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NMR Enantiodiscrimination of Polar and Apolar Substrates by Multifunctional Cyclodextrins

Gloria Uccello-Barretta,[a] Federica Balzano,[a] Giuseppe Sicoli,[a] Alessandro Scarselli,[a] and Piero Salvadori*[a]

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Mixed methylated/carbamoylated cyclodextrins constitute a new class of chiral complexing agents for NMR spectroscopy, which are able to induce anisochrony of enantiomeric mixtures of apolar trisubstituted allenes and polar derivatized compounds most of which are endowed with a π-acidic 3,5-dinitrophenyl ring. The differing contribution to enantiorecognition phenomena of the nature and location of the functional groups on the two cyclodextrin rims was shown. Some interesting aspects of the complexation phenomena, which are the basis of chiral recognition, have been underlined by NMR spectroscopic investigations.

Introduction

Cyclodextrins are cyclic oligosaccharides having a truncated cone shape determined by the strong network of hydrogen bonds between the secondary hydroxy groups of adjacent units. Their main distinctive feature is that the outer surface, which bears the hydroxy groups, is hydrophilic, whereas the inside is made apolar by the glycosidic oxygen atoms and the methine groups pointing inwards. This property is the basis of their ability to form inclusion complexes in aqueous solvents, as a consequence of the favourable process of displacement of the included solvent molecules by apolar moieties.[1,2] Due to the presence, in each glucopyranose unit, of three hydroxyls with strongly differentiated reactivities, particular attention was given to their derivatization,[1–6] in order to modulate their efficiency and versatility in molecular or chiral recognition processes by means of a suitable selection of the nature and location of the functional groups introduced onto the small and large rims of the cyclodextrins. Derivatized cyclodextrins have found efficient and widespread applications in several areas of chemical research.[7–9] Among these applications, their use as chiral solvating agents (CSAs) that are able to make enantiomeric mixtures distinguishable by NMR spectroscopy in solution has made an important contribution to the development of direct, rapid and reliable methods for the determination in solution of the enantiomeric compositions of chiral compounds,[10,11] complementing chromatographic techniques such as GC, HPLC and EC.[12–17] As a matter of fact, methylated cyclodextrins demonstrated considerable efficiency in the NMR enantiodiscrimination of substrates devoid of hydrogen-bond donor functional groups, such as trisubstituted allenes[18–20] and aromatic hydrocarbons.[20,21] Benzylated and benzoylated cyclodextrins, highly soluble in chloroform, were reported for the NMR enantiodiscrimination of derivatized chiral substrates, most of which bore a π-acidic aromatic ring.[22,23] In the NMR analyses of chiral polar substrates the potential use as CSAs of carbamate cyclodextrins, which have attracted so much attention in the chromatographic area,[24–27] was also suggested.[28–30] These results prompted us to investigate the use of the two mixed methylated/carbamoylated β-cyclodextrins, heptakis[2,3-di-*O*-methyl-6-*O*-(3,5-dimethylphenylcarbamoyl)]-β-cyclodextrin (**3**) and heptakis[6-*O*-methyl-2,3-di-*O*-(3,5-dimethylphenylcarbamoyl)]-β-cyclodextrin (**4**) (Scheme 1), as CSAs for NMR spectroscopy, with the potential of combining the enantiodiscrimination properties of the corresponding exhaustively permethylated (**1**) or percarbamoylated (**2**) cyclodextrins for the analysis of both apolar and polar substrates. Cyclodextrins **3** and **4** were compared with **1** and with the partially methylated cyclodextrins heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (**5**), heptakis(2,3-di-*O*-methyl)-β-cyclodextrin (**6**) and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (**7**) (Scheme 1) in the NMR differentiation of enantiomeric mixtures of apolar trisubstituted allenes, whereas the enantiodiscriminating capabilities of **3** and **4** towards polar chiral compounds were compared with exhaustively carbamoylated cyclodextrin **2** and mixed acetylated/carbamoylated cyclodextrinsheptakis[2,3-di-*O*-acetyl-6-*O*-(3,5-dimethylphenylcarbam-

[a] Dipartimento di Chimica e Chimica Industriale, Università di Pisa,
via Risorgimento 35, 56126 Pisa, Italy
Fax: +39-0502219409
E-mail: psalva@dcci.unipi.it

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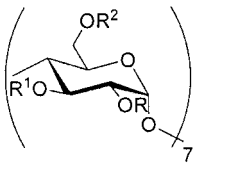
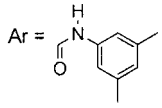
oyl)]-β-cyclodextrin (**8**) and heptakis[6-*O*-acetyl-2,3-di-*O*-(3,5-dimethylphenylcarbamoyl)]-β-cyclodextrin (**9**) (Scheme 1).



