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Heptakis[2,3-di-*O*-methyl-6-*O*-(L-valine-*tert*-butylamide-N^{α}-ylcarbonylmethyl)]-β-cyclodextrin: a New Multifunctional Cyclodextrin CSA for the NMR Enantiodiscrimination of Polar and Apolar Substrates

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Heptakis[2,3-di-*O*-methyl-6-*O*-(L-valine-*tert*-butylamide-N^{α}-ylcarbonylmethyl)]-β-cyclodextrin with methyl groups on the secondary sites and a diamide chiral selector on the primary ones showed considerable utility as a CSA in the NMR enantiodiscrimination of a wide spectrum of amino acid derivatives, while also allowing trisubstituted allenes to be enantiodiscriminated. Enantiodiscrimination processes were investigated by Diffusion-Ordered SpectroscopY (DOSY).

Introduction

NMR methods for the determination of enantiomeric composition and absolute configuration are based on the use of chiral auxiliaries that are capable of forming intrinsically anisochronous transient or stable diastereoisomeric associates of enantiomeric mixtures.[1–7] Chiral auxiliaries for NMR spectroscopy that possess enantiodiscriminating abilities based on solvation or complexation processes occurring in solution are known either as Chiral Solvating Agents (CSAs, diamagnetic) or as Chiral Lanthanide Shift Reagents (CLSRs, paramagnetic), whereas the use of Chiral Derivatizing Agents (CDAs) involves the formation of covalent bonds.[1–7] CSAs (or CLSRs) are particularly attractive from the applicative point of view, as their use requires only preparation, directly in the NMR tube, of their mixtures with the enantiomeric compounds to be analysed and acquisition of quick, routine NMR spectra. In the last twenty years, cyclodextrin CSAs have gained great popularity[1–40] by virtue of the effects of their unique structural features on complexation processes in solution. Cyclodextrins have a truncated cone shape, the inner part of which is predisposed to include apolar moieties. Located on the large and small rims, respectively, are secondary and primary hydroxy groups, which make the external surface polar and hence allow attractive interactions to be established through polar groups protruding from the cavity or by species interacting at the external surface. Importantly, the three hydroxy groups of each glucose unit of cyclodextrins have strongly differentiated reactivities and can be suitably derivatized, making it possible to modulate and to tailor cyclodextrin complexing and enantiodiscriminating features, in addition to their physicochemical properties.[41–46]

Our earlier studies[31–35] demonstrated the considerable efficiency of exhaustively methylated cyclodextrins as CSAs for enantiomeric purity determinations of chiral substrates devoid of hydrogen-bond donor groups, enantiodiscrimination of which by NMR methods constitutes a difficult challenge,[6] whereas carbamoylated cyclodextrins showed[36] great potential in the analysis of hydrogen-bond donor chiral substrates in halogenated solvents. More recently, derivatized cyclodextrins, bearing both methyl and carbamoyl groups, have been proposed[28,29,37] as CSAs capable of inducing nonequivalence in the NMR spectra of both classes of chiral substrates mentioned above. These results[28,29,37] indicate the need to have methyl groups at least on both cyclodextrin secondary sites in order to obtain NMR differentiation of enantiomers of trisubstituted allenes devoid of hydrogen-bond donor groups, and raised the problem of finding optimal derivatizing groups for introduction into primary sites in order to obtain the simultaneous efficient enantiodiscrimination of polar substrates.

Recently, a number of binary chiral stationary phases (CSPs) for enantioselective gas chromatography with an extended spectrum of enantioselectivity were synthesized[47–51] from L-valine-diamide and alkylated cyclodextrin selectors. These results prompted us to probe the potential of heptakis[2,3-di-*O*-methyl-6-*O*-(L-valine-*tert*-butylamide-N^{α}-yl-

