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Combining Magnetic Resonance Spectroscopies, Mass Spectrometry, and Molecular Dynamics: Investigation of Chiral Recognition by 2,6-di-*O*-Methyl-β-cyclodextrin

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Abstract: EPR spectroscopy has been employed to investigate the formation of complexes between heptakis-(2,6-*O*-dimethyl)-β-cyclodextrin (DM-β-CD) and different enantiomeric pairs of chiral nitroxides of general structure $PhCH_2N(O\cdot)CH(R)R'$. Accurate equilibrium measurements of the concentrations of free and included radicals afforded the binding constant values for these nitroxides. The relationship between the stereochemistry of the DM-β-CD complexes and the thermodynamics of complexation was elucidated by correlating EPR data with $^1H-^1H$ NOE measurements carried out on the complexes between DM-β-CD and the structurally related amine precursors of nitroxides. NOE data suggested that inclusion of the stereogenic center in the DM-β-CD cavity occurs only when the R substituent linked to the chiral carbon contains an aromatic ring. For these types of complexes, molecular dynamics simulation indicated that the depth of penetration of the stereogenic center into the cyclodextrin cavity is determined by the nature of the second substituent (R′) at the asymmetric carbon and is responsible for the observed chiral selectivity. Analysis of mass spectra showed that, for the presently investigated amines, electrostatic external adducts of CDs with protonated amines are detected by ESI−MS.

Introduction

Cyclodextrins[1] (CDs) are cyclic oligomers of 1,4-linked, α-D-glucose monomers that have become the focus of intense study by technologists interested in application of guest−host complexation[2] as well as by scientists interested in fundamental issues of molecular recognition.[3] Interest in these compounds derives from the fact that they act as host molecules to form inclusion complexes with a wide variety of guests. The cyclodextrins exist as single enantiomers, with the consequence that when they act as host molecules, interaction with a racemic guest will lead to the formation of diastereomeric complexes which may have different thermodynamic stability. The stereoselective complex formation of cyclodextrins has been applied to separate enantiomers in planar chromatography (TLC),[4] high performance liquid chromatography (HPLC),[5] gas−liquid-phase chromatography (GLC)[6] and capillary electrophoresis (CE).[7] Several investigations have also explored the selective precipitation of optically active compounds with cyclodextrins, and it has been found that precipitation with cyclodextrins may effectively lead to separation of some racemic compounds.[8]

Although chiral discrimination by cyclodextrins have been investigated in great detail, only relatively limited efforts have been devoted to understand the relationship between the stereochemistry of the complex and the complexation thermodynamic. By using computational techniques Lipkowitz and coworkers showed that in permethylated β-cyclodextrin enantiodifferentiating regions are at the interior rather than the exterior of the macrocycle and that the chiral discriminating forces are short-range dispersion forces.[9]

More recently, Rekharsky and Inoue investigated a wide variety of chiral compounds by employing isothermal titration calorimetry.[10] These papers represent the most important attempt to determine a direct relationship between thermodynamic parameters and chiral recognition by CDs.[11] Although a generalized pictured was not obtained, an important conclusion was that a direct correlation between the mode of penetration and chiral recognition is a fairly general rule applicable to a variety

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of cyclodextrin complexations. In particular, it has been shown that the spatial interaction between chiral guest and CD depends on the depth of guest penetration and therefore on the position of the most hydrophobic group around the chiral center of the guest.

However, these measurements are severely hampered by small enthalpic differences, which additionally are compensated by counteracting entropic contributions, leading to rather moderate differences in affinity. Moreover, in some cases, the cyclodextrins form complexes with higher stoichiometries that force one to explore a limited concentration range in order to obtain only 1:1 complexes.

Even if the complexation of a chiral guest with CDs gives rise to diastereomeric pairs,[12] EPR spectroscopy has only been rarely used to investigate the complexation of chiral radical species by CDs.[13] Recently, we have reported the EPR observation of the complexes between CDs and dialkyl nitroxide radicals in water.[14,15] Due to the shorter time scale of EPR spectroscopy compared to that of complexation processes, EPR spectra resulting from the superposition of separate signals from free and included species are usually observed when using free radicals as guest molecules. In particular, with the *tert*-butyl benzyl nitroxide radical the formation of an inclusion complex with CDs produces in the EPR spectrum both a decrease of the overall splitting and significant differences in the resonance fields of the $M_I(2H_\beta) = \pm 1$ lines, compared to the free species in water. These large spectral changes arise both from the decrease of the nitrogen hyperfine splitting, a(N), induced by the less polar environment of the CD host cavity, and from the strong reduction of the benzylic protons coupling, $a(2H_\beta)$, due to conformational changes occurring upon complexation.[16] Because of this favorable feature, the determination of the association constants is easily achieved by double integration of the EPR lines.

In contrast to EPR spectra, NMR spectra obtained from most CD complexes represent concentration-weighted averages since exchange between the free and complexed guest molecule is usually fast in the NMR time scale. Therefore, to determine association constants with CDs, non linear fitting of chemical shift changes as a function of concentration (the so-called NMR titration) is usually performed.[17]

On the basis of this, we decided to employ EPR spectroscopy to obtain accurate equilibrium constants for the formation of complexes between heptakis-(2,6-*O*-dimethyl)-β-CD (DM-β-CD) and different enantiomeric pairs of chiral nitroxides (**2a**−**2i**). The selected chiral nitroxides were chosen because they maintain both the favorable EPR features found in the related benzyl *tert*-butyl nitroxide and can be obtained easily as pure optical isomers by oxidation of the corresponding chiral amine precursors **1a**−**1i**. It will be shown that the reported chiral nitroxides are particularly suitable probes for the study of inclusion by cyclodextrins (and probably by other chiral complexing agents) by EPR spectroscopy, because their diastereomeric complexes are characterized by spectroscopic parameters differing significantly more than for any other type of probe previously used for this purpose. In addition, small differences in the affinity of the guest for the CDs cavity are reflected by a significant change of the EPR line intensity and formation of complexes with different stoichiometry give rise to additional EPR lines easily distinguishable from those of 1:1 complexes.[15]

Chart 1

(*R*)-**1** (*S*)-**1** (*R*)-**2** (*S*)-**2**

	R	R'	R''
a	CH_3	CH_2OH	CH_2Ph
a-d^4	CH_3	CD_2OH	CD_2Ph
b	CH_2CH_3	CH_2OH	CH_2Ph
b-d^2	CH_2CH_3	CH_2OH	CD_2Ph
c	$CH_2CH(CH_3)_2$	CH_2OH	CH_2Ph
c-d^4	$CH_2CH(CH_3)_2$	CD_2OH	CD_2Ph
d	Ph	CH_2OH	CH_2Ph
d-d^4	Ph	CD_2OH	CD_2Ph
d-d^9	Ph	CD_2OH	CD_2Ph-d^5
e	CH_2Ph	CH_2OH	CH_2Ph
e-d^4	CH_2Ph	CD_2OH	CD_2Ph
e-d^9	CH_2Ph	CD_2OH	CD_2Ph-d^5
f	3-Indolylmethyl	CH_2OH	CH_2Ph
f-d^4	3-Indolylmethyl	CD_2OH	CD_2Ph
g	Ph	CH_3	CH_2Ph
g-d^2	Ph	CH_3	CD_2Ph
g-d^7	Ph	CH_3	CD_2Ph-d^5
h	CH_2CH_3	CH_2OCH_3	CH_2Ph
h-d^2	CH_2CH_3	CH_2OCH_3	CD_2Ph
i	Ph	CH_2OCH_3	CH_2Ph
i-d^5	Ph	CH_2OCH_3	CH_2Ph-d^5
i-d^7	Ph	CH_2OCH_3	CD_2Ph-d^5

To fully clarify the relationship between the stereochemistry of the CD complexes and the complexation thermodynamics, EPR data were correlated with $^1H-^1H$ NOE measurements[17] carried out on the complexes between CDs and the structurally related amine precursors **1a**−**1i**.[18] Geometries inferred from NMR data were clarified by molecular dynamics simulations. Finally, the data obtained from magnetic resonance experiments were compared to those extracted from ESI−MS spectra.

Results

Preparation of the Optical Active Amines and Nitroxides. Chart 2 summarizes the synthetic methods used to prepare the optical pure amines. Briefly, the commercially available amino acids, amino alcohols and methylbenzylamine were reacted with

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(13) (a) Michon, J.; Rassat, A. *J. Am. Chem. Soc.* **1979**, *101*, 995−996. (b) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1992**, *114*, 2872−2874.

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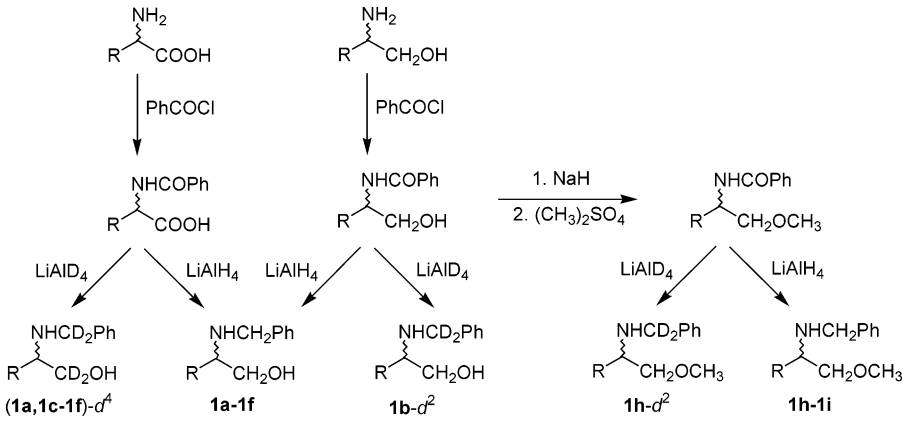
(15) Franchi, P.; Lucarini, M.; Pedulli, G. F. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 1842−1845.