	R	R^1	R^2
1	Me	Me	Me
2	Ar	Ar	Ar
3	Me	Me	Ar
4	Ar	Ar	Me
5	Me	Me	Si*t*BuMe$_2$
6	Me	Me	OH
7	Me	OH	Me
8	Ac	Ac	Ar
9	Ar	Ar	Ac



Scheme 1. Structures of cyclodextrins **1**–**9**.

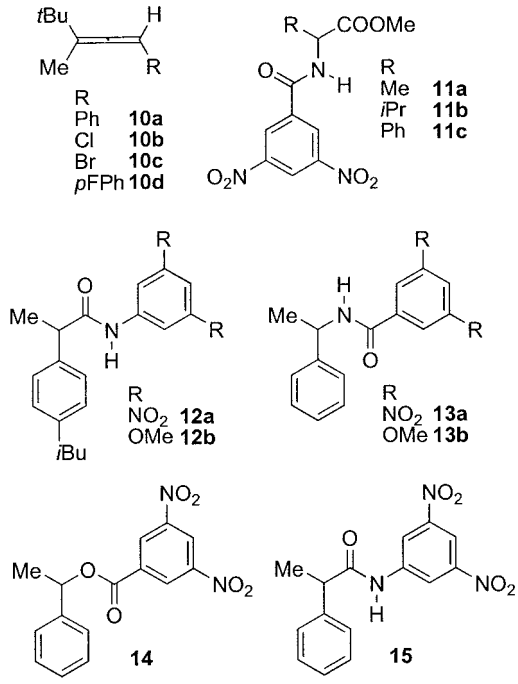
These studies were complemented by a careful NMR analysis of the complexation phenomena in solution, in order to investigate the molecular roots of chiral recognition.

Results and Discussion

The enantiodiscriminating efficiencies of partially methylated cyclodextrins **3**–**7** (Scheme 1) were evaluated in solutions containing equimolar amounts of the racemic allene **10a** (Scheme 2) and compared to that of **1**. The nature of the solvent played a critical role in these experiments, since no anisochrony of proton nuclei of **10a** was detected in chlorinated solvent, the best choices being CD_3OD and [D_6]-DMSO, in which, as already found for **1**,[18] nonequivalences (Table 1; see Supporting Information, Figure S1) were higher than they were in other solvents; satisfactory results were obtained in [D_6]acetone, in which, importantly, all the methylated chiral auxiliaries showed good solubility.

The presence of methyl substituents in the cyclodextrin derivatives and their location on at least both the secondary sites 2 and 3 were necessary for NMR enantiodiscrimination (Table 1). Thus, cyclodextrins **2**, **8** and **9**, which were devoid of methyl substituents, or **4**, which had them only on the primary sites, did not produce distinct resonances of the two enantiomers of **10a**. This was also the case for cyclodextrin **7**, with only one kind of methylated secondary site, which was not able to produce significant anisochrony of its enantiotopic nuclei.

Cyclodextrin **1** was the most efficient chiral auxiliary in the enantiodiscrimination of trisubstituted allenes; however, among 2,3-dimethylated cyclodextrins the nature of the



Scheme 2. Substrates **10**–**15**.

Table 1. Nonequivalences ($\Delta\delta$,[a] 300 MHz, 25 °C) of protons of (*R*,*S*)-**10a** (80 mM) in the presence of equimolar amounts of cyclodextrin **1**, **3**, **5** and **6**.

