Enantioselective recognition of amino acids by chiral peptido-calix[4]arenes and thiacalix[4]arenes†

Joackim Bois,[ab] Isabelle Bonnamour,[ab] Christian Duchamp,[ab] Helene Parrot-Lopez,[ab] Ulrich Darbost[ab] and Caroline Felix*[ab]

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In order to prepare new molecular hosts able to discriminate enantiomers of amino-acid derivatives, we have synthesized chiral thiacalix[4]arenes. Extensive studies using NMR and mass spectrometry techniques allow us to determine the major supramolecular interactions involved in the recognition process. Moreover, a titration study enables us to determine the binding constant between these new hosts and a series of amino-acid derivatives. Finally, we have also demonstrated that these calixarenes are able to discriminate enantiomers of amino-acid guests.

Introduction

Enantiomeric recognition is a fundamental step within biological systems. Chirality often impacts the behaviour of molecules. For example, enzymes are able to distinguish between two enantiomers by a selection from chemical rate constant of the reaction catalysis. The interest in mimicking chiral recognition, besides providing an understanding of natural living processes, also lies in its potential applications in various areas such as chemical asymmetric synthesis, catalysis, enzyme mimetics and analytical chemistry. Due to their high importance in the biological world and their key role in the synthesis of many pharmaceuticals, amino acids are among the most preferred candidates to evaluate the molecular recognition ability of an artificial host. Chiral recognition of α-amino acids has been extensively studied and is still of increasing interest. Considerable efforts have been devoted to the design and synthesis of artificial receptors for anion[1] or ammonium[2] binding. However, if much attention has been paid to the binding of ammonium or carboxylate derivatives of amino acids, it is important to emphasize that little progress has been made for the enantiodiscrimination of neutral amino acids.[3,4] Calix[*n*]arenes are a family of macrocyclic molecules consisting of *para*-substituted phenol units connected through methylene bridges in their *ortho* position.[5] These molecules and their derivatives have been extensively studied for the past ten years for their interesting and versatile properties *e.g.* complexation, formation of supramolecular assemblies, design of biomimetics. The calix[4]arene scaffold presents a rigid and structurally pre-organized cavity, which has previously and largely been used as a structural scaffold for metal, cation, anion or neutral molecule recognition. Recently, a new class of macrocycles, thiacalixarenes, has emerged. The presence of sulfur atoms (which possess lone pairs and vacant 3d orbitals) instead of methylene bridges modifies the electronic density of the phenyl rings and the conformer energies.[6]

Calixarenes can be rendered chiral by synthesizing inherently chiral derivatives in which the non-planarity of the molecules is exploited.[7] Another way consists to prepare chiral calixarenes by assembling an achiral calixarene with a chiral molecule. Such reaction is known as "non-covalent synthesis".[8] However, it is easier to generate chirality by appropriate substitution on the calixarene rings with suitable chiral chains. Hence, many chiral calixarene based receptors have been prepared.[1e,f,2b,7a,9,10] Chiral discrimination requires that the host and the guest form reasonably stable complexes. In order to lead to an efficient molecular recognition, the cooperative interaction of several weak forces between the host and the guest is required (*e.g.* hydrogen bonding, electrostatic or dipole–dipole interactions) that lead to molecular recognition. The fixing of the conformation of the host–guest complexes by effective bonding allows the chiral host molecules to make full use of their chiral centre to achieve optimum chiral differentiation. To this end, a "three points interaction" model has been proposed to favour chiral recognition. It considers hydrogen bonding, π–π overlapping and the chiral center. To improve this model, the conformational flexibility of the diastereomeric complexes has to be taken into account.[9h,11,12] In previous studies, we described the synthesis of chiral calix[4]arenes grafted with amino-acid units.[13,14] The binding abilities of these receptors with amino-acid carboxylate derivatives were performed. These studies confirmed the need and the effectiveness of hydrogen bonding and π–π stacking. We showed that a butylene spacer improved the binding ability of the ligand and that the presence of two chiral pendant chains were sufficient to obtain good binding constant values.

As a contribution to this area, we now report on the synthesis of new thiacalix[4]arene derivatives grafted with amino-acid units. Recognition ability and chiral differentiation towards neutral amino-acid derivatives were studied by ^{1}H NMR spectroscopy. The results were compared to those obtained with calix[4]arene based receptors previously described by our team.

[a] *Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, Chimie Supramoléculaire Appliquée (CSAp), CNRS, UMR 5246, 43 Boulevard du 11 Novembre 1918, F-69622, Villeurbanne, France. E-mail: caroline.felix@univ-lyon1.fr*
[b] *Université de Lyon, Lyon, Université Lyon 1, Villeurbanne*

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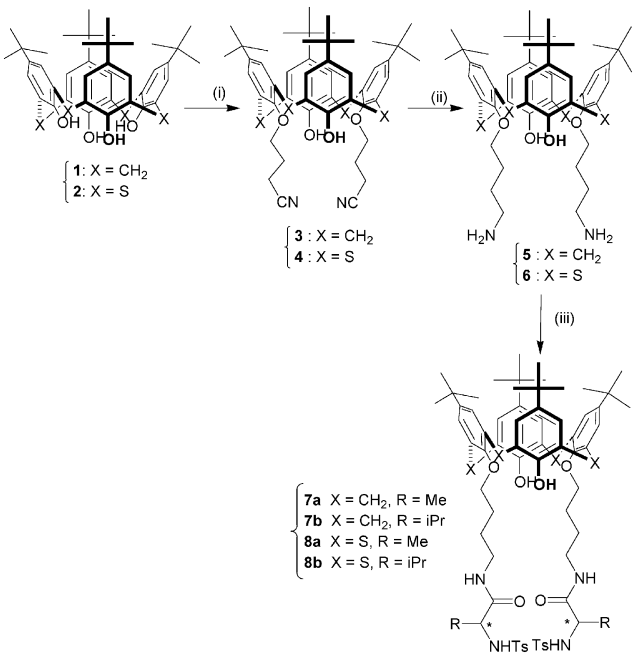
Results and discussion

Synthesis and characterisation

The preparation of ligands **7** has been previously reported.[13] Following the pathway outlined in Scheme 1, new chiral receptors **8** were prepared. Compound **4** was synthesized starting from native **2**. The reaction of **2** with an excess of butyronitrile bromide and caesium carbonate led to the nitrile derivative **4**. Then, reduction of the nitrile function was achieved with borane to give **6**. Finally, the synthesis of ligands **8** was accomplished *via* a condensation reaction of amino-acid chlorides with the diaminothiacalix[4]arene **6** in dry CH_2Cl_2 using Et_3N as a catalyst. The chiral receptors **8a,b** were obtained in high yields. Their structures were established by ^1H NMR, ^{13}C NMR, ESI data and by the presence of amide group absorption in the FT-IR spectrum.