(16) For other examples of application of benzyl *tert*-butyl nitroxide in supramolecular environments, see: (a) Franchi, P.; Lucarini, M.; Pedulli, G. F.; Sciotto, D. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 263−266. (b) Brigati, G.; Franchi, P.; Lucarini, M.; Pedulli, G. F.; Valgimigli, L. *Res. Chem. Intermed.* **2002**, *28*, 131−141.

(17) For reviews, see: (a) Schneider, H. J.; Hacket, F.; Rudiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 1755−1785 and references therein. (b) Schneider, H. J.; Yatsimirsky, A. P*rinciples and Methods in Supramolecular Chemistry*; Wiley and Sons: Chichester, 2000.

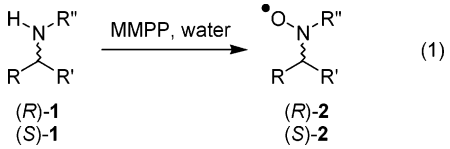
(18) Lucarini, M.; Mezzina, E.; Pedulli, G. F. *Eur. J. Org. Chem.* **2000**, 3927−3930.

Chart 2



benzoyl chloride to afford the corresponding amides which were then reacted with $LiAlH_4$ or $LiAlD_4$ to give the desired amines in yield ranging from 60% to 70%. To obtain the O-methylated derivatives (**1h**−**1i**) the corresponding amide alcohols were reacted in two steps with NaH and $(CH_3)_2SO_4$ before reduction. Products deuterated at the aromatic benzyl ring were obtained in a similar way by using d^5-PhCOCl. In all cases, the configuration at the chiral carbon in the starting amine was completely maintained in the final products.

The optically pure nitroxides were generated directly inside an EPR tube by oxidation of the corresponding chiral amines with the magnesium salt of monoperoxyphthalic acid (MMPP) in water (eq 1).

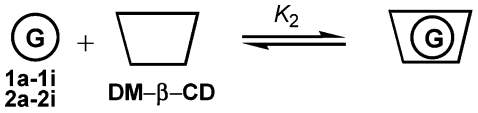


(1)

EPR Spectra. Good EPR spectra of nitroxides **2a**−**2i**, were obtained by oxidation of amines **1a**−**1i** (ca. 1.0 mM) with MMPP (ca. 1.0 mM) in water at 300 K. As an example in Figure 1a is reported the spectrum obtained by oxidizing the racemic mixture of amine **1e** in water at 300 K. All spectra were easily interpreted on the basis of the coupling of the unpaired electron with nitrogen, with the single proton bound to the chiral carbon and with the two diastereomeric benzylic protons (see Table 1). By recording the EPR spectra of the deuterated nitroxides labeled at the benzylic protons it was possible, in all cases, to assign the hydrogen hyperfine splitting constant (hfsc) to the single β-proton.

It should be remarked that for a given couple of enantiomeric amines, the oxidation of the two antipodes in an achiral medium, such as water, gave rise to the same EPR spectrum.[12]

When the EPR spectra of radicals **2a**−**2i** were recorded in the presence of DM-β-CD, additional signals were observed assigned to the radical included in the cavity of cyclodextrin, in equilibrium with the free nitroxide (eq 2). As an example Figure 1 reports the spectra of (*R*)-**2e** and (*S*)-**2e** recorded in the presence of 22.5 mM DM-β-CD.



(2)

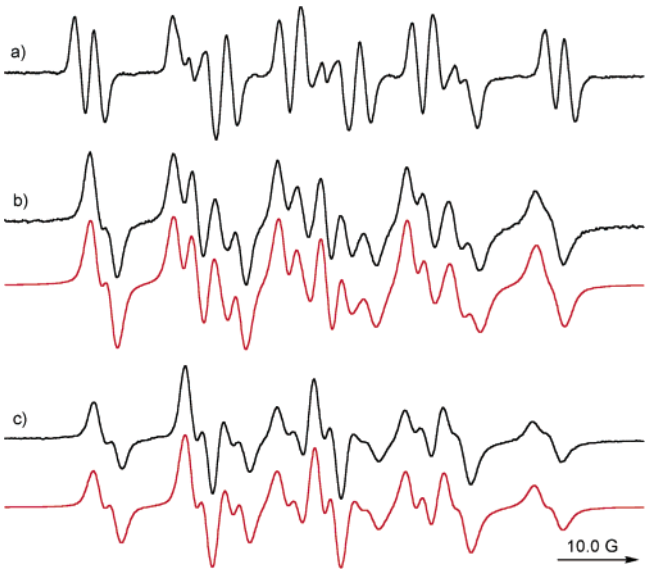


Figure 1. EPR spectra at 300 K of (a) racemic **2e** in water. (b) (*R*)-**2e** in the presence of DM-β-CD 22.5 mM. (c) (*S*)-**2e** in the presence of DM-β-CD 22.5 mM. In red are shown the corresponding simulations obtained by using the hfsc reported in Table 1.

By increasing the absolute concentration of DM-β-CD, the ratio between the included and the free species increased and eventually the spectrum of the 1:1 inclusion complex became dominant allowing for easy measurement of the spectroscopic parameters for the included nitroxides (see Table 1).

With heptakis-(2,3,6-*O*-trimethyl)-β-CD (TM-β-CD) no additional spectral lines were observed beside those due to the nitroxide radical in water solution, thus indicating that this cyclodextrin is not able to complex nitroxides **2a**−**2i**.

Inspection of Table 1 clearly indicates that the inclusion by the DM-β-CD chiral cavity of the (*R*) and (*S*) antipodes gave rise to diastereomeric inclusion complexes characterized by different EPR spectroscopic parameters. Actually, both a(N) and $a(2H_{benzylic})$ change significantly upon inclusion into the DM-β-CD cavity, thus giving rise to significant differences in the resonance fields for the $M_I(2H_\beta) = \pm 1$ lines of the complexed and free nitroxides. Due to this favorable feature, the ratio between the concentrations of the two species could be easily obtained by measuring the relative intensity of their low field EPR lines (see Figure 2). By plotting the ratio between the concentrations of the complexed and free species as function of the DM-β-CD concentration in water (see Figure 3) we could

Table 1. Hyperfine Splitting Constants for Free and Included Nitroxides (1:1 complex) in Water at 300 K

			Hpf splitting ($G = 0.1$ mT)[a]		
radical	enantiomer	host	**H**–C*	N–C***H**$_2$*–Ph	N
2a	racemic		3.71	10.82, 11.30	16.21
	(*S*)	DM-β-CD	3.55	7.34, 10.53	15.54
	(*R*)	DM-β-CD	3.63	7.33, 10.48	15.54
	(*S*)	β-CD	3.28	6.74, 10.74	15.70
	(*R*)	β-CD	3.34	6.64, 11.01	15.70
2b	racemic		2.76	11.45, 11.56	15.95
	(*S*)	DM-β-CD	2.69	7.69, 11.29	15.34
	(*R*)	DM-β-CD	2.68	7.63, 11.53	15.36
	(*S*)	β-CD	2.69	7.43, 11.27	15.44
	(*R*)	β-CD	2.70	6.97, 12.03	15.49
2c	racemic		2.29	10.98, 11.96	15.99
	(*S*)	DM-β-CD	2.21	7.24, 12.63	15.38
	(*R*)	DM-β-CD	2.01	7.06, 13.60	15.55
2d	racemic		6.13	10.39, 11.42	15.91
	(*S*)	DM-β-CD	7.00	9.24, 9.67	15.45
	(*R*)	DM-β-CD	6.80	9.65, 10.22	15.53
	(*S*)	β-CD[b]	7.04	9.63, 9.85	15.50
	(*R*)	β-CD[b]	7.18	9.88, 10.29	15.55
2e	racemic		2.24	11.63, 12.75	15.80
	(*S*)	DM-β-CD	1.80	10.97, 11.01	15.44
	(*R*)	DM-β-CD	1.72	9.98, 12.52	15.42
	(*S*)	β-CD	1.94	10.82, 10.85	15.59
	(*R*)	β-CD	1.85	10.43, 12.00	15.54
2f	racemic		2.16	11.69, 12.21	15.97
	(*S*)	DM-β-CD	1.29	10.22, 12.04	15.79
	(*R*)	DM-β-CD	1.46	9.55, 12.33	15.73
2g	racemic		7.79	10.62, 11.34	16.49
	(*S*)	DM-β-CD	8.66	8.93, 9.69	15.66
	(*R*)	DM-β-CD	8.59	8.62, 9.70	15.62
	(*S*)	β-CD	8.73	8.75, 10.09	15.80
	(*R*)	β-CD	8.63	8.68, 9.90	15.77
2h	racemic		2.76	11.55, 11.74	16.05
	(*S*)	DM-β-CD	2.45	8.10, 10.96	15.39
	(*R*)	DM-β-CD	2.44	7.67, 11.47	15.40
	(*S*)	β-CD	2.56	7.80, 10.86	15.52
	(*R*)	β-CD	2.57	7.32, 11.46	15.56
2i	racemic		5.50	10.28, 11.75	16.12
	(*S*)	DM-β-CD	5.95	9.00, 10.18	15.65
	(*R*)	DM-β-CD	5.66	9.58, 10.48	15.72

[a] The reported Hpf splitting constants refers to the nucleus indicated in bold. [b] T = 292 K.

determine the corresponding complex stability constants, K_2 (see Table 2). The reported uncertainties in the equilibrium constants were calculated as twice the standard error of the regression coefficient attained by analyzing up to 10 different EPR spectra recorded in the presence of variable concentrations of DM-β-CD. The uncertainties in the observed equilibrium constants are less than 4% except for the derivative **2f** for which the similarity of the spectroscopic parameters of the free and included species did not allow us to obtain well resolved spectra.

