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Enantiodiscrimination by inclusion phenomena inside a bis(ethyl lactate) *p-tert*-butylcalix[4]arene derivative

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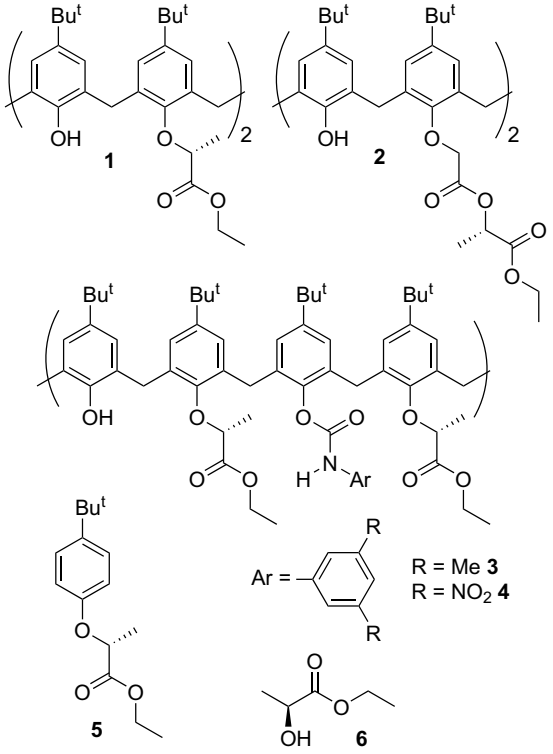
Abstract—An ethyl lactate derivative of *p-tert*-butylcalix[4]arene is able to differentiate the NMR spectra of 3,5-dinitrobenzoyl derivatives of amino acids methyl esters. The origin of enantiodiscrimination in solution was investigated by NMR spectroscopy and comparison with model chiral auxiliaries.

1. Introduction

Calixarenes are macrocyclic receptors[1–4] able to originate molecular recognition processes, which can be suitably addressed and controlled by derivatization of their lower rim hydroxyls. In particular, the introduction of chiral pendants or the synthesis of inherently chiral systems enables them to efficiently differentiate also enantiomeric mixtures as a result of the high level of molecular preorganization due to the cyclic structure. As a matter of fact, chiral calixarenes have found attractive applications in the development of enantiomer separation technologies using chromatography[5] and NMR spectroscopy.[6–8] As far as NMR spectroscopy is concerned, chiral calixarenes have been mainly employed as chiral solvating agents (CSAs) able to form diastereoisomeric solvates with enantiomeric pairs, that makes it possible to not only differentiate enantiomer NMR resonances, and hence evaluate enantiomeric purities by means of their integration, but also carry out NMR investigations into the stereochemistry, dynamics and thermodynamics of diastereoisomeric complexes in order to investigate the molecular basis of chiral discrimination. Several types of chiral pendants[1–4] have been proposed, amongst which, more recently, include tartaric ester moieties,[9] phenylalaninol[10] or 1,2-diphenyl-1,2-oxyamino residues.[11]

Herein we report enantiodiscrimination phenomena produced in solution by the chiral bis[(*R*)-ethyl lactate] derivative of *p-tert*-butylcalix[4]arene[12] **1** (Scheme 1) employed as CSA for the differentiation of the NMR spectra of enantiomeric mixtures of amino acid derivatives (Scheme 2), by



Scheme 1. CSAs **1**–**4** and model compounds **5** and **6**.

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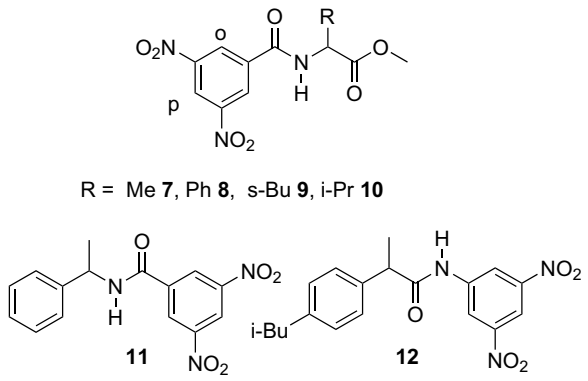


Scheme 2. Chiral substrates **7**–**15**.

exploiting several NMR investigation tools in order to elucidate the origin of enantiodifferentiation in solution.

As a further contribution to the understanding of chiral recognition processes we also probed calixarene derivative **2**, in which lactate moieties, and hence, chiral centres, are located further away from the calixarene structure. In order to ascertain the role of two residual hydroxyl groups of **1**, calixarene derivatives **3** and **4** (Scheme 1) were prepared, in which one hydroxyl function was derivatized to carbamate group endowed with a different kind of hydrogen-bond donor group in addition to aromatic moieties potentially able to address enantiomer interactions at the external surface of calixarene. Finally, the enantiodiscriminating efficiency of the simple acyclic models **5** and **6** (Scheme 1) was investigated, **5** being the ethyl lactate derivative of *p-tert*-butylphenol and **6** ethyl lactate itself.

2. Results and discussion

2.1. Synthesis and characterization of CSAs 1–4 and of the model compound 5

Following the procedure reported by Lazzarotto et al.,[12] chiral auxiliary **1** was prepared by 1,3-bis-substitution reaction of hydroxyls of *p-tert*-butylcalix[4]arene with ethyl (*S*)-(−)-*O*-tosyllactate via an S_N2 process leading to configurational inversion of the lactate stereogenic centre (Scheme 3).

Compound **2** was analogously synthesized starting from ethyl (*S*)-2-(2-chloroacetoxy)propanoate **III** and *p-tert*-butylcalix[4]arene (Scheme 3). The reaction of **1** with an excess of the suitable isocyanate gave carbamoyl derivatives **3** and **4**, in which the selective derivatization of a single free phenolic group occurred. Model compound *p-tert*-butylphenyl-[(*R*)-1-ethoxycarbonylethoxy] **5** was prepared by using the same procedure employed for **1** (Scheme 3). Chiral auxiliaries were characterized by comparing ^{1}H (600 MHz, 25 °C, $CDCl_3$) and ^{13}C (150 MHz, 25 °C, $CDCl_3$) NMR analyses of homonuclear (COrrelation SpectroscopY, COSY, and Rotating-frame Overhauser Enhancement SpectroscopY, ROESY) and heteronuclear (gradient Heteronuclear Single Quantum Coherence, gHSQC, and gradient Heteronuclear Multiple Bond Coherence, gHMBC) correlations (see Section 4).