The cone conformation of calix[4]arene derivatives is generally demonstrated by the AB splitting pattern of the methylene bridges, corresponding to a calixarene having C_{2v} symmetry. In the case of thiacalixarene derivatives, the absence of methylene bridge signals implies the use of COSY and NOESY experiments to determine the conformation of the ligands **8**. The ^1H NMR spectrum region corresponding to the thiacalixarene core is characterized by two sets of signals (Fig. 1): two singlets for the *tert*-butyl groups (0.82 and 1.36 ppm), and four doublets for aromatic protons (J = 2.4 Hz) and finally a singlet for the phenolic protons at downfield shift. Concerning the pendant chains, the CH_2–NH signal shows two pairs of multiplets showing non-equivalent protons. The chirality of the chiral center is transmitted through the planar amide function and the relative position of the chiral chain with respect to the macrocycle moiety is defined based on



Scheme 1 Synthesis of ligands **3–8**: *Reagents and conditions*: (i) Cs_2CO_3 for X = S or K_2CO_3 for X = CH_2, $Br(CH_2)_3CN$, acetonitrile; (ii) BH_3,THF; (iii) L-*N*-Ts-Ala-acyl chloride or L-*N*-Ts-Val-acyl chloride, Et_3N, DCM.

dipolar interactions for correlation of protons (NOESY experiment) (Fig. 1). The differences between the COSY and the NOESY spectra indicate which groups are close to each other. All the aromatic protons are correlated to the neighboring aromatic ring as shown by the corresponding spots on the NOESY spectrum of **8a** (and **8b**). Correlation peaks between neighboring *tert*-butyl groups are also present. Therefore, the four rings are placed on the same side. The NOESY spectrum also indicates interactions between the free hydroxyl groups and the methylene protons of the butylene spacer of adjacent units. On the basis of all these observations, we can conclude that the ligands **8** adopt a cone conformation and that no correlation was observed between the chiral center and the thiacalixarene core in the liquid state. Derivatives **7** have been previously characterized in the cone conformation in the liquid and solid states.[13]

^1H NMR enantiodifferentiation experiments

As a preliminary study for the rationalisation of chiral complexation properties of ligands **7** and **8**, we first investigated their ability to differentiate enantioselectively amino-acid derivatives. Before any complexation study, we checked that no dimerization of the ligand occured. The NMR analyses of ligands in the concentration range of 0.1 to 10 mM did not show significant variations of the chemical shift. To provide evidence of the formation of diastereomeric host–guest complexes, one equivalent of enantiomerically pure L-*N*-tosylphenylalanine (L-*N*-Ts-Phe) or D-*N*-tosylphenylalanine (D-*N*-Ts-Phe) was added to a solution of **7a** in $CDCl_3$ (Fig. 2). The sulfonamide resonance signal of phenylalanine derivative, which gives rise to a doublet at 5.05 ppm shifts in the presence of an equimolar amount of calixarene **7a**. The guest led to a downfield shift of the $NHSO_2$ signal suggesting that it is hydrogen bounded. It should be noted that the shielding effect is larger for the D enantiomer than for the L one, which was assigned to a stronger hydrogen bonding interaction. This observation was confirmed by the determination of the stability constant of both enantiomers. A further experiment was carried out on compound **7a** by using one equivalent of a racemic mixture of *N*-tosylphenylalanine. This led to the formation of two diastereomeric complexes. This result clearly indicates that the two enantiomers of *N*-tosylphenylalanine are discriminated by the ligand **7a**. This is in accordance with similar work described in the literature.[7b,9d]

Mass spectrometry (MS) investigations

MS can provide valuable data to support those obtained by NMR displacement.[15] In order to check the formation of the host–guest complexes, mass spectrometry analyses using the electrospray ionization technique (with source temperature at 200 °C) and negative ion detection were used. MS spectra data for compound **7b**–guest mixtures showed three significant peaks: assignable to the free guest, the free ligand (**7b**), and the 1 : 1 host–guest complex (see ESI† for an example). The MS data and NMR spectroscopy thus confirmed the stoichiometry of the complex.

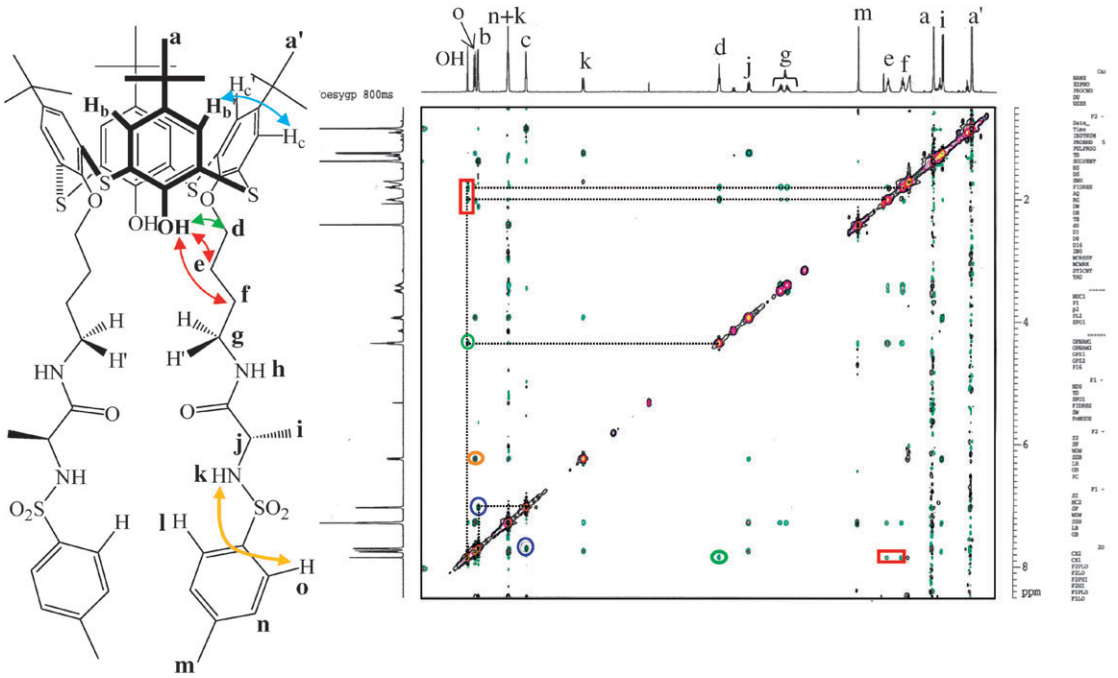


Fig. 1 NMR NOESY spectrum of **8a** (500 MHz, $CDCl_3$, 25 °C).