The enantioselectivity (ES) of DM-β-CD toward a given nitroxide enantiomeric couple was estimated quantitatively from the relative complex stability constant by means of eq 3

$$\text{Enantioselectivity (ES)} = \frac{|K_2(\text{S}) - K_2(\text{R})|}{K_2(\text{S}) + K_2(\text{R})} \times 100 \quad (3)$$

Further increase of DM-β-CD concentration (up to 0.1 M) did not lead to the appearance of any new EPR signals. This experimental observation was taken as evidence that DM-β-CD forms inclusion complexes with the investigated nitroxides with a unique geometry from the interaction between a single molecule of the guest and one host (1:1 complex). For comparison purposes, we also recorded the EPR spectra of some

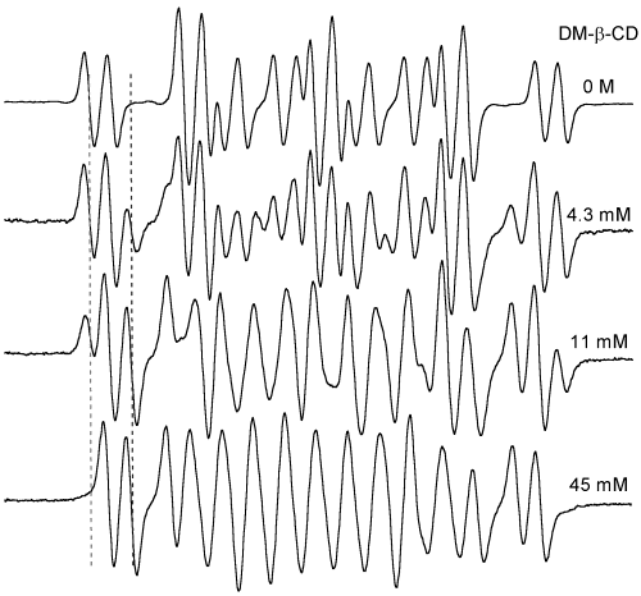


Figure 2. 300 K EPR spectra of (*R*)-**2b** in water in the presence of variable amount of DM-β-CD. The dotted lines correspond to the resonance field of the free and complexed species.

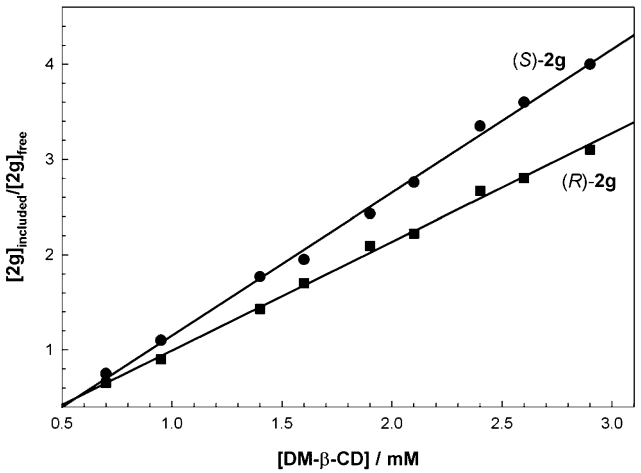


Figure 3. Plot of the concentration ratio between the 1:1 complex and the free nitroxide versus the concentration of DM-β-CD in water at 300 K for (*S*)-**2g** and (*R*)-**2g**.

representative nitroxides in the presence of the unmodified β-CD (see Table 1). Nitroxides having a second aromatic ring at R substituent (**2d**, **2e**, and **2g**), in the presence of β-CD at concentrations close to the solubility limit (13.0 mM), showed EPR spectra containing additional lines[19] beside those of the free and 1:1 complexed species (see the Supporting Information). These lines were attributed to a third radical species identified as an inclusion complex between one guest radical and two β-cyclodextrin units on the basis of experimental evidence similar to those reported previously for the dibenzyl nitroxide.[15] Because the driving force responsible for the β-CD dimer stabilization is the formation of intermolecular hydrogen bonds between the secondary hydroxyl groups of the two larger CD rings, the different behavior observed when using 2,6-*O*-dimethyl β-cyclodextrin as host is presumably due to the absence of some of the hydroxylic hydrogens. The results obtained with β-CD illustrates the ease by which complexes with stoichiom-

(19) Data to be published.

Table 2. Complex Stability Constants K_2 at 300 K for the Inclusion by DM-β-CD

guest	K_2/M^{-1}	ES/%
(*S*)-**2a**	249 ± 9[a]	
(*R*)-**2a**	245 ± 10	0.81
(*S*)-**2b**	277 ± 12	
(*R*)-**2b**	262 ± 11	2.78
(*S*)-**2c**	355 ± 15	
(*R*)-**2c**	341 ± 13	2.01
(*S*)-**2d**	244 ± 10	
(*R*)-**2d**	273 ± 11	5.61
(*S*)-**2e**	751 ± 37	
(*R*)-**2e**	678 ± 34	5.11
(*S*)-**2f**	560 ± 61	
(*R*)-**2f**	588 ± 59	2.43
(*S*)-**1g**	810 ± 156[b]	
(*R*)-**1g**	662 ± 106[b]	10.05
(*S*)-**2g**	1500 ± 64	
(*R*)-**2g**	1145 ± 48	13.42
(*S*)-**2h**	440 ± 17	
(*R*)-**2h**	409 ± 15	3.65
(*S*)-**2i**	459 ± 18	
(*R*)-**2i**	382 ± 15	9.16

[a] Errors are twice standard errors of equilibrium constants. [b] Determined by ^{1}H NMR titrations.

etries higher than 1:1 can be distinguished by their EPR spectra and lead us to the conclusion that these are not formed in the presence of DM-β-CD.

With nitroxides containing a phenyl and a CH_2OH (or CH_2-OCH_3) substituent at the chiral carbon (**2d** and **2i**) a marked selective broadening of the $M_i(2H_\beta) = +1$, $M_i(H^*_\beta) = -1/2$ or $M_i(2H_\beta) = -1$, $M_i(H^*_\beta) = +1/2$ lines was visible when their EPR spectra were recorded in the presence of DM-β-CD at room temperature (see Supporting Information). These line width effects[20] indicate that the rate of rotation about one of the two N−C_α bonds is significantly reduced with respect to the free species when nitroxides **2d** and **2i** are included inside the DM-β-CD cavity.[21] Although a decrease of the intramolecular flexibility upon inclusion by a model receptor has been already observed in paramagnetic guests,[22] to the best of our knowledge this is the first example of an internal motion restriction in an organic free radical included in a cyclodextrin cavity.

NMR Measurements. Room temperature ^{1}H NMR spectra of DM-β-CD were recorded in D_2O solutions in the presence of equimolar amounts of amines **1a**−**1i** (10−20 mM). In some cases, CD_3OD (10% v/v) was added to the sample to improve the amine solubility. The signals of the DM-β-CD recorded in the presence of the guest showed some differences with respect to those of the free CD. In particular, the internal H3 and H5 signals of the host underwent upfield shifts of about 0.11 and 0.17 ppm, respectively, in the presence of equimolar amounts of the guest molecules. This behavior has been taken as an indication of the occurrence of a "host−guest" interaction.[17] The formation of the DM-β-CD-guest complex is also confirmed by the observation of significant signal shifts of the amino guest protons. In no cases, however, did complexation by the cyclodextrin cavity produce the splittings of the aromatic protons of the *N*-benzylic substituent of the guest.

Heptakis-(2,3,6-*O*-trimethyl)-β-CD (TM-β-CD) was treated with amines **1a**−**1i** under the same experimental conditions for comparison. No significant spectral shifts of the cyclodextrin inner protons were observed, thus indicating that no complexation of amines **1a**−**1i** takes place at the cyclodextrin concentration employed.

Being interested in comparing the enantioselectivity of DM-β-CD toward nitroxides with respect to that of the parent amine measured by EPR, we tried to determine the affinity constants of some representative amines by NMR titrations.[17] Contrary to what was observed by EPR spectroscopy, the experimental NMR spectra represent the concentration-weighted average of the spectra of the amine in water and of the amine included in the CD cavity. Therefore, the association constant could be calculated by non linear fitting of the concentration dependence of the δ shift values of the guest or the host protons. To obtain accurate measurements of the affinity constants the measure of the chemical shift value corresponding to 100% complexation (complexation induced shift, CIS) is generally required but this is not always possible due to signal overlapping and formation of complexes with different stoichiometry. Moreover, recording of many ^{1}H NMR spectra is required in order to have relatively small uncertainties, which in turn would lead to a very time-consuming determination of the equilibrium constant.