2.2. ^{1}H NMR enantiodiscrimination experiments

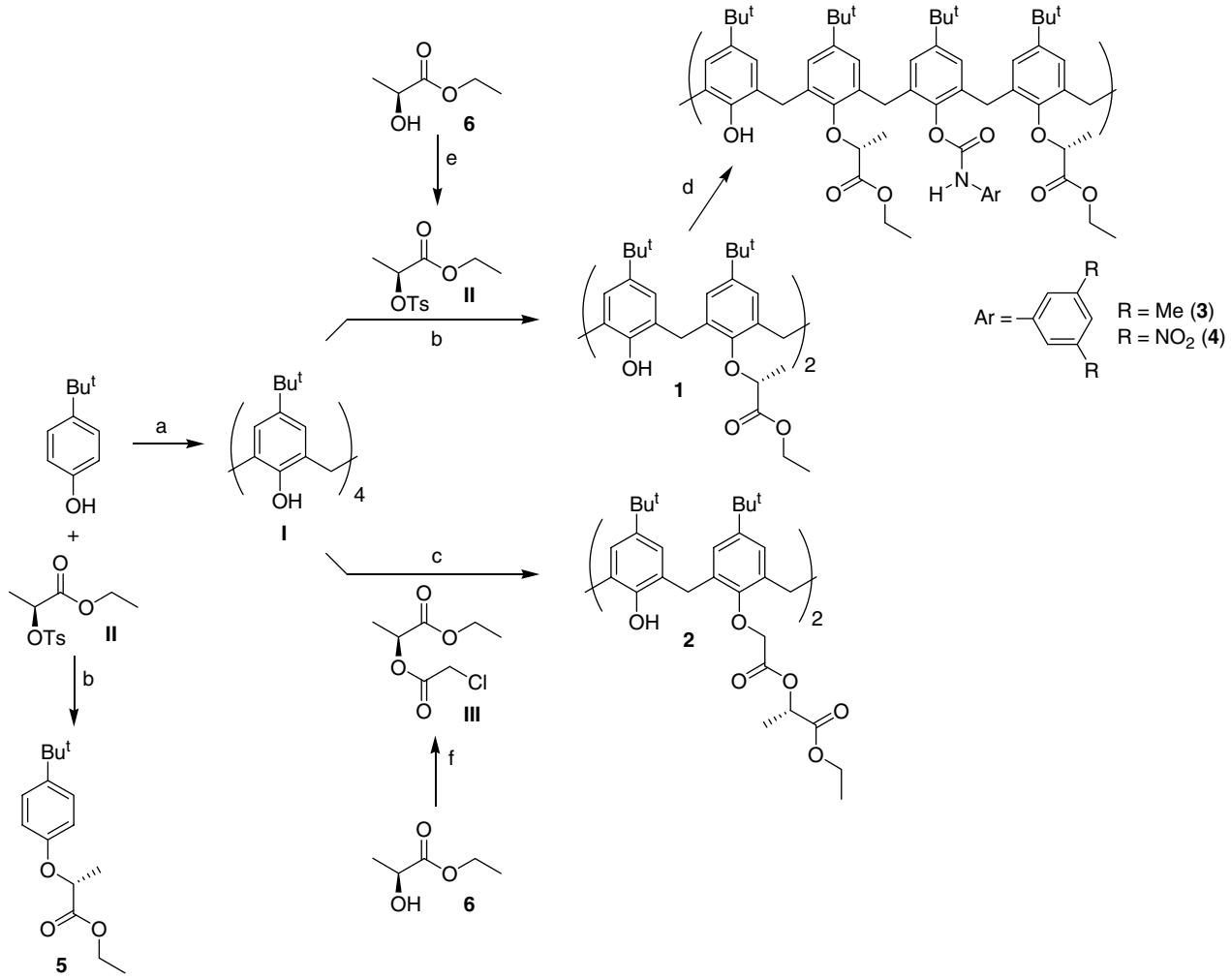
Calixarene **1** was probed as a CSA in $CDCl_3$ solution to differentiate the NMR nuclei of $CDCl_3$ soluble derivatives of amino acids **7**–**12** (Scheme 2). The presence of the π-acidic 3,5-dinitrophenyl moiety for compounds **7**–**12** was a prerequisite for observing enantiodiscrimination. NMR spectra of pure racemic substrates and their mixtures with the CSA were compared and the magnitudes of nonequivalences ($\Delta\delta$) were measured as the differences of the chemical shifts of the two enantiomers in the presence of the CSA, in order to evaluate the enantiodiscriminating efficiency of **1**. Amide resonance of alanine derivative **7**, which gives rise to a broad doublet at 7.22 ppm in the pure 80 mM compound (Fig. 1a), remarkably split by 0.12 ppm in the presence of equimolar amounts of calixarene **1** (Table 1, Fig. 1b). Amongst the other proton of **7**, the aromatic proton Hp of the 3,5-dinitrophenyl moiety also underwent remarkable splitting ($\Delta\delta = 0.07$ ppm, Table 1, Fig. 1b).

Analogous efficient differentiations of amide and Hp aromatic protons resonances of phenylglycine derivative **8** were observed, for which nonequivalences of 0.10 ppm and 0.06 ppm were measured, respectively, (Table 1). The enantiomers of valine **10** and isoleucine **9** were differentiated with lower efficiency, both giving nonequivalences of about 0.03 ppm and 0.01–0.02 ppm for the amide and Hp protons (Table 1). Thus the efficiency of enantiodiscrimination by **1** seems to be sensitive to steric factors generated by groups directly bound to the stereogenic centre of the amino acid.

Low-temperature (−20 °C) measurements of equimolar mixtures substrate/**1** at a four times lower concentration (20 mM) allowed us to obtain magnitudes of nonequivalences similar to that detected at 25 °C in the 80 mM equimolar solutions (Table 1).

Calixarene **2**, in which lactate pendant and calixarene structure are separated by a longer arm, gave remarkably lower nonequivalences with respect to **1** (Table 1). It caused significantly lower nonequivalences of 0.007 ppm for the amide proton of **7** (80 mM) and similarly low nonequivalences of its aromatic protons (Table 1, Fig. 1c). For amide and Hp protons of **8**, higher nonequivalences of 0.04 ppm and 0.05 ppm were, respectively, measured in the presence of **2**, which, in any case, were lower with respect to those measured in the mixtures containing **1** (Table 1). Calixarene **2** did not bring about enantiodiscrimination of derivatives **9** and **10** at all.

Further ^{1}H NMR enantiodiscrimination experiments have been carried out on compound **7** (80 mM) by using as CSAs acyclic model **5** and ethyl (*S*)-lactate **6** (Fig. 1d and e). Very low nonequivalences (less than 0.003 ppm) were measured on protons of racemic **7** even in the presence of 2 equiv of **5** or **6**, indicating the relevance of preorganization of the supramolecular architecture as in **1** in affecting enantiodiscrimination.