CD	Solvent	$\Delta\delta$		
		CH	*t*Bu	Me
1	[D_6]acetone	2.8	0.8	1.3
	CD_3OD	6.4	3.3	9.4
	[D_6]DMSO	7.9	1.9	3.2
3	[D_6]acetone	1.0	0.8	1.0
	CD_3OD[b]	1.1	0.7	1.0
	[D_6]DMSO	2.9	1.5	2.0
5	[D_6]acetone	1.7	0.6	0.6
	CD_3OD	4.3	1.2	1.7
6	[D_6]acetone	0.6	–	–
	CD_3OD	2.0	0.8	1.0
	[D_6]DMSO	2.8	0.8	0.6

[a] $\Delta\delta = |\delta_S - \delta_R|$, Hz. [b] 40 mM.

groups on the primary sites did not seem to affect the magnitude of nonequivalences significantly, as comparable nonequivalences of racemic **10a** were measured (Table 1) in the mixtures containing **3** (Figure 1), **5** and **6**, having carbamoylated, silylated and underivatized primary groups, respectively. Slightly lower nonequivalences produced by mixed carbamoylated/methylated cyclodextrin **3** in CD_3OD solutions (Table 1) were due to the reduced solubility of **3** in this solvent, which prevented us from performing enantiodiscrimination experiments at concentrations higher than 40 mM.

One of the advantages of the above-mentioned cyclodextrin derivatives is the fact that they allowed us to detect nonequivalences in three different kinds of deuterated solvents and hence made possible different ways of optimizing magnitudes of nonequivalences: in [D_6]DMSO and [D_6]acetone, in which chiral auxiliaries were very soluble, complex-

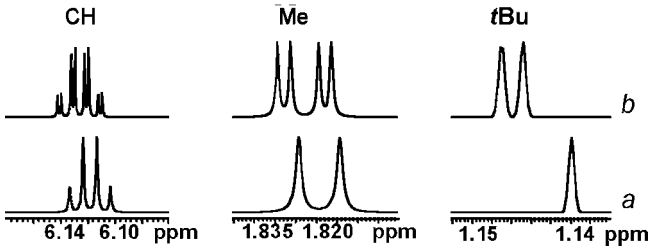


Figure 1. ^{1}H NMR (300 MHz, [D$_6$]acetone, 25 °C) spectral regions of **10a** resonances of: a) pure **10a** (80 mM) and b) an equimolar mixture of **10a** and **3**.

ation equilibria involved in the formation of diastereoisomeric solvates could be shifted towards the complexed forms by adding further equivalents of chiral auxiliaries to the solution containing **10a** (Table 2; see Supporting Information, Figure S2); in [D$_6$]acetone and CD$_3$OD, which had low freezing points, similar effects could be obtained by lowering the temperature to –40 °C (Table 2; see Supporting Information, Figure S3); at –20 °C nonequivalence increments analogous to those reached in solutions having a similar concentration of **10a** in the presence of three equivalents of the chiral auxiliaries were detected (Table 2).

Table 2. Nonequivalences ($\Delta\delta$,[a] 300 MHz, [D$_6$]acetone, 25 °C) of protons of (*R*,*S*)-**10a** (80 mM) in the presence of cyclodextrin **3** at different molar ratios of **10a/3** and at different temperatures.

T [°C]	**10a/3**	$\Delta\delta$		
		CH	*t*Bu	Me
25	1:1[b]	1.0 (2.9)	0.8 (1.5)	1.0 (2.0)
25	1:2	1.9	1.5	1.8
25	1:3[b]	2.8 (4.5)	2.0 (2.1)	2.6 (2.7)
0	1:1	1.9	1.5	1.7
–20	1:1	2.8	2.4	2.7
–40	1:1	3.4	3.2	3.5

[a] $\Delta\delta = |\delta_S - \delta_R|$, Hz. [b] $\Delta\delta$ measured in [D$_6$]DMSO are reported in parentheses.

Analogous results were obtained for the trisubstituted allenes **10b**–**d** (Scheme 2; see Supporting Information, Table S1).

The enantiodiscriminating efficiency and versatility of mixed methylated/carbamoylated cyclodextrins **3** and **4** towards polar chiral substrates was probed in solutions containing *N*-(3,5-dinitrobenzoyl)alanine methyl ester (**11a**) (Scheme 2), an amino acid derivative endowed with a π-acidic derivatizing group, towards which the corresponding exhaustively carbamoylated cyclodextrin **2** demonstrated remarkable enantiodiscriminating efficiency.[30]

Nonequivalences of **11a** measured in the mixtures containing **3** and **4** (Table 3) were compared to the ones produced by exhaustively carbamoylated cyclodextrin **2** and mixed acetylated/carbamoylated cyclodextrins **8** and **9**. CDCl$_3$ was selected for NMR measurements, as the chiral auxiliaries did not show significant solubility in less polar solvents such as C$_6$D$_{12}$ or CCl$_4$, which in principle could have favourably affected the magnitudes of the nonequivalences, whereas in polar protic or aprotic solvents including [D$_6$]acetone, [D$_6$]DMSO and CD$_3$OD no anisochrony was detected.