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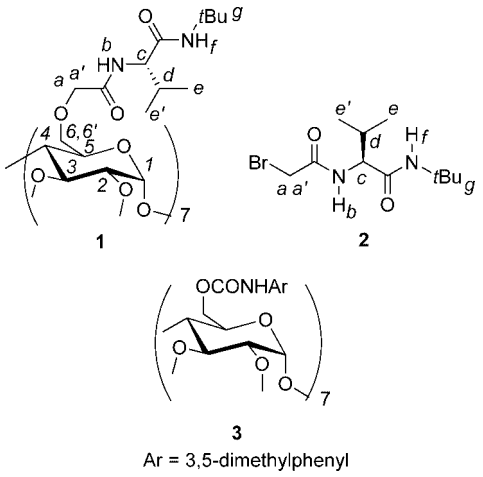
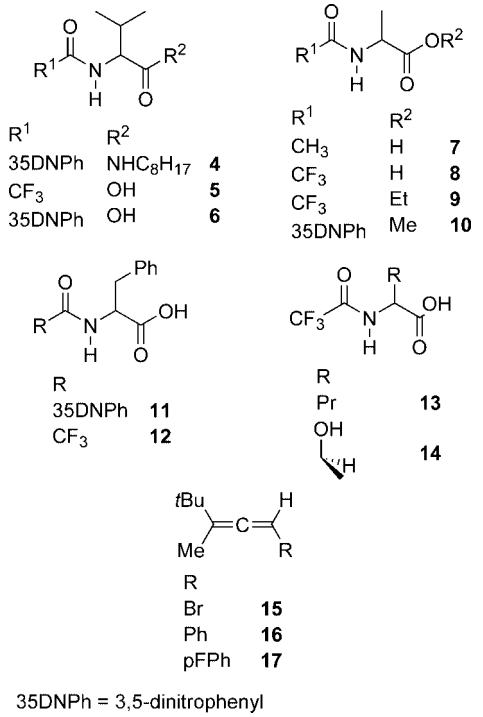
carbonylmethyl)]-β-cyclodextrin (**1**; Scheme 1), a cyclodextrin derivative containing both a diamide L-valine moiety and methyl groups, as a CSA for the NMR differentiation of enantiomeric mixtures of amino acid derivatives and trisubstituted allenes (Scheme 2), either endowed with hydrogen-bond donor groups or devoid of them. The efficiency of **1** was compared with that of *N*-bromoacetyl-L-valine-*tert*-butylamide (**2**; Scheme 1), employed in the synthesis of **1**, and also with that of the previously reported heptakis[6-*O*-(3,5-dimethylphenylcarbamoyl)-2,3-di-*O*-methyl]-β-cyclodextrin[37] **3** (Scheme 1), itself containing methylated secondary sites. Our investigations also involved extensive use of DOSY (Diffusion-Ordered SpectroscopY)[52–54] techniques in the analysis of enantiodiscrimination processes in solution.



Scheme 1. CSAs **1**–**3**.

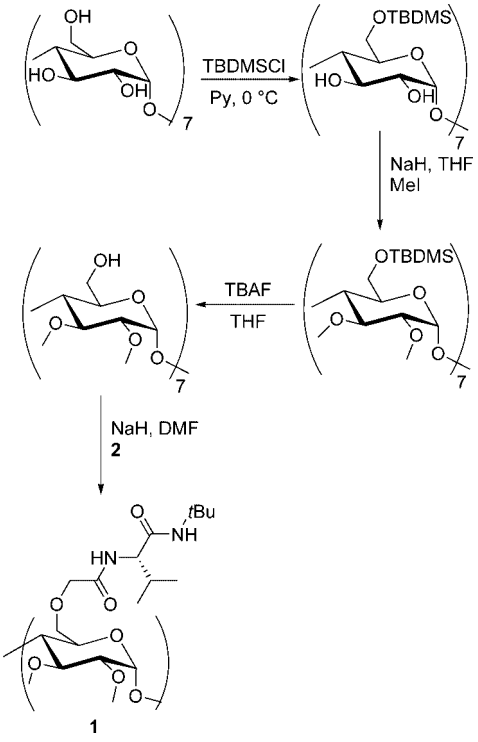


Scheme 2. Racemic substrates **4**–**17**.

Results and Discussion

Synthesis and Characterization of Chiral Auxiliaries

The chiral diamide *N*-bromoacetyl-L-valine-*tert*-butylamide (**2**) was synthesized according to a published procedure,[48] and cyclodextrin **1** was prepared from heptakis(2,3-di-*O*-methyl)-β-cyclodextrin by alkylation of the primary hydroxy groups with **2** (Scheme 3). The synthesis of heptakis(2,3-di-*O*-methyl)-β-cyclodextrin was carried out (Scheme 3) according to the classical procedure[55] of selective protection of the primary hydroxy groups as *tert*-butyldimethylsilyl (TBDMS) ethers, methylation of the secondary ones and, finally, deprotection of primary sites.



Scheme 3. Synthesis of CSA **1**.

^{1}H and ^{13}C NMR spectra of **1** were assigned by the 2D NMR techniques gCOSY, ROESY, HSQC and HMBC and the corresponding data are reported in the Experimental Section.

^{1}H NMR Enantiodiscrimination Experiments

Enantiodiscrimination experiments were carried out by comparing the NMR spectra of the racemic compounds **4**–**17** (Scheme 2) and those of their mixtures with the CSA **1**. Comparison with analogous mixtures containing pure enantiomers or their enantiomerically enriched mixtures confirmed the assignments of doubled resonances to the two enantiomers. Chemical shift nonequivalences ($\Delta\delta$s), which are the differences in the chemical shifts of corresponding nuclei of the two enantiomers in the presence of the CSA, were measured in order to evaluate the effectiveness of CSA **1**.

