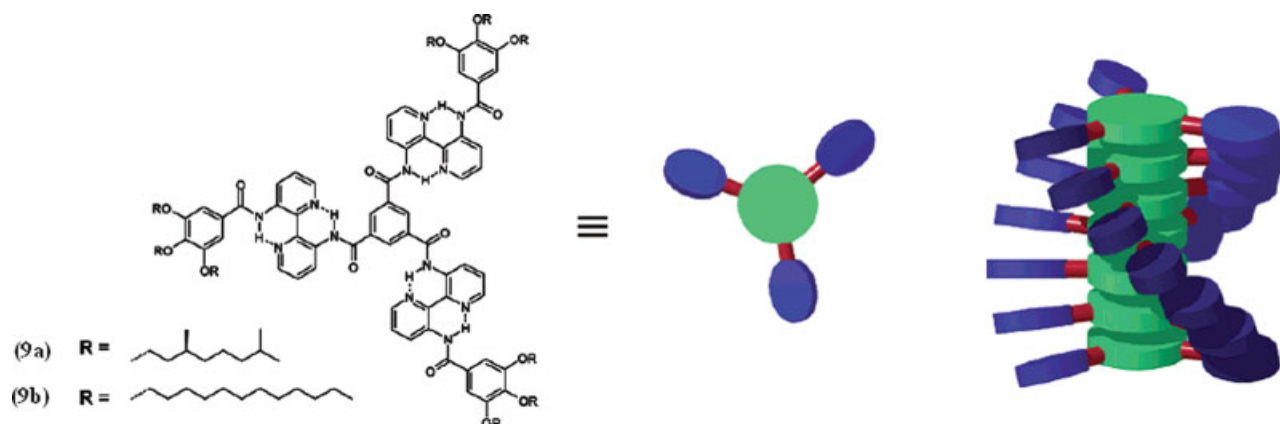


Fig. 9. Bipyridine based C₃-symmetrical disk-like molecules **9** and a cartoon representing their helical supramolecular stacking. (Reprinted with permission from Reference 37. van Gorp JJ, et al. *Journal of the American Chemical Society* 2002;124:14759–14769. Copyright 2002 American Chemical Society.)

eventually lead to the formation of gels⁴⁸ and lyotropic liquid crystalline mesophases.⁴⁹

The tetrameric arrangement schematized in Figure 13 (G-quartet) is the fundamental structure found in both telomere models and liquid crystalline mesophases. In the self-assembly process, the tetramers have a pronounced tendency to stack on top of one another and alkali metal ions are specifically involved. Similarly to the base pairs in double-stranded DNA, the tetramers do not stack in register, but are rotated with respect to each other to give an

overall helical structure; CD spectroscopy seems therefore to be the ideal technique to follow directly the self-assembly processes and to assess the importance of several factors, such as added ions, temperature, and solvent variations.⁵⁰

The guanine chromophore has a readily accessible absorption band in the 240–290 nm region, which is connected to two well-characterized π – π^* transitions at ca. 279 and 248 nm; the transitions are roughly short and long-axis polarized, respectively.⁵¹ The columnar structure

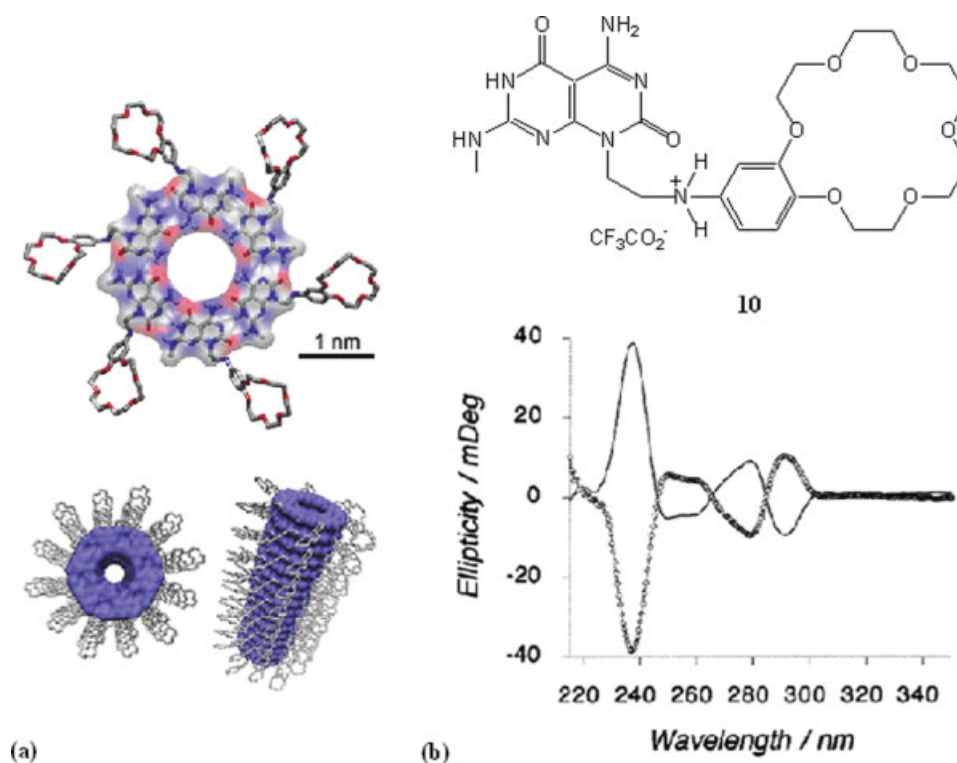


Fig. 10. Hierarchical self-assembly of **10** into a six-membered supermacrocycle (rosette) and resulting nanotube (a) and CD spectra of **10** + L-Ala (circles) or D-Ala (full line) (b). (Reprinted with permission from Reference 40. Fenniri H, et al. *Journal of the American Chemical Society* 2002;124:11064–11072. Copyright 2002 American Chemical Society.)

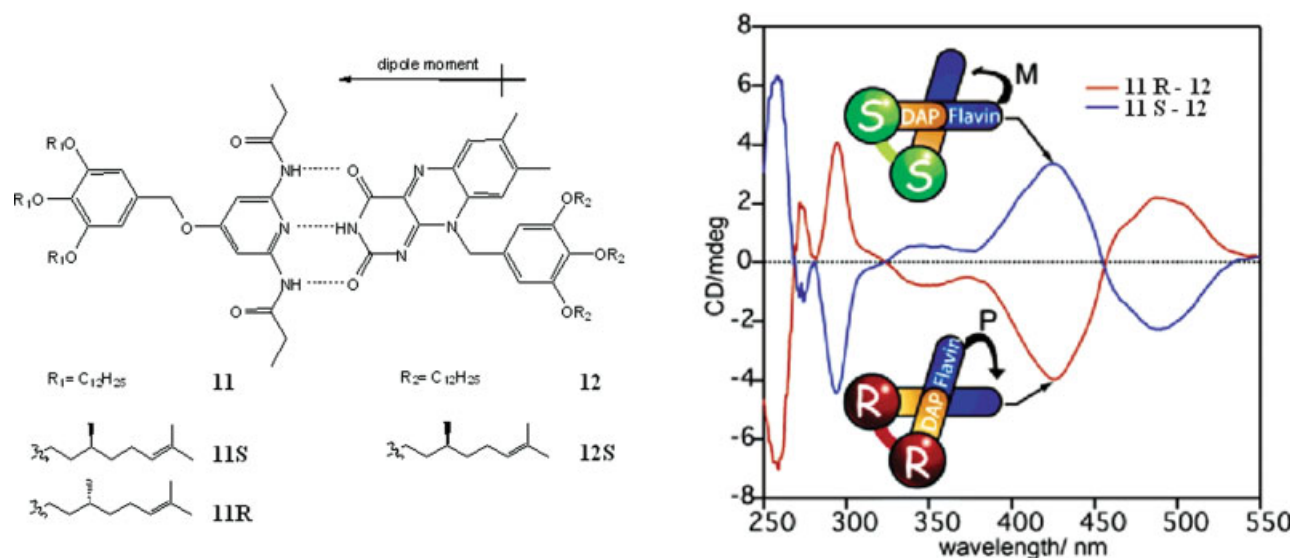


Fig. 11. CD spectra of **11S-12** and **11R-12** recorded at -10°C in hexane. (Reprinted with permission from Reference 41. Nakade H, et al. *Journal of the American Chemical Society* 2006;128:14924–14929. Copyright 2006 American Chemical Society.)

based on the piling of G-quartets was deduced by fibre diffraction work on polyguanylic acid, poly(G)⁵² and later confirmed by single crystal X-ray work on the G-rich oligonucleotide d(TG₄T).⁵³ This latter work gives more precise details of the structure: the four parallel strands form a right-handed helix, the quartets are highly planar and are connected to the backbone by glycosyl bonds that are exclusively *anti*. The quartets are stacked in a right-handed way and all have the same head to tail disposition sketched in Figure 13 (the G-quartets of Fig. 13 have in fact two nonequivalent faces). The quartets are perpendicular to the helix axis and sodium ions are located between the quartets.

Also in solution, poly(G) gives rise to an assembled four-stranded helical structure whose CD spectrum is reported in Figure 14. The CD spectrum is dominated by a nonconservative positive exciton couplet characterized by a positive band at ca. 260 nm with only a shoulder at ca. 290 nm, and a negative maximum at ca. 240 nm.

This spectrum can be reproduced satisfactorily by an exciton calculation with only near-neighbour interactions.^{54,55} Theoretically, one obtains higher CD intensity at ca. 290 nm by modifying the chromophoric geometry described earlier, such as by tilting or deforming the tetrameric planes.

The dinucleoside derivative d(GpG) **13** shows an instructive polymorphism of the assembled species according to the alkali metal ions present in solution (Fig. 15) (Scheme 2).

One can immediately notice how the spectra of the assembled species are different from those observed in very dilute solutions or at higher temperature (monomeric species). Furthermore, the shape and intensity of the spectra of the aggregates are considerably different in the presence of different metal ions: in the presence of an excess of K⁺ one observes a spectrum similar to that of poly(G)

(260 nm band), while, when Rb⁺ is added, the positive intensity is at 290 nm (290 nm band). With Na⁺ added, both bands are present.

As in the case of d(GpG) described here, CD bands centered around 260 and 290–300 nm have been consistently observed and discussed for G-rich oligonucleotides made of variable numbers of telomere repeats.⁵⁶ These systems, contrary to homoguanylates, which give aggregates composed of four parallel strands, can form G-quadruplexes by the assembly of four parallel or antiparallel strands, by intramolecular folding as for d(T₂AG₃)₄^{56,57} or by joining two hair-pin structures as for d(G₄T₄G₄).⁵⁸ It should be stressed that in the intramolecular folded or in the joined hair-pin structures, major modifications of the stereochemistry of the system with respect to that of poly(G) or in general the parallel tetraplexes have occurred.

In the structure of Figure 16 there is alternation of *syn* and *anti* conformations of the guanine residues around the glycosyl bond, both along the chains as well as around the G-tetrad.⁵⁸ The alternating *anti-syn* conformations in successive guanine tetrads lead to an inverted stacking arrangement of the bases and there are dinucleotide repeats along the polynucleotide chain in the stem region.^{57,58}

CD spectroscopy has been used empirically to distinguish between parallel and antiparallel (hair-pins are necessarily antiparallel) G-quartet structures: antiparallel structures show as their characteristic signature the 290 nm band, whereas parallel structures display the 260 nm band.⁵⁰

The “spectroscopic” interpretation does not take into account parallel or antiparallel structures as the sugar phosphate backbone is considered of minor importance in determining the CD spectrum around 280 nm; it focuses instead on the chromophoric tetrameric planes: regular structures with G-quartets in a head-to-tail disposition, per-

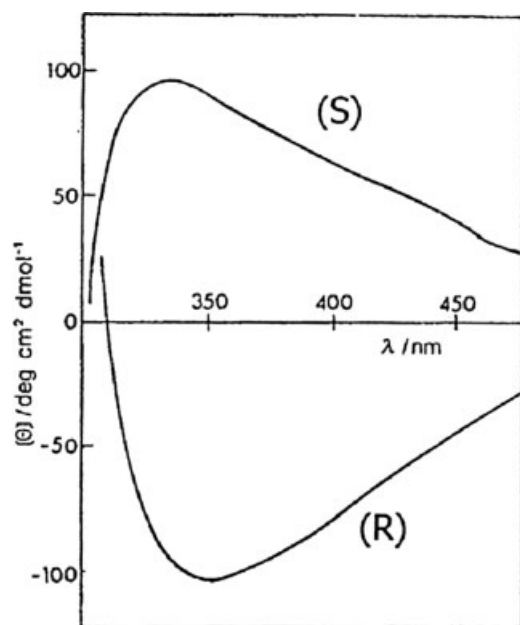


Fig. 12. CD spectra of the (*R*) and (*S*) enantiomers of 12-hydroxyoctadecanoic acid in CCl_4 in the gel state. (Reprinted with permission from Reference 17. Macmillan Publishers Ltd: *Nature* 278:578–579. Copyright 1979.)

pendicular to the helix axis, display the 260 nm band, whereas distorted structures are correlated to the 290 nm band. The spectral changes are therefore related not directly to the parallel or antiparallel orientation of the strands. In fact, there is no practical contradiction between these interpretations: in the first single-crystal X-ray structure of a hairpin dimer (290 nm CD band), the tetrameric planes seem to be rather distorted.⁵⁸

In Figure 17 the CD spectra of $d(\text{G}_{12})$, $d(\text{G}_4\text{TG}_4)$, $d(\text{G}_4\text{T}_2\text{G}_4)$, and $d(\text{G}_4\text{T}_4\text{G}_4)$ are reported as taken from Ref. 56; $d(\text{G}_4\text{T}_4\text{G}_4)$ is in a coupled hairpin quartet structure⁵⁸ and, accordingly, displays the 300 nm band. The other

three compounds are instead assembled with four parallel strands. One can notice immediately that, apart from the 300 nm positive band, the other part of the spectrum shows a nearly mirror-image relationship of the bands. Although this observation does not question the empirical use of the 260 and 290 nm bands in assigning the parallel tetraplex or the antiparallel hairpin structures, it seems to indicate that the chromophoric handedness of the quartets is opposite in the two cases, and this is probably due to the inversion of polarity of the quartets in hairpin structures.

To clarify this point, a careful inspection of the structure given in Ref. 58 for $d(\text{G}_4\text{T}_4\text{G}_4)$ is instructive. Even if the sugar-phosphate backbone is right-handed, the two internal chromophoric quartets give a strong negative contribution to the CD. This indicates again how the CD around 250 nm is related to the skew of the G-quartets and not directly to the handedness of the sugar-phosphate backbone. From the first information one can obtain the backbone handedness only if the stereochemistry of the glycosidic torsion is known.

The liquid crystal formation of guanosine derivatives may also be followed by CD. The formation of the cholesteric phase, characterized by pitches of ca. 20 μm (as determined by optical microscopy), can still be detected in aqueous solution of 3'-dGMP **14** (Scheme 3) by CD in the chromophoric absorption region. The spectrum gradually changes from the exciton shape, characteristic of the assembled helical species in isotropic solution, to a much stronger monosignate spectrum whose shape is very similar to the absorption (Fig. 18). The latter spectrum is due to the cholesteric phase whose presence was independently verified by optical microscopy. This CD spectrum allows the assignment of the handedness of the cholesteric phase.^{13,45} Cholesteric phases of guanosine derivatives have been detected by CD in aqueous^{59–62} (e.g., Fig. 18) and hydrocarbon solutions.⁶³ Furthermore, the effects of temperature and added salts on the formation of the cholesteric phase can be easily investigated.

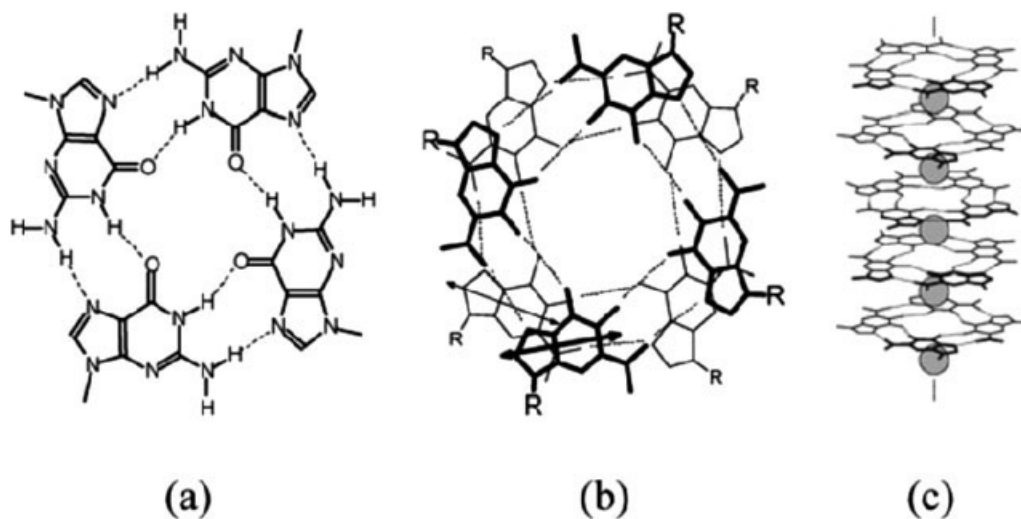


Fig. 13. The G-quartet motif (a), a head-to-tail right-handed stacking of two G-quartets (and the clockwise relationship between the transition moments) (b), and a right-handed columnar G-rich oligonucleotides (c).

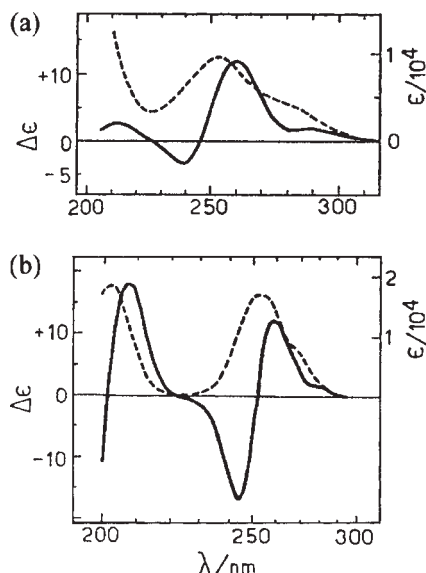


Fig. 14. (a) the absorption (dashed) and CD (solid) spectra of poly(G). (b) the simulated absorption (dashed) and CD (solid) spectra. (Reproduced from Reference 54. Gottarelli G, et al. *Gazzetta Chimica Italiana* 1990;120:101–107, with permission from Società Chimica Italiana.)

In the recent years the control of the supramolecular architectures obtained from guanosine derivatives soluble in organic solvents have been investigated: chemical modifications on the guanine and/or the sugar, the nature of solvent and/or cations possibly present may allow the generation of different types of supramolecular motifs.^{46,47,64–66}

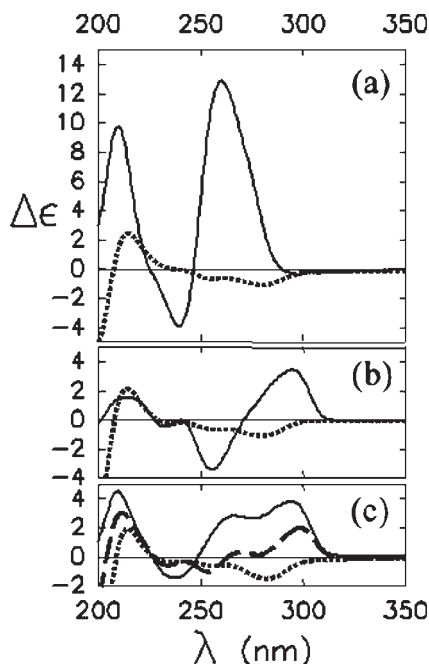
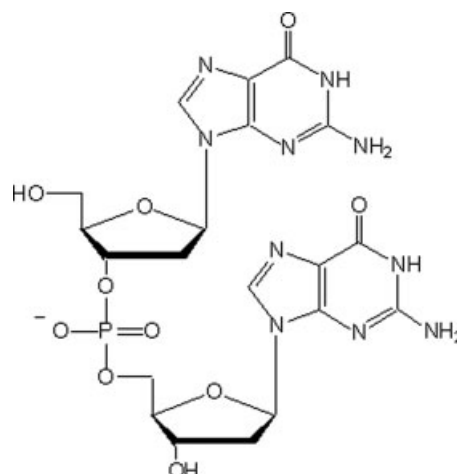


Fig. 15. CD spectra of d(GpG)Na at a guanine concentration of $1.5 \times 10^{-2} \text{ mol l}^{-1}$; frame (a) in KCl 0.05 mol l^{-1} recorded at 20 (full line) and 75°C (dotted line); frame (b) in RbCl 0.05 mol l^{-1} recorded at 25 (full line) and 50°C (dotted line); frame (c) in NaCl 0.05 mol l^{-1} recorded at 10°C (full line) and in water recorded at eight (dashed line); and 40°C (dotted line). (Reprinted with permission from Reference 50. Gottarelli G, et al. *Enantiomer* 1998;3:429–438. Copyright 1998 Taylor and Francis.)



d(GpG) 13

Scheme 2

Because of the molecular and supramolecular chirality of these systems, CD has been widely used in these studies.

For example, the tunable interconversion between discrete supramolecular assemblies of a lipophilic guanosine (LG 15, Scheme 4), from G-ribbons to G-quartet columns and vice versa, fueled by cation complexation and release has been reported.⁶⁷ The G-quartet structures are harnessed by the presence of a coordinated potassium cation and this offers the possibility of triggering a reversible ribbon–quartet interconversion by controlled sequential addition and removal of K^+ . The cryptate $[\text{K}^+ \subset 2.2.2]$ offers an efficient complexation of K^+ to yield the cryptate $[\text{K}^+ \subset 2.2.2]$. Upon protonation of one of the bridgehead nitrogens, the bound K^+ can be released, leading to the formation of $[\text{H}^+ \subset 2.2.2]$. CD spectroscopy has been successfully used to follow the interconversion: the CD spectrum of the G-ribbon in the region

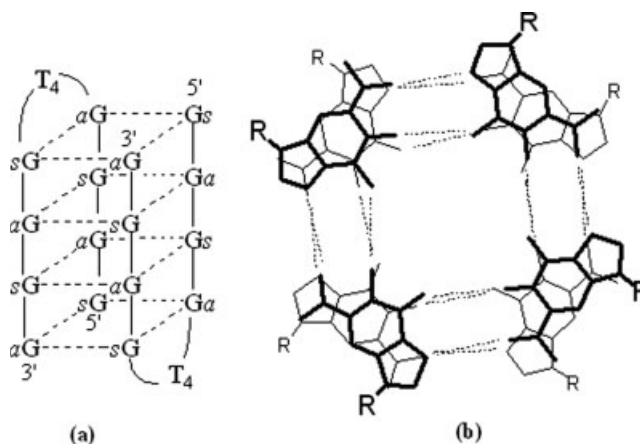


Fig. 16. (a) Schematic drawing of two joined hairpin of d(G₄T₄G₄). s indicates *syn* and a the *anti* conformation around the glycosyl bond. (b) A head-to-head (inverted) stacking of two G-quartets. (Reprinted with permission from Reference 50. Gottarelli G, et al. *Enantiomer* 1998;3:429–438. Copyright 1998 Taylor and Francis.)

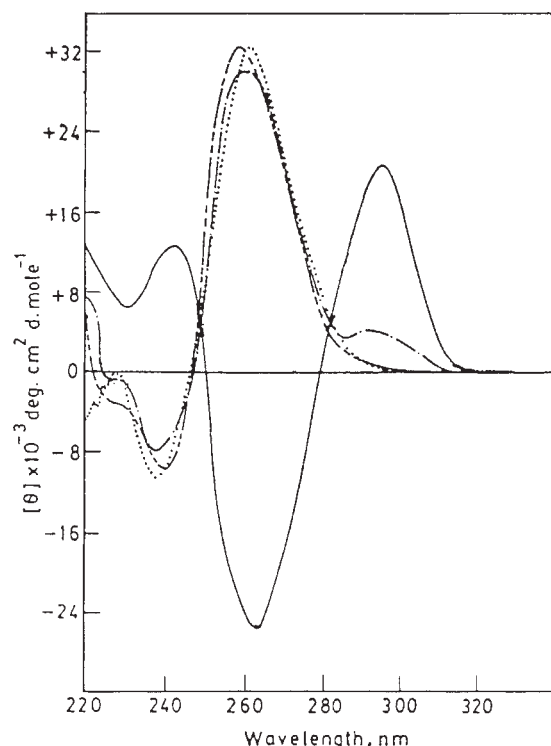
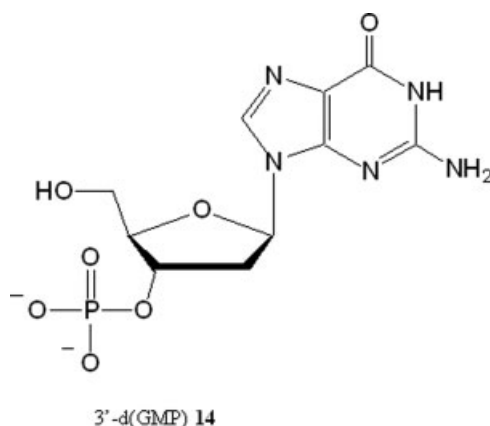


Fig. 17. CD spectra of d(G₄T₄G₄) (—), d(G₄T₂G₄) (---), d(G₄TG₄) (---), d(G₁₂) (····). (Reprinted from Reference 56: Balagurumoorthy P, et al. Hairpin and parallel quartet structures for telomeric sequences. *Nucleic Acid Research* 1992;20:4061–4067. By permission of Oxford University Press.)

of the intense $\pi\pi^*$ transitions of the guanine chromophore at ca. 260 nm is monosignate and weak (Fig. 19, trace *i*), the stabilization of stacked G-quartet-based structures induced by the K⁺ ion introduces instead a negative exciton signal (Fig. 19, trace *ii*) because of the rotation between adjacent quartets.

In recent years Kato and coworkers have investigated the self-assembly of folic acid derivatives. Folic acid contains the pterin moiety that is structurally similar to guanine and is able to form several different self-assembled nanoarchitectures depending on the conditions: few of them, based on quartets or ribbons, resemble the one



Scheme 3

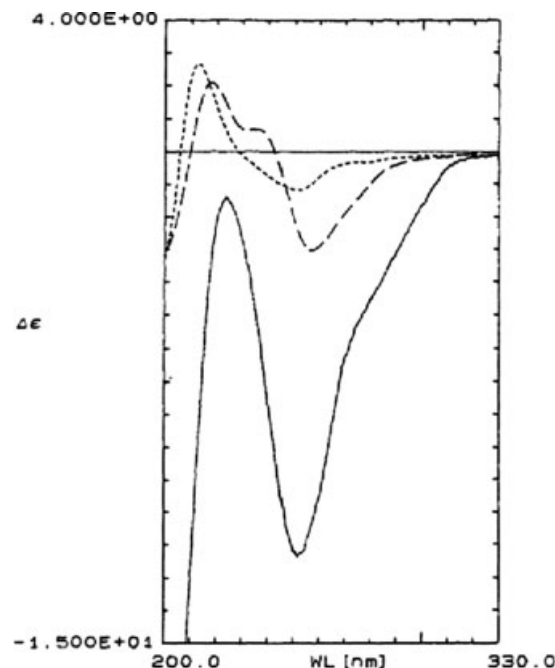
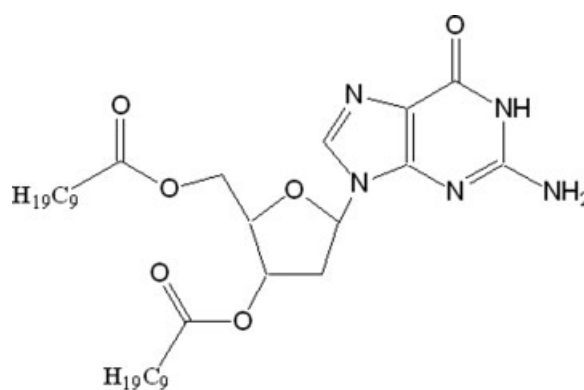


Fig. 18. CD spectra of 4% (w/w) ammonium 2'-deoxyguanosine-3'-phosphate 14 in water in the cholesteric (full line, $T = 1^\circ\text{C}$), isotropic assembled (long-dashed line, $T = 5^\circ\text{C}$) and isotropic disassembled (short-dashed line, $T = 30^\circ\text{C}$) states. (Reprinted with permission from Reference 59. Gottarelli G, et al. *Enantiomer* 1996;1:201–209. Copyright 1996 Taylor and Francis.)

exhibited by guanosine derivatives. Again, because of the chirality of folic acid, CD spectroscopy has been used to detect switching between different structure (For a recent paper see Ref. 68)

VIBRATIONAL CD

Until a few years ago, vibrational CD (VCD) was a technique used only by specialized research groups.^{69,70} Today, a few commercial instruments are available and the use of VCD seems promising for studying self-assembly processes and gel formation; in contrast to electronic CD, VCD, operating in the IR spectral range has the usual



Scheme 4

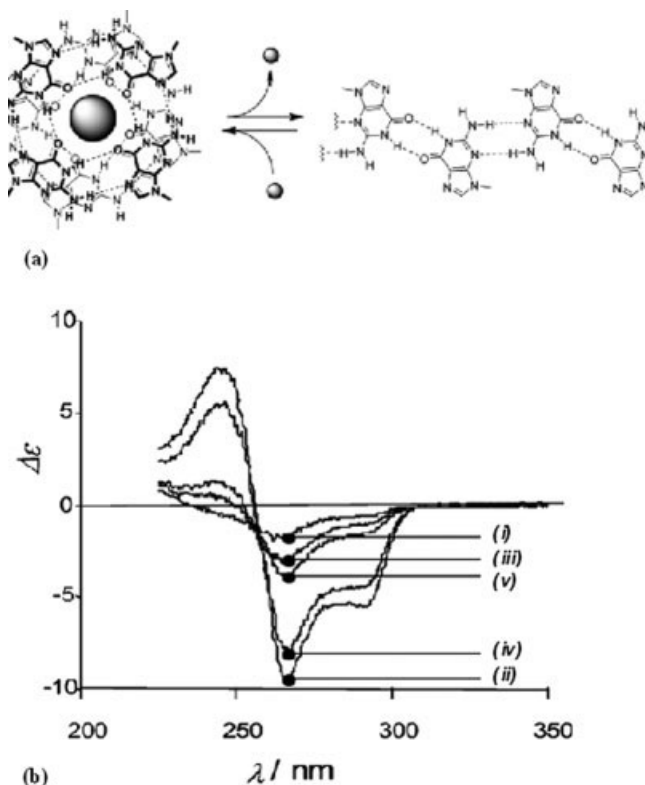


Fig. 19. (a) The tunable interconversion between two highly ordered supramolecular motifs (K^+ -templated G-quartet column and G-ribbon) of a lipophilic guanosine derivative (LG 14) fueled by cation complexation and release. (b) Observation of the reversible ribbon-octamer interconversion in a solution of LG in $CDCl_3$ by CD spectroscopy. (i) Initial sample (LG_n); (ii) after addition of potassium picrate ($K^+ LG_n$); (iii) after addition of cryptand [2.2.2] (LG_n); (iv) after addition of HTf ($K^+ LG_n$); and (v) after addition of Et_3N (LG_n). (Reprinted with permission from Reference 67. Pieraccini S, et al. *Organic Letters* 2006;8:3125. Copyright 2006 American Chemical Society.)

spectral advantage of a large number of resolved and relatively localized transitions, rather than the few broad bands found in the UV-vis region. Therefore VCD provide valuable information on the involvement of specific parts of molecules in the formation of chiral self-assemblies. For example, the self-assembly of brucine-porphyrin conjugates⁷¹ and of a chiral biphenyl bicarboxylic acid⁷² were followed studying the VCD in the $C=O$ at ca. 1655 cm^{-1} . More recently, VCD in combination with electronic CD and other techniques, has been employed to investigate the self-association and gel formation of guanosine-5'-hydrazide.⁷³

CONCLUSION AND OUTLOOK

The major technical and theoretical efforts in the development of electronic CD spectroscopy were carried out between 1960 and 1980; the technique is now mature and can be used to tackle more complex problems than those given by the study of small chiral molecules. CD spectroscopy is now routinely used to study biological macromolecules and is often employed in supramolecular chemistry. In particular the self-assembly processes leading to chiral supramolecular architectures can be appropriately moni-

tored by CD, which also provides useful structural information. Extension of the measurements to the IR region is now possible: a few applications in supramolecular chemistry have recently been reported and will, hopefully, become more common.

From the theoretical side, in the last few years considerable progress has been made in calculating the optical rotation and CD spectra of small molecules. The extension to larger systems seems highly desirable and should allow the obtainment of precise structural information for supramolecular systems.

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Chiral 1,1'-Binaphthyl-2,2'-Dithiol-Stabilized Gold Clusters: Size Separation and Optical Activity in the UV-vis

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT Gold particles covered with 1,1'-binaphthyl-2,2'-dithiol (BINAS) were prepared. Using size exclusion chromatography, it was possible for the first time to separate the sample into fractions with different sizes and colors. Transmission electron microscopy shows that the particles are very small, in the order of 1 nm or slightly above. The absorption spectra of the separated samples show rich structure. The particles show size-dependent optical activity in metal-based electronic transitions. The shape of both the absorption and circular dichroism spectra of one of the smallest fractions exhibits similarities with the spectra reported for Au₁₁ covered by 2,2'-bis(diphenylphosphino)-1,1'-biphenyl although the spectra are shifted to shorter wavelengths in the case of the dithiol. The anisotropy factors, $\Delta\epsilon/\epsilon$ of these particles are as large as 4×10^{-3} , which is larger than the values reported for gold particles stabilized by phosphines and water-soluble thiols. This indicates that BINAS is particularly well-suited to impart chirality on to gold particles. *Chirality* 20:486–493, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: circular dichroism; optical activity; 1,1'-binaphthyl-2,2'-dithiol; nanoparticles; gold; size separation

INTRODUCTION

Monolayer protected nanoparticles (MPNs) are fascinating hybrid materials composed of a metallic core surrounded by an organic monolayer.¹ Their physical and chemical properties can easily be tuned for custom requirements by changing their size and by modifying in a judicious way the organic shell. Their potential applications were reported in various fields such as catalysis,² sensing,^{3,4} therapy,⁵ electronics,⁶ and optics.⁷ Among the huge number of different particles, the subnanometer range of inorganic–organic hybrid nanoparticles has gained great interest both in fundamental and applied research. This class of material exhibits strong size-dependant properties.^{8,9} From a fundamental point of view, these particles can be understood as an intermediate state of matter between a single metal atom and a bulk metal. As such they serve as models to understand fundamental physical properties like quantum confinement and structural evolution. Their solubility in various solvents makes them amenable to size separation and spectroscopic techniques which are of great help in elucidating the size dependence of their physical properties. MPNs can also be viewed as the nanometer size analogue of self-assembled monolayers (SAMs) on extended metal surfaces and are a powerful tool for a better understanding of the interaction between metal surfaces and organic molecules.

Recently, the use of chiral passivating agents was considered. To probe the chiral nature of these particles,

chiroptical techniques such as circular dichroism (CD) and vibrational circular dichroism (VCD) are particularly helpful. Based on the comparison between the experimental and calculated VCD spectra, the preferential conformation of *N*-acetyl-cysteine¹⁰ and *N*-isobutyryl-cysteine¹¹ absorbed on gold particles was proposed. These results were in good agreement with those obtained from measurements on a SAM of the thiol on extended gold surfaces.¹² Furthermore, the use of a chiral capping agent leads to optical activity located in metal-based electronic transition.^{11,13–19} This shows that chirality can be bestowed to the metal. However, the question whether the metal core adopts an intrinsically chiral structure²⁰ or whether the chirality is only imposed on to the electronic structure of the metal core²¹ remains still unanswered.

The optical activity located in metal-based transitions is promising for applications in the field of asymmetric reactions catalyzed by small metal nanoparticles. Tamura and Fujihara have reported the asymmetric hydrosilylation of styrene with trichlorosilane catalyzed by chiral palladium

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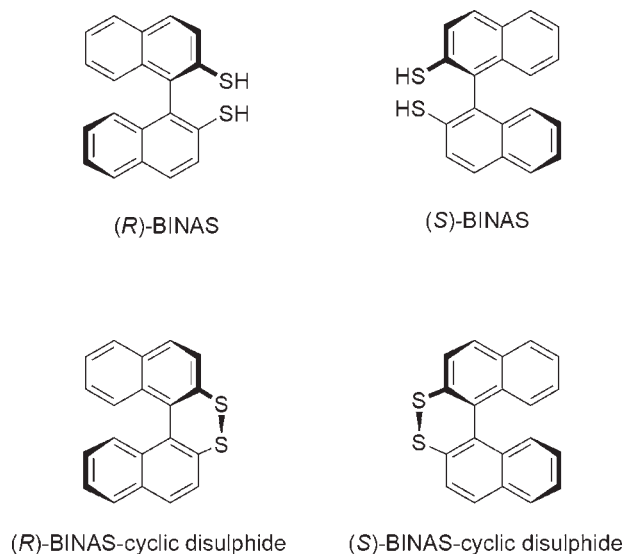


Chart 1. Structure of (R)-BINAS, (S)-BINAS, (R)-BINAS-cyclic disulphide, and (S)-BINAS-cyclic disulphide.

nanoparticles stabilized by optically active 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP).¹⁶

Catalysis by gold nanoparticles is evolving very rapidly. Gold is established to be effective as heterogeneous and homogeneous catalyst in hydrogenation, in selective oxidation, in nucleophilic addition to π systems and for many reactions for which a catalyst had not been previously identified.²² Thus chiral gold nanoparticles are expected to be of great interest in the field of enantioselective catalysis.

Here we report the preparation and chiroptical properties in the UV-vis of gold nanoparticles covered by the two enantiomeric forms of an atropisomeric dithiol: (R)-1,1'-binaphthyl-2,2'-dithiol [(R)-BINAS] and (S)-1,1'-C1 binaphthyl-2,2'-dithiol [(S)-BINAS]²³ (Chart 1). Size exclusion chromatography (SEC) allowed, for the first time, the separation of up to five different fractions with well-defined and distinctly different absorption spectra and colors. The five separated fractions showed strong optical activity in metal based electronic transitions. The origin of this optical activity is discussed.

MATERIALS AND METHODS

Materials

The preparation of enantiomerically pure (R)- and (S)-BINAS was reported elsewhere.²³ Deuterated chloroform (99.8%) was received from Cambridge Isotope Laboratories. Gold(III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 99.99%), sodium borohydride (NaBH_4 , 98%) and tetraoctylammonium bromide (TOAB, >98%) were purchased from Aldrich, chloromethylated styrene-divinyl benzene (99:1) resin was obtained from Bio-Rad, Richmond, CA (Bio-Beads SX1, 200–400 mesh). Water was purified with a Milli-Q system ($\geq 18 \text{ M}\Omega \text{ cm}$). Tetrahydrofuran (THF) and dichloromethane were distilled under nitrogen to

remove dissolved oxygen. All other chemicals were analysis grade and were used as received.

Synthesis of Gold BINAS MPNs

(R)-BINAS and (S)-BINAS MPNs were both prepared under anaerobic conditions following the two-phase method of Brust et al.²⁴ Briefly, to a vigorously stirred solution of tetrachloroauric acid (132 mg, 0.335 mmol) in 6 ml of deaerated Milli-Q (sonication under vacuum) was added 183 mg of TOAB (0.335 mmol) dissolved in 10 ml of distilled dichloromethane. The mixture was stirred for 30 min. The phase transfer of gold salts from water to dichloromethane results in a discoloration of the aqueous phase and imparts an orange color to the organic phase. The two phases were allowed to settle. Then, the orange organic phase was removed and added to 214 mg (0.67 mmol) of the corresponding enantiomer of BINAS dissolved in 100 ml of distilled dichloromethane. After 30 min, the solution was reduced by addition of a freshly prepared aqueous NaBH_4 solution (5 ml, 317 mg, 8.38 mmol) under vigorous stirring. The mixture was allowed to react over night under an argon atmosphere. The resulting dark organic solution was extracted and washed three times with water. The solution was dried over Na_2SO_4 and filtered using a 0.2- μm PTFE membrane to remove insoluble material. The filtrate was evaporated to near dryness at 40°C and the nanoparticles were precipitated several times with a large excess of hexane and filtered using the same 0.2 μm PTFE membrane. Purification of BINAS gold nanoparticles was repeated until no free BINAS, disulfur, or phase transfer catalyst were detected by ^1H NMR. We obtained finally 176 mg of gold nanoparticles protected with a monolayer of the corresponding enantiomer of BINAS.

Separation of Gold BINAS MPNs by SEC

The THF used for elution and for packing the column was freshly distilled under nitrogen and protected from atmospheric contamination. Bio-Beads SX1 beads were placed in THF (six times the resin weight). The resin was allowed to swell overnight and packed in a glass column (1.2 m length and 2 cm in diameter). The column was washed with several bed volumes of solvent after packing and between separations. For size separation, the sample (90 mg) was dissolved in a minimum amount of distilled THF and loaded on the column. The elution was done with THF at a flow rate of ca. 0.5 ml/min and 5 ml fractions were collected. Each fraction was controlled by UV-vis spectrometry and classified according to its absorption profile.

Characterization of the Particles by UV-vis, CD, and NMR Spectroscopy

^1H NMR spectra of the as prepared particles were measured on a Bruker Avance 400 MHz spectrometer at room temperature. Solutions were prepared in CDCl_3 at a concentration of $\sim 50 \text{ mg/ml}$. UV-vis and CD spectra, respectively, of the separated particles were collected on a

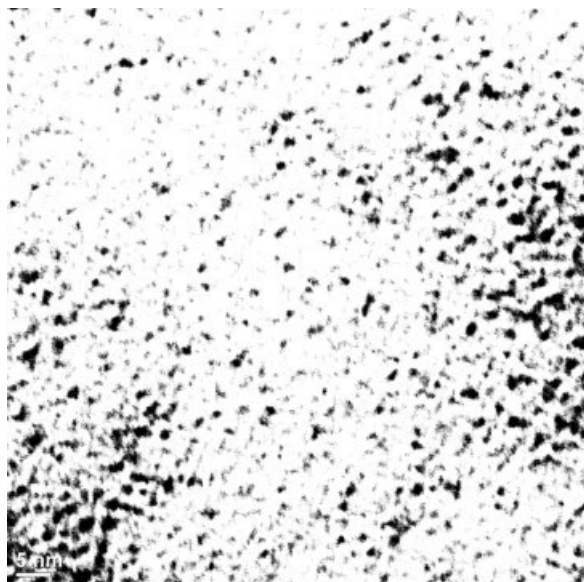


Fig. 1. TEM micrograph of gold BINAS MPNs fraction 1.

Cary 300 and a Jasco 710 spectrometer, respectively, using a quartz cell of 1-cm path length and solutions of about 0.3 mg/ml in THF.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) images were recorded with a Philips C200 electron microscope operated at 200 kV. The nanoparticles were dissolved in dichloromethane (~ 0.5 mg/ml). TEM samples were prepared by casting a drop of the solution onto a carbon-coated copper grid.

RESULTS AND DISCUSSION

Size Separation of Gold BINAS MPNs

The as-prepared product consists of gold BINAS MPNs with an average size smaller than 2 nm as shown by the TEM (see Fig. 1) and confirmed by the absence of a plasmon resonance band at around 520 nm in UV-vis (see Fig. 2). This type of small gold MPNs has a typical molecular weight range between 4 and 20 kDa,^{17,25} which corresponds well with the operating range of commercially available gels like Bio-Beads S-X1. Figure 3 shows a photograph of the SEC column during separation. Just after loading, the polydisperse sample gave rise to a black band about 2 cm-long. During the separation, this black band became increasingly broad showing that the average size of the nanoparticles is smaller than the exclusion limit of the beads that were used. Finally, five fractions with

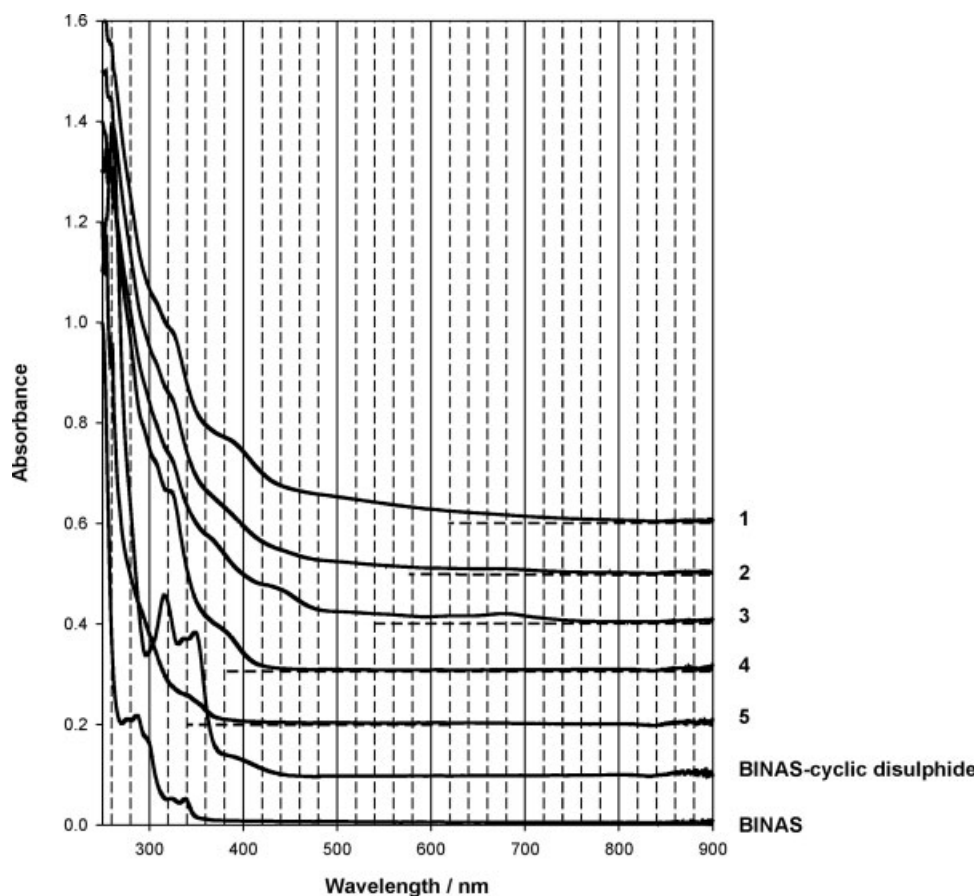


Fig. 2. UV-vis spectra of the separated fractions 1–5. Spectra are offset for clarity and normalized to one absorbance unit at 250 nm. The bottom spectrum corresponds to BINAS and the second one to BINAS-cyclic disulphide.

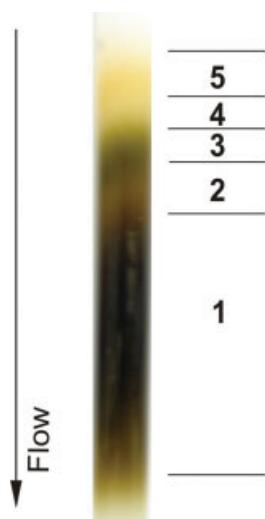


Fig. 3. Size exclusion chromatography separation of BINAS protected gold particles. The separated fractions are numbered from 1–5 according to their decreasing elution mobility. Horizontal lines show roughly the limit between adjacent fractions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

different color could be separated and were numbered 1–5 according to their elution order or their decreasing size. This shows that the size distribution is not homogenous and indicates the presence of magic number compounds, i.e., the high relative abundance of compounds with well-defined composition. The dependence of color on the size is a direct consequence of a quantum size effect. The latter is also responsible for the systematic red shift of the absorption onset for increasing particle size (from 5 to 1). Fractions 1, 3, and 5 are respectively brown, green, and yellow. Fraction 2 seems to be a mixture of the adjacent fractions 1 and 3 as judged from the UV-vis and CD spectra (vide infra).

Nuclear Magnetic Resonance of Gold BINAS Nanoparticles

The upper part of Figure 4 shows the aromatic region of ^1H NMR spectra for polydisperse gold nanoparticles covered with BINAS, free BINAS, and dinaphtho[2,1-c:1',2'-e] ^{1,2} dithiin (BINAS-cyclic disulphide) (Chart 1). The lower part of the Figure compares the aliphatic part of both TOAB and the as-prepared gold nanoparticles. In the aromatic region, the ^1H NMR spectrum of unbound BINAS displays six sets of signals with chemical shifts δ (ppm) = 7.89 (d, 2 H), 7.88 (d, 2H), 7.55 (d, 2 H), 7.41 (m, 2 H), 7.28 (m, 2 H), 6.98 (d, 2 H) and BINAS-cyclic disulphide shows five sharp signals, δ (ppm) = 7.90 (d, 2 H), 7.84 (2d, 2 H), 7.79 (d, 2 H), 7.44 (ddd, 2 H), 7.22–7.14 (m, 4 H). BINAS-cyclic disulphide is a major by-product observed during the synthesis of BINAS-gold nanoparticles. In contrast to the sharp signals observed for the latter compounds, the polydisperse gold nanoparticles are characterized by broad and shifted signals. This observation leads us to conclude that after purification the sample is free from BINAS-cyclic disulphide and BINAS impur-

ities. Previous ^1H NMR studies^{26–30} of MPNs have also described significant peak broadening and four main explanations have been put forward: (a) Rapid spin–spin relaxation from dipolar interaction; (b) dependence of chemical shifts on the size of nanoparticles and on the type of binding site (vertex, edges, terraces); (c) size-dependent rotation diffusion of the cluster leading to size-dependent spin–spin relaxation broadening; (d) the oxidation states of nanoparticles have a significant effect on NMR spectra of the monolayer ligand shell. The aliphatic part of the ^1H NMR spectra reveals the presence of tetraoctylammonium groups, which have a NMR signature significantly different from the one of free TOAB. This shows that there is no free TOAB. It has been shown by mass spectroscopy that the tetraoctylammonium groups balance the negative charge on the Au-thiolate cores.³¹

UV-vis Spectra

The UV-vis spectra in Figure 2 of the separated samples show no sign of a surface plasmon resonance characteristic for particles equal or larger to 3 nm, in agreement with the TEM measurements. However, the absorption spectra of the different fractions 1–5 show considerable structure. Also, the absorption spectra are distinctly different from the ones observed for the free BINAS and BINAS-cyclic disulphide ligands. There is also a clear trend in the spectra as concerns the absorption onset, which shifts to longer wavelengths with increasing particle size. This trend has already been observed for gold particles covered by glutathione^{14,25} and *N*-isobutyl-L-cysteine,¹¹ respectively, separated into different compounds by gel electrophoresis. For example, the onset of absorption occurred at about 400 nm for particles containing 10–12 gold core

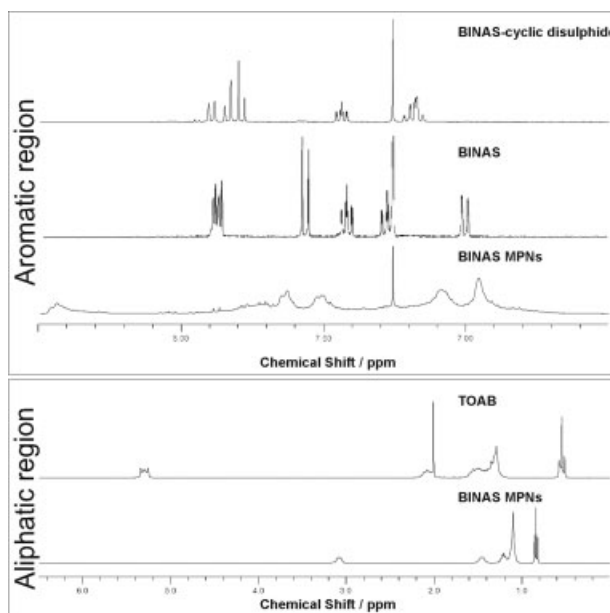


Fig. 4. ^1H NMR spectra (top) in the aromatic region of the polydisperse nanoparticles covered with BINAS, free BINAS and BINAS-cyclic disulphide and ^1H NMR spectra (bottom) in the aliphatic part of the polydisperse nanoparticles and of TOAB.

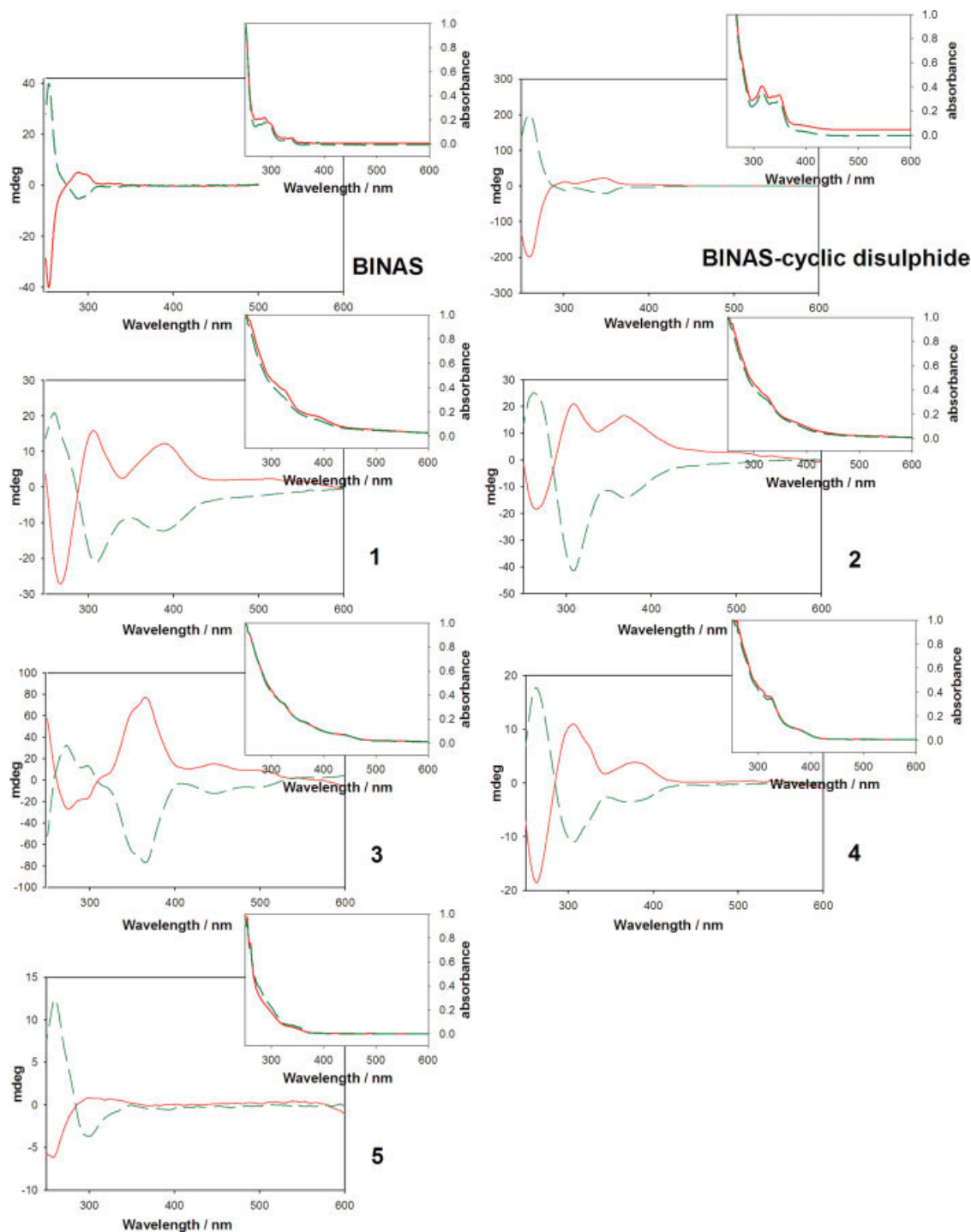
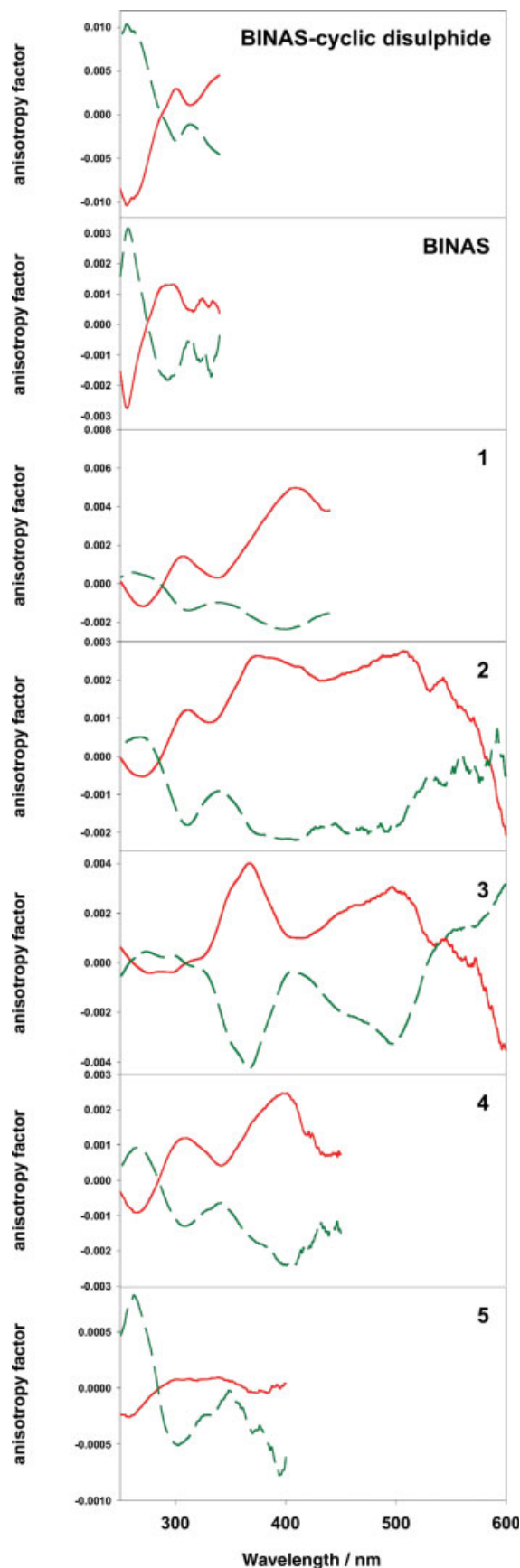


Fig. 5. CD and UV-vis spectra of separated fractions **1–5** of gold particles covered with *S*-BINAS (dashed) and *R*-BINAS (solid line). The UV-vis spectra were scaled to 1 absorbance unit at 250 nm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

atoms and above 800 nm for 25 gold core atoms. This indicates that the particles investigated here are very small, containing ten to a few tens of gold atoms only. Interestingly, the onset of absorption of particles containing 10 or 12 gold atoms depends strongly on the nature of the ligand. For Au₁₁ covered by phosphine ligands, the *Chirality* DOI 10.1002/chir

absorption onset is found at around 600 nm,¹⁷ whereas for gold particles of similar size covered by thiols it is found at around 400 nm.²⁵ This may be a consequence of the different nature of the gold–phosphorus and gold–sulfur bonds, the latter being considerably stronger. This likely has also an influence on the structure of the metal core. The struc-



ture of Au_{11} covered by phosphine ligands is well-known from X-ray crystallography,^{32,33} which reveals a compact metal core with a central gold atom surrounded by 10 peripheral gold atoms. The structure of gold particles of similar size covered by thiols might however be quite different. Indeed, density functional theory (DFT) calculations indicate that the structure of such small gold cores is strongly distorted by the adsorbing thiol. In particular, based on DFT calculations, the formation of cyclic thiolated gold clusters has been proposed.³⁴

Optical Activity in the UV-vis

Figure 5 shows the CD spectra of the separated fractions. The CD spectra of the gold particles covered with BINAS are distinctly different from the CD spectra of both free BINAS and BINAS-cyclic disulphide. It is, therefore, concluded that the optical activity observed for the gold particles covered by the BINAS is associated with electronic transitions within the metal core. It is also evident that the CD spectra depend on the particle size. This is particularly apparent from a comparison between the CD spectra of fractions **1** and **3**. None of the CD spectra is similar to those reported for small gold particles covered by the water-soluble thiols glutathione,¹⁴ penicillamine¹⁵ and *N*-isobutyl-cysteine,¹¹ respectively.

Optical activity of gold particles covered by BINAS has not yet been reported. However, two independent studies report the CD spectra of gold nanoparticles protected by BINAP.^{16,17} The protocol for the preparation of these particles was quite different in the two cases. In the procedure proposed by Hutchison and coworkers,³⁵ HAuCl_4 was reduced in the presence of the BINAP. This resulted in small particles with a size of 1.7 ± 0.4 nm, according to TEM. The other procedure started from a Au_2Cl_2 (BINAP) precursor that was subsequently reduced. This resulted selectively in Au_{11} clusters, as supported by electrospray ionization mass spectrometry and in agreement with TEM.¹⁷ The CD spectra reported for these two compounds are strikingly similar despite the fact that the average size of the particles in the former study is considerably larger. For the particles covered with the *R*-enantiomer of the BINAP, the CD spectra reported in both studies are characterized by a positive band at 440 nm, an about twice as strong positive band at 350 nm with a shoulder at longer wavelength and an even stronger negative band slightly below 300 nm. The striking similarity of these spectra indicates that the optical activity originates from similar particles within the two samples. Since the average size of the two samples is rather different and since in one case the sample consisted predominantly of Au_{11} clusters, it is proposed that the sample with the larger average size particles contains Au_{11} clus-

Fig. 6. Anisotropy factor of fractions **1–5** of BINAS gold nanoparticles, BINAS, and BINAS-cyclic disulphide. Dashed (solid) spectra are for the *S*-enantiomer (*R*-enantiomer). The anisotropy factor of fractions **1** and **5** is not symmetric for the two enantiomers because these fractions contain mixtures of different particles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ters, which leads to the observed optical activity. This furthermore implies that the larger particles, around 1.7 nm in size, do not contribute much to the optical activity of the sample.

Comparison of the CD spectra of the gold BINAP particles described above with the ones reported here for the gold BINAS particles shows for example that fraction **3** has a completely different CD spectrum. On the other hand fraction **4** exhibits a CD spectrum with similar shape and sign as that for Au₁₁ covered by BINAP, but shifted to shorter wavelength by about 50 nm. For the *R* enantiomer, a positive band at 380 nm, an about two and a half times as strong positive band at 300 nm with a shoulder at longer wavelengths and an even stronger negative band at 260 nm are observed. The corresponding UV-vis spectra also bear the same similarity with discrete bands shifted to longer wavelengths for the gold particles covered by the BINAS. For the gold particles covered by the BINAP (BINAS, fraction **4**) bands in the absorption spectra are observed at about 440 nm (380 nm), 370 nm (325 nm), and 350 nm (305 nm). These similarities could of course arise by chance, but they can also be an indication that fraction **4** contains Au₁₁ clusters covered by BINAS and that these Au₁₁ clusters have a similar structure as the ones covered by BINAP. The shift of the CD and absorption spectra has likely to do with the different nature of the gold-sulfur and gold-phosphorus bond. For such small clusters as Au₁₁ almost all the gold atoms reside on the surface of the metal particle and therefore the adsorbate has a strong influence on its electronic structure. For example, the latter is influenced by charge transfer between gold particles and adsorbate, which depends on the nature of the adsorbate.

It has been proposed that the optical activity of the Au₁₁ clusters covered by BINAP is due to an intrinsically chiral core.¹⁷ It is known that the structure of the Au₁₁ core depends on the type of the phosphine ligands.³⁶ This has probably to do with the fact that 10 of the 11 gold atoms reside on the surface of the metal core. The atoms are therefore unsaturated and relatively flexible. Adsorption of the BINAP leads to a deformation of the core to an asymmetric structure, which is at the origin of the optical activity in metal-based transitions. Such deformations are likely favored by molecules that contain two or more functional groups that can interact with the gold core. Our findings are in agreement with such a model. Since the gold-sulfur bond is stronger than the gold-phosphorus bond, the distortion of the gold core imposed by the adsorbed ligand is expected to be stronger in the case of adsorbed BINAS compared with adsorbed BINAP. This could also lead to a stronger deviation from a symmetric structure in the former case and therefore to a stronger chiroptical response. Indeed, the anisotropy factors ($\Delta\epsilon/\epsilon$) found here for fraction **4** of gold particles covered by BINAS are about a factor of two stronger than the ones reported for Au₁₁ covered by BINAP.¹⁷ In both cases, the band at the longest wavelength has the largest anisotropy factor (see Fig. 6), which is 1.2×10^{-3} in the latter and 2.5×10^{-3} in the former case. Note that anisotropy factors as large as 4.0×10^{-3} are found for fraction **3**. To the best of our knowl-

edge such large anisotropy factors were never observed for optically active gold particles.

In conclusion, we report the preparation of small gold particles passivated by BINAS. The as prepared and purified particles could be separated into several distinct fractions using size-exclusion chromatography. The different fractions exhibit different color, absorption and CD spectra. The absorption and CD spectra of one fraction bears some resemblance with the spectra previously reported for Au₁₁ particles stabilized by BINAP although the spectra are shifted to shorter wavelength in the case of the thiol. The anisotropy factors are as large as 4×10^{-3} , which is the largest reported so far for gold particles covered by chiral thiols. This indicates that BINAS is particularly well-suited to bestow chirality onto gold particles. The results show that the optical activity is enhanced by using a stronger gold-ligand bond. This is in good agreement with a model that ascribes the optical activity to an adsorbate-induced local deformation of the metal core, i.e., the formation of a "chiral-footprint."¹¹

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Circular Dichroism Study of DNA Binding by a Potential Anticancer Peptide Nucleic Acid Targeted Against the *MYCN* Oncogene

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT The interaction with DNA of a peptide nucleic acid (PNA) oligomer (16nt) conjugated with a nuclear localization signal (NLS) peptide, which was previously found to be able to inhibit tumor cell proliferation through block of transcription of the *MYCN* oncogene, was studied by UV and CD spectroscopy. While data obtained by UV were not conclusive, the use of circular dichroism gave clear-cut evidence of the formation of a PNA:DNA duplex of exceptionally high stability ($T_m \geq 90^\circ\text{C}$). Using the same approach, the effect of mutations on DNA:PNA stability was evaluated, and was found in accordance with that expected for a Watson–Crick interaction. The role of the NLS peptide was evaluated by using a PNA lacking of this part, which gave rise to less stable PNA:DNA duplexes. Finally, a competition experiment carried out with a 26mer dsDNA, containing the target 16mer sequence in its middle region, in the presence of PNA-NLS gave evidence for the formation of a ternary complex at 25° , while at higher temperature, the PNA:DNA duplex and the displaced homologous DNA strand were detected. The present results support the possibility of an analogous mechanism of action of this antitumor PNA in vivo. *Chirality* 20:494–500, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: PNA; DNA recognition; antitumor; antigene; circular dichroism; *MYCN*; strand-invasion

INTRODUCTION

The use of specific agents which block gene expression is one of the most promising approach for the control of different kinds of diseases.¹ This goal can be achieved in principle by different approaches (Fig. 1a): (i) blockade of splicing or translation from mRNA to protein by hybridizing with complementary oligonucleotides in the so called “antisense” approach,^{2,3} or in the very effective “RNA interference” (RNAi) strategy;⁴ (ii) blockade of transcription factors by saturating their binding site with oligonucleotides in the so called “decoy” strategy;⁵ (iii) blockade of transcription by binding the DNA itself through sequence-specific interactions, in the so called “antigene” approach.^{6,7}

Though the RNAi approach is currently considered as the most promising for regulation of gene expression and for drug development, the antigene approach remains one of the most intriguing and challenging goals, since candidate molecules should recognize in a sequence-selective way the source of genetic information itself, that is, the DNA double helix.