For these reasons, we decided to investigate only one enantiomeric pair of amines (*R*)-**1g** and (*S*)-**1g**, which by oxidation give aminoxyls displaying the highest enantiomeric discrimination (13.42%) toward DM-β-CD. These amines were also chosen because they undergo the most substantial signal shifts during complexation.

Titrations were carried out in D_2O solutions containing CD_3OD (10% v/v) at 25 °C by keeping the **1g** concentration constant (2.15 mM) and increasing gradually the amount of DM-β-CD. All solutions were prepared in a 0.1 M phosphate buffer adjusted at pH 7.4.

Experiments were performed by following the chemical shift change of the proton attached to the chiral carbon (this in the free amines falls at 4.22 ppm and after 100% of complexation is 0.30 and 0.26 ppm shifted toward lower frequencies for the (*S*) and (*R*) enantiomers, respectively). The calculated binding constants are $K_2(S) = 810 \pm 156\ M^{-1}$, $K_2(R) = 662 \pm 106\ M^{-1}$ leading to an ES ratio of 10.05% (see Figure 4). In the present case application of the fitting procedure yields equilibrium constants with uncertainties (calculated as twice the standard error) of about 16−18%. These large errors, which are quite common when the CIS value is introduced as adjustable parameters,[17b] reduce significantly the utility of NMR titrations in evaluating small differences of affinity constants.

The solution structures of the complexes between DM-β-CD and amines were investigated by ^{1}H−^{1}H NOE experiments in order to evaluate the geometry of the aggregates and to estimate the position of the amine chiral center within the cyclodextrin

(20) (a) Freed, J. H.; Fraenkel, G. K. *J. Chem. Phys.* **1963**, *39*, 326−348. (b) Hudson, A.; Luckhurst, G. R. *Chem. Rev.* **1969**, *69*, 191−225.

(21) It is possible to reproduce the observed line width alternating effect by considering the modulations of β hydrogen hyperfine splittings arising from exchange between two conformations which differ in the average value of $\cos^2\theta$, where θ is the dihedral angle between a β C−H bond and the axis of the $2p_z$ orbital on N. As an example, the experimental spectrum of the radical (*R*)-**2d** is well reproduced (see Supporting Information) by using for each conformer two sets of three β-hydrogen couplings whose sum is the same (26.7 G) and the averaged values are those reported in Table 1. To correctly simulate the alternating line width effects for one conformer the two C*H*$_2$−Ph couplings must be larger and the C*−*H* coupling smaller with respect to the other. However, determining the absolute values of these couplings would require us to record spectra at temperatures lower than 0 °C to freeze the internal rotation around the N−C bond in the EPR time scale, this being not possible in water solution.

(22) (a) Jäger, M.; Stegmann, H. B *J. Am. Chem. Soc.* **1996**, *35*, 1815−1818. (b) Jäger, M.; Schuler, P.; Stegmann, H. B.; Rockenbauer, A. *Magn. Reson. Chem.* **1998**, *36*, 205−210.

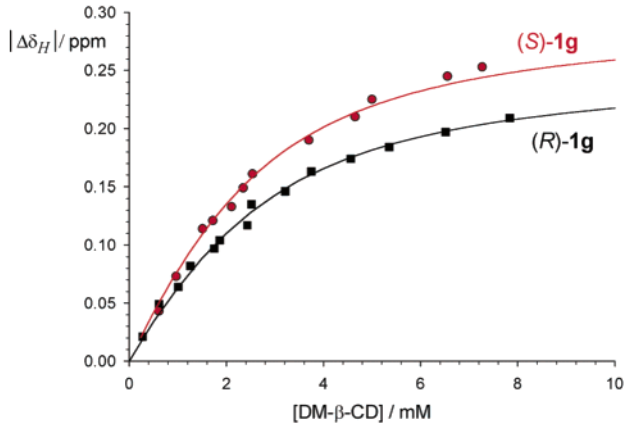
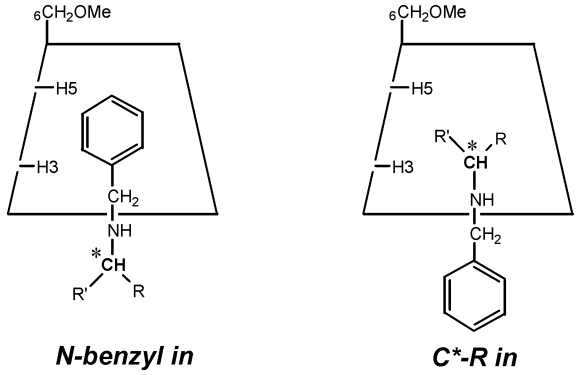


Figure 4. Plot of the complexation-induced chemical shift (CIS) of the hydrogen bound to the chiral carbon for (*S*)-**1g** and (*R*)-**1g** versus the concentration of DM-*β*-CD. The lines represent the theoretical dependence of CIS on the DM-*β*-CD concentration calculated by taking into account the formation of the inclusion complexes and were obtained by numerically fitting the experimental data by introducing as adjustable parameters the values of K_2 and CIS at 100% of complexation.

Chart 3



cavity. It should be pointed out that, for a given amine, each couple of enantiomers gives rise to similar ^{1}H−^{1}H NOE interactions.

Initially we studied the interactions of DM-*β*-CD with amines **1a**−**1c**, which differ only in the length of the alkyl chain (R, see Chart 1) bound to the chiral carbon. However the interpretation of the NMR spectra of the inclusion complexes was difficult to achieve, due to the overlapping of the cyclodextrin H2−H6 proton signals (3.3−4.0 ppm) with the benzylic proton signals and, to some extent, the C*H*$_2$OH proton signals of the amines. To overcome this problem, ^{1}H−^{1}H NOE interactions of the corresponding deuterated amines (**1a**,**c**)-*d*^{4} and **1b**-*d*^{2} (see Chart 1) were investigated in the presence of DM-*β*-CD. ROESY spectra of water solutions of the above-mentioned deuterated amines (15 mM) containing equimolar amounts of DM-*β*-CD showed significant interactions (see Table 3) of the aromatic protons of the guest molecule with the inner H3 (medium) and H5 (strong) cyclodextrin protons as well as with the H6 (medium) protons located at the smaller edge of the macrocyclic host (as an example see Figure 5). On the contrary only a weak interaction of the R alkyl group with the cyclodextrin H3 protons was observed. These results indicate that the inclusion complexes experience the same type of NOE interactions despite the different chain length of the R substituent bound to the chiral center. Moreover, from the strong interactions between the aromatic ring and the inner cyclodextrin protons, the geometry

Table 3. Cross Peaks Observed in the ROESY Spectra of Some Representative Amine/DM-*β*-CD Complexes in D_2O at 298 K (mixing times = 200 ms)

guest	guest protons			host protons			orientation of the complex
	R′	*N*-benzyl	other	H3	H5	H6	
1b-*d*^{2}		H_o, H_m, H_p		++[a]	+++	+	*N-benzyl in*
			C*H	[b]			
			Et	+			
1d-*d*^{9}	H_o, H_m, H_p			++	++	+	*C*−R in*
			C*H[c]				
1e-*d*^{9}	H_o			++	++	−	*C*−R in*
	H_m			+	+++	+	
	H_p			−	+	+	
			C*H	+	−	−	
	CH_2			+	−	−	
1f-*d*^{4} [d]	H4			+	+	−	*C*−R in*
	H5			−	++	−	
	H6			−	++	−	
	H7			+	+	−	
		H_o, H_m, H_p		++	−	−	
			C*H[c]				
			CH_2[c]				
1g-*d*^{7}	H_o, H_m, H_p			++	+++	+	*C*−R in*
			C*H[c]				
			CH_3	++			
1h-*d*^{2}		H_o, H_m, H_p		+	+++	++	*N-benzyl in*
			C*H	[a]			
			Et	+			
1i-*d*^{7}	H_o, H_m, H_p			++	+++	+	*C*−R in*
			C*H[c]				
			C*H*$_2$OMe[c]				
			OCH_3[c]				

[a] +++, strong; ++, medium; +, weak; −, no effect. [b] A cross-peak with H3 is recorded at higher mixing times. [c] The signal partially overlaps with some signals of DM-*β*-CD. [d] Measurement were carried out by using an amine and DM-*β*-CD concentration of 2.2 mM.

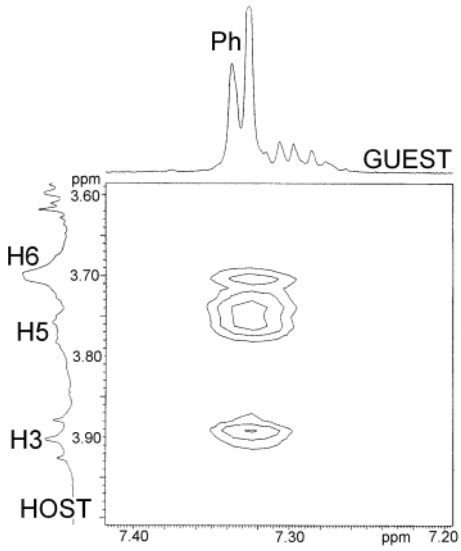


Figure 5. Portion of the ROESY spectrum of an equimolar solution (15 mM) of DM-*β*−CD/**1b**-*d*^{2} in D_2O at room temperature.

of the inclusion complexes can be inferred to be very similar in all cases with the *N*-benzyl part of the molecule deeply included inside the CD cavity. We call this orientation of the complex as *N-benzyl in* (see Chart 3).