Among the compounds analysed, we examined amino acids derivatives either with two hydrogen-bond donor groups (two amide functions as in **4** or one amide and one carboxyl function as in **5**–**8** and **11**–**14**) or with only one hydrogen-bond donor group (the amide function as in **9** and **10**), in order to identify the optimal combination of derivatizing functional groups for improving enantiodiscrimination. The compounds were analysed in $CDCl_3$, as their solubilities were too low in less polar solvents (CCl_4, C_6D_{14} or C_6D_{12}), which could, in principle, favour complexation processes by the chiral auxiliaries and hence enhance enantiodifferentiation. Nonequivalences of amino acid derivatives were not measured in solvents such as acetone, DMSO or methanol. Experiments with trisubstituted allenes were carried out in CD_3OD, which had been the optimal solvent in the previously reported[37] enantiodiscrimination experiments with cyclodextrin **3**. The use of methanol as solvent also made it possible to enhance the differentiation of enantiomer resonances through low-temperature experiments.

Starting our analysis from the racemic diamide derivative of valine **4**, we observed doublings of all its resonances in the presence of 1 equiv. of the CSA **1** (Supporting Information, Table S1). Nonequivalences were remarkably high (0.400 ppm at 20 mM; Figure 1d and Table 1) for the amide proton bonded to the chiral centre. The same amide proton was also strongly differentiated in the two enantiomers (0.078 ppm, Table 1 and Figure 1a) in progressively diluted equimolar solutions up to 1 mM, which is out of the concentration ranges commonly involved in the use of CSAs.

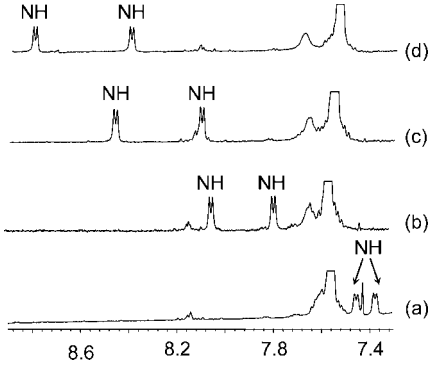


Figure 1. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) spectral regions corresponding to the amide proton bonded to the chiral centre of **4** in the presence of equimolar amount of **1** at 1 mM (a), 5 mM (b) 10 mM (c) and 20 mM (d).

Different kinds of derivatives of the same amino acid (Table 1, Figure 2) were efficiently enantiodifferentiated by **1**: in the case of the trifluoroacetyl derivative **5**, with the free carboxyl group, nonequivalence for the amide proton was 0.357 ppm in the equimolar 5 mM mixture, even higher than for the derivative **4** at the same concentration.

Interestingly, the magnitude of nonequivalence of the amide proton of **6** at 5 mM was about 0.232 ppm (i.e., very similar to the one measured at the same concentration for **4**; Table 1). Therefore, in view of the straightforward preparation of trifluoroacetyl derivatives of amino acids, we probed the enantiodiscriminating versatility of the CSA **1** towards trifluoroacetyl derivatives of different amino acids (**8**, **12**–**14**): chemical shift nonequivalences of the amide protons were similarly high in the case of the alanine derivative **8** (0.235 ppm, Table 1), as well as in the case of (trifluoroacetyl)threonine **14** (0.180 ppm), in which an OH group is also present. Differentiation was lower (0.043 ppm and 0.031 ppm, respectively) in the case of phenylalanine (**12**) and of norvaline (**13**) derivatives, in which a benzyl and an *n*-propyl group, respectively, is bonded to the chiral centre. It is noteworthy that measurements are carried out at very low concentrations and, hence, as demonstrated in the case of **4** (Figure 1), nonequivalences can be very simply optimized by increasing the total equimolar concentration of the analysed solutions.

Table 1. Nonequivalences ($\Delta\delta = |\delta_R - \delta_S|$, ppm; 600 MHz, $CDCl_3$) of NH protons of amino acid derivatives **4**–**8** and **11**–**14** in the presence of equimolar amount of CSA **1**.

Compound	mM	$\Delta\delta_{NH}$
4[a]	1	0.078
4[a]	5	0.258
4[a]	10	0.359
4[a]	20	0.400
5	5	0.357
6	5	0.232
7	5	0.269
8	5	0.235
11	5	0.068
12	5	0.043
13	5	0.031
14	5	0.180

[a] NH bonded to the chiral centre.