Peptide nucleic acids (PNAs, Fig. 2) are among the most potent tools for the control of gene expression by binding to complementary DNA or RNA.⁸ Their use as therapeutic agents has been proposed from early studies⁹ and recent advances in cellular delivery systems¹⁰ and in the so-called anti-gene strategy make them good candi-

dates for drug development.¹¹ Moreover, in the last decade, a great variety of experiments using PNA on different biological target have been described, ranging from bacteria,¹² to viruses,¹³ to endogenous genes connected with cardiovascular diseases,¹⁴ neurodegenerative disorders¹⁵ and tumors.¹⁶ Modified PNAs have also been designed to improve DNA binding and delivery efficiency.¹⁷ A unique property of homopyrimidine PNAs is their ability to form very stable PNA:DNA:PNA triplexes, through the formation of Watson–Crick and Hoogsteen hydrogen bonds;¹⁸ these structures can be formed by hybridization of PNAs on dsDNA by displacement of the homopyrimidine DNA strand; this process, known as “strand invasion,” can in principle be used for the anti-gene strategy, but is limited in use because of the necessity to have homopurine:homopyrimidine segments.¹⁹

Strand invasion of mixed sequences can be performed using two PNAs containing modified pseudocomplementary nucleobases (DNA openers), which are able to simul-

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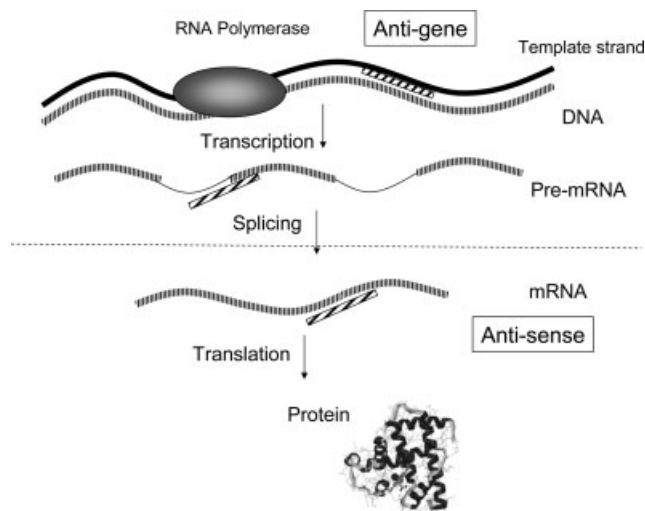


Fig. 1. Antisense and anti-gene strategies used for inhibiting gene expression by oligonucleotides and their analogs.

taneously bind to the two complementary strands, but do not form PNA:DNA duplexes.²⁰

Boffa et al. were the first to study cellular systems in which mixed-sequence unmodified PNAs (i.e., not triplex-forming) have been used to block gene expression through inhibition of transcription²¹; other examples using mixed sequence-PNAs have been subsequently reported,^{22,23} though the precise mechanism of action is still under investigation. Animal studies have been also recently described using this approach.²⁴

Recently, we have developed antisense²⁵ and anti-gene^{26,27} PNAs for the selective inhibition of the *N-myc* oncogene (*MYCN*) in pediatric tumors in which it is over-expressed, such as neuroblastoma (NB). A very efficient approach turned out to be the use of the 16mer anti-gene PNA **1**, conjugated with a nuclear localization signal (NLS) peptide (PKKRKR) and directed against the non-coding (antisense) strand of DNA. Inhibition of cell proliferation up to 90% was observed. In this work we present a detailed study by UV and CD spectroscopy on the stoichiometry and stability of the PNA:DNA duplex. The most convincing evidence comes from circular dichroism studies, confirming the importance of this technique in assessing biomolecular interactions, and support the proposed anti-gene mechanism previously proposed.

MATERIALS AND METHODS

Instrumentation

PNA automated synthesis was performed on a ABI 433A (Applied Biosystem) peptide synthesizer, following the synthetic Boc protocol provided by the company.

PNA analysis was performed by analytical HPLC-MS on a Waters 2695 separation module (Waters, Milford, MA) interfaced with a Micromass ZMD mass spectrometer (Micromass, Manchester, UK) equipped with an ESI interface and a single quadrupole analyzer.

Direct insertion mass spectra were obtained on the same mass spectrometer.

PNA purification was performed by semipreparative HPLC by using Waters 510 pumps, Waters automated gradient controller, Waters 759A UV-vis detector and a Waters 745 data module.

Absorption spectra and UV melting temperatures were measured on a Perkin-Elmer (Norwalk, CT) λ BIO 20 spectrophotometer, equipped with Peltier PTP 6 temperature programmer and a cell changer.

Circular dichroism spectra were recorded on a Jasco (Tokyo, Japan) J 715 spectropolarimeter equipped with a Peltier PTC 348 temperature controller unit.

Materials and Reagents

PNA *N*-Boc protected monomers, *N*-[1*H*-1,2,3-(benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU), dichloromethane and *N*-methylpyrrolidone (NMP) were purchased from Applied Biosystems (Foster City, CA).

Oligonucleotides (HPLC grade) were purchased from Thermo Electron, and used without further purification.

PNA Synthesis

The synthesis of PNA **1** and **2** was described previously.²⁶ PNA **3** was synthesized using the same procedure, by automatic synthesis on a MBHA resin with Boc chemistry, purified by RP-HPLC, and characterized by HPLC-ESI-MS. PNA **3**. Calculated: MH_3^{3+} : 1457.1; MH_4^{4+} : 1093.1; MH_5^{5+} : 874.6; MH_6^{6+} : 729.0; MH_7^{7+} : 625.0; MH_8^{8+} : 547.0. Found: 1457; 1093; 875; 729; 625; 547.

Spectral Measurements

Stock solutions of PNA **1-3**, and of DNA oligonucleotides (DNA **4-13**) were prepared in double distilled water and their actual concentration calculated by UV absorbance using the following ϵ_{260} ($M^{-1} cm^{-1}$) for the nucleobases: T 8600, C 6600, A 13700, G 11700. By using these concentrations, hybrid solutions containing 5 μM PNA-PNA or PNA-DNA duplexes were prepared. The measurements were performed using either a 1 cm or a 0.2 cm quartz cell, and spectra reported were normalized to that of the 1 cm cell. All the hybridisation experiments were carried out in 10 mM phosphate buffer, 100mM NaCl, 0.1 mM EDTA, pH = 7. All the hybrid samples reported were first incubated at 90°C for 5 min, then slowly cooled to room temperature. In the melting measurements, the samples were heated (1°C/min) and the UV signal variation at

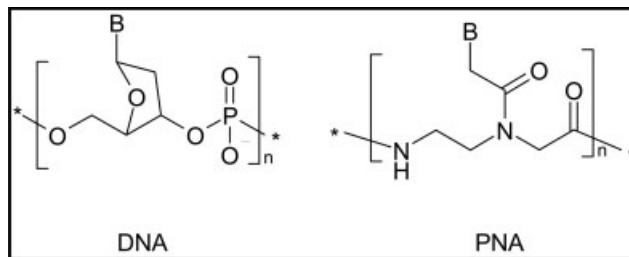


Fig. 2. Structure of DNA and PNA.

TABLE 1. PNA and DNA sequences used in the present work (in bold the target region of PNA is indicated, mutations are italic)

PNA/DNA	Sequence	Type ^a
PNA 1	H-ATG CCG GGC ATG ATC T -PKKKRKV-NH ₂	<i>MYCN</i> complementary with NLS peptide
PNA 2	H-GTG CCG AGC ATG <i>GTC</i> T -PKKKRKV-NH ₂	<i>MYCN</i> with three mutations and NLS peptide
PNA 3	H-ATG CCG GGC ATG ATC T -NH ₂	<i>MYCN</i> complementary without NLS peptide
DNA 4	5'-A GAT CAT GCC CGG CAT-3'	16mer, fully complementary to PNA 1
DNA 5	5'-A GAT CAT GCT CGG CAT-3'	16mer with one mutation
DNA 6	5'-A GAC CAT GCT CGG CAC-3'	16mer with three mutations
DNA 7	5'-CTT GCA GAT CAT GCC CGG CAT -3'	21mer containing target sequence for PNA 1
DNA 8	5'-CTT GCA GAT CAT GCC CGG CAT GGTGG-3'	26mer containing target sequence for PNA 1
DNA 9	5'-CTT GCA GAT CAT GCT CGG CAT GGTGG-3'	26mer with one mutation
DNA 10	5'-CTT GCA GAC CAT GCT CGG CAC GGTGG-3'	26mer with three mutations
DNA 11	5'-CC ACC ATG CCG GGC ATG ATC TGC AAG-3'	Complementary to DNA 8
DNA 12	5'-GGT GAA GCT AAC GTC GA-3'	17mer non complementary
DNA 13	5'-GCC CCT CAA CGT TAG CTT CAC CAA CAG-3'	27mer non complementary

^aFull matched DNA sequences were extracted from the *MYCN* gene sequence (Genbank accession no. M13241).

260 nm or CD signal at 264 nm were recorded. Melting temperatures were measured as the maximum of the first derivative of the melting curves. All the spectra were smoothed and treated with noise reduction software.

RESULTS AND DISCUSSION

UV Measurements

The PNAs and DNA studied in the present work are listed in Table 1. PNA 1 was designed in order to be complementary to the noncoding strand of *MYCN* gene at the initial part of hexone 2. In a previous study,²⁶ it was found that this PNA was more effective in inhibiting cellular growth in several tumor cell lines than its complementary sequence, which could act as an antisense agent (since it is complementary also to mRNA). These results suggested that PNA 1 could act by hybridizing the complementary

DNA strand in the cell genome, facilitated by the nuclear localization signal peptide attached to its C-terminal part. This hypothesis was further confirmed by the observation that an analogous PNA containing three mismatches (2) was ineffective under the same conditions, suggesting that the observed effect was based on sequence-selective DNA recognition. However, the sequence of PNA 1 is not homopyrimidinic and it is interesting to study why this PNA could invade DNA blocking transcription.

We first evaluated the stability of the PNA:DNA duplex by using UV melting curves at 260 nm, but the melting profile was anomalous (Fig. 3a), suggesting the formation of a duplex of very high stability. By measuring the PNA:DNA duplexes in the presence of one or three mismatches, we observed the T_m values reported in Table 2, without complete melting at 90°C, but with an inflection point at lower temperature, which was interpreted as the melting temperature.

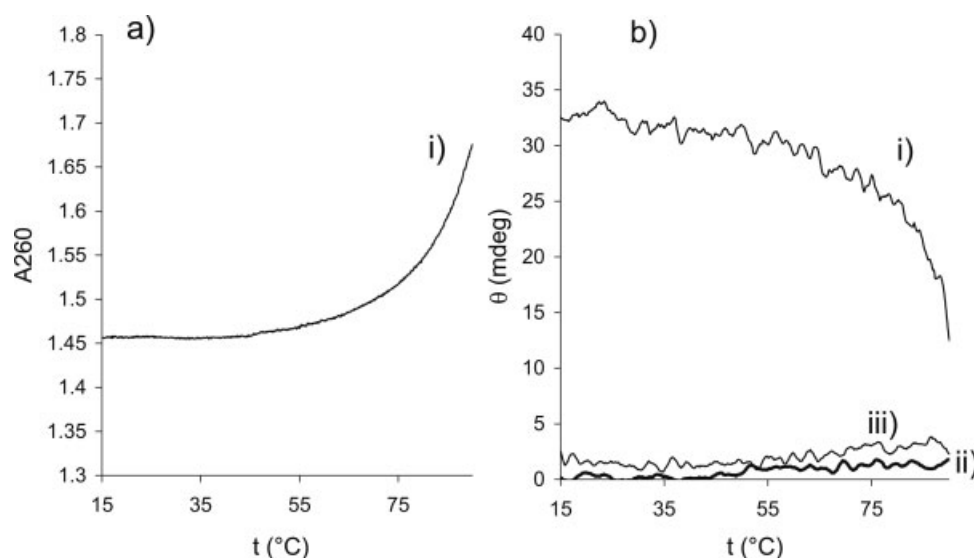


Fig. 3. Melting curves obtained for the duplex PNA 1: DNA 4 obtained by (a) UV at 260 nm; (b) CD at 264 nm: (i) PNA 1: DNA 4; (ii) PNA 1 alone; (iii) DNA 4 alone. Conditions: phosphate buffer (10 mM phosphate, 0.1 M NaCl, 0.1 mM EDTA); $c = 5 \mu\text{M}$ of each strand; scan speed $1^\circ\text{C}/\text{min}$.

TABLE 2. Melting temperatures obtained for different duplexes, as measured by UV and CD spectroscopy

Duplex	T_m	
	UV (260 nm)/°C	CD (263 nm)/°C
PNA 1:DNA 4	>90	>90
PNA 1:DNA 5	81	79
PNA 1:DNA 6	78	75
PNA 1:DNA 7	>90	>90
PNA 1:DNA 8	>90	>90
PNA 1:DNA 9	89	n.d. ^a
PNA 1: DNA 10	85	84
PNA 1: DNA 12	<15 ^b	<15 ^b
PNA 1: DNA 13	<15 ^b	<15 ^b
PNA 2: DNA 5	84	82
PNA 2: DNA 6	>90	>90
PNA 2: DNA 4	70	69
PNA 3: DNA 4	86	85
PNA 3: DNA 5	77	79
PNA 3: DNA 6	66	65
PNA 3: DNA 8	87	88
DNA 8: DNA 11	73	73

All values were obtained in phosphate buffer containing 0.1 M NaCl, at a concentration of 5 μ M of each strand.

^an.d.: Not determined.

^bTurbidity was observed in these samples.

We therefore evaluated if an hypochromic effect could be observed, in order to find evidence of the formation of the PNA:DNA duplex, but the results were not clear-cut (2.6% hypochromicity with standard deviation = 1.1%). This effect could be due to the self-association of the two strands (PNA and DNA) which attenuate the difference observed. In fact, self-melting was observed for both DNA 4 (T_m = 55°C) and PNA 1 (T_m = 70°C).

CD Measurements

The CD spectra of the PNA 1 of DNA 4 and of the full-match duplex are reported in Figure 4. The PNA, though it has a terminal chiral peptidic part (NLS), has practically negligible CD intensity in the 210–350 nm region, because of the flexibility of its backbone which prevents asymmetric disposition of the bases. The DNA 4 has a typical spectrum of a single-stranded oligonucleotide, with a crossover point at 264 nm. On the contrary, the mixture of the PNA 1 and DNA 4 has a spectrum with a maximum at 264 nm, typical of PNA:DNA duplexes. Therefore, in spite of the low hypochromicity observed in the UV spectrum, the CD spectrum clearly indicates that a PNA:DNA duplex is formed at 25°C. The large difference observed at 264 nm between the PNA:DNA complex and the two single strands allowed us to perform a Job-plot experiment, which showed that a 1:1 duplex was formed (Fig. 4b).

Since the CD of PNA:DNA was positive at 264 nm while that of the two strands was zero it was also possible to perform melting measurements by CD. The results are reported in Table 1 and in Figure 2b. The CD signal was still present at 90°C, with an intensity of about 50% of its value at 15°C, suggesting that the melting temperature is $\geq 90^\circ\text{C}$. The presence of one or three mismatches (DNA 5 and 6) gave a lower melting temperature (see Table 1), as expected for a Watson–Crick base pairing recognition scheme.

The same upper limit was found for longer oligonucleotides (DNA 7, 21mer and DNA 8, 26mer), thus ruling out the possibility that the C-terminal modification with NLS could destabilize the PNA:DNA duplex by creating steric hindrance with adjacent DNA monomers. On the contrary, a stabilizing effect was observed for mismatched sequences, suggesting attracting electrostatic interactions of the NLS peptide with the pending DNA tract.

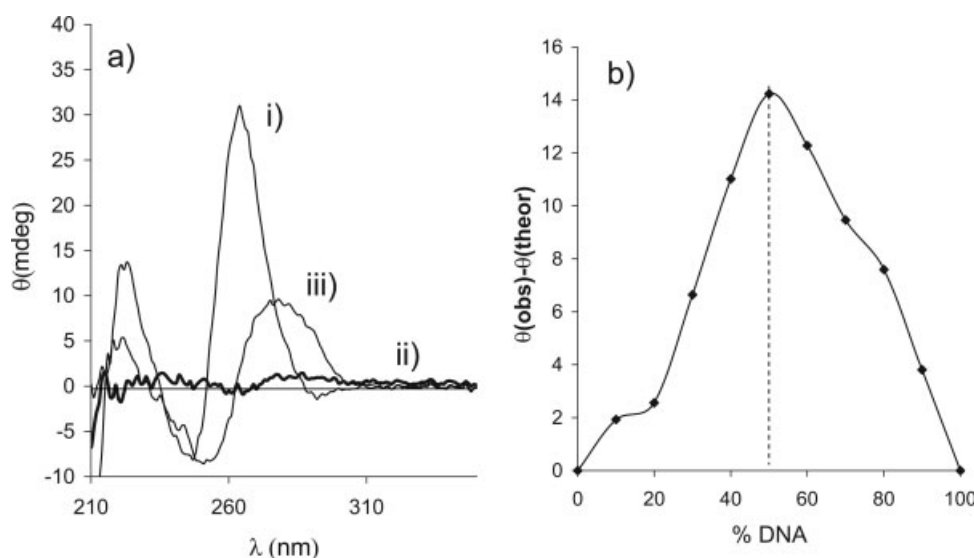


Fig. 4. (a) CD spectra obtained for (i) PNA 1: DNA 4; (ii) PNA 1 alone; (iii) DNA 4 alone. Conditions: phosphate buffer (10 mM phosphate, 0.1 M NaCl, 0.1 mM EDTA) c = 5 μ M of each strand. (b) Job-plot obtained from CD data of binary mixtures of PNA 1 and DNA 4, with total concentration = 10 μ M.

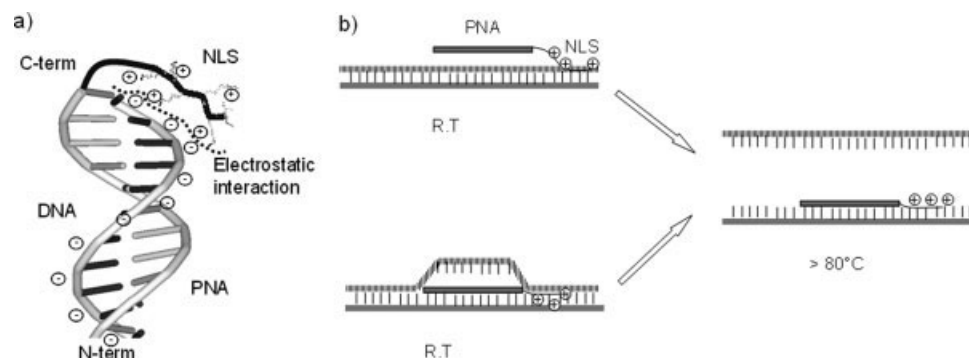


Fig. 5. (a) Pictorial model of the PNA:DNA interaction enhanced by the contribution by the NLS peptide. (b) Two possible type of interactions occurring in the ternary mixture. Upper model: external interaction of the PNA on the DNA double helix; lower model: strand invasion.

The interaction with non complementary sequences (DNA **12,13**) gave rise to very low duplex stability by UV (no melting different from self melt observed, Table 2), indicating that the major interaction mechanism is the Watson–Crick base pairing, although an unspecific interaction gave rise to partial precipitation of the DNA because of the positively charged NLS peptide.

Using a PNA containing three mismatches (PNA **2**), a similar trend was observed, with a very high melting temperature for the full match DNA **6** and a lower value for the mutated DNA **5** (two mismatches). This result is in line with the reported lower efficiency of the mismatched PNA **2** towards *MYCN* inhibition observed in cellular systems. It should be noted that although the melting temperatures reported are above 37°C for the mismatched duplexes in the experiments carried out on cellular systems it is likely that the effective concentration of the PNA will be significantly lower than that used in the medium (10 μ M), and that mismatched PNA (e.g., PNA **2** in this study) cannot successfully compete with the annealing of dsDNA (compare for example the T_m of dsDNA **8:11** with that of the PNA **2**:DNA **4**), and with other nuclear constituents (histones, DNA-binding proteins, transcription factors etc); therefore only PNA forming duplexes of very high stability (as the full-matched duplexes reported here) can be effective on cellular systems; this is in line with results from our previous studies, in which PNA **2** gave very low inhibition of cancer cell growth.

Both melting temperatures measured for the full-matched duplexes of PNA **1**:DNA **4** and PNA **2**:DNA **6** are much higher than those calculated according to a statistical model described in the literature for PNA:DNA duplexes²⁸ (70.3°C and 72.4°C respectively). This enhancement could be a consequence of the particular sequence used, or could be due to the additive contribution of the positively charged NLS peptide, which contains four lysine and one arginine residues. The role of the NLS peptide was therefore evaluated by synthesizing a PNA lacking of this part (PNA **3**). The melting temperatures measured in this case are reported in Table 1. Destabilization was observed for both the full-matched and for the mismatched duplexes, suggesting that the peptide has an unspecific stabilizing effect. Therefore, the presence of the NLS peptide has in this case two major consequences: it is

necessary in order to deliver the PNA into the cell nucleus and it further increases the stability of the PNA:DNA duplex by electrostatic interactions, as depicted in Figure 5a. Although the melting temperature measured for PNA **3**:DNA **4** is already high (higher than that predicted by the model), a further increase is important in view of its use *in vivo*, since many promising oligonucleotides analogues able to bind DNA *in vitro* are ineffective *in vivo*.

Competitive Experiments

In order to evaluate if PNA **1** was able to perform strand invasion, we used the 26mer DNA **8**:DNA**11** duplex as a model; this duplex was designed in order to have the target 16mer sequence flanked by a stretch of five oligonucleotides on both sides, corresponding to the sequences present in the *MYCN* gene. The melting curve of the dsDNA was measured both by CD and by UV and was found to be lower than the PNA:DNA duplex ($T_m = 73^\circ\text{C}$ in buffer containing 0.1 M NaCl), although in the latter only 16 out of 26 bases were hybridized.

At 25°C, the circular dichroism of the mixture PNA **1**:DNA **8**:DNA **11** has the CD spectrum reported in Figure 6a (compared with ssDNA, dsDNA, and PNA:DNA).

The spectrum of the ternary mixture was higher than that of both PNA **1**:DNA **8** and DNA **8**:DNA**11**.

Since the contribution of the PNA **1** alone is very weak (see spectrum in Fig. 4), it can be ruled out that the observed CD was the sum of dsDNA and PNA **1**. To evaluate if, on the contrary, it was the sum of PNA **1**:DNA **8** and of the ssDNA **1**, we compared the observed spectrum of the ternary mixture with the sum of these two components (Fig. 6b). Significant deviations were observed, notably at 274, 245, and 218 nm. We therefore concluded that under these conditions a ternary complex is formed.

The CD spectra recorded at 80°C for the same mixtures are reported in Figure 6c. The ternary mixture is clearly different from that of DNA **8**:DNA**11** (Fig. 6c). Comparison of the ternary mixture spectrum with that obtained from the sum of PNA **1**:DNA **8** and of DNA **11** showed good matching (Fig. 6d), suggesting that at this temperature, the ternary complex dissociates into the PNA **1**:DNA **8** duplex and ssDNA **11**. The presence of the complementary DNA **11** does not hamper the formation of the

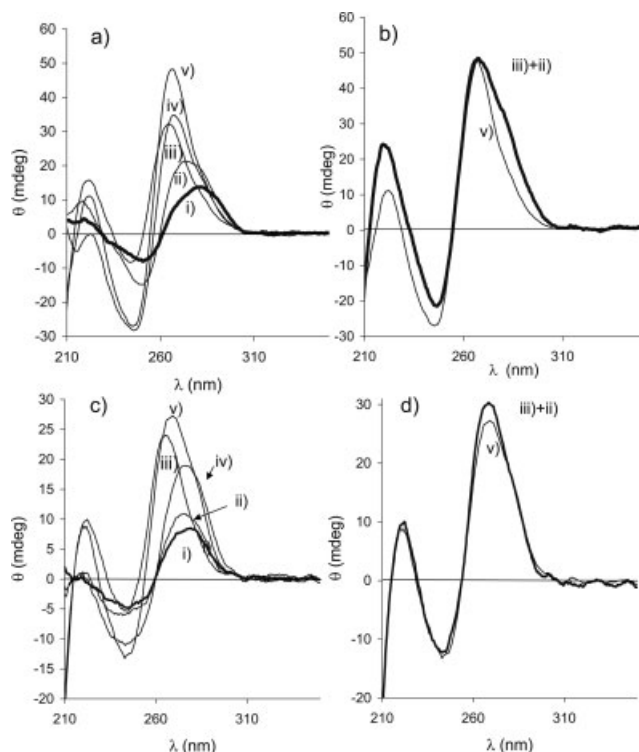


Fig. 6. Comparison of spectra obtained at different temperatures. (a) Spectra at 25°C and (c) spectra at 80°C; (i) DNA **8**; (ii) DNA **11**; (iii) PNA **1** + DNA **8**; (iv) DNA **8** + DNA **11**; (v) PNA **1** + DNA **8** + DNA **11**; (b) and (d) comparison of the spectra observed for the ternary mixture (v) and the sum of spectra of PNA **1** + DNA **8** and of the DNA **11**; (b) at 25°C and (d) at 80°C. Conditions: phosphate buffer (10 mM phosphate, 0.1 M NaCl, 0.1 mM EDTA); $c = 5 \mu\text{M}$ of each strand.

PNA:DNA duplex at high temperature, in agreement with the higher thermal stability of the former.

Two possible scenarios can be inferred from these data (Fig. 5b). In the first, the PNA:DNA:DNA interaction is external and becomes competitive only once the DNA:DNA complex is dissociated. In the second, a ternary complex, which has a CD spectrum different to that of DNA:DNA, is formed, and the thermal denaturation of this complex occurs by first losing the DNA strand competing with the PNA, a process which occurs at high temperature, and then by dissociation of the PNA:DNA, which occurs at temperatures higher than 90°C.

Although the *in vitro* measurements reported here are under conditions very different from those in cellular experiments, one major result is that PNA:DNA interaction is occurring at relatively high salt concentration (0.1 M NaCl), which normally stabilize dsDNA and prevent interaction with PNA.

This work demonstrates that the anti-tumor PNA **1** binds to its DNA target with a very high stability. The lack of a sigmoidal curve and the hypochromic effect in the UV spectrum of the duplex required confirmation of the UV data using circular dichroism spectroscopy. The latter allows better selection of the specific signal of the DNA:PNA duplex. The melting temperature observed is unexpectedly high, partly due to the favorable interactions

and the NLS peptide. The melting temperature is dependent on the sequence, thus indicating the formation of a duplex through Watson–Crick interactions. This high stability and selectivity can justify the efficiency of this particular PNA in cellular systems, in which an anti-gene effect was proposed. The competitive experiment studied herein by CD spectroscopy pointed out that at high temperature when the ternary mixture is analyzed, the DNA:DNA duplex melts, while the PNA:DNA duplex persists up to 90°C. The data at room temperature suggest two possible mechanisms for this process: external interaction of the PNA with the dsDNA or strand invasion by the PNA in the duplex. The contribution in the region of maximum CD intensity of the single strand DNA **11**, suggests that the latter mechanism is more likely. Further studies are in progress in order to investigate the structure of this ternary complex.

In both cases, the evidence supports the models proposed for the anti-gene mechanism. In fact the PNA could either be inserted into the DNA double helix, thus inhibiting transcription, or linked externally to the DNA double helix and replace the original DNA strand once this is subject to denaturation, which in the present study was obtained by raising the temperature, but could be carried out *in vivo* by the unwinding effect of the translational machinery.

These data are obtained with model systems and therefore may not be directly linked to mechanisms occurring in the cellular systems, in which interactions with other components (primarily with proteins) have to be taken into account. However, knowledge of the basic interactions occurring between PNA and DNA (single or double stranded) is necessary to build a more complete picture of the biomolecular interactions involved in the sequence-specific inhibition of gene expression.

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Determination of the Absolute Stereochemistry of Chiral Biphenanthryls in Solution Phase Using Chiroptical Spectroscopic Methods: 2,2'-Diphenyl-[3,3'-biphenanthrene]-4,4'-diol

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT Although the exciton coupling model has been widely used in the literature to suggest the absolute configuration using electronic circular dichroism (ECD) spectra, chiral biphenanthryl systems are one group of molecules where the application of the exciton model yields ambiguous conclusions. The use of 2,2'-diphenyl-[3,3'-biphenanthrene]-4,4'-diol, known as VAPOL, as a chiral ligand for enantioselective catalysis necessitates the reliable determination of its absolute configuration. Based on the X-ray diffraction data of its phosphoric amide derivative, the configuration of VAPOL has been determined in the literature to be (+)-(aS). The experimental vibrational circular dichroism, ECD and optical rotatory dispersion spectra, and corresponding quantum mechanical predictions of VAPOL are used in this manuscript to independently determine its absolute configuration. *Chirality* 20:501–510, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: VAPOL; absolute configuration; vibrational circular dichroism; electronic circular dichroism; optical rotation; vibrational spectra; density functional predictions

INTRODUCTION

Substituted biphenanthryl compounds are of importance, because the biphenanthryl group is present in a variety of natural and synthetic compounds; they provide expanded opportunities for chiral recognition and have the necessary skeleton needed for chiral molecular motors.^{1,2} For example, the 1,1',2,2',3,3',4,4'-octahydro-4,4'-biphenanthrylidine skeleton was used for a molecular motor.^{1,2} In addition, biphenanthryl molecules also belong to an important class of chiral polycyclic aromatic hydrocarbons with significant environmental interest. In chemical synthesis, compounds containing phenanthryl groups are considered to have an important role. In particular, 2,2'-diphenyl-[3,3'-biphenanthrene]-4,4'-diol, also known as VAPOL, and its derivatives have been identified^{3–5} as highly effective enantioselective catalysts and ligands. Their utility spans a range of asymmetric reactions such as the Diels-Alder,^{6,7} imino-aldol,⁸ and aziridination^{9–11} reactions. Numerous studies have solidified¹² the evidence that biphenanthryl compounds induce asymmetric reactions with a wide range of substrates and provide high yields. Enantiopure species and the knowledge of their absolute configurations are needed for applications in asymmetric syntheses.

The conformation (i.e., dihedral angle between the two phenanthryl groups) has not been determined unambiguously for substituted biphenanthryl molecules in general, although this information is necessary to establish the absolute configuration. As such there are two contradic-

tory assignments^{13–15} made for the absolute configurations of 3,3'-dihydroxy-4,4'-biphenanthryl and 3,3'-dimethoxy-4,4'-biphenanthryl. The absolute configuration of 3,3'-dihydroxy-4,4'-biphenanthryl was originally assigned¹³ as (–)-(aS), but was later determined¹⁵ to be (–)-(aR). The absolute configurations of 10,10'-dihydroxy-9,9'-biphenanthryl and 2,2'-dihydroxy-1,1'-biphenanthryl were determined^{16,17} to be (+)-(aR). The absolute configurations of 10,10'-dimethoxy-9,9'-biphenanthryl and 2,2'-dimethoxy-1,1'-biphenanthryl have also been reported^{16,17} in the literature. However, modern chiroptical spectroscopic methods have not yet been applied to any of these molecules.

In recent years, chiroptical spectroscopic methods have emerged^{18,19} as powerful tools to determine the absolute configuration and predominant conformations of chiral molecules in solution phase. These methods include vibrational circular dichroism (VCD), vibrational Raman optical activity, electronic circular dichroism (ECD), and optical rotatory dispersion (ORD). While all four methods need not be used simultaneously, the use of more than one

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method certainly helps reduce the uncertainty in the conclusions emerging from individual methods. Furthermore, as the sensitivity to conformational details varies among these methods, a complete understanding can only be obtained using multiple methods. The successful use of chiroptical spectroscopic methods depends on the reliable theoretical predictions of corresponding properties, and therefore quantum mechanical predictions of chiroptical properties are needed along with experimental measurements.

There are no prior VCD studies on biphenanthryl compounds. The molecule closest to biphenanthryl class of molecules that has been studied by VCD is 1-(9-phenanthryl)ethylamine.²⁰ In that work, the vibrational bands originating from methyl antisymmetric deformation and NH₂ deformations have been discussed, but those vibrations originating from phenanthryl group have not. Infrared spectra of phenanthrene single crystals were reported by several authors,^{21–24} but some uncertainties in vibrational assignments remain. The infrared spectra of phenanthrene and alkyl-substituted phenanthrenes in CS₂ or CHCl₃ were reported²⁵ to contain intense absorption bands at 1600 and 1500 cm^{–1}. Also the 1500–900 cm^{–1} region was reported to be dense with several bands appearing in this region. The bands at 870, 810, and 730 cm^{–1} were noted to be influenced by substitution. However, there is no discussion on the assignment of vibrations of phenanthrene group. The vapor-phase infrared spectra of phenanthrene was reported by Cane et al., and with the help of density functional theoretical predictions, vibrational assignments were suggested.²⁶ The infrared spectrum of phenanthrene predicted with B3LYP functional were compared to the gas-phase infrared spectrum²⁷ or single crystal infrared spectrum.²⁸ In these papers, the focus was on identifying the symmetry representation of the observed bands, but vibrational mode descriptions were not provided. Thus, there is a need for understanding the vibrational modes of phenanthrene itself. No literature reports could be found on the vibrational spectroscopy of biphenanthryl compounds.

The electronic spectra of phenanthrene have been analyzed^{29,30} in detail using C_{2v} point group symmetry. However, the irreducible representations of this point group are not used by the ECD community for specifying the electronic states. Instead, the spectroscopic notation of Platt^{31,32} is used for the analysis of ECD spectra. The five lowest energy electronic transitions, namely ¹L_b at 353 nm, ¹L_a at 303 nm, ¹B_b at 254 nm, ¹C_b at 212 nm, and ¹B_a at 187 nm, have been identified³¹ in the visible spectrum. Of these, ¹B_b transition is the most intense and is polarized along the long axis of phenanthrene. Thus to apply the exciton chirality ECD method³³ for chiral biphenanthryl compounds, one looks for ¹B_b absorption band and analyzes the associated bisignate ECD band. A positive bisignate ECD couplet associated with ¹B_b band indicates that the long axes of two phenanthryl groups make a right-handed screw sense; a negative bisignate ECD couplet indicates left-handed screw sense.³³ However, for practical applications, the identification of the ¹B_b band might not be easy when this band overlaps with those from other electronic transitions appearing in the

same wavelength range. The exciton chirality method³³ is useful for rigid molecules and when transitions associated with chromophores under consideration are isolated (not overlapped by the transitions from the remaining parts of the molecule). However, in a majority of cases, a given molecule may exist in different conformations; and the electronic transitions of chromophores under consideration need not be well separated from other transitions in the molecule. Thus, there are situations where exciton chirality method may not be easily applicable. Some of the substituted biphenanthryl molecules are such difficult examples. As an illustration of the difficulties in assigning the absolute configuration of phenanthryl compounds using exciton chirality ECD method, a few examples noted in the literature are summarized here. (1) Hattori et al.¹⁷ determined the configuration for 1,1'-biphenanthryl-2,2'-diol to be (+)-(aR). The (aR) configuration of 1,1'-biphenanthryl-2,2'-diol has two long axes oriented with negative chirality, and so the authors expected a negative ECD couplet for ¹B_b transition, but a positive ECD couplet was found for (+)-1,1'-biphenanthryl-2,2'-diol and its methoxy derivative. This discrepancy was thought to arise from the overlap with a second transition polarized along the short axis. As a result, the exciton chirality method is known to have limited applicability for the compounds containing phenanthrene chromophores. (2) Based on ¹H NMR studies, the absolute configuration of 1-(9-phenanthryl)-2-naphthoic acid and 1-(9-phenanthryl)-2-naphthalene methanol were assigned as (–)-(aS). The exciton chirality ECD method required several assumptions because the observed ECD spectra of these compounds gave complex ECD patterns, and it was not clear which ECD band corresponds to which electronic transition. Based on chemical correlation and X-ray results, the configurational assignment was reversed³⁴ as (+)-(aS). (3) For assigning the absolute configuration of 1-(9-phenanthryl)ethylamine, a bichromophoric derivative obtained by complexing 1-(9-phenanthryl)ethylamine with naphthimido chromophore was synthesized. The exciton chirality ECD method suggested the configuration of 1-(9-phenanthryl)ethylamine to be (+)-(aS), but this assignment had to be reversed²⁰ based on (a) the stereochemical outcome of catalytic hydrogenation; (b) VCD studies on 1-(9-phenanthryl)ethylamine; and (c) ECD studies on the Zn-porphyrin complex, where all three methods suggested (+)-(aR) assignment. Thus, the exciton chirality ECD method could not be used²⁰ for determining the absolute configuration of 1-(9-phenanthryl)ethylamine. To give a balanced view, it should be mentioned that exciton chirality ECD method did provide correct assignments for the absolute configuration in some cases. For 4,4' biphenanthrene-3,3'-diol and its thiol analogue, a simple negative ECD couplet was found to be associated with the ¹B_b absorption band, and this couplet correlated with absolute configuration.³⁵ Nevertheless, it should be remembered that this method is prone to give incorrect absolute configuration when (a) observed ECD is complicated (because ¹B_b band is overlapped with other electronic transitions); (b) it is not clear which electronic transition is responsible for which ECD band; and (c) the predominant conformation (dihedral

angle 3-4-4'-3' in the case of 4,4'-biphenanthryl) is not known.

In place of the exciton chirality ECD method, semiclassical theoretical models such as Devoe's polarizability model³⁶ have also been applied³⁵ for biphenanthryl compounds in the literature. However, these models are approximate in nature and do not enjoy the reliability required for sound interpretation in the current times.

Optical rotation at 589 nm is routinely reported by synthetic chemists whenever a compound is synthesized, but ORD data are not routinely reported. There are no prior experimental reports, nor theoretical studies, on the ORD spectra of any of the biphenanthryl compounds.

From this literature survey, it is clear that there are wide gaps in the spectroscopic and structural information on chiral molecules containing biphenanthryl groups. The latest developments in chiroptical spectroscopic methods^{18,19} can be used to fill some of these gaps. These developments include theoretical formalism³⁷⁻³⁹ and its subsequent implementation in the quantum mechanical programs⁴⁰⁻⁴⁴ within the density functional framework for VCD. Additionally, following the first quantum mechanical prediction of optical rotation,⁴⁵ remarkable advances have taken place in the prediction of ORD using density functional and coupled cluster theories.^{46,47} Around the same time, density functional theoretical methods for ECD predictions⁴⁸⁻⁵⁵ have appeared. However, density functional theoretical predictions of VCD, ECD, and ORD for biphenanthryl compounds have not been reported.

In this manuscript, we report structural investigations on one of the chiral biphenanthryl compounds, namely 2,2'-diphenyl-[3,3'-biphenanthrene]-4,4'-diol and known as VAPOL, using VCD, ECD, and ORD. The absolute configuration of VAPOL was previously determined³ as (+)-(aS) based on the X-ray diffraction structure of its phosphoric amide derivative. An independent determination of this assignment is reported here. In addition, it is useful to know the predominant conformation of VAPOL in solution phase, which may or may not be the same as that in crystalline phase.

CALCULATIONS

For biphenanthryl molecules with axial chirality, the structure is regarded as an elongated tetrahedron³³ and either one of the two pairs of ortho carbons is assigned the first two priorities using Cahn and Ingold rules, as shown in Figure 1. Keeping the lowest priority atom (No. 4) away from the observer, the sequence from high priority (No. 1) to lower priority atom (No. 3) shown in Figure 1 depicts counterclockwise rotation and hence (aS) configuration. Starting from the coordinates of the crystal structure,⁵⁶ the C_2 symmetric conformation shown in Figure 1, with (aS)-configuration, was used for geometry optimization, and for predicting VCD, ECD, and ORD properties. Selected parameters of the B3LYP/6-31G* optimized and crystal structures are summarized in Table 1. To obtain VCD, ECD, and ORD properties for (aR) configuration, those

obtained for (aS) configuration were multiplied by -1 . From the calculated vibrational frequencies, the C_2 symmetric conformation considered was found to represent the minima on the potential energy surface, as there are no imaginary frequencies. All calculation were undertaken with Gaussian program suite⁴⁰ using B3LYP functional and 6-31G* basis set. While VCD and ECD calculations required ~ 3 days of computing time on a desktop computer with a Pentium IV processor, ORD calculations required ~ 10 days of CPU time on an NCSA IBM P690 system with four processors.

The theoretical absorption and VCD spectra (see Fig. 2) were simulated with Lorentzian band shapes and 5 cm^{-1} half-width at half-peak height. The predicted vibrational band positions (Table 2) are normally higher than the experimental vibrational band positions, and therefore the calculated frequencies have been scaled by a factor of 0.9613. The theoretical ECD spectra (see Fig. 3) were simulated from the first 20 singlet $\rightarrow$ singlet electronic transitions using Lorentzian band shapes and 10 nm half-width at half-peak height. The predicted electronic transition wavelengths (Table 3) are reported as such without any scaling. The electronic absorption spectra (see Fig. 3) are presented as molar extinction coefficients (in $\text{L mol}^{-1}\text{ cm}^{-1}$), derived from dimensionless oscillator strength. The peak extinction coefficient of the i th band, ϵ_i^0 , is related to oscillator strength, f_i , as $\epsilon_i^0 = 7.369f_i \frac{\lambda_i^2}{\Delta_i}$, where Δ_i is the half-width at half-height of Lorentzian band.

EXPERIMENTAL MEASUREMENTS

Both enantiomers of VAPOL were purchased from Strem Chemicals and used as received. The absorption and VCD spectra were recorded on a Chiralir Fourier Transform VCD spectrometer in the $2000\text{--}900\text{ cm}^{-1}$ region. The VCD spectra were recorded with 1-h data collection time at 4 cm^{-1} resolution. Spectra were measured in CH_2Cl_2 solvent at 0.136 M concentration for (–)- and (+)-enantiomers, respectively. The sample was held in a demountable cell with BaF_2 windows and a path length of $100\text{ }\mu\text{m}$. In the absorption spectrum presented (see Fig. 2), the solvent absorption was subtracted out and the region $\sim 1294\text{--}1248\text{ cm}^{-1}$ has been removed from experimental traces due to interference from strong solvent absorption. Since the region blocked from solvent absorption is very small, no attempts were made to measure the spectra in CD_2Cl_2 solvent. The VCD spectrum presented here (see Fig. 2) is obtained as the difference between the VCD spectra of enantiomers and the result multiplied by 0.5.

The ECD spectra (see Fig. 3) were recorded on a Jasco J720 instrument, using a 0.01-cm path length quartz cell. The optical rotations at five discrete wavelengths (633, 589, 546, 436, 405 nm) have been measured (see Fig. 4) with a 1-dm cell using an Autopol IV polarimeter. ECD and ORD measurements were done at the respective concentrations of $9.28 \times 10^{-4}\text{ M}$ and 0.976 mg/ml in CH_2Cl_2 solvent.

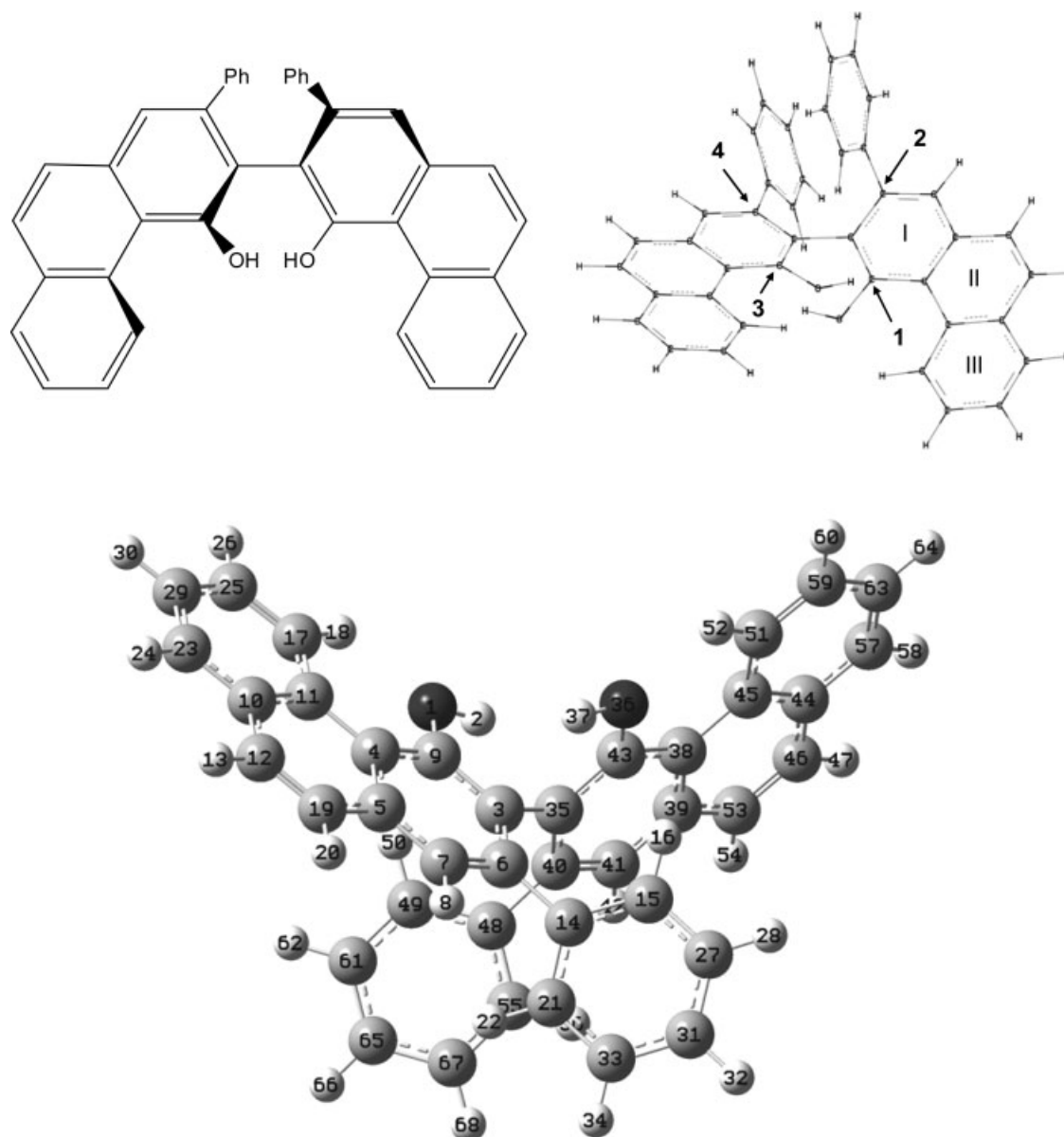


Fig. 1. Chemical structure of (aS)-2,2'-diphenyl-[3,3'-biphenanthrene]-4,4'-diol, (aS)-VAPOL (top left); numerical priority designations (top right) aiding the (aS) configurational assignment of the theoretical model and atomic numbering (bottom) for identifying the bond lengths and angles.

RESULTS AND DISCUSSION

Referring to the structural parameters in Table 1 and atom numbering in Figure 1, it will be noted that the C—O and C—C bond lengths are not significantly different among isolated VAPOL (B3LYP/6-31G* optimized) and crystal structures, but O—H bond lengths differed by ~ 0.14 Å. The O—H bonds are coplanar with the phenanthryl groups in isolated VAPOL molecule, while in the crystal structure, the O—H groups deviate from coplanarity by about 5° (compare the dihedral angle 2-1-9-3). The phenyl groups are oriented at $\sim 57^\circ$ (the dihedral angle, 3-6-14-15) relative to the phenanthryl groups in isolated molecule, while the crystal structure indicates this relative orientation to be $\sim 53^\circ$. The phenanthryl groups in isolated molecule are oriented at $\sim 84^\circ$ (the dihedral angle, 9-3-35-43), which is different from the value of $\sim 73^\circ$ obtained from the crystal structure. This comparison illustrates the influence of packing interactions in the crystal structure, because of which the crystal structure can deviate⁵⁷ from that of isolated molecule.

The experimental vibrational absorption spectrum in the $1700\text{--}900\text{ cm}^{-1}$ region is compared to the corresponding predicted spectrum in Figure 2. This mid-infrared region is dense with numerous vibrational bands, just as noted for phenanthrene.²⁵ There are 24 absorption bands that exhibit one-to-one correspondence between experimental and theoretical absorption spectra. Although there are dif-

ferences between experimental and theoretical absorption spectra, the overall agreement is good. The experimental spectrum shows a broad absorption band around 1600 cm^{-1} , which is well reproduced by the theoretical calculation. The agreement between experimental and theoretical spectra is particularly good in the $1500\text{--}1000\text{ cm}^{-1}$ region, where the theoretical calculation accurately predicts the positions and intensities of the absorption bands.

TABLE 1. Comparison between selected parameters of B3LYP/6-31G* optimized and crystal structure of (aS)-VAPOL

Parameter ^a		B3LYP/6-31G* optimized structure	Crystal structure
Bond angle	Atom nos		
H—O—C—C	D(2,1,9,3) = D(37,36,43,35)	−0.341	5.855
C—C—C—C	D(3,6,14,15) = D(35,40,48,49)	56.927	52.657
C—C—C—C	D(9,3,35,43)	84.319	73.061
H—O—C	A(2,1,9) = A(37,36,43)	107.396	109.419
O—H	B(2,1) = B(37,36)	0.977	0.838
C—O	B(1,9) = B(36,43)	1.366	1.372
C—C	B(3,35)	1.504	1.502

^aD, dihedral angle (deg); A, angle (deg); B, bond length (Å). See Figure 1 for atom numbers.

ferences in relative intensities among experimental and predicted absorption bands, the overall qualitative correlation is remarkably good. As the absorption spectra are sensitive to the conformation, the qualitatively satisfactory agreement seen among experimental and predicted vibrational absorption spectra can be used to suggest that the conformation used for the calculations is probably close to that adopted by VAPOL in CH₂Cl₂ solvent.

The assignment of absolute configuration depends on satisfactory comparison between experimental and calculated VCD spectra. The predicted VCD spectrum for (aR)-VAPOL is compared to the experimental VCD spectrum of (−)-VAPOL in Figure 2. There are 14 VCD bands that display one-to-one sign correspondence and show comparable relative intensities between the experimental and theoretical VCD traces. This satisfactory correlation among the VCD signals in experimental and predicted spectra leads to an unambiguous assignment that the (aR)-configuration corresponds to the (−)-enantiomer of VAPOL.

The vibrational assignments for molecules containing biphenanthryl groups have not been reported before. However, since there are numerous vibrational transitions, assignments are not reported for all of them in the interest of space. Instead, vibrational assignments are presented in Table 2 only for the major VCD bands, as predicted from B3LYP/6-31G* calculations and deduced from the Gauss-View program. The fused-rings of phenanthryl groups have been designated as I, II, and III (see Fig. 1) in order to facilitate the description of vibrational motions. Referring to Table 2 and Figure 2, the vibrational origins of dominant VCD bands can be deduced as follows. There are four transitions contributing to the region labeled No. 18. However, the large positive VCD band associated with No. 18 seen for (−)-VAPOL at 1493 cm^{−1} corresponds to two predicted vibrational modes at 1481 and 1472 cm^{−1}. Of these two, the former (originating from C—C stretching between the phenanthryl groups) has larger VCD intensity than the latter (originates from antisymmetric C—O—H bending). The large negative VCD band (No.

16) at 1444 cm^{−1} corresponds to two predicted vibrational modes both at ~1432 cm^{−1}, but one belonging to A symmetry representation and the other to B representation. They both originate from the C—C stretching and C—C—H bending motions of phenyl rings. The positive

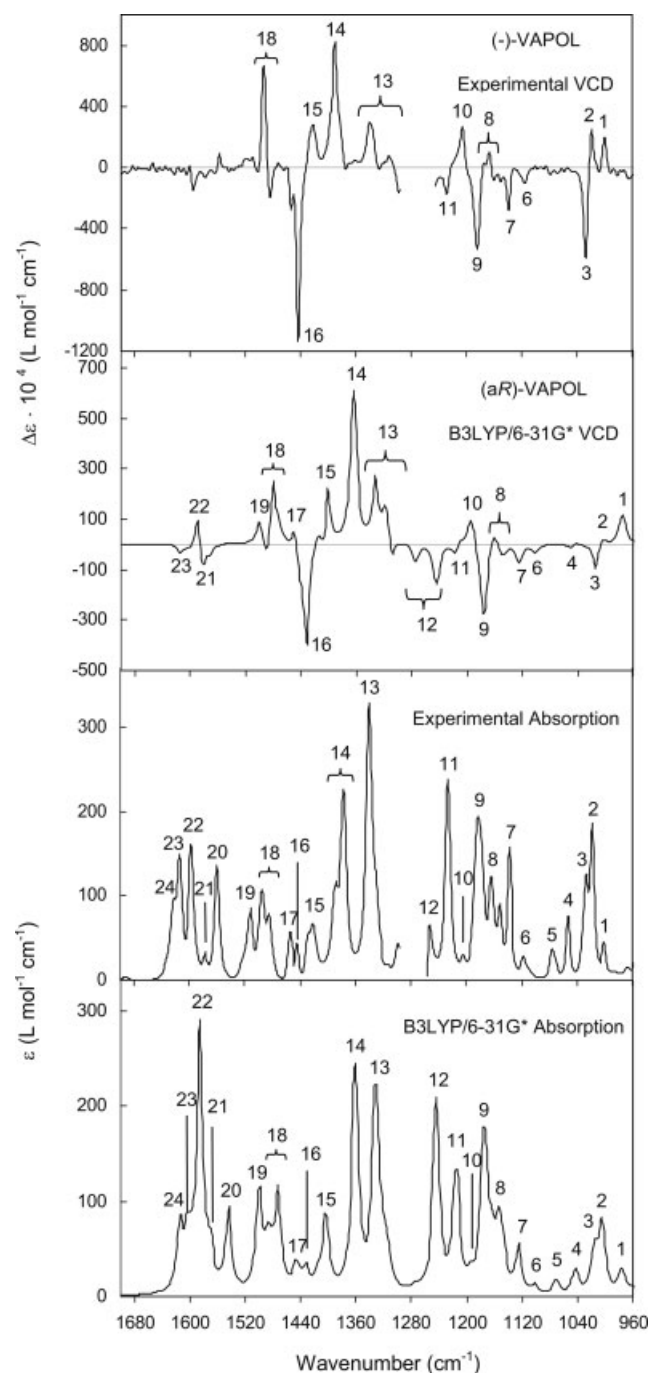


Fig. 2. Comparison of theoretical and experimental absorption spectra (bottom two traces) and theoretical and experimental VCD spectra (top two traces). The predicted traces of (aR)-VAPOL have been simulated using Lorentzian band shapes with 5 cm^{−1} half-width at one-half of the peak-height. The predicted frequencies were scaled by 0.9613. The experimental spectra were measured at the concentration of 0.136 M in CH₂Cl₂ solvent and 100-μm path length.

TABLE 2. Frequencies, intensities, and assignments for selected vibrational bands of (aR)-(-)-VAPOL

No. ^a	(aR)-VAPOL					(-)-VAPOL		Vibrational assignments
	Sym ^b	ν_{unscaled}^c	ν_{scaled}^d	IR ^e	R^f	ν_{abs}^g	ν_{VCD}^h	
3	B	1055.2	1014.4	16.544	-40.564	1026.0	(-), 1026.0	Antisymmetric breathing modes and C—O—H bending of phenanthryl groups.
	A	1060.3	1019.2	0.045	0.267			Symmetric breathing mode and C—H bending of phenyl rings.
	B	1061.0	1020.0	3.255	2.652			Antisymmetric breathing mode and C—H bending of phenyl rings.
9	A	1209.8	1163.0	16.596	19.896	1182.2	(-), 1184.2	Symmetric C—O—H bending; C—H bending of phenanthryl groups.
	B	1218.4	1171.2	0.456	0.894			Antisymmetric C—H bending of phenyl rings.
	A	1218.9	1171.8	3.504	8.162			Symmetric C—H bending of phenyl rings.
	B	1222.4	1175.1	62.759	-102.264			Antisymmetric C—O—H bending; C—C stretching and C—C—H bending of rings I; C—H bending of rings III.
14	B	1414.8	1360.1	66.299	3.829	1377.0, 1389.0	(+), 1390.5	Antisymmetric C—O stretching; C—C(O)—C bending of rings I.
	A	1419.0	1364.1	29.087	157.425			Symmetric C—O stretching; C—C(O)—C bending of rings I.
16	B	1489.5	1431.9	4.780	-67.121	1444.5	(-), 1444.5	Antisymmetric C—C stretching and C—C—H bending of phenyl rings.
	A	1490.1	1432.4	3.387	-37.905			Symmetric C—C stretching and C—C—H bending of phenyl rings.
18	B	1531.6	1472.3	35.951	13.298	1485.0, 1494.7	(-), 1483.0; (+), 1492.7	Antisymmetric C—O—H; C—C stretching and C—C—H bending of rings I and II; C—H bending of rings III and phenyl rings.
	A	1540.5	1480.8	8.529	68.538			C—C stretching between phenanthryl groups; symmetric C—O—H bending; C—C stretching and C—C—H bending of rings I and II; C—H bending of rings III and phenyl rings.
	B	1546.7	1486.9	10.390	-17.103			Antisymmetric C—C stretching between phenanthryl and phenyl groups; antisymmetric C—O—H bending; C=C stretching and C—C—H bending of phenyl rings.
	A	1549.0	1489.1	5.323	-14.714			Symmetric C—C stretching between phenanthryl and phenyl groups; symmetric C—O—H bending; C=C stretching and C—C—H bending of phenyl rings.

^aNumbers for individual or group of bands as given in Figure 2.^bSymmetry representation.^cB3LYP/6-31G* predicted unscaled frequencies in cm⁻¹.^dB3LYP/6-31G* predicted frequencies scaled with 0.9613 in cm⁻¹.^eB3LYP/6-31G* predicted IR intensities in km/mol.^fB3LYP/6-31G* predicted Rotational strengths in 10⁻⁴⁴ esu² cm².^gExperimental frequencies of absorption bands in cm⁻¹.^hExperimental signs and frequencies (in cm⁻¹) of VCD bands.

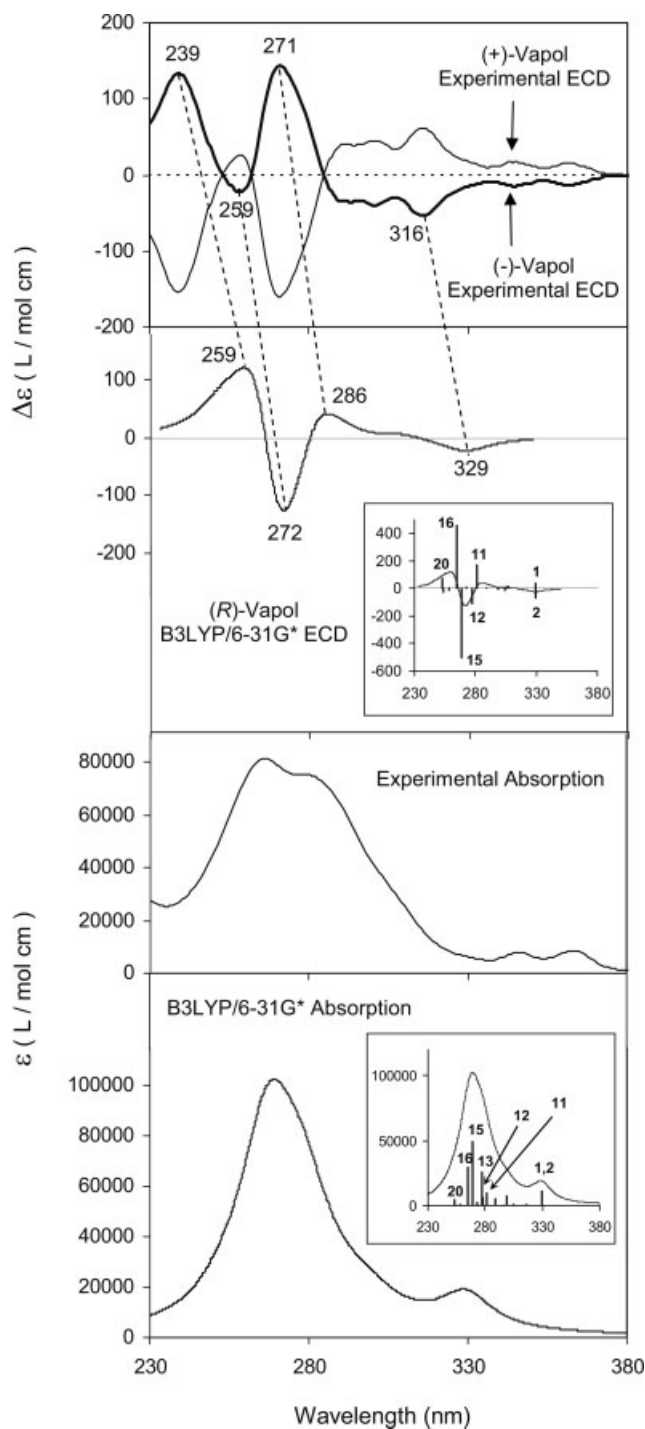


Fig. 3. Comparison of theoretical and experimental electronic absorption spectra (bottom two traces) and theoretical and experimental ECD spectra (top two traces) for VAPOL. Theoretical ECD spectrum for (aR)-VAPOL was simulated using Lorentzian band shapes with 10-nm half-width at one-half of the peak height. The experimental spectra were measured at the concentration of 9.28×10^{-4} M in CH_2Cl_2 solvent and 0.01 cm path length. The insets show the calculated intensities of individual transitions and their relation to the simulated spectra.

VCD band (No. 14) at 1390 cm^{-1} corresponds also to two predicted vibrational modes at 1364 and 1360 cm^{-1} , with the former originating from the symmetric C—O stretching vibration and latter from antisymmetric C—O stretch-

ing vibration. Simple conceptual models predict bisignate VCD associated with symmetric and antisymmetric C—O stretching vibrations. However, because of their coupling with other vibrational motions, the symmetric and antisymmetric C—O stretching vibrations here are predicted to have the same sign and to appear at nearly the same frequency. Also the symmetric C—O stretching mode is predicted to have much larger VCD than its antisymmetric counterpart. The larger negative VCD bands (Nos. 9 and 3) seen at 1184 and 1026 cm^{-1} for (–)-VAPOL appear to arise from the overlap of modes from phenanthryl and phenyl rings. In general, it is difficult to assign a given observed VA or VCD band to only one particular vibrational mode, as several vibrations can contribute to the same region of an observed VCD band.

The experimental electronic absorption spectrum (see Fig. 3) exhibits two resolved components with strong absorption intensity at $\sim 270\text{ nm}$. These components are not resolved in the predicted electronic absorption spectrum where one broad strong intensity band is present. Similarly the two resolved weak electronic absorption bands seen in the experimental spectrum between 330 and 380 nm appear as one broad weak unresolved absorption band at 330 nm in predicted spectrum. However, examination of Table 3 and Figure 3 indicates that the strong predicted absorption at $\sim 270\text{ nm}$ has contributions from several transitions, although transitions 15 and 16 are dominant; the weak absorption band at $\sim 330\text{ nm}$ has contributions from transitions 1 and 2. Except for the minor differences pertaining to resolution in the simulated spec-

TABLE 3. Symmetry, transition wavelengths, oscillator strengths, and rotational strengths of (aR)-VAPOL predicted with B3LYP/6-31G* for 20 electronic transitions

No.	Symmetry representation	Wavelength (nm)	Oscillator strength	Rotational strength ^a [10^{-40} erg esu cm)/Gauss]
1	A	329.4	0.0343	88.1
2	B	329.3	0.1411	–142.5
3	B	315.9	0.0061	–8.9
4	A	313.4	0.0023	8.9
5	A	306.3	0.0051	37.6
6	B	304.4	0.0133	–24.8
7	B	298.4	0.108	–8.9
8	A	296.0	0.0001	–0.1
9	A	289.2	0.0009	1.9
10	B	289.1	0.0831	11.3
11	A	281.2	0.1674	435.1
12	B	277.7	0.1069	–287.3
13	B	277.4	0.4494	11.5
14	A	272.9	0.0442	18.0
15	B	268.6	0.9184	–1336.6
16	A	264.6	0.5739	1239.6
17	B	258.4	0.0114	–23.3
18	A	255.1	0.0005	1.9
19	B	253.4	0.0339	–67.1
20	A	253.0	0.1002	199.9

^aVelocity representation.

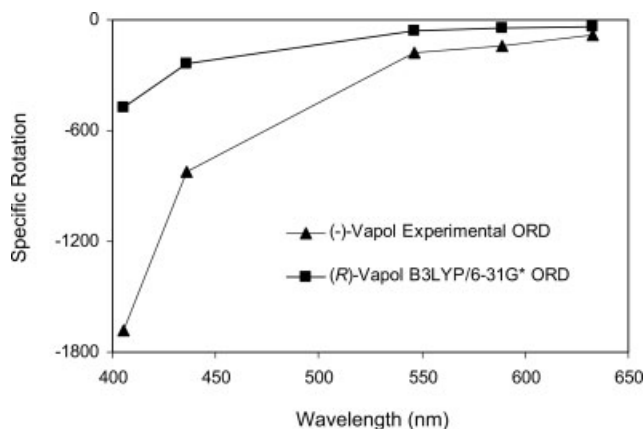


Fig. 4. Comparison of ORD predicted for (aR)-VAPOL with experimental ORD data of (-)-VAPOL. The measurements were done at the concentration of 9.76×10^{-4} g/ml in CH_2Cl_2 solvent and 1 dm pathlength.

tra, the predicted electronic absorption spectrum compares favorably with the experimental electronic absorption spectrum (see Fig. 3). This favorable comparison can be used to suggest that the conformation used for calculations is probably close to that adopted by VAPOL in CH_2Cl_2 .

ECD has been widely used in the literature, with the exciton chirality model, for assigning absolute configuration. However, as mentioned in the Introduction, this empirical method does not give an unambiguous conclusion when multiple bisignate ECD couplets are present in the ECD spectra. This turns out to be the situation for VAPOL, where the ECD spectrum of (-)-VAPOL (see Fig. 3) exhibits a negative band at 316 nm (which is very broad covering from 290 to 370 nm), a strong positive band at 271 nm, a weak negative band at 259 nm, and a strong positive band at 239 nm. In the absence of additional experimental information, one could interpret the experimental ECD spectrum to contain a negative couplet (316 (-)/271 (+)), a positive couplet (271 (+)/259 (-)), and a negative couplet (259 (-)/239 (+)). Depending on the way these couplets are construed, the empirical exciton model can yield contradictory conclusions about absolute configuration. On the contrary, the B3LYP/6-31G*-predicted ECD spectrum for (aR)-VAPOL faithfully reproduced the experimental ECD spectrum for (-)-VAPOL, as can be seen in Figure 3, where the correlation between band signs and positions is indicated by the dotted lines. Note that the electronic transitions predicted with the B3LYP/6-31G* basis set are known to appear⁵⁸ at longer wavelengths than those in the experimental spectra, and no attempt has been made to correct the predicted transition wavelengths. In the predicted ECD spectrum the positive band at 259 nm has contributions (see the inset in Fig. 3) from transitions 16 and 20, and the negative band at 272 nm has contribution from transition 15. Considerable cancellation occurs between positive and negative contributions from transitions 16 and 15, because of small wavelength difference between these transitions. Similar cancellation occurs between the ECD intensities associated with transi-

tions 11 and 12 and with transitions 1 and 2. Some contribution to the negative band at 272 nm also comes from transition 12. Transition 11 contributes to the positive band at 286 nm. The pleasing correspondence seen between the ECD spectra of (aR)-VAPOL and (-)-VAPOL verifies the absolute configuration as (-)-(aR) independent of conclusions from VCD spectra.

As a third independent verification of the configurational assignment, the experimental ORD data for (-)-VAPOL have been compared to the predicted ORD of (aR)-VAPOL in Figure 4. In principle, the wavelengths used for ORD predictions should be shifted⁵⁹ from those used for experimental ORD measurements, because the B3LYP/6-31G*-predicted electronic transition wavelengths appear at higher wavelengths⁵⁸ than those experimentally observed. However, no attempt has been made here to correct for the transition wavelength differences as these differences between predicted and observed transition wavelengths do not influence the conclusions here. This is because, the predicted ORDs for (aR)-VAPOL and experimental ORD of (-)-VAPOL are both monosignate at all wavelengths investigated, and these ORD spectra can be shifted along wavelength scale as a whole relative to each other without influencing any conclusions derived here. The experimentally obtained values of specific rotation are -78.6 at 633, -142.4 at 589, -177.6 at 546, -820.0 at 436, and -1681.0 at 405 nm. The predicted specific rotation values for (aR)-VAPOL are -36.9 at 633, -46.8 at 589, -62.7 at 546, -240.4 at 436, and -477.3 at 405 nm. There is a qualitatively satisfactory agreement between the experimental specific rotations for (-)-enantiomer and the corresponding values for (aR)-VAPOL. This agreement, in signs of optical rotation and the trend in ORD spectra (increasingly negative at shorter wavelengths), supports the conclusions about absolute configuration derived from VCD and ECD data. Thus, all three chiroptical spectroscopic methods independently suggest that the absolute configuration of VAPOL is (-)-(aR), which is in agreement with that derived from X-ray diffraction data of its phosphoric amide derivative.³

It is to be noted that while reliable VCD predictions for most molecules can normally be obtained at the B3LYP/6-31G* level of theory, ECD and ORD predictions require, quite often, higher levels of theory. Since all three predictions for VAPOL provided consistent configurational assignment, we have not attempted to perform ECD and ORD calculations at higher levels of theory.

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Intermolecular Chiral Recognition Processes Probed by Chiroptical Luminescence

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT Enantiopreferential energy transfer processes between dissymmetric lanthanide and transition metal complexes dissolved in acetonitrile are studied using chiroptical luminescence techniques. The energy donors (luminophores) in this study are a racemic mixture of $\text{Ln}(\text{dpa})_3^{3-}$ complexes (where $\text{Ln} = \text{Eu}^{3+}$ or Tb^{3+} and $\text{dpa} = 2,6\text{-pyridinedicarboxylate}$), and the energy acceptors (quenchers) are an enantiomerically-resolved population of $\text{Co}(\text{R,R-chxn})_3^{3+}$ (where $\text{R,R-chxn} = \text{trans-1R,2R-diaminocyclohexane}$) complexes. The luminophores are dissolved in acetonitrile as $(\text{NEt}_4)_3[\text{Ln}(\text{dpa})_3]$ (where $\text{NEt}_4 = \text{tetraethylammonium}$) and $(\text{NBu}_4)[\text{Ln}(\text{dpa})_3]$ (where $\text{NBu}_4 = \text{tetrabutylammonium}$) salts. The unquenched luminescence lifetimes are reported for both $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ in acetonitrile over the range 263–333 K, and these results are compared to luminescence lifetimes in aqueous solution. Time-resolved chiroptical luminescence measurements of enantiopreferential quenching kinetics are reported for samples with $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Co}(\text{R,R-chxn})_3^{3+}$ in acetonitrile over 263–333 K range. These results are analyzed using a phenomenological quenching kinetics model, and the results are compared to results in aqueous solution. These comparisons show that the overall Eu-Co luminescence quenching efficiency is reduced in acetonitrile vs. aqueous samples, because the salts of $(\text{NX}_4)_3[\text{Eu}(\text{dpa})_3]$ are not completely dissociated in acetonitrile. However, the enantioference exhibited is identical in acetonitrile vs. aqueous solution. *Chirality* 20:511–523, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: circularly polarized luminescence; chiral recognition; europium; terbium; luminescence lifetimes

INTRODUCTION

Chirality-dependent intermolecular interactions and chiral recognition in chemical and physical processes play an important role in chemistry and biology. Chiral recognition is demonstrated in many naturally occurring biochemical processes and is commonly used in syntheses and separations of complex chemical compounds. Considerable research effort has been devoted to understanding intermolecular chiral recognition processes and rationalizing the role of stereochemical structure and chirality-dependent properties in these processes.