Table 3. Nonequivalences ($\Delta\delta$,[a] 300 MHz, CDCl$_3$, 25 °C) of protons of (*R*,*S*)-**11a** (80 mM) in the presence of equimolar amounts of cyclodextrins **2**–**4**, **8** and **9**.

	4	**9**	**3**	**8**	**2**
Me	44.1	37.9	5.5	0	22.9
CH	55.1	36.0	5.4	2.0	8.3
NH	40.0	36.7	9.1	24.1	29.8
H_{ortho}	19.7	12.8	7.3	1.3	8.4

[a] $\Delta\delta = |\delta_S - \delta_R|$, Hz.

The data reported in Table 3 show that all the cyclodextrins containing carbamate groups differentiated the enantiomers of **11a**; exhaustively methylated cyclodextrin **1** did not produce any splitting of its resonances.

Enantiodiscriminating efficiency was affected by the location of the carbamate groups, greater splitting of **11a** resonances being caused by cyclodextrins with carbamoylated secondary sites (**4** and **9**) relative to cyclodextrins that had them only on the primary sites (Table 3). However, unlike the above-mentioned enantiodiscrimination phenomena of allenes, the nature of the derivatizing groups on the primary sites of 2,3-dicarbamoylated cyclodextrins seemed to affect the magnitudes of nonequivalences: the more apolar they were, the greater the nonequivalences. As a matter of fact, surprisingly, exhaustively carbamoylated cyclodextrin **2** caused doubling of **11a** resonances, which were half or even less than those produced by **4** and **9**, and between **4** and **9**, cyclodextrin derivative **4**, which contained methyl groups produced better results relative to **9**, which had acetyl moieties on the primary sites.

In order to investigate this problem further, we determined the association constants of the diastereoisomeric complexes formed in solution by either enantiomer of **11a** and each cyclodextrin derivative. Association constants (Table 4) were determined by analysing the NMR spectra of progressively diluted (from 150 mM to 0.5 mM) equimolar solutions containing (*S*)- or (*R*)-**11a** and the selected chiral auxiliary. Cyclodextrin derivatives **4** and **9**, with carbamoyl functions only on the secondary sites, were both more strongly bound to (*S*)-**11a** than they were to (*R*)-**11a**. Exhaustively carbamoylated cyclodextrin **2** behaved in a similar way, but it was able to complex both enantiomers more strongly than did **4** and **9**. The opposite trend was found for cyclodextrins **3** and **8** with carbamoylated primary sites, the association constants of which were higher for (*R*)-**11a** than they were for (*S*)-**11a**.

Thus, the carbamate groups on the primary sites stabilized preferentially (*R*)-**11a** and those on the secondary sites were more strongly bound to (*S*)-**11a**. The pattern of complexation shifts of the two enantiomers in the presence of all chiral auxiliaries, shown in Figure 2 for the methyl resonance of **11a**, revealed that cyclodextrin **4** produced high-frequency shifts of resonances for one enantiomer and op-

Table 4. Association constants (M^{-1}) of diastereoisomeric complexes formed in solution ($CDCl_3$) from **11a** and cyclodextrins **2**–**4**, **8** and **9**.

CSA	(*S*)-**11a**/CSA	(*R*)-**11a**/CSA
2[30]	35.7±1.5	14.1±0.5
4	18.1±1.5	9.9±0.8
9	10.1±0.4	6.6±0.5
3	5.9±0.3	21.4±1.5
8	8.1±0.3	11.9±0.5

posite effects for the other one, whereas cyclodextrins **3** and **8** shifted both enantiomers to higher frequencies. Hence the two kinds of carbamoyl groups, primary vs. secondary sites, produced opposite shifts of the resonances of only one enantiomer, which, in the percarbamate system **2**, brought about the reduction in the nonequivalences relative to cyclodextrins **4** and **9**.