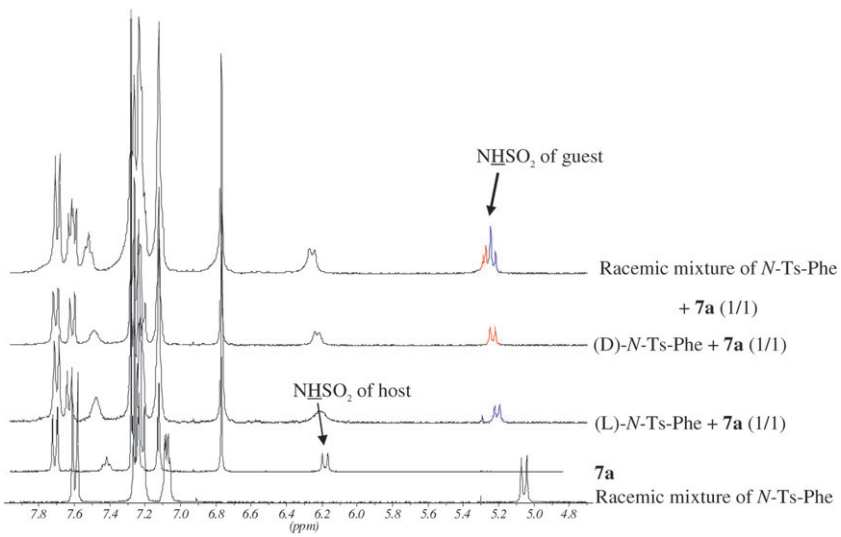


Fig. 2 ^{1}H NMR enantiodifferentiation experiments with ligand **7a** (10^{-2} M) and DL-*N*-Ts-Phe.

Complexation studies

^{1}H NMR spectroscopy conveniently provides information on the complexation mode of guests with the host. In all cases, the guest protons were observed as a single resonance signal due to the fast exchange between free and complexed guest on the NMR time scale. Ligands **7** and **8** were used for chiral recognition of various amino acids by ^{1}H NMR experiments in $CDCl_3$. To improve the solubility of amino acids in this solvent, *N*-tosylated derivatives of valine (Val), leucine (Leu) and phenylalanine (Phe) were prepared. The titration experiments showed that in all cases a gradually downfield shifting of the NH proton of the sulfonamide function of the host was observed upon addition of amino-acid derivatives (see Fig. 3 for an example). $CDCl_3$ solutions (10^{-2} M) of ligands were mixed with increasing amounts of *N*-tosylated amino-acid derivatives (0.2 to 1.6 eq.). These complexation studies were carried out at low concentration to ensure that no dimerization of the ligand took place. The variation of the $NHSO_2$ chemical shift of the host was determined directly from the ^{1}H NMR spectra. With regards to the 1 : 1 stoichiometry, which was determined by the molar ratio method (Fig. 4(a)), the complexation of chiral amino-acid derivatives (G) with calixarene derivatives (H) is expressed by the eqn (1):

$$H + G \overset{K}{\rightleftharpoons} H \cdot G \qquad (1)$$

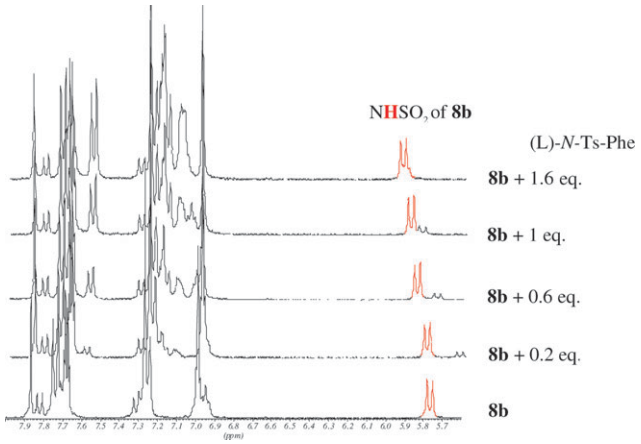


Fig. 3 $NHSO_2$ proton shift changes on the ^1H NMR spectra of **8b** upon addition of L-*N*-Ts-Phe in $CDCl_3$ (300 MHz, 25 °C).

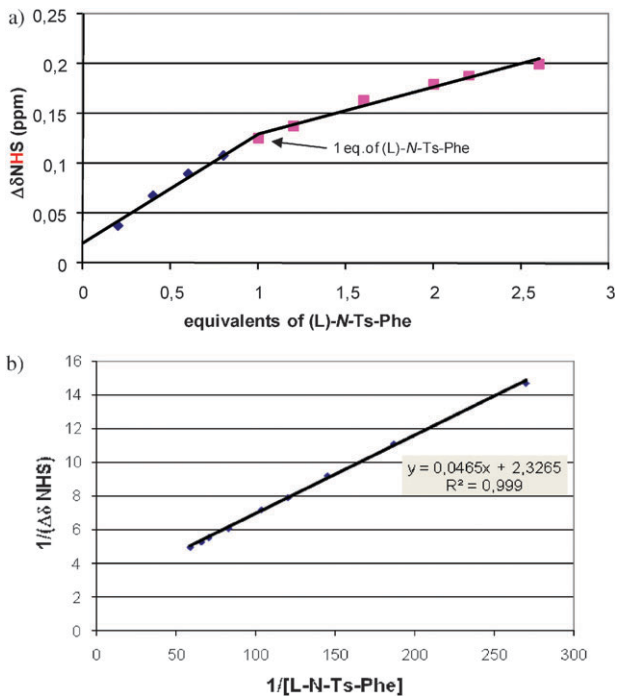


Fig. 4 (a) $NHSO_2$ proton shift changes on the ^1H NMR spectra of **8b** as a function of molar ratio in the titration of ligand **8b** (10^{-2} M) with compound L-*N*-Ts-Phe. (b) Corresponding Benesi–Hildebrand curve.