Scheme 3. Synthesis of CSAs **1**–**4** and of model compound **5**: (a) NaOH, HCHO, PhOPh; (b) K_2CO_3, acetone, Δ; (c) K_2CO_3, KI, acetone, toluene, Δ; (d) ArCNO, toluene, Δ; (e) TsCl, Py, −10 °C; (f) $ClCH_2COCl$, Py, CH_2Cl_2, 0 °C.

Finally, the derivatization of three of the four phenolic moieties of calixarenes, as obtained in **3** and **4**, is responsible for a significant reduction of the enantiodiscriminating efficiency and versatility, since they were only able to enantiodiscriminate compound **7** (80 mM) with very low nonequivalences (less than 0.001 ppm).

2.3. NMR investigation on enantiorecognition processes

As a basis for the rationalization of chiral recognition properties of calixarenes **1**–**4**, we first investigated their conformation in solution by detection of interproton dipolar interactions in the ROESY maps.

The ^1H NMR (600 MHz, $CDCl_3$, 25 °C) spectrum of **1** (Fig. 2) is constituted by three sets of signals: resonances of *tert*-butyl and methyl groups (1.8–0.8 ppm), multiplets due to the methylene groups and the methine proton on the chiral centre (4.8–3.2 ppm) and four doublets for aromatic protons and a singlet for the phenolic proton in the high-frequency region (7.2–6.6 ppm). Amongst the methylene groups, the two nonequivalent types of bridge methylene groups are diastereotopic producing doublets at 3.25 ppm (H-B′), 4.37 ppm (H-B), 3.29 ppm (H-A′) and 4.46 ppm (H-A). The analysis of the intramolecular dipolar interactions allowed us to establish that: (i) the signals at 3.29 and 3.25 ppm are due to the methylene protons in pseudo-equatorial arrangement and those at 4.46 and 4.37 ppm are due to the pseudo-axial ones, in agreement with the assignment reported by Lazzarotto et al.[12] on the basis of chemical shift correlations; (ii) the *tert*-butyl group resonating at 1.30 ppm is bound to the aromatic moiety producing resonances at 7.09 and 7.01 ppm, whereas the signals at 0.92, 6.72 and 6.79 ppm all arise from the other nonequivalent aromatic ring; (iii) all the aromatic rings are *syn*, with the *tert*-butyl groups facing in a cone arrangement as supported by the absence of intramolecular dipolar interactions between *tert*-butyl protons and OH or protons belonging to the lactate moiety. The assignment of the aromatic protons of the two different aromatic rings has been carried out by analysing long-range heteronuclear correlations of the phenolic protons in the gHMBC map. The cone structure of **1**, as herein established on the basis of detection of dipolar

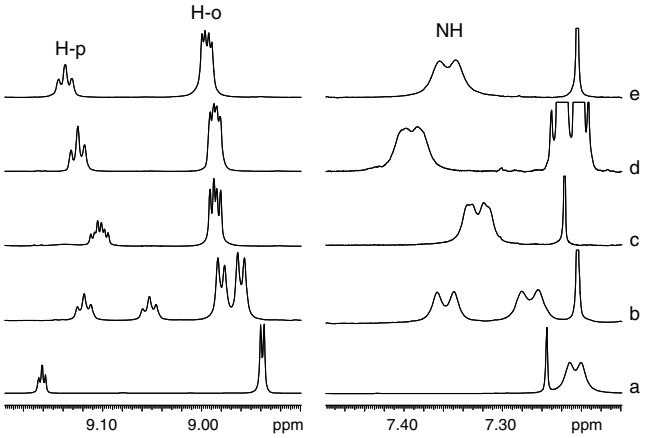


Figure 1. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) spectral regions corresponding to signals of the 3,5-dinitrophenyl moiety and NH protons of pure **7** (80 mM) (a) and of **7** (80 mM) in the presence of equimolar amounts of CSA **1** (b), **2** (c) and in the presence of 2 equiv of **5** (d) and of **6** (e).

Table 1. Nonequivalence data ($\Delta\delta$,[a] 300 MHz, $CDCl_3$) measured on protons of racemic **7**–**12** in the presence of equimolar amount of CSA **1** and **2**

		1 20 mM 25 °C	**1** 20 mM −20 °C	**1** 80 mM 25 °C	**2** 80 mM 25 °C
7	NH	n.d.[c]	0.079	0.117	0.007
	H-*o*	0.007	0.017	0.020	0.007
	H-*p*	0.024	0.052	0.066	0.004
8	NH	n.d.[c]	0.087	0.096	0.037
	H-*o*	0.006	0.014	0.019	0.021
	H-*p*	0.020	0.058	0.060	0.049
9	NH			0.028	0
	H-*o*			0.006	0
	H-*p*			0.016	0
10	NH			0.028	0
	H-*o*			0.012	0
	H-*p*			0.008	0
11[b]	NH	n.d.[c]			0[b]
	H-*o*	0.007			0[b]
	H-*p*	0.017			0.003[b]
12	NH	0.025	0.089	0.066	0.069
	H-*o*	0	0.019	0.007	0
	H-*p*	0	0.032	0.029	0.016

[a] $\Delta\delta = |\delta_R - \delta_S|$, difference between the chemical shifts (ppm) of the two enantiomers of the racemic compound in the presence of CSA.
[b] Nonequivalence data are measured at 20 mM due to the low solubility of compound **11**.
[c] n.d. = not determined.

correlations, confirmed the assignment previously reported[12] in solution and based on the high chemical shift differentiations of the bridge axial and equatorial protons.

The relative position of the chiral moiety with respect to the calixarene was defined on the basis of the interproton interactions detected in the ROESY traces of the methyl (H-T), methine (H-S) and ethyl (H-V) protons (Fig. 3): ethyl protons produce ROE on the pseudo-axial methylene H-B only (Fig. 3a), whereas methine proton H-S gives rise to intramolecular ROEs with both the pseudo-axial methylene protons H-B and H-A (Fig. 3b), the first being more

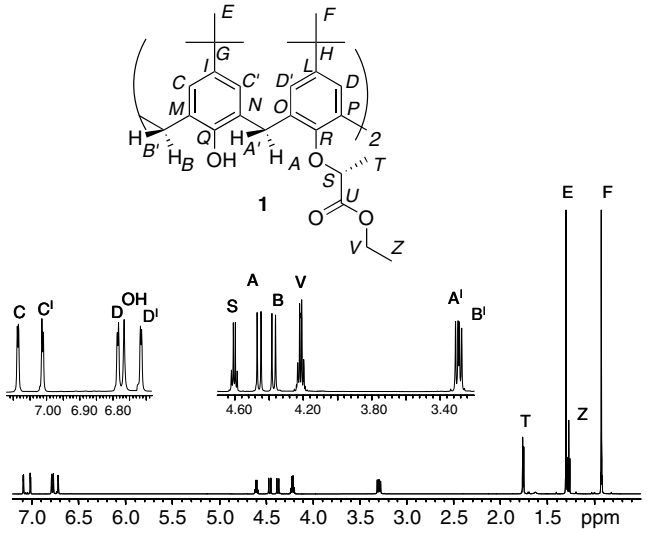


Figure 2. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) spectrum of **1** (65 mM).