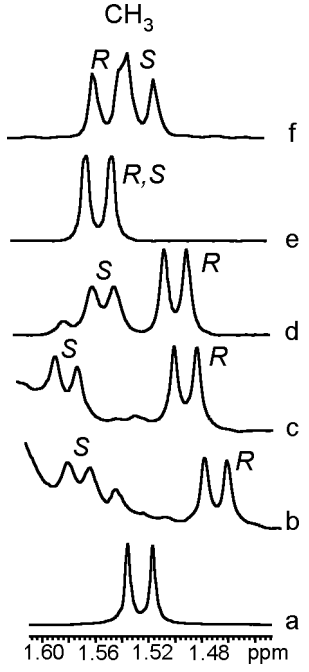


Figure 2. ^1H NMR (300 MHz, $CDCl_3$, 25 °C) spectral regions of the methyl resonances of: pure **11a** (80 mM) (a) and of equimolar mixtures of **11a/4** (b), **11a/9** (c), **11a/2** (d), **11a/8** (e), **11a/3** (f).

Finally, we would like to point out that the comparison of the association constants of **4** and **9** (Table 4), both carbamoylated on the secondary sites, but methylated and acetylated, respectively, on the primary ones, demonstrated that the differences between their enantiodiscriminating efficiencies were directly determined by the differences in their association constants with each enantiomer.

For the most efficient chiral auxiliary **4**, we also investigated the accuracy of the determinations of the enantiomeric compositions of samples of **11a**, which had known enantiomeric compositions ranging from 4% to 90% (see Supporting Information, Table S2). The results obtained by using **4** as a chiral solvating agent (on the basis of the integration of ester or 3,5-dinitrophenyl resonances of **11a**) were in optimal agreement with the values determined on the basis of optical rotation measurements.

Carbamate cyclodextrins showed analogous enantiodiscriminating capability towards polar substrates **11**–**15** (see Supporting Information, Tables S3 and S4).

In order to gain more insight into the origin of enantiodiscrimination by cyclodextrin derivatives having mixed carbamate and methylated or acetylated sites, we analysed the 2D ROESY (Rotating-frame Overhauser Enhancement SpectroscopY) maps of equimolar mixtures containing either enantiomer of **11a** and each cyclodextrin derivative, in order to detect the intermolecular dipolar interactions due to the spatial proximity of the proton nuclei of the complexed species. On the basis of association constant values (Table 4), we selected a value of the total concentration (120 mM) which guaranteed an observable percentage of the complexed species (about 30%).

The conformational features of the chiral auxiliaries have already been carefully and extensively analysed and discussed.[31]

In particular, we demonstrated[30,31] that the whole truncated cone shape, which is typical of underivatized cyclodextrins, was maintained in derivatized cyclodextrins endowed with hydrogen-bond donor-acceptor groups (such as carbamate functionalities or underivatized OH groups) on the secondary sites (cyclodextrins **2**, **4**, **9**), irrespective of the nature of the groups lying on the primary ones; this situation was probably favoured by the strong network of hydrogen bonds between adjacent units. In the above cases the glucose units were almost undistorted and the C–H_1 and C–H_4 bonds (see Supporting Information, Figure S4) of adjacent units were mainly coplanar. The 3,5-dimethylphenyl groups of carbamate functions produced an extension of the truncated cone shape, because of their greater distance from the outer surface of the cyclodextrin and from groups lying on the primary sites. A completely different situation was found[31] for cyclodextrins devoid of such functional groups on the secondary sites: cyclodextrins **3** and **8** were characterized by deviations of their glucopyranose units from the expected $^4C_1$ chair conformation, leading to skewed forms, as well as by tilting of the units due to rotations about the glycosidic linkages. In this way, carbamate groups on the primary sites were close to the groups on the secondary sites as a consequence of both unit distortions and tilting.

The conformation of the cyclodextrins did not undergo any change due to the presence of (*R*)- or (*S*)-**11a**.

Both enantiomers of amino acid derivative **11a** gave rise to prevailing intermolecular dipolar interactions with the carbamate moieties located on the primary or secondary sites and with glucopyranose protons directly bound to them, whereas, in some cases which will be discussed, only negligible NOEs were detected with methyl or acetyl groups. In particular, in the mixture containing cyclodextrin **4** and the (*S*)-enantiomer of **11a**, significant dipolar interactions were detected between the *ortho* protons of the 3,5-dinitrophenyl group of (*S*)-**11a**, and selectively, the *ortho*

protons of the 3,5-dimethylphenyl rings of the cyclodextrin located on the sites 2 (Figure 3b).