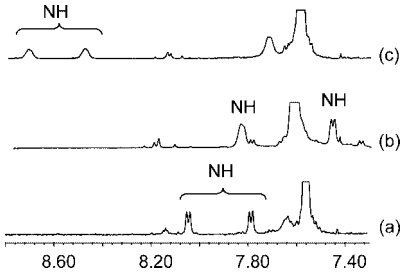


Figure 2. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C, 5 mM) spectral regions corresponding to the amide proton bonded to the chiral centre in **4** (a), **5** (b) and **6** (c) in the presence of equimolar amounts of **1**.

In the case of alanine, different derivatization strategies for the amino group were probed. Nonequivalence seemed to be quite insensitive to this kind of structural modification as the amide proton in the two enantiomers of **7** was differentiated by 0.269 ppm, which was quite similar to the value obtained for **8** under the same experimental conditions (Table 1).

On examination of the same kind of amino acid, remarkably lower nonequivalences were measured for its derivatives with a single hydrogen-bond donor group (Table 2): the nonequivalence measured for the amide proton of alanine derivative **9**, which has a trifluoroacetylated amino group as in **8**, but with an ester function in place of the

free carboxyl group, was significantly lower (0.061 ppm at 20 mM), indicating that efficient enantiodiscrimination by the CSA **1** required the presence of two hydrogen-bond donor groups.

Table 2. Nonequivalences ($\Delta\delta = |\delta_R - \delta_S|$, ppm; 600 MHz, $CDCl_3$) of NH protons of amino acid derivatives **9** and **10** in the presence of equimolar amounts of CSA **1** and comparison of enantiodiscriminating efficiency of **10** by CSA **3**.

Compound	CSA	mM	$\Delta\delta_{NH}$
9	**1**	20	0.061
10	**1**	20	0.050
10	**3**	80	0.030

Investigation of compound **10** confirmed the above conclusions: the magnitude of nonequivalence of its amide proton at 20 mM in the presence of 1 equiv. of **1** was 0.050 ppm (i.e., very similar to the value obtained at the same concentration in the case of **9**). The nature of the group bonded to the amide function is therefore not important, whereas the presence of a further hydrogen-bond donor group could play an important role in enantiodiscrimination processes.

CSA **1** also surpasses the corresponding mixed carbamoylated-methylated cyclodextrin **3** in the enantiodiscrimination of polar compounds, as evidenced in experiments involving **10** (Table 2). In spite of the fact that **10** was, among alanine derivatives (**7**–**10**), the least efficiently enantiodiscriminated in the presence of CSA **1** (Table 1), the magnitude of doubling obtained for its amide proton at 20 mM was significantly higher than it had been when the analogous mixed methylated-carbamoylated cyclodextrin **3**, itself with methyl groups on the secondary sites, had been used as CSA. Cyclodextrin **3** produced nonequivalence of the NH proton of **10** of 0.030 ppm (Table 2), in spite of the fact that it was employed at significantly higher equimolar concentrations (80 mM). Cyclodextrin **3** did not allow detection of any nonequivalence of compounds **8** and **4**, which were enantiodiscriminated efficiently by CSA **1**.

Finally, enantiodiscrimination experiments were performed on amino acid derivatives with the single diamide selector **2**, employed for the derivatization of primary sites in **1**. Among the substrates reported in Scheme 2, only enantiomers of diamide derivative of valine **4** were differentiated with high efficiency by **2**. The amide proton of **4** bonded to the 3,5-dinitrobenzoyl moiety was doubled by 0.250 ppm in the presence of 1 equiv. of **2** at 20 mM (Supporting Information, Figure S1). This value, already high, can be significantly increased (0.432 ppm) by addition of 4 equiv. of the CSA **2** (Supporting Information, Table S2). With regard to this last result, which might encourage the use of low molecular weight CSA **2** in place of CSA **1**, we stress that, among different kinds of amino acid derivatives, only the diamide **4** was enantiodiscriminated, whereas simple amino acid derivatives such as **5**–**14** were not enantiodiscriminated at all. Furthermore, CSA **2** did not show any enantiodiscriminating ability towards apolar substrates.

On changing over to enantiodiscrimination of substrates devoid of hydrogen-bond donor groups, we found that CSA **1** also allowed differentiation of enantiomers of trisubstituted allenes **15**–**17** (Scheme 2) in CD_3OD solutions (Table 3).

Table 3. Nonequivalences ($\Delta\delta = |\delta_R - \delta_S|$, ppm; 600 MHz, CD_3OD, 60 mM) of protons of allenes **15**–**17** in the presence of 1 equiv. of CSA **1**.