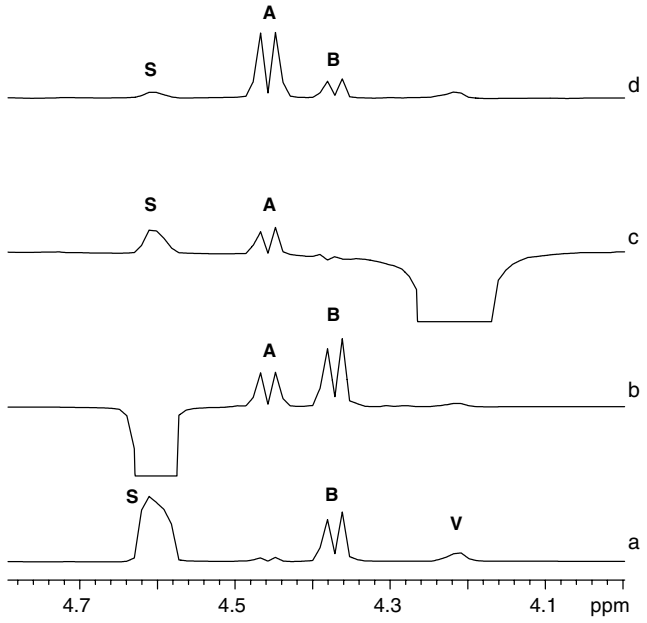


Figure 3. ROESY (600 MHz, $CDCl_3$, 25 °C, mix 0.8 s) traces corresponding to methyl (H-T) (a), methine (H-S) (b), methylene (H-V) (c) and OH (d) protons of **1** (65 mM).

intense than the latter; the methylene group of the ethyl chain produces ROE on the pseudo-axial protons H-A only (Fig. 3c). Taking into account the absolute configuration of the stereogenic centre (*R*), the two substituents bound to it are orientated at the external surface of the calixarene with the methyl group directed towards proton H-B and the methine in spatial proximity of both types of pseudo-axial protons, nearer to H-B than to H-A. The ethyl moiety is in spatial proximity of the pseudo-axial protons H-A, near to the OH protons, as confirmed by the more intense ROE OH–H-A than OH–H-B detected in the corresponding ROESY trace (Fig. 3d). In such a conformational arrangement (Fig. 4), a network of hydrogen-bond interactions involving the phenolic group and the ester moiety of adjacent units is possible.

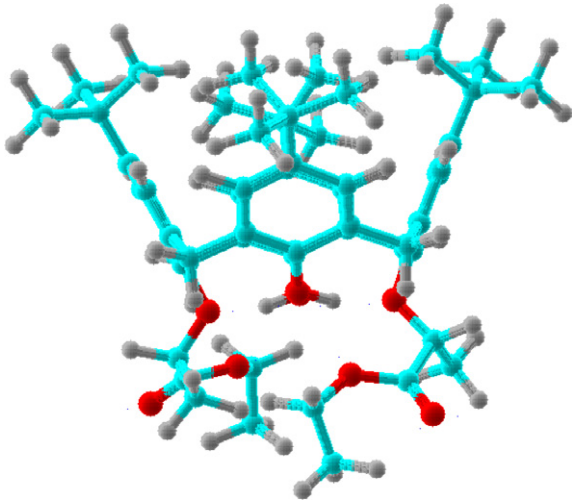


Figure 4. Graphical representation of the conformation assumed by **1** in $CDCl_3$ solution.

It is noteworthy that previously reported[12] X-ray analysis of **1** showed not only its slightly distorted cone conformation, but also that the lactate moieties spread away from one another with carbonyl groups outwards and residual hydroxyl groups pointing at ester oxygens, confirming the presence of hydrogen-bond networks involving them and ester oxygens.

Regarding calixarenes **2**–**4**, the cone arrangement was confirmed for all of them on the basis of following results: (a) chemical shift differences of axial and equatorial protons of each methylene bridge group were comparable (Table 2) to the differentiation found for analogous protons of **1**; (b) *tert*-butyl groups produced only reciprocal dipolar interactions and no interactions with lactate protons or, in the cases of **3** and **4**, with carbamate protons, were detected at all; (c) lactate or carbamate protons only gave dipolar interactions with external protons of calixarenes; (d) carbamate NH protons of **3** and **4** were in spatial proximity of the residual hydroxyl group, confirming the presence of a network of hydrogen bonds involving polar groups at the lower rim. These hydrogen-bond interactions can reasonably be assumed to be responsible for the stabilization of the cone conformation of all calixarenes investigated.

Table 2. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) chemical shift (δ in ppm referenced to TMS as an external standard) of the diastereotopic methylene protons of CSAs **1**–**4**

CSA	δ_{ax}	δ_{eq}	$\Delta\delta = \delta_{ax} - \delta_{eq}$
1	4.46	3.29	1.17
	4.37	3.25	1.12
2	4.45	3.33	1.10
	4.43	3.33	1.12
3	4.59	3.28	1.21
	4.49	3.23	1.26
	4.35	3.38	0.97
	4.19	3.32	0.86
4	4.38	3.40	0.98
	4.38	3.33	1.05
	4.25	3.33	0.92
	4.25	3.27	0.98

Enantiodiscrimination processes brought about by the more efficient chiral selector **1** were investigated in detail by analysis of mixtures containing **1**, and each enantiomer of **7**, in order to determine the stoichiometries, the association constants and the stereochemistry of the two diastereoisomeric adducts **1**/(*S*)- and **1**/(*R*)-**7**. Determinations of the complexation stoichiometry and of association constants were based on analysis of the dependence of chemical shift on molar ratios CSA/substrate and on concentration, respectively. Intra- and intermolecular proximity constraints were obtained by analysis of through space dipolar interactions in the ROESY maps and by analysis of complexation induced shifts (CISs, difference between chemical shifts of a selected nucleus in the presence and in the absence of the CSA).