The binding constant (K) of the supramolecular system formed was calculated according to the modified Benesi–Hildebrand equation[16] (eqn (2)):

$$\frac{1}{\Delta\delta} = \frac{1}{\Delta_0\delta K[G]_0} + \frac{1}{\Delta_0\delta} \qquad (2)$$

where $[G]_0$ denotes the total concentration of amino acid, $\Delta\delta$ is the variation of chemical shift of the ligand in the free and bound states and $\Delta_0\delta$ is the difference in chemical shift between that observed in the ligand and that observed in the host–guest complex. For all ligands used, plots of calculated $1/\Delta\delta$ values as a function of $1/[G]_0$ values gave good straight lines. A typical plot is shown for the complexation of ligand **8b** with *N*-Ts-Phe (Fig. 4(b)).The binding constants

Table 1 Binding constants (K) and enantioselectivities (K_L/K_D) for the complexation of receptors **7** and **8** with L/D guests in $CDCl_3$ at 25 °C

Entry	Host	Guest	K/M^{-1}	$\Delta_0\delta$/ppm	K_L/K_D
1	**7a**	L-*N*-Ts-Phe	33	0.22	
2	**7a**	D-*N*-Ts-Phe	36	0.25	0.92
3	**7a**	L-*N*-Ts-Leu	21	0.37	
4	**7a**	D-*N*-Ts-Leu	33	0.19	0.64
5	**7a**	L-*N*-Ts-Val	51	0.17	
6	**7a**	D-*N*-Ts-Val	36	0.20	1.42
7	**8a**	L-*N*-Ts-Phe	44	0.30	
8	**8a**	D-*N*-Ts-Phe	39	0.27	1.13
9	**8a**	L-*N*-Ts-Leu	15	0.19	
10	**8a**	D-*N*-Ts-Leu	31	0.19	0.48
11	**8a**	L-*N*-Ts-Val	26	0.23	
12	**8a**	D-*N*-Ts-Val	25	0.19	1.04
13	**7b**	L-*N*-Ts-Phe	59	0.39	
14	**7b**	D-*N*-Ts-Phe	47	0.51	1.26
15	**7b**	L-*N*-Ts-Leu	52	0.47	
16	**7b**	D-*N*-Ts-Leu	56	0.42	0.93
17	**7b**	L-*N*-Ts-Val	59	0.45	
18	**7b**	D-*N*-Ts-Val	61	0.43	0.97
19	**8b**	L-*N*-Ts-Phe	50	0.43	
20	**8b**	D-*N*-Ts-Phe	28	0.77	1.79
21	**8b**	L-*N*-Ts-Leu	42	0.52	
22	**8b**	D-*N*-Ts-Leu	37	0.58	1.14
23	**8b**	L-*N*-Ts-Val	37	0.63	
24	**8b**	D-*N*-Ts-Val	42	0.58	0.88

All data were corrected for dilution. Chemical shifts are measured with an accuracy of ±0.004 to ±0.008 ppm. The standard deviation of experiments is <10%.

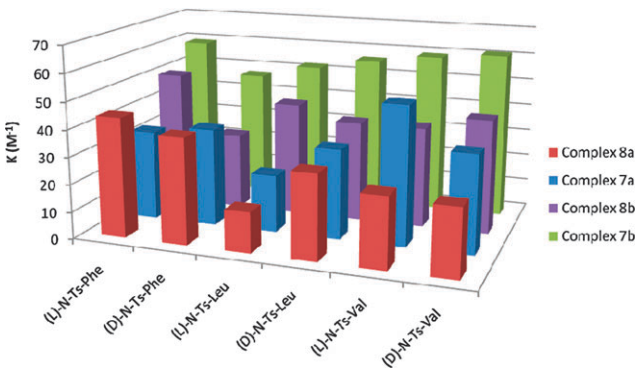


Fig. 5 Bar plots of the binding constants (K) of ligands **7** and **8** with L- or D-*N*-Ts-Phe, L- or D-*N*-Ts-Leu, and L- or D-*N*-Ts-Val.

(K) calculated from the slope and intercept are shown in Table 1 and Fig. 5, along with the enantioselectivity (K_L/K_D). Some experiments were conducted three times to verify the reproducibility of the measurements.

From the data shown in Table 1 we can deduce that all hosts had moderate binding constants compared with those obtained from anionic guests,[13,14] which may be due to the rather weak hydrogen bonding interaction between the host and the guest. Nevertheless, these values are in accordance with those described in the literature for neutral guests.[7b,9d] Ligands **7b** and **8b**, bearing valyl moieties, were the best complexants for all *N*-tosylated amino acids used (Fig. 5). Compared with calixarene based receptors **7**, thiacalixarenes **8** were less strongly complexing but gave the highest enantioselectivity (entries 9, 10 and 19, 20, Table 1). The highest chiral discriminations were obtained for receptor **8a** with *N*-Ts-Leu

and receptor **8b** with *N*-Ts-Phe. The size–fit relationship between host and guest is an essential requirement in molecular recognition. It has been previously reported that the structural rigidity of the host plays an important role in the association process and possibly for chiral discrimination.[1f] However, it is also known that the introduction of four sulfur atoms into the calix[4]arene skeleton induces considerable changes in the conformational behaviour of thiacalixarenes.[17] The cavity of thiacalix[4]arene is larger than classical calix[4]arene. The thiacalixarene derivatives also display remarkably higher flexibility in solution.[18] The highest ^{1}H NMR chemical shift displacement was for the NH–SO_2 signal in both thia and classical calix[4]arenes. This indicated that the binding modes were identical in both systems as previously observed by Lhotak *et al.*[19]

NMR investigation for the understanding of the host–guest complex conformation

As a contribution of chiral recognition processes of ligands **7** and **8**, we studied the conformation of a host–guest complex by detection of interproton dipolar interactions in the NOESY and ROESY spectra. These experiments were carried out using **7b** as ligand and L-*N*-Ts-Val as guest (1 : 1) in $CDCl_3$. The ^{1}H NMR (500 MHz, 25 °C) spectrum of **7b** is characterized by three sets of signals: in the range of 0.6 to 2.5 ppm, resonances of *tert*-butyl and methyl groups and also methylene groups of the spacer; in the range of 3.3 to 4.3 ppm, resonances of methylene bridges beside CH_2–O and CH_2–NH signals; from 5.7 to 7.8 ppm, resonances of the $NHSO_2$ signal, aromatic signals, NH from the amide function and OH. Amongst the methylene groups, many signals showed a multiplicity higher than that expected. The analysis of COSY and ROESY spectra of the **7b**/L-*N*-Ts-Val complex allowed us to establish that the signals at 3.25–3.4 ppm corresponded to the methylene protons of the bridge in pseudo-equatorial arrangement and those at 4.1–4.3 ppm were due to the pseudo-axial ones, in agreement with the literature (Fig. 6).[9d,i] The cone conformation of the host was also supported by the absence of correlation between the O*H* protons and *tert*-butyl protons and by the presence of interactions between the aromatic protons of two adjacent aromatic rings. The relative position of the chiral side chains in the complex was determined based on the interactions detected in the ROESY spectrum (Fig. 6). For the CH_2–O signal, the two hydrogens were not equivalent. One H produced ROE on the pseudo-axial methylene H of one bridge whereas the other produced ROE with the pseudo-axial methylene H of the adjacent bridge. The pseudo-equatorial methylene H gave correlation with the aromatic H of the calixarene core. The chiral moiety was in spatial proximity of OH function as confirmed by ROE correlation between this function and methylene H of the spacer and also with the H of the chiral center. Such results suggested that the side chains of the host are folded and directed outside of the calixarene core. Hence, the $NHSO_2$ function seems to be the better candidate for hydrogen bonding with the guest. A NOESY experiment of this complex suggested proton exchange between OH groups and NH of amide groups of the host, and also proton exchange between the NH of the guest (*N*-TsVal) and both the NH of the sulfonamide and OH function of the host.