Allene	Proton	$\Delta\delta$		
		25 °C	–20 °C	–40 °C
15	H	0.003	0.014	0.029
	Me	0	0.006	0.011
	*t*Bu	0	0.003	0.007
16	H	0.003	0.019	0.038
	Me	0.005	0.009	0.016
	*t*Bu	0	0.006	0.010
17	H	0.007	0.046	0.091
	Me	0.004	0.022	0.038
	*t*Bu	0.004	0.015	0.028

Nonequivalences, which ranged from 0.003 to 0.007 ppm at room temperature, increased significantly with decreases in temperature: nonequivalence of the allene proton of **15** changed from 0.003 ppm at room temperature to 0.014 ppm at –20 °C, reaching a value of 0.029 ppm at –40 °C (Figure 3 and Table 3). The above trend was confirmed in the case of racemic compound **16**, the allene proton of which was split by 0.003 ppm at room temperature and by 0.038 ppm at –40 °C (Table 3). Temperature gradients produced even more marked effects in the case of compound **17**, enantiomers of which were differentiated by 0.007 ppm in the presence of CSA **1** at room temperature, while a surprisingly high nonequivalence of 0.091 ppm was measured at –40 °C (Table 3).

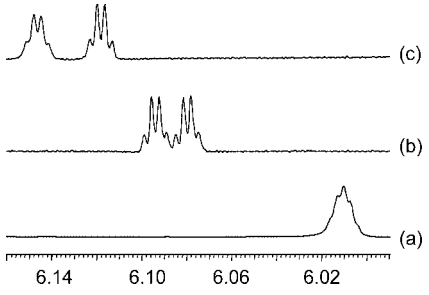


Figure 3. ^1H NMR (600 MHz, CD_3OD, 60 mM) spectral regions corresponding to the allene proton of **15** in the presence of equimolar amounts of **1** at 25 °C (a), –20 °C (b) and –40 °C (c).

Nonequivalences due to the presence of CSA **1** were comparable (Table 4) to those obtained by use of heptakis[6-*O*-(3,5-dimethylphenylcarbamoyl)-2,3-di-*O*-methyl]-β-cyclodextrin (**3**) at room temperature, but CSA **1** surpassed the mixed carbamoylated-methylated cyclodextrin **3**

Table 4. Comparison of nonequivalences ($\Delta\delta = |\delta_R - \delta_S|$, ppm; 600 MHz, CD_3OD, 60 mM) of the CH proton of allene **15** in the presence of CSAs **1** and **3**.

CSA	$\Delta\delta$	
	25 °C	–40 °C
1	0.003	0.028
3	0.003	0.017

at lower temperatures (Table 4). In this regard we would recall[37] that CSA **3** was the most efficient agent among mixed methylated-carbamoylated cyclodextrins with regard to trisubstituted allenes.

Analysis of Enantiorecognition Phenomena

Once the enantiodiscriminating versatility and effectiveness of a new chiral auxiliary for NMR have been established, a further important goal is to discern factors responsible for chiral recognition in solution. This last task is especially difficult for CSAs, as diastereoisomeric solvates are formed between CSA and both enantiomers. Furthermore, under the fast exchange conditions only one resonance set for each enantiomer in the complexed (δ_b) and free (δ_f) forms is detected [Equation (1), X_f and X_b are the molar fractions of the free and the complexed forms, respectively].

$$\delta = X_f\delta_f + X_b\delta_b \quad (1)$$

A precise approach to the problem is based on NMR investigations of mixtures containing the CSA and the single pure enantiomers in order to compare the stereochemistry, dynamics and thermodynamics of the diastereoisomeric complexes. In this way a true picture of the two diastereoisomers can be obtained, comparison of which allows us to pinpoint intermolecular interactions on which enantiodiscrimination is based. The above approach, which is more exhaustive and can also be complemented by computational methods,[56] can be applied only in cases in which sufficiently high molar fractions of the complexed forms can be obtained.

Alternatively, the relative contributions to detected NMR nonequivalences of stereochemical differentiation [δ_b in Equation (1)] and thermodynamics stabilization [X_b in Equation (1)] of the two enantiomers could be evaluated. These two contributions can be easily discriminated by analysis of the effect of a CSA on two spectral parameters of the two enantiomers in CSA/substrate mixtures: chemical shifts and diffusion coefficients. Chemical shifts represent local parameters that are very sensitive to environmental changes of enantiomeric substrates due to complexation. Diffusion coefficients (D) represent global size-dependent parameters and on the basis of the Stokes–Einstein[52–54] equation [Equation (2), k is the Boltzmann constant, η is the solvent viscosity and T is the temperature in K], can be correlated to the hydrodynamics radius (R_H).

$$D = \frac{kT}{6\pi\eta R_H} \quad (2)$$

By virtue of the above dependence, diffusion coefficients are extremely sensitive to variations in the apparent molecular sizes that are produced by complexation processes.