2.4. Stoichiometry and association constant of (*R*)-7/1 and (*S*)-7/1

Complexation stoichiometries were determined by the Job method[13,14] based on the analysis of mixtures at a constant total concentration but different molar ratios of the two components: the graph of $\Delta\delta\ X_{substrate}$ as a function of X_{CSA} (where $\Delta\delta$ is the difference between the chemical shift measured in the presence and in the absence of CSA, $X_{substrate}$ and X_{CSA} are the molar fractions of substrate and CSA, respectively) shows a maximum at the stoichiometric ratio. For both complexes (*R*)-**7**/**1** and (*S*)-**7**/**1** two considerably symmetrical curves were obtained with a maximum corresponding to 1 to 1 stoichiometries.

Association constants were determined by analysis of chemical shifts of (*R*)-**7**/**1** and (*S*)-**7**/**1** equimolar solutions progressively diluted from 120 mM to 1 mM and by non-linear fitting on the basis of Eq. 1, obtained by combining the expression of the chemical shift measured (δ_{obs}) in the fast-exchange condition (Eq. 2) and Eq. 3, giving the expression of the constant (K) for 1:1 adduct formation:

$$C = \frac{(\delta_{obs} - \delta_f)(\delta_b - \delta_f)}{K(\delta_b - \delta_{obs})^2} \quad (1)$$

$$\delta_{obs} = X_f\delta_f + X_b\delta_b \quad (2)$$

$$K = \frac{X_b}{C(1 - X_b)^2} \quad (3)$$

where δ_f and δ_b are the chemical shifts in the free and bound states, respectively; X_f and X_b are the molar fractions in the free and bound states and C is the initial concentration. Association constants of $7.1 \pm 0.4\ M^{-1}$ and $4.9 \pm 0.4\ M^{-1}$ for (*S*)-**7**/**1** and (*R*)-**7**/**1** were calculated, that is, low and only slightly differentiated for the two diastereoisomeric solvates.

2.5. Stereochemistry of the diastereoisomeric complexes (*R*)-7/1 and (*S*)-7/1

The interaction of **1** with both enantiomers of **7** did not affect the conformation of **1**. The only difference detected in the intramolecular dipolar interactions pattern of **1** in the presence of **7** is due to the occurrence of weak ROEs between the aromatic protons of one unit and the *tert*-butyl

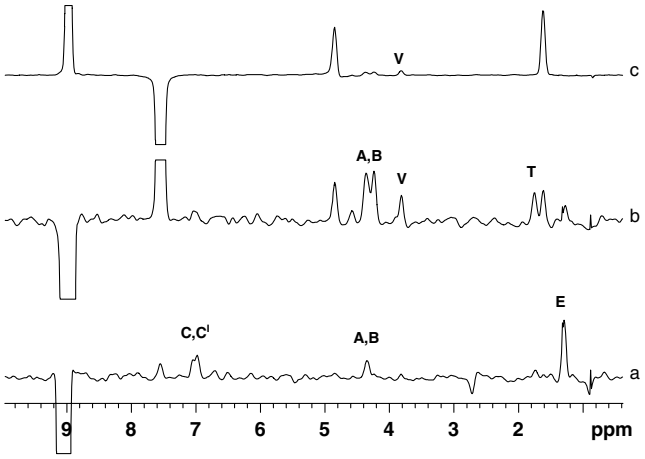


Figure 5. ROESY (600 MHz, $CDCl_3$, 25 °C, mix 0.8 s) analysis of the 1:1 mixture (*S*)-**7**/**1** (100 mM). Traces corresponding to *para* (a), *ortho* (b) and NH (c) protons of (*S*)-**7**.

protons belonging to the adjacent unit, supporting the hypothesis of a more rigid structure with the two nonequivalent aromatic moieties closer than in pure **1**, as a consequence of complexation with either (*R*)- or (*S*)-**7**.

The analysis of the intermolecular ROEs (Fig. 5) allowed us to demonstrate the occurrence of inclusion of the guest inside calixarene cavity as well as its orientation in the calixarene cavity.

Indeed, the *para*-proton of the 3,5-dinitrophenyl moiety of (*S*)-**7** produced sensitive ROE on the aromatic and *tert*-butyl protons belonging to the phenolic units (Fig. 5a), whereas the *ortho*- and NH protons of (*S*)-**7** produced weak ROEs on the pseudo-axial methylene protons H-A and H-B and on the chiral moiety at the narrow rim of the calixarene (Fig. 5b and c). The absence of intermolecular ROEs involving externally pointing pseudo-equatorial methylene protons (H-A′, H-B′) is evidence of the inclusion of (*S*)-**7** in the cavity of **1** with the 3,5-dinitrophenyl facing the phenolic unit and orienting its *para*-proton towards the *tert*-butyl group and their *ortho*- and NH protons towards the narrow rim near to the bridge methylene protons and the chiral moiety. Accordingly, the groups bound to the chiral centre of (*S*)-**7** produced ROEs

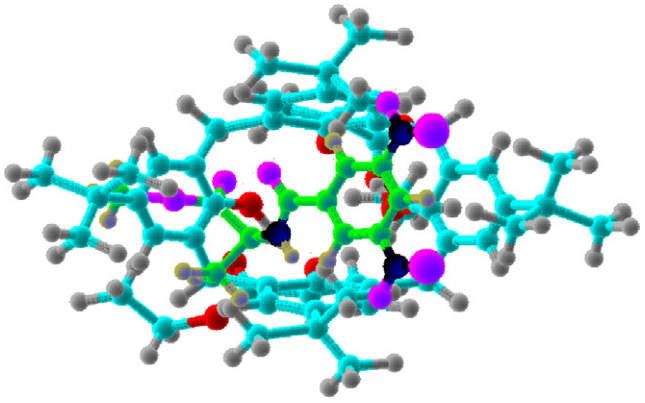


Figure 6. Graphical representation of the diastereoisomeric complex (*S*)-**7**/**1**.

Table 3. CISs ($\delta_{mixture} - \delta_{free}$, ppm; 600 MHz, $CDCl_3$, 25 °C) measured for protons of the two enantiomers of **7** (100 mM) in the presence of **1**

	$\delta_{mixture} - \delta_{free}$ (ppm)	
	(*R*)-**7**	(*S*)-**7**
H-*p*	0.055	0.173
H-*o*	−0.033	−0.071
NH	−0.232	−0.503
CH	−0.011	−0.021
OMe	0.027	0.043
Me	−0.017	−0.063

with the lactate moiety and/or with pseudo-axial protons H-A and H-B. In such a complexation stereochemistry (Fig. 6), the amide function of (*S*)-**7** can be involved in the network of hydrogen-bond interactions present at the narrow rim of the calixarene.