The amine **1h**-*d*^{2}, where the hydrophilic CH_2OH group has been converted to the corresponding CH_2OCH_3 group, showed NOE interactions similar to those of the structurally related aminoalchol **1b**-*d*^{2}. This result suggests that, also in this case, inclusion of the guest species into the CD cavity occurs from

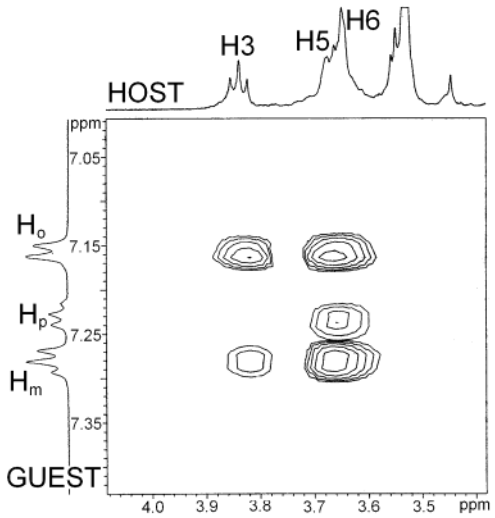


Figure 6. Portion of the ROESY spectrum of an equimolar solution (1 mM) of DM-β-CD/**1e**-d^9 in D_2O/CD_3OD (10:1 v/v) at room temperature.

the *N*-benzyl portion of the amine. However, the presence of an additional cross-peak due to a NOE interaction between the aromatic protons of (**1h**)-d^2 and the 6-OCH_3 protons of CD is indicative of a more profound interaction of the aminoether with the primary edge of the cavity when compared to the corresponding amino alcohol **1b**.

We then examined the amines containing two aromatic rings, the *N*-benzylic one and that one bound to the asymmetric carbon (**1d**, **1g**, and **1i**). To distinguish the aromatic protons of these two groups, amines completely deuterated at the *N*-benzylic substituent (**1d**-d^9, **1g**-d^7, and **1i**-d^7) were employed. ROESY experiments showed significant NOE interactions between the phenyl ring protons of the guest and the H3 (strong), H5 (strong), and H6 (medium) protons of DM-β-CD, thus indicating that complex formation occurs by the inclusion in the CD cavity of the phenyl ring directly linked to the stereogenic center. We call this orientation of the complex as *C*−R in* (see Chart 3). This behavior is opposite to that found with amines **1a**−**1c** and **1h** where NOE data suggested that the *N*-benzyl part of the molecule was complexed by the CD cavity.

Unfortunately, NMR data of the complex geometry concerning compounds **1d**, **1g** and **1i** did not provide information on the extent of inclusion of each amine into the cavity. This is due to the overlap of the signals for the aromatic *ortho*, *meta*, and *para* protons, that did not allow us to compare the single contributions of each kind of protons with the H3, H5, and H6 protons of DM-β-CD.

We also investigated the amino alcohol containing two benzylic groups (**1e**). Again the *N*-benzylic deuterated product (**1e**-d^9) was used to identify unambiguously the geometry of the complex. ^{1}H NMR spectra of the solution containing equimolar amounts of DM-β-CD and **1e**-d^9 showed separate signals for each proton of the guest. In particular, the benzyl and H−C* proton signals found under 3 ppm did not overlap with the CD protons, whereas the phenyl ring showed complete separation of the three distinct spin systems corresponding to the *ortho*, *meta*, and *para* protons. This favorable feature allowed us to assign unambiguously the most important NOE interactions. In the portion of the ROESY spectrum shown in Figure 6, the cross-peaks correlating the aromatic *ortho* and *meta* hydrogens with H3 protons of the host were found to have different intensities; in particular the larger cross-peak volume detected for H_o with respect to H_m and the absence of correlation between H_p and H3 indicates that H_p is directed toward the smaller primary rim of the cavity far from H3. Similar considerations hold in the analysis of the interactions of the aromatic protons with H5 and H6 of the host. Actually, the intensities of the NOE interactions between H_o, H_m, H_p, and H5, H6 ($H_m > H_o > H_p$) again indicate that the phenyl of the benzylic group bound to the chiral carbon is included inside the CD cavity. This conclusion is also confirmed by the presence of NOE interactions of benzylic and H−C* protons with H3 only (data not shown).

An analysis of the geometry of the complex involving amine **1f**-d^4 was carried out on a 2.1 mM solution containing an equimolar amount of DM-β-CD. In the spectrum of the complex recorded at this concentration each aromatic proton of the indole moiety shows distinct signals and the N-benzylic protons are easily distinguishable. The ROESY data can be summarized as follows: (a) protons H4 and H7 of the indolic group interact with both H3 and H5 of the host; (b) the indolic H5 and H6 show cross-peaks only with H5 of the host; (c) the aromatic protons of the *N*-benzylic moiety display correlations only with the internal H3 of the host (see Table 3). These results are consistent with a geometry in which the indolic fragment of the amine is complexed by the cavity of the cyclodextrin and the phenyl group is outside the wider rim.

Molecular Dynamics. To obtain a more detailed picture of the geometry of the complexes investigated by NOE experiments, stochastic dynamics (SD) simulations were performed by using the AMBER* force field of Macromodel 7.0 program.

For isolated guests **1a**−**1i**, a Monte Carlo conformational search was carried out by rotating all $C(sp^3)-C(sp^3)$, $Ar-C(sp^3)$, $O-C(sp^3)$, and $N-C(sp^3)$ bonds in the molecule. The most stable conformation found by this procedure was then used in the dynamic simulation. All computations were performed on the *S* enantiomer with the exception of amine (*R*)-**1g**.

The computational approach taken in this study is to dock the guest molecule inside the host cavity, energy minimize the complex, and carry out standard equilibrations and production runs to derive averaged distances. To accomplish this, we began with a 7-fold symmetric cyclodextrin with the guest molecule docked in its interior. We selected a symmetric CD only as a guide for the placement of the guest and with the understanding that these molecules will deform upon geometry optimization.[23] The origin of a Cartesian reference frame was placed at the center of mass of the CD with the *z* axis aligned with the C_7 symmetry axis of CD. A second reference frame was placed on the guest molecule with the *z* axis passing through the C*−N or $PhCH_2-N$ bond. The guest was translated along the *z* axis in order to have the nitrogen atom located in the plane passing through the seven acetal oxygens of the CD. For each complex only the experimental orientations previously found by NOE experiments were investigated (see Table 4). At the end of this procedure, minimization of the ensemble CD-amine lead to the starting geometry for stochastic dynamics (SD) simulations.

The simulations were run at 300 K with time steps of 1 fs and an equilibrium time of 500 ps before each dynamic run.

(23) (a) Lipkowitz, K. B. *Chem. Rev.* **1998**, *98*, 1829−1873. (b) Lipkowitz, K. B. *J. Org. Chem.* **1991**, *56*, 6357−6367.

Table 4. Calculated Averaged Energies (kJ mol^{-1}) and Distances (Å) at 300 K for the Inclusion Complexes between DM-β-CD and Amines **1a**−**1i**

guest	orientation of complex	energy of complex[a]	$\langle d_{C^*,H3}\rangle^b$	$\langle d_{C^*,H5}\rangle^c$	$\langle d_{Hp,H3}\rangle^b$	$\langle d_{Hp,H5}\rangle^c$
(*S*)-**1a**	*N-benzyl in*	1319.7	3.63	6.13		
(*S*)-**1b**	*N-benzyl in*	1318.1	1.70	4.20		
(*S*)-**1c**	*N-benzyl in*	1338.2	2.54	5.04		
(*S*)-**1d**	*C*−R in*	1340.2	1.78	4.28	4.60	2.10
(*S*)-**1e**	*C*−R in*	1335.0	1.44	3.94	4.63	2.13
(*S*)-**1f**	*C*−R in*	1331.3	2.54	4.94		
(*R*)-**1g**	*C*−R in*	1354.1	0.01	2.51	5.35	2.85
(*S*)-**1h**	*N-benzyl in*	1357.2	2.43	4.93		
(*S*)-**1i**	*C*−R in*	1368.0	0.93	3.43	4.69	2.19

[a] Potential energy of the entire complex averaged over the simulation time. [b] Distances to the plane defined by all H3 of DM-β-CD. [c] Distances to the plane defined by all H5 of DM-β-CD.

The total simulation time was set to 10 000 ps in order to achieve full convergence.[9a] During the simulation the distance between the chiral center of the guest and the H3 and H5 protons of CD were monitored. In the case of amines having a phenyl or benzyl group linked to the chiral center, the distances between the *para*-hydrogen of the guest and the H3 and H5 CD protons were also monitored.

As a measure of the degree of inclusion of the guests we chose the distances from the chiral center or from the *para*-hydrogen of the R group to the planes defined by all H3 and H5 protons of DM-β-CD within a particular complex. The results are summarized in Table 4.