Diffusion coefficients, which can be measured by NMR DOSY techniques,[52–54] represent, under conditions of fast exchange, the weighted average of their values in the complexed (D_b) and pure (D_f) forms [Equation (3)].

$$D = X_fD_f + X_bD_b \quad (3)$$

However, when the molecular size of the chiral auxiliary is significantly greater than that of complexed enantiomers, it is reasonable to assume that translational diffusion in solution is mainly controlled by the CSA. The diffusion coefficient of the bonded enantiomer could therefore be assumed to be equal to that of the CSA ($D_b \approx D_{CSA}$). This approximation has frequently been verified[57,58] when using cyclodextrin complexing agents. Thus, within the validity limits of the above approximation, bonded molar fractions can easily be extracted from Equation (3).

On the basis of the investigation protocol described above, the complexation-induced chemical shifts (CISs) ($\Delta\delta = \delta_{mix} - \delta_f$, where δ_{mix} and δ_f are the chemical shifts of the enantiomeric substrate in the presence and in the absence of the CSA, respectively) and the diffusion coefficient variations ($\Delta D = D_{mix} - D_f$, where D_{mix} and D_f are the diffusion coefficients of the enantiomeric substrate in the presence and in the absence of the CSA, respectively) of the selected substrates were measured. Corresponding data are shown in Table 5.

Table 5. Diffusion coefficients (D, 10^{10} m^2 s^{-1}) of the selected compounds (5 mM) in the absence (D_f) and in the presence (D_{mix}) of equimolar amounts of **1** and CISs ($\delta_{mix} - \delta_f$, ppm; 600 MHz, $CDCl_3$, 25 °C) measured for the NH protons of the same compounds.

Compound	D_f	D_{mix}	$\delta_{mix} - \delta_f$
4	7.3	6.6 (*S*), 7.1 (*R*)	0.300 (*S*),[a] 0.042 (*R*)[a]
5	11.4	5.8 (*S*), 5.8 (*R*)	1.102 (*S*), 0.744 (*R*)
6	8.6	4.1 (*S*), 4.1 (*R*)	1.942 (*S*), 1.710 (*R*)
7	13.2	7.3 (*S*), 7.3 (*R*)	0.911 (*S*), 0.642 (*R*)
8	12.8	5.2 (*S*), 5.2 (*R*)	1.161 (*S*), 0.926 (*R*)
9[b]	12.8	11.6 (*S*), 11.5 (*R*)	0.189 (*S*), 0.128 (*R*)
12	11.0	5.3 (*S*), 5.3 (*R*)	0.865 (*S*), 0.822 (*R*)
13	12.0	4.9 (*S*), 4.9 (*R*)	1.155 (*S*), 1.123 (*R*)

[a] NH bonded to the chiral centre. [b] At 20 mM.

For pure compound **4** at 5 mM we measured a diffusion coefficient of 7.3×10^{-10} m^2 s^{-1}, which slightly decreased in the presence of 1 equiv. of cyclodextrin **1** to 6.6×10^{-10} m^2 s^{-1} for (*S*)-**4** and 7.1×10^{-10} m^2 s^{-1} for (*R*)-**4**. The diffusion coefficient of the cyclodextrin (3.7×10^{-10} m^2 s^{-1}) was unaffected by the presence of **4**, which demonstrated that diffusion of the diastereoisomeric complexes is mainly controlled by large molecular species (i.e., cyclodextrin). From Equation (3), bonded fractions of the enantiomers [0.2 and 0.06 for (*S*)- and (*R*)-**4**, respectively] were found to be quite low but significantly different. Accordingly, significantly different CISs of 0.300 ppm for (*S*)-**4** and 0.042 ppm for (*R*)-**4** were measured. Therefore, the stereochemical differentiation caused by the complexation of the enantiomers with the CSA **1** is also accompanied by a significant differentiation of the stability constants of the two diastereoisomeric complexes.

The behaviour of amino acid derivatives with the free carboxyl function is very different from that of the diamide derivative **4**. The diffusion coefficients of the enantiomers in the mixtures are almost equal. The decrease in the abso-

lute values of the diffusion coefficients after addition of the CSA, however, is remarkable (Table 5). Accordingly, CISs for the enantiomers are always high (Table 5). As an example, the diffusion coefficient of pure *N*-acetylalanine (**7**; $13.2\times10^{-10}\,m^2s^{-1}$) decreases to $7.3\times10^{-10}\,m^2s^{-1}$ in the presence of **1** [(*S*)- or (*R*)-**7**/**1** 5 mM equimolar mixture], the diffusion coefficient of **1** remaining unchanged. These decreases in the diffusion coefficients were correlated to the very high bonded molar fractions [X_b; Equation (3)] of about 0.60 for the enantiomers. On the other hand, the CISs of the enantiomers are both very high and significantly differentiated (Table 5), resulting in the very high chemical shift nonequivalence (about 0.27 ppm) of the enantiomers of **7**. Therefore, the main contribution to the chemical shift nonequivalence is represented by the differences in the stereochemistries of the two diastereoisomeric complexes rather than by the differences in their thermodynamic stabilities.