A similar trend for the intermolecular dipolar interactions was observed in the (*R*)-**7**/**1** mixture, differing only in a weaker intensity than in the (*S*)-**7**/**1** mixture, reflecting the lower stability of this complex.

Therefore, (*S*)- and (*R*)-**7** interact with **1** with a similar inclusion stereochemistry according to the measured CISs (Table 3), whose trend is the same for the two diastereoisomeric complexes, with those of the (*S*)-**7**/**1** mixture higher than those of the (*R*)-**7**/**1** one.

3. Conclusion

The bis(ethyl lactate) derivative of *p*-*tert*-butylcalix[4]arene is able to bring about efficient enantiodiscrimination of simple amino acid derivatives. Enantiodiscrimination mainly depends on the stabilization of its rigid cone conformation, in which lactate moieties are put in proximity of the calixarene lower rim in virtue of an efficient hydrogen-bond network with residual phenolic hydroxyls. Such a remarkable stereochemical preorganization favours inclusion processes of enantiomeric substrates, simultaneously putting them in the proximity of the lactate stereogenic centre. In this way, even though the two enantiomers are included by the same stereochemistries relative to the calixarene cavity, groups bound to their stereogenic centres are, however, necessarily in different stereochemical environments with respect to lactate moieties at the lower rim. Accordingly, stability constants of the two diastereoisomeric complexes are slightly differentiated as stabilizing interactions must be quite similar.

The importance of inclusion phenomena is confirmed by the fact that none of the acyclic models **5** or **6** are able to bring about efficient enantiodiscrimination. The stereogenic centre of the lactate moiety being farther from the calixarene lower rim, as in **2**, probably does not make possible the cooperative processes involved in enantiodiscrimination by **1**. Finally, the stereoelectronic features of polar groups introduced at the calixarene lower rim play a fundamental role, since in the cases of carbamate derivatives **3** and **4**, competitive interaction processes probably address substrate interactions at the external surface instead of at

its internal cavity, in spite of the fact that calixarene cone conformation is not perturbed.

4. Experimental

4.1. General methods

NMR measurements were performed on spectrometers operating at 300 and 600 MHz for ^{1}H and at 75 and 150 MHz for ^{13}C, respectively. The temperature was controlled to ±0.1 °C. All ^{1}H and ^{13}C NMR chemical shifts are referenced to TMS as an external standard. The 2D NMR spectra were obtained by using standard sequences. The gCOSY spectra were recorded in the absolute mode acquiring 4 scans with a 5 s relaxation delay between acquisitions for each of 256 FIDs. The ROESY spectra were recorded by employing a mixing time of 0.8 s. The gradient ^{1}H, ^{13}C-gHSQC spectra were obtained in 16 transients per increment into a 2048 × 128 point data matrix. The gradient HMBC experiments were optimized for a long-range ^{1}H–^{13}C coupling constant of 8 Hz. The spectra were acquired with 256 time increments, 16 scans per t_1 increment and a 3.5 ms delay period for suppression of one-bond correlation signals. No decoupling during acquisition was used.

The stoichiometries of (*R*)-**7**/**1** and (*S*)-**7**/**1** complexes were determined by measuring the chemical shifts of substrate **7** in solutions prepared by mixing different volumes of stock solutions of each component having the same molar concentration M ($M = 0.01$) to obtain a prefixed volume directly in the NMR tube.

Optical rotations were measured using a JASCO-DIP 360 polarimeter.

4.2. Materials

p-*tert*-Butylphenol, ethyl (*S*)-lactate, chloroacetyl chloride, *p*-toluensulfonyl chloride, 3,5-dimethylphenyl isocyanate and 3,5-dinitrophenyl isocyanate were purchased from Aldrich.

5,11,17,23-Tetra-*tert*-butylcalix[4]arene-25,26,27,28-tetraol **I**, ethyl (*S*)-2-(*p*-toluensulfonyloxy)propanoate **II** and ethyl (*S*)-2-(chloroacetoxy)propanoate **III** were prepared as described in Refs. 15,16 and 17, respectively. 5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(*R*)-1-ethoxycarbonylethoxy]-26,28-dihydroxycalix[4]arene **1** was synthetized as described by Lanzarotto et al.[12]

Pyridine was distilled over KOH, acetone was dried over K_2CO_3 and then distilled and toluene was distilled over Na.

4.2.1. 5,11,17,23-Tetra-*tert*-butylcalix[4]arene-25,26,27,28-tetraol I.[15] ^{1}H NMR ($CDCl_3$) δ 1.19 (36H, s, Me_3C), 3.48 and 4.24 (8H, d, J 14.3, CH_2), 7.03 (8H, s, Ar-H), 10.32 (4H, s, OH); ^{13}C NMR ($CDCl_3$) δ 31.4 (Me_3C), 32.6 (CH_2), 34.0 (Me_3C), 125.9 and 127.7 (Ar); aromatic quaternary C: 125.9, 144.4, 146.7. Anal. Calcd for $C_{44}H_{56}O_4$: C, 81.44; H, 8.70. Found: C, 81.38; H, 8.64.

4.2.2. Ethyl (*S*)-2-*p*-toluensulfonyloxypropanoate II.[16] ^{1}H NMR ($CDCl_3$) δ 1.17 (3H, t, J 7.4, $MeCH_2$), 1.47 (3H, d, J 7.2, $MeCH$), 2.41 (3H, s, *Me*-Ar), 4.08 (2H, q, J 7.4, $MeCH_2$), 4.89 (1H, q, J 7.2, CH), 7.31 (2H, d, J 8.4, Ar-H), 7.78 (2H, d, J 8.4, Ar-H); ^{13}C NMR ($CDCl_3$) δ 13.9 ($MeCH_2$), 18.4 ($MeCH$), 21.6 (*Me*-Ar), 61.8 ($MeCH_2$), 74.1 (CH), 128.0 (Ar), 129.7 (Ar); quaternary carbons: 133.4, 145.0, 169.0 (CO). Anal. Calcd for $C_{12}H_{16}O_5S$: C, 52.93; H, 5.92. Found: C, 53.01; H, 5.95. $[\alpha]_D^{26} = -30$ (c 1.0, CH_2Cl_2).