Mass Spectra. The chiral discrimination of amines **1a**−**1i** by DM-β-CD has been also investigated by using the mass spectrometry (MS)-enantiomer labeled guest method.[24] This method consists of using an isotopically labeled enantiomer of a selected guest to distinguish the diasteromeric host−guest complex ions in a mass spectrum. A 1/1 mixture of a labeled and an unlabeled guest enantiomer (i.e., *R*-d^n + *S* or *R* + *S*-d^n) is complexed with a target chiral host. The enantioselectivity of the host DM-β-CD toward a given racemic mixture is estimated quantitatively from the relative peak intensity value (I_R/I_{S-dn} or I_S/I_{R-dn}) of the two host−guest diastereomeric complex ion peaks in the mass spectrum. In our case, electrospray ionization mass spectrometry (ESI−MS) was employed.

A 1:1 racemic solution of enantiomer guests was prepared by mixing an equal amount of a water/ACN solution (2/1 v/v) of each enantiomer (10 μM) in the presence of DM-β-CD 40 μM. A typical ESI−MS spectrum recorded in the region 1542−1556 *m*/*z* units for the mixture (*R*)-**1g** and (*S*)-**1g**-d^7 is shown in Figure 8.

Analyses of the ESI−MS peak intensities led to an ES ratio smaller than 1% for all investigated amines (**1a**−**1i**). The apparent absence of chiral discrimination resulting from ESI−MS experiments contrasts with the enantioselectivity ratios found when using magnetic resonance spectroscopies (see discussion).

We also recorded ESI−MS spectra of amine **1g** in a water/ACN solution (2/1 v/v) containing an equimolar mixture of DM-β-CD and TM-β-CD for comparison. The ESI-MS spectra showed besides the signal of the DM-β-CD-guest complex also a strong signal due to the TM-β-CD-guest complex (see Supporting Information).

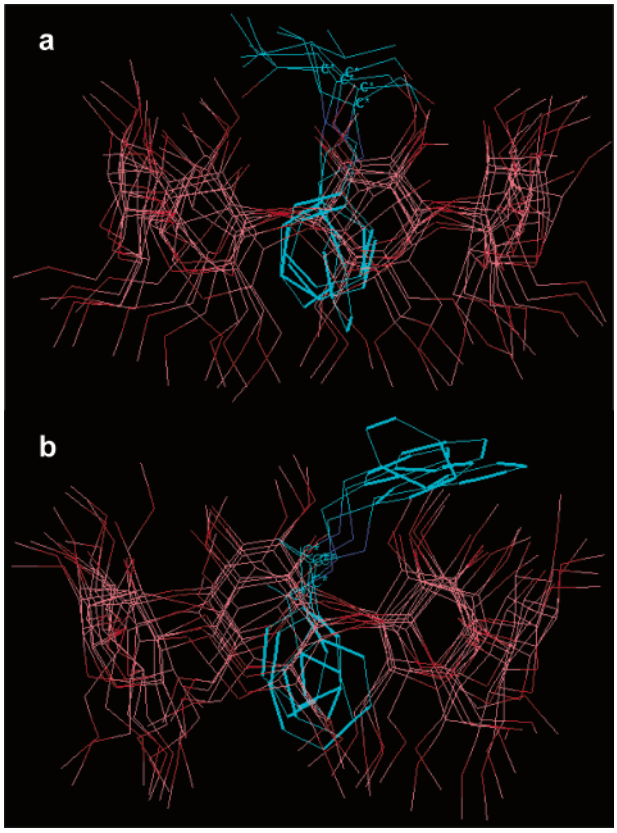


Figure 7. Clustered molecular display. Dynamics of the complex of DM-β-CD with **1c** (a) and **1g** (b). The drawings include only 5 structures that refer to the time interval of simulation between 5000th to the 6000th ps. Hydrogen atoms have been omitted for clarity.

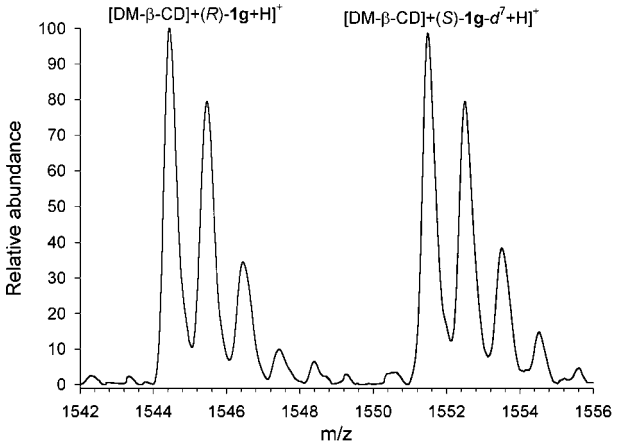


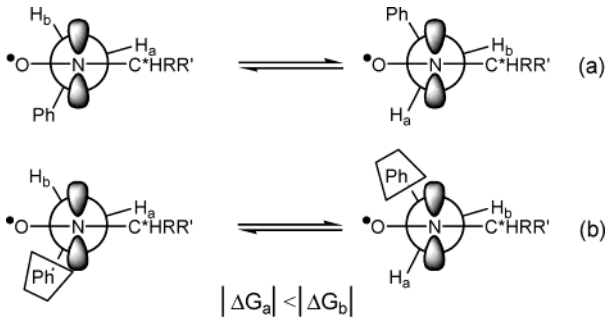
Figure 8. Positive ESI spectra of water/ACN solution (2/1) containing an equimolar amount of (*R*)-**1g** and (*S*)-**1g**-d^7 (15 μM) in the presence of DM-β-CD 40 μM.

Discussion

EPR Parameters. Inspection of Table 1 shows that the value of a(N) decreases significantly when the aminoxyl is located in the less polar environment of the host cavity. This reduction is in the range from −0.21 G for the indolyl derivative (**2f**) to −0.85 G for **2g**. The reduction of a(N), reported in previous

(24) (a) Fales, H. M.; Wright, G. J. *J. Am. Chem. Soc.* **1977**, *99*, 2339−2340. (b) Sawada, M.; Takai, Y.; Yamada, H.; Hirayama, S.; Kaneda, T.; Tanaka, T.; Kamada, K.; Mizooku, T.; Takeuchi, S.; Ueno, K.; Hirose, K.; Tobe, Y.; Naemura, K. *J. Am. Chem. Soc.* **1995**, *117*, 7726−7736. (c) Sawada, M.; Shizuma, M.; Takai, Y.; Adachi, H.; Takeda, T.; Uchiyama, T. *Chem. Commun.* **1998**, 1453−1454. (d) Sawada, M. *Mass Spectrom. Rev.* **1997**, *16*, 73−90. (e) So, P. M.; Wan, T. S. M.; Dominic Chan, T.-W. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 692−695.

Chart 4



studies carried out with different nitroxide radicals[14,15,25] has been attributed to the larger weight of the nitroxide mesomeric forms in media of low polarity in which the unpaired electron is localized on the oxygen rather than on the nitrogen atom.

If we consider the nitroxides forming an inclusion complex with the *C**−R in* orientation and take the calculated distances from the chiral center to the planes defined by all H3 (see Table 4) as a measure of the degree of inclusion (a larger inclusion corresponds to a more hydrophobic environment), an acceptable correlation ($r = 0.94$) is obtained between this distance and the reduction of the nitrogen splitting ($a(N)_{water} - a(N)_{CD}$) measured by EPR (see Supporting Information).

The value of the hyperfine splitting constant at the β-protons in alkyl nitroxides is sensitive to conformational changes and depends on the spin population in the $2p_z$ orbital of nitrogen (ρ_N) and on the dihedral angle (ϑ) between the symmetry axis of this orbital and the N−C−H_β plane according to the Heller−McConnel equation[26]

$$a(H_\beta) = \rho_N(A_N + B_N\langle\cos^2\vartheta\rangle) \qquad (4)$$

The nitroxides investigated in this work have two types of β-protons: the two benzylic protons and the single proton bound to the chiral carbon. As far as the benzylic protons are concerned, analysis of the corresponding hyperfine splitting constants allowed us to differentiate the behavior of the nitroxide forming *N-benzyl in* complexes from those giving rise to *C**−R in* complexes. In the former complexes (**2a**−**2c**, **2h**), the difference in benzylic couplings ($\Delta a_{2H\beta}$) is much larger in the radical included in the DM-β-CD cavity than in the free nitroxide (in water the average $\Delta a_{2H\beta}$ is 0.44 G, whereas in the complexes with DM-β-CD is 4.05 G; see Table 1). On the other hand, nitroxides (**2d**−**2g**, **2i**) affording *C**−R in* complexes, are characterized by differences in the value of the two benzylic couplings similar to those observed in the free paramagnetic species (in water the average $\Delta a_{2H\beta}$ is 0.97 G, whereas in the complexes with DM-β-CD it is 1.16 G; see Table 1).

In the investigated nitroxide radicals, the lower energy conformations around the $PhCH_2$−N bond are those where the chiral center avoids the phenyl group (see Chart 4, equation a). Due to the presence of the carbon chiral center, the spatial orientation of the two β-hydrogens with respect to both the phenyl ring and the C* substituents will differ for the two rotamers. Consequently, the methylene protons will be magnetically nonequivalent and the energies associated with the two rotamers will likewise be different. When the interconversion between the two nonequivalent conformers is fast on the EPR time scale the observed β-hydrogen splittings will be time averaged over the residence time in each conformation. Because the two conformations have different energies the two splittings will be different, their values being closer to those of the favored conformer.[27]

On the basis of these considerations the increased magnetic non equivalence of the benzylic protons observed in the *N-benzyl in* complexes should be attributed to an increase of the energy difference for the two rotamers when the nitroxides are included by DM-β-CD (Chart 4, equation b). This means that the differences in the two rotamers concerning the spatial orientation of the two β-hydrogens with respect to both the phenyl ring and the C* substituents are enhanced when the benzylic aromatic ring is included in the DM-β-CD cavity.