In the case of the *N*-(trifluoroacetyl)alanine derivative **9**, with an ester function, it should be remarked that the diffusion coefficients of the enantiomers in the presence of 1 equiv. of CSA **1** ($11.5–11.6\times10^{-10}\,m^2s^{-1}$), even at four times the equimolar concentration (20 mM), are very close to that of pure **9** ($12.8\times10^{-10}\,m^2s^{-1}$), so only weak diastereoisomeric complexes, with very similar association constants, are formed. The origin of the observed chemical shift nonequivalence of the enantiomers of **9** therefore resides in the different stereochemistry of the diastereoisomeric associates (*R*)-**9**/CSA-**1** and (*S*)-**9**/CSA-**1**.

Conclusions

Chiral diamide moieties and alkyl groups constitute excellent combinations for the exhaustive derivatization of β-cyclodextrin to provide a new versatile and efficient CSA for NMR spectroscopy. The novel CSA heptakis[2,3-di-*O*-methyl-6-*O*-(L-valine-*tert*-butylamide-N^{α}-ylcarbonylmethyl)]-β-cyclodextrin (**1**) is able to differentiate enantiomeric mixtures of substrates either possessing hydrogen-bond donor groups (i.e., amino acid derivatives) or devoid of them (trisubstituted allenes), surpassing the previously reported[37] mixed methylated-carbamoylated cyclodextrin heptakis[6-*O*-(3,5-dimethylphenylcarbamoyl)-2,3-di-*O*-methyl]-β-cyclodextrin (**3**) in the enantiodiscrimination of amino acid derivatives and, at least at low temperatures, also in the enantiodiscrimination of trisubstituted allenes. In the enantiodiscrimination of amino acid derivatives CSA **1** also surpassed *N*-bromoacetyl-L-valine-*tert*-butylamide (**2**), which had been employed to synthesize **1**.

Importantly, a wide range of amino acid derivatives were enantiodiscriminated by CSA **1**, among which compounds with two hydrogen-bond donor groups are quite generally not only strongly enantiodiscriminated, but also strongly bonded, so low-concentration measurements with a remarkable saving in CSA can be performed.

Finally, the great potential of DOSY techniques for measurement of diffusion coefficients both in complexation and enantiorecognition investigations were confirmed: the stabilities of complexes or solvates formed by cyclodextrins can be very quickly compared, which constitutes an important contribution to the understanding of enantiodiscrimination mechanisms.

Experimental Section

General Methods: NMR measurements were performed with spectrometers operating at 600 and 150 MHz for ^1H and ^{13}C, respectively. The temperature was controlled to ±0.1 °C. ^1H and ^{13}C NMR chemical shifts are referenced to TMS as external standard. The 2D NMR spectra were obtained by standard sequences. Proton gCOSY 2D spectra were recorded in the absolute mode with acquisition of four scans with a 3 s relaxation delay between acquisitions for each of 256 FIDs. The ROESY (Rotating-frame Overhauser Enhancement SpectroscopY) spectra were recorded in the phase-sensitive mode, by employing a mixing time of 0.3 s. The pulse delay was maintained at 5 s; 256 increments of four scans and 2 K data points each were collected. The data matrix was zero-filled to 2 K × 1 K and sinebell and sinebell-shifted functions were applied for processing in both dimensions. The gradient ^1H,^{13}C-gHSQC spectrum was obtained in 32 transients per increment into a 2048 × 128 point data matrix. The gradient HMBC (Heteronuclear Multiple Bond Correlation) experiment was optimized for a long-range ^1H,^{13}C coupling constant of 8 Hz. The spectra were acquired with 256 time increments, 32 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. DOSY experiments were carried out by use of a stimulated echo sequence with self-compensating gradient schemes, a spectral width of 8000 Hz and 64 K data points. Typically, a value ranging from 50 to 190 ms was used for Δ, 1.0 ms for δ, and g was varied in 30 steps (16 transients each) to obtain an approximately 90–95% decrease in the resonance intensity at the largest gradient amplitudes. The baselines of all arrayed spectra were corrected prior to processing the data. After data acquisition, each FID was apodized with 1.0 Hz line broadening and Fourier-transformed. The data were processed with the DOSY macro (involving the determination of the resonance heights of all the signals above a pre-established threshold and the fitting of the decay curve for each resonance to a Gaussian function) to obtain pseudo two-dimensional spectra with NMR chemical shifts along one axis and calculated diffusion coefficients along the other.