4.2.3. 5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(*R*)-1-ethoxycarbonylethoxy]-26,28-dihydroxycalix[4]arene 1.[12] ^{1}H NMR (600 MHz, $CDCl_3$, 65 mM, 25 °C) δ 0.92 (18H, s, H-F), 1.27 (6H, t, J 7.1, H-Z), 1.30 (18H, s, H-E), 1.76 (6H, d, J 6.7, H-T), 3.25 (2H, d, J 13.7, H-B′), 3.29 (2H, d, J 13.7, H-A′), 4.22 (4H, q, J 7.1, H-V), 4.37 (2H, d, J 13.7, H-B), 4.46 (2H, d, J 13.7, H-A), 4.60 (2H, q, J 6.7, H-S), 6.72 (2H, d, J 2.3, H-D′), 6.77 (2H, s, OH), 6.79 (2H, d, J 2.3, H-D), 7.01 (2H, d, J 2.3, H-C′), 7.09 (2H, d, J 2.3, H-C); ^{13}C NMR (150 MHz, $CDCl_3$, 65 mM, 25 °C) δ 14.1 (C-Z), 17.9 (C-T), 31.0 (C-F), 31.6 (C-BB′), 31.7 (C-E), 32.2 (C-AA′), 33.8 (C-G, C-H), 61.2 (C-V), 80.0 (C-S), 124.9 (C-C), 125.1 (C-C′), 125.2 (C-D), 126.0 (C-D′), 127.4 (C-M), 128.3 (C-N), 132.0 (C-P), 132.8 (C-O), 141.3 (C-I), 146.7 (C-L), 149.0 (C-R), 150.6 (C-Q), 171.6 (C-U). Anal. Calcd for $C_{54}H_{72}O_8$: C, 76.38; H, 8.55. Found: C, 76.29; H, 8.50. $[\alpha]_D^{22} = +65$ (c 1.0, CH_2Cl_2).

4.2.4. Ethyl (*S*)-2-(chloroacetoxy)propanoate III. ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J 7.1, $MeCH_2$), 1.50 (3H, d, J 7.3, $MeCH$), 4.12 (2H, m, $ClCH_2$), 4.19 (2H, q, J 7.1, $MeCH_2$), 5.15 (1H, q, J 7.3, CH); ^{13}C NMR ($CDCl_3$) δ 14.0 ($MeCH_2$), 16.7 ($MeCH$), 40.5 ($ClCH_2$), 61.6 ($MeCH_2$), 70.1 (CH); quaternary carbons: 166.7 and 169.9 (CO). Anal. Calcd for $C_7H_{11}O_4Cl$: C, 43.20; H, 5.70; Cl, 18.22. Found: C, 43.11; H, 5.76; Cl, 18.20. $[\alpha]_D^{26} = -42.6$ (c 1.0, CH_2Cl_2).

4.2.5. 5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(*S*)-(1-ethoxycarbonylethoxy)carbonylmetoxy]-26,28-dihydroxycalix[4]-arene 2. A mixture of **I** (1.0 g, 1.54 mmol), **III** (0.82 g, 4.22 mmol), K_2CO_3 (0.21 g, 1.54 mmol) and KI (0.51 g, 3.08 mmol) in dry acetone (40 mL) and toluene (15 mL) was refluxed under N_2 for 12 h. The solvent was evaporated under reduced pressure and the residue was treated with H_2O, extracted with CH_2Cl_2, dried over Na_2SO_4 and finally concentrated. After precipitation (MeOH), **2** was obtained (yield 75%): ^{1}H NMR (600 MHz, $CDCl_3$, 65 mM, 25 °C) δ 0.96 (18H, s, H-F), 1.25 (6H, t, J 7.3, $MeCH_2$), 1.28 (18H, s, H-E), 1.57 (6H, d, J 6.9, $MeCH$), 3.33 (4H, d, J 13.1, H-A′ and H-B′), 4.20 (4H, m, $MeCH_2$), 4.43 (2H, d, J 13.1, H-A), 4.45 (2H, d, J 13.1, H-B), 4.75 (2H, d, J 15.8, $OCHHCO$), 4.83 (2H, d, J 15.8, $OCHHCO$), 5.26 (2H, q, J 6.9, CH), 6.80 (4H, s, H-D), 7.04 (2H, s, OH), 7.04 (4H, s, H-C); ^{13}C NMR (150 MHz, $CDCl_3$, 65 mM, 25 °C) δ 14.0 ($MeCH_2$), 16.9 ($MeCH$), 31.0 (C-F), 31.7 (C-E), 31.8 (2C, $ArCH_2Ar$), 33.8 and 33.9 (C-G, C-H), 61.5 ($MeCH_2$), 69.3 (CH), 72.1 (OCH_2CO), 125.1 (C-C), 125.7 (C-D), 127.8 (C-M), 127.9 (C-N), 132.3 (C-P), 132.4 (C-O), 141.5 (C-I), 147.2 (C-L), 150.4 (C-R), 150.6 (C-Q), 168.5 (CO), 170.3 (CO). Anal. Calcd for

$C_{58}H_{76}O_{12}$: C, 72.12; H, 7.94. Found: C, 72.21; H, 7.98. $[\alpha]_D^{24} = -7.1$ (*c* 1.0, CH_2Cl_2).

4.2.6. Synthesis of calixarenes 3 and 4. A solution of **1** (1.18 mmol) and the opportune isocyanate (2.60 mmol) in dry toluene (35 mL) was refluxed under N_2 for 72 h for **3** and 24 h for **4**. The toluene was evaporated under reduced pressure and the residue was suspended in CH_2Cl_2 and filtered over a PTFE (0.45 μm) filter to recover **3** (83% yield) and **4** (95% yield).