A number of enantiopreferential intermolecular excited-state energy-transfer studies have been reported for systems in which the luminophores are dissymmetric lanthanide complexes and the quenchers include dissymmetric transition metal complexes,^{1–14} and biomolecules.¹⁵ Most of these studies have focused on correlations between enantiopreferential quenching and the electronic and stereochemical structural properties of the luminophore and quencher molecules. However, the results obtained from some of these studies indicate that a significant influence is exerted on the enantiopreferential quenching processes by variations in sample properties such as solvent type, temperature, ionic strength and solution viscosity. In

most of these studies, the solutions were prepared from amphiprotic solvents (e.g., H_2O , D_2O , and methanol). In this article, results are presented from a study of intermolecular chiral recognition processes in solution samples where the solvent is acetonitrile (an aprotic solvent system). As in previous studies,^{3,9,14} the luminophores are complexes of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ (where $\text{dpa} = 2,6\text{-pyridinedicarboxylate}$), and the chiral quencher is an optical diastereomer of $\text{Co}(\text{R,R-chxn})_3^{3+}$ (where $\text{R,R-chxn} \equiv \text{trans-1R,2R-diaminocyclohexane}$).

The structural and spectroscopic properties of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ complexes in aqueous solution are well-characterized^{16–23}, but, these properties have not yet been characterized for these complexes in acetonitrile solution. Because the sodium salts of $\text{Ln}(\text{dpa})_3^{3-}$ complexes are insoluble in acetonitrile, the samples used in the present study are prepared from two different tetraal-

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kylammonium salts of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ which were shown by Harrowfield and coworkers²⁴ to be soluble in acetonitrile solution. Optical and chiroptical spectroscopic measurements were performed in the ${}^7\text{F}_{0,1,2} \leftarrow \text{D}_0$ (emission) and ${}^7\text{F}_{0,1} \rightarrow {}^5\text{D}_1$ (excitation) transition regions of $\text{Eu}(\text{dpa})_3^{3-}$ and in the ${}^7\text{F}_5 \leftarrow {}^5\text{D}_4$ (emission) transition region of $\text{Tb}(\text{dpa})_3^{3-}$. The results are compared to those obtained from samples of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ in aqueous solution.

In addition, studies on the temperature dependence of the emitting-state lifetimes of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$, dissolved in acetonitrile, are reported. Previous studies have demonstrated that the emitting-state lifetimes of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ are very sensitive to solvent composition in solution samples.¹⁰ In studies carried out on samples dissolved in D_2O versus H_2O , the $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ emitting-state lifetimes show significant differences, in which the $\text{Eu}(\text{dpa})_3^{3-}$ lifetime varies by almost a factor of two. The emitting-state lifetimes obtained in the present study also show a significant solvent dependence.

Several studies of enantiopreferential luminescence quenching in samples that contain $\text{Ln}(\text{dpa})_3^{3-}$ ($\text{Ln} = \text{Eu}^{3+}$ or Tb^{3+}) as luminophores and resolved optical isomers of $\text{Co}(\text{R,R-chxn})_3^{3+}$ as the chiral quencher species have demonstrated efficient quenching and show a high degree of enantioselectivity in the quenching actions. The influence of solvent on enantiopreferential quenching kinetics has been characterized for $\text{Ln}(\text{dpa})_3^{3-}\text{-Co}(\text{R,R-chxn})_3^{3+}$ systems in several amphiprotic solvents.^{5-8,10} The results from previous studies combined with the results obtained in the present study provide an opportunity to compare the chirality-dependent quenching kinetics of $\text{Ln}(\text{dpa})_3^{3-}\text{-Co}(\text{R,R-chxn})_3^{3+}$ systems dissolved in an aprotic (CH_3CN) vs. an amphiprotic (H_2O) solvent.

The primary focus of the work presented in this article is to determine the sensitivity of quenching rate parameters (and enantioselectivity) to a change in solvent type (amphiprotic vs. aprotic solvent), and to changes in the cation of the $\text{Ln}(\text{dpa})_3^{3-}$ salt (where $\text{Ln} = \text{Eu}^{3+}$ or Tb^{3+}). Time-resolved chiroptical luminescence (TR-CL) measurement techniques are used to observe the $\text{Eu}(\text{dpa})_3^{3-}\text{-Co}(\text{R,R-chxn})_3^{3+}$ and $\text{Tb}(\text{dpa})_3^{3-}\text{-Co}(\text{R,R-chxn})_3^{3+}$ quenching processes, and the results of these studies are analyzed using a phenomenological model of the enantiopreferential quenching kinetics.²⁵ In addition, the temperature-dependent quenching rate parameters and chirality-dependent rate parameters are determined for acetonitrile solution samples of $\text{Eu}(\text{dpa})_3^{3-}\text{-Co}(\text{R,R-chxn})_3^{3+}$. These results are compared with results from previous studies of enantiopreferential quenching kinetics of $\text{Eu}(\text{dpa})_3^{3-}\text{-Co}(\text{R,R-chxn})_3^{3+}$ systems in aqueous solution.^{3,9,10}

Stereochemical Structure and Spectroscopic Properties of the Luminophores

The coordination complexes, $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$, have trigonal dihedral (D_3) symmetry, and the three bicyclic chelate rings in these complexes form either a left-handed (Λ) or right-handed (Δ) three-bladed propeller arrangement about the threefold axis. All of the structural chirality in these complexes is derived from the helical dis-

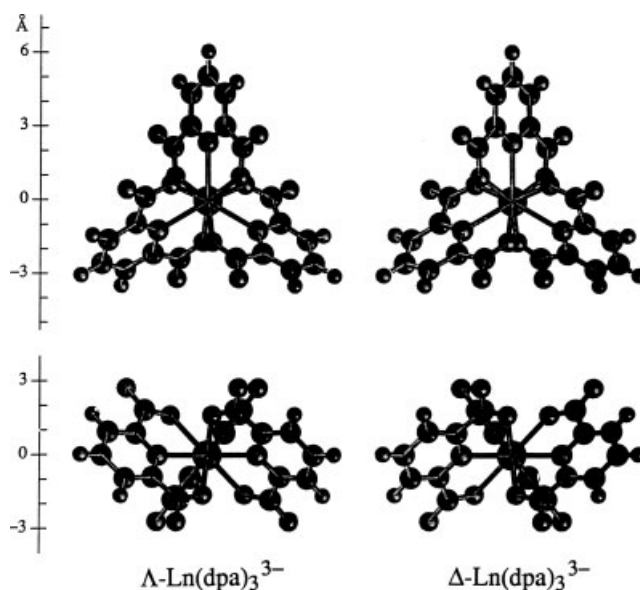


Fig. 1. Structural representations of $\Lambda\text{-Ln}(\text{dpa})_3^{3-}$ and $\Delta\text{-Ln}(\text{dpa})_3^{3-}$ enantiomers. The top structures are viewed along the trigonal symmetry axis, and the bottom structures are viewed along one of the digonal (C_2) symmetry axes. The dimensions of the structures are indicated by the angstrom ($\text{\AA}$) scales shown on the left-hand side of the figure.

tribution of the chelate rings about the lanthanide ion, because the individual ligands and their chelate rings have achiral structures. Following standard practice, we label the structural enantiomers of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ as Λ or Δ , which refer to the helicity of the chelate rings about the trigonal (C_3) symmetry axis. However, although the Λ enantiomer is defined to have left-handed chirality about its trigonal axis, it has right-handed chirality about each of its three twofold (C_2) symmetry axes. Conversely, the Δ enantiomer is defined to have right-handed chirality about its trigonal axis, and left-handed chirality about each of its twofold axes. Therefore, the absolute configuration of an enantiomer is uniquely specified by the label Λ or Δ , but the “handedness” of its structure is dependent upon the choice of reference axis. Structural representations of Λ - and $\Delta\text{-Ln}(\text{dpa})_3^{3-}$ enantiomers are shown in Figure 1, with views along the trigonal axis and one of the twofold axes.

In aqueous solution, the $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ complexes exist as racemic mixtures of interconverting ($\Lambda \leftrightarrow \Delta$) enantiomers. Using TR-CL measurement techniques, the enantiomer interconversion kinetics of $\text{Eu}(\text{dpa})_3^{3-}$ dissolved in acetonitrile were found to be slow over the time scale of our experiments (i.e., slow compared to excited-state quenching rates), and therefore, the enantiomer interconversion rates were not factored into our analysis of the enantiopreferential quenching kinetics.

The optical excitation and emission properties of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ are well-characterized in both aqueous solution and single-crystal samples.²¹⁻²³ Analyses of these properties have produced detailed characterizations of the crystal-field energy-level structures of $\text{Eu}^{3+}(4f^6)$ and $\text{Tb}^{3+}(4f^8)$ in $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$. For $\text{Eu}(\text{dpa})_3^{3-}$ in aqueous solution, the most intense luminescence is observed in the ${}^7\text{F}_2 \leftarrow {}^5\text{D}_0$ transition region, but

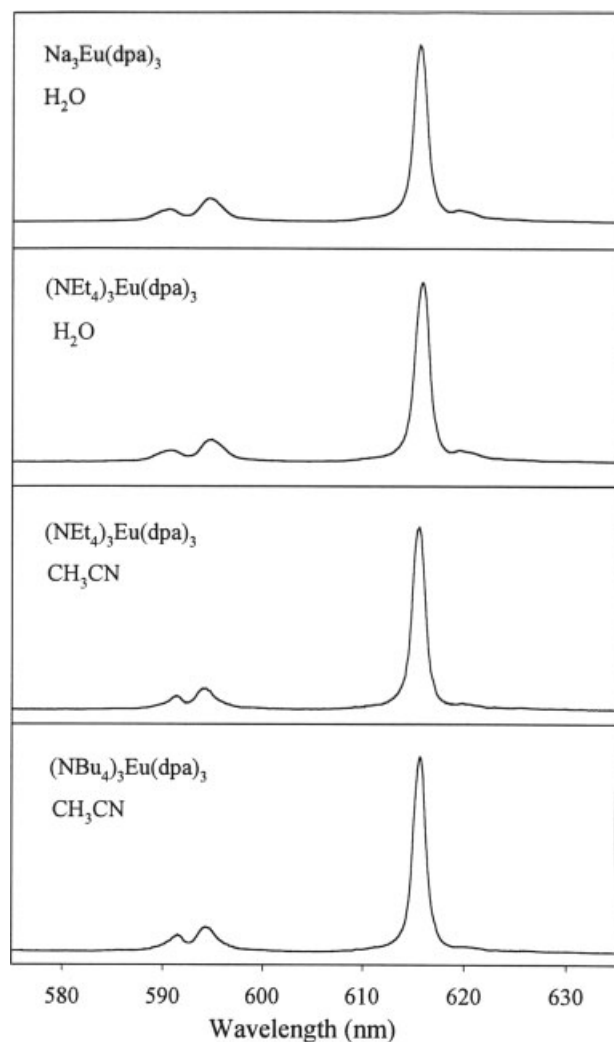


Fig. 2. Comparison of optical emission spectra in the ${}^7F_{0-2} \leftarrow {}^5D_0$ transition regions of $\text{Eu}(\text{dpa})_3^{3-}$ dissolved in aqueous and acetonitrile solutions. All of the spectra were obtained on solution samples at 293 K. The 465.8 nm line of an argon ion laser was used for excitation.

the strongest chiroptical properties are observed in the ${}^7F_1 \leftarrow {}^5D_0$ transition region. The most intense luminescence and strongest chiroptical properties for $\text{Tb}(\text{dpa})_3^{3-}$ in aqueous solution are observed in the ${}^7F_5 \leftarrow {}^5D_4$ transition region.

The TR-CL measurements were carried out on samples of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ luminophores dissolved in acetonitrile. Therefore, the optical and chiroptical properties of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ in acetonitrile need to be characterized in order to properly analyze and interpret the quenching results. Figure 2 shows results from optical emission spectra recorded in the ${}^7F_2 \leftarrow {}^5D_0$ transition regions of $\text{Eu}(\text{dpa})_3^{3-}$ dissolved in aqueous solution and acetonitrile. Figure 2 shows emission spectra recorded for samples containing three different $\text{Eu}(\text{dpa})_3^{3-}$ salts: $\text{Na}_3\text{Eu}(\text{dpa})_3$ in H_2O , $[\text{N}(\text{C}_2\text{H}_5)_4]_3\text{Eu}(\text{dpa})_3$ in H_2O and acetonitrile; and $[\text{N}(\text{C}_4\text{H}_9)_4]_3\text{Eu}(\text{dpa})_3$ in acetonitrile. The relative intensities and locations of the transitions are essentially identical in all of the spectra shown, indicating

that the coordination structure and optical properties of $\text{Eu}(\text{dpa})_3^{3-}$ are relatively invariant to changes in solvent type and salt (i.e. $\text{Na}_3\text{Eu}(\text{dpa})_3$ vs. $[\text{N}(\text{C}_2\text{H}_5)_4]_3\text{Eu}(\text{dpa})_3$ vs. $[\text{N}(\text{C}_4\text{H}_9)_4]_3\text{Eu}(\text{dpa})_3$). Figure 3 shows the circularly-polarized excitation (CPE) spectra in the ${}^7F_{0,1} \rightarrow {}^5D_1$ transition regions obtained on solution samples of $\text{Eu}(\text{dpa})_3^{3-}$ in acetonitrile. The spectral features shown in Figure 3 are essentially identical to the spectral features observed in CPE spectra (in the ${}^7F_{0,1} \rightarrow {}^5D_1$ transition regions) obtained on samples of $\text{Eu}(\text{dpa})_3^{3-}$ in aqueous solution.¹

The spectroscopic results shown in Figures 2 and 3 indicate that the stereochemical structures and optical properties are essentially identical for the sodium, tetraethylammonium, and tetrabutylammonium salts of $\text{Eu}(\text{dpa})_3^{3-}$ dissolved in water or acetonitrile. The stereochemical structures and optical properties of $\text{Tb}(\text{dpa})_3^{3-}$ are also assumed to be independent of salt and solvent.

Stereochemical Structure and Spectroscopic Properties of the Quencher

Complexes of $\Delta(\lambda\lambda\lambda)\text{-}[\text{Co}(\text{R,R-chxn})_3]^{3+}$ have been used previously as quencher molecules in studies of enantioselective energy-transfer processes.^{3,9,10} The details of the structural properties of $\Delta(\lambda\lambda\lambda)\text{-}[\text{Co}(\text{R,R-chxn})_3]^{3+}$ have been described in other studies,^{3,9,10} and for the purpose of this article, we shall limit the discussion to structural properties that define the elements of chirality in the complex. The complex has a tris (bidentate) chelate structure of trigonal dihedral (D_3) symmetry. The three chelate rings in this structure form a three-bladed propeller with a right-handed (Δ) screw sense about the trigonal symmetry axis. This arrangement of the chelate rings about the trigonal axis defines the configurational chirality of the

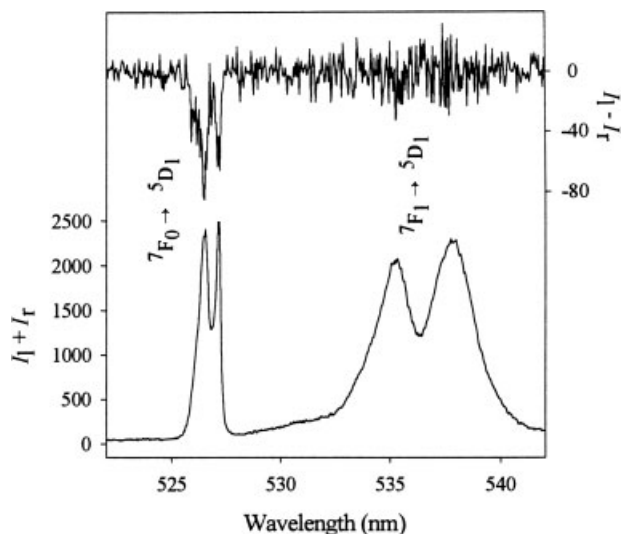


Fig. 3. CPL (top, $I_L - I_R$) and TL (bottom, $I_L + I_R$) excitation spectra for 10 mM racemic $\text{Eu}(\text{dpa})_3^{3-}$ dissolved in acetonitrile. These spectra were recorded by measuring the CPL and TL intensity at 594.3 nm (which corresponds to the ${}^7F_1 \leftarrow {}^5D_0$ transition region) and scanning the right-circularly polarized dye laser through the excitation region. The spectra span the ${}^7F_0, {}^7F_1 \leftarrow {}^5D_0$ transition regions. The spectra are not corrected for the wavelength dependence of the dye laser excitation intensity.

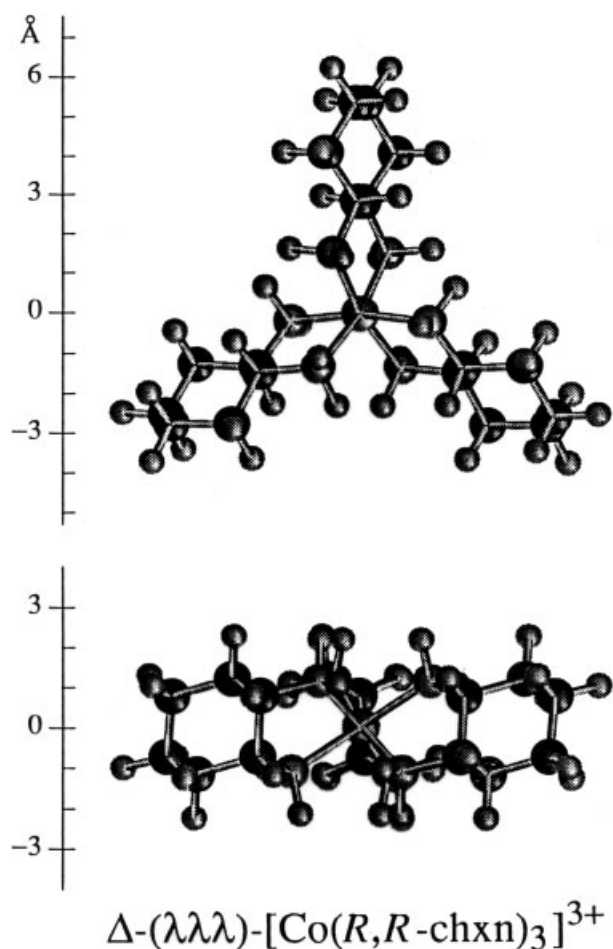


Fig. 4. Structural representations of the $\Delta-(\lambda\lambda\lambda)-[\text{Co}(\text{R},\text{R}-\text{chxn})_3]^{3+}$ complex, with views along the trigonal symmetry axis (top structure) and along one of the digonal (C_2) symmetry axes (bottom structure). The dimensions of the complex are indicated by the angstrom (Å) scales shown on the left hand side of the figure.

complex. Each of the five-membered chelate rings has a nonplanar, chiral conformation in which the twist of the C—C bond in the ring has a left-handed (λ) screw sense about an axis that runs through the two nitrogen donor atoms in the ring. This conformational chirality (λ in each chelate ring) is dictated by the absolute configuration of the two asymmetric carbon atoms in the 1*R*,2*R*-diaminocyclohexane ligand. The presence of asymmetric atomic centers in the ligands of a metal complex is often referred to as vicinal chirality.^{26,27} Therefore, the overall structural chirality of the $\Delta-(\lambda\lambda\lambda)-[\text{Co}(\text{R},\text{R}-\text{chxn})_3]^{3+}$ complex is specified by its Δ configurational chirality, $\lambda\lambda\lambda$ conformational chirality, and the vicinal chirality derived from two asymmetric carbon atoms (1*R*,2*R*) in each ligand. Structural representations of $\Delta-(\lambda\lambda\lambda)-[\text{Co}(\text{R},\text{R}-\text{chxn})_3]^{3+}$ are shown in Figure 4.

The relevant electronic and vibronic states of $\Delta-(\lambda\lambda\lambda)-[\text{Co}(\text{R},\text{R}-\text{chxn})_3]^{3+}$ are those that can provide acceptor levels for Eu^* -to- Co and Tb^* -to- Co energy transfer processes (where Eu^* and Tb^* denote $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ complexes in their $^5\text{D}_0$ and $^5\text{D}_4$ excited states, respec-

tively, and Co denotes $\Delta-(\lambda\lambda\lambda)-[\text{Co}(\text{R},\text{R}-\text{chxn})_3]^{3+}$). An absorption spectrum of $\Delta-(\lambda\lambda\lambda)-[\text{Co}(\text{R},\text{R}-\text{chxn})_3]^{3+}$ over the 300–640 nm wavelength range is shown in Figure 5, along with those parts of the Eu^* and Tb^* emission spectra that fall within this wavelength region. Figure 5 shows that the absorption band centered at ~ 475 nm completely overlaps with the highest-energy Tb^* emission band ($^7\text{F}_6 \leftarrow ^5\text{D}_4$) and partially overlaps with the next-highest-energy Tb^* emission band ($^7\text{F}_5 \leftarrow ^5\text{D}_4$). This shows that parts of the energy-level manifold of Co^{3+} satisfy the resonance conditions for Tb^* - Co electronic energy transfer.

Figure 5 shows that there is negligible overlap between the absorption band (ca. 475 nm) and any of the Eu^* emission lines ($^7\text{F}_{1,2} \leftarrow ^5\text{D}_0$ transition regions), and therefore, indicates that this Co^{3+} energy-level manifold does not contain suitable acceptor levels for Eu^* - Co energy-transfer processes. However, studies by Wilson et al. have shown that Co^{3+} complexes have additional very weak absorption bands at longer wavelength that do overlap the Eu^* emission lines.²⁸ These absorption bands are the result of spin-forbidden transitions (within the Co^{3+} energy-level manifold) which are too weak to support a Förster type energy-transfer mechanism, but do not exclude an exchange mechanism in the Eu^* - Co energy transfer process.

Theory and Measurement Methodology

The enantiopreferential excited-state quenching studies were performed on samples containing a racemic mixture of chiral luminophore molecules (ΔL and ΔL) and an enantiomerically resolved concentration of chiral quencher molecules (Q). In these experiments, a pulse of racemic (unpolarized) light is used to produce a racemic excited-state population of luminophores, and luminescence from

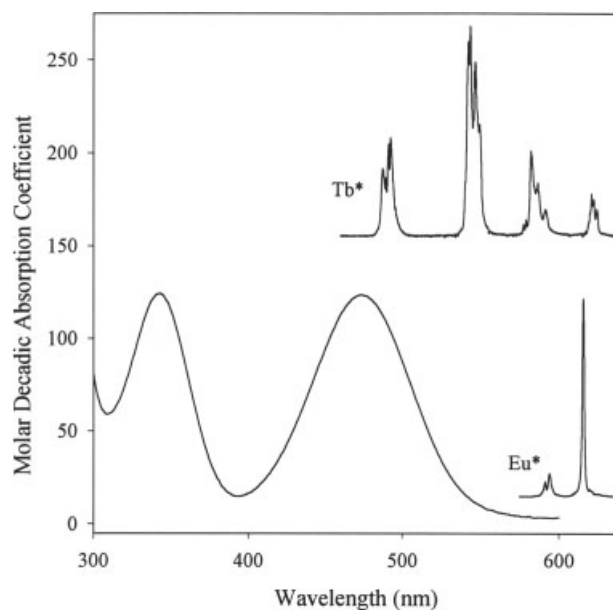


Fig. 5. Absorption spectrum of $\Delta-(\lambda\lambda\lambda)-[\text{Co}(\text{R},\text{R}-\text{chxn})_3]^{3+}$ over the 300–625 nm wavelength region. The inset spectra show $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ emission lines that fall within this region. All spectra were obtained on acetonitrile solution samples at 293 K.

this excited-state population is measured as a function of time. The luminescence is measured as sums and differences of left- and right-circularly polarized components of the intensity, and ratios of the sums and differences are calculated. The time-dependent observables in these measurements are expressed as

$$I(\lambda', t) = I_L(\lambda', t) + I_R(\lambda', t) \quad (1)$$

$$\Delta I(\lambda', t) = I_L(\lambda', t) - I_R(\lambda', t) \quad (2)$$

$$g_{\text{em}}(\lambda', t) = \frac{2\Delta I(\lambda', t)}{I(\lambda', t)} \quad (3)$$

where λ' denotes emission wavelength, t denotes time after excitation, and I_L and I_R denote left- and right-circularly polarized emission intensities, respectively. Throughout the remainder of this article, I is referred to as the total luminescence (TL) intensity, ΔI as the circularly polarized luminescence (CPL) intensity, and g_{em} as an emission dissymmetry factor.

In the absence of enantio-preferential quenching, the excited-state decay rates of ΛL^* and ΔL^* are identical, and the excited-state population of luminophores remain racemic at all times. However, if the $\Lambda L^* - Q$ and $\Delta L^* - Q$ quenching rates differ, the emitting population of luminophores can become nonracemic over time, and is indicated in the luminescence measurements by the appearance of CPL (ΔI) intensity and emission dissymmetry (g_{em}). The development of an enantiomeric excess (η_{L^*}) in the emitting-state population of luminophores can be followed by measuring the time-dependent emission dissymmetry factor, $g_{\text{em}}(\lambda', t)$. The relationship between $\eta_{L^*}(t)$ and $g_{\text{em}}(\lambda', t)$ is given by

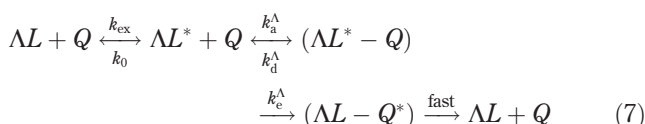
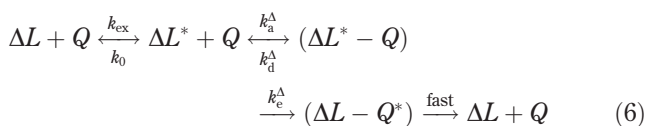
$$g_{\text{em}}(\lambda', t) = g_{\text{em}}^{\Lambda}(\lambda')\eta_{L^*}(t) = -g_{\text{em}}^{\Delta}(\lambda')\eta_{L^*}(t) \quad (4)$$

where $g_{\text{em}}^{\Lambda}(\lambda')$ and $g_{\text{em}}^{\Delta}(\lambda')$ denote emission dissymmetry factors for enantiomerically pure ΛL^* and ΔL^* , respectively, and $\eta_{L^*}(t)$ is defined as

$$\eta_{L^*}(t) = \frac{[\Lambda L^*]^t - [\Delta L^*]^t}{[\Lambda L^*]^t + [\Delta L^*]^t} \quad (5)$$

where $[\Lambda L^*]^t$ and $[\Delta L^*]^t$ are time-dependent concentrations of ΛL^* and ΔL^* , respectively.

The mechanistic pathways for the $\Lambda L^* - Q$ versus $\Delta L^* - Q$ quenching rate processes are



where the arrows labeled k_{ex} in eqs. 6 and 7 represent the excitation processes that produce an excited-state lumino-phore population. In addition to the rate processes associated with quencher (Q) actions, the ΛL^* and ΔL^* decay kinetics directly depend upon the intrinsic emitting-state decay processes, $\Lambda L^* \rightarrow \Lambda L$ and $\Delta L^* \rightarrow \Delta L$ (with rate constants denoted by k_0^{Λ} and k_0^{Δ}). In the absence of any chirality-dependent perturbation (e.g., enantiopreferential quenching), these excited-state decay rates are the same for ΛL^* and ΔL^* enantiomers (and $k_0^{\Lambda} = k_0^{\Delta} \equiv k_0$). Contributions from both radiative (k_r) and nonradiative (k_{nr}) decay processes of L^* are included in the intrinsic emitting-state decay rate constant ($k_0 = k_r + k_{\text{nr}}$) shown in eqs. 6 and 7. The unimolecular enantiomer interconversion ($\Lambda L^* \leftrightarrow \Delta L^*$) processes may also affect the relative ΛL^* and ΔL^* decay kinetics. However, the enantiomer interconversion rates for the systems examined in this study are slow on the time scale of the emission decay kinetics in these experiments, and therefore, enantiomer interconversion rates will not be considered in the analysis.

The L^* -to- Q energy-transfer processes presented in eqs. 6 and 7 are assumed to involve short-range (contact) interaction mechanisms,²⁵ and occur entirely within ($L^* - Q$) encounter complexes. The formation kinetics of the ($\Lambda L^* - Q$) and ($\Delta L^* - Q$) encounter complexes are identified in eqs. 6 and 7 with the association rate constants, k_a^{Λ} and k_a^{Δ} (where Λ or Δ indicates the enantiomer of the luminophore). The luminophore-quencher encounter complexes can either dissociate with rate constants identified by k_d^{Λ} and k_d^{Δ} , or undergo energy transfer within the encounter complexes (rate constants denoted by k_e^{Λ} and k_e^{Δ}). In the systems examined in this study, the rate of $Q^* \rightarrow Q$ deactivation is fast compared to the energy-transfer rate from the excited quencher (Q^*) back to the ground-state luminophore (L). The rates of formation and dissociation of encounter complexes are considered to be fast compared to the rate of energy transfer within the ($L^* - Q$) complexes (e.g., $k_d^{\Lambda} \ll k_e^{\Lambda}$), and therefore we define the quasi-equilibrium constants, $K_a^{\sigma} = k_a^{\sigma}/k_d^{\sigma}$ (where $\sigma = \Lambda$ and Δ). The excited-state quenching rate constants can now be expressed as²⁵

$$k_q^{\Lambda} = K_a^{\Lambda} k_e^{\Lambda}, \quad (8)$$

$$k_q^{\Delta} = K_a^{\Delta} k_e^{\Delta} \quad (9)$$

where k_q^{Λ} and k_q^{Δ} are the rate constants for $\Lambda L^* - Q$ and $\Delta L^* - Q$ quenching, respectively.

The overall kinetics of the competing $\Lambda L^* - Q$ and $\Delta L^* - Q$ quenching processes can be analyzed by measuring the time evolution of the observables defined in eqs. 1–3. Based on the rate processes shown in eqs. 6 and 7 and the conditions specified in the previous two paragraphs and eqs. 8 and 9, these time-dependent observables can be written as

$$I(\lambda', t) = \frac{I(\lambda', 0)}{2} [e^{-k_1 t} + e^{-k_2 t}] \quad (10)$$

$$\Delta I(\lambda', t) = \frac{I(\lambda', 0)g_{\text{em}}^{\Lambda}(\lambda')}{4} [e^{-k_1 t} - e^{-k_2 t}] \quad (11)$$

$$g_{\text{em}}(\lambda', t) = g_{\text{em}}^{\Lambda}(\lambda') \tanh \left[\frac{(k_q^{\Lambda} - (k_q^{\Lambda})[Q]t)}{2} \right] \quad (12)$$

where $k_1 = k_0 + k_q^{\Lambda}[Q]$, $k_2 = k_0 + k_q^{\Lambda}[Q]$, $[Q]$ is the concentration of optically-resolved quenchers, $I(\lambda', 0)$ is the TL at $t = 0$, and $\tanh$ denotes a hyperbolic tangent function.

EXPERIMENTAL PROCEDURES

Preparation of the Luminophores and Quenchers

The tetraethylammonium salts of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ were prepared according to the methods of Harrowfield and coworkers.²⁴ $\text{Ln}(\text{OH})_3$ ($\text{Ln} = \text{Eu}$ or Tb) was prepared by addition of NaOH to an aqueous solution containing LnCl_3 until a pH value >10 was attained. Three equivalents of dipicolinic acid and one equivalent of $\text{Ln}(\text{OH})_3$ were dissolved in aqueous solution under gentle warming, and tetraethylammonium hydroxide was added until a pH value of ~ 6 was attained. The solution was evaporated to a pink viscous oil. This oil-like product was stirred with acetone and methanol, and the solvent was subsequently evaporated. The crude product was then heated in boiling methanol, filtered, and washed with acetone, twice. The solvent was evaporated under vacuum to form $(\text{N}(\text{C}_2\text{H}_5)_4)_3[\text{Ln}(\text{dpa})_3]$ as a white solid. The tetrabutylammonium salts of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ ($(\text{N}(\text{C}_4\text{H}_9)_4)_3[\text{Ln}(\text{dpa})_3]$ where $\text{Ln} = \text{Eu}$ or Tb) were prepared following a slightly modified procedure from the tetraethylammonium salts.²⁹

The hexafluorophosphate (PF_6^-) salts of $\Delta-(\lambda\lambda\lambda)$ - $[\text{Co}(\text{R},\text{R}\text{-chxn})_3]^{3+}$ complexes were prepared by slowly adding ammonium hexafluorophosphate to an aqueous solution containing $\Delta-(\lambda\lambda\lambda)$ - $[\text{Co}(\text{R},\text{R}\text{-chxn})_3]\text{Cl}_3$ until a precipitate was formed. The orange precipitate was then collected and washed with water.

Sample Preparation

Stock solutions were prepared containing 10 mM $\text{X}_3[\text{Ln}(\text{dpa})_3]$ (where $\text{Ln} = \text{Eu}$ or Tb , and $\text{X} = \text{N}(\text{C}_2\text{H}_5)_4^+$ or $\text{N}(\text{C}_4\text{H}_9)_4^+$) dissolved in spectrophotometric-grade acetonitrile, and a stock solution was prepared containing 257 μM $\Delta-(\lambda\lambda\lambda)$ - $[\text{Co}(\text{R},\text{R}\text{-chxn})_3](\text{PF}_6)_3$ dissolved in spectrophotometric-grade acetonitrile. The concentration of the $\Delta-(\lambda\lambda\lambda)$ - $\text{Co}(\text{R},\text{R}\text{-chxn})_3^{3+}$ solution was determined spectrophotometrically and with atomic absorption. Samples used in the quenching studies were prepared by diluting the stock solutions concentrations of 5 mM $\text{Ln}(\text{dpa})_3^{3-}$ and 128.5 μM $\Delta-(\lambda\lambda\lambda)$ - $\text{Co}(\text{R},\text{R}\text{-chxn})_3^{3+}$. Samples of 10 mM $\text{Eu}(\text{dpa})_3^{3-}$ or $\text{Tb}(\text{dpa})_3^{3-}$ dissolved in acetonitrile were used in the measurements of temperature-dependent unquenched luminescence lifetimes. A small amount of white precipitate formed in almost all of the prepared samples over the time period of our spectroscopic measurements.

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Instrumentation and Measurement Techniques

Absorption measurements were made on a Cary Model 5 UV/VIS/NIR absorption spectrophotometer. The emission and TR-CL measurements were made on instrumentation constructed at the University of Virginia (in F.S. Richardson's laboratory). An argon ion (Ar^+) laser was used for sample excitation at 488.0 nm ($\text{Tb}(\text{dpa})_3^{3-}$) and 465.8 nm ($\text{Eu}(\text{dpa})_3^{3-}$). The excitation beam was chopped by a variable-speed chopper (Stanford Research Systems SR-540) operating in the frequency range 60–120 Hz. The sample was held in a water-jacketed 1-cm fluorescence cuvette, and a circulating water/ethylene glycol bath (Haake model F3) was used to maintain sample temperatures of 253–353 K. The emission was collected at 90° to the excitation direction and passed through a photoelastic modulator (Hinds International Model PEM-80) and linear-polarizer combination, which together serve as a circular polarization analyzer. The emission was focused on a 0.75 m double-grating monochromator, and the luminescence intensity was measured using laboratory-constructed photon-counting instrumentation. The gated photon-counting device was referenced to the modulation frequency of the photoelastic modulator (PEM) to determine the temporal distribution of the photon pulses, both within the PEM cycle (which determines the CPL and TL content of the emission) and along a train of PEM cycles after the excitation pulse (which determines the decay characteristics of the emission). The PEM was operated at a frequency of 50 kHz, and the temporal resolution of this system was 20 μs . A computer is used to store the TL, CPL, and time information acquired in our TR-CPL measurements.

To achieve the large signal-to-noise ratios required for the observation of small TR-CPL signals, millions of emission decays are summed. The time-resolved measurements reported in this study were collected over a period of hours. Sample stability was checked by measuring emission decay characteristics both at the start and end of a spectral run.

Data Manipulation and Analysis

Marquardt-based non-linear-least-squares fitting routines were used to fit the $I(\lambda', t)$, $\Delta I(\lambda', t)$, and $g_{\text{em}}(\lambda', t)$ data to eqs. 10–12 modified to account for the dark counts which are present in the $I(\lambda', t)$ data. The equations for $I(\lambda', t)$ and $g_{\text{em}}(\lambda', t)$, including dark count corrections, can be written as

$$I(\lambda', t) = \frac{I(\lambda', 0)}{2} [e^{-k_1 t} + e^{-k_2 t}] + \text{dc} \quad (13)$$

$$g_{\text{em}}(\lambda', t) = g_{\text{em}}^{\Lambda}(\lambda') \tanh \left[\frac{(k_q^{\Lambda} - k_q^{\Lambda})[Q]t}{2} \right] \times \frac{I(\lambda', t) - \text{dc}}{I(\lambda', t)} \quad (14)$$

where dc is the instrumental dark count correction term, and $I(\lambda', t)$ is the experimentally-determined TL. Dark

counts will contribute to the noise in the $\Delta I(\lambda', t)$ measurement, but not to the $\Delta I(\lambda', t)$ magnitude.

The presence of precipitate in the spectroscopic samples can affect the magnitude of $\Delta I(\lambda', t)$ but not $I(\lambda', t)$. Therefore, all of the quenching kinetics results presented in this study were derived from fits of the $I(\lambda', t)$ data to eq. 13. Fits of the $\Delta I(\lambda', t)$, and $g_{em}(\lambda', t)$ data to eqs. 11 and 14 provided results that were slightly different than those presented in this study.

RESULTS

Luminescence Lifetimes

Time-resolved luminescence measurements were performed on samples of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ complexes dissolved in acetonitrile in order to determine the emitting-state luminescence lifetimes. In the absence of chiral quencher molecules, the g_{em} and CPL (ΔI) will remain zero at all times, and the TL will decay according to

$$I(\lambda', t) = I(\lambda', 0)e^{-k_0 t} \quad (15)$$

where k_0 is the emitting-state decay rate constant, and $k_0 = 1/\tau_0$ (where τ_0 is the emitting-state lifetime). The results of these measurements are presented in Figures 6 and 7. Figure 6 shows results of measurements of the $^5\text{D}_0$ excited-state lifetime (τ_0) of $\text{Eu}(\text{dpa})_3^{3-}$ dissolved in acetonitrile over the temperature range 253–333 K. These results are compared with results obtained from samples in aqueous solution over the temperature range 273–353 K. The τ_0 ($^5\text{D}_0$) for $\text{Eu}(\text{dpa})_3^{3-}$ is considerably longer in acetonitrile samples. However, neither the aqueous samples nor the acetonitrile samples show very large variations in τ_0 over the temperature ranges presented in Figure 6.

The τ_0 ($^5\text{D}_4$) values for $\text{Tb}(\text{dpa})_3^{3-}$ dissolved in acetonitrile and water over the 253–353 K temperature range are shown in Figure 7. Results are shown for samples of both $(\text{NEt}_4)_3[\text{Tb}(\text{dpa})_3]$ (where $\text{NEt}_4 = \text{N}(\text{C}_2\text{H}_5)_4 \equiv$ tetraethyl-

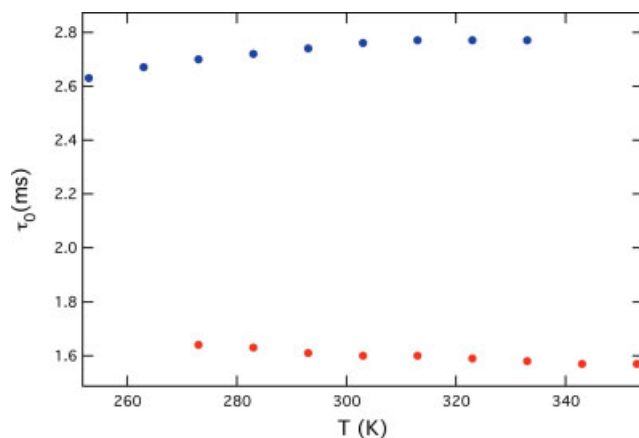


Fig. 6. Plots of τ_0 vs. temperature for samples of $\text{Eu}(\text{dpa})_3^{3-}$ dissolved in water (red circles) and acetonitrile (blue circles). The uncertainty in τ_0 is ca. 0.004 ms. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

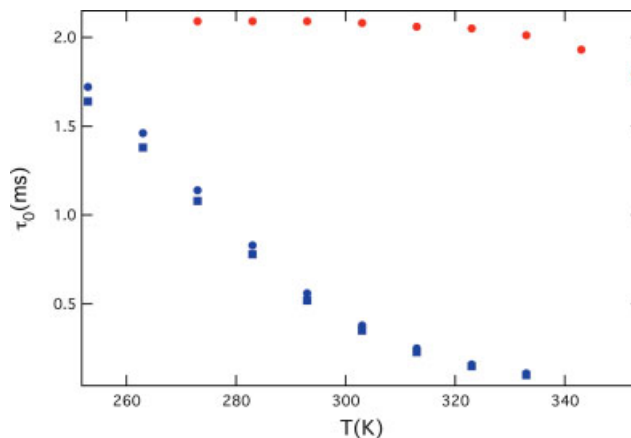


Fig. 7. Plots of τ_0 vs. temperature for samples of $\text{Tb}(\text{dpa})_3^{3-}$ dissolved in water (red circles) and $(\text{NEt}_4)_3[\text{Tb}(\text{dpa})_3]$ (blue circles) and $(\text{NBU}_4)_3[\text{Tb}(\text{dpa})_3]$ (blue squares) dissolved in acetonitrile. The uncertainty in τ_0 is ca. 0.004 ms. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ammonium) and $(\text{NBU}_4)_3[\text{Tb}(\text{dpa})_3]$ (where $\text{NBU}_4 = \text{N}(\text{C}_4\text{H}_9)_4 \equiv$ tetra-*n*-butylammonium) dissolved in acetonitrile. In acetonitrile solution, the τ_0 ($^5\text{D}_4$) values for $\text{Tb}(\text{dpa})_3^{3-}$ rapidly decrease as the sample temperature increases, and are significantly smaller than the τ_0 values determined for aqueous solution samples.

Quenching Results

An example of time-resolved total luminescence (I), CPL (ΔI), and emission dissymmetry (g_{em}) data is shown in Figure 8. Figure 8 shows TR-CL results obtained on a sample that contains $(\text{NEt}_4)_3[\text{Eu}(\text{dpa})_3]$ and $\Delta-(\lambda\lambda\lambda)\text{-Co}(\text{R,R-chxn})_3^{3+}$ dissolved in an acetonitrile solution. The CPL and g_{em} shown in Figure 8 exhibits an initial value of zero, reflecting an initially racemic excited-state population of luminophores (Eu^*) prepared by the excitation pulse. Note that g_{em} grows in magnitude with a time dependence that reflects the evolution of an enantiomeric excess in the excited-state luminophore population (due to enantioselective quenching kinetics). The time dependences of the TL (labeled by $I_L + I_R$) and CPL (denoted as $I_L - I_R$) follow the double-exponential rate expressions given by eqs. 10 and 11.

The $I(\lambda', t)$, $\Delta I(\lambda', t)$, and $g_{em}(\lambda', t)$ data were analyzed according to the phenomenological quenching kinetics model represented in eqs. 10–14. From these analyses, we can determine k_q^Δ , the rate constant representing $\Delta L^* - Q$ quenching, and k_q^Λ , the rate constant representing $\Delta L^* - Q$ quenching. It is also useful to introduce another parameter that provides a direct measure of both the degree and sense of enantioselectivity in the quenching processes. This enantioselectivity parameter is defined by

$$E_q = \frac{k_q^\Delta - k_q^\Lambda}{k_q^\Delta + k_q^\Lambda} \quad (16)$$

and it can have values between +1 (for purely $\Delta L^* - Q$ quenching) and −1 (for purely $\Lambda L^* - Q$ quenching).

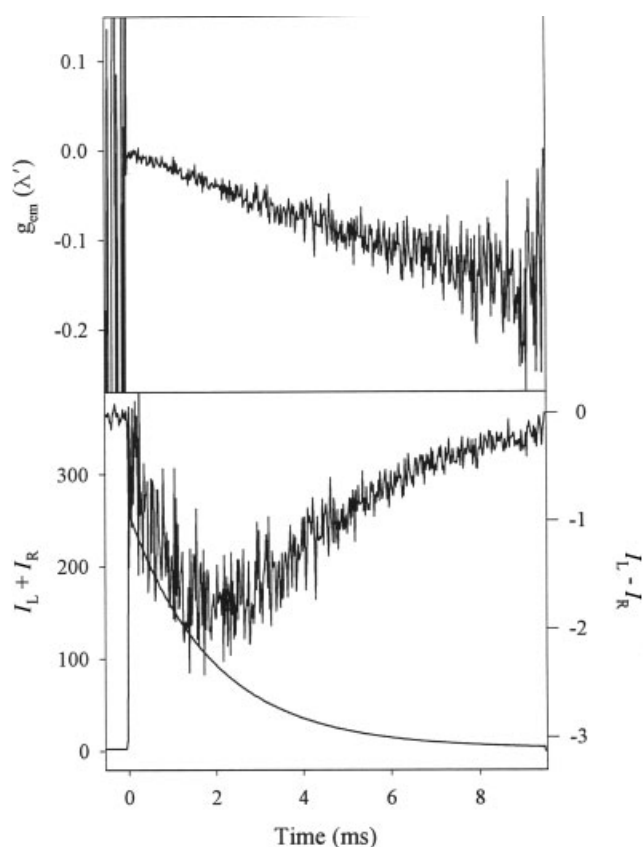


Fig. 8. Time-resolved chiroptical luminescence data for 5 mM $(\text{NEt}_4)_3[\text{Eu}(\text{dpa})_3]$ with 128.5 μM $\Delta-(\lambda\lambda\lambda)\text{-Co}(R,R\text{-chxn})_3^{3+}$ dissolved in acetonitrile. The bottom frame shows the time-resolved TL ($I_L + I_R$, smooth trace) and CPL ($I_L - I_R$, noisy trace). The top frame shows the time-resolved emission dissymmetry data calculated from the CPL and TL data given in the bottom frame. The sample was excited with chopped radiation at 465.8 nm, and the emission intensity was monitored at 594.3 nm. Sample temperature was 313 K.

Phenomenological quenching kinetics parameters were obtained from analyses of the temperature-dependent quenching ($L^* - Q$) measurements of $\text{Eu}(\text{dpa})_3^{3-}$ com-

plexes. However, the analogous temperature-dependent quenching measurements of $\text{Tb}(\text{dpa})_3^{3-}$ complexes as the luminophores did not allow satisfactory analysis and interpretation. The kinetics parameters determined from our temperature-dependent quenching measurements are given in Table 1. These results were obtained on samples containing $(\text{NEt}_4)_3[\text{Eu}(\text{dpa})_3]$ or $(\text{NBu}_4)_3[\text{Eu}(\text{dpa})_3]$ and $\Delta-(\lambda\lambda\lambda)\text{-Co}(R,R\text{-chxn})_3^{3+}$ dissolved in acetonitrile over the temperature range 273–333 K. Below 273 K, the quenching rate is too slow (compared to the excited-state decay rate) to measure in our experiments. The results tabulated in Table 1 include the enantiopreferential quenching rate constants (k_q^A and k_q^B), the mean quenching rate constant ($k_q = (k_q^A + k_q^B)/2$), and the enantioselectivity parameter (E_q) determined for the $(\text{Eu}^*\text{-Co})$ quenching samples, and the unquenched emitting-state decay constant of the luminophores. In Table 1, both the enantiopreferential quenching rate constants and the mean quenching rate constants are seen to decrease with temperature, but the enantioselectivity parameters remain relatively unchanged.

The enantiopreferential $(\text{Eu}^*\text{-Co})$ quenching results obtained for samples dissolved in acetonitrile, along with results previously obtained for aqueous solution samples offer the opportunity to make comparisons that demonstrate the solvent dependence of enantiopreferential quenching processes. Comparisons of mean quenching rate constants and enantioselectivity parameters obtained for $\text{Eu}(\text{dpa})_3^{3-}/\Delta-(\lambda\lambda\lambda)\text{-Co}(R,R\text{-chxn})_3^{3+}$ systems dissolved in water and acetonitrile are presented in Table 2. The results are reported over the temperature range 273–333 K. The mean quenching rate constant (k_q) values are two orders of magnitude smaller in acetonitrile than in water. However, the enantioselectivity parameters determined for the acetonitrile samples are only slightly smaller than the values determined for aqueous samples. These results suggest that the overall efficiency of $\text{Eu}^*\text{-Co}$ quenching is much less in acetonitrile versus aqueous solution samples, but the enantioselectivity is similar and considerably less sensitive to the change in solvent (H_2O vs. CH_3CN).

TABLE 1. Temperature dependence of quenching parameters for samples of $(\text{NEt}_4)_3[\text{Eu}(\text{dpa})_3]$ or $(\text{NBu}_4)_3[\text{Eu}(\text{dpa})_3]$ and $\Delta-(\lambda\lambda\lambda)\text{-Co}(\text{chxn})_3^{3+}$ dissolved in acetonitrile

T (K)	k_0 (s^{-1})	$k_q^A \times 10^{-5}$ ($\text{M}^{-1} \text{s}^{-1}$) ^a		$k_q^B \times 10^{-5}$ ($\text{M}^{-1} \text{s}^{-1}$)		$k_q \times 10^{-5}$ ($\text{M}^{-1} \text{s}^{-1}$) ^b		E_q ^c	
		NEt_4	NBu_4	NEt_4	NBu_4	NEt_4	NBu_4	NEt_4	NBu_4
333	361	23.3 (0.2)	20.3 (0.1)	8.0 (0.1)	10.7 (0.1)	15.7	15.5	−0.48	−0.31
323	361	20.9 (0.2)	15.7 (0.1)	7.0 (0.1)	7.1 (0.1)	13.9	11.4	−0.50	−0.38
313	361	18.8 (0.2)	11.1 (0.2)	6.3 (0.1)	5.6 (0.2)	12.5	8.4	−0.50	−0.33
303	362	15.5 (0.3)	8.6 (0.2)	6.2 (0.2)	3.6 (0.1)	10.8	6.1	−0.43	−0.41
293	365	14.2 (0.3)	5.4 (0.4)	5.0 (0.2)	2.9 (0.4)	9.6	4.2	−0.48	−0.29
283	368	10.0 (0.4)	5.3 (0.4)	3.4 (0.3)	2.2 (0.4)	6.7	3.7	−0.49	−0.42
273	370	7.6 (0.6)	3.1 (0.7)	1.6 (0.4)	1.7 (0.7)	4.6	2.4	−0.64	−0.30

^aThe numbers shown in parentheses are the fitting errors in the parameter values.

^bThe mean quenching rate constant, k_q , is defined as $k_q = (k_q^A + k_q^B)/2$.

^cDefined in eq. 16.

TABLE 2. Comparison of the mean quenching rate constants and enantioselectivity parameters determined in samples of $\text{Eu}(\text{dpa})_3^{3-}/\Delta-(\lambda\lambda\lambda)\text{-Co}(\text{R},\text{R-chxn})_3^{3+}$ dissolved in water and acetonitrile^a

<i>T</i> (K)	(NEt ₄) ₃ [Eu(dpa) ₃] in CH ₃ CN		(NBu ₄) ₃ [Eu(dpa) ₃] in CH ₃ CN		Na ₃ [Eu(dpa) ₃] ^b in H ₂ O	
	<i>k_q</i> × 10 ^{−5} (M ^{−1} s ^{−1})	<i>E_q</i>	<i>k_q</i> × 10 ^{−5} (M ^{−1} s ^{−1})	<i>E_q</i>	<i>k_q</i> × 10 ^{−5} (M ^{−1} s ^{−1})	<i>E_q</i>
333	15.7	−0.48	15.5	−0.31	1130	−0.65
323	13.9	−0.50	11.4	−0.38	1020	−0.63
313	12.5	−0.50	8.4	−0.33	954	−0.63
303	10.8	−0.43	6.1	−0.41	870	−0.61
293	9.6	−0.48	4.2	−0.29	802	−0.59
283	6.7	−0.49	3.7	−0.42	727	−0.56
273	4.6	−0.64	2.4	−0.30	654	−0.52

^aParameters defined according to eq. 16 and the corresponding text.^bFrom Ref. 10.

The temperature-dependent enantiopreferential quenching constants (k_q^σ , where $\sigma = \Lambda$ or Δ) shown in Table 1 are analyzed in terms of Arrhenius ($\ln k_q^\sigma$ vs. $1/T$) and Eyring ($\ln(k_q^\sigma/T)$ vs. $1/T$) plots over the 263–333 K temperature range. The k_q^σ data were fit to the Arrhenius and Eyring equations

$$\ln k_q^\sigma = \ln A^\sigma - \frac{E_a^\sigma}{RT} \quad (17)$$

$$\ln\left(\frac{k_q^\sigma}{T}\right) = \ln\left(\frac{k_B}{h}\right) + \frac{\Delta S^\#}{R} - \frac{\Delta H^\#}{RT}, \quad (18)$$

respectively, where E_a^σ , $\Delta S^\#$, and $\Delta H^\#$ are the activation parameters associated with the $\Lambda\text{L}^* - Q$ and $\Delta\text{L}^* - Q$ quenching processes. Figure 9 shows the Eyring plots of the quenching results obtained from samples of (X)₃[Eu(dpa)₃] (where X = NEt₄ or NBu₄) and $\Delta-(\lambda\lambda\lambda)\text{-Co}(\text{R},\text{R-chxn})_3^{3+}$ dissolved in acetonitrile. The lines shown on the plots are from least-squares fits of the k_q^σ data to equation (18). The Eyring (see Fig. 9) analyses of the k_q^σ data obtained from (NBu₄)₃[Eu(dpa)₃] samples yielded linear plots, while data from (NEt₄)₃[Eu(dpa)₃] samples

show distinctly nonlinear plots over the temperature range studied. The activation parameters derived from the Arrhenius and Eyring analyses of the quenching data obtained from (NBu₄)₃[Eu(dpa)₃]/ $\Delta-(\lambda\lambda\lambda)\text{-Co}(\text{R},\text{R-chxn})_3^{3+}$ samples are given in Table 3. The $\Delta G^\#$ activation parameters shown in Table 3 are defined as $\Delta G^\# = \Delta H^\# - T\Delta S^\#$, where $T = 298$ K. The ΔE_a , $\Delta\Delta S^\#$, $\Delta\Delta H^\#$, and $\Delta\Delta G^\#$ quantities shown in Table 3 are defined as differences between activation parameters associated with $\Lambda\text{L}^* - Q$ versus $\Delta\text{L}^* - Q$ quenching processes: $\Delta E_a = E_a^\Delta - E_a^\Lambda$; $\Delta\Delta S^\# = \Delta S^\#_\Delta - \Delta S^\#_\Lambda$; $\Delta\Delta H^\# = \Delta H^\#_\Delta - \Delta H^\#_\Lambda$; and $\Delta\Delta G^\# = \Delta G^\#_\Delta - \Delta G^\#_\Lambda$. Also shown in Table 3 are the activation parameters determined from previous analyses of quenching rate data obtained on aqueous solution samples of $\text{Eu}(\text{dpa})_3^{3-}$ and $\Delta-(\lambda\lambda\lambda)\text{-Co}(\text{R},\text{R-chxn})_3^{3+}$.

DISCUSSION

Luminescence Lifetimes

The results presented in Figure 6 show that the luminescence lifetime of $\text{Eu}(\text{dpa})_3^{3-}$ is sensitive to the solvent composition of our solution samples, and that in going from H₂O to CH₃CN solutions, the $\tau_0(^5\text{D}_0)$ for $\text{Eu}(\text{dpa})_3^{3-}$ increases. It is important to note that the spectral dispersion characteristics of the luminescence spectra of $\text{Eu}(\text{dpa})_3^{3-}$ are nearly identical in aqueous and acetonitrile solution samples. Therefore, it is unlikely that the solvent dependence of τ_0 can be attributed to perturbations of the energy-level structure or inner-sphere coordination properties of the $\text{Eu}(\text{dpa})_3^{3-}$ complexes. It is more likely that this dependence reflects effects due to bulk solvent properties or properties of solvent molecules that reside in the outer-coordination sphere of the complexes.

Previous studies of the luminescence lifetimes of $\text{Eu}(\text{dpa})_3^{3-}$ in aqueous solution indicate that the emitting-state ($^5\text{D}_0$) decay rate process is dominated by H₂O-mediated nonradiative decay processes ($k_{\text{nr}}(\text{H}_2\text{O})$).^{10,25} Therefore, the results in Figure 6 ($k_0(\text{CH}_3\text{CN}) \ll k_0(\text{H}_2\text{O})$) indicate that CH₃CN-mediated nonradiative decay processes are less efficient than H₂O-mediated nonradiative decay processes ($k_{\text{nr}}(\text{CH}_3\text{CN}) \ll k_{\text{nr}}(\text{H}_2\text{O})$).

The results presented in Figure 7 show the solvent dependence of the luminescence lifetime of $\text{Tb}(\text{dpa})_3^{3-}$ in aqueous vs. acetonitrile solution. The $\tau_0(^5\text{D}_4)$ values for

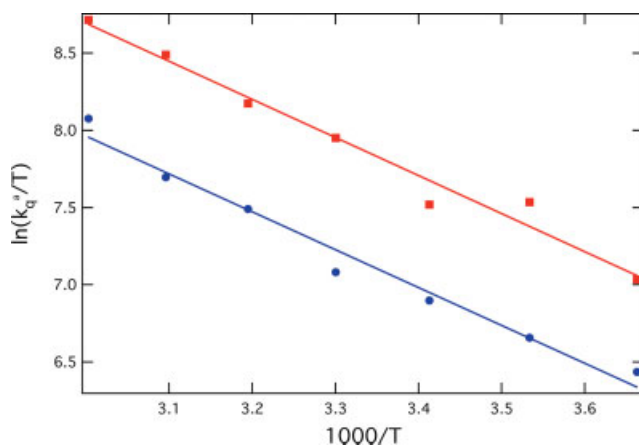


Fig. 9. Plot of $\ln(k_q^\Lambda/T)$ (red squares) and $\ln(k_q^\Delta/T)$ (blue circles) versus $1000/T$ for quenching results obtained from measurements on (NBu₄)₃[Eu(dpa)₃]/ $\Delta-(\lambda\lambda\lambda)\text{-Co}(\text{R},\text{R-chxn})_3^{3+}$ dissolved in acetonitrile over the 273–333 K temperature range. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 3. Comparison of parameters obtained from Arrhenius and Eyring analysis of the temperature-dependent (Eu³⁺-Co) quenching data of samples dissolved in acetonitrile and water

		(NBu ₄) ₃ [Eu(dpa) ₃]/ CH ₃ CN ^a	Eu(dpa) ₃ ³⁻ / H ₂ O ^b
Arrhenius	E_a^Λ (kJ/mole)	23.1 ± 1.5	7.4 ± 0.1
	E_a^Δ (kJ/mole)	23.0 ± 1.3	1.9 ± 0.4
	ΔE_a (kJ/mole)	-0.03 ± 2.0	-5.5 ± 0.4
	$\ln A^\Lambda$ (M ⁻¹ s ⁻¹)	22.8 ± 0.6	21.7 ± 0.1
	$\ln A^\Delta$ (M ⁻¹ s ⁻¹)	22.1 ± 0.6	18.1 ± 0.2
Eyring	$\Delta H_A^\ddagger$ (kJ/mole)	20.6 ± 1.4	4.9 ± 0.1
	$\Delta H_\Delta^\ddagger$ (kJ/mole)	20.5 ± 1.3	-0.6 ± 0.4
	$\Delta \Delta H^\ddagger$ (kJ/mole)	-0.03 ± 1.9	-5.5 ± 0.4
	$\Delta S_A^\ddagger$ (J/K mole)	-63.6 ± 4.8	-73.0 ± 0.4
	$\Delta S_\Delta^\ddagger$ (J/K mole)	-69.7 ± 4.5	-102.9 ± 1.5
	$\Delta \Delta S^\ddagger$ (J/K mole)	-6.1 ± 6.6	-29.9 ± 1.6
	$\Delta G_A^\ddagger$ (kJ/mole)	39.5 ± 2.0	26.6 ± 0.2
	at 298 K		
	$\Delta G_\Delta^\ddagger$ (kJ/mole)	41.3 ± 1.9	30.1 ± 0.6
	at 298 K		
	$\Delta \Delta G^\ddagger$ (kJ/mole)	1.8 ± 2.8	3.5 ± 0.6
	at 298 K		

^aParameters are defined according to eqs. 17 and 18, and the corresponding text.

^bFrom Ref. 10.

Tb(dpa)₃³⁻ decrease significantly in going from aqueous to acetonitrile solution. Previous studies of the luminescence lifetimes of Tb(dpa)₃³⁻ in aqueous solution indicate that the emitting-state (⁵D₄) decay rate process is dominated by radiative decay processes.^{10,25} An explanation for the solvent-dependent behavior of the τ_0 (⁵D₄) values is not readily apparent, but this could be due, in part, to the complexity of the energy levels in the ⁵D₄ excited-state manifold, and their intrinsic radiative spontaneous emission probabilities.

Enantiopreferential Quenching

The results presented illustrate the sensitivity of the enantiopreferential quenching kinetics of Eu(dpa)₃³⁻/Δ-(λλλ)-[Co(R,R-chxn)₃]³⁺ systems to changes in both salt (NEt₄ vs. NBu₄) and solvent (CH₃CN vs. H₂O). Results from previous studies of the quenching kinetics of Eu(dpa)₃³⁻/Δ-(λλλ)-[Co(R,R-chxn)₃]³⁺ systems in aqueous solution samples indicate that the rate of diffusion to form Eu³⁺-Co encounter complexes is greater than the rate of electronic energy transfer.¹⁰ Because the viscosity of acetonitrile is less than that of water, the Eu³⁺-Co quenching rate in acetonitrile solution is not expected to be diffusion-controlled. Therefore, the solvent dependence of the quenching kinetics is due to the influence of the solvent molecules on the stability (and therefore transient lifetimes) of the Eu³⁺-Co encounter complexes and possibly the interactions that govern energy transfer in these complexes.

The quenching parameters for Eu³⁺-Co quenching processes in aqueous and acetonitrile solutions over the 273–333 K temperature range are shown in Table 2. Comparison of the quenching parameters shown in Table 2 reveal

that the mean quenching rate constants (k_q) and the enantioselectivity parameters (E_q) obtained for aqueous solution samples are larger than the k_q and E_q values obtained for acetonitrile solution samples. Furthermore, the k_q and E_q values differ for the different (NEt₄ and NBu₄) salts of Eu(dpa)₃³⁻ used in the acetonitrile solution samples. The most striking difference in the quenching parameters shown in Table 2 is the >75-fold increase in the k_q values upon changing the solvent from acetonitrile to water. For all of the Eu³⁺-Co systems considered in Table 2, it is assumed that the quenching is a bimolecular process that follows the mechanistic pathways shown in eqs. 6 and 7, and that the differences in the quenching kinetics are due to influences of the solvent molecules and the cations upon the Eu³⁺-Co quenching processes.

As discussed earlier, the quenching processes for the systems of interest in this study are mediated by a quasi-equilibrium in the formation/dissociation of the Eu³⁺-Co encounter complexes, followed by a slow energy-transfer step. This is reflected in eqs. 8 and 9, which we rewrite here as

$$k_q^\sigma = K_a^\sigma k_e^\sigma \quad (19)$$

where $\sigma = \Lambda$ or Δ . Following previous work by Glover-Fischer et al., eq. 19 can be expressed as¹⁰

$$k_q^\sigma \approx \left[10^{-24} \left(4\pi N_A \hat{R}_\sigma^3 / 3 \right) \exp(-\hat{U}_\sigma / k_B T) \right] \hat{k}_e^\sigma \quad (20)$$

where N_A is the Avogadro constant, $\hat{R}_\sigma$ is the Ln-Co metal-metal separation distance in the encounter complex (expressed in nm), $\hat{k}_e^\sigma$ is the electronic energy-transfer rate constant at Ln-Co separation distance $\hat{R}_\sigma$, k_B is the Boltzmann constant, and $\hat{U}_\sigma$ is the work done in forming the $L^* - Q$ complex from free L^* and Q in solution. Assuming that electrostatic charge ($z_L e$)-charge ($z_Q e$) interactions will make the dominant contributions to $\hat{U}_\sigma$, we can write the work function in the following approximate form:

$$\begin{aligned} \hat{U}_\sigma &\approx \frac{10^9 z_L z_Q e^2}{4\pi \epsilon_0 \hat{D} \hat{R}_\sigma (1 + \beta \hat{R}_\sigma)} \\ &\approx (2.307 \times 10^{-19}) \frac{z_L z_Q}{\hat{D} \hat{R}_\sigma (1 + \beta \hat{R}_\sigma)} \end{aligned} \quad (21)$$

where $\hat{U}_\sigma$ is expressed in joules (J), $\hat{R}_\sigma$ is expressed in nm, z_L and z_Q denote the ionic valences of the lumiphore and quencher species, respectively, D is the dielectric constant of the solvent medium, and β has units of nm⁻¹, and is defined by

$$\beta = 10^{-9} (2F^2 \rho_s \mu / \epsilon_0 D N_A k_B T)^{1/2} \approx 15.90 (\rho_s \mu / D T)^{1/2} \quad (22)$$

where F is the Faraday constant (9.648×10^4 C/mol), ρ_s is the solvent density (expressed in kg/m³), and μ is the ionic strength of the solution sample (expressed in mol/kg).

In our luminophore/quencher systems, the quencher has a net charge ($z_Q e$) of $+3e$ in both aqueous and acetonitrile solution, and the net charge ($z_L e$) of the luminophore is $-3e$ in aqueous solution. However, conductivity measurements indicate that the NEt_4^+ and NBU_4^+ ions are not fully dissociated from the $\text{Eu}(\text{dpa})_3^{3-}$ ion in acetonitrile solution, and the absolute value of the effective charge of the luminophore is most likely less than 3 (unpublished results). Therefore, the decrease in k_q values observed for the acetonitrile solution samples (compared to aqueous solution samples), as shown in Table 2, can be attributed to the association of the cations with the $\text{Eu}(\text{dpa})_3^{3-}$ complexes, and the resulting reduction in the absolute magnitude of the charge-product ($z_L z_Q$) of the luminophore/quencher encounter pairs. It is important to note that the ratios of $k_q(\text{H}_2\text{O})$ to $k_q(\text{CH}_3\text{CN})$ shown in Table 2 decrease as the temperature increases. This temperature-dependent behavior is indicative of association between the cations and the $\text{Eu}(\text{dpa})_3^{3-}$.

The enantioselectivity parameters (E_q) for the quenching processes in acetonitrile solution are smaller in magnitude than those observed for aqueous solution samples. However, the sense of the enantiopreference is the same in acetonitrile and water. The quenching results obtained for acetonitrile solution samples indicate that the cations are affecting the quenching processes by reducing the stability of the encounter complexes. This decreased stability of the encounter complexes results in shorter transient lifetimes, which allows less time for the enantiopreferential recognition to occur. Therefore, the decrease observed in the $|E_q|$ values between aqueous and acetonitrile solution is likely due to the reduced stability of the encounter complexes in acetonitrile.

As shown in eq. 19, the enantiopreferential quenching rate constant (k_q^σ) is dependent upon the “stability” of the encounter complexes (as gauged by K_a^σ) and the rate of electronic energy transfer (as determined by $\hat{k}_e^\sigma$). In the preceding discussion of the enantiopreferential quenching processes and in eqs. 20–22, we have focused almost entirely on the relationship between K_a^σ and k_q^σ . Following previous work by Glover-Fischer et al., the electronic energy-transfer rate constant ($\hat{k}_e^\sigma$) can be expressed as¹⁰

$$\hat{k}_e^\sigma = \kappa_e^\sigma v_\sigma^\dagger e^{-\Delta G_\sigma^\ddagger/k_B T} \quad (23)$$

where $\Delta G_\sigma^\ddagger$ represents the free-energy of activation for electronic energy transfer in the Ln^*-Q encounter complexes, κ_e^σ represents the probability for electronic energy transfer in the activated $(\text{Ln}^*-Q)^\ddagger$ species, and $v_\sigma^\dagger$ denotes the frequency of activation. If eq. 23 is substituted for $\hat{k}_e^\sigma$ in eq. 20, then k_q^σ may be expressed as

$$k_q^\sigma \approx 10^{-24} \left(4\pi N_A \hat{R}_\sigma^3 / 3 \right) \kappa_e^\sigma v_\sigma^\dagger \exp \left[- \left(\hat{U}_\sigma + \Delta G_\sigma^\ddagger \right) / k_B T \right] \quad (24a)$$

$$\approx \left(2.523 \hat{R}_\sigma^3 \kappa_e^\sigma k_B T / h \right) \exp \left[\Delta S_\sigma^\ddagger / k_B \right] \times \exp \left[- \left(\hat{U}_\sigma + \Delta H_\sigma^\ddagger \right) / k_B T \right] \quad (24b)$$

where eq. 24b follows from eq. 24a by making the “classical approximation” for the activation frequency factor, $v_\sigma^\dagger = k_B T / h$, and by setting $N_A = 6.022 \times 10^{23}$ and $\Delta G_\sigma^\ddagger = \Delta H_\sigma^\ddagger - T \Delta S_\sigma^\ddagger$.