It should be pointed out that the different behavior of the *N-benzyl in* and *C**−R in* complexes originates from conformational effects rather than from a different affinity for the cyclodextrin cavity of the related nitroxides. Thus, a large variation of the spectroscopic parameters when passing from the free to the included species does not depend on the affinity of the guest for the CD cavity.[29]

Also, the variation of the hyperfine splitting of the hydrogen bound to the chiral center $a(C^*-H_\beta)$ observed when passing from the free to the complexed species, depends to some extent on the nature of the inclusion complexes. With nitroxides forming *N-benzyl in* complexes an averaged decrease of 0.16 G is observed by passing from the free to the complexed radical while with nitroxides giving rise to *C**−R in* complexes a larger average difference (0.55 G) is found. Since the value of the coupling for the single β-hydrogen depends on the conformation adopted around the N−C* bond, inclusion of the chiral part of the molecule has a larger influence on its value.

A final consideration concerns the value of hyperfine splitting of the single proton bound to the chiral carbon which is much larger in nitroxides where the R substituent is a phenyl group (**2d**, **2g**, and **2i**). For a methyne group the lowest energy conformation, i.e., that one in which the carbon−hydrogen bond is eclipsed by the benzylic group, is characterized by a small value of the H_β splitting (Chart 5, structure a). With nitroxides where R═Ph, the conformation having the phenyl group eclipsed by the p_z orbital on the nitrogen atom (Chart 5, structure b) seems to be important, thus leading to the higher value of the H_β splitting observed experimentally.

Comparison between Amine and Nitroxides Guests. To correlate the geometries of the amine/DM-β-CD complexes

(25) (a) Kotake, Y.; Jansen, E. *J. Am. Chem. Soc.* **1988**, *110*, 3699−3701. (b) Kotake, Y.; Jansen, E. *J. Am. Chem. Soc.* **1989**, *111*, 5138−5140. (c) Fathallah, M.; Fotiadu, F.; Jaime, C. *J. Org. Chem.* **1994**, *59*, 1288−1293. (d) Pérez, F.; Jaime, C.; Sánchez-Ruiz, X. *J. Org. Chem.* **1995**, *60*, 3840−3845.

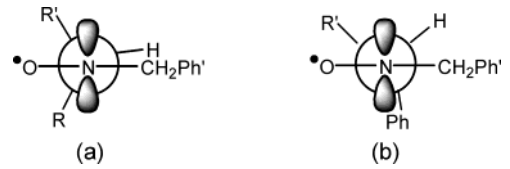
(26) Heller, C.; McConnell, H. M. *J. Chem. Phys.* **1960**, *32*, 1535−1539.

(27) It should be pointed out that even in the case that all conformations were equally populated, magnetic nonequivalence would still be observed since in each conformer the two methylene splittings can be coincident only by accident. The residual nonequivalence still present when all conformations are energetically degenerate, is called intrinsic nonequivalence. We have recently demonstrated that the magnetic nonequivalence of diastereomeric β-protons in the EPR spectra mainly arise from the population difference of the various conformations.[28]

(28) Franchi, P.; Lucarini, M.; Pedulli, G. F.; Bandini, E. *J. Chem. Soc., Chem. Commun.* **2002**, 560−561.

(29) This behavior has already been observed by NMR spectroscopy. See for examples: (a) Dodziuk, H.; Ejchart, A.; Lukin, O.; Vysotsky, M. O. *J. Org. Chem.* **1999**, *64*, 1503−1507. (b) Barretta-Uccello, G.; Balzano, F.; Caporusso, A. M.; Iodice, A.; Salvadori, P. *J. Org. Chem.* **1995**, *60*, 2227−2231. (b) Barretta-Uccello, G.; Balzano, F.; Caporusso, A. M.; Salvadori, P. *J. Org. Chem.* **1994**, *59*, 836−839.

Chart 5



determined by NMR or computed by MD experiments, with the enantiomeric discrimination of DM-β-CD toward the corresponding nitroxides obtained by EPR spectroscopy, we point out that the structure of the inclusion complexes is similar for the nitroxide radicals and their amine precursors. This is true for the DM-β-CD complexes of some achiral secondary amines and of the corresponding nitroxides, as it has been demonstrated previously by us when comparing NMR[18] and EPR[14b] data.

In the series of products investigated here, the complexes of compounds **1** and **2** have closely related structures. Support for this assumption comes from the similar enantioselectivity ratio measured by NMR for the amine **1g** (ES = 10.05%) and by EPR for the corresponding nitroxide **2g** (ES = 13.42%). This result, besides suggesting a similar geometry of inclusion for the two guests, indicates that the change of hybridation on the nitrogen atom on passing from **1** (sp^3) to **2** (sp^2) is not an important factor for chiral discrimination.

Additional evidence in this direction comes from the analysis of the variation of hfsc of the benzylic protons when the nitroxides are included inside the CD cavity. With amines forming *N-benzyl in* complexes the corresponding nitroxides show a large increase in the difference of the two β-splittings. On the other hand, amines forming *C*−R in* complexes give rise by oxidation to nitroxides that show small variations in the benzylic hfsc upon inclusion. These conclusions strongly support the assumption that the structure of the amine/DM-β-CD complex is likely to also represent the association mode of the nitroxide radicals.

Geometry of the Inclusion Complexes. We have previously emphasized that the same guest can give rise to two type of complexes: those having the *N*-benzyl side of the guest inside the cyclodextrin cavity (*N-benzyl in*) and those in which the portion of the guest included inside the cavity bears the chiral center (*C*−R in*).

Calculated molecular models for complexes belonging to the first group (**1a**, **1b**, **1c**, and **1h**) show that the guest is inserted with the *N*-benzyl group in the smaller primary rim of the CD and the alkyl group R placed near the larger secondary rim of the host (see Figure 7a). In this arrangement, the stereogenic center interacts weakly with the cyclodextrin interior, being located in the middle of the larger rim. In addition, the other R′ substituent (CH_2OH) at the chiral carbon presumably behaves as a hydrogen bond donor toward one of the oxygen atoms present in the larger rim of DM-β-CD, with a consequent poor penetration of the guest into the host cavity. These results are probably the main reason for the lack of chiral discrimination of the corresponding chiral nitroxide radicals (Table 2). Due to the geometry of these complexes an increase in the substituent R lipophilicity (R = Me, Et, *i*-Bu) does not affect the enantiomeric discrimination.

Conversion of CH_2OH to a CH_2OMe group (compound **1h**) allows for a better insertion of the guest (presumably due to the loss of hydrogen bonding) as indicated by the larger affinity constants found by EPR for the corresponding nitroxide. However, poor enantiodiscrimination is again observed due to the small interaction between the stereogenic center of the guest and the chiral group of the CD-cavity as suggested by the calculated distance between the chiral center and the plane containing the cyclodextrin H3.

We can conclude that the poor enantiodiscrimination of amines and nitroxides forming *N-benzyl in* complex is due to a favorable interaction of the host with the benzylic phenyl ring which is far from the stereogenic center. Thus, any change on the alkyl substituents bound to the stereogenic carbon is not able to affect substantially the chiral recognition process.

The situation is reversed in the case of guests where R is or contains an aromatic ring (**1d**−**1g**, **1i**) giving *C*−R in* complexes, independently of the nature of the aliphatic group R′ (CH_2OH, CH_2OMe, CH_3). In this case, the host cavity shows a greater affinity for the aromatic system bonded to the chiral center than for the *N*-benzyl group, forcing the chiral carbon to be included within the cyclodextrin cavity. With this geometry, the nature of the other substituent at the asymmetric carbon determines the depth of penetration of the stereogenic center into the cyclodextrin cavity and thus is responsible for the observed chiral selectivity. In particular with nitroxides, where the R substituent is a phenyl group, as in **2d**, **2i**, and **2g**, the degree of enantioselection increases proportionally to the hydrophobicity of the R′ group (R′ = CH_2OH, CH_2OMe, CH_3, respectively), the best value of chiral discrimination being that found with nitroxide **2g** (ES = 13.42%).

Molecular dynamics calculations also indicate that increasing of the R′ substituent hydrophobicity gives rise to a deeper penetration of the chiral carbon into the receptor cavity. Actually, the average distance between the guest chiral center and the plane defined by all H3 protons of DM-β-CD increases from 0.01 Å for R′ = CH_3 to 0.93 and 1.78 Å for R′ = CH_2OCH_3 and R′ = CH_2OH, respectively. The more pronounced penetration of the guest with R′ = CH_3 (**1g**) with respect to **1d** and **1i** is also confirmed by the larger distance, computed in the inclusion complex of **1g**, between the *para*-hydrogen of the Ph−C* group and the plane defined by all H3 protons (see Table 4).

Molecular dynamics simulations show that amine **1e**, containing a methylene unit between the phenyl group and the chiral center, behaves similarly to **1d**. Actually, the stereogenic center of the guest interacts weakly with the cyclodextrin interior leading to the low enantiodiscrimination observed in the corresponding nitroxides. However, the distance of the *para*-H from the plane passing through the H3 protons of cyclodextrin is similar to that found for **1d**, implying that the aromatic ring is more tilted in **1e** than in **1d**.