4.2.6.1. 5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(*R*)-1-ethoxycarbonylethoxy]-26-(3,5-dimethylphenylcarbomoyloxy)-28-hydroxycalix[4]arene 3. ^{1}H NMR (600 MHz, $CDCl_3$, 65 mM, 25 °C) δ 0.81 (9H, s, Me_3C), 0.83 (9H, s, Me_3C), 1.11 (6H, t, *J* 7.2, *Me*CH_2), 1.33 (3H, d, *J* 6.4, *Me*CH), 1.36 (18H, s, Me_3C), 1.39 (3H, d, *J* 6.4, *Me*CH), 2.32 (6H, s, MeAr), 3.23 and 4.49 (2H, 2d, *J* 13.7, Ar*CH*$_2$Ar), 3.28 and 4.59 (2H, 2d, *J* 13.7, Ar*CH*$_2$Ar), 3.32 and 4.19 (2H, 2d, *J* 13.7, Ar*CH*$_2$Ar), 3.38 and 4.35 (2H, 2d, *J* 13.7, Ar*CH*$_2$Ar Hz), 3.79–3.96 (4H, m, Me*CH*$_2$), 4.29 (1H, q, *J* 6.4, CH), 4.39 (1H, q, *J* 6.4, CH), 6.51 and 6.53 (2H, 2d, *J* 2.3, Ar-H), 6.54 and 6.57 (2H, 2d, *J* 2.3, Ar-H), 6.66 (1H, s, H-*para*), 6.81 (1H, s, OH), 7.12 and 7.13 (2H, 2d, *J* 2.3, Ar-H), 7.21 (2H, s, Ar-H), 7.33 (2H, s, H-*ortho*), 8.33 (1H, NH, s); ^{13}C NMR (150 MHz, $CDCl_3$, 65 mM, 25 °C) δ 14.1 (2C, *Me*CH_2), 17.9 and 18.1 (*Me*CH), 22.6 (*Me*Ar), 30.9 (2C, *Me*$_3$C), 31.6 (3C, *Me*$_3$C and 2 Ar*CH*$_2$Ar), 31.7 (*Me*$_3$C), 31.8 (Ar*CH*$_2$Ar), 31.9 (Ar*CH*$_2$Ar), 33.6 (Me$_3$*C*), 33.7 (Me$_3$*C*), 33.9 (Me$_3$*C*), 34.3 (Me$_3$*C*), 60.9 (2C, Me*CH*$_2$), 79.5 (CH), 80.4 (CH), 116.1 (C-*ortho*), 123.9 (C-*para*); Ar-H: 125.1, 125.2 (2C), 125.3, 125.4, 125.5 (3C); quaternary Ar-C: 127.9, 128.2, 131.7, 131.8, 132.0, 132.3, 135.3, 136.0, 138.1, 138.8, 141.8, 145.0, 148.1, 149.6, 150.1, 150.2; CO: 152.0, 171.3, 171.5. Anal. Calcd for $C_{63}H_{82}NO_9$: C, 75.95; H, 8.19; N, 1.41. Found: C, 75.86; H, 8.23; N, 1.44.

4.2.6.2. 5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(*R*)-1-ethoxycarbonylethoxy]-26-(3,5-dinitrophenylcarbomoyloxy)-28-hydroxycalix[4]arene 4. ^{1}H NMR (600 MHz, $CDCl_3$, 65 mM, 25 °C) δ 0.78 (9H, s, Me_3C), 0.86 (9H, s, Me_3C), 1.10 (3H, t, *J* 7.1, *Me*CH_2), 1.11 (3H, t, *J* 7.1, *Me*CH_2), 1.31 (3H, d, *J* 6.6, *Me*CH), 1.36 (9H, s, Me_3C), 1.37 (9H, s, Me_3C), 1.38 (3H, d, *J* 6.6, *Me*CH), 3.27 and 4.25 (2H, 2d, *J* 13.7, Ar*CH*$_2$Ar), 3.33 and 4.38 (2H, 2d, *J* 13.7, Ar*CH*$_2$Ar), 3.33 and 4.25 (2H, 2d, *J* 13.7, Ar*CH*$_2$Ar), 3.40 and 4.38 (2H, 2d, *J* 13.7, Ar*CH*$_2$Ar), 3.85–4.00 (4H, m, Me*CH*$_2$), 4.23 (1H, q, *J* 6.6, CH), 4.31 (1H, q, *J* 6.6, CH), 6.48 and 6.54 (2H, 2d, *J* 2.4, Ar-H), 6.60 and 6.63 (2H, 2d, *J* 2.4, Ar-H), 6.98 (1H, s, OH), 7.11 and 7.17 (2H, 2d, *J* 2.4, Ar-H), 7.24 (2H, s, Ar-H), 8.70 (1H, br s, H-*para*), 9.00 (2H, d, *J* 1.9, H-*ortho*), 9.17 (1H, s, NH); ^{13}C NMR (150 MHz, $CDCl_3$, 65 mM, 25 °C) δ 14.2 (2C, *Me*CH_2), 18.3 and 18.4 (*Me*CH), 30.9 (*Me*$_3$C), 31.1 (*Me*$_3$C), 31.6 (Ar*CH*$_2$Ar), 31.7 (Ar*CH*$_2$Ar), 31.8 (Ar*CH*$_2$-Ar), 31.9 (2C, *Me*$_3$C), 32.0 (Ar*CH*$_2$Ar), 33.9 (Me$_3$*C*), 34.0 (Me$_3$*C*), 34.1 (Me$_3$*C*), 34.6 (Me$_3$*C*), 61.3 (Me*CH*$_2$), 61.5 (Me*CH*$_2$), 79.9 (CH), 80.6 (CH), 111.78 (C-*para*), 118.5 (C-*ortho*); Ar-H: 125.5, 125.6 (3C), 125.7, 125.9, 126.0 (2C); quaternary Ar-C: 127.7, 128.3, 131.8, 131.9, 132.0, 132.2, 135.2, 135.9, 142.2, 142.5, 144.7, 146.6, 147.1, 148.9, 149.3, 149.9, 150.0, 150.2; CO: 152.3, 171.2, 171.6. Anal. Calcd for $C_{61}H_{75}N_3O_{13}$: C, 69.23; H, 7.14; N, 3.97. Found: C, 69.30; H, 7.19; N, 4.01.

4.2.7. *p-tert*-Butylphenyl-[(*R*)-1-ethoxycarbonylethoxy] 5. A mixture of *p-tert*-butylphenol (0.59 g, 3.93 mmol), **II** (1.62 g, 5.89 mmol) and K_2CO_3 (0.81 g, 5.9 mmol) in dry acetone (35 mL) was refluxed under N_2 for 5 days. The solvent was evaporated under reduced pressure and the residue was treated with H_2O and extracted with CH_2Cl_2. The organic layer was washed with a solution of HCl (10%), brine, dried over Na_2SO_4 and finally concentrated. After chromatographic purification (SiO_2, hexane/AcOEt 90:10), **5** was obtained (yield 78%): ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) δ 1.25 (3H, t, *J* 7.6, *Me*CH_2), 1.28 (9H, s, Me_3C), 1.60 (3H, d, *J* 6.6, *Me*CH), 4.22 (2H, q, *J* 7.6, Me*CH*$_2$), 4.71 (1H, q, *J* 6.6, CH), 6.80 (2H, d, *J* 9.0, Ar-H), 7.27 (2H, d, *J* 9.0, Ar-H); ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C) δ 14.1 (*Me*CH_2), 18.6 (*Me*CH), 31.5 (*Me*$_3$C), 34.1 (Me$_3$*C*), 61.1 (Me*CH*$_2$), 72.8 (CH), 114.6 (C-Ar), 126.3 (C-Ar); quaternary C: 144.2, 155.4, 172.4 (CO). Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.01; H, 8.89. $[\alpha]_D^{26} = +26.6$ (*c* 1.0, CH_2Cl_2).

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