In performing Eyring analyses of our temperature-dependent quenching rate-constant data, we used the following expression for k_q^σ ,

$$k_q^\sigma = (k_B T / h) \exp \left[\Delta S_\sigma^\ddagger / N_A k_B \right] \exp \left[- \Delta H_\sigma^\ddagger / N_A k_B T \right], \quad (25)$$

and from plots of $\ln(k_q^\sigma / T)$ versus $1/T$ we were able to obtain values for the $\Delta S_\sigma^\ddagger$ and $\Delta H_\sigma^\ddagger$ parameters in this expression (see results presented in Table 3 for the Eu^* -Co quenching kinetics). These parameters may be related to the various parameters (and other quantities) that appear in eq. 24b according to

$$\Delta S_\sigma^\ddagger = N_A \left[k_B \ln(2.523 \hat{R}_\sigma^3 \kappa_e^\sigma) + \Delta S_\sigma^\ddagger \right] \quad (26)$$

$$\Delta H_\sigma^\ddagger = N_A \left[\hat{U}_\sigma + \Delta H_\sigma^\ddagger \right] \quad (27a)$$

$$\approx (1.389 \times 10^5) \frac{z_L z_Q}{D \hat{R}_\sigma (1 + \beta \hat{R}_\sigma)} + N_A \Delta H_\sigma^\ddagger \quad (27b)$$

where eq. 27b follows from eq. 27a if the work function $\hat{U}_\sigma$ is defined according to eq. 21 and is expressed in joules (per interacting $L^* - Q$ pair). Note that the $\Delta H_\sigma^\ddagger$ parameter in eqs. 24b, 27a, and 27b corresponds to the enthalpy of activation required for L^* -to- Q electronic energy transfer within an $(L^* - Q)$ encounter complex, and if we assume that this involves an elementary rate process, without any significant volume change in the encounter complex, then the value of $\Delta H_\sigma^\ddagger$ will always be ≥ 0 . On the other hand, for the oppositely charged luminophore ($z_L < 0$) and quencher ($z_Q > 0$) molecules examined in this study, the sign of the charge-product quantity ($z_L z_Q$) is negative, and, therefore, the contributions of $\hat{U}_\sigma$ to $\Delta H_\sigma^\ddagger$ will always be < 0 . Thus, the $\Delta H_\sigma^\ddagger$ parameters derived from our analyses of the temperature-dependent k_q^σ rate-constant data reflect algebraic sums of $\Delta H_\sigma^\ddagger$ values (always ≥ 0) and $\hat{U}_\sigma$ values (all < 0 , with magnitudes modulated largely by $|z_L z_Q|$ and the relevant $\hat{R}_\sigma$, D , and β parameters). Recall that $\hat{U}_\sigma$ is related entirely to the energy associated with the formation of $(L^* - Q)$ complexes from free L^* and Q species in solution, and it is reasonable to assume that this function will be quite sensitive to solvent properties. However, $\Delta H_\sigma^\ddagger$ reflects activation processes within the $(L^* - Q)$ complexes, and these processes are not expected to be influenced strongly by the solvent.

We note from Table 3 that the values of $\Delta H_\sigma^\ddagger$ and $\Delta H_\Delta^\ddagger$ determined for the acetonitrile solution samples are considerably larger than those determined for aqueous solution samples. The $\Delta H_\sigma^\ddagger$ parameters are expected to have only small solvent- and salt-dependence, and, therefore, the differences in the $\Delta H_\sigma^\ddagger$ values are due to the solvent- and salt-dependence reflected in the $\hat{U}_\sigma$ functions

($\hat{U}_\sigma(\text{H}_2\text{O}) < \hat{U}_\sigma(\text{CH}_3\text{CN}) < 0$). As indicated in results discussed previously, the solvent- and salt-dependence of $\hat{U}_\sigma$ is largely dependent upon the charge-product ($z_1 z_Q$) of the luminophore/quencher encounter pairs (in aqueous versus acetonitrile solution), which follows the relationship: $z_1 z_Q(\text{H}_2\text{O}) < z_1 z_Q(\text{CH}_3\text{CN}) < 0$.

CONCLUSION

TR-CL measurement techniques have been used in many recent studies of intermolecular excited-state energy-transfer processes between dissymmetric lanthanide complexes and dissymmetric transition-metal complexes in solution.^{1–14} Several of these studies indicate significant solvent mediation of the chirality-related (enantio-preferential) energy-transfer processes in solution samples in which the solvent is amphiprotic (e.g. H_2O , D_2O , CH_3OH , ...). However, none of these studies involved solution samples prepared from an aprotic solvent (e.g. CH_3CN , DMF, DMSO, ...).

In this article, we have presented results obtained from studies of intermolecular excited-state energy-transfer processes between dissymmetric $\text{Ln}(\text{dpa})_3^{3-}$ complexes ($\text{Ln} = \text{Eu}$ or Tb) as energy donors (luminophores) and dissymmetric cobalt(III) complexes ($\Delta(\lambda\lambda\lambda)\text{-Co}(\text{R,R-chxn})_3^{3+}$) as energy acceptors (quenchers) dissolved in acetonitrile. The luminophores were dissolved in acetonitrile as both the $(\text{NEt}_4)_3[\text{Ln}(\text{dpa})_3]$ and $(\text{NBu}_4)_3[\text{Ln}(\text{dpa})_3]$ (where $\text{Ln} = \text{Eu}$ or Tb) salts. Optical spectroscopic measurements indicate that the stereochemical structures of $\text{Ln}(\text{dpa})_3^{3-}$ complexes dissolved in acetonitrile are essentially identical to those of $\text{Ln}(\text{dpa})_3^{3-}$ in aqueous solution. However, the unquenched luminescence lifetimes (τ_0) obtained for samples of $\text{Eu}(\text{dpa})_3^{3-}$ and $\text{Tb}(\text{dpa})_3^{3-}$ dissolved in acetonitrile are significantly different than the τ_0 values determined for these complexes in aqueous solution.

TR-CL measurement techniques were used to examine the enantio-preferential quenching kinetics in samples containing $\text{Eu}(\text{dpa})_3^{3-}$ or $\text{Tb}(\text{dpa})_3^{3-}$ in acetonitrile solution with $\Delta(\lambda\lambda\lambda)\text{-Co}(\text{R,R-chxn})_3^{3+}$ complexes. The results obtained from the measurements of $\text{Eu}^*\text{-Co}$ quenching were analyzed in terms of a phenomenological quenching kinetics model, and the enantio-preferential quenching parameters were determined over the temperature range 263–333 K. Comparisons of the results presented in this article with those obtained in previous studies of $\text{Eu}^*\text{-Co}$ quenching kinetics in aqueous solution indicate that while the overall $\text{Eu}^*\text{-Co}$ quenching efficiency is reduced in acetonitrile versus aqueous solution samples, the sense of enantio-preference ($\Delta\text{Eu}^*\text{-Co}$ vs. $\Delta\text{Eu}^*\text{-Co}$ quenching) is identical in the two solvent systems.

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Entropy Production in Chiral Symmetry Breaking Transitions

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT It is now well known that nonequilibrium chemical systems may reach conditions that spontaneously generate chiral asymmetry. One can find a host of model reactions that exhibit such behavior in the literature. Among these, models based on one originally devised by Frank have been studied extensively. Though the kinetic aspects of such model reactions have been discussed in great detail, the behavior of entropy in such systems is rarely discussed. In this article, the rate of entropy production per unit volume, σ , in a modified Frank model is discussed. It is shown that the slope of σ changes at the point at which the asymmetric states appear, behavior similar to that observed in second-order phase transitions. *Chirality* 20:524–528, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: asymmetry generation; thermodynamics; entropy; chiral asymmetry

INTRODUCTION

Classical thermodynamics, as formulated in the 19th century by Carnot, Clausius, Kelvin, and others, is a theory of states. The entire theory is a description of the thermodynamic states, which are specified by state variables such as pressure, p , temperature T , and amounts of each component N_k . The laws of thermodynamics are statements about energy, U , and entropy, S , two fundamental functions of state. In the famous words of Clausius, “The energy of the universe is a constant. The entropy of the universe approaches maximum.” Though natural processes that cause changes in thermodynamic states are implicit in the theory, they are not explicitly included in the classical formulation. The rates of processes do not appear in the theory. In fact, classical thermodynamics does not concern time. Changes in entropy are calculated using the concept of infinitely slow reversible processes for which $dS = dQ/T$, where dQ is the heat exchanged by the system. For irreversible processes that take place in a finite time, there is only an inequality as follows:

$$dS > dQ/T \quad (1)$$

In this theory of states, there is no relationship between rates of changes in entropy and processes such as heat conduction and chemical reactions.

Thermodynamics was reformulated in the 20th century by Onsager,¹ De Donder and Van Rysselberghe,^{2,3} Prigogine,^{4,5} and others as a theory of irreversible processes by obtaining explicit expressions that relate entropy and irreversible processes. In this “modern” formulation, dS is expressed as a sum of two terms:

$$dS = d_e S + d_i S \quad (2)$$

The term $d_e S$, is the entropy change due to exchange of energy and matter; it equals dQ/T for closed systems. $d_i S$

is the entropy change due to irreversible processes. In modern thermodynamics, irreversible processes are described in terms of thermodynamic forces F_k and thermodynamic flows J_k .^{6,7} The irreversible process of heat conduction, for example, is associated with a thermodynamic force $F_h = \nabla(1/T)$ and a flow which is the heat current J_h . Table 1 lists a few thermodynamic forces and flows. We shall use s to represent entropy per unit volume, in which case $ds = d_e s + d_i s$ refer to entropy changes per unit volume. In general, s is a function of local state variables that are functions of position and time. In the modern formulation it is shown that $d_i s$, the entropy produced by irreversible processes per unit volume in time dt , can be written as

$$d_i s = \sum_k F_k J_k dt \quad (3)$$

In this expression the product $F_k J_k$ is a scalar. The Second Law of thermodynamics takes the form $d_i s > 0$. Expression (3) relates entropy to irreversible processes and time, dt . In classical thermodynamics, heat exchanged, dQ , is treated as an “imperfect differential” because Q is not a function of state. Since processes that transport energy and matter are an integral part of the modern thermodynamics, there is no need to treat heat-exchanged dQ as “imperfect differential”: dQ is the heat exchanged in time dt . That is, dQ is well defined in terms of the heat flow J_h .

In this article, we compute the entropy generated by chemical reactions. De Donder identified affinity as the

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TABLE 1. Examples of thermodynamic flows and the forces that drive them

Irreversible process	Thermodynamic force F_k	Thermodynamic flow J_k
Heat flow	$\nabla(1/T)$	Heat current J_h
Matter flow	$\nabla(\mu/T)$	Diffusion current J_k
Chemical reaction, $S + T \rightleftharpoons X$	Affinity, $A = \mu_S + \mu_T - \mu_X$	Reaction velocity, $v = k_f[S][T] - k_r[X]$

Heat flow is driven by the spatial gradient of $(1/T)$; Diffusion and chemical reactions are described in terms of chemical potential μ . Diffusion is driven by spatial gradient of (μ/T) . For a reaction $S + T \rightleftharpoons X$, the thermodynamic driving force is the affinity $A = \mu_S + \mu_T - \mu_X$.

thermodynamic force that drives chemical reactions.^{2,3} For example, for the reaction



the affinity is defined as:

$$A = \mu_A + \mu_B - \mu_X \quad (5)$$

in which μ_A , μ_B , and μ_X are the chemical potentials of A, B, and X, respectively. If $R_f = k_f[A][B]$ and $R_r = k_r[X]$ are the forward and reverse reaction rates, the corresponding thermodynamic flow is defined to be the velocity of reaction

$$v = R_f - R_r = K_f[A][B] - k_r[X] \quad (6)$$

The rate of entropy production per unit volume, σ , is then given by⁵⁻⁷:

$$\sigma = \frac{d_i s}{dt} \equiv \frac{A}{T} v \quad (7)$$

In a system that has many reactions indexed by k :

$$\sigma \equiv \frac{d_i s}{dt} = \sum_k \frac{A_k}{T} v_k \quad (8)$$

If the reaction mechanism of every reaction k is known as a sequence of elementary steps with forward rate R_{fk} and reverse rate R_{rk} , then it can be shown that⁷:

$$\sigma = R \sum_k (R_{fk} - R_{rk}) \ln(R_{fk}/R_{rk}) \quad (9)$$

in which R is the universal gas constant. With this formalism we can compute the rate of entropy production in any nonequilibrium chemical system. In what follows we apply it to a model reaction that exhibits spontaneous chiral symmetry breaking, a well-known modification of the Frank model. We show that for this model, the nonequilibrium transition to asymmetric state is associated with a sharp change in behavior of the rate of entropy production σ .

METHODS

One of the early model reaction schemes to demonstrate how chiral asymmetry could arise spontaneously as a result of chiral autocatalysis was devised by Frank.⁸ There are several other variants of this model that have been discussed during the last decades,⁹⁻¹⁴ but thermodynamic properties such as entropy production were not ana-

lyzed in this work. These models and their properties have been reviewed by Bonner¹⁵ and Plasson et al.¹⁶

A modification of Frank's basic model⁸ which enables the study of this reaction scheme in the context of symmetry breaking transitions in nonequilibrium transitions was introduced by Kondepudi and Nelson.^{17,18} This model considers a system with an inflow and an outflow, (Fig. 1) and an autocatalytic chiral product X. The reaction scheme is the following:

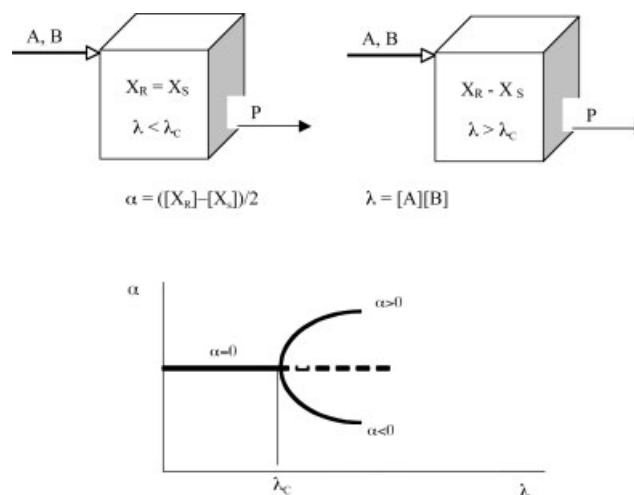


Fig. 1. Spontaneous breaking of chiral symmetry in a far from equilibrium chemical system whose reaction steps are shown in (9)–(14). The upper figure shows an open system in which there is an inflow of the reactants A and B and an outflow of the product P. The inflow and outflow keep the system in a nonequilibrium state. These flows can maintain the concentrations of A and B at any given value. The parameter $\lambda = [A][B]$ represents the nonequilibrium constraint the system is under. The transition to asymmetric states is described using the variable $\alpha = ([X_R] - [X_S])/2$. The graph of α versus λ shows the symmetry breaking transition.

Reactants A and B flow into the system and react to form chiral species X, in one of the two enantiomeric forms X_R or X_S (reactions 10 and 12). The species X is assumed to be chirally autocatalytic (reactions 11 and 13), and the two enantiomers react to form an inactive product P. In the model, it is assumed that P is “removed” from the system through a suitable outflow; this is equivalent to the assumption that X_R and X_S can react to form compounds that play no further reactive role in the mechanism. The above reaction scheme is symmetric in terms of the interchange of the two enantiomers. To investigate the behavior of the system close to and far from thermodynamic equilibrium, all the reactions in the above scheme are essential. For the purposes of calculating the rate of entropy production, small parity violating differences in the energies and reaction rates due to electro-weak interactions are ignored.

We assume each of the reactions (10)–(14) is an elementary step and write the reaction rates as follows:

$$R_{1f} = k_{1f}[A][B] \quad R_{1r} = k_{1r}[X_R] \quad (15)$$

$$R_{2f} = k_{2f}[X_R][A][B] \quad R_{2r} = k_{2r}[X_R]^2 \quad (16)$$

$$R_{3f} = k_{3f}[A][B] \quad R_{3r} = k_{3r}[X_S] \quad (17)$$

$$R_{4f} = k_{4f}[X_S][A][B] \quad R_{4r} = k_{4r}[X_S]^2 \quad (18)$$

$$R_{5f} = k_{5f}[X_R][X_S] \quad R_{5r} = k_{5r}[P] \quad (19)$$

For the rate constants, chiral symmetry requires $k_{1f} = k_{3f}$, $k_{1r} = k_{3r}$, etc. We also assume the flow can maintain the concentrations of A, B, and P at a fixed value. With these assumptions, the rate equations for $[X_R]$ and $[X_S]$ can be written as follows:

$$\frac{d[X_R]}{dt} = R_{1f} - R_{1r} + R_{2f} - R_{2r} - R_{5f} + R_{5r} \quad (20)$$

$$\frac{d[X_S]}{dt} = R_{3f} - R_{3r} + R_{4f} - R_{4r} - R_{5f} + R_{5r} \quad (21)$$

In this set of equations, $[A]$, $[B]$, and $[P]$ are parameters. A detailed theoretical analysis of this set of equations gives the following results.^{17,18} When the flow rates are zero, the system evolves to the equilibrium state in which the amount of X_R equals the amount of X_S , a chirally symmetric state. The system can be driven away from thermodynamic equilibrium by the inflow of A and B and an outflow of P. In the mathematical analysis of such a system, it is convenient to represent the constraint imposed by the flow with a parameter $\lambda = [A][B]$. As λ increases, the system is forced away from equilibrium. When λ exceeds a critical value, λ_C , the symmetric state becomes unstable and it makes a transition to an asymmetric state in which $[X_R] \neq [X_S]$, breaking the chiral symmetry of the chemistry DOI 10.1002/chir

cal reactions. For the analysis of this transition from a symmetric state to an asymmetric state, both the direct production (reactions 10 and 12) and the autocatalytic production (reactions 11 and 13) are essential. The critical point λ_C of the transition is given by^{17,18}:

$$\lambda_C = \frac{s + \sqrt{s^2 - 4k_{2f}^2 k_{1r}^2}}{2k_{2f}^2} \quad \text{in which} \quad s = 2k_{2f}k_{1r} + \frac{4k_{2r}^2 k_{1r}}{k_3 - k_{2r}} \quad (22)$$

This expression shows that it is important to include the inverse reaction of 10 and 12. If it is ignored by setting $k_{1r} = 0$, the critical point $\lambda_C = 0$; i.e., if this reaction is ignored, the system is always above the critical point, since $\lambda = [A][B] > 0$. In this case, one would not be able to analyze the system's behavior as it makes a transition from symmetric state, $\lambda < \lambda_C$ to an asymmetric state $\lambda > \lambda_C$.

A general quantitative theory could be developed for such transitions using group theory.¹⁷ The asymmetry could be quantified using the variable $\alpha = ([X_R] - [X_S])/2$. In the vicinity of the critical value λ_C , the time evolution of α can be described by a general equation of the form as follows:

$$\frac{d\alpha}{dt} = -U\alpha^3 + V(\lambda - \lambda_C)\alpha \quad (23)$$

in which the coefficients U and V depend on the chemical kinetic constants. Beyond the transition point, i.e. when $\lambda > \lambda_C$, the system will make a transition to a state in which $\alpha > 0$ or to a state in which $\alpha < 0$. In one state the amount of R-enantiomer is larger, in the other the amount of S-enantiomer is larger. In the absence of any chiral influences that favor one of these two states, both states, $\alpha > 0$ and $\alpha < 0$, will arise with equal probability. Which one will be realized is left entirely to chance. For a given value of λ , the value of α system will settle into is obtained by equating $d\alpha/dt$ in (23) to zero and solving for α , i.e., by the steady states of (23). These steady states are graphically represented in Figure 1. A detailed study of this system, including explicit expressions for the calculation of the coefficients U and V may be found in Ref. 17.

With this background about the model under consideration, we now turn to its thermodynamic aspects and chiral symmetry. The rate of entropy production per unit volume for the model can be calculated using the relation:

$$\sigma = R \sum_{k=1}^5 (R_{kf} - R_{kr}) \ln(R_{kf}/R_{kr}) \quad (24)$$

in which (15)–(19) are used for the reaction rates R_{kf} and R_{kr} . It is not possible to find an analytic solution for the system of coupled equations (20) and (21). However numerical solutions could be obtained using Mathematica numerical differential equation solving routines. The solutions generate the concentrations $[X_R]$ and $[X_S]$ as functions of time. Using these, the rates R_{kf} and R_{kr} can be

obtained as a function of time, and subsequently σ as a function of time can be calculated using (24).

RESULTS

In the numerical solutions, though the units are essentially arbitrary, the units of time can be assumed to be seconds and that of concentrations to be millimolar. The following numerical values were used for the computation of σ . The value of $\lambda = [A][B]$ was in the range of 0.001–0.009, and it was assumed $[A] = [B]$. Rate constants: $k_{1f} = k_{3f} = 1.0$, $k_{1r} = k_{3r} = 0.1 \text{ s}^{-1}$, $k_{2f} = k_{4f} = 20$, $k_{2r} = k_{4r} = 0.02$ as required by chiral symmetry. For the reaction (19), $k_{5f} = 5.0$, $k_{5r} = 1.0 \times 10^{-12}$, and the concentration of $[P] = 1.0 \text{ M}$; these values ensure that the reverse rate is negligible. The initial values were: $[X_R] = 0.001$, $[X_S] = 0.0$ which gives a small advantage to the R-enantiomer. If $\lambda < \lambda_C$ this difference will decrease and eventually the system will evolve to a steady state in which $[X_R] = [X_S]$; if $\lambda > \lambda_C$ this difference will increase and eventually the system will evolve to a steady state in which $[X_R] \gg [X_S]$.

With the above numerical values it was found that for $\lambda < 0.005$, the system evolved to a symmetric state, $[X_R] = [X_S]$, which corresponds to $\alpha = ([X_R] - [X_S])/2 = 0$ in Figure 1 and equation (23). The time evolution of $[X_R]$, $[X_S]$, and σ for $\lambda = 0.004$ is shown in Figure 2. The value of σ reaches a steady state in about 30 units of time, and in about 100 units of time the concentrations of $[X_R]$ and $[X_S]$ converge and become equal.

A typical time evolution of the asymmetric state, when $\lambda = 0.008$, is shown in Figure 3. Because of its small initial excess, the R-enantiomer dominates and reaches a value of 3.00 while the S-enantiomer is suppressed to a very small value, 0.0005. The same figure also shows σ , which increase in step with the concentration of $[X_R]$. This increase in entropy production is due to the autocatalytic production of X_R , which drives the system to the asymmetric state. The steady states are reached in about 250 time units. Thus, for every value of λ there is a steady state value of σ , $[X_R]$, and $[X_S]$.

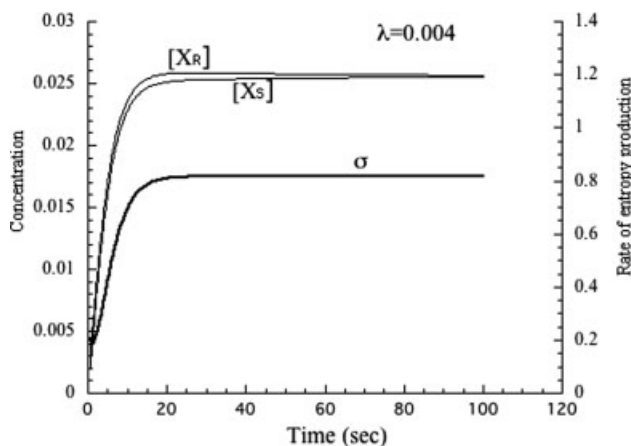


Fig. 2. Time variations of concentrations $[X_R]$ and $[X_S]$ (left axis) and rate of entropy production σ (right axis) when $\lambda = 0.004$. The units of time are seconds and concentration in millimolar.

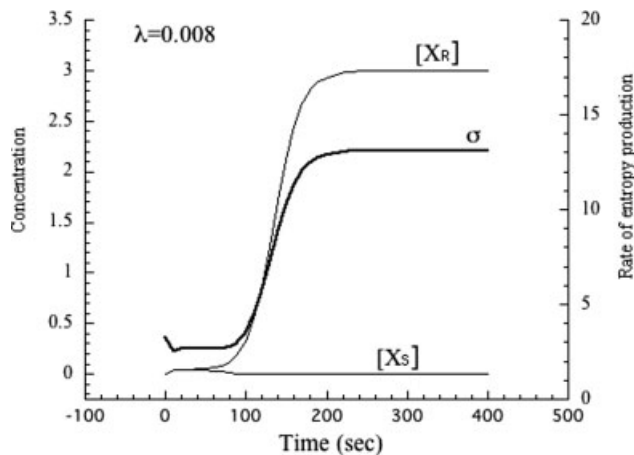


Fig. 3. Time variations of concentrations $[X_R]$ and $[X_S]$ (left axis) and rate of entropy production σ (right axis) when $\lambda = 0.008$. The units of time are seconds and concentration in millimolar.

The steady state of σ and $([X_R] - [X_S])$ as a function of λ are summarized in Figure 4. It clearly shows a sharp change in the slope of σ at the point where the transition to asymmetry begins, the point at which $([X_R] - [X_S])$ begins to deviate from zero.

DISCUSSION AND CONCLUSIONS

Spontaneous breaking of symmetry can occur both in equilibrium and nonequilibrium systems. In equilibrium systems it is associated with phase transitions. The associated changes in entropy are well established. For first-order transitions, molar entropy changes discontinuously. For second-order transitions, the slope of entropy changes discontinuously. Second-order phase transitions are generally symmetry-breaking transitions such as magnetization, in which the isotropy is broken by the direction of magnet-

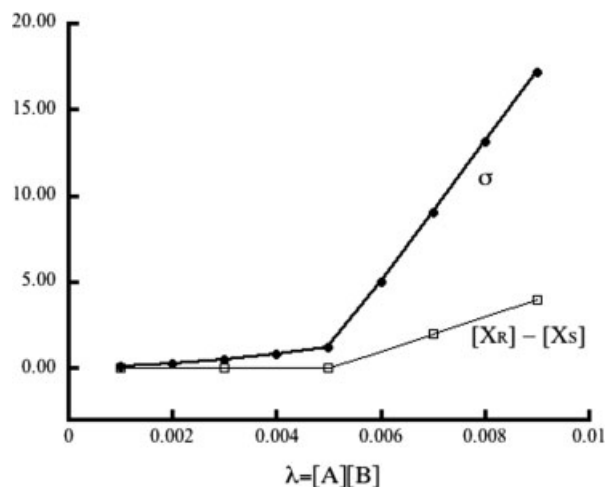


Fig. 4. The steady state rate of entropy production σ and the difference $([X_R] - [X_S])$ as a function of the parameter λ . The sharp change in slope indicates the onset of asymmetric states. The increase in σ is due to the autocatalytic reaction (11) and (13). The units of σ are $\text{JK}^{-1} \text{ m}^{-3}$.

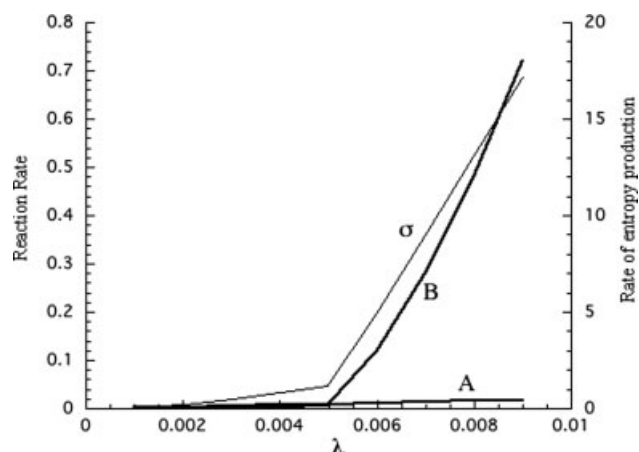


Fig. 5. The steady-state values of the rate of entropy production σ ($\text{JK}^{-1} \text{m}^{-3}$) and the rates of production of X (mM s^{-1}) as a function of the critical parameter λ . Curve A is the rate of production of X through the direct reactions (10) and (12). Curve B is the rate of production of X through autocatalytic reactions (11) and (13). The curves show a sharp increase in the rate of autocatalytic production of X at the onset of asymmetric state.

ization. If such a system is not isotropic but only has a two-fold symmetry, then the magnetization takes place on one of the two directions. It is similar to chiral symmetry breaking.

Nonequilibrium chemical system is characterized by the nonzero rate of entropy production, σ (at equilibrium $\sigma = 0$). Entropy production for several nonequilibrium systems have been investigated.^{19–21} Our investigations show that the onset of symmetry-breaking states reveals interesting features. As shown in Figure 4, when the steady-state entropy production is plotted as a function of a “critical parameter” λ , we see that the onset of asymmetry is accompanied by a sharp change in the slope of σ . The parameter λ is a nonequilibrium constraint on the system; it may be considered as a measure of how far the system is from thermodynamic equilibrium. Hence, as λ increases, the rate of entropy production, σ , increases but when $\lambda > \lambda_c$, the slope of σ changes.

The change in the slope of σ is essentially because of the increased autocatalytic reaction. When $\lambda < \lambda_c$, the rate of production of X is mainly due to direct reactions (10) and (12). When $\lambda > \lambda_c$ one of the autocatalytic reactions, (11) or (13), becomes dominant. This is clearly seen when the steady-state reaction rates of the direct production and the autocatalytic production are compared. This comparison is shown in Figure 5. The rate of autocatalytic reaction sharply increases beyond the critical point. The increased concentration of X_R increases the entropy production rates of all reactions in which it is involved such as the reverse reaction of (13).

The change in the slope also depends on the relative rates of these two reaction rates. In our simulations, the rate constants used were $k_{1f} = k_{3f} = 1.0$ and $k_{2f} = k_{4f} = 20.0$. When all concentrations equal one unit, then the autocatalytic reaction $A + B + X_R \rightarrow 2X_R$ is 20 times faster than the direct rate $A + B \rightarrow X_R$. At this stage it is not evident if all model reactions that exhibit chiral symmetry breaking will show the same behavior. This is an interesting aspect that requires further investigation.

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Review Article

Analysis of Efficacy of Chiral Adrenergic Agonists

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT The origin of terms, affinity, intrinsic activity (or efficacy) and spare receptors has been reviewed. The Easson–Stedman theory (1933) in relation to the activation of adrenoceptors by agonists proved to be useful in the analysis of affinity and efficacy. Eudismic ratios of agonists provided critical information about the receptor-mediated activation. The evidence from circular dichroism spectroscopy with a fluorescent-tagged adrenoceptor agonist indicates a stereoselective interaction with the receptor. Thus, the simplest definition of efficacy may include the rate of change of the specific conformation of the receptor by the agonist, leading to the organized response. The functional groups of the potent enantiomer are postulated to interact in a “preferred” sequence with the receptor. The 7TM GPCR protein crystal structure of bovine rhodopsin was used as a model to construct the agonist interacting amino acid residues for α_{1A} - and β_1 -adrenoceptors. It was observed that both $-NH_3^+$ group and chiral $-OH$ group of (–)-epinephrine interact with Asp¹⁰⁶ TM III of α_{1A} -adrenoceptor. Similar interactions were observed for (+)-epinephrine but critical differences were observed. Enantiomers of epinephrine and oxymetazoline were also docked in the position at β_1 -adrenoceptor to elucidate the conformational changes. Some unique information has emerged about the activation of adrenoceptors by agonists. The differences in the pharmacological efficacy of the enantiomers compare favorably with the dynamics of conformational changes by the agonist at α_{1A} - and β_1 adrenoceptors. *Chirality* 20:529–543, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: adrenergic agonist; ionization; efficacy; functions; enantiomers of catecholamines; imidazolines; binding sites; 7TM α - β adrenoceptors; initial events; activation sites; molecular modeling; conformations

HISTORICAL PERSPECTIVES ON EFFICACY

Nearly 100 yr ago, the sympathomimetic hormone from the adrenal gland was isolated and characterized as adrenaline or epinephrine.¹ Pure enantiomers were resolved.² Naturally occurring (–)-epinephrine, then called suprarenin, was a more potent pressor substance than the (+)-form.³ Around the same time, the concept of drug–receptors was introduced.⁴ The interest in the new synthetic structure–activity studies emerged. Structural molecular basis for differences in the pharmacological activity between enantiomers of epinephrine was simply explained by comparing the pressor activity of (+)-epinephrine with epinine. The latter symmetrical molecule without the benzylic hydroxyl group is pharmacologically equiactive to (+)-epinephrine. These facts led Easson and Stedman to postulate the importance of functional groups around an asymmetric center for the higher biologic activity of the (–)-epinephrine over the (+)-form.⁵ The validity of the steric structure–activity relationship in the Easson–Stedman hypothesis for the sympathomimetic class of substances has been investigated.⁶ On both α - or β -adrenoceptor-

containing preparations, the order of potency in terms of EC₅₀ of these catecholamines were (–)-enantiomer > (+)-enantiomer = desoxy catecholamine (Fig. 1). In organs with spare receptors the maximum effects at their highest concentrations of these agonists appeared equal. Particularly, in the vascular system after reduction of the spare α -adrenoceptors, the order of the maximum effect, an expression of efficacy was (–)-enantiomer > (+)-enantiomer = desoxy analogue. In other words, the enantiomers not only differed in affinity but also in intrinsic activity.

In part presented as a poster titled “Efficacy in relation to the physical–chemical properties and molecular chirality” at the first IUPHAR conference on receptor mechanisms: Principles of Agonism. July 23–25, 1998, Merano, Italy.

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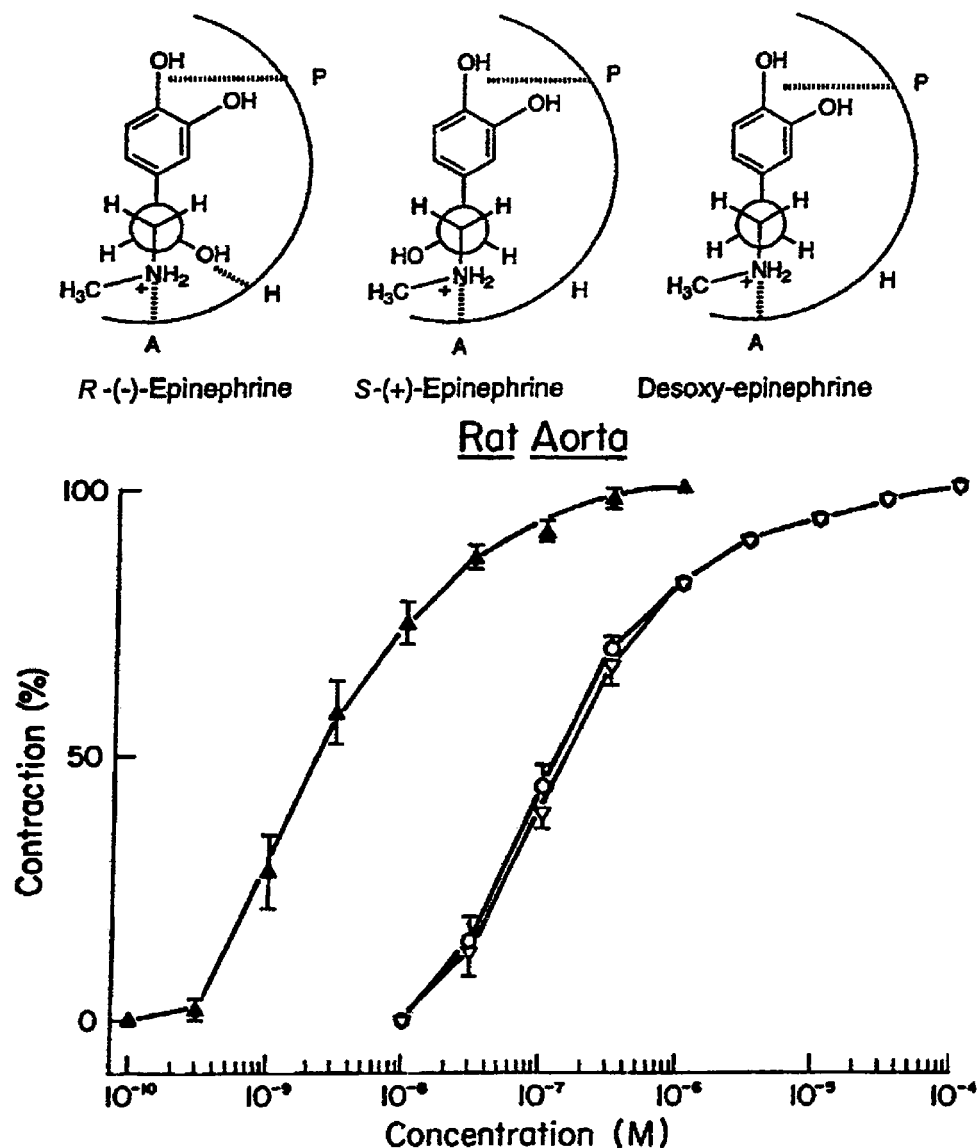


Fig. 1. Neuman projection of agonists. The Easson-Stedman hypothesis indicated that the high pharmacological activity of (-)-epinephrine was due to the sterically correct orientation of the benzylic OH with the receptor.⁹ In (+)- form or in desoxy epinephrine (Epine) such an interaction might be missing, resulting in low activity. The concentration response curve for rat aorta (α -adrenoceptor mediated response) is indicative of the greater higher activity of (-)-epinephrine (▲) and the low but equal potency of (+)-epinephrine (▽) and epine (○).^{7,8}

Ariëns⁹ investigated the concentration-response relationship of a series of agonists on isolated organs. The terms affinity and intrinsic activity were used to explain drug action at the receptors. The maximum response at the highest concentration was referred to as the intrinsic activity. In an important publication, Stephenson recognized the fact that only a small number of total receptors in the organ were required by a potent drug to achieve maximum response.¹⁰ The terms efficacy, spare receptors, and partial agonists were introduced. Intrinsic activity and efficacy are considered similar in their meaning. A molecule with low efficacy could interact with all receptors in the organ to produce a maximum response much below that produced by a highly potent agonist with low receptor occupancy. Such a drug is referred as a partial agonist.

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Thus, the EC_{50} of the partial agonist may represent the affinity parameter because the drug-receptor saturation kinetics can occur. But the EC_{50} of the highly potent molecule in the organ with spare receptors does not represent the affinity parameter because saturation of receptors at the maximum response does not occur.¹¹ Furchgott¹² inactivated a certain population of the receptors in an organ with various concentrations of the irreversible blocker dibenamine and observed a rectangular hyperbola of the agonist concentration-response curves. Subsequently, a unique method to calculate affinity and relative efficacies of agonists in organs with spare receptors was introduced.¹³ The organ is treated with an irreversible blocker in such a way that the "fraction" of available receptors can be activated to produce a reduced concentration response

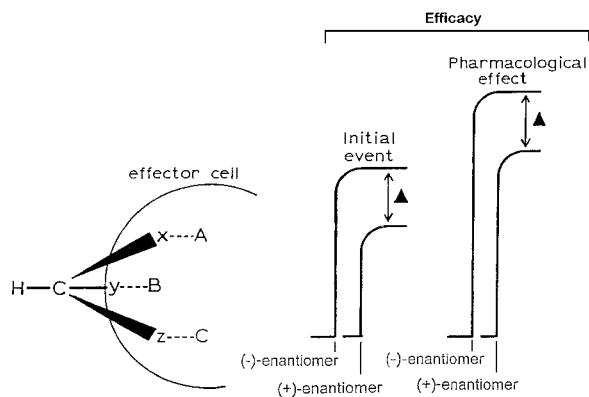


Fig. 2. Differential initial events and the subsequent mechanical pharmacologic effects produced by interaction of enantiomeric agonist with the hypothetical sites of the receptor. If both enantiomers activate two of the three interactive sites, then initial event or efficacy as $\blacktriangle$ = $\blacktriangle$ pharmacological effect can be postulated. On the isolated organs the efficacy is measured as the functional pharmacologic effect.^{16,17}

for the so-called potent agonist. The saturation of the limited capacity of free receptors is possible and EC_{50} under these conditions represents the affinity of the drug for the receptor. The relative intrinsic efficacy can be calculated. The absolute intrinsic efficacy of a single agonist cannot be expressed in thermodynamic units. No such method is available so far. Differences in relative maximum effects of (–) and (+)-enantiomers observed in the concentration response curves generally represent differences in efficacy at the receptors.

Colquhoun¹⁴ has carefully analyzed the problem of “affinity” and “efficacy” of agonists for the ion channel receptor. Accordingly the affinity constants determined in ligand binding experiments do not measure the receptor-related conformational changes that are intrinsically linked to the efficacy. He defines efficacy as simply the set of all the other microscopic equilibrium (or rate) constants, which describe all the transduction events that follow the initial binding reactions of receptor activation by the agonist. Such a definition encompasses all aspects of the transduction process. But, the molecular evolution of receptors indicates that the initial conformational change of the receptor by the agonist appears to be the most crucial one.

Thus, efficacy can be significantly related to the rate of change of specific conformation of the receptor and coupled cellular processes by the agonist in the physiologic milieu. A detailed understanding of the molecular events, as well as quantitative changes of receptor conformation by the agonist, is yet to be accomplished. Efficacy differences between enantiomers of epinephrine in all probability originate from nonlinear global conformational changes at the receptor. The signal is then coupled for the enlarged mechanical response via several second messengers collectively referred to as the transducer.¹⁵ Interdependence of second messengers is spatially and kinetically complex. A simplified concept for the drug–receptor-related efficacy of enantiomers is illustrated in Figure 2. It is assumed that both enantiomers interact with the similar sites of the receptor. The enantiomeric activity ratios (or

Eudismic ratios) provide an important parameter to discriminate adrenoceptor subtypes in various organs.^{18–20} Snail neurons were more sensitive to dopamine, than either enantiomers of norepinephrine.²¹ Dopamine-sensitive adenylate cyclase in mammalian neurons provided evidence for the dopamine receptor in substantia nigra.²² (–)-Methoxamine is a potent α -adrenoceptor activator but blocks the β -adrenoceptor. Dobutamine enantiomers provide unusual pattern in the activation of the adrenoceptor.²³ Thus, in many cases, prediction of affinity and efficacy of catecholamine derivatives has been a difficult task. Therefore, steric structure–activity studies in the search of potent agonists remained empirical.

Stereoselective changes in the circular dichroism spectra of rat lung containing β -adrenoceptor protein provoked by enantiomers of epinephrine has been observed. Pharmacologically active (–)-epinephrine produced specific changes in the 205–220-nm region of the spectra indicating that the receptor helices may be perturbed.¹⁶ The fluorescent tagged β -adrenoceptor also exhibits stereoselective changes with the enantiomers of isoproterenol.²⁴ The receptor-preferred conformation of the agonist, as well as resting conformation of the membrane receptor, are vital for the optimum efficacy. Over-expression of β_1 -adrenoceptors in the heart of mouse by genetic engineering produces higher resting heart rate. It was concluded that the receptor spontaneously isomerized to the activated state without the agonist.^{25,26} Such an abnormal base line cannot be used to compare the optimum efficacies of two agonists. Certainly a pathological condition is expressed.

MOLECULAR SPECIES IN ACTIVATION OF THE RECEPTOR

In addition to chirality, the initial events in drug–receptor interaction are governed by many factors. Ganellin²⁷ provided the most important insight of catecholamines, which can exist in four ionic species. Epinephrine has two pK_a values, one for the catechol (8.39) and the other for the amino group (9.85). At pH 7.4 the majority ($\geq 95.1\%$) of the amine exists as charged amino cation which may be responsible for the activation of the α -adrenoceptor.²⁸ The concentration of the zwitterionic species is about 3%. Is the latter species responsible for the interaction with the β -adrenoceptor? (see Fig. 3). It is important to note that the amino group of sympathomimetic drugs is not absolutely essential for the activation of the β -adrenoceptor. Kaumann et al.²⁹ observed the activation of the β -adrenoceptor in heart cells by 3'-4'-dihydroxy-2-methylpropiphenone, an agonist without nitrogen. The efficacy was about half that of the potent agonist (–)-isoproterenol. Earlier, Larsen suggested a formation of the quinone methide species of catecholamines for the activation of α and β -adrenoceptors.³⁰ The interesting speculations are difficult to confirm.

Electron withdrawing fluorine (F) substitution at the 2 and 6 positions of the phenyl ring of norepinephrine significantly altered the receptor activating properties of the molecule. 6-F-($\pm$)norepinephrine maintained high po-

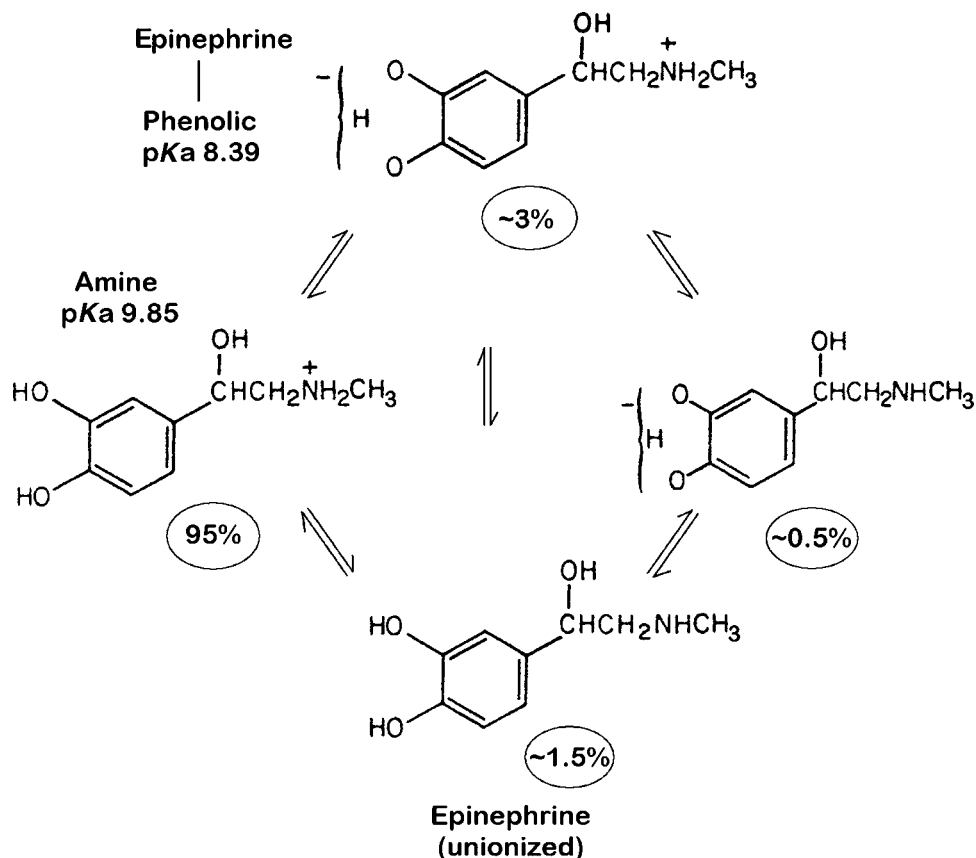


Fig. 3. Epinephrine with two pK_a values can exist in four different species in solution at the physiologic pH. Data represented from the report by Ganellin.²⁷

tency at α -adrenoceptor with little β -adrenoceptor activating property. 2-F-($\pm$)norepinephrine maintained the β -adrenoceptor activity with little α -adrenoceptor activity. The fluoro substitution alters the ionization characteristics of the meta- and parahydroxyl groups of the catecholamine, norepinephrine. This latter agonist is equally potent in activating α - and β -adrenoceptors. Liposolubility is increased by the fluorine substitution and it also uncovers the importance of ionization of specific catechol groups in differentiating the efficacy at the adrenoceptor.³¹ Enantiomers of 6-F, and 2-F-($\pm$)norepinephrine were resolved and adrenoceptor binding assays of enantiomers with α_1 - α_2 , β_1 and β_2 adrenoceptors were performed. Depending on the position of F-substitution, *R*-form had a marked effect in promoting adrenoceptor affinity. The effects of F-substitution for the *S*-forms were less predictable.³² Catecholimidazolines and desoxy catecholimidazolines were substituted with F at the 2, 5, and 6 positions of the catechol ring were investigated. Either in vivo or in vitro, the vascular activity mediated by α -adrenoceptors was significantly increased in 5-F-catecholimidazoline when compared with other substitutions. Because catecholimidazolines are poor activators of β -adrenoceptor, the F-substitution provided inconclusive improvements of the efficacy at the cardiac receptor.³³⁻³⁵ The efficacy predictions from the F substitution of norepinephrine cannot be

extended to the catecholimidazolines containing apparently similar functional groups.

Liposolubility, ionization, and solution conformation of catecholamines and imidazoline sympathomimetic drugs provided the most valuable information about the activation of the receptor in the biophase. The $\log P_c$ values of oxymetazoline, naphazoline, tetrahydrozoline, and tolazoline are 4.8, 3.8, 3.5, and 2.6, respectively. These imidazolines are highly liposoluble as compared with water soluble catecholamines with $\log P_c \leq 1$. Chemical structures of catecholamines and some imidazoline type of agonists are presented in Figure 4. The imidazolines will diffuse to the membrane receptor biophase much faster than a catecholamine, like norepinephrine. It is proposed that one of the charged nitrogens of the imidazoline ring interacts with a specific site on the α -adrenoceptor. Which of the two nitrogens is preferred by the site at the receptor? The conformational flexibility of the imidazoline ring is such that there may be equal probability of either nitrogen for the interaction with the receptor site. The aromatic functional group of imidazolines appear to be a sequentially secondary site for the activation of the α -adrenoceptor. Thus, for the initial activation of the α -adrenoceptor by tolazoline-like molecules, a two-site interaction is expected. In all probability, a charged nitrogen interacts first with the receptor, and this initial interaction flips the aromatic group

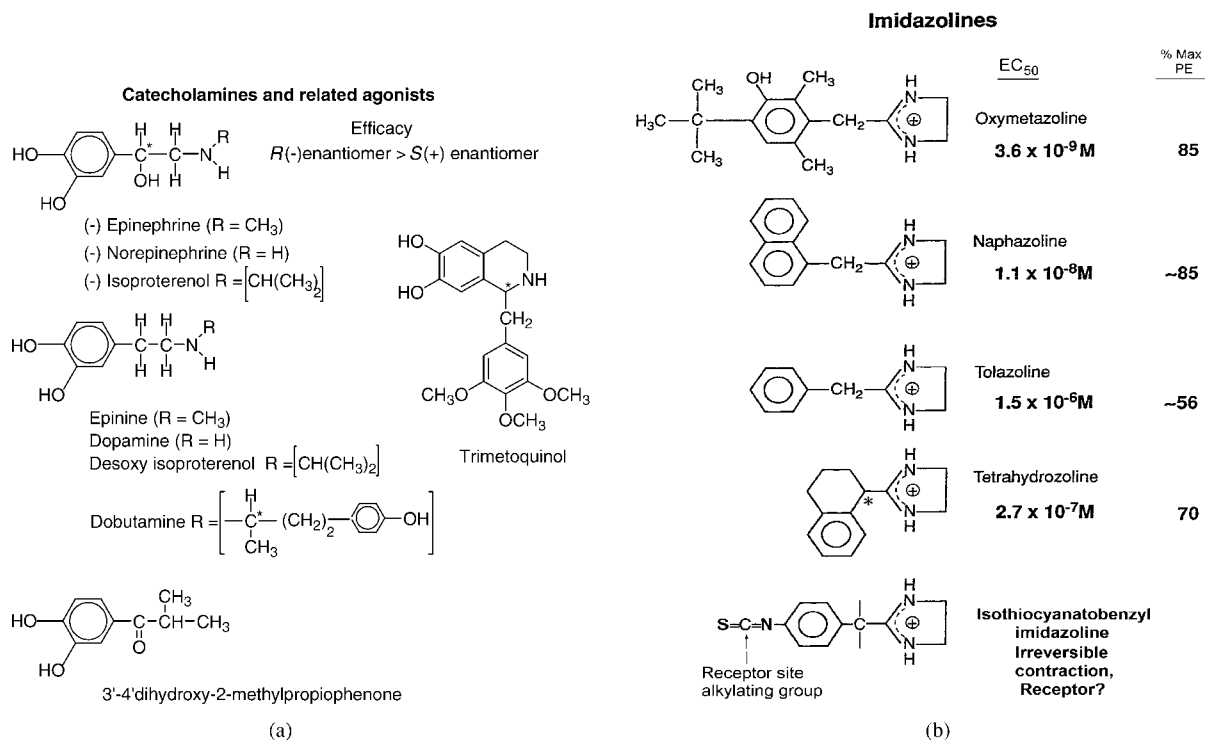


Fig. 4. (a, b) Representative chemical structures of catecholamines and imidazoles and related molecules. Chirality indicated by *. Note the partial agonist activity of imidazoles relative to phenylephrine (PE) on the vascular system.³⁶ Isothiocyanatobenzyl imidazoline (SCN-tolazoline) appears as an "irreversible agonist" on the vascular system.

of the agonist to the second site. The partial agonist activity³⁶ may be related to partial conformational change of the receptor due to interaction "hesitancy" of a nitrogen of the imidazoline ring. Based on steric-structure activity studies with catecholamines and imidazoles, it is postulated that the sequence of functional groups of (-)-norepinephrine for interaction with the α -adrenoceptor appears as a charged amino group first, catechol-phenyl second, and benzylic hydroxyl the last to produce the maximum effective conformational change for high efficacy.^{37,38} The low efficacy of (+)-norepinephrine and dopamine may be due to the lack of the interaction of the benzylic hydroxyl with the receptor. It must be emphasized that the priority of sequence of group designation around asymmetric center of catecholamines by the well-known Cahn, Ingold, and Prelog rule may not follow the group sequence for the activation of the receptor by a given series of agonists.

In elucidating the potency of catecholimidazoles, Miller et al.³⁹ observed the most fascinating relative order of potency of agonists for the activation of α -adrenoceptor: (-)-catecholimidazoline = desoxycatecholimidazoline > (+) - catecholimidazoline. The molecular geometry for activation of the α -adrenoceptor was defined. The pattern of the activation of the receptor by imidazoles was different than that of catecholamines. In catecholamidines (CA) where two free NH_2 groups are linked to the carbon, the rank order of potency for the activation of the α -adrenoceptor was desoxy CA > (-) CA > (+) CA.⁷ The benzylic hydroxyl group is of little value in the efficacy (Fig. 5). As

compared with catecholamines, catecholimidazoles are very weak ($\text{EC}_{50} > 100 \mu\text{M}$) activators of β -adrenoceptor. This property also extends to other noncatechol imidazoles. On the basis of these observations a clear structural distinction between catecholamines and imidazoles is evident in activating α and β adrenoceptors.

Thermodynamic analysis of drug-receptor interactions provided some insight into the efficacy parameter. Large efficacy differences in the mechanical and pharmacological effects of enantiomers of epinephrine indicate that the higher efficacy of (-)-form related to the enthalpy parameter associated with the benzylic hydroxyl group must be highly efficient. The benzylic hydroxyl group of the (-)-enantiomer appears to act like an arrow from the bow in search of the correct target site on the receptor, so that specific conformational change occurs. To maintain the evolutionary efficiency, the energy utilization for the interaction must be small. Rice et al. calculated Gibb's free energy ΔG° from the dissociation constants, the values -8.1 and -6.2 kcal/mol for (-)- and (+)-enantiomer of epinephrine, respectively.⁴⁰ This indicates that interactions of enantiomers is largely entropy driven, there is a small contribution from enthalpy.

EFFICACY, DESENSITIZATION AND CROSS-DESENSITIZATION

Repeated application of a sympathomimetic agonist at short intervals reduces its pharmacological effects. The

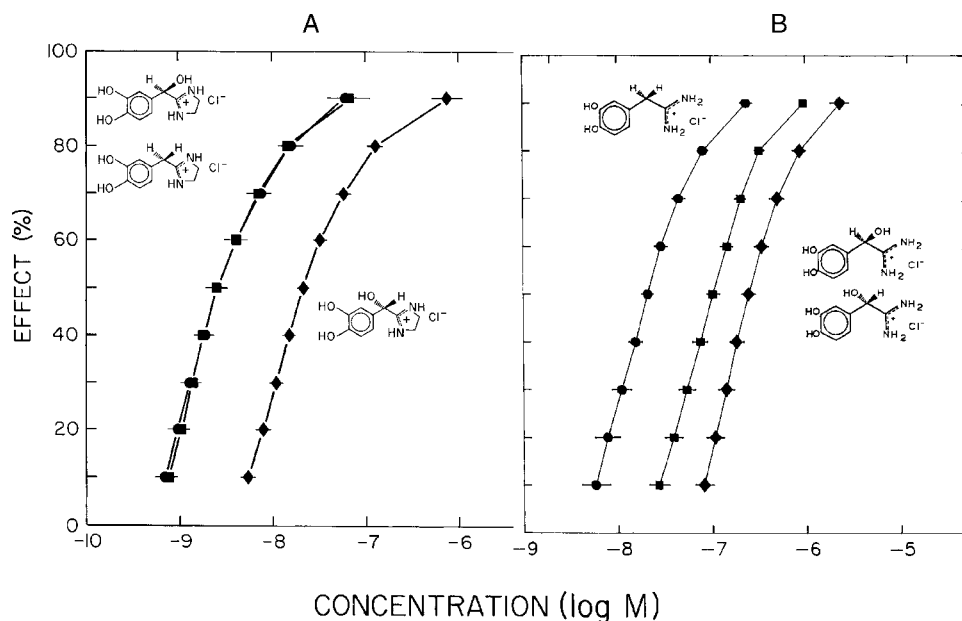


Fig. 5. Concentration response curves of catecholimidazoles-5A and catecholamidines-5B their desoxy analogs obtained on rat aorta containing α -adrenoceptor. The rank order of activity is desoxy form (■) = (-) enantiomer (●) > (+) enantiomer (◆). In catecholamidines (CA) series 5B, the rank order of potency is desoxy CA (●) > (-) CA (■) > (+) CA (◆).⁷ In both types of molecules the pattern of α -adrenoceptor mediated activity differs from that of the Easson-Stedman postulate.

receptor-related mechanisms are not entirely clear. Receptor conformation, second messenger and/or related regulatory ionic mechanisms may be altered. Biophase drug disposition mechanisms may also be altered. On the isolated rat vas deferens, oxymetazoline, (10 μ M) application at 20-min intervals, almost abolished the α -adrenoceptor-mediated contractile response by the seventh application. During the desensitized state, the contractile response to other α -adrenoceptor agonists like (-)-phenylephrine, (-)- and (+)-norepinephrine, dopamine, epinine, ($\pm$)-methoxamine, and ($\pm$)- α -methyl norepinephrine is preserved.²³ The contractile response to other imidazolines was abolished during the oxymetazoline desensitized state. (see Fig. 6). This interesting, puzzling observation indicates dual possibilities for the control of efficacy of two types of sympathomimetic substances. The receptor system desensitized to oxymetazoline can be activated by (-)-norepinephrine by interaction with different sites of the receptor. Two types of agonists may activate different subtypes of adrenoceptors. Both molecules may activate the same type of receptor, which may connect to different G-protein transduction pathways for the expression of pharmacological efficacy.

Miller and associates⁴¹⁻⁴³ synthesized a fascinating affinity label SCN-substituted tolazoline. At a narrow range of relatively high concentrations, the affinity reagent produces long lasting vascular contraction. A classical α -adrenoceptor blocker does not reduce the response significantly. On guinea pigs, stomach smooth muscle SCN-tolazoline will also produce a contractile response. The tissue, however, develops both self-blockade and cross-blockade to other agonists. We refer to such a molecule as "an irreversible agonist" for which the receptor, as well as the

mechanism of induction of efficacy is not settled. It was assumed that efficacy of all classical agonists was related to the rapid association and dissociation of the agonist with the specific receptor. The SCN-substituted imidazoline presumably does not easily dissociate from the receptor because the SCN-group interacts irreversibly. It must also be emphasized that total stimulus output after activation of unknown cellular receptor mechanisms may produce the contractile response by several mechanisms. Furthermore, repeated doses of oxymetazoline produced an

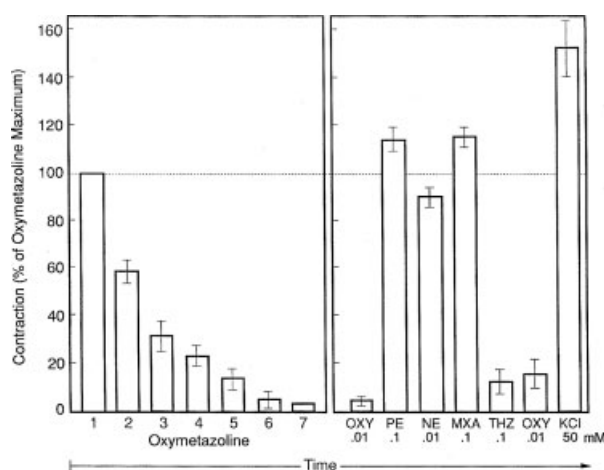


Fig. 6. Rat vas deferens desensitized to the repeated doses of oxymetazoline (oxy) (10 μ M) at 20-min intervals maintains its response to other α -adrenoceptor agonists like phenylephrine (PE), norepinephrine (NE), and methoxamine (MXA). The response to KCl maintained whereas that of tetrahydrozoline (THZ) is abolished (redrawn from Ref. 23).

expected reduced vascular response. During this phase of desensitization, SCN-substituted tolazoline maintained its normal contractile efficacy. The mechanism of efficacy of the two types of molecules must be different (Patil, P.N. unpublished report).

ENANTIOMERIC AGONIST COMBINATIONS IN RELATION TO EFFICACY

Racemates contain equal amounts of each enantiomer. Thus, as compared with the biologic activity of a single potent enantiomer, the activity of the racemate will be half. If the other enantiomer is "inert" nothing could be stated about its efficacy. There are a few examples in which the so-called "inactive" form may be "inert."⁴⁴ For several reasons, it appears doubtful if (+)-cobefrin (erythro α -methyl norepinephrine) is active at all. Its apparent activity may be totally due to contamination with the active form. The weak vascular α adrenergic activators (–)- and (+)-isoproterenol have nearly equal EC_{50} values but different maximal effects. Desoxyisoproterenol behaves similar to (+)-isoproterenol, confirming the predictions of the Easson-Stedman theory.⁴⁵ From the analysis of the dose–response curve, it is implied that both (–)- and (+)-isoproterenol must also interact with the receptor but with different efficacy.

The intrinsic activities of dobutamine isomers present the most fascinating variation in pairs of catecholamine series of drugs where the asymmetric center is at the N-substituted carbon.⁴⁶ The (–)-enantiomer is a potent α -adrenergic agonist and also a weaker β -adrenoceptor agonist when compared with the (+) enantiomer. The latter enantiomer, however, is a stronger β -adrenoceptor activator and lacks α -adrenoceptor agonist activity. But, it blocks α -adrenoceptor mediated activity of the (–)-form. Maximal inotropic effects of the racemate are greater than that of either enantiomer. The rationality based on the cardiac receptor studies in vitro indicates that a racemate may be better than a single enantiomer. These enantiomers provide an important tool to analyze receptor mediated complexities in efficacies.

PHARMACOLOGIC QUANTITATION OF EFFICACIES OF ENANTIOMERS

α -Adrenoceptors

An accurate comparison of the receptor-related efficacies of agonists after fractional inactivation of spare receptors by an irreversible blocker can be made on isolated tissues containing functionally intact systems.¹³ On the rabbit aorta, Kim et al.⁴⁷ determined the dissociation constants of (–)- and (+)-epinephrine for α -adrenoceptor activation as 0.3 and 10.8 μ M, respectively. The intrinsic efficacy of (+)-epinephrine relative to the potent enantiomer was 0.44. The low efficacy of the (+)-form or the desoxy form was confirmed in a receptor protection experiment. It is important to note that due to spare receptors, maximum contractions of both enantiomers appear equal in normal tissue. The latter observation can be misinterpreted as the

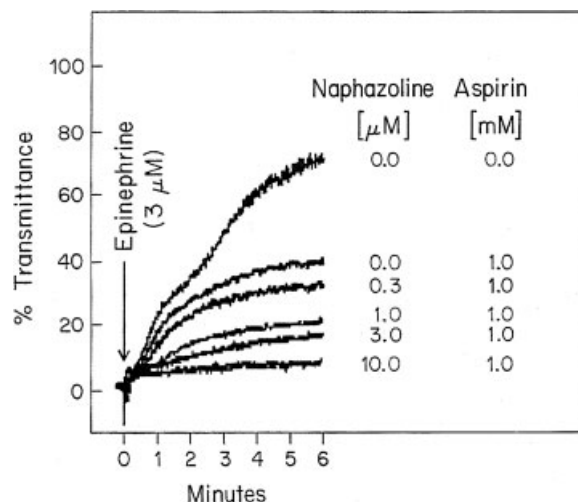


Fig. 7. Two phases of platelet aggregation by (–)-epinephrine. Inhibition of secondary phase by aspirin and the primary phase by naphazoline (data from Feller and coworkers³⁵ and personal communication, 1995).

enantiomers having equal intrinsic activity. Such an interpretation in relation to the conformational changes of receptors can be confusing. Rice et al.^{40,48} extended investigations about intrinsic activities of enantiomers of catecholamines various organs of rat. Expected differences in efficacy between epinephrine enantiomers were seen in spleen, portal vein, and aorta. The values were nearly equal in the vas deferens where spare receptor numbers, as well as receptor mediated coupling efficiency, appear to be very high.

Drugs, which are partial agonists in the normal functional organs, readily exhibit differences in the maximum response or efficacy. Enantiomers of tetrahydrozoline and hydroxytolazoline show marked differences in the α -adrenoceptor-mediated pharmacologic activity in blood vessels.^{49,50} Intrinsic efficacy differences must arise at the adrenoceptor. In comparing enantiomers for efficacies in organ systems with multiple receptors, there is no guarantee that both enantiomers will prefer only a single type of receptor. S(+)-hydroxytolazoline produces two types of contractile responses. Only one component is blocked by the α -adrenoceptor antagonist prazosin.⁵⁰

Feller and co-investigators^{35,51–54} completed the most comprehensive studies on α_2 -adrenoceptor-mediated platelet aggregation by a series of enantiomeric agonists. The primary and secondary phases of platelet aggregation are induced by the catecholamine or the catecholimidazoline. The second phase is prostaglandin-pathway dependent because it is selectively blocked by acetylsalicylic acid treatment. Naphazoline, which is a partial agonist on the vascular α_1 -adrenoceptor system, inhibits the primary phase of aggregation of epinephrine in the acetylsalicylic acid pre-treated platelets (Fig. 7). Adenosine diphosphate, thromboxane A_2 and serotonin-dependent pathways of platelet aggregation are also recognized. In platelets from some human donors, both enantiomers of norepinephrine produced concentration dependent aggregation, whereas

TABLE 1. Human platelet aggregatory and anti-aggregatory properties of enantiomers of catecholamines and their desoxy analogs^{52,63}

Catecholamine	EC ₅₀ ^a (μM)	K _B (μM)
(-)-Epinephrine	0.46	–
(+)-Epinephrine	18.2	NT
Epinephrine (desoxy epinephrine)	40.7	NT
(-)-Norepinephrine	1.3	–
(+)-Norepinephrine	19.1	54 ^b
Dopamine (desoxy norepinephrine)	22.4	25 ^b
(-)-Isoproterenol	– ^c	204
(+)-Isoproterenol	– ^c	309
Desoxy-isoproterenol	– ^c	73

n, 3–6; NT, not tested.

^aMaximal aggregation was equal for all drugs. K_B determined against (–)epinephrine-induced aggregation.

^bPlatelets from some donors do not exhibit aggregation⁵²; therefore, anti-aggregatory effects (K_B) were quantitated.

^cNot active at concentrations up to 1 mM.

the response to (+)-norepinephrine was absent in preparations obtained from other donors. The reason for the complete lack of aggregation or efficacy of a lesser active enantiomer is not known. Perhaps, the initial component, which triggers the stereoselective aggregation is missing. (+)-Norepinephrine, however, blocks the aggregatory effect of the (–)-norepinephrine. The blocking activity of the enantiomer measured as K_B of 54 μM is statistically equal to the EC₅₀ for the aggregatory effect of ~20 μM. Dopamine, which lacks the benzylic hydroxyl group, shows identical behavior to (+)-norepinephrine (Table 1). The pattern of the activity conforms to that of the Easson–Stedman postulate.