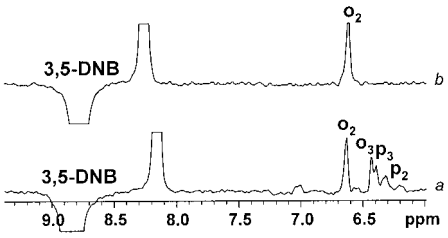


Figure 3. 2D ROESY (300 MHz, $CDCl_3$, 25 °C, τ_m = 0.3 s) traces of the 3,5-dinitrophenyl protons of: a) (*R*)- and b) (*S*)-**11a** in the presence of **4**.

The methine proton of (*S*)-**11a** produced NOEs on the external proton 2-H only, whereas dipolar interactions were detected between its ester methyl group and *ortho* protons of both kinds of 3,5-dimethylphenyl rings located on the sites 2 and 3, together with comparable effects on the internal proton 3-H and external protons 2-H and 1-H (Figure 4). The effect of the methyl group bound to the chiral centre of the amino acid derivative on cyclodextrin protons 2-H and 3-H was weak. Interestingly, the NH proton of (*S*)-**11a** or of cyclodextrin carbamate functions did not produce reciprocal dipolar interactions or exchange effects.

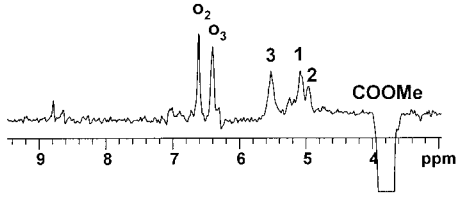


Figure 4. 2D ROESY (300 MHz, $CDCl_3$, 25 °C, τ_m = 0.3 s) trace of the COOMe protons of (*S*)-**11a** in the presence of **4**.

Consequently, the 3,5-dinitrophenyl moiety of (*S*)-**11a** faced the aromatic group on site 2 of the cyclodextrin, and its methyl, methine and ester groups were directed towards the large rim of the cyclodextrin, with the methyl and methine groups mainly external to the cavity and the ester function located between two adjacent carbamate groups near the internal proton 3-H and hence partially included in the cavity (Figure 5A). Given the proximity of the ester methyl and the proton 1-H, the two above-mentioned adjacent carbamate groups should belong to adjacent glucopyranose rings. As a result, a stabilizing π–π attractive interaction between the aromatic moiety of (*S*)-**11a** and that of the cyclodextrin located on site 2 probably occurred, which could have been facilitated by attractive interactions between their complementary NH-CO moieties. The partial inclusion of the ester function could be favoured by a hydrogen-bonding interaction with the carbamate lying on site 3 of the adjacent glucopyranose ring.

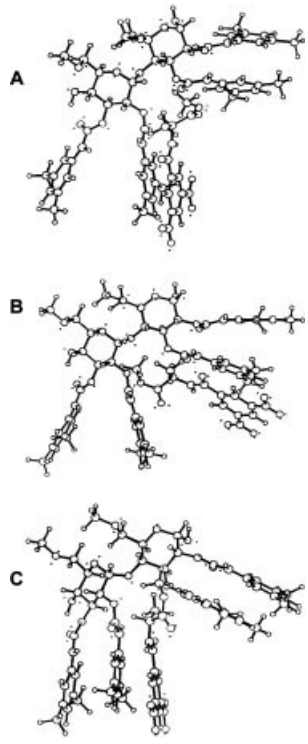


Figure 5. Stereochemical representation of the diastereoisomeric adducts formed by **4** and (*S*)-**11**a (A) or (*R*)-**11a** (B and C).

Interestingly, dipolar interactions detected in the mixtures containing the (*R*)-enantiomer were quite different, since the *ortho* protons of its 3,5-dinitrophenyl ring produced NOEs on both kinds of cyclodextrin carbamate groups on sites 2 and 3 (Figure 3a), and not only near their *ortho* protons, but also near the *para* ones. The methine and methoxy protons of (*R*)-**11a** produced NOE patterns analogous to those detected for (*S*)-**11a**. The methyl group bound to the chiral centre of (*R*)-**11a** produced quite strong effects on the two kinds of carbamate groups in addition to significant effects on the protons 3-H, 2-H and 1-H of the cyclodextrin (Figure 6). Therefore, the interaction with (*R*)-**11a** involved its 3,5-dinitrophenyl moiety as well as both cyclodextrin carbamate moieties on sites 2 and 3 of adjacent glucopyranose rings. Moreover, both the ester and the methyl group of (*R*)-**11a** were directed towards the internal part of the large rim (Figure 5B,C), where the latter could give rise to steric repulsive interactions responsible for the lower stability of the complex formed with the (*R*)-enantiomer relative to the complex formed with (*S*)-**11a** (Table 4).