Finally, we observed chemical shift changes in the CH_3 signals of the isopropyl groups of **7b** upon addition of increasing amounts of L-*N*-Ts-Val (Fig. 7). The two doublets are upfield shifted. With the results of all these considerations, we can conclude that the host and the guest are complexed by

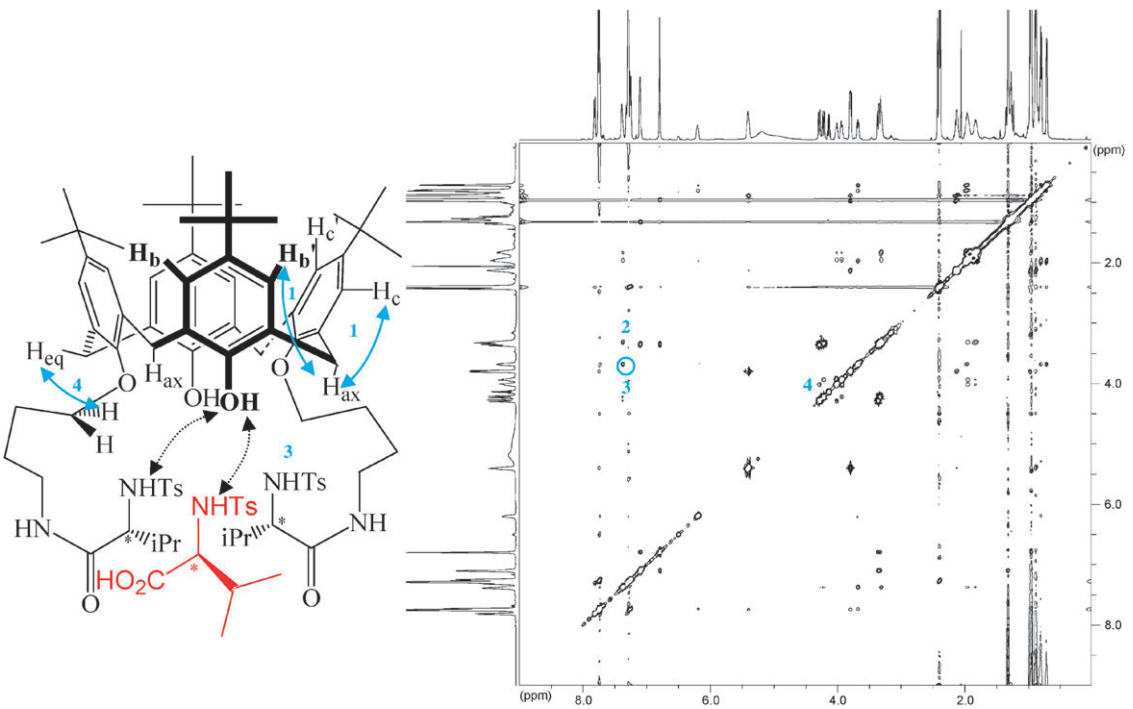


Fig. 6 ROESY spectrum of the complex **7b**/L-*N*-Ts-Val (1 : 1), (500 MHz, $CDCl_3$, 25 °C) (red arrows represent exchange protons observed on NOESY).

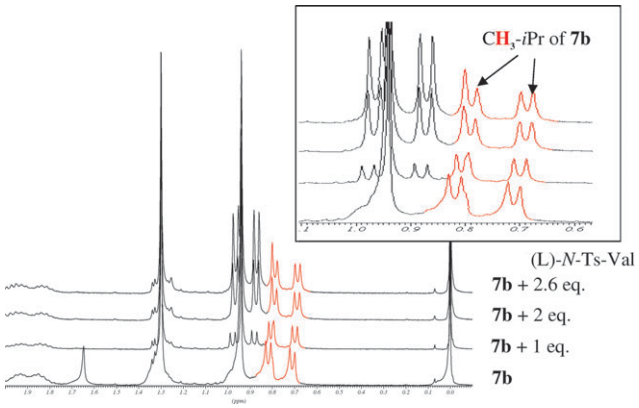


Fig. 7 Shift on the CH_3-*i*Pr ^{1}H NMR spectrum of **7b** upon addition of L-*N*-Ts-Val ($CDCl_3$, 300 MHz, 25 °C).

an *exo*-binding mode. The chiral centre was also deeply implied in the recognition process (as shown by the ^{1}H NMR displacements of the groups directly attached to the stereogenic carbon).

Conclusion

We have prepared novel chiral thiacalix[4]arene–amino acid conjugates. We verified that these hosts adopt a rigid cone conformation by ^{1}H NMR NOESY and ROESY experiments. The introduction of amino-acid moieties into the lower rim of calix and thiacalix[4]arenes placed the chiral groups distant from the internal aromatic cavity of the calixarene core, which allowed secondary interactions with chiral guests. NMR studies and mass spectrometry have demonstrated the formation of 1 : 1 complexes. NMR NOESY and ROESY experiments gave rise to a better understanding of the binding mode between the host and the guest. The side chains of the host were folded and the guest was bound through hydrogen bonding with the $NHSO_2$ functions of the host. With regards to the chemical shift of this group (downfield shifted), we can assume that it is a hydrogen bond donor. The *N*-tosylated calix[4]arenes and thiacalix[4]arenes **7** and **8** might be suitable for the synthesis of hydrophobic neutral amino-acid receptors which are able to bind neutral *N*-tosylated amino-acid derivatives through hydrogen bonding. The chiral center was also implied in the recognition process. We showed that **7** and **8** were able to discriminate between L and D neutral amino-acid derivatives. The highest chiral discriminations were obtained with thiacalix[4]arene derivatives, receptor **8a** with *N*-Ts-Leu and receptor **8b** with *N*-Ts-Phe. The binding constant value and even the chiral recognition depended on the shape of the receptor, the bulkiness of the guest, the ability to link through hydrogen bonding and also π–π stacking. However, the recognition process seems to be also subject to a mutual conformational rearrangement leading to the most favorable complex.