In the platelet preparation desoxycatecholimidazoline appears to be a partial agonist. In the vascular preparation, however, maximal effects, as well as EC₅₀ of the desoxycatecholimidazoline are equal to that of (–)-catecholimidazoline.³⁷ The vascular contractile response to the catechol derivative is not affected by acetylsalicylic acid treatment. The human platelet preparation appears to be the most useful system to elucidate the molecular mechanisms of efficacy so that specific antiplatelet aggregatory drugs for therapeutic benefit can be developed.

The maximal effects of (–) and (+)-epinephrine were investigated on rabbit aorta with functional antagonistic α- and β-adrenoceptors. The percent maximum contraction was equal for each form. The enantiomeric activity difference at EC₅₀ or ED₅₀ was 1.8 log units. When the vascular tone was induced by histamine, the maximal β-adrenoceptor-mediated relaxation of each enantiomer was ~50% of the initial contraction by the spasmogen. Because of the limited receptor and/or the functional reserve of the β-adrenoceptor, unequal efficacy related differences of the enantiomers were expected in the vascular system. The enantiomeric activity difference for the relaxation at EC₅₀ was 1.74 log units, close to 1.8 log units obtained for α-adrenoceptor-mediated contraction (Patil, unpublished data). Thus, elucidating the role of affinity and efficacy parameters in relation to the receptor mediated functional

control of the responses by enantiomeric substances still presents a challenge.

β-Adrenoceptors

Initial molecular events associated with the activation of the β-adrenoceptor by catecholamines are well studied.^{55–59} DeSantis et al.⁶⁰ measured the rise in cAMP after (–) and (+)-norepinephrine in fat cells. The enantiomeric activity difference for lipolysis measured at EC₅₀ was 300. The ratio for rise in second messenger cAMP was also 300. Similar co-relations were obtained for the activation of β-adrenoceptor by enantiomers of isoproterenol.^{57,59} These important quantitative observations indicated that initial activation of β-adrenoceptor-mediated efficacies of the enantiomers were stereoselective. No decrease in the maximum cardiac response to a β-adrenergic agonist is observed after intensive treatment with an irreversible β-adrenoceptor blocker.⁶¹ The rat heart contains a very high receptor reserve with an extremely efficient coupling mechanism. Even the residual cardiac receptors are capable of producing maximal effects of an agonist.

In well-designed experiments Birnbaum et al.⁶² measured the increase in atrial rate as well as cAMP rise after (–), or (+)-isoproterenol. The concentration–effect curve for the production of mechanical effects was at least 100-fold to the left of that for the production of cAMP. The chronotropic response was greatly amplified. At the EC₅₀, the enantiomeric differences for both parameters were ~2500 fold. The maximal chronotropic effects, as well as cAMP production, were also equal for the enantiomers. Because of spare receptors and/or related spare function, no differences in the efficacy for enantiomers were observed. In rat uteri, maximally stimulated levels of cAMP were 52, ± 3.8 and 40.5, ± 4.5 pmol/mg of protein by (–) and (+)-isoproterenol, respectively. The difference in the production of second messenger by the enantiomers in part reflects an unequal efficacy in the transduction cascade. Dithiothreitol, which selectively reduces sulfhydryl groups of the receptors presumably altering conformational changes of the β-adrenoceptor, clearly reduces atrial rate after (+)-norepinephrine over that of the (–)-norepinephrine.⁶⁰

Steric structure activity studies for the series of agonists for the β-adrenoceptor-mediated glycerol release from the rat fat cells indicated that (–)-isoproterenol was 300 times more potent than the (+)-isoproterenol. The intrinsic activity, as indicated by the maximal glycerol release by each enantiomer, was equal. Desoxyisoproterenol, however, had low intrinsic activity of 0.57. (–)-Tetrahydroisoquinoline was about 1000 times more potent than the (+)-form with intrinsic activity of 0.71.⁵⁵ In guinea pig tracheal smooth muscle, β-adrenoceptor mediated muscle relaxant activity of (–)-trimetoquinol was ~20% greater than the (+)-enantiomer. Maximum cyclic AMP produced by the latter enantiomer was nearly half that produced by the (–)-form. Both enantiomers, however, produced equal maximal rate accelerating effect on the guinea pig atria. The onset of action of these agonists was relatively slow for the functional response. As much as 10–15 min were

required to reach the equilibrium in the atria at a given concentration of the agonist. A 1000-fold concentration range of the agonist was needed to complete the dose-response curve in the heart. In spite of such technical difficulties, about 18% difference was observed in the maximal effect of the potent *S*(-)-tetrahydropapaveroline over that of the *R*(+)-form. In all probability, differences in the maximal effect reflect the differences in the intrinsic efficacy at the β -adrenoceptor.

SIGNIFICANCE OF STEREOSELECTIVE DRUG BINDING

The stereoselective quantitative pharmacologic effects of catecholamines on α and β -adrenoceptor in various organs were well defined. Even the intracellular increase in second messengers associated with the mechanical pharmacologic effect was well correlated with activation of the β -adrenoceptors. The adrenoceptor containing membranes from the organs were separated. The availability of high specific activity radiolabeled high affinity β -adrenoceptor antagonist provided a powerful tool to investigate the specific interactions of catecholamines in competition with the labeled blocker. Structurally related enantiomers of agonists and antagonists exhibited marked stereoselectivity in competition for the equilibrium binding with the labeled blocker.⁶⁴⁻⁶⁶ For the agonists the degree of stereoselectivity roughly paralleled to that for the rise in cAMP, the indicator of the pharmacologic response. The linear relationship between the agonist affinity in terms of binding in homogenates to that of the efficacy parameter in the organ remained a difficult task.

De Robertis and coworkers extracted α -adrenoceptors from bovine spleen. The proteolipids containing the receptor were incorporated into an artificial membrane. In this preparation, norepinephrine produced stereospecific dose-dependent membrane conductance changes and this effect was antagonized by the α -adrenoceptor blocker phentolamine. The concentrations of the agonist and antagonist to produce this effect were relatively high 10^{-5} – 10^{-4} M, well above EC_{50} of the agonist in the organ containing α -adrenoceptor.⁶⁷ A subcellular fraction from rat vas deferens was isolated, where labeled dihydroazapetine (an α -adrenoceptor blocker) exhibited saturable binding. The affinity constants of five different imidazolines showed excellent positive correlations to their α -adrenoceptor activating property in the isolated organ. Oxymetazoline was the most potent in the series. Paradoxically, the binding of (-)-methoxamine, (-)-norepinephrine and (-)-phenylephrine was increased over the (+)-enantiomers. Different modes of interaction of two types of agonists with the α -adrenoceptor were postulated.⁶⁸ These observations prompted us to design new pharmacologic experiments in the rat vas deferens. In an oxymetazoline-desensitized organ, catecholamines and methoxamine still maintained the contractile effect or the receptor-related efficacy. The mode of binding and/or related events of two types of agonists must be different.²³ When the competition of norepinephrine enantiomers and dopamine was investigated with labeled clonidine, an imidazoline type of agonist, or WB

4101, a benzodioxan analogue with α -adrenoceptor blocking properties, the inhibition of binding followed an interesting pattern. For the competition with the agonist clonidine the order of potency was (-)-norepinephrine > dopamine > (+)-norepinephrine, whereas that against WB 4101 was as predicted from the Easson-Stedman hypothesis where (-)-norepinephrine > (+)-norepinephrine = dopamine.⁶⁹ All these binding studies on membranes containing α -adrenoceptors indicate that relationship between the binding constant and efficacy of the agonist in intact organ system is a nonlinear complex process. The primary cellular event after activation includes increase in inositol phosphate and Ca^{++} which is needed for efficacy.⁷⁰

The receptor-interacting conformation of catecholamines was elucidated to understand the efficacy of potent agonists in the system. Conformationally, restricted *cis* and *trans*-2-(3,4-dihydroxyphenyl)-cyclobutylamines were synthesized to investigate the dopamine receptor-interacting conformation of the agonist. The *trans*-form had 20 times the affinity of the *cis*-form. The efficacy promoting functional groups of several adrenergic and dopaminergic agonists are in the *trans*-interactive conformation.²⁸

AGONIST SPECIFIC INTERACTING SITE AT THE ADRENOCEPTORS AND ITS EFFICACY IMPLICATIONS

Since 1948, Ahlquist's classification of α and β adrenoceptors is well accepted. On the basis of the relative pharmacologic potencies of agonists, β -adrenoceptor subtypes β_1 (heart) β_2 (lungs) were defined.⁷¹ The neuronal prejunctional α -adrenoceptor, which inhibits the release of the transmitter and the postjunctional receptor were subclassified as α_2 and α_1 -adrenoceptor subtypes, respectively.⁷² Between 1986 and 1994, Dixon, Strader, Lefkowitz and Perez et al., cloned and characterized the pharmacological properties of the major subtypes of adrenoceptors.⁷³

For the seven transmembrane domain activated receptors, many components of the cellular organization undergo synergistic interaction before a mechanical response is observed: G-proteins, extracellular and intracellular Ca^{++} , inositol triphosphate, cAMP, and prostaglandins to name a few. In homogenates or cloned receptors, the agonist-activated cascade of transduction is likely to be distorted to provide an accurate efficacy parameter of an agonist observed in an organ. Even in intact cells, in the absence of the synaptic transmitter cloned receptors may not acquire the same conformation as in intact vertebrate organs. These investigations, however elegant, should be viewed with caution in understanding efficacy of sympathomimetic amines. At a recent meeting Milligan reported that G-protein-coupled receptors may form dimers or oligomers which may regulate receptor function and related efficacy of agonists.⁷⁴ In this review, receptor monomer simplicity is maintained to explain efficacy.

Some time ago, Furchgott et al.⁷⁵ observed in vitro the vascular relaxation in response to light. The observation has been reinvestigated in relation to physiologic adapta-

tion. The superficial blood vessels in skin react to light with relaxation whereas deep muscle blood vessels do not. The mechanism of light activated transduction leading to vascular relaxation is unknown. The apparent similarity of the light sensitive 7TMG protein receptor of the eye and that of the vascular system response should not be ignored. The mechanisms of efficacy or alterations by photons in the two systems may be different.

All these observations in various organ systems provided the foundation for a new inquiry with better methods to understand transduction by agonists at receptor sites. Information from organ systems provides the best tool to guide additional studies.

β -Adrenoceptor

Strader et al.⁷⁶ elucidated the β -adrenoceptor agonist and the antagonist-interacting sites within the seven transmembrane G-protein linked architecture of the receptor. Site-directed mutagenesis experiments revealed that Asp¹¹³ of the transmembrane helix III is critical for interaction of the $^+\text{NH}_3$ cation of norepinephrine or other catecholamines. Asp⁷⁹ in helix II, if substituted with Ala in the β_2 -adrenoceptor, resulted in a decrease in affinity and efficacy without significant effect on the binding of antagonists. Ser²⁰⁴ and Ser²⁰⁷ in the transmembrane helix V are postulated to form hydrogen bonds with the catechol group of the agonist. If these Ser residues are substituted with Ala, the affinity of catecholamine agonists is decreased 10 fold with 50% decrease in efficacy. Phe²⁹⁰ in the VIth transmembrane helix presumably stabilized the aromatic ring of the catecholamine. Wieland et al.⁷⁷ recognized Asn²⁹³ of the TM VI as the probable site for the interaction of the benzylic OH group of the more potent enantiomer of the catecholamine. A fluorescein maleimide probe attached at Cys²⁶⁵ of the intracellular loop of the β_2 -adrenoceptor was used to investigate conformational changes after activation of the receptor by structurally related agonists.⁷⁸ It was deduced that (–)-norepinephrine causes:

1. Rapid changes in domain V due to interaction of the catechol groups with two of the three Ser^{203,204,207} residues.
2. The chiral OH then interacts with Asn²⁹³ of the domain VI.
3. The charged amino group ($-\text{NH}_3^+$) then interacts with the Asp¹¹³ of the domain III. This change appears to be slow.

According to the studies of Lohse⁷⁹ the second phase coincides with a conformational change of the receptor. Based on the steric structure-action studies on isolated organs, similar sequential activation of the β -adrenoceptor was proposed.³⁷

α -Adrenoceptor

Piascik and Perez⁸⁰ reviewed α -adrenoceptor interaction topography for the agonists. Ser¹⁸⁸ and Ser¹⁹² are involved in the interaction of the catechol group of the agonist. These investigators identified Phe³⁰⁸ and Phe³¹² of the

TM-VII domain, as the most important sites for α_1 -adrenoceptor blockers and partial agonist imidazolines.

Perez and co-investigators indicated the presence of a salt bridge constraint in the α_{1B} -adrenoceptor.^{81,82} At the critical site, the carboxyl group of Asp¹²⁵ of the III TM domain and the $^+\text{NH}_3$ cation of the Lys³³¹ of the VII TM domain form a bridge. Sympathomimetic activity of catecholamines or imidazolines results from disruption of the bridge by the charged nitrogen of the agonist. Depending on the molecular nature of the agonist, specific conformational change of the receptor presumably occurs after initial activation at this site. Second messenger changes also occur as the result of the disruption of the salt bridge.

The α_{2A} -adrenoceptor has a cysteine residue at position 201 (corresponding to position 203 on the β_2 -adrenoceptor). Molecular modeling and site-directed mutagenesis shows that this cysteine, as well as Ser²⁰⁰ and Ser²⁰⁴, plays an important role in the interaction of the catechol oxygens with TM helix V of the α_{2A} -adrenoceptor.^{83,84} The aromatic ring is postulated to interact with TM helix VI through Tyr³⁹⁴ and Phe³⁹¹ as well as TM III through Val.¹¹⁴ Consistent with this premise, the mutation of Tyr³⁹⁴ to Phe results in a 60-fold reduction in the affinity of (–)-norepinephrine (P. Hieble, unpublished data).

Benzylic OH Group

The site of interaction between the chiral benzylic hydroxyl group of catecholamines with α -adrenoceptors is not established. Ser¹⁶⁵ in TM IV was initially suggested as a favorable location for the potential stereoselective interaction between a hydroxyl group and the β_2 -adrenoceptor.⁷⁶ The investigators were unable to express the β_2 -adrenoceptor mutant where Ser¹⁶⁵ was replaced by alanine to test this hypothesis. However, subsequent studies showed this mutation to have no effect on the affinity of the β_2 -adrenoceptor for isoproterenol.⁷⁷ Similarly, this mutation had no effect on the affinity of the α_{2A} -adrenoceptor for norepinephrine or epinephrine.⁸⁵ Molecular modeling and/or site directed mutagenesis has suggested possible interaction with amino acids on TM II, TM VI, and TM VII.⁸⁴ Evaluation of the binding affinity of a series of agonists with the α_{2A} -adrenoceptor, combined with molecular modeling, suggests that the benzylic hydroxyl group interacts with the same aspartic acid Asp¹¹³. This interaction is postulated to occur via hydrogen bonding between a hydroxyl group and one of the side-chain oxygen atoms. Molecular modeling of the agonist-receptor complex shows that this interaction cannot occur for the (+)-enantiomer.

Molecular Modeling of Adrenoceptors

In our own modeling efforts, both homology models of α_{1A} and β_1 adrenoceptors were built with the MODELLER 9v1 program by using bovine rhodopsin (PDB ID 1F88) as the template. (–)-Epinephrine, (+)-epinephrine and oxymetazoline were docked into the agonist binding sites by using AutoDock v4 β .^{86,87} The Lamarckian genetic algorithm (LGA) for ligand conformational searching was selected because it has enhanced performance relative to

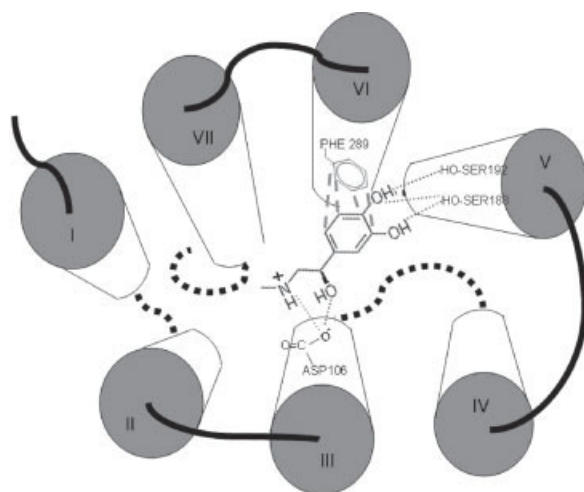
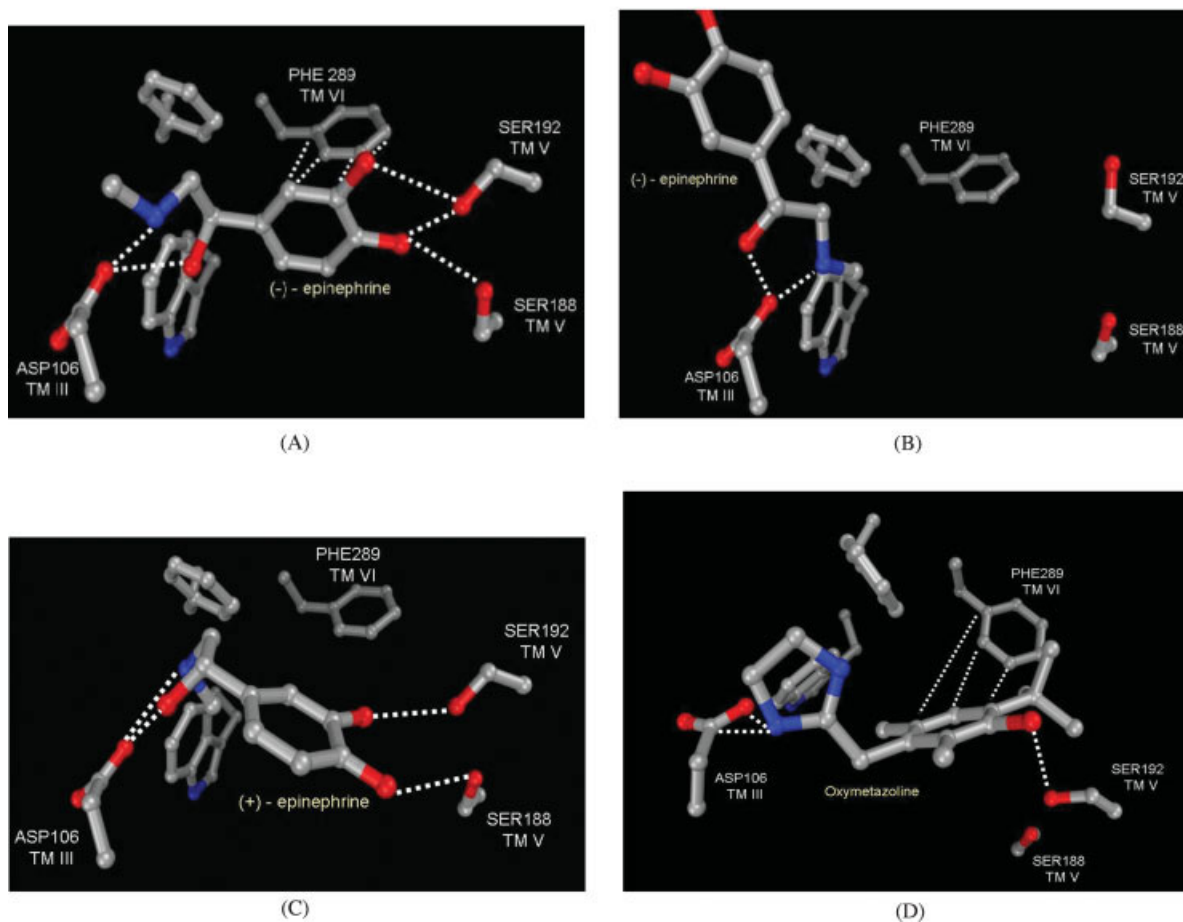
(-)Epinephrine docking at α_{1A} adrenoceptor

Fig. 8. Modeled binding modes of (-)-epinephrine, (+)-epinephrine and oxymetazoline to α_{1A} adrenoceptor. (A) Initial anchoring of (-)-epinephrine with Asp¹⁰⁶; (B, C, D) (-)-epinephrine, (+)-epinephrine, and oxymetazoline stable binding modes. Note in Figures 8b and 8c $-^+NH_3$ and chiral $-OH$ of the enantiomers interact with Asp¹⁰⁶ of the adrenoceptor. But in (+)-epinephrine, interaction with PHE²⁸⁹ of TM VI domain is lacking. The modeling of epinephrine remains to be examined.

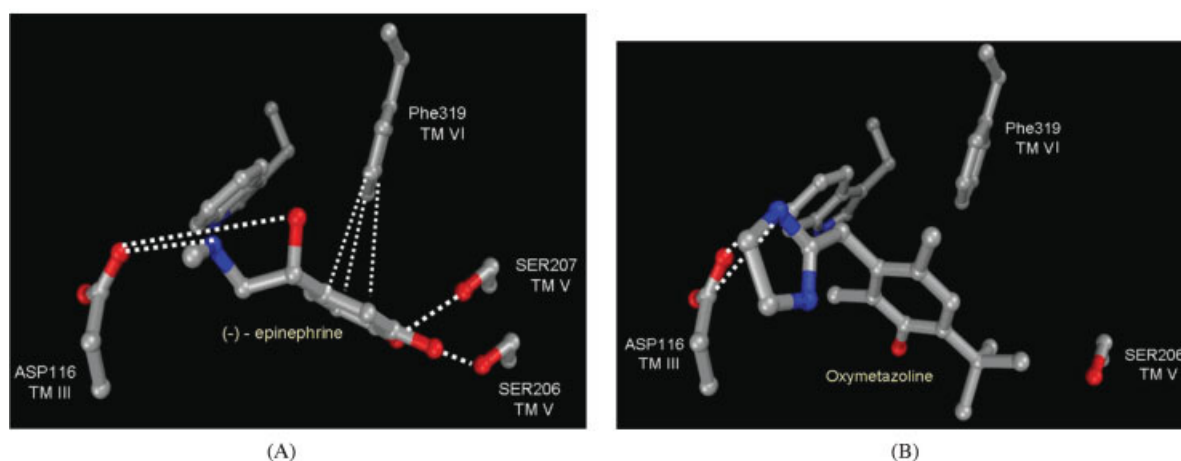
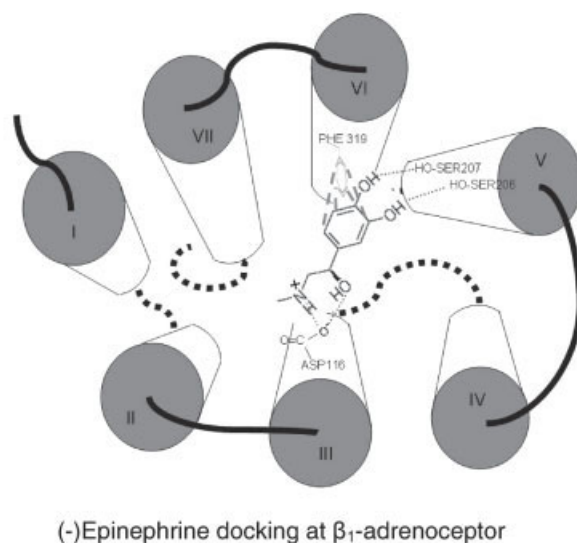


Fig. 9. Modeled binding modes of (-)-epinephrine (A) and oxymetazoline (B) to β_1 adrenoceptor. The imidazoline fails to interact significantly with the Ser residues to activate the receptor.

simulated annealing or the generic genetic algorithm. Besides using fully flexible, we allowed the side chains of ligand contacting residues to be flexible also, in order to simulate the binding process and the induced-fit effect. Amino acids in TM III, V and VI domains of α_{1A} and β_1 -adrenoceptor were numbered as for human protein receptors.

In the docking simulation of (-)-epinephrine to the α_{1A} adrenoceptor, two binding modes were observed. Figures 8 and 8A show the initial anchoring of (-)-epinephrine to the receptor by electrostatic and hydrogen bonding interactions between the positively charged amine group of (-)-epinephrine and Asp¹⁰⁶ at TM III. Figure 8B shows the final binding mode of (-)-epinephrine. Besides the well-preserved interactions between the amine of (-)-epinephrine and Asp¹⁰⁶, the para-hydroxyl group of (-)-epinephrine forms hydrogen bonds with both Ser¹⁸⁸ and Ser¹⁹² on TM V (three H-bonds). The phenyl ring of (-)-epinephrine has aromatic interactions with Phe²⁸⁹ and the Ser¹⁸⁸ hydroxyl side chain flips upon final binding. Surpris-

ingly, the chiral hydroxyl group appears to form hydrogen bond with Asp¹⁰⁶ also. So the binding starts with the amine anchoring to Asp¹⁰⁶ and the chiral hydroxyl group, and then proceeds to form hydrogen bonds with Ser¹⁸⁸ and Ser¹⁹² and an aromatic interaction with Phe²⁸⁹. From initial anchoring to final binding, the gain on free energy of binding is only 0.7 Kcal/mol. It appears that the final binding mode is to trigger the global conformational change of the receptor for activation, which is associated more with efficacy than affinity. Figure 8C shows the binding of (+)-epinephrine to the α_{1A} adrenoceptor. Compared to (-)-epinephrine, its binding energy is 1.3 Kcal/mol weaker, which is comparable to the experimental difference of 1.9 Kcal/mol. Even though the absolute binding of both agonists was proposed to be mainly entropy-driven, the difference here is mainly enthalpic because (+)-epinephrine loses the aromatic interaction with Phe²⁸⁹ and para-hydroxyl group forms one hydrogen bond with Ser¹⁹² (two H-bonds instead of three of the catechol groups). So (+)-epinephrine is weaker than (-)-epinephrine in both

affinity and efficacy. The modeling of epinine will be explored.

Figure 8D shows oxymetazoline binding to α_{1A} adrenoceptor. The imidazoline ring shows strong electrostatic and hydrogen bonding interactions with Asp¹⁰⁶. Aromatic and hydrophobic interactions play important roles in this case.

For the β_1 adrenoceptor, again both (–)-epinephrine and (+)-epinephrine bind similarly except the chiral hydroxyl group of the latter does not interact. This observation with (+)-epinephrine is in contrast to that for the α_{1A} -adrenoceptor. Figures 9 and 9A show the binding mode of (–)-epinephrine. The amine group shows both electrostatic and hydrogen bonding interactions with Asp¹¹⁶ of TM III. The meta-hydroxyl group forms a hydrogen bond mainly with Ser²⁰⁶ instead of Ser²⁰⁷/Ser²¹⁰ on TM V. However, an alternate binding mode does show the para-hydroxyl group forming hydrogen bond with Ser²⁰⁴ (unpublished data and Ref. 76). This hydrogen bonding seems very important. For epinephrine, the docked mode is consistent and conserved; whereas the oxymetazoline docking mode is not consistent and the phenyl ring flips around due to lost anchoring with Ser²⁰⁶. Therefore, oxymetazoline is not able to trigger global conformation change and does not activate β_1 adrenoceptors.

CONCLUDING REMARKS

In discussing evolutionary changes such as specificity, regulation and cooperativity in enzymes, Koshland⁸⁸ indicated that these large molecules became programmed to allow one substrate to exhibit negative cooperativity whereas a second substrate may show positive cooperativity. The so-called “transition state” of the enzyme–substrate interaction is viewed as a near attack conformation.⁸⁹ Parallel events may occur at the active sites of the adrenoceptor and its subtypes, for activation by the chiral agonists. The crystal structure of the β_2 -adrenoceptor was reported.⁹⁰ Our model was similar in both overall structure and active site residue orientations.

There is no doubt that resolution of a single active enantiomer of old and new drugs can result in clinical and therapeutic benefit in humans. The detailed study of their efficacy differences is equally fascinating for understanding the initial molecular events in the activation of the receptor. The efficacy at the receptor is ultimately expressed at the clinical level in patients.

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Review Article

Recent Advances in Spirochalcogenurane Stereochemistry—A Mini Review

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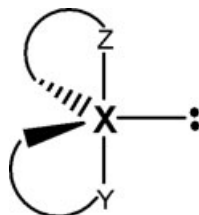
Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT A summary of the present knowledge of the stereochemistry of the hypervalent spirochalcogenuranes, especially the role of the central atom (S, Se, Te) for the chemical and configurational stability and the chiroptical and X-ray crystallographic techniques available for the determination of absolute configuration, is given. *Chirality* 20:544–551, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: hypervalent compounds; C_2 -symmetry; resolution; X-ray crystallography; vibrational and electronic CD spectroscopy; exciton coupling; absolute configuration

INTRODUCTION

Because of their hypervalent structure, spirochalcogenuranes have been subject to great interest and many experimental investigations. Their general structure (**I**) shows a trigonal bipyramid arrangement around the central atom $X = S, Se, \text{ or } Te$ with the lone pair lying in the equatorial plane and the most electronegative substituents (here represented by Y and Z) taking apical positions. Structure **I** is chiral and consequently exists as a racemate. In the case where Y differs from Z , the structure has no symmetry element (C_1 case), if $Y = Z$ (and spirorings are identical) it has C_2 symmetry.



Since the prediction in 1969 by Musher¹ of a trigonal bipyramidal sulfurane structure with a three-center four-electron bonding configuration, and the synthesis of the first spiro-sulfurane[†] of structure **I** ($X = S, Y = Z = -OCO-$) in 1971 by Kapovits and Kálmán,² a number of reviews on the chemistry and properties of hypervalent chalcogenuranes has appeared.^{3–12} This mini review will therefore focus mainly on results from stereochemical investigations obtained during the last two decades.

SYNTHETIC ROUTES

The easiest way to obtain a compound of structure **I** is by a cyclodehydration of the corresponding X-oxide, as shown in Scheme 1. When five-membered spirorings are formed, this reaction occurs spontaneously for $X = Se$ and

Te , whereas for $X = S$ it requires a dehydrating agent of some sort. The first synthesis of this kind (of **1b**, Scheme 1) was carried out in 1914 by Lesser and Weiss,¹³ and this compound was reinvestigated in 1972 by Dahlén and Lindgren.¹⁴ The sulfur analog **1a**, prepared and investigated in 1971,² was followed in 1995 by the tellurium analog **1c**, synthesized by Takaguchi and Furukawa.¹⁵ The relative ease of formation in the series **1a–c** is further shown by the fact that the **1b** can be directly prepared from the selenide precursor by the use of hydrogen peroxide, while **1c** is formed directly from the telluride even in the absence of any added oxidation agent.¹⁵

In 1979 Kapovits et al. described a synthesis of diaryldia-cyloxyspirosulfuranes by a reaction of the parent sulfide with a halogenating agent, like chloramine-T, chlorine, or bromine, and a variety of spiro-systems were prepared in this way¹⁶ and by the sulfoxide dehydration technique.¹⁷

Another route to isolable spiro-sulfuranes, used by Martin and coworkers,^{18,19} is shown in Scheme 2. The stability of **2**, which follows the series **2a** < **2b** < **2c** < **2d**, is a good example of steric and electronic substituent effects, particularly the *gem*-dialkyl effect.

Even nitrogen atoms in the axial positions can give rise to comparatively stable spiro-sulfuranes.^{20,21} The synthesis of compound **3** below is quite similar to the method described in Scheme 3.

The ready formation of spiro-systems from selenoxides was demonstrated in 1970 by the synthesis of 4,4'-spirobi(4-selena-4-butanolide) by the oxidation of 3,3'-selenodipropionic acid.²²

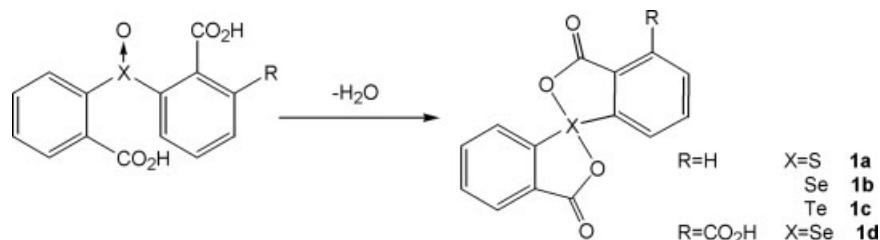
[†]A spiro- λ -sulfane according to more recent nomenclature.

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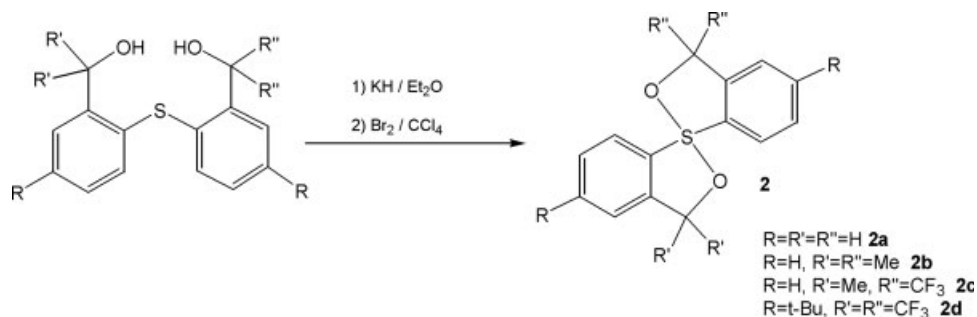
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Scheme 1. Spiro- λ^4 -chalcogenuranes prepared via the cyclodehydration route.



Scheme 2. Martin's method to achieve spirocyclization.

EARLY RESOLUTION ATTEMPTS

In 1978 the first resolution of a spiro-sulfuranone was reported.²³ Kapovits²⁴ later made use of the same enantiopure sulfoxide precursor to obtain (–)-**4** as shown in Scheme 4.

This technique of dehydrocyclization of an enantiopure sulfoxide was later used to obtain compounds **5** and **6**.²⁵ However, already in 1972 the enantioenrichment of the spiro-selenurane **1d** had been achieved in attempts of classical resolution.²⁶

Enantiomeric enrichment of **2d** was achieved by recrystallization of the racemate with optically active 2,2'-dihydroxy-1,1'-binaphthol because of preferential formation of an inclusion complex with one of the enantiomers.²⁷

In 1993 the first chromatographic resolution of a spiro-sulfuranone racemate (**1a**) was made.²⁸ Although performed on an analytical scale, it permitted characterization of the pure enantiomers by circular dichroism (CD) spectroscopy.

ENANTIOSELECTIVE LIQUID CHROMATOGRAPHY

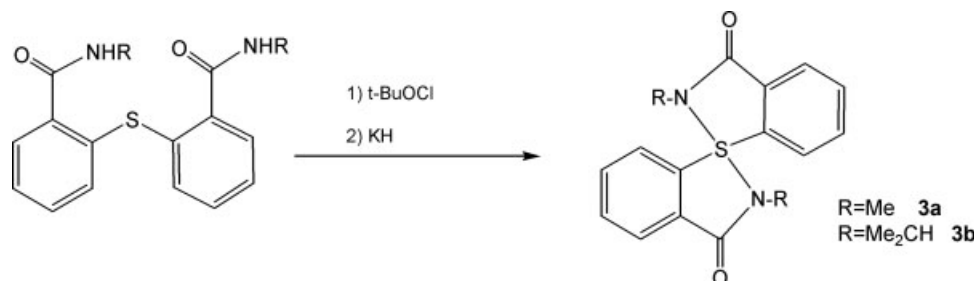
Ready access to enantiopure spirochalcogenuranes was given by preparative or semipreparative liquid chromatography, and a variety of resolutions (Scheme 5) have been

performed in this way.^{29,30} Chromatographic data is collected in Table 1.

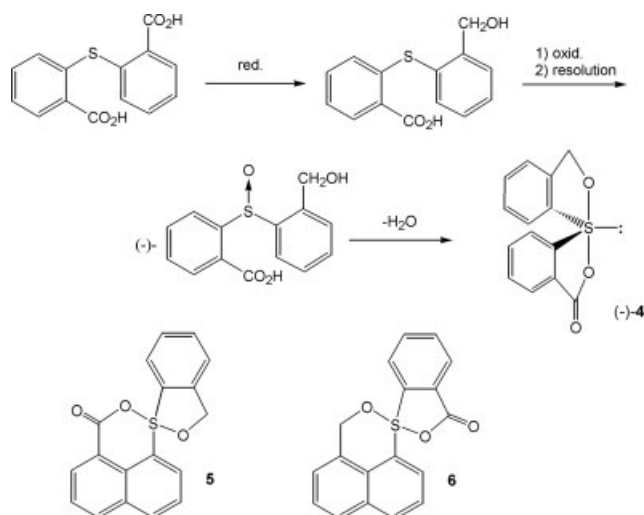
CHEMICAL AND CONFIGURATIONAL STABILITY

The pioneering work on spiro-sulfuranones carried out by Martin and coworkers^{18,37–40} and by Kapovits and coworkers^{2,40–43} clearly demonstrated the significantly increased chemical stability of the spirocyclic sulfuranones compared to their open-chain analogs. Thus, compound **2d** (Scheme 2), which can be regarded as a spiroketal of a sulfoxide, was found to be stable in boiling aqueous acid or base,³⁷ whereas an open-chain analog underwent rapid hydrolysis.^{38,40} Similarly, compound **1a**, in contrast to its noncyclic analogs, is quite stable toward hydrolysis.

The size of the spiro-rings are of course of fundamental importance and in addition to compounds **1a** and **8**, spiro-sulfuranones with rings of different sizes within the molecule have been prepared (Scheme 6).⁴² Kinetic studies of their acid-catalyzed hydrolysis⁴³ showed **14** to react about 60 times faster than **13** in dioxane/2.5% water at 25°C and **8** and **13** about 50 times faster than **1a** in dioxane/10% water at 25°C. Thus, the reactivity is determined by the size of the



Scheme 3. Synthetic route to spirodiazasulfuranones.



Scheme 4. Optically active spiro-sulfuranes prepared via prior resolution of an unsymmetrical sulfoxide.

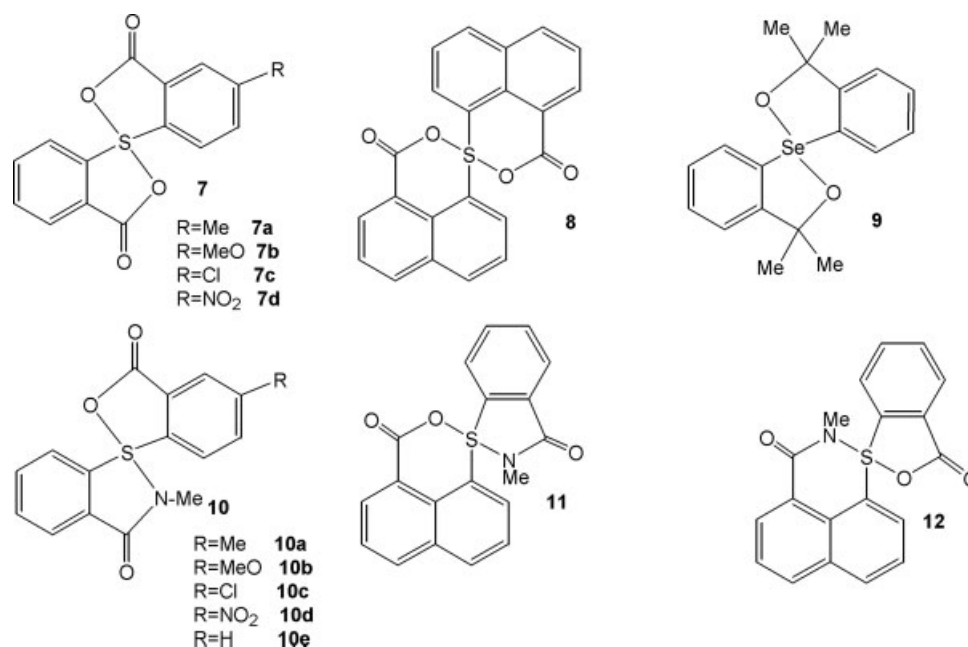
largest spiro-ring, which is most easily opened but does not take a significant part in any ring-closure equilibrium.

The hydrolysis was found to follow the eqn.: $k = k_0 + k_{\text{cat}}[\text{H}_3\text{O}^+]$. In acid, ring opening takes place after the protonation of a sulphur-bound oxygen, yielding a cyclic acyloxysulfonium ion and a free carboxyl group originating from the largest spiro-ring. The solvent isotope effect, $k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}}$, determined for **1a**, was found to be 0.56 for the acid-catalyzed reaction and 1.66 for the uncatalyzed reaction (dioxane/20% H_2O or D_2O used). ^{18}O -labeling experiments showed the subsequent step to be attack by water on the sulfonium sulfur present after the ring opening.

The stability toward hydrolysis increases with the size of the central atom; thus, for **1a–c** in the series **1a** < **1b** < **1c**. This parallels the well-known tendency of the oxides to exist as hydrates in water solution because of stabilization of the chalcogen–oxygen single bonds.^{44–46} While sulfoxides do not form hydrates, the corresponding telluroxides are completely hydrated in water. The selenoxides are stabilized by hydrogen bonding to a water molecule, as demonstrated by both X-ray crystallography and DFT calculations.⁴⁵

It was early recognized that spirochalcogenuranes could readily undergo a Berry pseudorotation⁴⁷ (BP) process, since a variable temperature ^{19}F -NMR spectrum of compound **15a** (Scheme 7) showed four resonance signals below -100°C but only two at higher temperatures.⁴⁸ This signal collapse was attributed to a fast exchange within the exo- and the endo-located trifluoromethyl groups, respectively. Since both these processes have the lone-pair fixed in an equatorial position, the interconversion barrier is relatively low, i.e. $\Delta G_{173\text{K}}^\ddagger = 31$ kJ/mol. Other examples are found in compounds **15b** and **15c**, where low-temperature ^{13}C NMR displayed two resonance signals in each case.^{49,50} The interconversion barriers were determined as $\Delta G_{161\text{K}}^\ddagger = 33$ kJ/mol and $\Delta G_{145\text{K}}^\ddagger = 29$ kJ/mol, respectively. Compound **15c**, however, was found to give a single resonance peak above room temperature, which cannot be rationalized by a BP process unless the lone-pair is allowed to take an axial position, which is considered a high-energy pathway. A mechanistic alternative, proposed by Martin, is the cuneal inversion route, which passes via a square planar transition state.¹⁹ Another possibility would be via a dissociative mechanism, meaning opening of a Te–O bond followed by inversion and ring closure.

One should keep in mind that the compounds studied here (**15**), all have a higher symmetry than those with



Scheme 5. Some of the racemates resolved by chiral liquid chromatography.

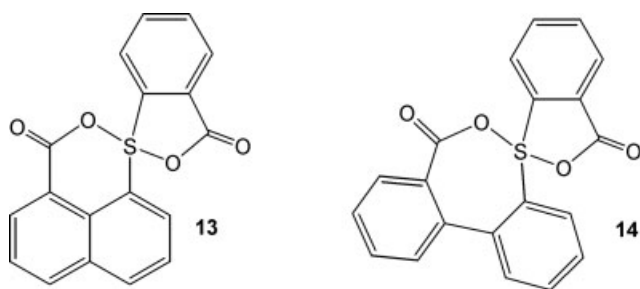
TABLE 1. Liquid chromatographic resolutions of spirochalcogenuranes reported

Compound	Column ^a	Mobile phase	α	Elution order	Ref. no.
1a	CHI-DMB	Hexane/2% 2-propanol	1.96	(+) first	28
1b	CHI-DMB (20 × 250 mm)	Hexane/20% dioxane	1.32	(+) first	31
1c	CHI-DMB	Hexane/30% dioxane	1.34 ^b	(+) first	32, 33
4	CHI-DMB	Hexane/30% dioxane	1.18		30
7a	CHI-DMB	Hexane/20% dioxane	1.62		30
7b	CHI-DMB	Hexane/20% dioxane	1.88		30
7c	CHI-DMB	Hexane/20% dioxane			30, 34
7d	CHI-DMB	Hexane/30% dioxane	1.59		30
8	CHI-DMB	Hexane/20% dioxane (anhydrous)	1.53	(+) first	35
9	CP-AS	Hexane/1.4% 2-propanol	2.1 (ca)		36
10a	CHI-DMB	Heptane/70% dioxane	1.13		30
10b	CHI-DMB	Heptane/70% dioxane	1.16		30
10c	CHI-DMB	Heptane/70% dioxane	1.24		30
10d	CHI-DMB	Heptane/70% dioxane	1.10		30
10e	CHI-DMB	Heptane/70% dioxane	1.09		30
11	CHI-DMB	Hexane/20% dioxane	1.88		30
12	CHI-DMB	Hexane/20% dioxane	1.82		30

^aCHI-DMB, Kromasil CHI-DMB; CP-AS, Daicel Chiralpak AS.^bAt about −10°C.

axial substituents, differing from the equatorial ones (such as the diaryldiacloxy compounds **1a–c** or the diaryldiacylamino compounds **3**). Therefore, since all interconverting ligands are identical, compounds **15** require only one pseudorotation to effect an inversion of configuration, i.e. an enantiomerization process. For the C_2 -symmetric compounds **1a–c** and **3** enantiomerization via pseudorotation will require a number of steps passing over high-energy intermediates with an axial lone-pair.^{19,51}

Resolution of the racemates **1a–c** by enantioselective liquid chromatography revealed that the spirotellurane **1c** underwent a fast enantiomerization process in the mobile phase at room temperature and that the temperature had to be about −20°C for this process to be slow enough to permit isolation of the enantiomers.³² Figure 1 shows a chromatogram of **1c** at 0°C and the experimental and simulated (dotted line) chromatograms at −4.9°C, from which an apparent enantiomerization rate constant of $3 \times 10^{-4} \text{ s}^{-1}$, corresponding to an activation energy $\Delta G_{268\text{K}}^\ddagger = 83 \text{ kJ/mol}$, could be calculated.^{32,33} The mechanism of this enantiomerization process has not yet been elucidated.

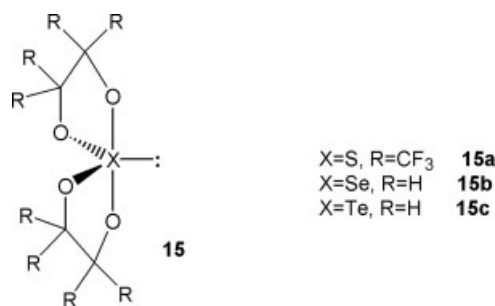


Scheme 6. Compounds used for a study of the effect of ring-size on the rate of hydrolysis.

DETERMINATION OF ABSOLUTE CONFIGURATION ECD and VCD Spectroscopy

The rigid structure of spirochalcogenuranes makes them suitable for ab initio calculations of chiroptical properties. Moreover, the aryl groups present in many of the compounds described earlier are suitable chromophores for the observation of exciton coupling in electronic CD (ECD) spectra. The small amount needed for an ECD spectrum is readily obtained by analytical chiral LC, and the progress in this field has greatly assisted the determination of absolute configuration. Figure 2 shows the experimental³⁴ (a) and calculated (b) ECD spectra of spirothiurane **4**. The qualitative resemblance between the (–)-(S)-enantiomer (dotted line) in (a) and the calculated spectrum for the (S)-configuration in (b) is apparent. The long wavelength absorption band at ~240 nm in the exciton couplet is well reproduced.

Comparison of calculated and experimental ECD spectra has enabled the determination of absolute configuration (AC) of quite a number of spirochalcogenuranes, and some results are given in Table 2.



Scheme 7. Tetraalkoxyspirochalcogenuranes used for studies of pseudorotation processes.

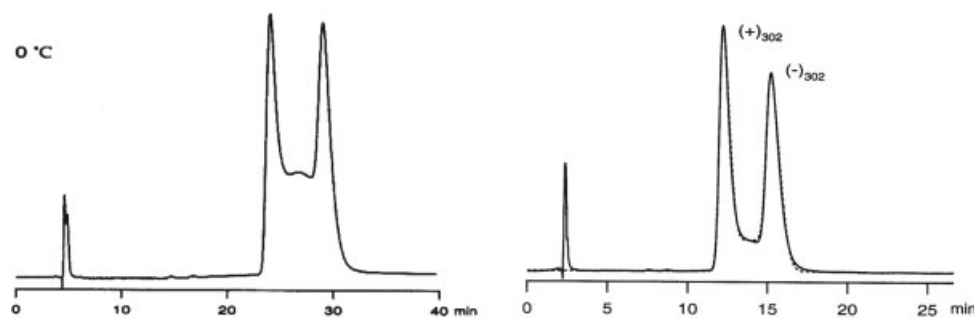


Fig. 1. Demonstration of the stereochemical lability of spirotellurane **1c**.

Recently, vibrational circular dichroism (VCD) spectroscopy has established itself as an extremely powerful method for AC determination.^{55–58} Although an experimental VCD spectrum requires a substantial amount of optically active material, the technique has the advantage that the calculation of a spectrum is less complicated, since no excited states need to become involved. Thus far,

only a few applications of VCD to the AC elucidation of spirochalcogenuranes have been made. Claeson and Holmén used VCD to confirm the ACs of compounds **1a** and **1b**,⁵⁹ and Petrovic et al. showed by VCD that the selenium analog of **2b** was (–)₅₈₉-(S)-correlated.⁶⁰

X-ray Crystallography

The absolute configurations of compounds **4** and **5** were determined by single crystal X-ray crystallography to be (+)-(R). Both compounds gave orthorhombic crystals of space group $P2_12_12_1$.²⁵ The structure determination of these unsymmetrical spiro-sulfuranes gave interesting information insofar as the lengths of the axial bonds were found to be quite unequal as also found for the corresponding racemates.⁶¹ Thus, the S–O–(alkyl) bond lengths in **4** and **5** were found to be 1.707 and 1.661 Å, respectively, whereas the lengths of the corresponding S–O–(acyl) bonds were as much as 2.052 and 2.129 Å. The O–S–O arrangement was found to be almost linear in both cases (angle 180° and 173°, respectively), demonstrating the trigonal bipyramidal geometry. The long S–O–(acyl) bonds reflect a high degree of polarization close to a zwitterionic structure (Fig. 3).

Recently, the Se-oxide of compound **9** was obtained in enantiopure form by chiral liquid chromatography and the AC was found to be (–)-(R) by single crystal X-ray crystallography.⁶² Figure 4 shows the significantly bent trigonal bipyramidal structure of the compound (O–Se–O, 167.7°).

In connection with an investigation on the stereochemistry of the hydrolysis of compounds **16a–c** under acidic and basic conditions^{63–65} the ACs of **16a** and **16c** were determined by X-ray crystallography (Scheme 8).

Interestingly, it was found that hydrolysis of spiro-sulfurane **16a** under acidic or neutral conditions gave the sulfide with Ss configuration, whereas basic conditions gave the opposite sulphur configuration Rs. The mechanisms responsible for this opposite stereochemical behavior have not been fully elucidated, although a difference in the first dissociate step (S–O–alkyl bond opening in acid versus S–O–acyl bond opening in basic medium) has been proposed.^{63,64}

Recently, spirocyclic Se and Te systems **17**, containing four-membered rings were investigated.^{66,67} In these cases a considerable distortion of the trigonal bipyramidal struc-

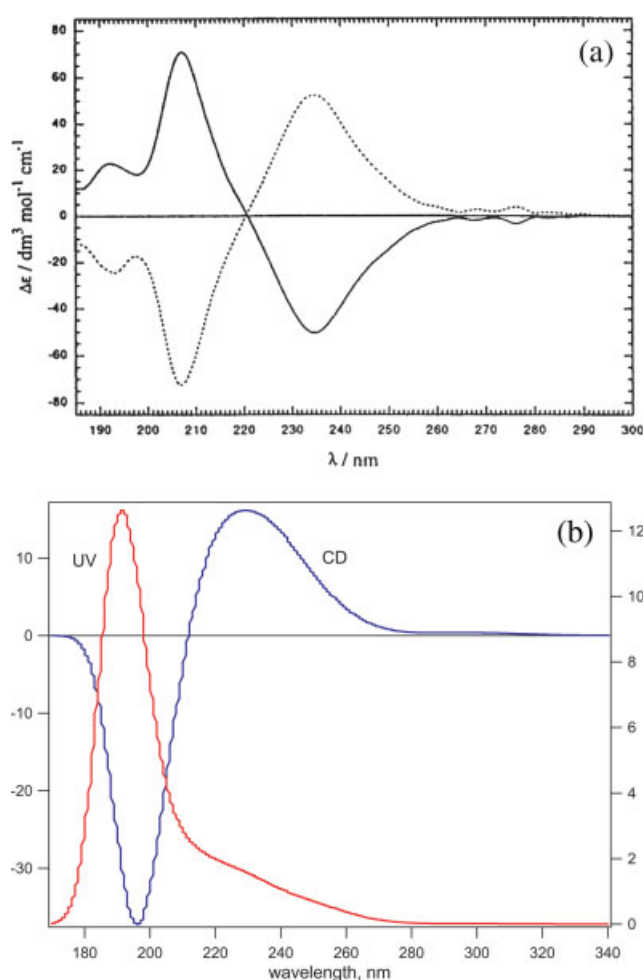


Fig. 2. (a) ECD spectra of the isolated enantiomers of **4** in acetonitrile. Solid line: (+)-(R)-, dotted line (–)-(S)-form. Reprinted from Ref. 34, with permission from Taylor and Francis. (b) UV and CD spectra calculated for (S)-**4** (B3PW91/6-31G(d,p) used).

TABLE 2. Absolute configurations determined or verified from ECD spectra

Compound	Enantiomer	Spec. rotation	Abs. config.	Calc. method/couplet sign ^a	Ref.
1a	(+)-form	$[\alpha]_{546} = +27.3$ (MeCN)	<i>S</i>	B3PW91/6-31G(d,p)/positive	28, 34 ^b
1b	(+)-form (at 302 nm)	n.d.	<i>S</i>	B3PW91/6-31G(d,p)/positive	28 ^b
1c	(+)-form (at 302 nm)	n.d.	<i>S</i>	B3PW91/6-31G(d,p)/positive	28 ^b
7c	(+)-form	n.d.	<i>S</i>	B3PW91/6-31G(d,p)/positive	34
4	(-)-form	$[\alpha]_{546} = -37.5$ (DMF)	<i>S</i> ^c	B3PW91/6-31G(d,p)	34 ^b
5	(-)-form	$[\alpha]_{546} = -158$ (DMF)	<i>S</i> ^c		34
6	(+)-form	$[\alpha]_{546} = +852$ (DMF)	<i>S</i> ^c		34
11	(+)-form	n.d.	<i>R</i>		52, 53
12	(+)-form	n.d.	<i>S</i>		52, 53

^aOf the ¹L_a transition dipole moment vectors.^bS. Allenmark (to be published).^cAccording to the convention given by Martin and Balthazor (Ref. 54).

ture was found; the O—Se—O and O—Te—O angles were as low as 155.3° and 148.1°, respectively (Scheme 9).

An interesting method based on the use of a chiral dirhodium tetracarboxylate for complexation and evaluation of the NMR spectra of the complexes, as first described by Duddeck,⁶⁸ has been applied to the spirochalcogenuranes gave diastereomeric complexes with the dirhodium tetracarboxylate formed with Mosher's acid, and both the ¹H and ¹³C signal dispersions ($\Delta\nu$) from the diastereomers were large and of uniform sign, permitting not only enantiomer differentiation but possibly also a determination of absolute configuration by a convenient rule. The assumption that the rhodium ligand is bound to the central chalcogen atom (by its lone pair) has been supported by semiempirical (PM3) as well as DFT calculation methods.

SUMMARY AND CONCLUSIONS

Recent advances in methods to obtain enantiopure spirochalcogenuranes, together with *ab initio* techniques for

the calculation of structure and chiroptical data, have led to a better understanding of the dynamics and chemical and spectroscopic behavior of these hypervalent systems. X-ray crystallography of compounds with different heteroatoms in the axial positions has demonstrated a large difference in the axial interatomic distances, such that the less electronegative atom forms an essentially normal covalent bond, whereas the other is part of a more elongated bond.^{70,71} Good agreement between experimental

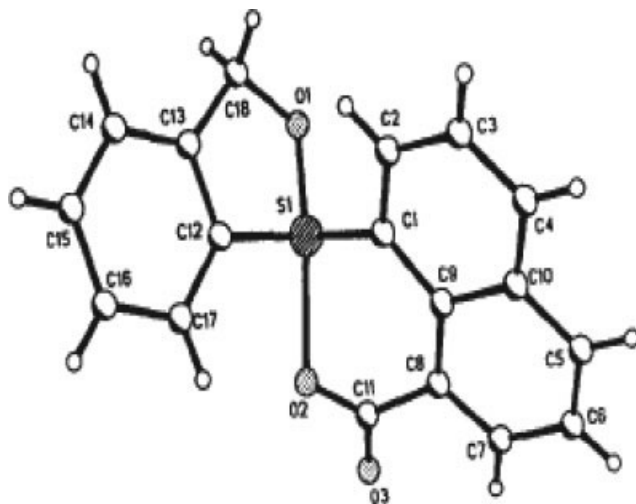


Fig. 3. X-ray structure of compound **5**. Reprinted from Ref. 25 (copyright 1997), with permission from Elsevier.

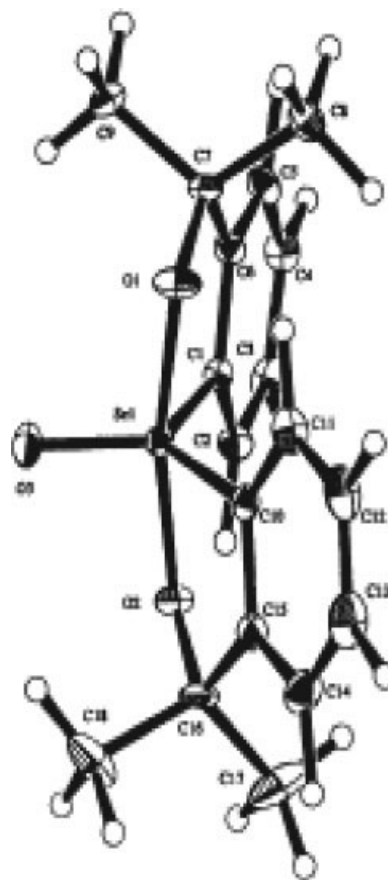
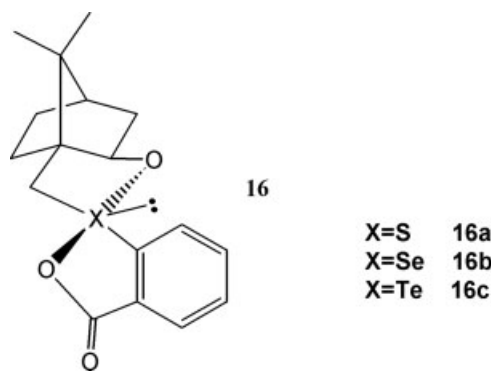
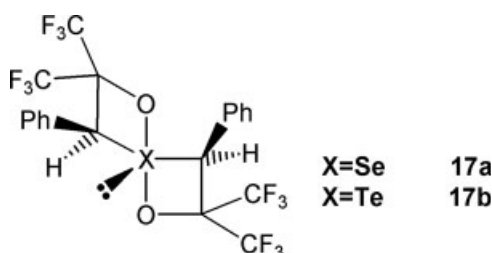


Fig. 4. X-ray structure of the (-)-(R)-Se-oxide of **9**. Reprinted from Ref. 62, with permission from Wiley.



Scheme 8. Spirochalcogenuranes synthesized from the chiral pool and containing an *exo*-norbornanol moiety providing an axial alkoxy group.



Scheme 9. Two 1,5-dioxaspiro[3.3]heptanes with selenium and tellurium centers, respectively.

and calculated structures and chiroptical spectra has been found.

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Species-Dependent Stereoselective Drug Binding to Albumin: A Circular Dichroism Study

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT Drug binding to albumins from different mammalian species was investigated to disclose evidence of species-dependent stereoselectivity in drug-binding processes and affinities. This aspect is important for evaluating the reliability of extrapolating distribution data among species. The circular dichroism (CD) signal induced by drug binding to the albumins [human serum albumin (HSA), bovine serum albumin (BSA), rat serum albumin (RSA), and dog serum albumin (DSA)] were measured and analyzed. The binding of selected drugs and metabolites to HSA significantly differed from the binding to the other albumins in terms of affinity and conformation of the bound ligands. In particular, phenylbutazone, a marker of site one on HSA, showed a higher affinity for binding to BSA with respect to RSA, HSA, and DSA, respectively. In the case of diazepam, a marker of site two on HSA, the affinity decreased in order from HSA to DSA, RSA, and BSA. The induced CD spectra were similar in terms of energy and band signs, suggesting almost the same conformation for the bound drug to the different albumins. Stereoselectivity was high for the binding of ketoprofen to HSA and RSA. A different sign was observed for the CD spectra induced by the drug to the two albumins because of the prevalence of a different conformation of the bound drug. Interestingly, the same induced CD spectra were obtained using either the racemic form or the (S)-enantiomer. Finally, significant differences were observed in the affinity of bilirubin, being highest for BSA, then decreasing for RSA, HSA, and DSA. A more complex conformational equilibrium was observed for bound bilirubin. *Chirality* 20:552–558, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: protein binding; molecular recognition; HSA; BSA; RSA; DSA; induced CD

INTRODUCTION

Drug binding to plasma and tissue proteins is crucial to the overall pharmacological activity of a drug. Human serum albumin (HSA) is the most important plasma protein, acting as a drug carrier with pharmacokinetic implications, namely drug distribution.^{1–4}

As the actual volume of distribution and concentration of a drug at the receptor site are strictly related to the unbound drug fraction, a full characterization of the binding mechanism to plasma proteins such as HSA has become essential to understand the pharmacokinetic, pharmacodynamic, and toxicological profile of any drug. This is particularly true for highly protein bound drugs, for which small changes in the bound fraction have a significant impact on the circulating-free fraction. As an example, a 4% difference between 99 and 95% bound fraction corresponds to a fivefold difference in the free fraction, whereas the same 4% difference between 92 and 88% bound fraction corresponds to a 1.5-fold difference in the free fraction.

HSA binds a number of relatively insoluble endogenous and exogenous compounds and a wide variety of drugs and facilitates their transport throughout the body. However, a high affinity for plasma proteins reduces the free concentration of the drug, so that a high affinity for plasma

proteins may be a benefit or a drawback for their efficacy, depending on the target and the drug.

In the case of lipophilic drugs, their HSA-binding results in improved solubility and sometimes in decreased toxicity.¹ The free drug concentration can change once administered because of its interactions with other drugs and endogenous factors binding to HSA, or significant alterations of the serum carrier concentration.^{4,5}

Furthermore, enantioselective binding can occur if the drug is chiral, because of the different stability of two diastereomeric adducts formed between the single enantiomers and the protein. In this situation, the single enantiomers act as distinct compounds, their metabolism, distribution, and elimination differing in principle. Taking into account that protein binding is not a simple transport mechanism, the impact of stereoselectivity on biological activity becomes evident. This accounts for the growing in-

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terest in the molecular mechanisms involved in stereodiscrimination by serum proteins. HSA is known to possess the highest enantioselectivity amongst plasma proteins. Because of its rather exceptional enantioselectivity, comparable to that displayed by a specific drug target, HSA has also been regarded as a "silent receptor."⁶ Stereoselectivity is generally linked to the high-affinity (or primary) binding site on HSA. At therapeutic concentrations, it is this primary site that binds the drug and regulates its transport. X-ray analysis well defined the sites I and II on HSA.⁷ In particular, the binding of phenylbutazone and diazepam to sites I and II, respectively, confirms the reliability of using these drugs as selective markers. Furthermore, X-ray crystallography gives detailed information on the most stable conformation of bound phenylbutazone and diazepam. Site I is complex and several subsites can be recognized. However, a quite high adaptability of the binding cavities should be considered as due mainly to local conformational changes. The shape of the pockets and the distribution of basic and polar residues on the largely hydrophobic interior walls of the pockets determine the binding specificities. These polar and basic residues are involved in hydrogen-bonding interaction and charge neutralization.

Thus, the binding to the serum carriers, namely HSA, is becoming essential to characterize the distribution phase, amongst ADME (absorption, distribution, metabolism, excretion) parameters, in the frame of the design and development of new drugs. Most of the distribution experiments carried out *in vivo* are performed on animals. Hence it is interesting to determine the binding of new compounds to albumins from different species to evaluate the reliability of extrapolating the distribution data obtained in animals to humans.

Many techniques are suitable to determine the binding parameters of drugs to serum albumins, such as equilibrium dialysis,¹ biochromatography,⁸⁻¹² spectroscopy,¹³⁻²² and optical biosensors.²³⁻²⁵ This study used CD (circular dichroism) spectroscopy not only to determine the affinity constants of selected ligands to albumins from different species, but also to gain information on the stereochemistry of the bound drug. CD is well suited to monitoring the signal associated with the formation of the drug/protein complex, yielding direct information on the binding interaction without interfering with the equilibrium process. In particular, CD can shed more light on the stereochemistry of the protein/drug adduct, and thus on the mechanism of binding. In addition, CD usually allows selective determination of the binding parameters related to the highest affinity-binding site, being the only stereoselective one in most cases. CD proved suitable for monitoring stereoselectivity in the binding of drugs and metabolites to albumins from different species and determining the affinity constants to the higher affinity-binding sites.

EXPERIMENTAL

Materials

HSA, dog serum albumin (DSA), bovine serum albumin (BSA), rat serum albumin (RSA), bilirubin, phenylbutazone, (S)-ketoprofen, *rac*-ketoprofen, and HPLC-grade 1-

propanol were purchased from Sigma-Aldrich (Milan, Italy). Diazepam was kindly supplied by Prof. Lucacchini, University of Pisa, Italy. Water was doubly distilled, and buffers were filtered through a 0.22- μ m membrane filter. All other materials were of analytical grade and were used without further purification.

CD Measurements

Circular dichroism (CD) spectra were recorded using a Jasco J-810 spectropolarimeter (Jasco, Tokyo, Japan). All measurements were carried out at room temperature using cells with path length ranging from 5 cm to 0.01 cm. The instrument was interfaced to a personal computer to acquire and process data. The same instrumental parameters were employed to reduce the errors: time constant 4 sec, scan speed 20 nm/min, resolution 0.2 nm, SbW 1 nm. Solutions of the proteins were prepared in phosphate buffer (pH 7.4, 67 mM) immediately before use. The markers (diazepam, phenylbutazone *rac*-ketoprofen, (S)-ketoprofen, and bilirubin) were selected for their selective binding to the main binding areas on HSA. Stock solutions of the drugs were prepared in 1-propanol. Their concentrations ranged from 3×10^{-3} to 54×10^{-3} M. The stock solutions of bilirubin were prepared dissolving it in 0.1 M KOH (20% of the final volume) and then diluting this solution with K_2HPO_4 0.1 M. The stock solutions of bilirubin were 3×10^{-3} in the stereoselective-binding investigations and 15×10^{-3} M in the affinity constant determination experiments. The stock solutions of bilirubin were protected from light and prepared immediately before use. The concentration of 1-propanol in the stereoselective-binding investigations never exceeded 1% v/v, while it was always lower than 2% v/v in the affinity constant determination experiments. Bilirubin-albumin solutions did not contain any organic modifier. Induced CD spectra were studied on [albumin]/[analite] 1/1 complexes, using 1-cm cell. Albumin and marker were kept constant, 30 μ M. *rac*-ketoprofen and (S)-ketoprofen complexes with HSA and RSA were also studied at different molar ratios. When bilirubin and phenylbutazone were the markers, the experiments were carried out using 100- μ M ligand and protein concentration, 1-mm cell. Affinity constants were determined by analyzing the change in the induced CD signal for a 1/1 [marker]/[HSA] complex, upon changing its concentration. The absorption was maintained constant by using different cells for the different concentrations. The induced CD spectra were obtained by subtracting the albumin solution spectrum recorded in the same experimental conditions.

RESULTS AND DISCUSSION

Difference CD Spectroscopy: Stereoselective Binding

Difference CD spectroscopy was used to characterize the binding properties of selected ligands to different albumins measuring the CD spectra induced by four drugs [phenylbutazone, diazepam, *rac*-ketoprofen, and (S)-ketoprofen], and a metabolite [bilirubin] complexed to the albumins [HSA, DSA, BSA, and RSA]. The selected ligands are known to bind to specific-binding areas on

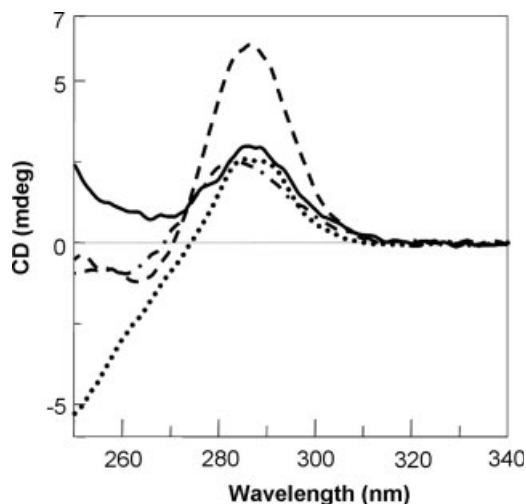


Fig. 1. Induced CD spectra of 1/1 molar ratio complexes of phenylbutazone bound to HSA (—), BSA (---), RSA (.....), and DSA (— · — ·). [albumin] = 100 μ M in phosphate buffer 67 mM, pH 7.4, path length 0.1 cm.

HSA as the highest affinity-binding sites.^{1,26} In particular, phenylbutazone binds in the large hydrophobic cavity of subdomain IIA at site I, following the notation by Sudlow et al.²⁷ Diazepam, *rac*-ketoprofen, and (*S*)-ketoprofen bind in the hydrophobic cavity of subdomain IIIA at site II, according to Sudlow et al.²⁷ Bilirubin also binds in the large hydrophobic cavity of subdomain IIA.^{1,28} All the ligands used except (*S*)-ketoprofen are achiral or racemic, and so the interaction with albumin is responsible for the observed CD signal, which arises only from the complexed ligand and will selectively reflect the stereoselective binding.