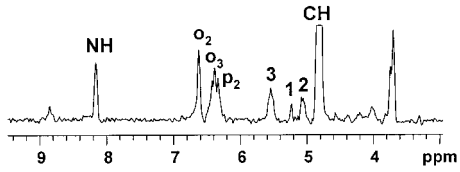


Figure 6. 2D ROESY (300 MHz, $CDCl_3$, 25 °C, τ_m = 0.3 s) trace of the methyl protons of (*R*)-**11a** in the presence of **4**.

The optimal fit between cyclodextrin carbamate groups and (*S*)-enantiomer, leading to a significant degree of im-

mobilization of (*S*)-**11a**, was presumably responsible for the significant broadening of the resonances of the (*S*)-enantiomer, which was greater than the broadening for the (*R*)-enantiomer (Figure 2b).

In the mixtures formed by cyclodextrin **3**, carbamoylated on the primary sites and methylated on the secondary sites, the two enantiomers gave rise to similar dipolar interactions with the carbamate functions lying on the primary site and with the methylene protons of the primary group of the cyclodextrin. No interaction was detected with the internal proton 5-H (see Supporting Information, Figure S5), bound to the same glucopyranose carbon atom as the carbamate functions. The methylene protons on the primary sites gave rise to the expected reciprocal dipolar interactions with the π-acidic aromatic ring of **11a** (see Supporting Information, Figure S6). These effects suggested once again the occurrence of attractive interactions near the carbamate groups, but on the outer surface of the cyclodextrin far from its cavity, as shown in Figure 7.

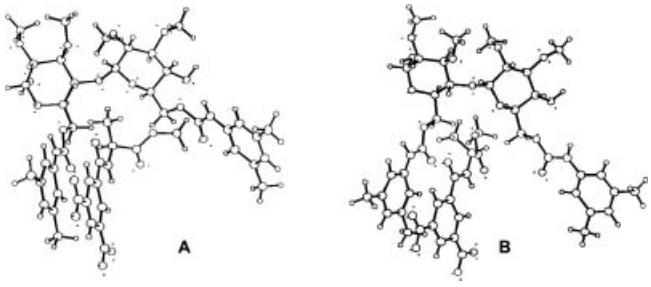


Figure 7. Stereochemical representation of the diastereoisomeric adducts formed by **3** and (*S*)-**11a** (A) or (*R*)-**11a** (B).

Finally, the NOE effects produced by the 3,5-dinitrophenyl protons of **11a** on the cyclodextrin methoxy groups on the secondary sites (see Supporting Information, Figure S5) were probably due to the presence of skew glucopyranose rings[31] and to the rotation of the glucopyranose rings about the glycosidic linkages, both bringing primary groups of one unit close to the secondary groups of the adjacent one. Furthermore, enantiodiscrimination could arise simply from the variation in the relative positions of the groups bound to the chiral centre of the two enantiomers of **11a**, because of the configurational change causing these groups to be in different environments with respect to each pair of adjacent glucopyranose units.

The interaction mechanism involving percarbamate cyclodextrin has already been discussed.[30] In this case it was demonstrated that secondary sites stabilized both enantiomers, whereas primary ones interacted preferentially with the (*R*)-enantiomer, which was in optimal agreement with the values of the association constants summarized in Table 4.