Experimental

General methods

Solvents were purified and dried by standard methods prior to use. All reactions were carried out under nitrogen. Column chromatography was performed using silica gel (Kieselgel-60, 0.040–0.063 nm, Merck). Reactions were monitored by TLC on POLYGAM® SIL G/UV_{254} (Macherey-Nagel) silica gel plates and visualized by UV light. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz ($CDCl_3$) on a Bruker Avance DRX 300 spectrometer unless otherwise noted. 2D NMR (COSY or NOESY or ROESY) were recorded at 500 MHz ($CDCl_3$) on a Bruker Avance DRX 500. Mass spectra were acquired on a ThermoFinnigan LCQ Advantage ion trap instrument, detecting positive ions (+) or negative ions (−) in the ESI mode. Samples (in methanol–dichloromethane–water, 45 : 40 : 15, v/v/v) were infused directly into the source (5 μL min^{-1}) using a syringe pump. The following source parameters were applied: spray voltage 3.0–3.5 kV, nitrogen sheath gas flow 5–20 arbitrary units. The heated capillary was held at 200 °C. High-resolution mass spectra were acquired on a THERMOQUEST Finnigan MAT 95 XL. Optical rotations were measured on a Perkin Elmer 241 polarimeter at a wavelength of 589.3 nm. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. FT-IR spectra were recorded on a Perkin-Elmer spectrophotometer.

Syntheses

N-Ts-amino-acid chlorides and ligands **7a** and **7b** were prepared as previously described.[13]

5,11,17,23-Tetra-*tert*-butyl-25,27-di(cyanopropoxy)-26,28-dihydroxythiacalix[4]arene (4). To a suspension of tetra-*tert*-butylthiacalix[4]arene (3.47 g, 4.8 mmol) and caesium carbonate (1.83 g, 5.59 mmol) in dry acetonitrile (40 mL) were added NaI (3.04 g, 20 mmol) and 4-bromobutyronitrile (2.4 mL, 19 mmol). The reaction mixture was refluxed for 7 days. After cooling, the solvent was removed and the white suspension was quenched with HCl (2 M, 30 mL) and extracted with chloroform (200 mL). The organic layers was washed with water (2 × 100 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed in vacuum and the resulting residue was then recrystallized from $CHCl_3$–MeOH (250 mL–100 mL) to obtain **4** (2.38 g, 58%) as a white powder: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (s, 18H, ^{t}Bu), 1.33 (s, 18H, ^{t}Bu), 2.33–2.42 (m, 4H, CH_2–CH_2–CH_2), 2.93 (t, 4H, J = 7.1 Hz, CH_2CN), 4.52 (t, 4H, J = 5.7 Hz, OCH_2), 7.06 (s, 4H, *H*–Ar), 7.66 (s, 4H, *H*–Ar), 7.74 (s, 2H, O*H*); ^{13}C NMR (75 MHz, $CDCl_3$): 14.9 (*C*H_2), 26.9 (*C*H_2–CN), 31.2 (*C*H_3), 31.9 (*C*H_3) 34.5 (*C*$(CH_3)_3$), 73.9 (O–*C*H_2), 120.0 (–*C*N), 122.0, 129.0, 133.9, 135.0, 143.5, 149.0, 156.2 (Ar); FT-IR: 3363 (–OH), 2965, 2870 (C–H), 2251 (–CN), 744 (C–S) cm^{-1}; LRMS (ES^-) m/z 853.4 ($[M - H]^-$).

5,11,17,23-Tetra-*tert*-butyl-25,27-di(aminobutoxy)-26,28-dihydroxythiacalix[4]arene (6). To a solution of thiacalix[4]arene **4** (0.994 g, 1.16 mmol) in THF (60 mL) was added BH_3 (1M in THF) (20 mL). The colourless solution was stirred at 80 °C for 4 days under nitrogen. After cooling, the solution was quenched with HCl (1 M, 30 mL) and stirred at room temperature for 1 h (H_2 release). Solvent was removed in vacuum and the resulting product was stirred for 4 h at room temperature in HCl (20 mL, 6 M). HCl was then removed and the product was solubilised in CH_2Cl_2 (100 mL); and NaOH

(2M, 50 mL) was added. The organic layer was washed with water (2 × 40 mL) and brine (40 mL) and then dried over Na_2SO_4. Evaporation of the solvent resulted in a white powder (0.906 g, 90%): ^{1}H NMR (300 MHz, $CDCl_3$): 0.79 (s, 18H, ^{t}Bu), 1.32 (s, 18H, ^{t}Bu), 1.65–1.96 (m, 4H, CH_2–CH_2–NH_2), 2.17–2.23 (m, 4H, CH_2–CH_2–O), 3.36 (m, 4H, CH_2–NH_2), 3.56–3.72 (t, 4H, CH_2–O), 4.31 (s, 4H, NH_2), 6.96 (s, 4H, *H*–Ar), 7.66 (s, 4H, *H*–Ar), 7.76 (s, 2H, O*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.9 (*C*H_2), 27.9 (*C*H_2), 30.7 (*C*H_3, ^{t}Bu), 31.9 (*C*H_3, ^{t}Bu), 34.1 (*C*, ^{t}Bu), 34.4 (*C*, ^{t}Bu), 45.7 (*C*H_2NH), 76.9 (*C*H_2O), 122.7 (*C*, Ar), 123.6 (*C*, Ar), 130.5, 134.4, 135.1, 136.6 (*C*H, Ar), 138.9, 141.7, 147.8, 156.0, 157.7, 164.8 (*C*, Ar); FT-IR: 3387–3261 (OH, NH_2), 2964, 2868 (C–H), 1649 (C=C), 742 (C–S) cm^{-1} (LRMS (ES$^+$) *m*/*z* 863.4 ([M − H]$^+$); HRMS (ES$^+$) for $C_{48}H_{66}N_2O_4S_4H^+$, calc. 863.3984, found 863.3983.

General procedure for the preparation of the amino-acid thiacalixarene derivatives 8a and 8b. The solution of amino-acid chloride prepared in dry CH_2Cl_2[13] (10 mL) was added dropwise to a solution of *p*-*tert*-butyl-thiacalix[4]arene diamine **4** (0.20 mmol) and triethylamine (1.00 mmol) in dry CH_2Cl_2 (35 mL) at 0 °C. The mixture was stirred at room temperature for 18 h, washed with 1 M HCl (2 × 25 mL) and water (25 mL), and dried over Na_2SO_4. The solvent was removed in vacuum and the solid residue was purified by column chromatography to give **8a** and **8b**.