Albumin-bound phenylbutazone showed a positive CD band at about 290 nm for all the albumins analyzed (Fig. 1). The behavior of the induced CD spectra was very similar for HSA, DSA, and BSA phenylbutazone 1/1 complexes,

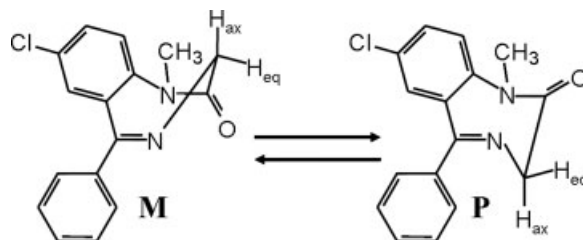


Fig. 3. The M and P conformers of diazepam.

whereas the RSA-bound drug showed a negative trend of the CD signal at wavelengths shorter than 270 nm. The BSA-bound phenylbutazone showed the highest intensity-induced CD signal at about 285 nm.

Also, in the case of diazepam, the induced CD spectra were very similar for the bound drug with all the albumins, but the intensities of the CD spectra differed significantly (Fig. 2), suggesting a species dependency in the binding of benzodiazepines to the serum proteins. In particular, the induced CD of diazepam bound to protein increased for RSA, DSA, BSA, and HSA, respectively (Fig. 2). The observed CD spectra reflect the stabilization of one of the possible conformations of diazepam in solution. In fact, this drug was present in two mirror-image conformations, M and P (Fig. 3), in rapid exchange between each other. The M conformer prevailed for chiral benzodiazepines²⁹ with the (*S*) absolute configuration at C3 of the seven-membered ring. Thus the M conformation prevailed when diazepam was bound to the albumins. This stereoselectivity has already been shown for HSA,^{30–32} presenting a well-defined induced CD spectrum upon drug binding to its stereo-specific site, closely resembling to that of a chiral benzodiazepine with the (*S*)-absolute configuration.

Species dependency of stereoselective drug binding to albumins was impressive using ketoprofen as the marker. Ketoprofen is known to bind at site II as the high affinity-

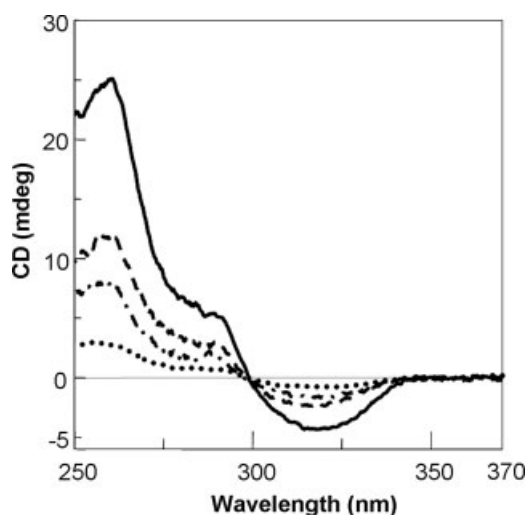


Fig. 2. Induced CD spectra of 1/1 molar ratio complexes of diazepam bound to HSA (—), BSA (---), RSA (.....), and DSA (— · — ·). [albumin] = 30 μ M in phosphate buffer 67 mM, pH 7.4, path length 1 cm.

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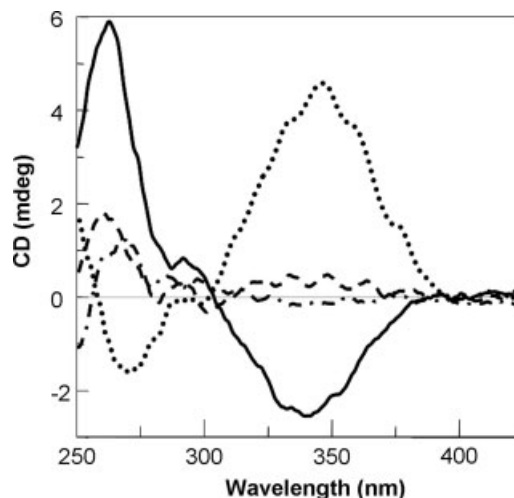


Fig. 4. Induced CD spectra of 1/1 molar ratio complexes of *rac*-ketoprofen bound to HSA (—), BSA (---), RSA (.....), and DSA (— · — ·). [albumin] = 30 μ M in phosphate buffer 67 mM, pH 7.4, path length 1 cm.

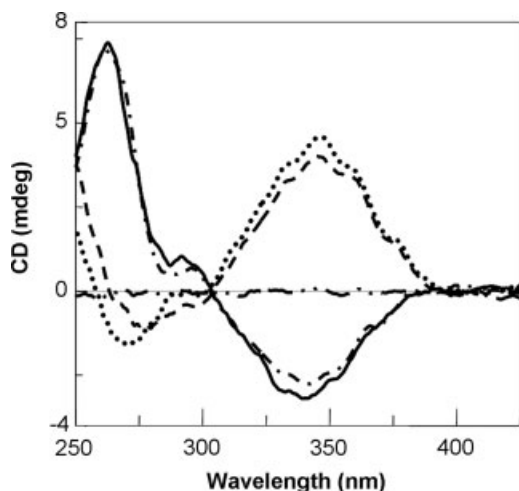


Fig. 5. CD spectrum of (S)-ketoprofen 30 μM (---), and induced CD spectra of 1/1 molar ratio complexes of *rac*-ketoprofen/HSA (—), *rac*-ketoprofen/RSA (●●●●), (S)-ketoprofen/HSA (—●—●), and (S)-ketoprofen/RSA (—●—●). [albumin] = 30 μM in phosphate buffer 67 mM, pH 7.4, path length 1 cm.

binding site on HSA.^{28,33} The induced CD spectrum was not significant, in the range 270–400 nm, for the [*rac*-ketoprofen]/[DSA] 1/1 complex, while the same 1/1 stoichiometry gave a very low-intensity CD signal for *rac*-ketoprofen/[BSA].³⁴ In the cases of [*rac*-ketoprofen]/[RSA] and [*rac*-ketoprofen]/[HSA], the induced CD spectra were of very high intensity, but reversed in sign of the observed CD bands (Fig. 4). The same behavior was observed using (S)-ketoprofen as the marker, instead of the racemate. The induced CD spectrum was positive at about 340 nm for the 1/1 [*rac*-ketoprofen]/[RSA] and [(S)-ketoprofen]/[RSA] complexes, while a negative sign of the CD band at the same wavelength was observed in the cases of [*rac*-ketoprofen]/[HSA] and [(S)-ketoprofen]/[HSA] complexes

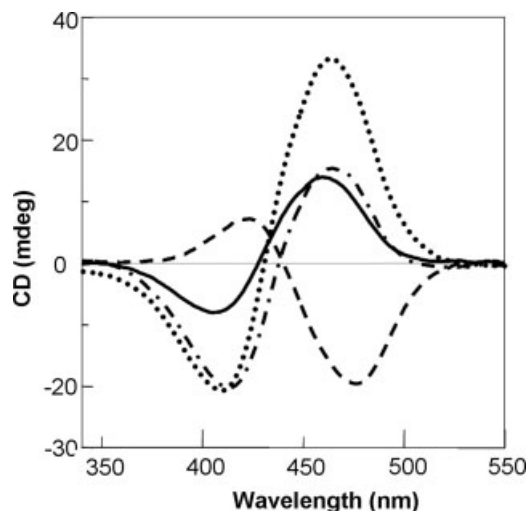


Fig. 7. Induced CD spectra of 1/1 molar ratio complexes of bilirubin bound to HSA (—), BSA (---), RSA (●●●●), and DSA (—●—●). [albumin] = 100 μM in phosphate buffer 67 mM, pH 7.4, path length 0.1 cm.

(Fig. 5). In both cases, the induced CD spectra due to the $n \rightarrow \pi^*$ electronic absorption band show a much higher intensity than that of the free (S)-ketoprofen.^{34,35} This behavior suggests a conformational change of the benzophenone moiety from a quasi-symmetric to a chiral arrangement.

Increasing the ratio of the complexes up to [(S)-ketoprofen]/[RSA] 2/1, the induced CD signal increased (Fig. 6A), while in the case of HSA the intensity almost peaked for the 1/1 stoichiometry, suggesting a higher affinity or site II specificity of the drug to the protein (Fig. 6B).

Last, bilirubin binding to the various albumins was investigated, using this metabolite as a marker of the HSA-binding site reported as the bilirubin-binding site.^{1,28} Bilirubin is achiral so that it does not show any CD signal in aque-

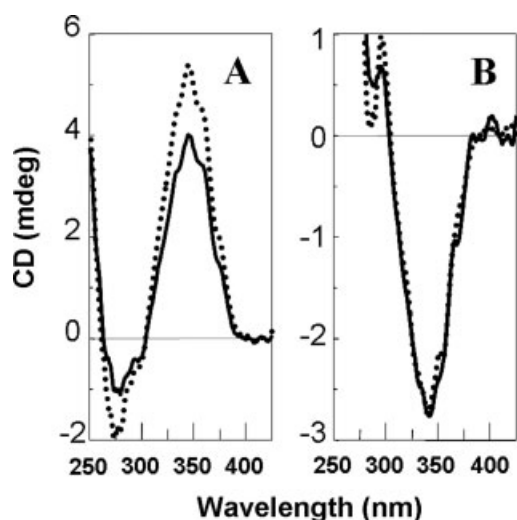


Fig. 6. A: Induced CD spectra of (S)-ketoprofen/RSA complexes of 1/1 (—) and 2/1 (●●●●) molar ratio. B: Induced CD spectra of (S)-ketoprofen/HSA complexes at 1/1 (—) and 2/1 (●●●●) molar ratio. [albumin] = 30 μM in phosphate buffer 67 mM, pH 7.4, path length 1 cm.

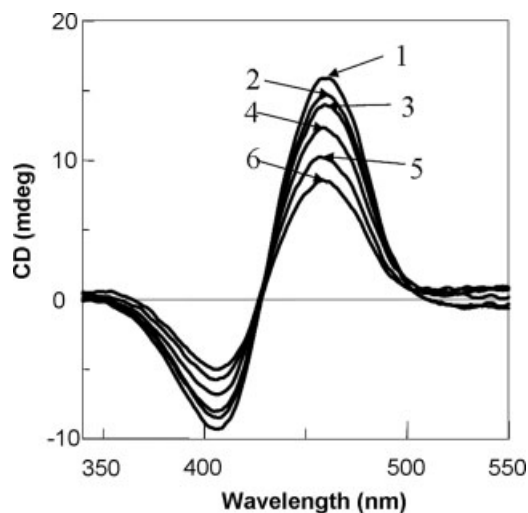


Fig. 8. Induced CD spectra of 1/1 molar ratio complex of bilirubin bound to HSA at different concentrations and cell path length. (1) [HSA] = 1 mM, $l = 0.01$ cm; (2) [HSA] = 200 μM , $l = 0.05$ cm; (3) [HSA] = 100 μM , $l = 0.1$ cm; (4) [HSA] = 10 μM , $l = 1$ cm; (5) [HSA] = 5 μM , $l = 2$ cm; and (6) [HSA] = 2 μM , $l = 5$ cm. Protein and bilirubin were in phosphate buffer 67 mM, pH 7.4.

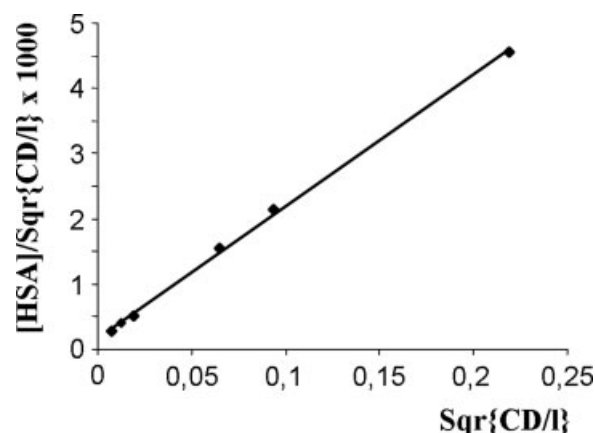


Fig. 9. Linear fitting for 1/1 [bilirubin]/[HSA] complex at 457 nm (X units, $\text{cm}^{-1/2}$; Y units, $\text{M cm}^{-1/2}$; slope, 0.0202; intercept, 2.0×10^{-4}).

ous solution. However, a well-defined induced CD spectrum was observed upon binding to the stereo-specific site on HSA.^{8,14,20,21,36} Bilirubin exists in aqueous solution as an isoenergetic mixture of two conformational isomers of P- and M-helicity.^{36–38} The appearance of a bisignate CD spectrum when bilirubin binds to HSA has been interpreted as HSA bound bilirubin adopting a prevailing conformation of P helicity.^{36–38} The same CD behavior observed for the 1/1 [bilirubin]/[HSA] complex was revealed for the 1/1 [bilirubin]/[RSA] and [bilirubin]/[DSA] complexes, whereas the 1/1 [bilirubin]/[BSA] complex showed a reversed sign CD couplet, for negative at lower energy and positive at higher energy (Fig. 7).³⁹ A different bilirubin conformation thus prevails for the BSA-bound bilirubin, again suggesting a significant species dependency of the ligand binding to the albumin. In particular, the inversion of the bisignate CD spectrum was related to the prevailing M-helicity conformation for the BSA bound bilirubin, while the P-helicity conformation prevailed for the other albumin bound bilirubin complexes.

Difference CD Spectroscopy: Affinity Constants Determination

Different methods have been developed for the determination of binding parameters by modifying the spectroscopic features of the small molecule upon binding to HSA.^{1,8–25} One possible approach to determine the affinity constants is the quantitative analysis of the stereo-specific

interactions by means of dilution curves. According to the Beer-Lambert law, if a complex is diluted and the spectrometric cell path length is proportionally increased, the resulting decrease of the signal is uniquely due to the effect of dilution, which depends on the affinity constant of the binding site. As an example, the change in the induced CD signal by diluting the [bilirubin]/[HSA] 1/1 complex is reported in Figure 8. For CD analysis, which only measures stereo-specific interactions, the following linear equation (eq. 1) was derived for a 1/1 stoichiometric interaction^{19,32}:

$$pl^{1/2} \text{CD}^{-1/2} = \Delta\epsilon^{-1} l^{-1/2} \text{CD}^{1/2} + K^{-1/2} \Delta\epsilon^{-1/2} \quad (1)$$

where p is the protein concentration, CD is the measured signal, l is the cell pathlength, K is the affinity constant, and $\Delta\epsilon$ is the molar coefficient of dichroic absorption. By changing p and l , and measuring CD , K , and $\Delta\epsilon$ can be obtained by linear fitting, that is, measuring the affinity and the stereospecificity of the interaction.^{19,32}

The analysis of the induced CD signals for the [bilirubin]/[HSA] 1/1 complex is shown in Figure 9: the slope allows the $\Delta\epsilon$ to be determined, while the affinity constant can be obtained from the intercept. The values of the affinity constants and $\Delta\epsilon$ values for the bound ligands to the different albumins are reported in Tables 1 and 2, respectively. The highest affinity value of phenylbutazone was obtained with BSA, and then RSA, HSA, and DSA, in agreement with biochromatography data,^{40,41} whereas HSA and RSA showed a reverse order in the PBU binding using equilibrium dialysis.⁴² The obtained affinity constant determined for the HSA/PBU complex is also in agreement with the previous reports using CD spectroscopy.¹⁴ The affinity constant values determined by CD spectroscopy may differ from those obtained by other methods, because the CD technique selectively monitors only binding to the stereoselective-binding sites.

The binding of diazepam disclosed a high affinity for the HSA and DSA, while much lower values were obtained for the RSA and BSA samples. The induced CD value is not sufficient to determine the scale in the affinity for the various proteins. For example, the affinity constant of diazepam for BSA binding is relatively low (Table 1), while the comparison of induced spectra registered for the 1/1 complexes (Fig. 2) did not confirm this. This is due to the high intensity of the $\Delta\epsilon$ associated with BSA/DZP: the significant differences observed for the various complexes (Table 2) are probably due to differences in the prevailing

TABLE 1. Binding constant of the markers to the albumins [K_A , M^{-1} (analysis λ , nm)]

Ligand	HSA	BSA	RSA	DSA
Phenylbutazone	5.2×10^4 (286)	2.8×10^5 (286)	2.1×10^5 (286)	2.0×10^4 (284)
Diazepam	2.6×10^5 (315)	2.1×10^4 (315)	1.9×10^4 (315)	2.4×10^5 (315)
	2.8×10^5 (260)	1.6×10^4 (260)	3.0×10^4 (260)	2.4×10^5 (260)
Ketoprofen	9.2×10^5 (347)	N.D.	3.8×10^5 (343)	N.D.
Bilirubin	3.8×10^5 (407)	4.2×10^{6a}	2.3×10^6 (409)	3.2×10^5 (414)
	5.1×10^5 (457)	1.0×10^{7b}	3.7×10^6 (463)	1.6×10^5 (467)

^aPositive maximum of each spectrum.

^bNegative maximum of each spectrum.

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TABLE 2. $\Delta\epsilon$ of the main dichroic bands of the complexes [$\Delta\epsilon$ (analysis λ , nm)]

Ligand	HSA	BSA	RSA	DSA
Phenylbutazone	15.9 (286)	22.1 (286)	9.7 (286)	15.4 (284)
Diazepam	7.1 (315)	7.6 (315)	3.1 (315)	5.2 (315)
	40.4 (260)	37.2 (260)	9.4 (260)	26.5 (260)
Ketoprofen	2.2 (347)	N.D.	5.3 (343)	N.D.
Bilirubin	29.5 (407)	23.7 ^a	69.4 (409)	78.7 (414)
	49.5 (457)	62.5 ^b	109 (463)	69.0 (467)

^aPositive maximum of each spectrum.^bNegative maximum of each spectrum.

conformation of the bound drug. Thus, species dependency is confirmed in the ligand binding to the protein, both for the affinity and for the stereochemistry of the bound drug.

In addition to diazepam, *rac*-ketoprofen, a drug also known to bind selectively to site II on HSA was investigated, and the affinity constant determined for the different albumins. A high affinity was observed for its binding to RSA and HSA, the highest being for HSA (Table 1). This result was suggested by the high saturability of the HSA-binding site (Fig. 6). Instead, the $\Delta\epsilon$ value was much higher for the [*rac*-ketoprofen]/[RSA] complex (Table 2). A reliable determination of the affinity constant and delta epsilon values for the binding of *rac*-ketoprofen to BSA and DSA was precluded by the very low values of the induced CD signals.

Quite high values of the affinity constants were measured for the binding of bilirubin to the albumins (Table 1). The highest affinities were observed for BSA and RSA, whereas the $\Delta\epsilon$ values were much higher for the [bilirubin]/[RSA] and [bilirubin]/[DSA] complexes (Table 2). The agreement with literature data is quite good, but some differences in the induced CD signal intensity and affinities were observed, probably due to the different experimental conditions adopted. In addition to being inverted with respect to all the signs of the other complexes, the induced CD spectrum for [bilirubin]/[BSA] showed a significant blue shift of the bisignate spectrum upon decreasing the concentration, suggesting a more complex conformational equilibrium of the ligand.³⁹ Furthermore, spectroscopic studies of mutant HSA/bilirubin complexes revealed that despite the similarity of the K_A values, dramatic changes had occurred in the structure of the adducts.³⁷

CD therefore is a useful and reliable method for screening drug-binding parameters to albumins from different mammals. As animals are used for preclinical studies, it is important to collect serum carrier-binding data using albumins from different species to ensure the reliability of extrapolating distribution data among the species.

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Investigation of the Stereoselectivity of an Anti-Amino Acid Antibody Using Molecular Modeling and Ligand Docking

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT The structure of the binding site of the stereoselective anti-D-amino acid antibody 67.36 was modeled utilizing web antibody modeling (WAM) and SWISS-MODEL. Although docking experiments performed with an aromatic amino acid as model ligand were unsuccessful with the WAM structure, ligand binding was achieved with the SWISS-MODEL structure. Incorporation of side-chain flexibility within the binding site resulted in a protein structure that stereoselectively binds to the D-enantiomer of the model ligand. In addition to four hydrogen bonds that are formed between amino acid residues in the binding site and the ligand, a number of hydrophobic interactions are involved in the formation of the antibody-ligand complex. The aromatic side chain of the ligand interacts with a tryptophan and a tyrosine residue in the binding site through π - π stacking. Fluorescence spectroscopic investigations also suggest the presence of tryptophan residues in the binding site, as ligand binding causes an enhancement of the antibody's intrinsic fluorescence at an emission wavelength of 350 nm. Based on the modeled antibody structure, the L-enantiomer of the model ligand cannot access the binding site due to steric hindrance. Additional docking experiments performed with D-phenylalanine and D-norvaline showed that these ligands are bound to the antibody in a way analogous to the D-enantiomer of the model ligand. *Chirality* 20:559–570, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: antibody stereoselectivity; anti-hapten antibody; computer-modeling; fluorescence enhancement; ligand-docking; protein-ligand interaction

INTRODUCTION

Physiological processes depend on highly specific interactions between biological binding partners. It has long been known that proteins such as enzymes, membrane receptors, and antibodies have the ability to discriminate between different stereoisomeric forms of their ligands.^{1–4} As a consequence of this stereoselectivity, the enantiomers of exogenous compounds (e.g., drugs, nutrients, and pesticides) may exhibit dissimilar biological effects.^{5,6} Increasing awareness of the stereoselectivity of drug action has caused regulatory agencies such as the FDA to institute guidelines for the development and testing of stereoisomeric drugs.⁷ Although structural analysis has provided some insight into the molecular basis of the stereoselectivity of catalytically active proteins, that is, enzymes and catalytic antibodies,^{8,9} the cause of stereoselective binding of proteins to ligands in their ground state remains poorly understood. A better comprehension of the structural factors that govern specificity and stereoselectivity would be valuable for the rational design of more effective drugs and the development of tailor-made chiral selectors for the precise determination of bioactive chiral molecules and their purification.

Our current conception of chiral discrimination by proteins is still strongly influenced by the three-point attach-

ment model,^{10,11} first proposed for receptor-ligand interactions by Easson and Stedman in 1933.¹⁰ According to this model, optimal binding requires the interaction between three sites in the receptor (A' , B' , C') and complementary groups (A , B , C) in the ligand. Simultaneous interaction between all three corresponding groups is only possible if the ligand has the "correct" configuration; mirror-image like orientation of A , B , and C on the other hand limits the points of interaction with the receptor to a maximum of two. Thus, the "correct" enantiomer (eutomer) will be bound with a higher affinity and be more active with regard to its physiological effects than its mirror image (distomer). The major drawbacks of the three-point attachment model are the two-dimensionality of the protein binding site, and the notion that all interactions between receptor and ligand are attractive. As has been demonstrated by Fersht and others, however, the relative specificity of a

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certain protein toward two structurally similar ligands is determined by both favorable (attractive) and unfavorable (repulsive) forces, which account for the overall difference in binding energies.^{12,13}

Mesecar and Koshland introduced a four-point location model to explain the binding of two of the four possible stereoisomers of isocitrate to the enzyme isocitrate dehydrogenase.¹⁴ X-ray crystallographic results revealed that three points of interaction between the two stereoisomers and the enzyme are identical, while the location of a fourth group appears to be responsible for chiral discrimination. Although this work clearly illustrates the importance of the three-dimensional arrangement of the potential points of interaction, the generality of the deduced model has been questioned.¹⁵ Although binding of (2*R*, 3*S*)-isocitrate to the enzyme is dependent on the presence of Mg²⁺ ions, the complex of the (2*S*, 3*R*)-stereoisomer and isocitrate dehydrogenase is only formed under metal-free conditions.

Recently, Sundaresan and Abrol proposed a general model for protein stereoselectivity that is based on the number of substrate locations through which each stereogenic center may interact with a receptor-binding site; these interactions can be binding, nonbinding, or even repulsive.¹⁵

A number of techniques have been utilized to examine the interaction of a variety of chiral ligands with their respective protein binding partners. Among these, X-ray crystallography has been used to study not only stereoselective enzymes^{8,14,16–18} but also transport proteins¹⁹ and antibodies.^{9,20,21} More recently, molecular modeling has emerged as a powerful new method to investigate stereoselective protein-ligand interactions. In cases where structural information is available from X-ray crystallography or NMR studies, molecular modeling may be used to gain insight into a protein's ability to discriminate enantiomers,^{22–25} or to predict the effect of structure-guided site-directed mutagenesis in ligand docking and molecular dynamics experiments.^{26,27} If structural information is not readily available, protein modeling can be combined with ligand docking and molecular dynamics to investigate experimentally observed stereoselectivity.^{28,29}

The objective of this study was to elucidate the molecular basis of the stereoselectivity exhibited by the monoclonal anti-D-amino acid antibody 67.36 (anti-D-AA 67.36) using molecular modeling and ligand docking. Although the utility of this antibody for enantiomer detection and separation was previously demonstrated with a number of analytical techniques including immunoassays, biosensors, LC, and LC-MS,^{30–39} fluorescence spectroscopy was employed here to obtain additional information on the antibody's binding characteristics.

MATERIALS AND METHODS

Antibody Production and Characterization

The monoclonal anti-D-amino acid antibody anti-D-AA 67.36 was produced as previously described.³⁰ In brief, 8-wk-old BALB/c mice were immunized with conjugates of keyhole limpet hemocyanin (KLH; Sigma, Rehovot, Israel) *Chirality* DOI 10.1002/chir

and *p*-amino-D- and -L-phenylalanine (Sigma, Deisenhofen, Germany), respectively, prepared by diazotization. The hapten-protein ratios of the azo-proteins were 8 residues per functional protein subunit (51 kDa) for *p*-azo-D-phenylalanine-KLH and 11 residues per subunit for *p*-azo-L-phenylalanine-KLH. Booster injections of immunogens were administered twice at intervals of 2 weeks. Four and 3 days before fusion, final boosts were given intraperitoneally. Splenocytes of mice showing strong immune responses were fused with NS0 myeloma cells using polyethylene glycol. Hybridomas were selected in hypoxanthine/aminopterin/thymidine medium, and supernatants were screened by noncompetitive enzyme-linked immunosorbent assay (ELISA) as described below. Hybridomas producing stereoselective antibodies were cloned at least twice by limiting dilution. Large quantities of the anti-D-amino acid antibody secreted by clone 67.36 (anti-D-AA 67.36) were obtained by the preparation of ascites fluid. The antibody was purified by ammonium sulfate precipitation followed by ion-exchange chromatography on DEAE-Sephacel using a gradient of 0–400 mM NaCl in 10 mM tris(hydroxymethyl)aminomethane (TRIS) pH 8.5 for elution.

Antibody stereoselectivity was first verified in noncompetitive ELISAs on 96-well microtiter plates (Nunc, Rochester, NY) using conjugates of bovine serum albumin (BSA) and *p*-amino-D- or -L-phenylalanine, prepared by diazotization, or underivatized BSA, as solid-phase coatings (100 μ l/well; 1 μ g/ml in 50 mM carbonate buffer, pH 9.6; 14 h at 4°C). Hapten-protein ratios of the BSA conjugates were eight residues per protein molecule (66.43 kDa) for *p*-azo-D-phenylalanine-BSA, and nine residues per molecule for *p*-azo-L-phenylalanine-BSA. Unoccupied adsorption sites in microtiter wells were blocked with 1% gelatin in PBS/0.05% Tween 20 (250 μ l/well; 2 h at 37°C). Antibody samples (100 μ l) in varying dilutions were incubated for 2 h at 37°C. Bound antibody was detected with horseradish peroxidase-conjugated anti-mouse antibody (Jackson, West Grove, PA; 1/10,000 in PBS; 100 μ l/well; 2 h at 37°C) utilizing the enzymatic conversion of *o*-phenylenediamine (Sigma, St. Louis, MO). Absorbance was read in a FLUOstar plate reader (BMG Lab Technologies, Offenburg, Germany) at 492 nm after acidification with 1 N sulfuric acid. Absorbance values obtained on the BSA coating were subtracted from those obtained on the *p*-azo-D-phenylalanine-BSA coating and the *p*-azo-L-phenylalanine-BSA coating, respectively.

In competitive ELISA procedures, antibody (50 μ l/well) at a fixed concentration was incubated together with varying concentrations of inhibitor (50 μ l/well). All other steps were analogous to the noncompetitive test. Absorbance values were converted to % inhibition using the equation: % inhibition = $[1 - (A/A_0)] \times 100$, where *A* represents values in the presence of competitor, while *A*₀ is the absorbance without competitor.

The binding characteristics of anti-D-AA 67.36 were further examined utilizing tryptophan (Trp) fluorescence. Tests were performed in Reacti-Bind White Opaque 96-well Plates (Pierce Biotechnology, Rockford, IL) blocked with 1% gelatin in PBS/0.05% Tween 20 (250 μ l/well; 2 h

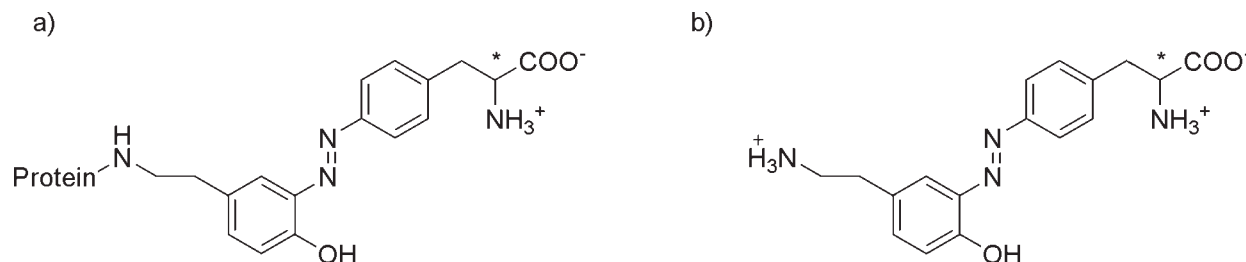


Fig. 1. (a) The immunogen used to raise the stereoselective anti-D-amino acid antibody 67.36 contains an azo-structure linked to a protein carrier. The amino acid functional grouping with the stereogenic center is exposed on the surface and is accessible for interaction with the antibody binding site. (b) Structure of the model ligand used for docking experiments. The ligand comprises a *p*-aminophenylalanine portion linked to tyramine via diazotization to yield the depicted azo-compound.

at 37°C). Samples containing antibody (50 μ l/well) in a fixed concentration and amino acids used as ligand in serial dilutions (50 μ l/well) were preincubated at RT before Trp fluorescence was measured in the FLUOstar plate reader. The excitation wavelength was 280 nm, and emission was detected at 350 nm.

Sequence Analysis of Antibody Heavy and Light Chains

The mRNA encoding the heavy- and light-chain variable domains, respectively, of anti-D-AA 67.36 was isolated from the corresponding hybridoma cells. Hybridoma cells (10^8) were homogenized, and the mRNA was isolated using an oligo(dT) spin column (Qiagen, Valencia, CA). Reverse transcriptase was then used in a first-strand cDNA reaction to create cDNA from the isolated mRNA. The degenerate primers described by Wang et al.⁴⁰ were used for the amplification of antibody heavy (~340 bp) and light (~325 bp) chain DNA. Heavy- and light-chain DNA was ligated into the plasmid pCR2.1 for transfection into *E. coli* Top 10 (Invitrogen, Carlsbad, CA). DNA sequences were determined by the NIU Plant Molecular Biology Laboratory at Northern Illinois University (DeKalb, IL). The nucleotide sequences were analyzed using Sequencher (Gene Codes, Ann Arbor, MI) and translated into protein sequences; open-reading frames were determined using the web-based translation tool on the ExPASy Proteomics Server.⁴¹ The program Basic Local Alignment Search Tool (BLAST)⁴² was utilized to verify the origin of the sequences by comparing them with sequences in the National Center for Biotechnology Information's (NCBI) nonredundant (nr) database. The validity of the sequence was recently confirmed by high-resolution X-ray crystallography (unpublished results). The structures of the most sequence-homologous proteins were retrieved from the Protein Databank (PDB)⁴³ for modeling.

Antibody Modeling

WAM. Web antibody modeling (WAM)^{44,45} was used to model the heavy- and light-chain variable domains of anti-D-AA 67.36. The heavy- and light-chain sequences were aligned according to the complementarity determining region (CDR) and framework (FR) rules on the WAM website. The verification of the sequence alignment and the assignment of the canonical classes were carried out using WAMPredict.⁴⁶ The sequence was modeled using

dead-end elimination for side-chain building and the VFF energy option for screening. General options included database searches only for the noncanonical non-H3 loops as well as the option to use the tentative sequence structure rules for H3 loops.

Swiss model. The variable domains of anti-D-AA 67.36 were also modeled using the SwissModel Project Mode on the SWISS-MODEL website.^{47,48} The template file employed for the alignment of anti-D-AA 67.36 was created using only the variable domains of chain D of PDB 1BLN (for the heavy chain) and chain L of PDB 2BRR (for the light chain), respectively.^{49,50} Two domain structures were combined to create a template structure for alignment with anti-D-AA 67.36 using PyMol (DeLano Scientific, Palo Alto, CA). The template PDB was checked visually for backbone or side-chain clashes. The sequence of anti-D-AA 67.36's variable domains was then aligned to the template structure file in DeepView.⁵¹ Because of the difference in length between CDR-H3 of anti-D-AA 67.36 and CDR-H3 of 1BLN, multiple alignments of the variable domains were modeled separately. Resulting structures were scored using ANOLEA,⁵² GROMOS,⁵³ and Verify-3D,⁵⁴ and the structure with the best overall scores was used as the model for anti-D-AA 67.36.

Modeling of the Model Ligand

PDB files for the D- and L-enantiomers of the model ligand shown in Figure 1b were created using the Dundee PRODRG2 Server.^{55,56} The orientation of the two aromatic rings within the ligands was verified using GaussView 3.0 and Gaussian 03.⁵⁷ The lowest energy structure, which contained a planar azo-moiety, was used for modeling.

Docking

Docking studies were performed with the program Discovery Studios Modeling-SBD 1.2 (DSM 1.2; Accelrys, Burlington, MA). The binding site was determined using LigandFit in DSM 1.2. Monte Carlo-based docking was carried out using a nonflexible ligand and a torsional step size of 30°. The maximum number of poses retained was 10 with thresholds set to 1.5 Å root mean square deviation (RMSD) and an energy difference of 20 kcal/mol. For energy minimization, 10 iterations of rigid body steepest descent minimization and one hundred iterations of rigid

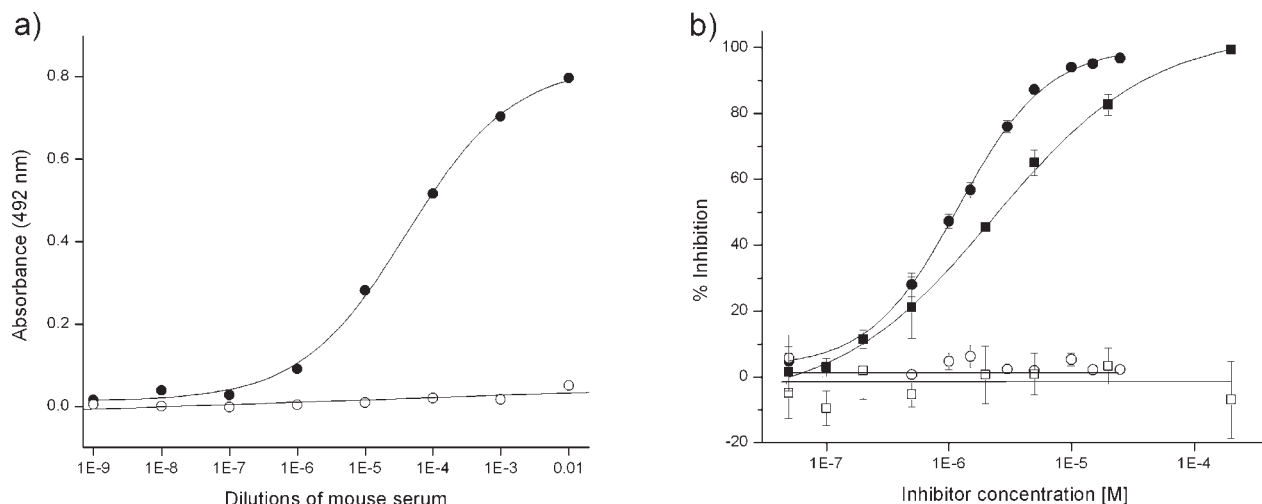


Fig. 2. (a) Immunization of a mouse with *p*-azo-D-phenylalanine-KLH resulted in the production of stereoselective antibodies as verified in a noncompetitive ELISA using serial dilutions of serum on microtiter wells coated with *p*-azo-D-phenylalanine-BSA (●) and *p*-azo-L-phenylalanine-BSA (○), respectively. Results obtained on control-wells coated with underivatized BSA were subtracted. (b) As determined by competitive ELISA, the monoclonal antibody anti-D-AA 67.36 stereoselectively binds to the D-enantiomer of the azo-structure (●) used in varying concentrations as competitor, but not to the L-enantiomer (○). Competitive tests with D-Phe (■) and L-Phe (□) show that the antibody's affinity for the D-enantiomer of Phe is slightly lower compared to the azo-structure. Data points represent means of triplicate determinations. Missing error bars are obscured by symbols. Experimental details are given in Materials and Methods.

body Broyden-Fletcher-Goldfarb-Shanno (BFGS) minimization were performed. An RMSD threshold for the ligand/site match of 2.0 Å was used for shape fitting. No poses were rejected based on the DockScore, and resulting structures were not clustered. The ligand was scored using LigScore,⁵⁸ PLP1,⁵⁹ PLP2,⁶⁰ Jain,⁶¹ PMF,⁶² and Ludi3^{63,64} scoring functions.⁶⁵ All results were inspected for clashes and hydrogen bonds between ligand and modeled protein structures. For docking studies, using the WAM model, the volume of the binding site was varied between 441 and 13,309 Å³, and hydrogen atoms were added to the structure after defining the binding site. Using the SWISS-MODEL structure, the volume of the binding site was varied from 1000 Å³ to 2500 Å³. Docked structures were analyzed using the program Ligplot.⁶⁶ Docking of other amino acid enantiomers used as ligands was performed accordingly.

RESULTS AND DISCUSSION

The stereoselective anti-amino acid antibody investigated in this study was previously produced by first immunizing mice with an immunogen that had been synthesized by coupling the D-enantiomer of the hapten *p*-aminophenylalanine to the protein-carrier KLH via diazotization.³⁰ This reaction links the hapten primarily to tyrosine residues on the protein, thus forming an azo-structure with the free α -amino acid functional grouping being exposed on the surface of the immunogen (Fig. 1a). Diazotization is a well-established method for the synthesis of hapten-carrier conjugates and has been used for the production of antibodies since the late 1920s^{3,67}; since then, azo-structures are known to elicit good immune responses in animals. Immunization of mice with the KLH conjugate of *p*-amino-D-phenylalanine resulted in the formation of

highly stereoselective antibodies as tested by ELISA (Fig. 2a). The monoclonal antibody anti-D-AA 67.36 was produced by the fusion of mouse splenocytes with myeloma cells following standard protocols. Anti-D-AA 67.36, which is of the immunoglobulin G1 (IgG1) subclass, was also found to display exquisite stereoselectivity for the azo-structure as determined in competitive ELISA experiments (Fig. 2b). The IC₅₀ value (i.e., the concentration causing 50% inhibition) deduced from such assays indicated that the D-enantiomer is bound with an affinity in the low-micromolar range; the L-enantiomer, on the other hand, is not bound by the antibody. Although the antibody exhibits strongest binding to the D-enantiomer of the azo-structure, additional competitive ELISAs showed that it also binds stereoselectively to structurally different α -amino acids. As expected, anti-D-AA 67.36 binds more strongly to D-amino acids having aromatic or bulky side chains than to aliphatic amino acids. For example, aromatic amino acids such as D-Phe (Fig. 2b), D-Tyr, and *p*-amino-D-Phe are bound with slightly weaker affinities than the azo-structure (i.e., with low micromolar affinities), while the interaction of the antibody with the D-enantiomers of aliphatic amino acids is weaker by several orders of magnitude and falls into the high micromolar to millimolar range. However, regardless of the side chain, interaction with L-amino acids is negligible. The binding characteristics of anti-D-AA 67.36, which have also been confirmed with other analytical techniques using this antibody as chiral selector,^{31,34,38} thus, are overall comparable to those previously described for polyclonal anti-D-amino acid antibodies.^{30,68}

To elucidate the molecular basis of the exquisite stereoselectivity exhibited by anti-D-AA 67.36, it was necessary to first determine the amino acid sequence of its binding site. IgGs are composed of four polypeptides, two identical "heavy chains" and two identical "light chains," which

Antibody Light Chains (Kappa)

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<-----FR1-----><-----CDR-L1-----><-----FR2-----><-----CDR-L2-----><-----FR3----->
AHO 1 10 20 30 40 50 60 70 80 90 100
67.36 DIVLTQTAIMSASPWEKVTMTCSRAS--SSVS-----SSYLHWYQKSGASPKLWIYS-----TSNLASGVPARFSGSGSG--TSYSLTISSEVEDAATYYC

<-----CDR-L3-----><-----FR4----->
AHO 110 120 130 140 149
67.36 QQYSG-----TPLTFGAGTKLELKR

```

Antibody Heavy Chains

```

<-----FR1-----><-----CDR-H1-----><-----FR2-----><-----CDR-H2-----><-----FR4----->
AHO 1 10 20 30 40 50 60 70 80 90 100
67.37 EVQLEES-GGGLVKPGGSLKLSCAASG-FTFSN-----YDMSWVRQTPEKRLIEWVTSISSGG-----YTYYPDSVKGRFTISRDNVRNLYLQMSSLRSED