Materials: Heptakis(2,3-di-*O*-methyl)-β-cyclodextrin was prepared according to a literature procedure[55] and its ^1H and ^{13}C NMR are in accordance with those previously reported.[59] *N*-Bromoacetyl-L-valine-*tert*-butylamide (**2**) was synthesised as already described elsewhere.[48] (*RS*)-*N*-{2-methyl-1-[(octylamino)carbonyl]propyl}-3,5-dinitrobenzamide (**4**) was prepared according to the procedure reported by Pirkle).[60] 3,5-Dinitrobenzoyl[61] and *N*-trifluoroacetyl[62] derivatives of amino acids and *N*-(trifluoroacetyl)alanine ethyl ester (**9**)[48] were synthesised as described elsewhere.

Heptakis[2,3-di-*O*-methyl-6-*O*-(L-valine-*tert*-butylamide-N^{α}-ylcarbonylmethyl)]-β-cyclodextrin (1): A solution of heptakis(2,3-di-*O*-methyl)-β-cyclodextrin (1.08 g, 0.82 mmol) in dry DMF (5 mL) was added under N_2 at 0 °C to NaH (60%, 3.55 g, 89 mmol) and the mixture was stirred until the evolution of H_2 had ceased. This mixture was added carefully at 0 °C to a solution of *N*-bromoacetyl-L-valine-*tert*-butylamide (**2**, 1.83 g, 6.25 mmol) in dry DMF (5 mL). The reaction mixture was stirred at room temperature overnight.

An excess of MeOH was added to the solution at 0 °C until the evolution of H_2 subsided. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 and washed several times with brine and H_2O and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (EtOAc→EtOAc/EtOH 20:1) to give **1** (0.44 g, yield 20%). ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 0.90 (d, J = 7.4 Hz, 21 H, e-H), 0.92 (d, J = 7.4 Hz, 21 H, e′-H), 1.32 (s, 63 H, g-H), 2.04 (m, 7 H, d-H), 3.18 (dd, $J_{2,3}$ = 9.8, $J_{2,1}$ = 3.6 Hz, 7 H, 2-H), 3.49 (dd, $J_{3,2}$ = 9.8, $J_{3,4}$ = 8.9 Hz, 7 H, 3-H), 3.50 (s, 21 H, OMe-2), 3.58 (dd, $J_{4,5}$ = 9.5, $J_{4,3}$ = 8.9 Hz, 7 H, 4-H), 3.63 (s, 21 H, OMe-3), 3.73 (br d, $J_{6,6'}$ = 11.5 Hz, 7 H, 6-H), 3.81 (br dd, $J_{5,4}$ = 9.5, $J_{5,6'}$ = 4.0 Hz, 7 H, 5-H), 3.92 (dd, $J_{6',6}$ = 11.5, $J_{6',5}$ = 4.0 Hz, 7 H, 6′-H), 4.01 (d, $J_{a,a'}$ = 15.5 Hz, 7 H, a-H), 4.08 (d, $J_{a',a}$ = 15.5 Hz, 7 H, a′-H), 4.08 (dd, $J_{c,b}$ = 8.8, $J_{c,d}$ = 8.2 Hz, 7 H, c-H), 5.15 (d, $J_{1,2}$ = 3.6 Hz, 7 H, 1-H), 6.35 (s, 7 H, f-H), 7.56 (d, $J_{b,c}$ = 8.8 Hz, 7 H, b-H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): δ = 18.9 (C-e), 19.3 (C-e′), 28.9 (C-g), 31.2 (C-d), 51.5 (CMe_3), 58.7 (OMe-2), 59.3 (C-c), 61.6 (OMe-3), 70.6 (CH_2-aa′), 70.9 (CH_2-66′), 71.2 (C-5), 80.9 (C-4), 81.8 (C-3), 82.3 (C-2), 99.6 (C-1), 170.2 (CONH-b), 170.3 (CONH-f) ppm.

Supporting Information (see footnote on the first page of this article): Nonequivalences (600 MHz, $CDCl_3$) of protons of **4** in the presence of CSA **1** (Table S1). Nonequivalences (600 MHz, $CDCl_3$) of NH proton of **4** in the presence of CSA **2** at different molar ratio CSA/substrate (Table S2). Comparison of enantiodiscrimating ability of CSA **1** and **2** towards **4** (Figure S1).

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