5,11,17,23-Tetra-*tert*-butyl-25,27-di(*N*-tosyl-L-alanylamino-butoxy)-26,28-dihydroxythiacalix[4]arene (8a). The product was purified by column chromatography on silica gel eluting with EtOAc–heptane (6 : 4) to obtain **8a** (131 mg, 51%) as a white powder: $[\alpha]_D^{20}$ = −3.1 (*c* 1, $CHCl_3$); mp 105–109 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82 (s, 18H, ^{t}Bu), 1.23 (d, 6H, *J* = 7.1 Hz, CHC*H*$_3$), 1.36 (s, 18H, ^{t}Bu), 1.76–1.81 (m, 4H, C*H*$_2$CH$_2$NH), 1.97–2.00 (m, 4H, C*H*$_2$CH$_2$O), 2.40 (s, 6H, C*H*$_3$Ar), 3.39 (m, 2H, NHC*H*$_2$), 3.47 (m, 2H, NHC*H*$_2$), 3.90 (m, 2H, NHC*H*), 4.33 (t, 4H, *J* = 6.1 Hz, C*H*$_2$O), 6.22 (d, 2H, *J* = 8.5 Hz, SO_2N*H*), 7.01 (d, 2H, *J* = 2.4 Hz, *H*–Ar), 7.02 (d, 2H, *J* = 2.4 Hz, *H*–Ar), 7.27 (m, 6H, SO_2Ar–*H*, N*H*CO), 7.68 (d, 2H, *J* = 2.4 Hz, *H*–Ar), 7.69 (d, 2H, *J* = 2.4 Hz, *H*–Ar), 7.73 (d, 4H, *J* = 8.2 Hz, SO_2Ar–*H*), 7.84 (s, 2H, O*H*); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19.9 (*C*H_3), 22.0 (*C*H_3–Ts), 26.4 (*C*H_2), 27.4 (*C*H_2), 31.2 (*C*H_3, ^{t}Bu), 31.9 (*C*H_3, ^{t}Bu), 34.5 (*C*, ^{t}Bu), 34.7 (*C*, ^{t}Bu), 39.7 (*C*H_2NH), 53.0 (*C*HNH), 77.4 (*C*H_2O), 122.3 (*C*, Ar), 122.4 (*C*, Ar), 127.6 (*C*H, Ar), 128.8, 129.0, 130.1 (*C*H, Ar), 133.6 (*C*H, Ar), 134.4 (*C*H, Ar), 137.7, 143.5, 148.6, 156.0, 156.7, 172.6 (*C*O). FT-IR 3364 (NH, OH), 2957, 2866, 1652 (CO) cm^{-1}; LRMS (ES$^+$) *m*/*z* 1313.3 ([M + H]$^+$), 1335.5 ([M + Na]$^+$); HRMS (ES$^+$) for $C_{68}H_{88}N_4O_{10}S_6Na^+$, calc. 1335.4722, found: 1335.4725.

5,11,17,23-Tetra-*tert*-butyl-25,27-di(*N*-tosyl-L-valylamino-butoxy)-26,28-dihydroxythiacalix[4]arene (8b). The product was purified by column chromatography on silica gel eluting with EtOAc–heptane (1 : 1) to obtain **8b** (126 mg, 46%) as a white powder: $[\alpha]_D^{20}$ = −10 (*c* 0.1, $CHCl_3$); mp 156–158 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 (d, 6H, *J* = 6.6 Hz, CHC*H*$_3$), 0.80 (s, 18H, ^{t}Bu), 0.81 (d, 6H, *J* = 6.6 Hz, CHC*H*$_3$), 1.34 (s, 18H, ^{t}Bu), 1.61–1.69 (m, 4H, C*H*$_2$CH$_2$NH), 1.85–1.96 (m, 4H, C*H*$_2$CH$_2$O), 2.00–2.10 (m, 2H, C*H*(CH$_3$)$_2$), 2.36 (s, 6H, C*H*$_3$Ar), 3.25 (m, 4H, NHC*H*$_2$), 3.64 (m, 2H, NHC*H*), 4.33 (t, 4H, *J* = 6.1 Hz, C*H*$_2$O), 5.82 (d, 2H, *J* = 8.5 Hz, SO_2N*H*), 6.96 (m, 6H, *H*–Ar, N*H*CO), 7.23 (d, 4H, *J* = 8.1 Hz, SO_2Ar–*H*), 7.66 (d, 2H, *J* = 2.4 Hz, *H*–Ar), 7.68 (d, 2H, *J* = 2.4 Hz, *H*–Ar), 7.73 (d, 4H, *J* = 8.3 Hz, SO_2Ar–*H*), 7.84 (s, 2H, O*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.2 (CH*C*H$_3$), 19.2 (CH*C*H$_3$), 21.5 (*C*H$_3$–Ts), 25.5 (*C*H$_2$), 27.0 (*C*H$_2$), 30.7 (*C*H$_3$, ^{t}Bu), 31.5 (*C*H$_3$, ^{t}Bu), 34.0 (*C*, ^{t}Bu), 34.2 (*C*, ^{t}Bu), 39.2 (*C*H$_2$NH), 62.1 (*C*HNH), 76.3 (*C*H$_2$O), 121.9, 122.0, 126.4 (*C*H, Ar), 127.4, 128.5, 129.6 (C*H*, Ar), 133.0 (C*H*, Ar), 134.4 (C*H*, Ar), 136.9, 143.0, 148.2, 155.5, 156.1, 172.6 (*C*O); FT-IR: 3357–3260 (NH, OH), 2957, 2866, 1650 (CO) cm^{-1}; LRMS (ES$^+$) *m*/*z* 1369.0 ([M + H]$^+$), 1391.2 ([M + Na]$^+$); HRMS (ES$^+$) for $C_{72}H_{96}N_4O_{10}S_6H^+$, calc. 1369.5529, found: 1369.5513.

^{1}H NMR titrations

The complexation abilities of the hosts were studied by ^{1}H NMR in $CDCl_3$ solutions at 300 K. In these experiments, *N*-Ts-amino acids (Phe, Leu, Val) were employed as guests. Binding studies were carried out by adding increasing amounts of the appropriate guest solution (20 μL, *c* = 0.05 M) to a solution of the host (0.5 mL, *c* = 0.01 M). All data were corrected for dilution. Having verified a 1 : 1 stoichiometry complex by the molar ratio method, the binding constants (*K*), based on monitoring of the SO_2N*H* signal, were calculated. Results are summarized in Table 1.