<-----CDR-H3-----><-----FR4----->
AHO 110 120 130 140 149
67.36 TAMYYCARALYDYT-----TSPWFAYWGQGLTVTVS-

```

Fig. 3. Sequence alignments of the heavy- and light-chain variable domains of anti-D-AA 67.36 according to the AHO-numbering scheme. The beginning and end of each CDR and FR are marked. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

form an overall Y-shaped molecule. An IgG antibody comprises two binding sites, each of which is formed by three loops within the variable domain of a light chain and three loops within the variable domain of a heavy chain. Although most of an antibody's structure is highly conserved (i.e., it is homologous with other antibodies, which typically exhibit different specificities), the sequence and three-dimensional structure of its binding site has a higher degree of variability and is decisive for its distinctive binding properties. The highest degree of sequence variability is found within the aforementioned six loops, the so-called complementarity determining regions (CDRs); it is via amino acids in these loops that an antibody specifically interacts with a binding partner. The CDRs are flanked by relatively conserved framework regions (FRs). The exact location of the CDRs and FRs within an antibody sequence depends on the positioning of key conserved residues found in all antibodies; these key residues, which are located at the beginning and the end of each CDR, are important reference points in the various numbering schemes that have been proposed to unambiguously identify each amino acid position within an antibody sequence.^{45,69–73} To determine the sequences of the heavy and light chains of anti-D-AA 67.36, the mRNA of antibody-secreting hybridoma cells was isolated and converted into cDNA employing reverse transcriptase. Degenerate primers were then used to amplify the sequences encoding the antibody's heavy and light chain, respectively, and their amino acid sequences were deduced from the nucleotide sequences as determined by DNA sequencing. The basic local alignment search tool (BLAST)⁴² was applied to confirm that the sequences indeed encode for antibody chains and to identify homologous sequences. The sequence most homologous to the heavy chain of anti-D-AA 67.36 was found to be that designated as 1BLN (E value 4×10^{-44} , bit score 179), whereas the sequence most homologous to its light chain was 2BRR (E value 5×10^{-34} , bit score 145); the overall sequence identities were 82 and 93%, respectively. Crystal structures of both of the corresponding antibodies had previously been determined^{49,50} and are available at the PDB website.⁴³

Once the sequences of the heavy and light chains of anti-D-AA 67.36 were confirmed to be antibody sequences, the variable regions had to be identified and numbered. Although several numbering schemes for immunoglobulin variable domains have been proposed, we primarily used the AHO numbering scheme by Honegger and Plückthun.⁷³ Figure 3 shows the sequences of the heavy- and light-chain variable domains, respectively, of anti-D-AA 67.36 according to this numbering scheme.

The sequence information was used to predict the three-dimensional structure of the antibody by protein modeling. In general, two different approaches may be utilized to model protein structures—homology modeling and ab initio modeling. In homology modeling, a likely protein structure may be found based on comparison with known structures of proteins that have similar (i.e., homologous) sequences. Because the FRs in the variable domains of antibodies are well conserved, they can be readily modeled using sequence-homologous structures.⁷⁴ In addition, five of the six CDRs (CDRs-L1-3, CDR-H1, and CDR-H2) have been found to assume only a limited number of possible backbone conformations, the so-called canonical classes, which can typically be modeled based on homology. The canonical classes depend on the length of the individual loops and the nature and presence of certain residues within the CDR and FR, respectively.⁷⁵ Because of its considerable variability with regard to overall length and amino acid composition, CDR-H3 is the only noncanonical CDR and is generally less amenable to homology modeling. However, the energetically most favorable structure of CDR-H3 may be found based on energy-minimization ab initio calculations, which are also useful for the determination of side-chain conformations or structures of sequences for which homologous structures are not available.

Here, two separate modeling programs, WAM^{44,45} and Swiss-Model,^{47,48} were used to model the structure of anti-D-AA 67.36. WAM models the backbone and side chains of the FRs and the backbone and side chains of conserved residues within the five canonical CDRs using the most homologous structures; due to the length of CDR-H3 its

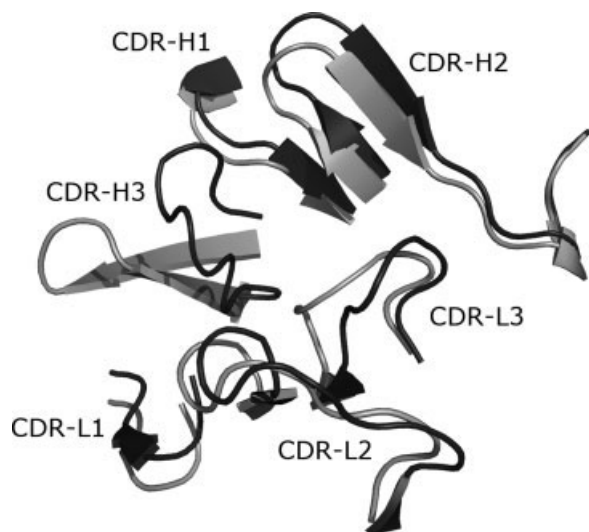


Fig. 4. Overlay structures of the variable heavy- and light-chain domains of anti-D-AA 67.36 as modeled with WAM (light gray) and SWISS-MODEL (black). Although both structures overall show a high degree of complementarity, differences in the conformation and orientation of CDR-H3 are evident.

backbone is modeled using CAMAL.^{72,76} Side chains of nonconserved residues in the CDRs are modeled using the dead-end elimination algorithm. The modeled structure is then screened using the valence force field energy option.⁷⁷

The structure of anti-D-AA 67.36 created by WAM is shown in Figure 4 in light gray color. The CDRs fall into the following canonical classes: L1 is class 7, while L2, L3, H1, and H2 each fall into class 1. The CDR-H3 loop contains 13 residues and was modeled as a long hair pin type structure protruding away from the binding site. Very few residues of CDR-H3 appear to be involved in the overall binding site topography. Because numerous crystallographic studies have shown that CDR-H3 typically plays a significant role in the interaction of antibodies with low-molecular weight binding partners,⁷⁸ the accuracy of the WAM-modeled CDR-H3 of anti-D-AA 67.36 has to be questioned.

In an attempt to overcome the drawbacks of the WAM model, SWISS-MODEL was used to determine the structure of anti-D-AA 67.36. SWISS-MODEL is a web-based homology-modeling site that can be used to model sequences of interest upon manually prealigning them with a template; alternatively, the sequence can be modeled based on an automated alignment. Because the automated alignment allows modeling of only one domain at a time, it is not possible to model both the variable heavy- and variable light-chain domains of antibodies simultaneously. Therefore, the sequences of the variable domains of anti-D-AA 67.36's heavy and light chains were manually aligned with the corresponding sequences of 1BLN and 2BRR, respectively.

Although in SWISS-MODEL, the backbone atom positions are averaged based on homology,^{47,48} regions containing amino acid additions or deletions cannot be modeled

using the template structure. Because the CDR-H3 of anti-D-AA 67.36 is three residues longer than the template sequence (13 residues vs. 10), the respective part of the sequence was modeled by constraint space modeling (CSP). CSP is a function of SWISS-MODEL, which determines the position of individual amino acid residues depending on neighboring residues. Upon creation of the backbone structure, the side chains of the template's amino acids were replaced with the side chains of anti-D-AA 67.36, and the most probable position of individual side chains was determined by comparison with backbone-dependent rotamer libraries. As a final step, the modeled structure of anti-D-AA 67.36 was energy minimized using GROMOS96.⁵³ Analysis of the modeled structure of anti-D-AA 67.36 (shown in black in Fig. 4) using Verify3D, GROMOS, and ANOLEA gave satisfactory results overall. Only the sequence SPWF within CDR-H3 gave somewhat less satisfactory results in GROMOS and ANOLEA, which is probably based on the fact that the 1BLN template has two residues (SP) less in this region. Thus, neither the backbone nor the side chains of these two residues of anti-D-AA 67.36 could be modeled based on homology but needed to be generated by CSP.

To directly compare the two structures of anti-D-AA 67.36 modeled by WAM and SWISS-MODEL, respectively, they were overlaid in PyMol (see Fig. 4); the overall RMSD value was determined to be 0.998 Å. A comparison of the modeled FRs gave an RMSD value of 0.766 Å. The RMSD values for the individual CDRs are as follows: CDR-L1 0.630 Å; CDR-L2 0.392 Å; CDR-L3 1.705 Å; CDR-H1 0.092 Å; CDR-H2 0.376 Å; CDR-H3 3.861 Å.

In general, RMSD values of 1 Å or less are considered to be indicative of high structural similarity, while an RMSD value greater than 6 Å correlates with a low degree of similarity.⁷⁹ Thus, the two structures are overall very similar, and the two programs primarily differ in the way they model the CDR-H3. As mentioned earlier, the WAM structure shows CDR-H3 as a long hairpin containing a single β -turn that points outward away from the binding site. In contrast, the CDR-H3 modeled by SWISS-MODEL is folded inward and is positioned in such a way that it participates in the formation of the binding site. The apex of the loop assumes a backbone conformation resembling the letter "W;" this structure is stabilized by a hydrogen bond between Y113 of the heavy chain (Y113H) and S132H.

Both the WAM and the SWISS-MODEL structures were used in molecular-docking experiments to investigate the stereoselectivity of anti-D-AA 67.36. As a model ligand, the low-molecular weight azo-compound shown in Figure 1b was used. This compound is analogous to the structure the antibody was raised against (Fig. 1a) and is bound stereoselectively by anti-D-AA 67.36 as shown in competitive ELISA experiments. The three-dimensional structures of both the D- and the L-enantiomer of the ligand were created using the Dundee PRODRG2 server and optimized with Gaussian 03.⁵⁷ Molecular-docking studies were performed with both enantiomers using DSM 1.2. DSM 1.2 follows the basic progression of molecular-docking programs, that is, first a protein's binding site is defined, then

the ligand position and orientation in this site are optimized, and finally the docked structure is evaluated utilizing different scoring functions.

DSM 1.2 identified one possible binding site within the modeled WAM structure that was formed by the six CDRs and had a volume of $441 \text{ \AA}^3$. However, after adding hydrogen atoms to the modeled structure, DSM 1.2 was unable to expand the site, and all attempts to dock either enantiomer of the ligand failed. Because it is known from experimental results that anti-D-AA 67.36 does bind the ligand in a stereoselective manner, it was concluded that the WAM structure was not correct.

A single binding site formed by the six CDRs was also found within the SWISS-MODEL structure. This site could be expanded upon addition of hydrogen atoms, and the D-enantiomer of the ligand was successfully docked to the site with a volume of $1178 \text{ \AA}^3$. At this stage, DSM 1.2 identified four hydrogen bonds between the ligand and amino acid residues in the binding site. One is between the hydroxyl group of Y40 of the light chain (Y40L) and the hydroxyl group of the tyramine portion of the ligand (H-donor-acceptor distance $1.61 \text{ \AA}$), two are between the backbone carbonyl of S132H and the ligand's α -amino group (H-donor-acceptor distances 1.10 and $2.29 \text{ \AA}$, respectively), and one is between the phenol hydroxyl group of Y44L and the ligand's α -carboxyl group (H-donor-acceptor distance $2.42 \text{ \AA}$). Expansion of the binding site above $2500 \text{ \AA}^3$ allowed docking of the ligand in an unlikely orientation, so that for all further docking experiments the binding site was kept at values between 1000 and $2500 \text{ \AA}^3$. Because the antibody was raised against a hapten-KLH conjugate that has the amino acid functional grouping being exposed on the surface of the immunogen, it has to be assumed that it is this part of the hapten that is facing the antibody binding site; the moiety through which the hapten is attached to the carrier protein (i.e., tyrosine residues in the KLH or the analogous tyramine portion of the model ligand), however, needs to be oriented away from the antibody binding site to avoid steric hindrance. Using the same docking parameters, the L-enantiomer of the model ligand could also be docked, albeit with only two hydrogen bonds between ligand and antibody. These are the hydrogen bonds between the backbone carbonyl of S132H and the ligand's α -amino group (H-donor-acceptor distances 1.89 and $1.93 \text{ \AA}$, respectively). Because this finding is not consistent with the experimental results, which do not show any evidence for the L-enantiomer being bound by anti-D-AA 67.36, the binding site was further optimized by taking into account localized side-chain flexibility. This was accomplished by investigating possible rotamer conformations of the binding site residues Y40L, H42L, Y44L, Q107L, Y109L, L137L, S132H, W134H, and F135H utilizing the rotamer library and mutagenesis function in PyMol. A random combination of all possible rotamers that did not result in any significant side-chain clashes yielded five structures, which allowed stereoselective docking of the ligand. The best results were obtained with the structure containing different rotamers of Y40L and L137L (designated anti-D-AA 67.36_rotamer_Y40L-L137L). It is noteworthy that side-chain movements within

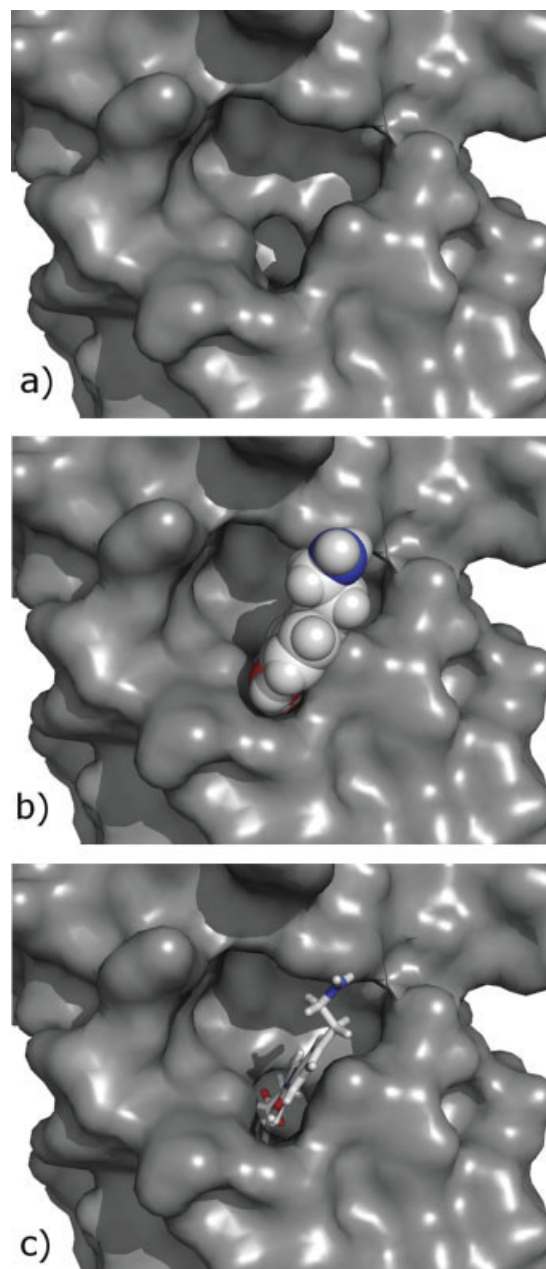


Fig. 5. Surface contour images of the modeled anti-D-AA 67.36_rotamer_Y40L-L137L structure (a) without the ligand, (b) with the docked ligand in a spherical representation, and (c) with the docked ligand in a stick representation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

binding sites are common upon ligand binding, typically involving three residues or less.⁸⁰

Docking experiments using the anti-D-AA 67.36_rotamer_Y40L-L137L structure with a defined binding site volume of $1200 \text{ \AA}^3$ resulted in stereoselective binding of only the D-enantiomer of the ligand in the presumed correct orientation. No other docking results were obtained. A large portion of this ligand is buried in the cavity of the binding site, resulting in a good fit between protein and ligand (see Fig. 5). DSM 1.2 identified three hydrogen bonds;

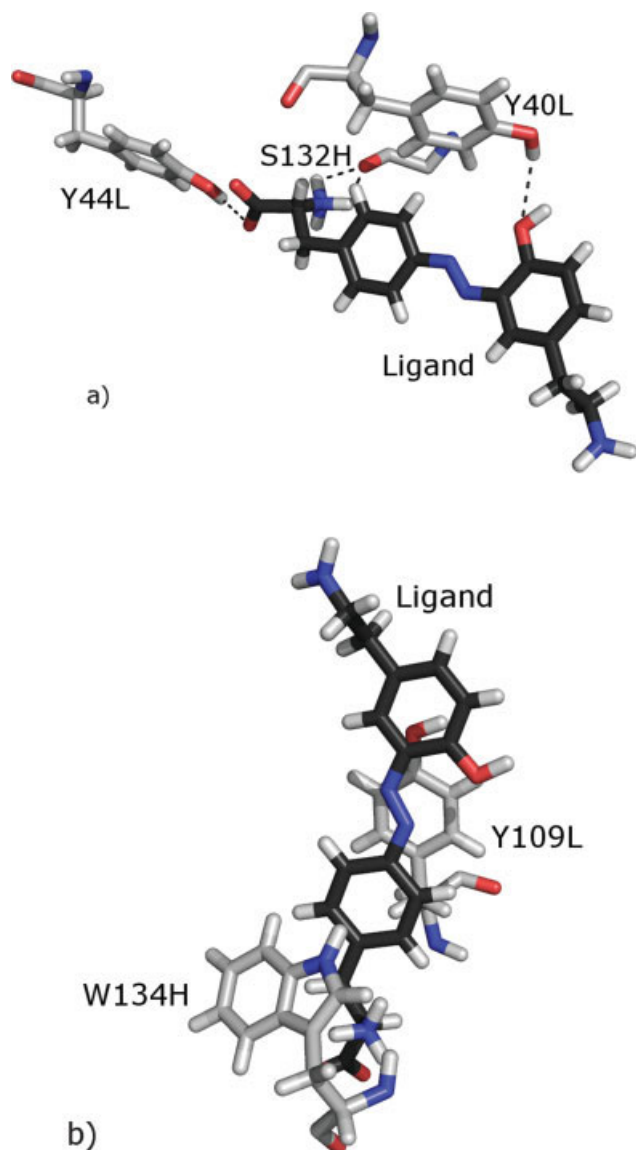


Fig. 6. Antibody-ligand interactions as predicted by DSM 1.2 and Ligplot. The ligand is shown in black; nitrogen and oxygen atoms are shown in blue and red respectively. (a) The binding site residues Y40L, Y44L, and S132H form hydrogen bonds with appropriate functional groups on the ligand. (b) The binding site residues W134H and Y109L interact with the aromatic side chain of the ligand through π - π stacking. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

one is between the phenol hydroxyl group of Y44L and the ligand's α -carboxyl group (H-donor-acceptor distance 2.33 Å), and two are between the backbone carbonyl of S132H and the ligand's α -amino group (H-donor-acceptor distances 1.59 and 2.29 Å, respectively). Ligplot analysis of the antibody-ligand complex indicated the formation of hydrogen bonds between S132H and the α -amino group of the ligand (H-donor-acceptor distance 2.22 Å) and between the phenol hydroxyl group of Y40L and the hydroxyl group of the tyramine moiety of the ligand (H-donor-acceptor distance 3.26 Å). Predicted hydrophobic contacts between ligand and antibody involved residues

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W134H, F135H, H42L, Y109L, and L137L. Y109L and W134H interact through π - π stacking with the ligand, which is sandwiched between these two residues. Although the modeled structure does not position W134H perfectly parallel to the ligand, the degree of tilting falls well within the range of commonly observed orientations of stacked aromatic residues.⁸¹ Figure 6 displays the points of interaction between the D-enantiomer of the model ligand and the anti-D-AA 67.36_rotamer_Y40L-L137L structure that were proposed by DSM 1.2 and Ligplot. The LUDI score^{82,83} for the interaction between the D-enantiomer of the ligand and the anti-D-AA 67.36_rotamer_Y40L-L137L structure was 532; this corresponds to an equilibrium dissociation constant of about 4.8 μ M, which, based on experimental results, is reasonable.

Docking of the D-enantiomer of the ligand to the SWISS-MODEL structure overall occurs in the same area within the antibody-binding site regardless of the rotamer conformation of Y40L and L137L. However, while the position of the amino acid functional grouping is not significantly changed in the anti-D-AA 67.36_rotamer_Y40L-L137L structure (when compared with the original structure), the ligand's azo-ring system is moved by about 1.5 Å (see Fig. 7).

To rationalize the stereoselectivity of anti-D-AA 67.36, the L-enantiomer of the model ligand (which could not be docked to anti-D-AA 67.36_rotamer_Y40L-L137L) was manually superimposed onto the D-enantiomer docked to the binding site using the program PyMol. As shown in Figure 8, the hydroxyl group of the tyramine moiety of the ligand clashes with the phenol ring of residue Y40L at the entrance of the binding site; in addition, more clashes occur deeper inside the binding pocket, in which the α -amino group interferes with the indole ring of W134H and the phenyl side chain of F135H. Thus, it appears that residues Y40L, W134H, and F135H all cause severe steric

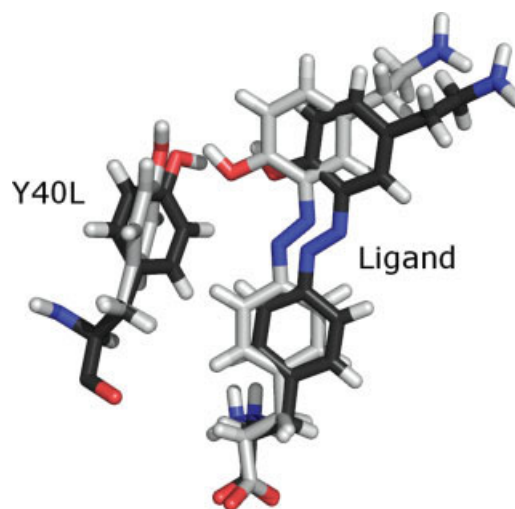


Fig. 7. Overlay structures showing the position of the D-enantiomer of the model ligand in the original SWISS-MODEL structure of anti-D-AA 67.36 (gray) and in the anti-D-AA 67.36_rotamer_Y40L-L137L structure (black). Note the different orientation of the binding site residue Y40L relative to the ligand. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

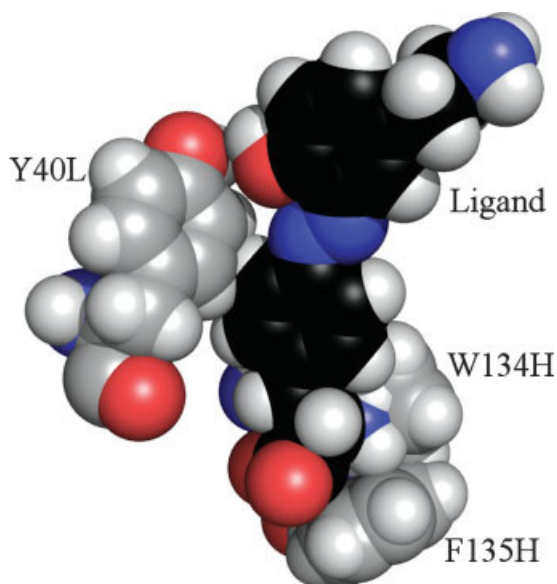


Fig. 8. Manually placing the L-enantiomer of the model ligand in the antibody binding site in an orientation analogous to that of the D-enantiomer, it becomes obvious that clashes between the ligand and the protein occur. The representation was created using PyMol. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

hindrance and prevent the L-enantiomer of binding to the anti-D-AA 67.36_rotamer_Y40L-L137L structure.

Additional docking experiments were performed with the aromatic ligand D-phenylalanine and the aliphatic ligand D-norvaline to test whether such compounds could dock with orientations similar to that of the azo-structure and whether predicted affinities are comparable with the experimental results obtained with these amino acids. As shown in Figure 9, all three ligands are positioned in the binding site in such a way that their stereogenic center is basically superimposable; furthermore, the phenyl side chain of D-phenylalanine overlays with the corresponding moiety of the azo-structure and is sandwiched between residues Y109L and W134H of the binding site. The LUDI score for the interaction between D-phenylalanine and the anti-D-AA 67.36_rotamer_Y40L-L137L structure was 504,

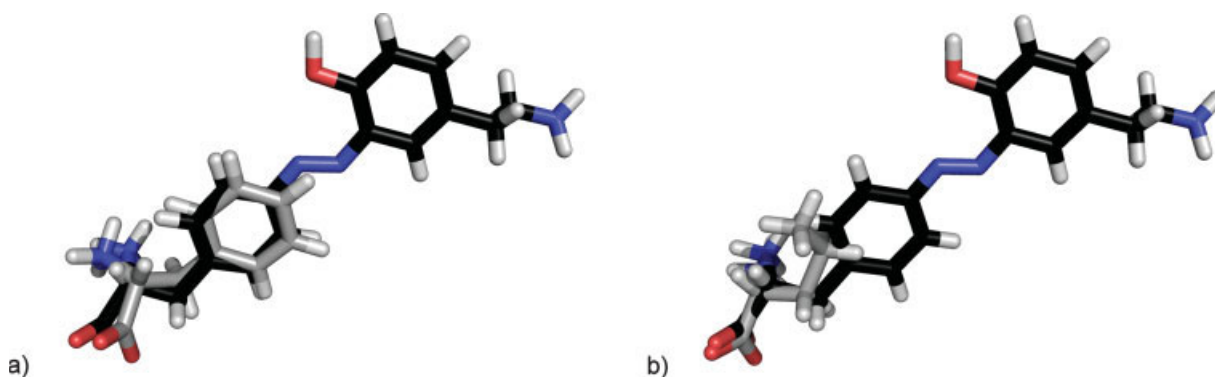


Fig. 9. Docking results obtained with (a) D-phenylalanine and (b) D-norvaline (both in gray) were overlaid with the D-enantiomer of the azo-structure (black) bound to anti-D-AA 67.36 (binding-site residues omitted). The orientation of all ligands is very similar, and the position of the stereogenic center is practically identical. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

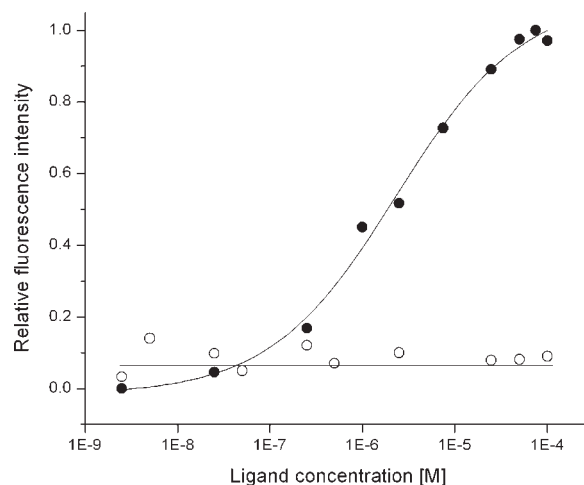


Fig. 10. Binding of D-phenylalanine (●) to anti-D-AA 67.36 causes a concentration-dependent enhancement of the protein's intrinsic Trp fluorescence, while no such effect is observed using L-phenylalanine (○) in varying concentrations. Experimental details are given in Materials and Methods.

while that for D-norvaline was 222; these values correspond to equilibrium dissociation constants of about 9 μ M and 6 mM, respectively, which seems reasonable.

Because computer modeling and docking experiments indicated the presence of a Trp residue in the binding site of the antibody, fluorescence spectroscopy experiments were performed to provide additional experimental evidence to support the computer-modeling results. Fluorescence spectroscopy, which has been widely used to investigate protein-ligand interactions,⁸⁴ is based on the change of the quantum yield of either the ligand or the protein upon complex formation. In cases where Trp residues are located in or near the binding site of a protein, ligand binding can either quench or enhance the protein's intrinsic fluorescence, which can be followed at emission wavelengths around 350 nm. Binding curves obtained with increasing concentrations of ligand can be used to obtain, for example, affinities. Here, anti-D-AA 67.36 was incubated with a number of amino acid enantiomers in varying concentrations, and the protein's intrinsic Trp fluorescence

TABLE 1. EC₅₀ values determined with anti-D-AA 67.36 and various amino acids using fluorescence spectroscopy

General structure		
		
Amino acid	Side chain R	EC ₅₀
D-Phenylalanine (a)		1.4 μM
D-Cyclohexylalanine (b)		9.1 μM
D-Histidine (c)		219.4 μM
D-Norleucine (d)		355.7 μM
D-Leucine (d)		1.8 mM
D-Norvaline (d)		3.2 mM

EC₅₀ values represent means of triplicate determinations. Fifty percent inhibition was not reached with the opposite enantiomer using maximum concentrations as follows: (a) 0.5, (b) 5, (c) 10, and (d) 20 mM.

upon excitation at 280 nm was detected at 350 nm. As exemplified in Figure 10 for the interaction of anti-D-AA 67.36 with the enantiomers of Phe, binding of the D-enantiomer of amino acids caused a concentration-dependent increase in intrinsic Trp fluorescence, while no such effect was observed with L-amino acids. Table 1 summarizes the affinities, expressed as EC₅₀ values (i.e., the concentration needed to obtain 50% of the maximal response), of anti-D-AA 67.36 toward a number of D-amino acids investigated by fluorescence spectroscopy. The results obtained confirm that the antibody binds more strongly to aromatic and bulky amino acids than to aliphatic ligands; the fact that the absolute affinities are in the same range as those predicted from docking experiments furthermore corroborates the validity of the modeled results. Differences between absolute and predicted affinities are to be expected and may be based on the inability of computer models to take into account, for example, the participation of water molecules or ions in the interaction between protein and ligand. Although proteins represent the structurally most diverse group of biological macromolecules, it has been found that, based on the amino acid sequence, only a limited number of preferred secondary and super-secondary structures are formed in the folding process.^{85,86} As the number of protein structures deposited into databanks constantly increases, it becomes more feasible to identify sequence-homologous proteins useful for homology-based modeling of proteins of interest. Because a large portion of their structure is conserved, antibodies are particularly amenable to homology modeling. However, because of its sequence variability and flexibility, *Chirality* DOI 10.1002/chir

modeling of the CDR-H3 loop within the binding site remains challenging. In this study, the availability of homologous structures of both the variable heavy- and light-chain domains of anti-D-AA 67.36 greatly facilitated modeling of the binding site of this stereoselective antibody. Because antibodies are well known to undergo conformational changes upon ligand binding,^{78,87,88} it is advisable to consider protein flexibility in the modeling process. Here, the incorporation of side-chain flexibility allowed us to create a structure with which the experimentally observed high degree of stereoselectivity of anti-D-AA 67.36 can be rationalized.

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Enantioselective HPLC of Potentially CNS-Active Acidic Amino Acids with a Cinchona Carbamate Based Chiral Stationary Phase

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ABSTRACT A *tert*-butylcarbamoylquinine-based chiral stationary phase (Chiralpak QN-AX) has been employed for the enantiomer separation of underivatized chiral acidic amino acids, viz. 4-carboxyphenylalanine (4-CPHE), 1-aminoindan-1,5-dicarboxylic acid (AIDA), 2-(5-carboxy-3-methyl-2-thienyl)glycine (3-MATIDA), 2-(4-carboxy-5-methyl-2-thienyl)glycine (5-MATIDA), and 2-(2'-carboxy-3'-phenylcyclopropyl)glycine (PCCG). Some of the acidic amino acids have potential activity on the central nervous system and are thus of great interest. A stereoselective HPLC method that allows the baseline resolution of all the five test solutes has been developed. For that purpose the mobile phase composition (pH, organic modifier, and type) and flow rate were optimized. The final method makes use of mild elution conditions, namely methanol – 0.8 M ammonium acetate buffer (97.5:2.5; v/v) pH 5.5 which are also compatible with mass spectrometric detection. *Chirality* 20:571–576, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: chiral separation; HPLC; *tert*-butylcarbamoylquinine; chiral stationary phase; Chiralpak QN-AX; 4-carboxyphenylalanine (4-CPHE); 1-aminoindan-1,5-dicarboxylic acid (AIDA); 2-(5-carboxy-3-methyl-2-thienyl)glycine (3-MATIDA); 2-(4-carboxy-5-methyl-2-thienyl)glycine (5-MATIDA); 2-(2'-carboxy-3'-phenylcyclopropyl)glycine

INTRODUCTION

Quinine-based chiral stationary phases (QN-CSPs) (Fig. 1) successfully provide the direct HPLC enantioseparation of N-protected amino acids, peptides, and other different types of chiral acidic compounds of pharmaceutical relevance.^{1–4} Various kinds of protecting groups (e.g. FMOC, Boc, Z) or N-labeling reagents (Sanger's reagent, NBD-F) can be successfully used.^{5,6} However, in general N-3,5-dinitrobenzoyl (DNB) derivatives usually give the best enantiodiscrimination for amino acids with primary amino groups.⁷ High stereoselectivity can be achieved for N-derivatized amino acids both in the reversed-phase or polar organic mode using buffered hydro-organic or purely organic acid/base-containing eluents.

For these anion-exchange type CSPs, ionic interactions with complementary charged chiral analytes represent the main driving forces of the enantiorecognition process. These attractive intermolecular interactions have to be accompanied by other interactions such as hydrogen bonds, π - π -interactions, and van der Waals type interactions between the two binding molecular species (selector and analyte) to support the stereodiscrimination process.^{1–4}

On the contrary, the enantiomer separation of free amino acids with nonderivatized (nonprotected) amino groups is much more challenging. LC methods of choice

for this task make use of teicoplanin or teicoplanin aglycone CSPs (Chirobiotic T and TAG, respectively),^{8–11} chiral ligand exchange chromatography systems,^{12–15} or chiral crown ether CSPs.^{16–18} Underivatized amino acids endowed with specific physicochemical features such as the presence of an additional ionizable group and/or particularly bulky portions can also be resolved on quinine and quinidine carbamate CSPs (Chiralpak QN-AX and QD-AX, respectively). However, there is only one example reported in the literature, viz the enantiomer separation of underivatized thyroxine and triiodothyronine.¹⁹ An important prerequisite for successful enantiorecognition is the interaction capability of the amino acid side chain.

In this study, we have developed an enantioselective method for the chiral discrimination of some potentially CNS-active acidic amino acids (Fig. 2) with a cinchona carbamate based chiral stationary phase.

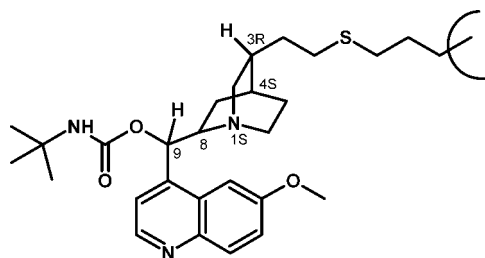
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CHIRALPAK® QN-AX: (8*S*,9*R*) Quinine derived
(Chiral Technologies Europe)

Fig. 1. Structure of the employed CSP.

EXPERIMENTAL

Materials

With the exception of 4-CPHE (4-carboxyphenylalanine) which was a research sample from our laboratory all the remaining compounds, viz 1-aminoindan-1,5-dicarboxylic acid (AIDA),^{20,21} 2-(5-carboxy-3-methyl-2-thienyl)glycine (3-MATIDA),²² 2-(4-carboxy-5-methyl-2-thienyl)glycine (5-MATIDA) [unpublished], (2*S*,1'*S*,2'*S*,3'*S*)- and (2*R*,1'*R*,2'*R*,3'*R*)-2-(2'-carboxy-3'-phenylcyclopropyl)glycine (PCCG)²³ were kindly provided by Prof. Roberto Pellicciari of the Department of Chemistry and Technology of Drugs at the University of Perugia, Italy.

The enantioselective column employed in the present study was a prototype of the commercially available Chiralpak QN-AX column (Fig. 1) (from Chiral Technologies Europe, Illkirch, France) (120 Å pore size, 5 µm particle diameter and column dimension of 150 × 4 mm I.D.). Its selector density was about 0.36 mmol/g CSP.

Acetonitrile (MeCN) and methanol (MeOH), both of HPLC grade were obtained from Fisher Scientific (Loughborough, UK). Glacial acetic acid (AcOH) and ammonium acetate (NH₄AcO) were purchased from Fluka (Buchs, Switzerland). The eluent pH was measured and adjusted in the hydro-organic mobile phase mixture and thus represents the apparent pH.

Chromatographic Instrumentation

All the measurements have been performed on a LaChrom Merck Hitachi HPLC equipped with a quaternary gradient pump L-7150, programmable autosampler L-7250, a diode array detector L-7455, an interface D-7000 (all from VWR International, Vienna, Austria). The data acquisition and processing of the chromatograms was carried out with Merck Hitachi Model D-7000 Version 4.0 chromatography data station software. The HPLC column was kept at a constant temperature of 25°C with a column thermostat from W.O. Electronics (Langenzersdorf, Austria). Unless otherwise stated, the flow rate was 1.0 ml/min. The detection wavelength was set at 250 nm.

RESULTS

Mobile phase composition (buffer type and concentration, pH, type and percentage of organic component, ionic strength, etc.) can drastically affect the enantioselective selector-analyte association thus influencing the overall chro-

matographic performances.^{19,24,25} The most important parameters in the present ion-exchange system are pH and buffer concentration which control the actual ion-exchange capacity and thereby retention. Reversed-phase mode (buffered hydro-organic eluents with MeOH and MeCN as organic modifiers), which may smoothly change to a hydrophilic interaction chromatography (HILIC) mode at high organic modifier content, has been adopted. While the common highly aqueous reversed-phase media may favor hydrophobic interactions, these are largely off-set at high organic content while ionic interactions, hydrogen bonding, dipole-dipole interactions are strengthened owing to the reduced dielectric shielding in media with lower dielectric constants.

Effect of eluent pH

It is well known that in an ion-exchanging environment, the eluent pH heavily influences the chromatographic behavior of ionizable analytes.^{19,26}

Over the pH-range investigated presently (5.0–7.0), the quinuclidine ring is protonated throughout and thus the WAX-type CSP has a reasonable anion-exchange capacity. Moreover, in this pH range also the carboxylic acid functionalities of the analytes (*pK_a*-values of COOH groups < 4.6; *pI* values of the acidic amino acids range between ~4.6 and ~5.6) are ionized thus enabling attractive electrostatically driven selector-solute interactions. To fine-tune the pH of the eluent, the impact of pH on retention and enantioselectivity was evaluated employing methanol-0.1 M ammonium acetate buffer (80:20; v/v) as mobile phase. As a confirmation, the above two chromatographic parameters were found to be affected by varying the eluent acidity as follows.

Figure 3 displays the effect of the eluent pH on retention (*k*), enantioselectivity (*α*), and resolution (*R_S*) for the whole set of investigated compounds. Retention (*k*) of both of the enantiomers was found to increase as the eluent pH was reduced from 7.0 to 5.5 and then decreased with further lowering of pH (Fig. 3a). This means that maximal retention is observed close to the isoelectric point of the analytes. This general trend is observed for all investigated solutes. (Note, however, the inversion of elution order for 3-MATIDA and 5-MATIDA (*S* before *R*) in relation to the other test compounds (*R* before *S*). For

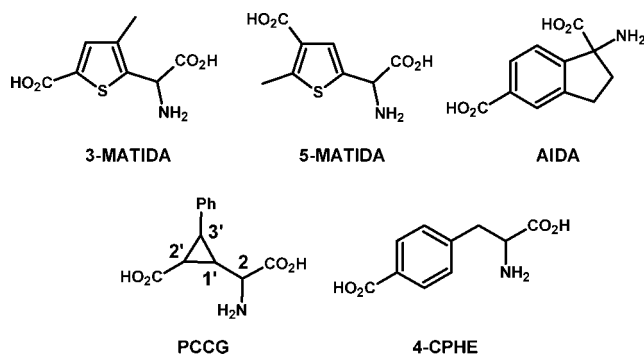


Fig. 2. Structure of the analytes investigated.

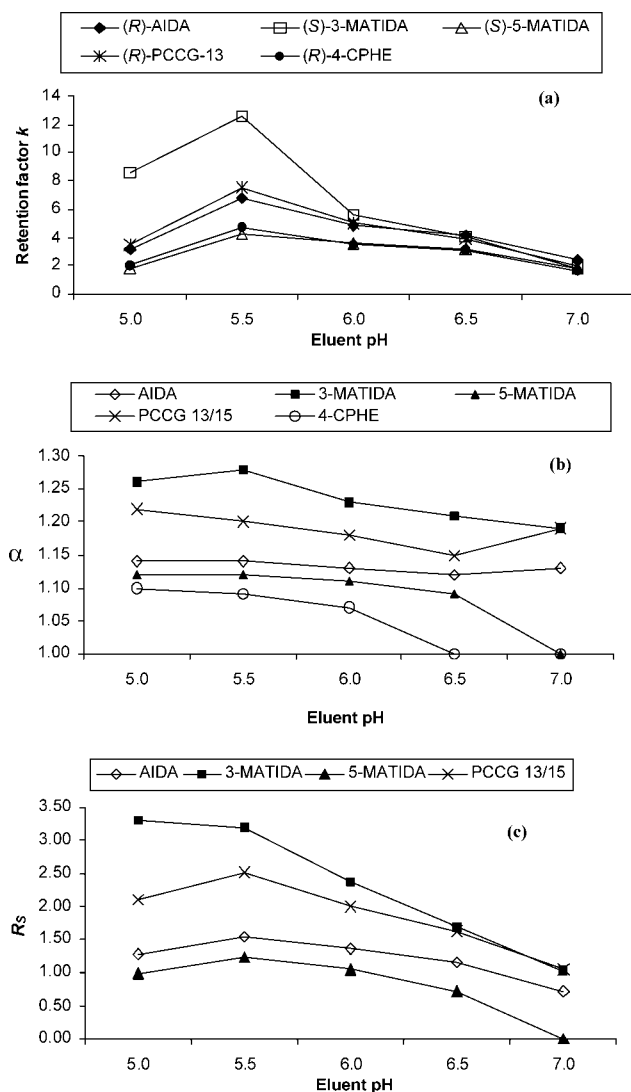


Fig. 3. Effect of eluent pH on (a) retention (k) of the first eluted peak; (b) enantioselectivity (α); and (c) resolution factor (R_s). Eluent, MeOH/0.1 M NH_4AcO (80:20; v/v); pH adjusted in the mixture to the respective pH value by addition of glacial acetic acid or ammonia; flow rate, 1.0 ml/min; temperature, 25°C.

these solutes, the priorities of the substituents in the absolute configuration assignment have been changed which is due to the presence of the sulfur atom. However, for these two solutes, the *R*-enantiomer corresponds to the *L*-isomer while for all the other solutes the *S*-enantiomer corresponds to the *L*-enantiomer.²⁷

All compounds showed better separation factors α at lower pH values (optimal α at 5.0 or 5.5) except for AIDA which maintained a nearly constant α -value over the entire investigated pH range (Fig. 3b). It is worth mentioning the more pronounced enantioselective dependence of 5-MATIDA and 4-CPHE enantiomers from the eluent pH. Indeed, there was no enantiodiscrimination for the former at pH 7.0 or for the latter at either pH 6.5 or 7.0.

Like retention, also resolution factors (R_s) showed a similar trend for all solutes (Fig. 3c). With the only excep-

tion of 3-MATIDA, which underwent a progressive gain in terms of resolution towards lower pH values, maximal values were observed for all the remaining analytes at pH 5.5. The resolution factors for the 4-CPHE enantiomers were not large enough to be correctly calculated and are therefore not reported in Figure 3c.

Type and content of organic modifier

The type and content of organic modifier in the reversed phase-mode play a remarkable role, affecting mainly hydrophobic and dispersive interactions as well as the specific solvation of functional groups involved in selector-solute interactions. Generally, the polar protic MeOH or the dipolar aprotic MeCN, which are complementary in their solvatochromic properties and thus often in chromatographic selectivities, represent the preferential choices as organic modifiers.

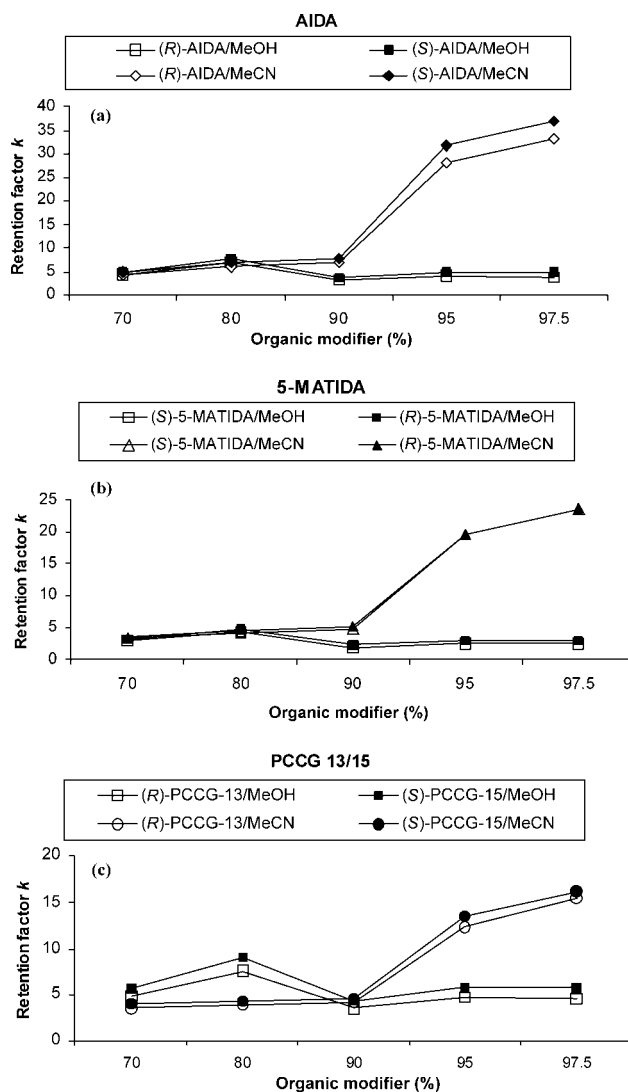


Fig. 4. Dependence of retention factors on the percentage of organic modifiers (MeOH or MeCN) for (a) AIDA, (b) 5-MATIDA, and (c) PCCG 13/15 enantiomer pairs. Eluent: MeOH or MeCN/aqueous NH_4AcO (C_{tot} 0.02 M), pH 5.5, flow rate 1.0 ml/min, 25°C.

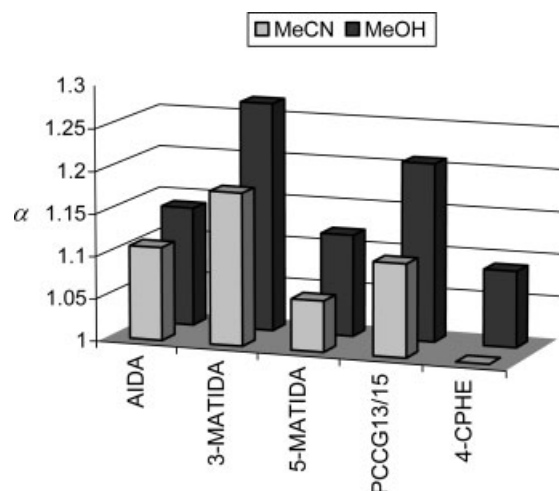


Fig. 5. Effect of organic modifier type on enantioselectivity. Eluent: MeOH or MeCN/0.1 M aqueous NH_4AcO (80/20; v/v) (C_{tot} 0.02 M), pH 5.5, flow rate 1.0 ml/min, 25°C.

A subset of pairs of enantiomers (AIDA, 5-MATIDA, and PCCG) has been selected in order to evaluate the effect exerted by a different amount of the two organic modifiers on retention, enantioselectivity, and resolution.

Figure 4 shows that within the range of investigated modifier percentages, typical RP-behavior was not

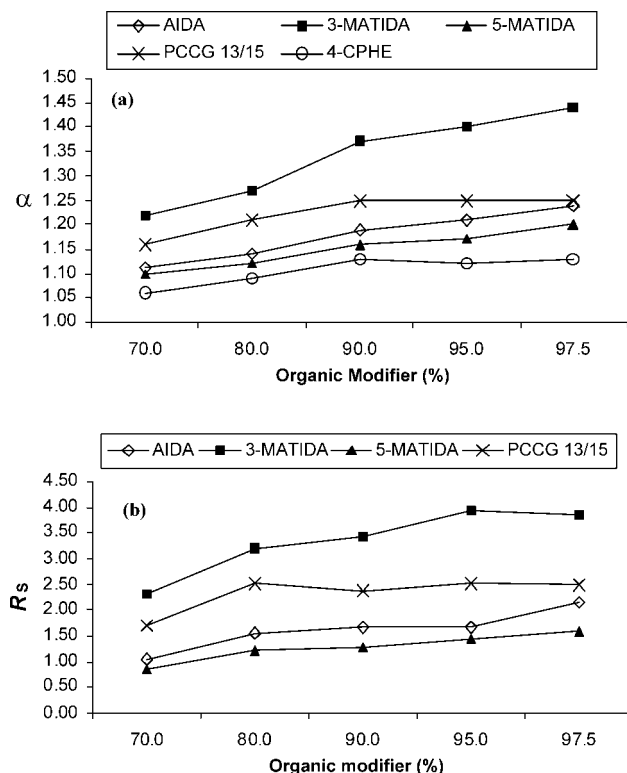


Fig. 6. Dependence of (a) enantioselectivity, and (b) resolution factors on the percentage of MeOH. Eluent: MeOH/aqueous NH_4AcO (C_{tot} 0.02 M), pH 5.5, flow rate 1.0 ml/min, 25°C.

Chirality DOI 10.1002/chir

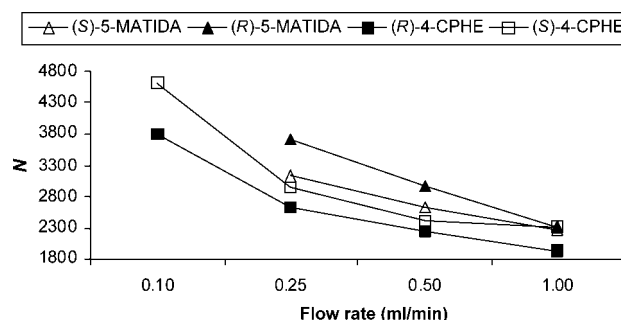


Fig. 7. Plot of number of theoretical plates (N) for 5-MATIDA and 4-CPHE enantiomers vs eluent flow rate (ml/min). Eluent, MeOH/0.8 M aqueous NH_4AcO (97.5:2.5; v/v); pH 5.5; flow rate, 1.0 ml/min; temperature, 25°C.

observed which means that hydrophobic interaction contributions are negligible (note that in this series of experiments the total buffer concentration in the mobile phase was kept constant). This holds essentially for acetonitrile, but to a minor extent for methanol as well. In contrast, the HILIC mode is evident with acetonitrile, but much less so with methanol. A clear indication for the activation of such an elution mode is the increase of retention with increasing percentage of acetonitrile (in the range 90.0%–97.5%). It is also typical of the HILIC mode that methanol displays a stronger elutropic effect than acetonitrile in the most abundant organic mobile phases employed.

The higher elution strength of methanol-rich eluents (compared with the analogous acetonitrile-containing ones) can be explained on the basis of their more pronounced protic nature.²⁶ The protic modifier is a significantly stronger competitor for intermolecular selector-solute hydrogen bonding.

More importantly, different solvatochromic solvent properties of methanol- and acetonitrile-containing mobile phases may lead to significant differences in enantioselectivity as a result of distinct solvation of functional groups

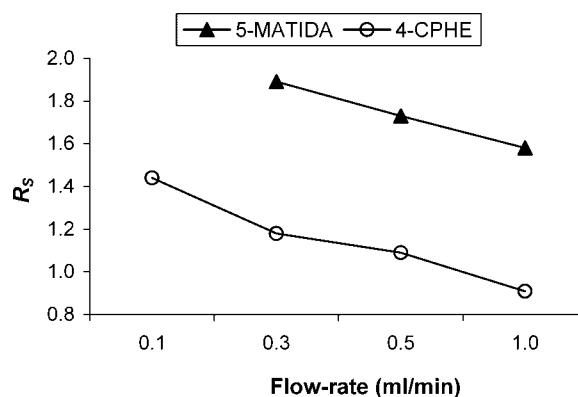


Fig. 8. Plot of resolution factor (R_s) for 5-MATIDA and 4-CPHE enantiomeric couples vs eluent flow rate (ml/min). Eluent, MeOH/0.8 M aqueous NH_4AcO (97.5:2.5; v/v); pH 5.5; flow rate, 1.0 ml/min; temperature, 25°C.

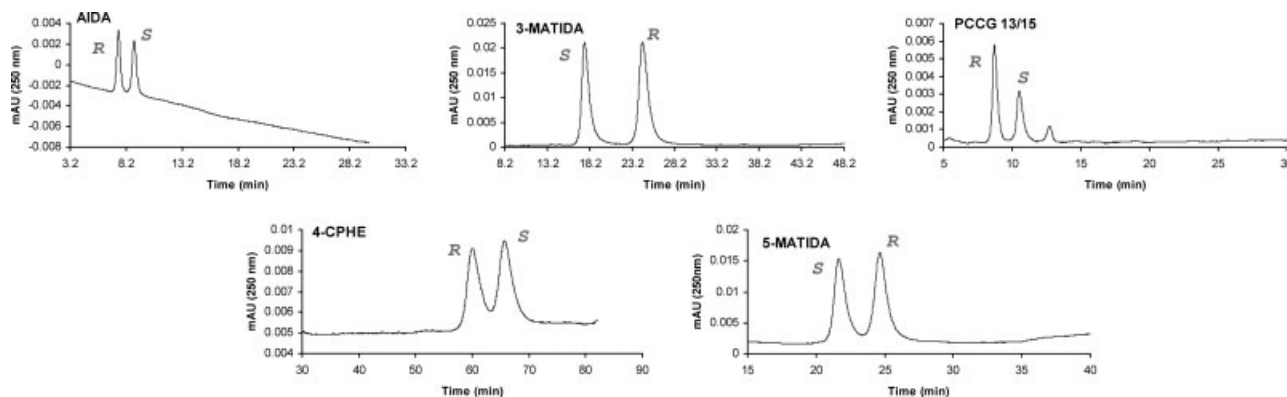


Fig. 9. Chromatograms of the investigated enantiomers. Eluent, MeOH/0.8 M aqueous NH_4AcO (97.5:2.5; v/v); pH 5.5; flow rate, 1.0 ml/min (0.25 ml/min for 5-MATIDA and 0.1 ml/min for 4-CPHE); temperature, 25°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

and binding sites.²⁶ Figure 5 depicts the different solvatochromic effects of the two distinct organic modifiers in the mobile phase, MeOH or MeCN/0.1 M ammonium acetate (80:20; v/v) pH 5.5, on enantioselectivities.

It was observed that, while MeOH generally enabled reasonable enantioseparations of all the compounds tested, MeCN afforded lower separation factors despite stronger retention and was completely unsuccessful in enantiomer distinction of 4-CPHE. Consequently, MeOH was the first choice modifier component for the following experiments (Fig. 5).

Having identified MeOH as the preferred organic solvent, the effect of its percentage on enantioselectivity (α) and resolution (R_S) was evaluated.

Figure 6 shows that slight modifications in terms of organic modifier content can result in noticeable variations of the overall chromatographic performances. As a confirmation, an improvement in terms of both enantioseparation and resolution factors was observed for almost all the compounds as the MeOH content was increased in the range from 70.0% to 97.5% (Fig. 6). Yet, the enantiomer separation factors α of PCCG and 4-CPHE do not vary substantially by increasing the methanol content above 90.0%.

The dependence of resolution on the MeOH content were essentially resembles those of the separation factors. Thus, AIDA, 5-MATIDA, and 3-MATIDA displayed a slight gain in terms of resolution factors when a higher MeOH content was employed. In contrast, PCCG enantiomers were almost equally resolved in the range of 80–90% organic modifier. As a consequence of the experimental observations, the overall most effective eluent was composed of MeOH/0.8 M aqueous ammonium acetate (97.5:2.5; v/v) (pH 5.5).

Flow rate

Even after optimization of the mobile phase composition, full baseline separation could not be accomplished for 4-CHPE at a flow rate of 1.0 ml/min. Therefore, the effect reducing the eluent flow rate was investigated for 4-CHPE and 5-MATIDA. A significant gain in efficiency resulted when the linear flow velocity was lowered from

1.0 to 0.1 ml/min (Fig. 7) because of the slow mass transfer in the CSP, well known for enantioselective chromatography systems with its heterogeneous adsorption sites.²⁶

In the present case, an improvement of the plate counts by a factor of about 1.5 could be achieved for 5-MATIDA by reducing the eluent flow rate from 1.0 to 0.25 ml/min. For 4-CPHE enantiomers the efficiency gain was even more noteworthy, namely a factor of about 2. These enhancements of the plate numbers translated into resolution gains of about 1.2 for 5-MATIDA (optimal $R_S = 1.9$), and 1.6 for 4-CPHE (optimal $R_S = 1.4$) (Fig. 8).

Figure 9 depicts the chromatograms of all investigated (racemic or nonracemic) mixtures of enantiomers of the test solutes obtained under optimized conditions.

DISCUSSIONS

An ion-exchange type HPLC method for the stereoselective analysis of underivatized acidic amino acids has been proposed. The method makes use of a *tert*-butylcarbamoylquinine-modified silica as chiral stationary phase (Chiralpak QN-AX) and hydro-organic or polar organic mobile phases containing organic salts as displacers. It turned out that the hydro-organic mode with a high content of organic modifier (97.5% methanol) is advantageous and a pH around 5.5 yields the best separations. Baseline separations for all of the five distinct test solutes could be achieved. The chromatographic separation method has the advantage of using mild conditions and the eluents are compatible with mass spectrometric detection. In general, the HPLC method could also be of value for the stereoselective preparation of the enantiomers of the presently investigated amino acids because of its general capability of a high loading capacity and mild eluents.

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Factors Determining the Pattern of a Hydrogen-Bonding Network in the Diastereomeric Salts of 1-Arylethylamines with Enantiopure P-Chiral Acids

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007

ABSTRACT In order to clarify factor(s) determining the pattern of a hydrogen-bonding network in the diastereomeric salts of 1-arylethylamines (**1**) with enantiopure P-chiral acids, three kinds of enantiopure P-chiral alkylphenylphosphinothioic acids (**3**) were synthesized, and their chiral recognition abilities for racemic **1** were examined. The characteristics of the diastereomeric salt crystals of **1** with **3** were then studied on the basis of their X-ray crystallographic analyses. Statistical analysis on the molecular conformations observed in the diastereomeric salts with **3** as well as those with P-chiral *O*-alkyl arylphosphonothioic acids (**2**), which have a chemical structure similar to that of **3**, and molecular orbital calculations for **2** and **3** in a gas phase revealed that the torsion angle between the aromatic plane and the P–O(alkoxy in **2**) or P–C(alkyl in **3**) plays an important role in determining the pattern of a hydrogen-bonding network in the diastereomeric salts, either a closed globular cluster or an infinite 2_1 column type. The calculations also indicated that there is a hydrogen-bonding-like interaction between the ammonium hydrogen atom of 1-arylethylammonium cations and the P–O(alkoxy) oxygen atom of phosphonothioate anions in the clusters. *Chirality* 20:577–584, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: enantiopure P-chiral acids; enantioseparation; hydrogen-bonding network; torsion angle; X-ray crystallographic analysis; statistical analysis; molecular orbital calculation

INTRODUCTION

The enantioseparation of a racemate through diastereomeric salt formation with a resolving agent is one of the most attractive methods for obtaining an enantiopure compound, with advantages such as its simplicity in operation, recyclability of the chiral source, and applicability on an industrial scale.^{1,2} However, the choice of a proper resolving agent for a target racemate requires time-consuming trial-and-error effort, because the chiral recognition mechanism of the diastereomeric salt formation at present is not clear. In order to overcome the situation, we have been studying chiral recognition mechanisms by analyzing the difference in stability/solubility between pairs of less- and more-soluble diastereomeric salts on the basis of their X-ray crystallographic analyses. The knowledge accumulated through such efforts was successfully applied to the proper selection of resolving agents for target racemates and to the rational design of resolving agents.^{3–5}

Some 1-arylethylamines (**1**) are important intermediates for the synthesis of pharmaceuticals.⁶ Therefore, the development of an efficient method for obtaining the enantiopure forms of **1** is required from academic as well as industrial standpoints. Moreover, the amines **1** are suitable

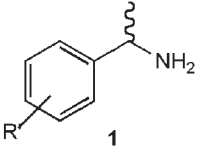
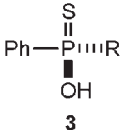
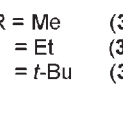
for the elucidation of the chiral recognition mechanisms of enantioseparation through the diastereomeric salt formation, because the amines can be serialized as simple chiral amino compounds by systematically changing the substituent on the phenyl group. Recently, we have reported the synthesis of enantiopure *O*-alkyl phenylphosphonothioic acids (**2**) and their application to the enantioseparation of **1**.^{7–9} The acids **2** have a chiral functional group which interacts with an amino group to afford less-soluble diastereomeric salts with a hydrogen-bonding network of either 2_1 column or cluster; in some of the less-soluble diastereomeric salts of **1** with **2**, there exists a closed hydrogen-bonding network, composed of four phosphonothioate anion molecules and four ammonium cation molecules to give a globular molecular cluster (cluster), while a hydrogen-bonding network of an infinite helical column with a twofold screw axis in the center (2_1 col-

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TABLE 1. Chiral recognition ability of enantiopure **1**

					
R' = H (1a) = o-Me (1b) = m-Me (1c) = p-Me (1d) = p-Cl (1e)		R = Me (3a) = Et (3b) = t-Bu (3c)			
1a	1b	1c	1d	1e	
3a	58% yield ^a (Sp) ^b 29% ee ^c (S) ^d CH ₃ CN ^e (1.0) ^f	94% yield (Sp) 20% ee (R) Ether (10.0)	not crystallized	89% yield (Sp) 34% ee (S) Ether (1.0)	51% yield (Sp) 27% ee (S) Ether (1.0)
3b	78% yield (Sp) 20% ee (S) AcOEt (10.0)	94% yield (Rp) 80% ee (S) Ether (3.0)	not crystallized	not crystallized	70% yield (Rp) 92% ee (R) Ether (2.0)
3c	63% yield (Rp) 92% ee (S) H ₂ O (7.0)	46% yield (Rp) 7% ee (S) Ether (3.0)	76% yield (Rp) 0% ee Ether/Hexane (3.0/10.0)	62% yield (Rp) 76% ee (S) Ether (1.0)	57% yield (Sp) 76% ee (R) Ether (4.0)

^aYield of the crystallized diastereomeric salt based on a half amount of the racemic amine.^bAbsolute configuration of enantiopure **3** used.^cEnantiomeric excess (ee) of the liberated amine, which was determined by a HPLC analysis.^dAbsolute configuration of major enantiomer, which was determined by a X-ray crystallographic analysis and/or deduced from the order of elution in a HPLC analysis.^eSolvent used in the enantioseparation.^fAmount of the solvent used. Normalized for 1.0 mmol scale.

umn) is constructed in the other less-soluble salts. Although a **2**₁ column is universally formed in the less-soluble diastereomeric salts of chiral primary amines with enantiopure carboxylic acids such as mandelic acid and its derivatives,^{10–13} such a cluster was observed for the first time in the less-soluble diastereomeric salts with **2**. Moreover, the enantiomeric excess of **1** incorporated in the corresponding less-soluble diastereomeric salt with **2** was always very high when a cluster formed in the salt crystal. However, the formation of a cluster from **1** and **2** strongly depends on the shape of **1**,⁸ and factor(s) determining the pattern of the hydrogen-bonding network, cluster or **2**₁ column, in the less-soluble diastereomeric salt crystals have not yet been clarified. Then, in order to clarify the factor(s), we tried to use enantiopure alkylphenylphosphinothioic acids (**3**), which have a chemical structure similar to that of **2**, in the enantioseparation of **1**; the acids **3** have an sp³ carbon in the place of the ester oxygen atom of **2**.

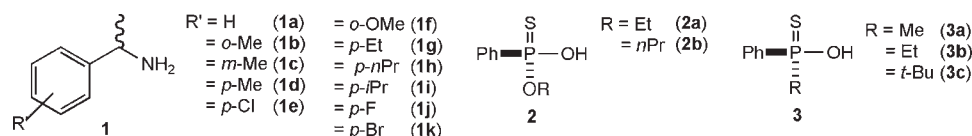
In this article, we report the synthesis and chiral recognition ability of **3** and the characteristics of the diastereomeric salt crystals examined by X-ray crystallography. Plausible factor(s) determining the pattern of the hydrogen-bonding network in the crystals are also discussed on the basis of a statistical analysis of the conformations of

the acid component in the crystals as well as those in the diastereomeric salt crystals with **2**.

EXPERIMENTAL

A General Procedure for the Enantioseparation of Racemic **1** with Enantiopure **3**

To a solution of racemic **1** (1 mmol) in ether or ether/hexane (see Table 1) was added an equimolar amount of enantiopure **3**, and the mixture was just stirred at rt for 15 h. When CH₃CN, AcOEt, or H₂O was used as the solvent, the mixture was heated at a reflux temperature, and then the solution was slowly cooled down to rt. The crystalline salt precipitated was collected by filtration and washed with the used solvent (For **1a-3a**, **1a-3b**, **1a-3c**, **1b-3b**, **1d-3c**, **1e-3b**, **1e-3c**, 1 ml; for **1b-3a**, **1b-3c**, **1c-3c**, **1d-3a**, **1e-3a**, 10–15 ml). The yield was calculated based on a half amount of the racemic amine. To determine the enantiomeric excess of the amine incorporated in the salt, a small amount of the salt crystals was dissolved/suspended in water, and the solution/suspension was basified with 1 M NaOH aq. The resultant solution was extracted with ether (2 ml), and the extract was dried over anhydrous magnesium sulfate. After removal of the solvent



Formulae



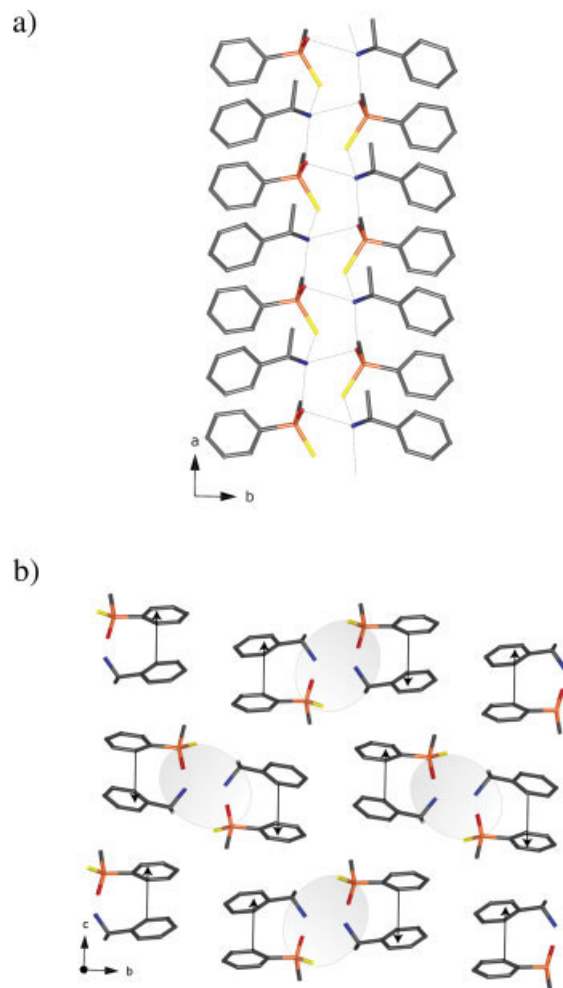


Fig. 1. Crystal structure of the less-soluble (S)-1a·(Sp)-3a salt. (a) Side view of the 2_1 column. Dotted lines show the hydrogen-bonding network. (b) Viewed down the axis of the 2_1 column (the a axis). Gray circles show the hydrogen-bonding networks, and arrows indicate intra-columnar CH/ π interactions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

very difficult to unify the solvent system. Therefore, the solvent giving the highest enantioselectivity for the amine incorporated in the salt was used. The results are summarized in Table 1.

The phosphinothioic acids **3a–c** could not recognize the chirality of the *m*-methylated amine **1c** at all, indicating that **3a–c** would be unsuitable for the enantioseparation of *m*-substituted 1-arylethylamines. However, the phosphinothioic acid **3a** showed a chiral recognition ability for the other amines **1a**, **1b**, **1d**, and **1e**, although the ability was only moderate. In contrast, the phosphinothioic acids **3b** and **3c** showed chiral recognition abilities obviously higher than **3a** for particular amines. The enantiomeric excesses of **1b** and **1e** obtained by the enantioseparation with **3b** were good/excellent, while the enantioseparations of **1a** and **1d** with **3b** resulted in low chiral recognition and no crystallization, respectively. On the other hand, the enantiomeric excesses of **1a**, **1d**, and **1e**, afforded by the enantioseparation with **3c**, were excellent/

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good, although the enantiomeric excess of **1b** was very low. It is noteworthy that the chiral recognition abilities of **3b** and **3c** are thus complementary to each other.

Characteristics of the Diastereomeric Salt Crystals of **1** with Enantiopure **3**

To understand the chiral recognition mechanism for the enantioseparation with enantiopure **3**, we carried out X-ray crystallographic analyses of the diastereomeric salts. Single crystals were grown from ethyl acetate solutions by the slow diffusion of hexane. The crystal structures of the less-soluble (S)-**1a**·(Sp)-**3a**, [Crystal data for (S)-**1a**·(Sp)-**3a**: $C_{15}H_{20}N_1O_1P_1S_1$, $M = 293.36$, Orthorhombic, space group $P2_12_12_1$, $a = 6.4230(3)$ Å, $b = 15.5910(10)$ Å, $c = 16.0710(2)$ Å, $V = 1609.4(2)$ Å³, $Z = 4$, $D_c = 1.211$ mg m⁻³, $R = 0.0420$, $R_w = 0.0520$][†] (S)-**1a**·(Sp)-**3b**, [Crystal data for (S)-**1a**·(Sp)-**3b**: $C_{16}H_{22}N_1O_1P_1S_1$, $M = 323.39$, Orthorhombic, space group $P2_12_12_1$, $a = 6.4560(2)$ Å, $b = 15.3640(7)$ Å, $c = 17.2640(9)$ Å, $V = 1712.42(13)$ Å³, $Z = 4$, $D_c = 1.254$ mg m⁻³, $R = 0.0470$, $R_w = 0.0640$] and (S)-**1d**·(Rp)-**3c** [Crystal data for (S)-**1d**·(Rp)-**3c**: $C_{19}H_{28}N_1O_1P_1S_1$, $M = 349.47$, Orthorhombic, space group $P2_12_12_1$, $a = 7.1770(3)$ Å, $b = 13.4740(9)$ Å, $c = 21.4810(2)$ Å, $V = 2077.3(3)$ Å³, $Z = 4$, $D_c = 1.117$ mg m⁻³, $R = 0.0440$, $R_w = 0.0510$] salts are shown in Figures 1–3, respectively. A columnar hydrogen-bonding network (2_1 column), consisting of phosphinothioate anions and ammonium cations, is commonly constructed in the three salt crystals. The lengths of the hydrogen-bonds formed in the (S)-**1a**·(Sp)-**3a** and (S)-**1a**·(Sp)-**3b** salts are very close to each other; the N...O distances are in a range of 2.78–2.94 Å, and the N...S distances are both 3.32 Å. In contrast, the distances in the (S)-**1d**·(Rp)-**3c** salt are quite different from those in (S)-**1a**·(Sp)-**3a** and (S)-**1a**·(Sp)-**3b**; one of the N...O distances is a little short at 2.71 Å, and the other N...O and N...S distances are obviously long (3.10 and 3.46 Å). The formation of such a distorted 2_1 column in (S)-**1d**·(Rp)-**3c** is probably due to steric repulsion arising from the bulky *tert*-butyl group of (Rp)-**3c**. The 2_1 columns of the **1·3** salt crystals are topologically similar to those in the **1·2** salt crystals. However, the packing mode of the columns in the **1·3** salt crystals is largely different from those of the **1·2** salt crystals; no remarkable interaction is observed between the columns in the **1·3** salt crystals, while there exists CH/ π interaction between the columns in the **1·2** salt crystals, which contributes to the stabilization of the three-dimensional crystals. Therefore, the lesser chiral recognition ability of enantiopure **3** than that of **2** arises from nothing other than the van der Waals interaction between the columns in **1·3** salt crystals.

Although the crystal structures of more-soluble diastereomeric salts are very informative for the elucidation of the chiral recognition mechanism during the diastereomeric salt formation, it is generally difficult to solve the

[†]CCDC 641430, CCDC 641431, CCDC 641432 and CCDC 641433 contain the supplementary crystallographic data for this article. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

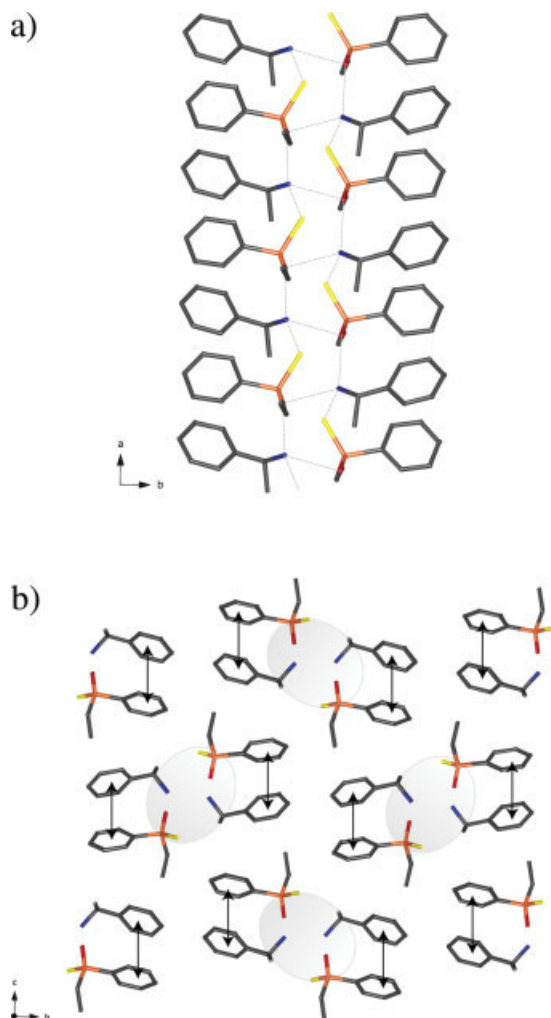


Fig. 2. Crystal structure of the less-soluble (*S*)-**1a**·(*Sp*)-**3b** salt. (a) Side view of the 2_1 column. Dotted lines show the hydrogen-bonding network. (b) Viewed down the axis of the 2_1 column (the *a* axis). Gray circles show the hydrogen-bonding networks, and arrows indicate intra-columnar cooperative CH/ π interactions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

crystal structures of more-soluble salts due to their poor crystallinity. However, fortunately, we could obtain a single crystal of the more-soluble (*R*)-**1a**·(*Sp*)-**3a** [Crystal data for (*R*)-**1a**·(*Sp*)-**3a**: $C_{15}H_{20}N_1O_1P_1S_1$, $M = 293.36$, *Monoclinic*, space group $P2_1$, $a = 13.324(4)$ Å, $b = 15.091(5)$ Å, $c = 16.911(5)$ Å, $\beta = 104.0902(11)^\circ$, $V = 3298.0(18)$ Å³, $Z = 8$, $D_c = 1.182$ mg m⁻³, $R = 0.0435$, $R_w = 0.0400$] salt with satisfactory quality and succeed in its X-ray crystallographic analysis. The crystal structure is shown in Figure 4. The hydrogen-bonding network in the more-soluble (*R*)-**1a**·(*Sp*)-**3a** salt crystal is surprisingly different from that in the corresponding less-soluble (*S*)-**1a**·(*Sp*)-**3a** salt crystal; the network is of a globular molecular cluster consisting of four acid molecules and four amine molecules, as was observed often in the less-soluble salts with **2**.^{7–9} The distances are in the range 2.74–2.82 Å for the N...O bonds and 3.24 Å for the N...S bond, which are very similar to those in the clusters formed with **2**.

However, the symmetry of the cluster is very low, so that the shape of the cluster is far from the cubic form of the less-soluble salts with **2**, which is essential for the effective packing of the clusters, so that there is no remarkable interaction between the clusters.

Thus, the less-soluble (*S*)-**1a**·(*Sp*)-**3a** and more-soluble (*R*)-**1a**·(*Sp*)-**3a** salt crystals are composed of different hydrogen-bonding networks with one- and zero-dimensions (2_1 column and cluster), respectively, and commonly have no strong interaction between the columns/clusters. The resultant chiral recognition of **1a** with 29% ee by (*Sp*)-**3a** would arise from the less difference in stability/solubility between the columnar and globular hydrogen-bonding networks. In the other pairs of the diastereomeric salts

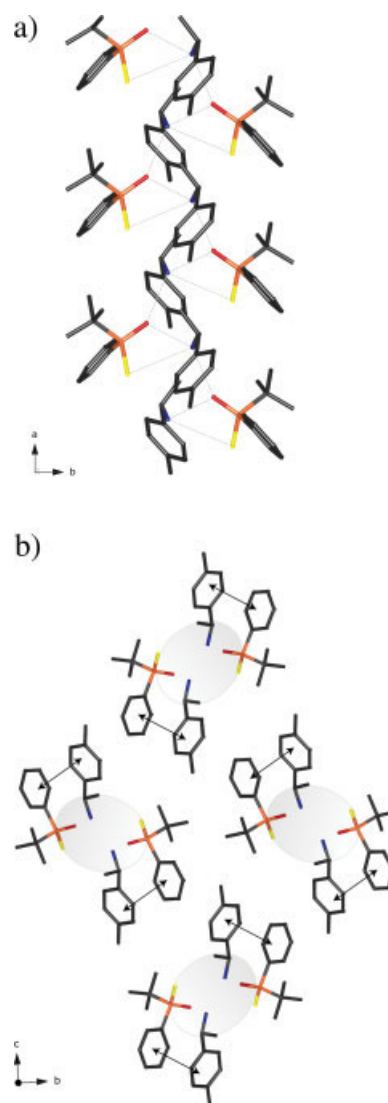


Fig. 3. Crystal structure of the less-soluble (*S*)-**1d**·(*Rp*)-**3c** salt. (a) Side view of the 2_1 column. Dotted lines show the hydrogen-bonding network. (b) Viewed down the axis of the 2_1 column (the *a* axis). Gray circles show the hydrogen-bonding networks, and arrows indicate intra-columnar cooperative CH/ π interactions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

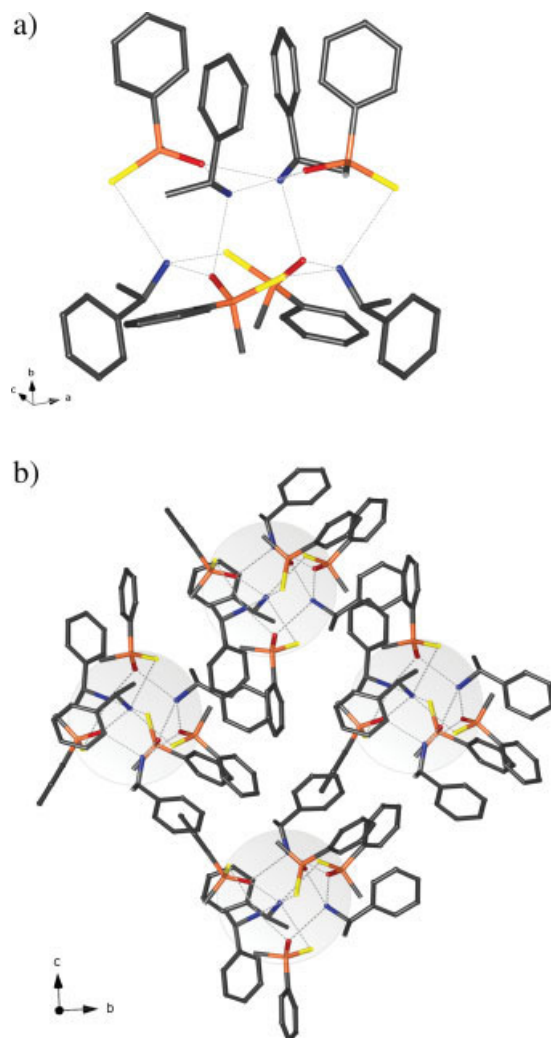


Fig. 4. Crystal structure of the more-soluble (*R*)-**1a**·(*Sp*)-**3a** salt. (a) Side view of the cluster structure. Dotted lines show the hydrogen-bonding network. (b) Viewed down the axis of the 2_1 column (the *a* axis). Gray circles show hydrogen-bonding networks. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

with **3**, there would exist no remarkable interaction between the columns to result in the chiral recognition of particular **1** with **3**, depending on the efficiency of van der Waals interaction between the columns.

Factors Determining the Pattern of the Hydrogen-Bonding Network in the Less-Soluble Diastereomeric Salts

There exists a hydrogen-bonding network of 2_1 column or cluster in the crystals of the less-soluble diastereomeric salts with the phosphinothioic acids **2**⁸ as well as those with the phosphinothioic acids **3**. To elucidate factor(s) determining the pattern of the hydrogen-bonding network in the less-soluble diastereomeric salts, the crystal structures of the diastereomeric salts were statistically analyzed in detail. At first, we focused on the torsion angle between

the aromatic plane and the ester group of **2** or alkyl group of **3**, because this angle should be important to fix the molecular conformation of the acid components in the crystals. Figure 5 shows the torsion angles observed in the less-soluble diastereomeric salts of **1** with **2/3**, of which the crystal structures were solved so far. Although there are two kinds of conformations in the cluster crystals, the torsion angles are close to each other and average values are presented in Figure 5. Large torsion angles are observed for the 2_1 column crystals; 61.3° in the salt crystals with **2** and 76.7° with **3**. On the other hand, the torsion angles in the cluster crystals are relatively small, (33.6°). To explain this difference in torsion angle between the cluster and 2_1 column crystals, the most relaxed structures of the acids **2** and **3** in a gas phase were determined by molecular orbital calculations. The geometry optimizations for the acids in three different states (anion, free acid, and tautomer of free acid) were carried out with B3LYP/cc-pVDZ. Table 2 summarizes the torsion angles between the aromatic plane and P—O(alkoxy in **2**)/P—C(alkyl in **3**) and the bond lengths of the P—O(alkoxy in **2**)/P—C(alkyl in **3**) bonds. The conformations of **2** and **3** observed in the crystals are considerably different from those of the anion forms optimized by the calculations. The observed conformations of **2** in the 2_1 column crystals, **2** in the cluster crystals, and **3** in the 2_1 column crystals are rather close to those of the calculated **2a**-tautomeric acid, **2a**-free acid, and **3**-free acids, respectively, from the viewpoints of the torsion angles and P—C bond lengths; the electron densities of the anions of **2** and **3** would be reduced by strong hydrogen-bonding interaction between the ammonium hydrogen atom and the thioate anion to bring the conformations of **2** and **3** to those similar to the tautomeric or free acid forms in the crystals. It is noteworthy that a small torsion angle derived from the most relaxed molecular conformations is highly correlated with the construction of a cluster in the less-soluble diastereomeric salt crystals. Thus, the P-chiral acids **2** and **3**