Mixed acetylated/carbamate cyclodextrins **8** and **9** gave analogous results, as both enantiomers of **11a** showed a strong selectivity towards the carbamate functions, but in the case of **9**, which had carbamoylated secondary sites, only (*S*)-**11a** produced NOEs on the internal proton 3-H (see Supporting Information, Figure S7), demonstrating its ability to give rise to partial inclusion in addition to π–π attractive interactions between its 3,5-dinitrophenyl ring and aromatic moieties of cyclodextrin carbamate functions, whereas (*R*)-**11a** seemed to remain preferentially external to the cavity. Probably in this case acetyl functions on the primary sites, extending towards the external surface of the host, and the ester function of the amino acid derivative produced reciprocal repulsive interactions, which could be responsible for the lower degree of stabilization of both enantiomers relative to **4**. For the analogous cyclodextrin **8**, which had acetylated secondary sites and carbamoylated primary groups, once again only superficial interactions with the carbamate groups were detected (see Supporting Information, Figure S8).

Finally, the analogous investigation carried out on solutions containing the trisubstituted allene **10a** and cyclodextrin **3** did not lead to well-defined interaction mechanisms, as dipolar interactions of all allene protons with the methyl groups on the secondary sites were revealed, which indicated a multimodal interaction mechanism involving mainly the secondary sites, but it did not exclude primary ones, as some effects were detected on the carbamate groups. We would point out that, as already discussed, the effects on the carbamate moieties could be due to the significant degree of conformational distortion that the cyclodextrin undergoes, bringing groups on the secondary sites close to groups lying on the primary ones.

Conclusions

Cyclodextrins constitute an important class of chiral molecules for projecting multimodal versatile chiral solvating agents for NMR spectroscopy; their hydroxy functions on the primary and secondary sites can be modified in different ways in order to introduce functional groups that are able to address the interactions of different classes of chiral substrates selectively towards the secondary and primary rims. Carbamate and alkyl groups represent very promising derivatizing groups: when they are both introduced into the cyclodextrin, they act independently of each other and enable derivatized cyclodextrins to discriminate enantiomers of polar and apolar substrates, combining the enantiodiscriminating properties of exhaustively carbamoylated and alkylated cyclodextrins.

The results discussed above clearly suggested that the rational design of more efficient chiral auxiliaries for use as CSAs for NMR, as well as in several other analytical areas, above all in chromatography, requires greater understanding of the molecular basis of chiral recognition. To this end, NMR spectroscopy represents one of the most efficient and versatile tools of investigation,[32] allowing us to gather several kinds of information on dynamics, thermodynamics and stereochemistry. It should, however, be stressed that, above all in the cases of cyclodextrins, for which understanding of enantiorecognition phenomena often constitutes a considerable challenge, the contributions from complementary analytical methods, such as molecular model-

ling, X-ray crystallography, mass spectrometry, IR spectroscopy or microcalorimetric measurements, should not be underestimated.[33,34]

Experimental Section

General Methods: NMR measurements were performed on a spectrometer operating at 299.94 MHz for ^{1}H and the temperature was controlled to ±0.1 °C. All ^{1}H NMR chemical shifts are referenced to TMS as external standard. The pulse-repetition period was 10 s, 128 scans were collected into 32 K data points. The spectral width was 3326 Hz with the transmitter offset at δ = 1.52 ppm. Adequate baseline correction and phasing was performed after Fourier transformation. The 2D NMR spectra were obtained by using standard sequences. The ROESY spectra were recorded in the phase-sensitive mode, by employing a mixing time of 0.3 s. The spectral width used was 3326 Hz in both dimensions. The pulse delay was maintained at 5 or 10 s; 512 increments of 8 scans and 2 K data points each were collected. The data matrix was zero-filled to 2 K × 1 K, and a Gaussian function was applied for processing in both dimensions. In the association constant determinations,[19] the nonlinear fitting of the chemical shift dilution data was performed by using KaleidaGraph 3.09. The stereochemical representations were obtained with the PCModel 6.0 program (MMx force field).

Materials: Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (**1**) and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (**7**) were purchased from Sigma. Heptakis[2,3,6-tri-*O*-(3,5-dimethylphenylcarbamoyl)]-β-cyclodextrin (**2**) was prepared according to ref.[30] Cyclodextrins **3**–**6**, **8** and **9** were prepared following the procedures reported in ref.[31]

Supporting Information (see footnote on the first page of this article): NMR enantiodiscrimination spectra of allene **10a**; nonequivalence data of allenes **10b**–**d**; accuracy of the NMR determination of the enantiomeric purities; nonequivalence data of compounds **11**–**15**; schematic representation of two adjacent undistorted glucose units of cyclodextrin; ROESY traces of (*R*)- or (*S*)-**11a** in the presence of **3**, **8** and **9**.

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