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References

1 See for example: (*a*) A. Hamdi and J. Vicens, *J. Inclusion Phenom. Macrocycl. Chem.*, 2008, **60**, 193–196; (*b*) N. Singh, G. Woo Lee and D. Ok Jang, *Tetrahedron*, 2008, **64**, 1482–1486; (*c*) Y. K. Kim, H. N. Lee, N. J. Singh, H. J. Choi, J. Y. Xue, K. S. Kim, J. Yoon and M. H. Hyun, *J. Org. Chem.*, 2008, **73**, 301–304; (*d*) E. Quilan, S. E. Matthews and T. Gunnlaugsson, *J. Org. Chem.*, 2007, **72**, 7497–7503; (*e*) A. V. Yakovenko, V. I. Boyko, V. I. Kallchenko, L. Baldini, A. Casnati, F. Sansone and R. Ungaro, *J. Org. Chem.*, 2007, **72**, 3223–3231; (*f*) G. Y. Qing, Y. B. He, F. Wang, H. J. Qin, C. G. Hu and X. Yang, *Eur. J. Org. Chem.*, 2007, 1768–1778; (*g*) F. Troisi, A. Russo, C. Gaeta, G. Bifulco and P. Neri, *Tetrahedron Lett.*, 2007, **48**, 7986–7989; (*h*) Z. Rodriguez-Dcampo, S. I. Pascu, S. Kubik and S. Otto, *J. Am. Chem. Soc.*, 2006, **128**, 11206–11210.

2 See for example: (*a*) H. Tsukube and H. Sohmiya, *J. Org. Chem.*, 1991, **56**, 875–878; (*b*) M. Durmaz, S. Alpayadin, A. Sirit and M. Yilmaz, *Tetrahedron: Asymmetry*, 2007, **18**, 900–905; (*c*) Y. Turgut, N. Demirel and H. Hosgören, *J. Inclusion Phenom. Macrocycl. Chem.*, 2006, **54**, 29–33; (*d*) M. Karakaplan and T. Aral, *Tetrahedron: Asymmetry*, 2005, **16**, 2119–2124; (*e*) W. M. Hassen, C. Martelet, F. Davis, S. P. J. Higson, A. Abdelghani, S. Helali and N. Jaffrezic-Renault, *Sens. Actuators, B*, 2007, **124**, 38–45.

3 A. Galan, D. Andreu, A. Echevarren, P. Prados and R. de Mendoza, *J. Am. Chem. Soc.*, 1992, **114**, 1511–1512.

4 B. Botta, I. D'Acquarica, L. Nevola, F. Sacco, Z. Valbuena Lopez, G. Zappia, C. Fraschetti, M. Speranza, A. Tafi, F. Caporuscio, M. C. Letzel and J. Mattay, *Eur. J. Org. Chem.*, 2007, 5995–6002.

5 (*a*) C. D. Gutsche, in *Calixarenes Revisited*, Royal Society of Chemistry, Cambridge, 1998; (*b*) C. D. Gutsche, in *Calixarenes 2001*, Kluwer Academic Publishers, Dordrecht, 2001.

6 I. Mohamed-Ziegler and F. Billes, *J. Inclusion Phenom. Macrocycl. Chem.*, 2007, **58**, 19–42.

7 (*a*) S. Bozkurt, M. Durmaz, M. Yilmaz and A. Sirit, *Tetrahedron: Asymmetry*, 2008, **19**, 618–623; (*b*) S. Shirakawa, A. Moriyama and S. Shimizu, *Org. Lett.*, 2007, **9**, 3117–3119; (*c*) F. Narumi, T. Hattori, N. Matsumura, T. Onodera, H. Katagiri, C. Kabuto, H. Kameyama and S. Miyano, *Tetrahedron*, 2004, **60**, 7827–7833.

8 S. A. Fernandes, F. F. Nachtigall, M. Lazzarotto, F. Y. Fujiwara and A. J. Marsaioli, *Magn. Reson. Chem.*, 2005, **43**, 398–404.

9 See for example: (*a*) E. Kocabas, M. Durmaz, S. Alpaydin, A. Sirit and M. Yilmaz, *Chirality*, 2008, **20**, 26–34; (*b*) J. Y. Gu, W. P. He, X. F. Shi and L. N. Ji, *Chem. Res. Chinese Univ.*, 2008, **24**, 106–109; (*c*) S. P. Bew, R. A. Brimage, N. L'Hermite and S. V. Sharma, *Org. Lett.*, 2007, **9**, 3713–3716; (*d*) G. Ucello-Barretta, M. G. Berni and F. Balzano, *Tetrahedron: Asymmetry*, 2007, **18**, 2565–2572; (*e*) F. Liu, G. Y. Lu, W. J. He, M. H. Liu and L. G. Zhu, *Thin Solid Films*, 2004, **468**, 244–249; (*f*) C. Gaeta, M. De Rosa, M. Fruilo, A. Soriente and P. Neri, *Tetrahedron: Asymmetry*, 2005, **16**, 2333–2340; (*g*) A. Karakucuk, M. Durmaz, A. Sirit, M. Yilmaz and A. S. Demir, *Tetrahedron: Asymmetry*, 2006, **17**, 1963–1968; (*h*) Y. S. Zheng and C. Zhang, *Org. Lett.*, 2004, **6**, 1189–1192; (*i*) K. Jennings and D. Diamond, *Analyst*, 2001, **126**, 1063–1067; (*j*) M. Lazzarotto, F. F. Nachtigall, I. Vencato and F. Nome, *J. Chem. Soc., Perkin Trans. 2*, 1998, 995–998.

10 U. Darbost, X. Zeng, M. Giorgi and I. Jabin, *J. Org. Chem.*, 2005, **70**, 10552–10560.

11 X. X. Zhang, J. S. Bradshaw and R. M. Izatt, *Chem. Rev.*, 1997, **97**, 3313–3361.

12 S. Ahn, J. Ramirez, G. Grigorean and C. B. Lebrilla, *J. Am. Soc. Mass Spectrom.*, 2001, **12**, 278–287.

13 S. Ben Sdira, C. P. Felix, M. B. Giudicelli, P. Seigle-Ferrand, M. Perrin and R. J. Lamartine, *J. Org. Chem.*, 2003, **68**, 6632–6638.

14 S. Ben Sdira, R. Baudry, C. P. Felix, M. B. Giudicelli and R. J. Lamartine, *Tetrahedron Lett.*, 2004, **45**, 7801–7804.

15 (*a*) E. Da Silva, C. Valmalle, M. Becchi, C. Y. Cuilleron and A. Coleman, *J. Inclusion Phenom.*, 2003, **46**, 65–69; (*b*) M. Hamdan, O. Curcuruto, H. Molinari, L. Zetta and L. Ragona, *J. Mass Spectrom.*, 1996, **31**, 1261–1264.

16 (*a*) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703–2707; (*b*) L. Fielding, *Tetrahedron*, 2000, **56**, 6151–6170.

17 P. Lhotak, *Eur. J. Org. Chem.*, 2004, 1675–1692.

18 H. Dvorakova, J. Lang, J. Vlach, J. Sykora, M. Cajan, M. Himl, M. Pojarova, I. Stibor and P. Lhotak, *J. Org. Chem.*, 2007, **72**, 7157–7166.

19 P. Zlatuskova, I. Stibor, M. Tkadlecova and P. Lhotak, *Tetrahedron*, 2004, **60**, 11383–11390.