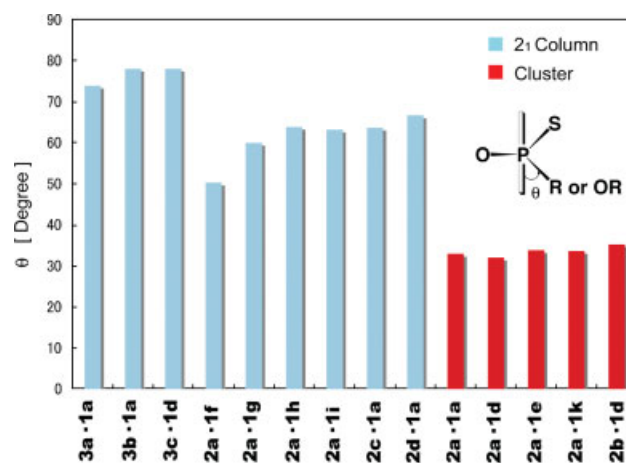


Fig. 5. Torsion angle (θ) between the aromatic plane and the alkyl group of the phosphinothioic acids **3** or the ester group of the phosphinothioic acids **2** in the less-soluble salts of 1-arylethylamines. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 2. Optimized structures of **2a** and **3** with B3LYP/cc-pVDZ and the comparison with the X-ray structures

		a)	b)	c)
			θ (degree)	d (Å)
Anion	2a		92.1	1.711
	3a		101.9	1.867
	3b		95.5	1.879
	3c		99.7	1.925
Free acid	2a		33.5	1.652
	3a		74.5	1.838
	3b		73.4	1.839
	3c		86.0	1.840
Tautomer	2a		67.6	1.648
	3a		127.2	1.831
	3b		109.0	1.846
	3c		112.9	1.891
Analyzed by X-ray Crystallography	2_{ave} (2 ₁ column)		61.3	1.602
	2_{ave} (cluster)		33.6	1.591
	3a		73.9	1.810
	3b		78.1	1.809
	3c		78.1	1.834

tend to exist in the conformations of free acids, in their diastereomeric salt crystals which would be favorable from an energetic viewpoint. In general, **2** and **3** give cluster and 2₁ column crystals, respectively. The acids **2**, however, would be able to change their conformation in diastereomeric salt crystals from the free acid forms into the tautomeric acid forms by the influence of some weak inter- and/or intramolecular interaction to afford 2₁ column crystals without large energy loss.

Role of the Oxygen Atom of the P–O(alkoxy) Bond in **2**

Even though the only structural difference between **2** and **3** is the presence or absence of the oxygen atom

attached to the phosphorus atom, the P–O(alkoxy) bond lengths observed in the actual cluster and 2₁ column crystals are shorter than those of the calculated **2a**-tautomeric and free acids, respectively. This means that there is some factor shortening the bond length for **2a**. In order to understand the factor, additional calculations were carried out for two possible models with an arbitrarily fixed conformation as shown in Figure 6. One model is **2a**-anion interacting with an NH₄⁺ group. The initial position of the NH₄⁺ group was arranged at that of the ammonium nitrogen atom in a cluster crystal determined by an X-ray analysis, and the positions of the N atom and the H atoms were optimized with B3LYP/cc-pVDZ by maintaining the distances between the ammonium nitrogen atom and the sulfur atom of **2a**, and between the ammonium nitrogen atom and the ester oxygen atom of **2a**. The other model is **2a**-anion interacting with a H⁺, which was fixed at the optimized position of the NH₄⁺ group. The former model shows that the P–O(alkoxy) bond length is slightly shortened by the interaction with the NH₄⁺ group, but the value of 1.688 Å is still longer than those observed in the actual crystals. However, the value of 1.652 Å, which was obtained from the latter model, is closer to the experimental values. Thus, these calculations would suggest that the P–O(alkoxy) bond length is strongly influenced by the interaction with an ammonium group, especially with a proton; there would exist some interaction between the proton of the ammonium group in a 1-arylethylammonium cation and the lone pair electrons of the oxygen atom of the P–O(alkoxy) in a phosphonothioate anion. The present explanation is strongly supported by the fact that the negative charges on the oxygen atoms in the two models are smaller than that in the relaxed **2a**-anion (The Mulliken charges of the oxygen atom of the P–OEt bond in **2a**-anion, (**2a**-anion + NH₄⁺), and (**2a**-anion + H⁺) are –0.437, –0.434, and –0.410, respectively). Although the X-ray crystallographic analyses indicate that the three protons of the ammonium cation interact with only the acidic functional group, the calculations suggest that the occupancy of the protons of the ammonium cation is not localized over the acidic functional group but delocalized even over the oxygen atom of the P–O(alkoxy) bond; the verification by experiment could only be done by an accurate neutron diffraction analysis.

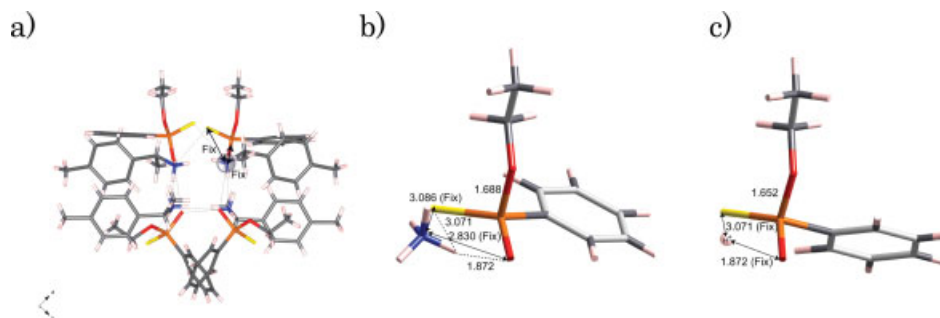


Fig. 6. Cluster determined by a X-ray crystallographic analysis, and model molecules extracted from the crystal and optimized with B3LYP/cc-pVDZ. (a) (*R*)-**1c**·(*Sp*)-**2a** salt, (b) **2a** anion interacting with NH₄⁺, and (c) **2a** anion interacting with H⁺. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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Exploring the Scope of Redox-Triggered Chiroptical Switches: Syntheses, X-ray Structures, and Circular Dichroism of Cobalt and Nickel Complexes of *N,N*-Bis(arylmethyl)methionine Derivatives

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT *N,N*-Bis(arylmethyl)methionine derivatives are chiral ligands whose complexes with metal ions may show molecular helicity that can be modulated by defined structural processes. It was shown previously that exciton-coupled circular dichroism (ECCD) spectral amplitude could be modulated by one-electron copper redox chemistry in copper complexes of these ligands. Here we describe the further development of novel systems that show conformational changes resulting in the inversion of exciton chirality. The phenomenon was probed in a *N,N*-bis(arylmethyl)methionine derivative containing quinoline/pyridine moieties and a methionine carboxylate moiety. The sign of the ECCD of the complex formed between this ligand and CoCl₂ is negative, which suggests that the deprotonated carboxylate oxygen coordinates to the metal, but the sulfur atom does not. The sign of the ECCD inverts to positive upon addition of ascorbic acid, which can be turned back to negative upon further treatment with persulfate. X-ray quality crystals of three cobalt complexes and one nickel complex were obtained. The ascorbate-treated cobalt complex of the ligand and the same ligand with nickel, however, vary from the behavior expected from their X-ray crystal structures. It is clear that the solution and crystallographic structures of these complexes differ in several cases. *Chirality* 20:585–591, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: exciton coupling; UV/vis spectroscopy; coordination chemistry; cobalt; nickel; copper; X-ray crystallography

INTRODUCTION

Chiroptical molecular switches attract substantial interest because of their stereochemical novelty and potential applications.^{1,2} For example, such compounds have been used for absolute configurational assignment of organic compounds,³ helical induction in liquid crystals,⁴ molecular electronics applications,⁵ and mechanical manipulation.⁶ We are particularly interested in redox-triggered molecules whose molecular helicity can be modulated by defined structural processes.³ Our approach to this problem grew out of our studies of conformationally biased, labile coordination complexes.⁷ Central to these studies was the observation that tripodal ligands with a single chiral center in one of the arms experience a bias affording an additional element of helical chirality.⁸ The propeller-like conformation adopted fixes the orientation of appended or inherent chromophores, resulting in exciton-coupled circular dichroism (ECCD).^{9,10} Additionally, we showed previously that ECCD spectral amplitude could be modulated by one-electron copper redox chemistry, and the spectroscopic change was correlated with conformational dynam-

ics of the ligand in Cu^I and Cu^{II} states.^{11,12} It then became of interest to find a way to invert the handedness of the helicity of the complexes by inducing a change in the conformation of the ligand.

It was found that methionine reacts with bromomethyl-quinoline to form ligands that complex Cu^{II} and show strong ECCD couplets.^{13,14} The CD-active product is illustrated in Figure 1. Tetradentate metallochelates form involving the Cu^{II} ion, the tertiary amine, the two quinolines, and the carboxylate. The stereocenter of the amino acid arm dictates the orientation of the quinoline groups by a “gear” with the methylenes of the achiral arms, such

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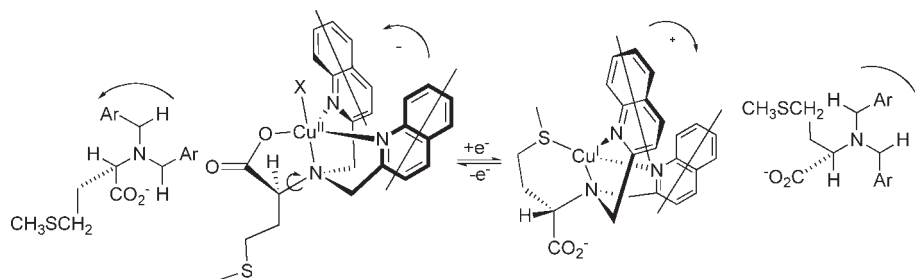


Fig. 1. *N,N*-Bis(2-quinolylmethyl)-L-methionine complexes with Cu^{II} and Cu^{I} . The chiral center of the amino acid dictates the orientation of the quinoline chromophores via a gearing mechanism as illustrated (Ar = 2-quinolyl or 2-pyridyl). The transition dipoles in the quinolines in the two complexes invert in the sense of absolute orientation and therefore give opposite ECCD spectra.

that a propeller forms from the planar carboxylate and quinoline groups (Fig. 1). The Cu^{I} complex gave a propeller complex with the opposite configuration because of the preference of Cu^{I} for coordination with the sulfur atom. As shown in Figure 1, for the sulfide to come proximal to the metal center, the amino acid arm must pivot about the C—N bond. This inverts the gearing, and therefore the orientation, of the quinoline moieties, leading to an ECCD couplet with the opposite sign. Synthetic and spectroscopic details plus further structural characterization of Zn^{II} and Cu^{II} complexes of (*S*)-*N,N*-bis(2-pyridylmethyl)-methionine ((*S*)-**1**) and its quinoline-containing analog (*S*)-*N,N*-bis(2-quinolylmethyl)-methionine ((*S*)-**2**) have also been reported.¹⁴ Crystal structures of Zn^{II} and Cu^{II} complexes showed that these metal ions coordinate with the carboxylate instead of the sulfide.¹⁴ NMR spectra of the Cu^{I} complexes demonstrated Cu^{I} —S coordination. Other amino acid derivatives showed similar behavior with Cu^{I} , Cu^{II} , and Zn^{II} .^{15,16}

In search of other metal ions that might exhibit interesting redox-dependent ligation modes with such ligands, we prepared several cobalt and nickel complexes and investigated their CD properties and their X-ray structures. Cobalt and nickel were selected because of the accessibility of other oxidation states. Given our reasoning that it is the softness of the Cu^{I} in the known systems that drives the reorganization of the ligands, cobalt and nickel seemed to offer some potential to exhibit similar phenomena compared to other transition metal redox couples.

MATERIALS AND METHODS

All reagents and solvents were purchased from commercial sources and used as received, unless noted otherwise. Cyclic voltammetry experiments were carried out in a standard three-electrode apparatus, with a glassy carbon working electrode, a nonaqueous reference electrode (0.1 M AgNO_3 in acetonitrile), and a platinum wire auxiliary electrode. Experiments were run in 0.1 M *n*- Bu_4NPF_6 as supporting electrolyte in acetonitrile or methanol. FT-IR spectra (KBr pellets) were recorded on a Nicolet 750 spectrophotometer (Magna-IR 750). Electrical conductivity measurements of the complexes at room temperature in acetonitrile solutions were carried out using a YSI conductivity instrument (model 3200) with a YSI dip cell (model 3256, cell constant 0.1 cm^{-1}), while room-temperature

magnetic susceptibility was measured using an MSB-auto magnetic susceptibility balance (Johnson Matthey), which was calibrated with $\text{Hg}[\text{Co}(\text{SCN})_4]$. A Kratos MALDI-TOF I mass spectrometer or a Bruker X-Flex MALDI-TOF using the matrix α -ACHC was used for mass spectrometry. Unless stated otherwise, UV-vis spectra were obtained on a Perkin-Elmer Lambda-5 instrument, and CD spectra (0.1 cm path length) were taken on an Aviv 212 CD instrument at ambient temperature in methanol. The synthesis of (*S*)-*N,N*-bis(2-pyridylmethyl)-methionine ((*S*)-**1**) and (*S*)-*N,N*-bis(2-quinolylmethyl)-methionine ((*S*)-**2**) were reported elsewhere.¹⁴ The optical rotation of these ligands was determined as follows: (*S*)-**1**, $[\alpha]_{\text{D}}^{21} = -19^\circ$ (*c* 0.086, 4:1 acetonitrile:water) and (*S*)-**2**, $[\alpha]_{\text{D}}^{21} = -75^\circ$ (*c* 0.0049, methanol).

$[\text{Co}^{\text{II}}(\text{1})]\text{ClO}_4$

Caution! Perchlorate salts of metal complexes with organic ligands are potentially explosive. They should be handled in small quantities and with caution. This compound was prepared by precipitation from methanol in a manner similar to $[\text{Zn}(\text{1})]\text{ClO}_4$.¹⁴ To a methanolic solution of $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (55.3 mg, 0.151 mmol in 10 ml methanol) was added a solution of (*S*)-**1** (49.9 mg, 0.151 mmol in 10 ml methanol) and stirred for 30 min. The resultant precipitate was filtered under suction and dried under vacuum to yield 58.4 mg (80%) of the complex, which was green in color. Crystals suitable for X-ray diffraction studies were obtained from methanol/water by slow evaporation over several days. The crystallographic structure obtained was for racemic material, and it was found that the crystals showed no optical activity. Anal. Calcd for $\text{C}_{17}\text{H}_{20}\text{N}_3\text{O}_6\text{SCoCl}$: C, 41.77; H, 4.12; N, 8.60. Found: C, 42.00; H, 4.02; N, 8.74. FT-IR (cm^{-1}): 1608 (CO); 1556 (CoN); 1099 (ClO_4). MS (*m/e*): 389 (CoL)⁺, mp 250° .

$[\text{Co}^{\text{II}}(\text{2})\text{Cl}]$

To a methanolic solution of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (179 mg, 0.75 mmol in 6 ml of methanol) was added a solution of (*S*)-**2** (220 mg, 0.51 mmol in 6 ml methanol) and stirred for 2 h at 50°C . A greenish-blue precipitate was filtered under suction and dried under vacuum to yield 174 mg (65%) of the complex. Crystals suitable for X-ray diffraction studies were obtained from methanol/water by slow evaporation. Anal. Calcd for $\text{C}_{25}\text{H}_{24}\text{N}_3\text{O}_2\text{SCoCl} + \text{HCl}$: C, 53.49; H, 4.49; N, 7.49. Found: C, 53.76; H, 4.56; N, 7.65. FT-IR (cm^{-1}):

TABLE 1. Crystal data and structure refinement

	Complex			
	(±)-Co ^{II} (1)ClO ₄	Co ^{II} ((S)- 2)Cl	Co ₂ ^{II} ((S)- 2) ₂ (C ₂ O ₄)	[Ni ₄ ^{II} ((S)- 2) ₄](ClO ₄) ₄
Empirical formula	C ₁₇ H ₂₀ ClCoN ₃ O ₆ S	C ₅₂ H ₄₈ Cl ₂ Co ₂ D ₈ N ₆ O ₆ S ₂	C ₂₆ H ₂₄ CoN ₃ O ₄ S	C ₁₀₄ H ₁₀₂ Cl ₄ N ₁₄ Ni ₄ O ₂₄ S ₄
Formula weight	488.80	1121.96	533.47	2436.88
Wavelength (Å)	0.71073	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic	Tetragonal	Monoclinic
Space group	<i>P</i> 2(1)/ <i>n</i>	<i>P</i> 2(1)	<i>P</i> 4 ₁ 2 ₁ 2	<i>P</i> 21
<i>a</i> (Å)	9.493(2)	6.924(1)	14.656(1)	14.397(2)
<i>b</i> (Å)	9.327(2)	28.835(5)	14.656(1)	15.650(2)
<i>c</i> (Å)	24.008(5)	13.517(2)	31.999(4)	23.871(3)
α (°)	90	90	90	90
β (°)	100.859(3)	90.013(3)	90	98.881(3)
γ (°)	90	90	90	90
Volume (Å ³)	2087.6(7)	2698.7(8)	6873(1)	5314.0(12)
<i>Z</i>	4	2	8	2
Density (calculated) (mg/m ³)	1.555	1.381	1.031	1.523
Absorption coefficient (mm ⁻¹)	1.089	0.844	0.587	0.957
<i>F</i> (000)	1004	1156	2208	2520
Crystal habit and color	green, block	violet, needle	orange, block	blue, block
Crystal size (mm ³)	0.21 × 0.20 × 0.18	0.31 × 0.13 × 0.07	0.28 × 0.13 × 0.13	0.28 × 0.24 × 0.20
Theta range for data collection	1.73–25.06°	0.71–27.53°	1.53–25.09°	1.55–25.06°
Reflections collected	12,629	24,206	23,853	35,882
Independent reflections	3699	11,552	6102	18,496
Observed reflections	3168	10,946	3166	14,295
Completeness to theta = 25.06°	99.8%	99.3%	99.7%	99.6%
Data/restraints/ parameters	3699/106/313	11,552/5/638	6102/2/300	18,496/71/1464
GOF	1.036	1.008	1.012	1.058
<i>R</i> ₁ , <i>wR</i> ₂ [<i>I</i> > 2σ(<i>I</i>)]	0.0536, 0.1253	0.0502, 0.1347	0.0847, 0.2149	0.0583, 0.1312
<i>R</i> indices (all data)	0.0648, 0.1306	0.0529, 0.1363	0.1377, 0.2360	0.0844, 0.1447
Flack parameter		0.047(18)	0.17(4)	0.022(13)

1617 (CO); 1504 (CoN). MS (*m/e*): 489.2 (CoL)⁺, mp 189°, [α]_D²¹ = −236° (*c* 0.058, methanol).

[Ni(**2**)]ClO₄

To a methanolic solution of Ni(ClO₄)₂·6H₂O (275 mg, 0.75 mmol in 6 ml of methanol) was added a solution of (S)-**2** (220 mg, 0.51 mmol in 6 ml methanol) and stirred for 2 h at 50°C. A greenish precipitate was filtered under suction and dried under vacuum to yield 207 mg (69%) of the complex. Crystals suitable for X-ray diffraction studies were obtained from methanol/water by slow evaporation. Anal. Calcd for C₂₅H₂₄N₃O₂SCoCl₂·CH₃OH·H₂O: C, 48.90; H, 4.54; N, 6.70. Found: C, 48.85; H, 4.60; N, 6.58. MS (*m/e*): 489 (NiL)⁺, [α]_D²¹ = −46° (*c* 0.062, methanol).

X-ray Crystallographic Analysis

A very small crystal was placed onto the tip of a 0.1-mm diameter glass capillary and mounted on a Bruker SMART Platform CCD diffractometer for a data collection at 173(2) K. A preliminary set of cell constants was calculated from reflections harvested from three sets of 20 frames. These

initial sets of frames were oriented, such that orthogonal wedges of reciprocal space were surveyed. This produced initial orientation matrices determined from various numbers of reflections. The data collection was carried out using Mo K α radiation (graphite monochromator). A randomly oriented region of reciprocal space was surveyed to the extent of 1.5 hemispheres and to a resolution of 0.84 Å. Four major sections of frames were collected with 0.30° steps in ω at four different ϕ settings and a detector position of −28° in 2θ . The intensity data were corrected for absorption and decay (SADABS).¹⁷ Please refer to Table 1 for additional crystal and refinement information. Unless otherwise noted, the structures were solved using SHELXS-86¹⁸ or SHELXS-97¹⁹ and refined using SHELXL-97.¹⁹ A direct-methods solution was calculated, which provided most nonhydrogen atoms from the E-map. Full-matrix least squares/difference Fourier cycles were performed which located the remaining nonhydrogen atoms. All nonhydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were placed in ideal positions and refined as riding atoms with relative isotropic displacement parameters.

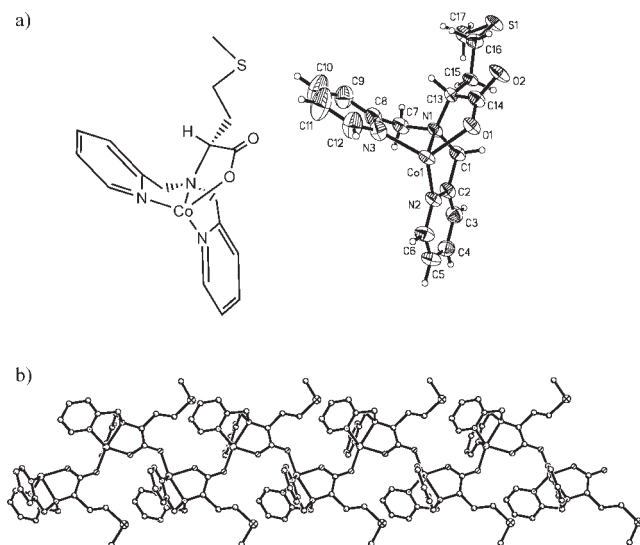


Fig. 2. (a) ORTEP view of the cation portion of the complex $\text{Co}^{\text{II}}(\mathbf{1})\cdot\text{ClO}_4$. Atom O2' from an adjacent complex is excluded for clarity. (b) Helical chain formed by bridging carboxylate groups in the solid state. One enantiomer is shown here, but crystallographic structure is of the racemate.

RESULTS AND DISCUSSION

The coordination chemistry of these ligands was explored with cobalt and nickel salts because of the accessibility of oxidation states that might give ligand reorganization. Generally, the metal salts were reacted with the ligands to produce complexes that gave precipitates that were characterized by elemental analysis. Some of the complexes were then reacted with ascorbic acid as a reductant, which had previously worked well in the reduction of Cu(II) to produce Cu(I) complexes.^{13,20}

$\text{Co}^{\text{II}}(\mathbf{1})\text{ClO}_4$

This complex was prepared by precipitation. The structure is analogous to the structures of recently published of Zn^{II} and Cu^{II} complexes of ligand **1**.¹⁴ An ORTEP²¹ view of the complex is shown in Figure 2. In this structure, Co^{II} is five-coordinated and possesses a trigonal bipyramidal geometry. The ligand **1** coordinates to the metal through two pyridine nitrogens, the tertiary amine nitrogen and the deprotonated carboxylate oxygen atoms. Two pyridine nitrogens and one carboxylate oxygen form the trigonal plane with the central Co^{II} atom, while a tertiary amino nitrogen and a second carboxylic oxygen from the neighboring molecule occupy the apical positions leading to a one-dimensional helical polymer. The cobalt-oxygen bond distances are very similar; that for Co—O1 is 2.010(3) Å, and that for Co—O2 (from an adjacent ligand) is 1.999(3) Å. The branch containing the sulfur atom, which is not coordinated to the metal ion, is modeled as disordered over two positions (76:24). The perchlorate counter ion is modeled as disordered over three positions (83:8:9). As with the Cu(**1**) and Zn(**1**) complexes, the complex forms a one-dimensional polymer in the solid state resulting from bridging of carboxylate groups (Fig. 2b). Such aggregates dissociate in the polar solvent used for solution measure-

ments. The 1D polymer has a supramolecular helical structure. Interestingly, the crystal is racemic, with racemization having occurred during the slow crystallization process.

$\text{Co}^{\text{II}}(\mathbf{2})\text{Cl}$

An ORTEP view of the complex is shown in Figure 3. The space group was determined to be $P2_1$. The structure is a pseudomerohedral twin with a twin index of 1. Although the crystal system is monoclinic based on the data, the β angle is 90.013(3)°, which allows for the cell to transform to the orthorhombic system via a rotation about the a -axis. The ratio of the main to the twinned component is 59:41 by mass. The structure was also tested for inversion twinning, but nothing significant was found.

In this structure, Co^{II} is five-coordinated and possesses a trigonal bipyramidal geometry. The ligand (*S*)-**2** coordinates to the metal through two quinoline nitrogens, the tertiary amine nitrogen and the deprotonated carboxylate oxygen atoms and the chloride. Two quinoline nitrogens and one carboxylate oxygen form the trigonal plane with the central Co^{II} atom, while a tertiary amino nitrogen and the chloride occupy the apical positions. The sulfur atom does not coordinate the cobalt. There is only one chloride per cobalt in the crystal, consistent with a Co^{II} oxidation state. The asymmetric unit consists of two independent cobalt molecules and two deuterated methanol molecules, one of which is disordered over two positions (76:24). Per unit cell, there is also a solvent channel parallel to the a -axis (Fig. 3b) of 206 Å³ which accounts for 78 electrons, as calculated by program PLATON, function SQUEEZE.^{22,23} However, the largest residual peak is only 1.2 electrons per Å³, suggesting either extreme disorder or that the solvent can escape readily via the channel. Also noteworthy is that while this structure forms channels, the previous structure forms chains.

$\text{Co}_2^{\text{II}}(\mathbf{2})_2(\text{C}_2\text{O}_4)$

This complex was prepared from $[\text{Co}^{\text{II}}(\mathbf{2})\text{Cl}]$ by reaction with ascorbic acid. The structure shows the presence of an oxalate dianion bridging two cobalt atoms. Oxalate is an oxidation product of ascorbic acid.^{24,25} An ORTEP view of the complex is shown in Figure 4. The space group $P4_12_12$ was determined based on systematic absences and intensity statistics. The structure solution refines as an inversion twin in a ratio of 83:17, indicating that partial racemization occurred under the conditions of crystallization. One half of the oxalate-bridged dimer is unique, with the other half being generated by a twofold axis which runs along C1 and C2. The end of the sulfur branch is modeled as disordered over two positions (50:50). Highly disordered solvent was detected which could not be modeled. The data were corrected to remove this electron density using program PLATON, function SQUEEZE,²³ which determined that there were 827 electrons in 2697 Å³ per unit cell. The tremendous amount of solvent may also explain the large thermal ellipsoids, since the molecules were in a "loose" crystalline lattice, thus allowing more movement than expected for a data collection at 173 K. Constraints (AFIX 66) were used on the phenyl rings (C7–C12,

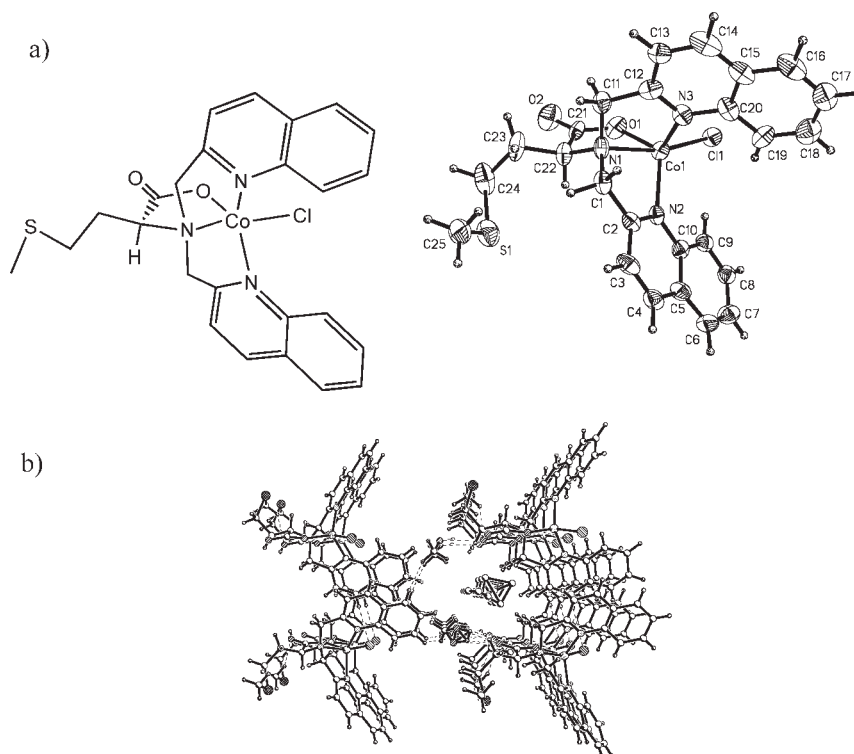


Fig. 3. (a) ORTEP view of the complex $\text{Co}^{\text{II}}(\mathbf{2})\text{Cl}$. (b) Solvent channel parallel to the a -axis in the crystal of $\text{Co}^{\text{II}}(\mathbf{2})\text{Cl}$.

C17–C22) to make them ideal. It must be noted that, because of the use of function SQUEEZE, various fields in Table 1 (empirical formula, formula weight, $F(000)$, etc.) are incorrect. In this structure, Co^{II} is six-coordinated and possesses octahedral geometry. Ligand **2** coordinates to the metal through two quinoline nitrogens, the tertiary amine nitrogen and the deprotonated carboxylate oxygen from the parent-amino acid moiety and two other oxygen atoms from the oxalate (the deprotonated oxygen on one carbon and the carbonyl oxygen on the other carbon from the oxalate). The other two oxygen atoms coordinate another cobalt that is ligated to another molecule of (*S*)-**2** to form a dimer. The sulfur atoms do not coordinate to the metal ions.

$[\text{Ni}_4^{\text{II}}(\mathbf{2})_4](\text{ClO}_4)_4$

An ORTEP view of the complex is shown in Figure 5. The structure was solved using SIR923 and refined using SHELXL-97. There are also four ClO_4^- and two MeCN molecules per main molecule. The structure is a tetranuclear aggregate of four metal ions and four ligands. In this structure, Ni^{II} is six-coordinated and possesses octahedral geometry. The ligand (*S*)-**2** coordinates to the metal through two quinoline nitrogens, the tertiary amine nitrogen and the deprotonated carboxylate oxygen from the parent-amino acid moiety and two other oxygen atoms from the carboxylate of a second (*S*)-**2**. The two oxygen atoms from the carboxylate of the first ligand coordinate to a second nickel.

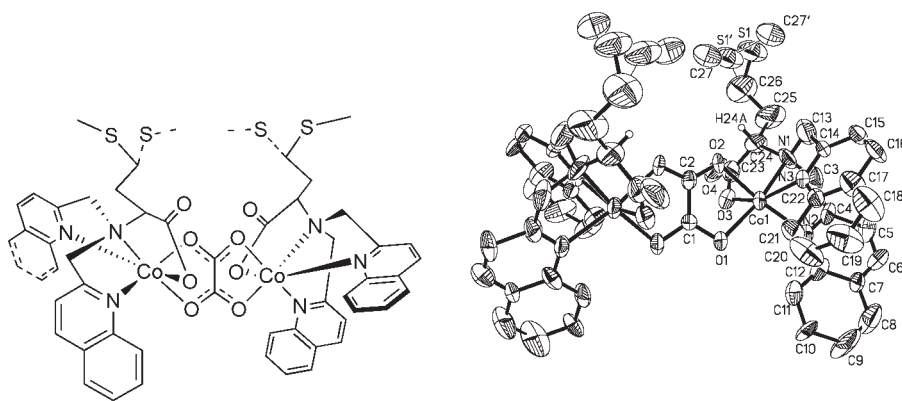


Fig. 4. ORTEP view of the complex $\text{Co}_2^{\text{II}}((L)\text{-}\mathbf{2})_2(\text{C}_2\text{O}_4)$. There is one crystallographic unique $\text{Co}(\mathbf{2})$ unit and a second, present by symmetry, included in this diagram. The disorder of the thiomethyl moiety is also depicted.

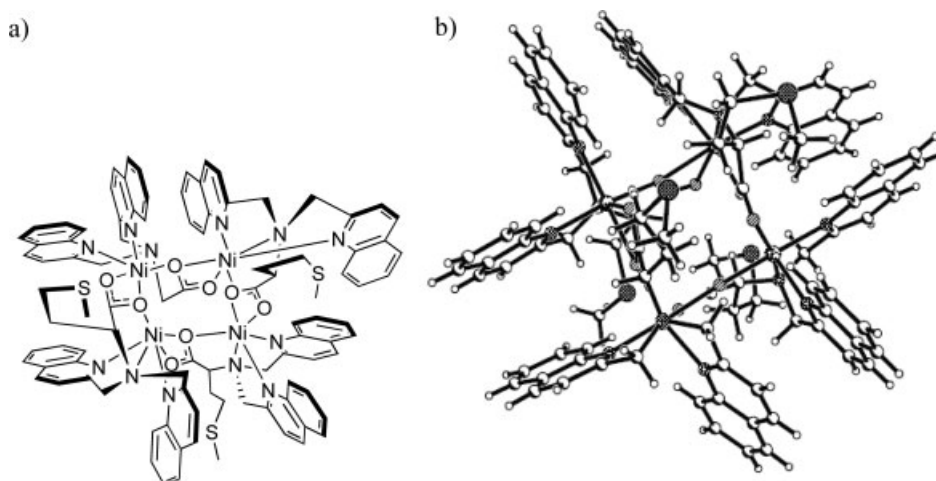


Fig. 5. (a) View of the cation portion of the complex $[\text{Ni}_4^{\text{II}}(\text{2})_4](\text{ClO}_4)_4$. (b) ORTEP view of the Ni atoms and atoms within their inner coordination sphere.

The nickel cationic unit is quadrupled via carboxylate-oxygens linkages. The sulfur atoms do not coordinate to the metal ions.

UV and CD Studies

The UV spectra of the quinoline-containing compound (*S*)-**2** and Cu^{II} , Cu^{I} , and Zn^{II} complexes were reported previously,¹⁴ and the peaks in their absorption bands were assigned. Upon addition of CoCl_2 to this ligand, the $^1\text{B}_g$ bands of the quinoline moieties blueshift slightly by 4 nm with a $\lambda_{\text{max}} = 231$ nm. Addition of ascorbic acid has a very small effect on this band ($\lambda_{\text{max}} = 230$ nm). Subsequent treatment with ammonium persulfate reverts the miniscule change ($\lambda_{\text{max}} = 230$ nm; Fig. 6). Generally, the UV-vis spectra shown here exhibit the same properties as the related Cu^{II} and Zn^{II} compounds, which have been discussed in detail. The free ligand (*S*)-**2** exhibited almost no CD signal. Addition of CoCl_2 resulted in intense CD signals which display nonconservative ECCD spectra (Fig. 6). Elemental analysis showed that the complex formed is the HCl adduct of $\text{Co}(\text{2})\text{Cl}$, although the elemental analysis could also be interpreted as the $\text{Co}^{\text{III}}(\text{2})\text{Cl}_2$ complex, but the preponderance of other data, including X-ray crystallography, indicated that it must be the HCl salt of a Co^{II} complex. The sign of the ECCD of this salt is negative, similar to the Cu^{II} and Zn^{II} complexes of (*S*)-**2**.¹⁴ The sign of the ECCD inverted to positive upon addition of ascorbic acid, and was turned back to negative upon further treatment with ammonium persulfate. Sodium thiosulfate was also used successfully as reductant, and gave more reproducible results than ascorbate. This behavior is consistent with a structural hypothesis in which the original complex formed by addition of CoCl_2 to (*S*)-**2** is a Co^{II} complex. Addition of reductant might result in a reduced cobalt (Co^{I}) species,^{26–28} but may also yield a transient intermediate such as an adduct with the reductant or a byproduct. Addition of oxidant would then return either transient reduced species to the Co^{II} oxidation state.

The complexes formed by mixing (*S*)-**2** and $\text{Ni}(\text{ClO}_4)_2$ exhibit ECCD with a positive sign as is shown in Figure 7, *Chirality* DOI 10.1002/chir

which is opposite to that expected if the carboxylate were coordinated to the metal. However, the crystallographic structure of $\text{Ni}((\text{S})\text{-2})\text{ClO}_4$ shows a carboxylate-coordinated tetramer with the ligand in two different conformations. In one conformation, the quinolines show a very small dihedral angle and would therefore be expected to give only a weak contribution to the ECCD spectrum. In the other ligand conformation, the quinoline moieties are oriented in a negative chiral sense, which would be expected to give a negative ECCD couplet. It is possible that the ECCD spectrum may derive from interaction between quinolines from different ligands within this aggregated structure. However, both positive and negative relative pairwise quinoline orientations can be identified in the crystal structure, and so there is no obvious prediction that can be made regarding the ECCD sign of the couplet. Additionally, such aggregates often dissociate into other species in solution,¹⁴ further complicating the analysis.

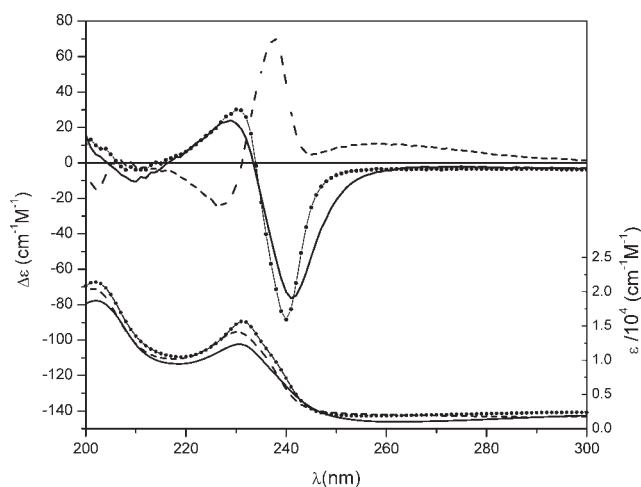


Fig. 6. UV (bottom) and CD (top) spectra of $[\text{Co}^{\text{II}}(\text{2})\text{Cl}]$ itself (solid curve) in methanol ($c = 0.1$ mM), after it was treated with 1 equiv of ascorbic acid (dash curve) and after the previous mixture was subsequently treated with 1 equiv of ammonium persulfate (dot-on-line curve).

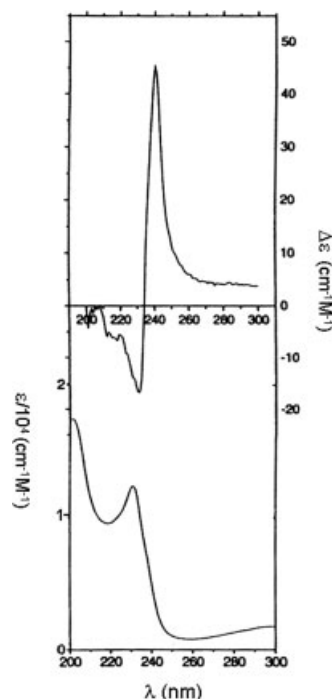


Fig. 7. UV (bottom) and CD (top) spectra of $[\text{Ni}(\mathbf{2})]\text{ClO}_4$ in methanol ($c = 0.1 \text{ mM}$).

CONCLUSIONS

This report describes the further development of a novel system that shows conformational changes resulting in the inversion of chromophoric exciton chirality upon one-electron change. In these propeller-shaped molecules, this spectroscopic phenomenon is best rationalized by the inversion of the axial chiral element defined by the orientation of the two chromophores. Previous work with Zn^{II} , Cu^{II} , and Cu^{I} yielded a consistent picture of the conformational properties and redox-induced ligand reorganization in this system. Certain $\text{Co}(\text{II})$ complexes showed similar behavior to the previously studied copper complexes and gave inversion of chirality when reacted with reducing agents, but $\text{Ni}(\text{II})$ complexes did not show the expected CD spectra. It is clear that the solution and crystallographic structures of these complexes differ in several cases.

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Enantioselective Oxidation of Sulfides Catalyzed by Titanium Complexes of 1,2-Diarylethane-1,2-Diols: Effect of the Aryl Substitution

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT The effects of the substitution on the aryl moiety on the asymmetric oxidation of sulfides mediated by Ti-complexes of chiral 1,2-diarylethane-1,2-diols were investigated. The substitution of the aryl ring of the diol with both EWG and EDG substituents generally decreased the enantioselectivity with respect to the use of unsubstituted 1,2-diphenylethane-1,2-diol (**1a**). Only in the presence of 1,2-di(4-*t*-butyl)phenyl-1,2-diol (**1g**) were higher ee's obtained with aryl methyl and aryl benzyl sulfides affording ee's up to 90 and 99%, respectively. Lower ee's were achieved with larger naphthyl and aryl alkyl sulfides. Contrary to the other Ti-alcoholates used in the oxidation of sulfides, the Ti-complex of diol **1g** was soluble in hexane, allowing us to perform the process with high enantioselectivity in this solvent, with great environmental advantages over the commonly used chlorinated solvents. *Chirality* 20:592–596, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: sulfoxides; asymmetric synthesis; chiral diols; enantioselective catalysis; asymmetric oxidation

INTRODUCTION

The enantioselective oxidation of prochiral sulfides is one of the most efficient and practical procedures to achieve optically active sulfoxides,^{1–5} compounds of great synthetic interest as chiral auxiliaries,⁶ synthetic intermediates and bioactive compounds.⁷ Enantioselective oxidations of sulfides can be performed either by biocatalysis^{8,9} or by chemical oxidation, the latter occurring in the presence of chiral oxidizing species, or by using achiral oxidants and chiral metal complexes.^{1–5,7} The most successful enantioselective oxidation procedure, which also has industrial application,^{10–13} requires the use of achiral hydroperoxides in the presence of stoichiometric amounts of a chiral Ti(IV) complex. This approach dates back to the seminal work of Kagan and coworkers^{14–17} and Modena and coworkers¹⁸ who, independently, reported the use of alkyl hydroperoxides in conjunction with a stoichiometric amount of a Ti-complex formed in situ from Ti(*i*-PrO)₄ and (*R,R*)-diethyl tartrate (DET). Since then many efforts have been devoted to the search for catalytic procedures to achieve highly enantioenriched sulfoxides using only catalytic amounts of Ti-complexes, with great economical and practical advantages. In fact, the purification step of sulfoxides from the large amount of Ti salts produced during the workup is a serious drawback of this approach, which can greatly limit its use on large or industrial scale. Although a number of approaches using catalytic Ti-complexes of chiral diols have been described in the literature,^{19–24} many of these cannot be considered

truly catalytic, either because they employ only substoichiometric amounts of Ti and diol (up to 0.4 equiv)²¹ or give good enantioselectivity not in the enantioselective sulfide oxidation process, but mainly thanks to a kinetic resolution process²⁵ determined by a concomitant enantioselective overoxidation of the sulfoxide to sulfone.^{19,23,26–32} In the latter case, although the ee's of sulfoxides are high, this goal is reached with significant reduction of chemical yields, which often do not exceed 50%. Some years ago we described a real catalytic oxidation of sulfides in which the use of 5% mol of a catalyst formed in situ by reacting Ti(*i*-PrO)₄, enantiopure 1,2-diphenylethane-1,2-diol (**1a**), and water gave the optically active sulfoxide in good to high ee's avoiding the overoxidation of sulfoxide to sulfone and kinetic resolution.^{33–36} The main advantages of our approach reside in the use of a catalytic amount of the metal complex, in the high chemical yields achievable, and in the unprecedented high ee's (up to 99%) obtained with benzyl alkyl sulfoxides. Thanks to its reliability and efficiency, this procedure has been employed by other groups in the preparation of valuable chiral drugs,^{37,38} and

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it constitutes the key step of an efficient protocol for the preparation of optically active dialkylsulfoxides by substitution at sulfur.^{39,40} Furthermore, by the same procedure a large class of alkyl aryl sulfoxides, having different structure, have been prepared allowing us to set up efficient methods of analysis of their chiroptical properties and to develop new nonempirical approaches for the assignment of absolute configuration of chiral sulfoxides.^{41–48}

In search of further improvement to our sulfide oxidation procedure, aimed at enhancing the enantioselectivity for aryl alkyl sulfoxides and to discover reaction conditions suitable for industrial scale up, we investigated the effect of the diol structure on the enantioselectivity. In particular, in this article the electronic and steric effects of substituents on the diol aryl moiety are investigated with the aim of achieving a more enantioselective process and to obtain deeper insight into the oxidation mechanism.

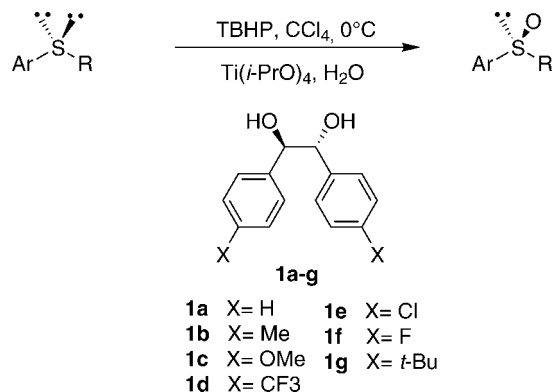
MATERIALS AND METHODS

General

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. ¹H NMR (300 MHz) and ¹³C NMR (75 MHz) spectra were recorded in CDCl₃ on a Bruker Aspect 300 spectrometer using TMS as internal standard. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Enantiomeric excesses of the sulfoxides were determined by HPLC analysis performed with a Hewlett Packard series 1050 pump with a Varian 2550 UV detector and Daicel Chiralcel OD or Chiralcel OJ column.³⁶ Hexane was freshly distilled prior to use on sodium benzophenone ketyl under a nitrogen atmosphere. CCl₄ was distilled from CaH₂ and stored over activated 4-Å molecular sieves. Ti(*i*-PrO)₄ was distilled prior to use under N₂ atmosphere. Commercially available *tert*-butyl hydroperoxide (TBHP) (70% in water) was purchased (Aldrich) and used without further purification. Phenyl methyl sulfide, *p*-tolyl methyl sulfide, and phenyl benzyl sulfide were used as purchased (Aldrich), *p*-nitrophenyl methyl sulfide (Aldrich) was recrystallized from hexane prior to use. The other aryl alkyl sulfides were prepared by alkylation of the corresponding thiols. Enantiomerically pure (*R,R*)-1,2-diarylethane-1,2-diols **1a–g** were prepared by asymmetric dihydroxylation of the corresponding (*E*)-1,2-diarylethenes as described elsewhere.^{49–51} Racemic sulfoxides were prepared by oxidation of the corresponding sulfides with H₂O₂/trifluoroacetone according to a literature procedure.⁵² Analytical and preparative TLC were performed, respectively, on 0.2- and 2.0-mm silica gel plates Merck 60 F-254 and column chromatography was carried out with silica gel Merck 60 (80–230 mesh).

General Procedure for the Catalytic Oxidation of Sulfides

To a suspension of (*R,R*)-diol (0.16 mmol) in CCl₄ (5 ml) were dropwise added in sequence Ti(*i*-PrO)₄ (23.6 μl, 0.08 mmol) and H₂O (28.8 μl, 1.6 mmol) (Scheme 1). To the resulting homogeneous solution was added the sulfide (1.61 mmol), stirring at room temperature for 15 min. The solution was then cooled at 0°C and TBHP (70% in water, 440 μl, 3.22 mmol) added. The mixture was left



Scheme 1

stirring at 0°C for 2 h, then diluted with CH₂Cl₂ and dried over Na₂SO₄ for a few minutes. After filtration and evaporation of solvent the residue was immediately purified by column chromatography (EtOAc) isolating the sulfoxide.

RESULTS AND DISCUSSION

In a preliminary investigation on the influence of the diol aryl moiety, carried out some years ago,³⁵ we observed that the *para* substitution of the diol with both a OCH₃ and a CF₃ group decreases the enantioselectivity of the sulfide oxidation and that unexpectedly, the CF₃ substitution reverses the absolute configuration of the sulfoxide obtained. These results, although not easy to interpret, clearly show that the presence on the aryl moiety of substituents exerting either an electronic, a coordinating or simply a steric effect, can deeply affect the stereochemical outcome of the sulfide oxidation. We, therefore, started the present investigation aimed at clarifying the effect of diol substitution on enantioselectivity.

To study the influence of both electronic and steric effects, we prepared diols (*R,R*)-**1b,c**, *para* substituted with electron donating groups (EDG's) CH₃ and OCH₃, respectively, diols (*R,R*)-**1d–f**, substituted with electron withdrawing groups (EWG's), and diol (*R,R*)-**1g**, where the *t*-Bu substitution should give rise to interactions mainly steric in nature. The preparation of (*R,R*)-**1b,c**,^{49,50} (*R,R*)-**1d–f**,⁵¹ and (*R,R*)-**1g**⁵³ was performed according to literature by the enantioselective Sharpless dihydroxylation of the corresponding *E*-alkenes,⁵⁴ in turn obtained by the Wittig reaction or Mc Murry coupling.⁵⁵

The diols **1b–g** were then tested as chiral ligands in the enantioselective oxidation of *p*-tolyl methyl sulfide representative of aryl methyl sulfides, comparing the results with those obtained with the unsubstituted diol **1a** (Table 1). The oxidations were carried out following the best standard conditions established in our previous work,^{33,34} which use reagent ratios of sulfide/diol/Ti/H₂O = 1.0/0.1/0.05/1.0, and 2 equiv of TBHP 70% in water as oxidant, carrying out the reaction at a temperature of 0°C in CCl₄, under N₂ for 2 h. In our experience these optimized conditions are the best compromise in order to limit sulfone for-

TABLE 1. Enantioselective oxidation of *p*-tolyl methyl sulfide with diols **1a–g^a**

Run	Diol	X	Yield (%) ^b	ee (%) ^c	A.C. ^d
1	(<i>R,R</i>)- 1a	H	62	80	S
2	(<i>R,R</i>)- 1b	4-Me	48	40	S
3	(<i>R,R</i>)- 1c	4-OMe	55	48	S
4	(<i>R,R</i>)- 1d	4-CF ₃	70	26	R
5	(<i>R,R</i>)- 1e	4-Cl	55	36	S
6	(<i>R,R</i>)- 1f	4-F	65	39	S
7	(<i>R,R</i>)- 1g	4- <i>t</i> Bu	70	90	S

^aReaction conditions: sulfide/(*R,R*)-**1a–g**/Ti(*i*-PrO)₄/H₂O = 1.0/0.1/0.05/1.0 in CCl₄ at 0°C under N₂, reaction time 2 h, of 70% TBHP in water as oxidant.

^bIsolated yields.

^cDetermined by HPLC on a Daicel Chiralcel OB column, see Ref. 36

^dDetermined by comparison of [α]_D with literature values, see Ref. 34.

mation (<10%), to have a satisfactory conversion and good to high ee's.

As inferred from Table 1, the use of (*R,R*) configured diols always afford (*S*)-tolyl methyl sulfoxide, except for the CF₃ substituted diol (*R,R*)-**1d** which, as already reported,³⁵ provides the (*R*) sulfoxide in low ee. The presence of both EDG and EWG as substituents, albeit maintaining a moderate to good chemical yield (50–70%), always results in a sharp lowering of the ee obtained, with respect to the results obtained with **1a**, such decreases appearing most relevant with **1d–f**, bearing EWG substituents. Much to our surprise, the *t*-Bu substituted diol **1g** afforded good chemical yield and enantioselectivity higher than that obtained with the parent diol **1a**.

We then decided to further investigate the potentiality of this new promising diol in the oxidation reaction, comparing the results obtained with those coming from **1a**. Several aryl alkyl and aryl benzyl sulfides were then submitted to enantioselective oxidation employing diol **1g** as chiral ligand under the standard conditions described before (Table 2). The oxidation of phenyl substituted methyl sulfides with **1g** in CCl₄ always gave (entries 1,3,5,6,8), comparable yields and higher enantioselectivity with respect to **1a**, with an average ee increment of 10%. In particular, the oxidation of aryl benzyl sulfides with **1g** (entries 14 and 15) afforded the corresponding sulfoxides with ee > 99%. On the contrary, the oxidation of more hindered sulfides like the 1- and 2-naphthyl substituted ones (entries 9, 11, and 17) and those having an alkyl moiety larger than methyl (entries 12 and 13), gives rise to lower ee's than **1a**. In summary, the Ti-complex of diol **1g** displays higher substrate selectivity than **1a** in the sulfide oxidation, increasing enantioselectivity with aryl methyl and aryl benzyl sulfides and decreasing ee with more hindered sulfides. Such selectivity can be interpreted as due to the presence of large hindering groups on the diol like *t*-Bu, which give the complex a narrower active site able to fit only medium size sulfides properly.

As previously reported³⁴ the diol **1a** is only slightly soluble in CCl₄ and completely insoluble in hexane. Its Ti-complex dissolves in both solvents, but further addition of water affords a homogeneous solution in CCl₄, and precipitation of a white solid in hexane. The enantioselectivity in both solvents was comparable while the homogeneity of the mixture in CCl₄ provided higher yields. For this reason the latter solvent was chosen as best for our standard proce-

TABLE 2. Enantioselective oxidation of sulfides (ArSR) with diols **1g and **1a**^a**

entry	Ar	R	solvent	Diol 1g		Diol 1a ^b	
				Yield (%) ^c	ee ^d (%) (AC) ^e	Yield (%) ^c	ee ^d (%) (AC) ^e
1	Ph	Me	CCl ₄	65	82(S)	63	80(S)
2	Ph	Me	hexane	73	82(S)	–	–
3	<i>p</i> -MeC ₆ H ₄	Me	CCl ₄	70	90(S)	62	80(S)
4	<i>p</i> -MeC ₆ H ₄	Me	hexane	70	86(S)	–	–
5	<i>p</i> -MeOC ₆ H ₄	Me	CCl ₄	46	80(S)	55	69(S)
6	<i>p</i> -BrC ₆ H ₄	Me	CCl ₄	48	78(S)	58	67(S)
7	<i>p</i> -BrC ₆ H ₄	Me	hexane	52	80(S)	–	–
8	<i>p</i> -NO ₂ C ₆ H ₄	Me	CCl ₄	70	60(S)	74	44(S)
9	2-Naphthyl	Me	CCl ₄	65	60(S)	65	73(S)
10	2-Naphthyl	Me	hexane	68	74(S)	–	–
11	1-Naphthyl	Me	CCl ₄	63	46(S)	70	57 ^f (S)
12	Ph	Et	CCl ₄	61	36(S)	71	70(S)
13	Ph	<i>i</i> -Pr	CCl ₄	55	18(S)	60	22(S)
14	Ph	PhCH ₂	CCl ₄	65	>99(S)	73	>99(S)
15	<i>p</i> -MeOC ₆ H ₄	PhCH ₂	CCl ₄	70	>99(S)	60	92(S)
16	<i>p</i> -MeOC ₆ H ₄	PhCH ₂	hexane	72	>99(S)	–	–
17	2-Naphthyl	PhCH ₂	CCl ₄	72	16(S)	75	92(S)

^aConditions: sulfide/(*R,R*)-diol/Ti(*i*-PrO)₄/H₂O = 1.0/0.1/0.05/1.0 at 0°C under N₂ atmosphere, reaction time 2 h, 2 equiv of 70% TBHP in water as oxidant.

^bData from Ref. 34.

^cIsolated yields.

^dDetermined by HPLC on a Daicel Chiralcel OB or OJ column, see Ref. 36.

^eDetermined by comparison of [α]_D with literature values, see Refs.34 and 36.

^fData from Ref. 36.

ture.³⁴ We now noticed that **1g** was, on the contrary, very soluble both in CCl₄ and hexane and that its Ti-complex was still soluble in hexane also after addition of water. This finding prompted us to investigate the enantioselective oxidation of sulfides with the Ti-complex **1g** in this solvent also, much less toxic and more environmentally friendly than CCl₄ and other chlorinated solvents used so far. As inferred from Table 2, the use of diol **1g** allows oxidations in hexane (entries 2, 4, 7, 10, 16) with similar yields and ee's than those obtained in CCl₄ disclosing the way to a "greener" oxidation protocol.

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Fluorous “Racemic” Mixture Synthesis: Polysaccharide-Based Chiral Columns for Simultaneous Demix and Enantioseparation of Racemic Fluorous Tagged Compounds

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Dedicated to Professor Nina Berova on the occasion of her significant anniversary and her receiving the Chirality Medal 2007.

ABSTRACT The proof of concept experiments of fluorous “racemic” mixture synthesis (FRMS) is shown using polysaccharide-based chiral stationary phases. The mixture of racemic *O*-benzoylmandelate derivatives bearing different lengths of fluorous cleavable tags undergoes sequential reactions to provide individual derivatives as well as their enantiomers resolved on polysaccharide-based chiral HPLC columns (DAICEL CHIRALCEL[®] and CHIRALPAK[®] series). *Chirality* 20:597–603, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: chiral HPLC column; combinatorial chemistry; enantioseparation; fluorous racemic mixture synthesis (FRMS); fluorous tag

The development of technologies for analysis and purification of organic compounds in enantiopure forms is of critical importance in modern synthetic organic chemistry.^{1,2} Especially in combinatorial chemistry,^{3–11} rapid synthesis of a library^{12–30} of analogues, and efficient separation and identification of target analogues are essential. For these purposes, the technology for separation using perfluoroalkyl ponytails, the so-called fluorous tags, has recently been developed.^{31–34}

We have already developed an efficient synthetic and purification process for enantiopure compounds from racemic substrates with fluorous tags (In collaboration with Takeuchi, we have also reported the asymmetric reduction of fluorous ketones but not fluorous cleavable tagged, as the antecedent of prochiral substrates),^{35–39} that is, *fluorous “racemic” mixture synthesis* (FRMS), and demonstrated the efficiency of FRMS by using chiral β -cyclodextrin (β -CyD) HPLC columns (SUMICHIRAL[®] OA-7000 series⁴⁰)[†] (Scheme 1b). We report here the application of FRMS using polysaccharide-based chiral HPLC columns^{41–46} (DAICEL CHIRALCEL[®] and CHIRALPAK[®] series⁴⁷)[‡] (Scheme 1a). In FRMS (Scheme 2), two possible types of starting materials (1) racemic ($S^1/R^1 - S^n/R^n$) and (2) prochiral ($Pc^1 - Pc^n$) substrates, with cleavable fluo-

rous tags ($F^1 - F^n$) can be used. After a segmented reaction sequence (m), enantiopure products ($R^1 - R^{nm}$; $S^1 - S^{nm}$) can be obtained simultaneously solving the enantioseparation, and identification problems in conventional solution-phase mixture synthesis.

DAICEL chiral HPLC columns (CHIRALCEL[®] OD-H and CHIRALPAK[®] AD-H) can also be employed to efficiently enantioseparate fluorous tagged racemates in particular with highly polar functionality (Scheme 1a). The stationary phases of these columns consist of the tris(phenylcarbamate) derivatives of polysaccharides (OD-H: tris(3,5-dimethylphenylcarbamate) derivatives of cellulose; AD-H: tris(3,5-dimethylphenylcarbamate) derivatives of amylose). The DAICEL chiral HPLC columns show excellent enantiodiscrimination of a variety of racemates with polar functional groups in particular, through hydrogen bonding (Scheme 1a).

This FRMS method was first applied to racemic starting materials (Scheme 3a), in the context of the synthesis of *O*-benzoylmandelate derivatives with important biological properties.^{48,49} Racemic mandelic acid derivatives as starting materials were tagged with different fluorous alcohols, $C_nF_{2n+1}CH_2OH$ ($n = 1, 3, 7$) by the standard (DCC/DMAP) method.

To produce fluorous-tagged mandelic acid derivatives directly from prochiral glyoxylate and achiral aromatic

[†]SUMICHIRAL OA-7000 series are sold by Sumika Chemical Analysis Service. SUMICHIRAL OA-7000 is the column with unmodified β -CD bonded to the silica gel via saccharide (glucuroylgluconoyl group) spacer; SUMICHIRAL OA-7100 is the column with unmodified β -CD bonded to the silica gel via alkyl group spacer; SUMICHIRAL OA-7500 is the column with β -CD, in which hydroxyl group is protected by methyl group, bonded to the silica gel via alkyl group spacer.

[‡]DAICEL CHIRALCEL[®] and CHIRALPAK[®] series are sold by DAICEL CHEMICAL INDUSTRIES, CPI COMPANY.

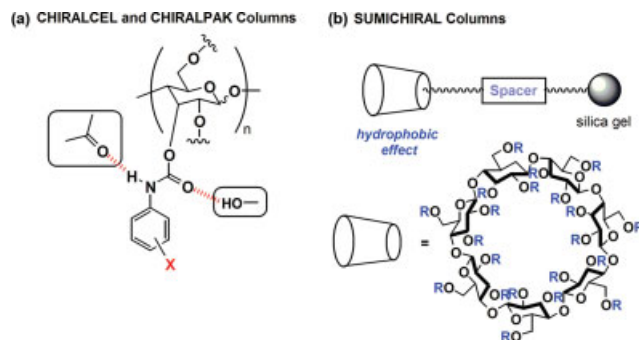
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Scheme 1. Comparison of chiral stationary phases. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

compounds (Scheme 3b), the Friedel-Crafts carbonyl alkylation^{50–55} of aromatic compounds such as isobutylbenzene was also exploited with fluororous-tagged glyoxylate in the presence of Lewis acids (TiCl_4 or racemic BINOL-Ti catalysts).

Each tagged compound n (three) was mixed and split into m (three) portions and then reacted with m (three) types of benzoylchloride derivatives to give $n \times m$ (“3-mix/3-split”) tagged products (**1a–i**).

The resulting “3-mix/3-split” mixture of *O*-benzoylmandelate derivatives and their enantiomers were simultaneously enantioresolved using AD-H column to give $3 \times 3 \times 2$ enantiopure *O*-benzoylmandelate derivatives (Fig. 1); each mixture of *O*-benzoylmandelate derivatives was eluted in order to decrease the length of fluororous tags starting from C_7F_{15} to end up with CF_3 . This order of the decreasing length of fluororous tags is in sharp contrast to the order of the increasing length of fluororous tags eluted from the chiral β -cyclodextrin columns based on hydrophobic interaction with the fluororous tags.

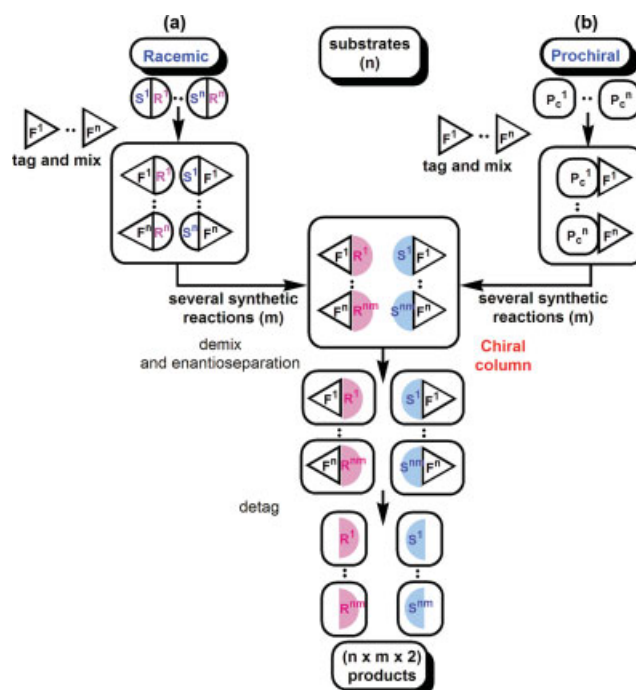
Each enantiomer pair was simultaneously resolved using AD-H column with (S)-(-)-enantiomers as the faster eluents. The enantioresolution of each enantiomer was further improved by introducing a *p*-substituent into the *O*-benzoyl group. In the case of *p*-Me and *p*- NO_2 substituents, each enantiomer was completely separated (Figs. 1b and 1c). The configuration of the enantiomers can be determined by circular dichroism (CD) (JASCO CD-995 (1595)) and/or optical rotation (OR) (JASCO OR-1590) HPLC spectroscopy (circular dichroism/ultraviolet (CD/UV) or optical rotation/refractive index unit (OR/RIU) HPLC spectroscopy to determine the enantiomeric excesses of products).^{56–61} $n \times m \times 2$ mixture of enantiopure *O*-benzoylmandelate derivatives and their enantiomers can thus be separated.

We further applied FRMS for the derivative of natural products, e.g., mappicine^{62,63} **4** bearing polar fluororous ester tags rather than less polar silyl ether tags, by using chiral AD-H and OD-H columns. Each enantiomer of mappicine **4** with fluororous ester tag was baseline separated on both columns under the normal conditions (Fig. 2). The high level of enantiodiscrimination is probably ascribed to the polar hydrogen bonding interaction between phenyl-

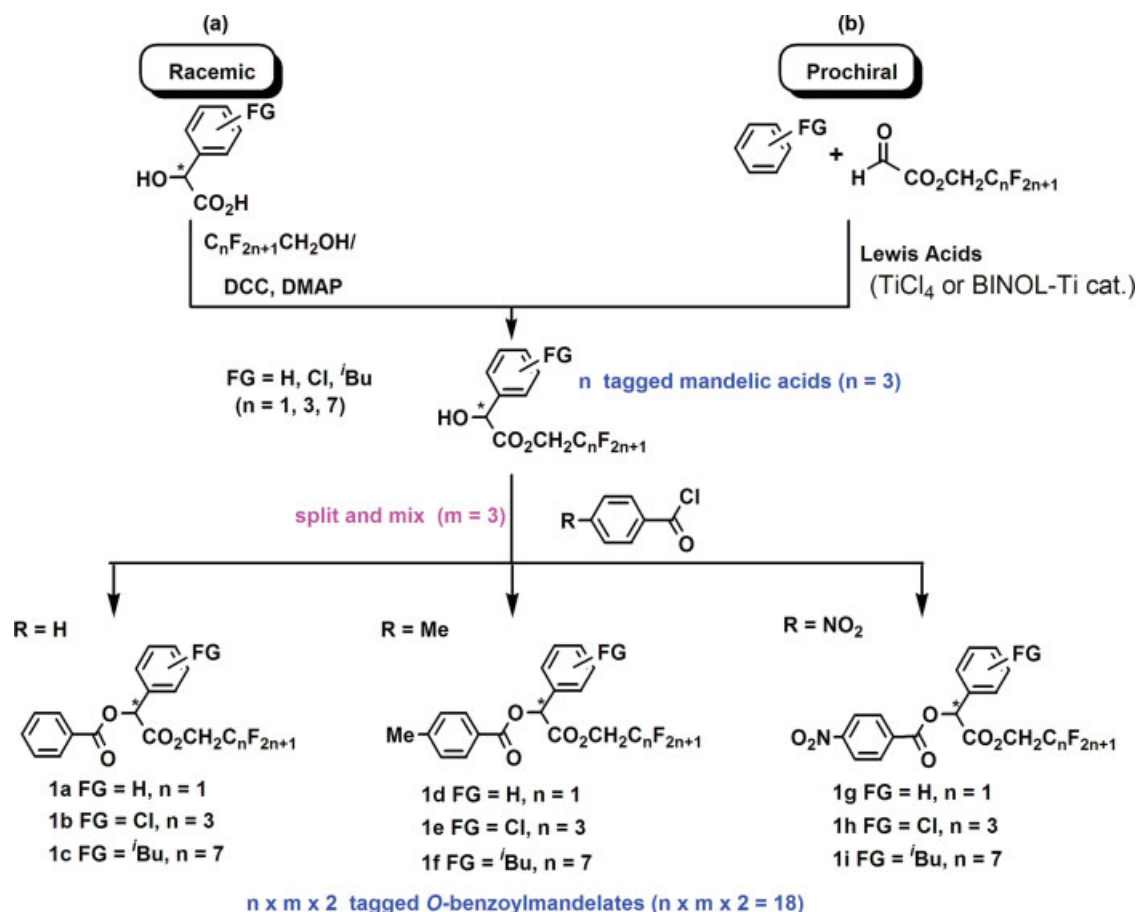
carbamate group in the polysaccharide-based stationary phases of DAICEL columns and fluororous ester tags. This shows that FRMS is potentially applicable to a wide range of natural products and their derivatives by sorting a fluororous tag which is suitable for the structure of chiral stationary phases.

Fluorous techniques such as “quasiracemic synthesis”⁶⁴ employ enantiopure starting material by tagging the enantiomers with different lengths of fluororous tags: (1) the separation of enantiomers is thus enhanced by the tags; (2) the structure of the enantiomers cannot be confirmed by CD-HPLC but has increased predictability. However, as reported, racemization during the synthesis is often problematic. In contrast, racemization is not problematic in FRMS. A chiral polysaccharide-based HPLC columns such as chiral DAICEL HPLC (AD-H and OD-H) columns, can efficiently enantiodiscriminate racemates by introduction of fluororous ester tags. The advantages of the FRMS strategy are (Table 1): (1) enantiopure starting materials are not necessary; (2) racemization in a reaction can be disregarded; (3) two enantiomeric products can be simultaneously obtained by single fluororous tag; and (4) the efficiency of the present FRMS can be maximized by the auxiliary use of circular dichroism (CD) and/or optical rotation (OR) HPLC spectroscopy to determine the absolute configuration of products.^{65–68}

In conclusion, we have reported the proof of concept experiments of FRMS (fluorous “racemic” mixture synthesis). By using a polysaccharide-based chiral column to enantioresolve racemates bearing fluororous tags, we have



Scheme 2. Fluorous racemic mixture synthesis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]



Scheme 3. Fluorous racemic mixture synthesis of *O*-benzoylmandelate library. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

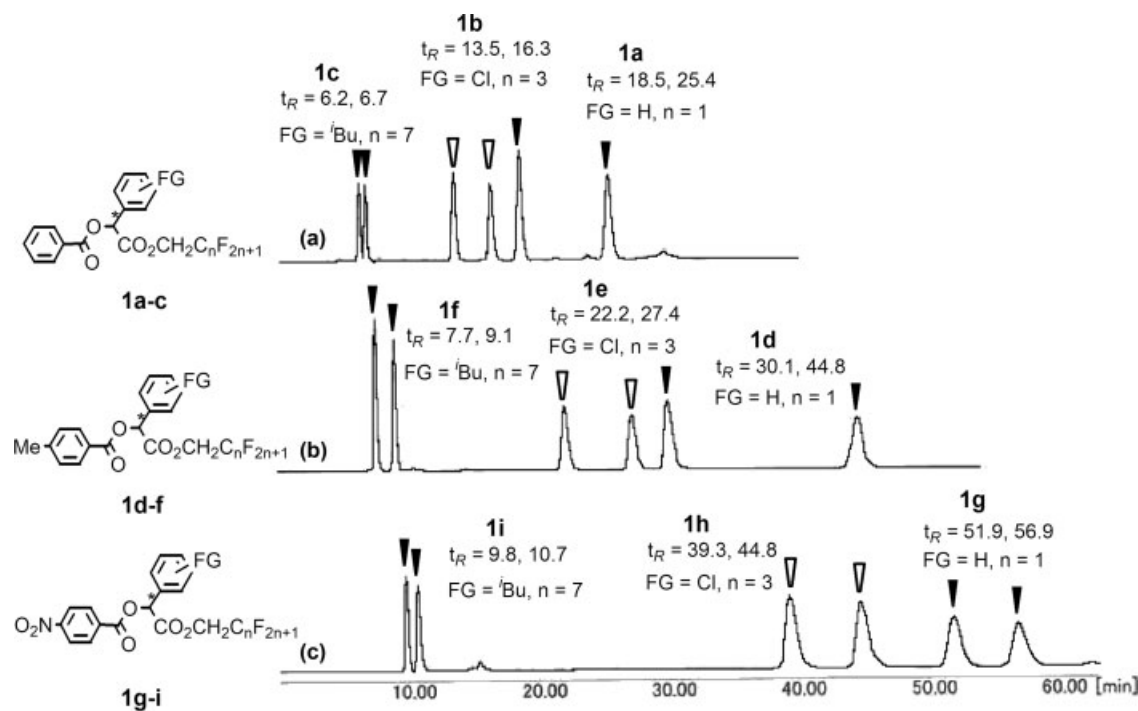


Fig. 1. Enantioseparation of *O*-benzoylmandelate derivatives (1a–i) and their enantiomers.

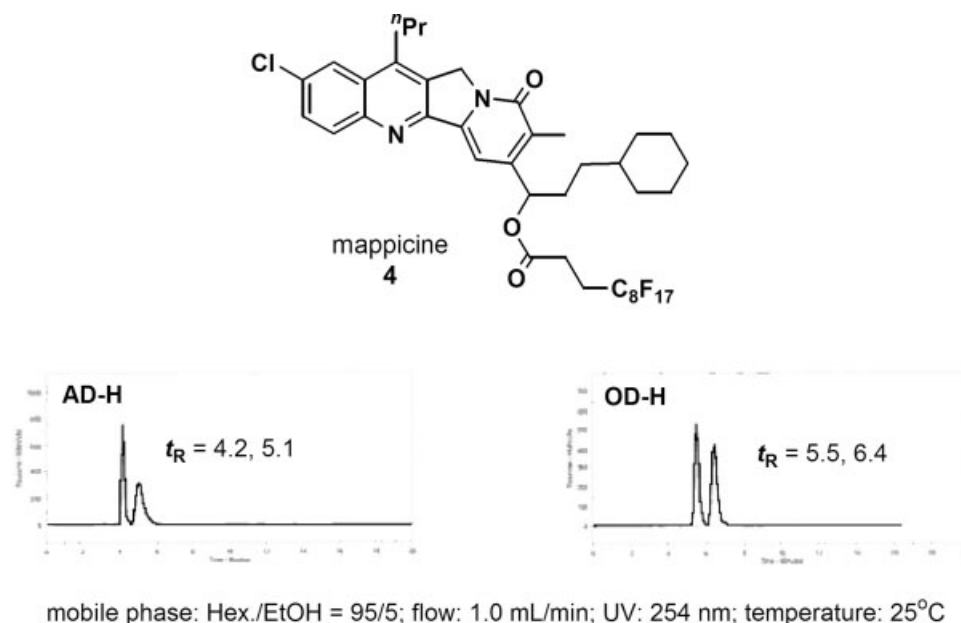


Fig. 2. Enantioseparation of mappicine derivative bearing fluorous tags.

exemplified the wide applicability of FRMS to a variety of chiral natural and unnatural compounds.

TYPICAL CHIRAL HPLC ANALYSIS

HPLC analysis was performed on polysaccharide-based chiral columns (DAICEL CHIRALCEL[®] OD-H, 25 cm $\times$ 4.6 mm i.d.; CHIRALPAK[®] AD-H, 25 cm $\times$ 4.6 mm i.d.) with a GL cart (Inertsil SIL100-5) as a guard column and a JASCO HPLC system (pump: PU-980, gradient unit: LG-980-02, degasser: DG-980-50, column oven: CO-960, UV and CD detector: UV-975 and CD-1595, auto sampler: AS-950). Peak area was calculated by JASCO-BORWIN as an automatic integrator. HPLC grade hexane and IPA were purchased from Kanto Chemical.

($\pm$)-2,2,2-Trifluoroethyl O-benzoylmandelate (1a)

¹H NMR (300 MHz, CDCl₃) δ 4.53 (q, $J = 8.4$ Hz, 2H), 6.22 (s, 1H), 7.46 (m, 5H), 7.59 (d, $J = 6.3$ Hz, 2H), 8.13 (d, $J = 8.7$ Hz, 2H).

¹⁹F NMR (282.4 MHz, CDCl₃) δ -74.0 (t, $J = 6.2$ Hz, 3F).

DAICEL CHIRALCEL AD-H, UV detector 254 nm, temperature 20°C, hexane/*i*PrOH = 98/2, flow rate 0.7 ml/min, $t_R = 18.5$ min, 25.4 min.

($\pm$)-4-Chloro-2,2,3,3,4,4,4-heptafluorobutyl O-benzoylmandelate (1b)

¹H NMR (300 MHz, CDCl₃) δ 4.65 (qd, $J = 5.1, 12.0$ Hz, 2H), 6.20 (s, 1H), 7.41–7.64 (m, 7H), 8.12 (d, $J = 7.2$ Hz, 2H).

¹⁹F NMR (282.4 MHz, CDCl₃) δ -127.6 (s, 2F), -120.5 (m, 2F), -80.9 (t, $J = 9.0$ Hz, 3F).

DAICEL CHIRALCEL AD-H, UV detector 254 nm, temperature 20°C, hexane/*i*PrOH = 98/2, flow rate 0.7 ml/min, $t_R = 13.5$ min, 16.3 min.

($\pm$)-4-Isobutyl-1*H*,1*H*-perfluorooctyl O-benzoylmandelate (1c)

¹H NMR (300 MHz, CDCl₃) δ 0.90 (d, $J = 6.7$ Hz, 6H), 1.86 (sept, $J = 6.7$ Hz, 1H), 2.50 (d, $J = 7.2$ Hz, 2H), 4.64 (sext, 2H), 6.18 (s, 1H), 7.21 (d, $J = 8.1$ Hz, 2H), 7.44–7.63 (m, 5H), 8.12 (d, $J = 9.9$ Hz, 2H).

¹⁹F NMR (282.4 MHz, CDCl₃) δ -126.1 (s, 2F), -123.3 (s, 2F), -122.8 (s, 2F), -122.1 (s, 4F), -119.4 (m, 2F), -80.7 (t, $J = 9.3$ Hz, 3F).

DAICEL CHIRALCEL AD-H, UV detector 254 nm, temperature 20°C, hexane/*i*PrOH = 98/2, flow rate 0.7 ml/min, $t_R = 6.2$ min, 6.7 min.

TABLE 1. Comparison between FRMS and quasiracemic synthesis

	FRMS	'Quasiracemic' synthesis
Starting material	Even racemic and prochiral	Enantiopure only
Racemization	No problem	Problematic (racemization can occur)
The number of fluorous tag	One fluorous tag for a pair of racemates	Two fluorous tags for a pair of racemates
Enantiomer identification	Auxiliary methods are required	Identified by the order of elution

(±)-2,2,2-Trifluoroethyl O-(4-methyl) benzoylmandelate (1d)

¹H NMR (300 MHz, CDCl₃) δ 2.43 (s, 3H), 4.53 (q, *J* = 8.1 Hz, 2H), 6.21 (s, 1H), 7.27 (d, *J* = 7.8 Hz, 2H), 7.45 (m, 3H), 7.60 (m, 2H), 8.02 (d, *J* = 8.1 Hz, 2H).

¹⁹F NMR (282.4 MHz, CDCl₃) δ -73.8 (t, *J* = 8.8 Hz, 3F).

DAICEL CHIRALCEL AD-H, UV detector 254 nm, temperature 20°C, hexane/ⁱPrOH = 98/2, flow rate 0.7 ml/min, *t*_R = 30.1 min, 44.8 min.

(±)-4-Chloro-2,2,3,3,4,4,4-heptafluorobutyl O-(4-methyl) benzoylmandelate (1e)

¹H NMR (300 MHz, CDCl₃) δ 2.43 (s, 3H), 4.63 (tt, *J* = 4.8, 12.6 Hz, 2H), 6.17 (s, 1H), 7.27 (d, *J* = 6.9 Hz, 2H), 7.42 (d, *J* = 8.1 Hz, 2H), 7.52 (d, *J* = 6.9 Hz, 2H), 8.00 (d, *J* = 8.1 Hz, 2H).

¹⁹F NMR (282.4 MHz, CDCl₃) δ -127.7 (s, 2F), -120.5 (m, 2F), -80.9 (t, *J* = 8.8 Hz, 3F).

DAICEL CHIRALCEL AD-H, UV detector 254 nm, temperature 20°C, hexane/ⁱPrOH = 98/2, flow rate 0.7 ml/min, *t*_R = 22.2 min, 27.4 min.

(±)-4-Isobutyl-1*H*,1*H*-perfluorooctyl O-(4-methyl) benzoylmandelate (1f)

¹H NMR (300 MHz, CDCl₃) δ 0.90 (d, *J* = 6.9 Hz, 6H), 1.87 (sept, *J* = 6.9 Hz, 1H), 2.42 (s, 3H), 2.50 (d, *J* = 7.2 Hz, 2H), 4.65 (sext, 2H), 6.16 (s, 1H), 7.20 (d, *J* = 7.8 Hz, 2H), 7.25 (d, *J* = 7.8 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 8.02 (d, *J* = 8.4 Hz, 2H).

¹⁹F NMR (282.4 MHz, CDCl₃) δ -126.1 (s, 2F), -123.3 (s, 2F), -122.8 (s, 2F), -122.1 (s, 4F), -119.4 (m, 2F), -80.8 (t, *J* = 9.3 Hz, 3F).

DAICEL CHIRALCEL AD-H, UV detector 254 nm, temperature 20°C, hexane/ⁱPrOH = 98/2, flow rate 0.7 ml/min, *t*_R = 7.7 min, 9.1 min.

(±)-2,2,2-Trifluoroethyl O-(4-nitro) benzoylmandelate (1g)

¹H NMR (300 MHz, CDCl₃) δ 4.54 (q, *J* = 8.1 Hz, 2H), 6.25 (s, 1H), 7.46 (m, 3H), 7.57 (m, 2H), 8.32 (m, 4H).

¹⁹F NMR (282.4 MHz, CDCl₃) δ -73.8 (t, *J* = 6.5 Hz, 3F).

DAICEL CHIRALCEL AD-H, UV detector 254 nm, temperature 20°C, hexane/ⁱPrOH = 98/2, flow rate 0.7 ml/min, *t*_R = 51.9 min, 56.9 min.

(±)-4-Chloro-2,2,3,3,4,4,4-heptafluorobutyl O-(4-nitro) benzoylmandelate (1h)

¹H NMR (300 MHz, CDCl₃) δ 4.65 (tt, *J* = 3.3, 13.5 Hz, 2H), 6.22 (s, 1H), 7.50 (m, 4H), 8.32 (m, 4H).

¹⁹F NMR (282.4 MHz, CDCl₃) δ -127.7 (s, 2F), -120.5 (m, 2F), -80.8 (t, *J* = 8.2 Hz, 3F).

DAICEL CHIRALCEL AD-H, UV detector 254 nm, temperature 20°C, hexane/ⁱPrOH = 98/2, flow rate 0.7 ml/min, *t*_R = 39.3 min, 44.8 min.

(±)-4-Isobutyl-1*H*,1*H*-perfluorooctyl O-(4-nitro) benzoylmandelate (1i)

¹H NMR (300 MHz, CDCl₃) δ 0.90 (d, *J* = 10.8 Hz, 6H), 1.87 (sept, *J* = 6.9 Hz, 1H), 2.51 (d, *J* = 7.2 Hz, 2H), 4.65

(sext, 2H), 6.21 (s, 1H), 7.23 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 8.23–8.36 (m, 4H).

¹⁹F NMR (282.4 MHz, CDCl₃) δ -126.1 (s, 2F), -123.3 (s, 2F), -122.7 (s, 2F), -122.1 (s, 4F), -119.4 (m, 2F), -80.7 (t, *J* = 8.5 Hz, 3F).

DAICEL CHIRALCEL AD-H, UV detector 254 nm, temperature 20°C, hexane/ⁱPrOH = 98/2, flow rate 0.7 ml/min, *t*_R = 9.8 min, 10.7 min.

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