Contrary to the other enantiomers, (*R*)-**2f** and (*S*)-**2f** (R′ = 3-indolilmethyl) which contain aromatic substituent at the chiral carbon, are not selectively recognized by DM-β-CD (ES = 2.43%). Both the small change of the EPR nitrogen coupling when passing from the free to the complexed form and the MD simulations indicate that only the indolic ring is included inside the CD cavity while the *N*-benzylic group is exposed to solvent. This geometry of the complex rationalizes the large distance between the chiral center and the interior of the CD-cavity and thus the observed lack of enantiodiscrimination.

On the basis of the above discussion, it can be concluded that better chiral recognition is obtained when the chiral center of the guest interacts strongly with the internal chiral field of the cyclodextrin. If we assume that a greater intermolecular interaction between the chiral groups of guest and host is operating at the center of the cyclodextrin cavity, the dependence of the chiral discrimination on the depth of guest penetration could be modeled by means of a Gaussian distribution centered at the acetalic-*O* plane. Figure 9 shows that a good fit of the experimental data is obtained by means of this model by using a half width σ equal to 1.7 Å.

This simplified model implies that the mechanism of enantiomeric discrimination is determined by the mode of guest penetration, with the magnitude of enantioselection dependent upon the degree of intermolecular contact inside the cyclodextrin cavity. Application of this hypothetical model implies that, after reaching a maximum in the enantiodiscrimination process, accommodation of the chiral center at the smaller rim of the cavity should lead to a decrease in enantiodiscrimination. Design of additional chiral probes is required to confirm this hypothesis.[30]

ESI−MS Results. Analysis of the ESI−MS peak intensities of the signals due to labeled and unlabeled guest enantiomers (i.e., *R*-d^n + *S* or *R* + *S*-d^n) complexed with DM-β-CD, provided an ES ratio smaller than 1% for all of the investigated amines. The poor chiral discrimination resulting from ESI−MS experiments could be attributed to the fact that expected inclusion complexes are not formed in the gas phase but instead only external electrostatic adducts of DM-β-CD with protonated amines are formed. This result is, perhaps, not surprising since binding contributions from solvophobic interactions are absent in the gas phase and purely hydrophobic adducts can hardly survive in ESI conditions.[31]

The statement that hydrophobic interactions play an important role in controlling the stabilities of the complexes between our probes and DM-β-CD comes from the analysis of the affinity constants that depend strongly on the relative hydrophobicities of the substituents at the chiral carbon. For instance, the affinity constant increases in the series **2a** < **2b** < **2c** due to the increasing lipophilicity of the R substituent which changes from CH_3, to CH_2CH_3 and to $CH_2CH(CH_3)_2$ in this series. Similarly, K_2 is larger in **2h** than in **2b** due to the replacement of the CH_2-OH substituent by CH_2OCH_3; in the series **2d** < **2i** < **2g** the value of the affinity constant parallels the lipophilicity of the R′ substituent, i.e., CH_2OH < CH_2OCH_3 < CH_3.

Additional support for this view comes from the spectra of **1g** recorded in the presence of two different cyclodextrins: DM-β-CD, which in water solution is able to include the phenyl ring bound to the chiral carbon, and TM-β-CD for which no complexation was observed by EPR and NMR spectroscopies. Nevertheless, the ESI−MS peak intensity of the [TM-β-CD + **1g** + H^+] is comparable to that of [DM-β-CD + **1g** + H^+].

On the basis of these observations we may conclude that in the case of amines **1a**−**1i**, ESI−MS detects electrostatic external adducts of CDs with protonated amines which are more stable in the gas-phase[32] instead of the expected inclusion complexes with CDs preformed in solution.

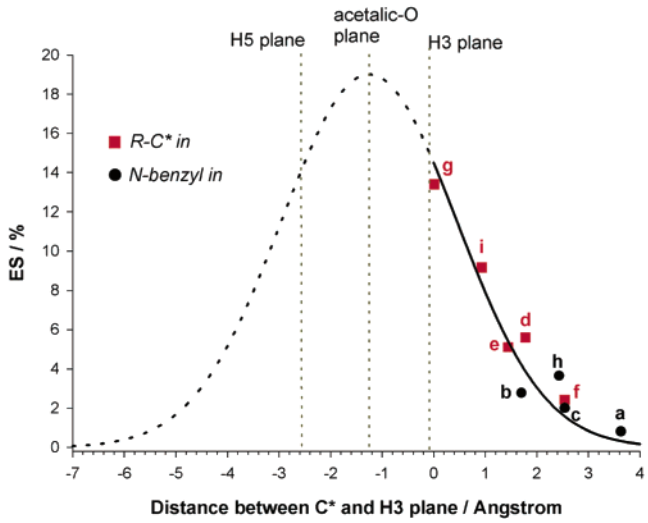


Figure 9. Plot of chiral discrimination versus the distance between the guest chiral carbon and the H3 plane in DM-β-CD/amine complexes. The line represents a hypothetical Gaussian behavior with the maximum centered at the acetalic-*O* plane and was obtained by numerical fitting of the experimental data by introducing the half width σ as an adjustable parameter.

Conclusions

By using specifically designed EPR spin probes and their corresponding diamagnetic precursors, we could clearly distinguish different geometries of inclusion complexes and succeed in identifying the factors responsible for chiral recognition by DM-β-CD. The use of EPR and NMR spectroscopies together with molecular dynamics provides a detailed description of supramolecular complexes in solution. Work to extend this approach to other supramolecular systems is in progress.

Experimental Section

EPR Measurements. EPR spectra were obtained using a Bruker ESP300 spectrometer equipped with an NMR gaussmeter for field calibration and a Hewlett-Packard 5350B microwave frequency counter for the determination of the *g*-factors, which were referred to that of the perylene radical cation in concentrated H_2SO_4 (g = 2.002 58). The sample temperature was controlled with a standard variable temperature accessory and was monitored before and after each run using a copper−constantan thermocouple. The instrument settings were as follows: microwave power 5.0 mW, modulation amplitude 0.05 mT, modulation frequency 100 kHz, scan time 180 s. Digitized EPR spectra were transferred to a personal computer and were analyzed using digital simulations carried out with a program developed in our laboratory and based on a Monte Carlo procedure.[14b] Nitroxide radicals were generated by mixing a solution of the corresponding amine (ca. 1 mM) with a solution of the magnesium salt of monoperoxyphthalic acid (Aldrich, technical grade) (ca 1 mM). To achieve a sufficiently large radical concentration, the mixed solution was sometimes heated at 60 °C for 1−2 min. Aliquots from a concentrated CD solution were added to the solution of nitroxide to yield the required concentrations. Samples were then transferred in capillary tubes (1 mm i.d.) and the EPR spectra were recorded.

(30) We selected a Gaussian function because the variation in the magnitude of enantioselection can be correctly reproduced by a bell shape function, with the understanding that the real function can be determined only by studying additional chiral probes which are able to accommodate the chiral center in the smaller rim of the cavity.

(31) Cunniff, J. B.; Vouros, P. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 437−447.

(32) It has been reported[33] that protonated amino acids form inclusion complexes with TM-β-CD in the gas-phase. The existence of the included structure even in the gas-phase has been attributed to the "three-point attachment" interaction in which $-NH_3^+$ and −COOH groups are inside the CD cavity.

(33) (a) Lebrilla, C. B. *Acc. Chem. Res.* **2001**, *34*, 653−661. (b) Ramirez, J.; Ahn, S.; Grigorean, G.; Lebrilla, C. B. *J. Am. Chem. Soc.* **2000**, *122*, 6884−6890.

NMR Measurements. All 1D and 2D NMR spectra were recorded at 298 K on Varian Inova spectrometers operating at 300 and 600 MHz, respectively, and on a Varian Mercury instrument operating at 400 MHz. NMR experiments were performed in D_2O solutions using residual HOD as an internal standard (4.76 ppm). In some cases CD_3OD (10% v/v) was added in order to improve the amine solubility. Chemical shifts are reported in parts per million (δ scale).

Amine titrations with DM-β-CD were carried out on a Varian Inova 300 at 298 K. NMR determination of the binding constants of diastereomeric complexes of (*R*) and (*S*)-**1g** were performed on 2.15 mM solutions of the guest containing increasing amounts of DM-β-CD (0−20 mM), in D_2O/CD_3OD (10:1 v/v). All solutions were prepared in a 0.1 M phosphate buffer adjusted at pH 7.4.

ROESY data were collected on a Varian Mercury 400 and on a Varian Inova 600, using a 90° pulse width of 13.5 μs and 6.1 μs and a spectral width of 4000 and 6000 Hz in each dimension, respectively. The data were recorded in the phase sensitive mode using a CW spin-lock field of 2 kHz, without spinning the sample. Acquisitions were recorded at mixing times 50−400 ms. Other instrumental settings are: 256 increments of 2 K data points, 8 scans per t_1, 2.5 s delay time for each scan.

Dynamic Simulations. SD simulations were carried out using the MacroModel 7.0 program. Extended nonbonded cutoff distances were set to 8 Å and 20 Å for the van der Waals and electrostatic interactions. All C−H and O−H bond lengths were held fixed using the SHAKE algorithm. Translational and rotational momentum were removed every 0.1 ps.

ESI−MS Measurements. Mass spectra were recorded with Micromass ZMD ESI−MS spectrometer by using the following instrumental settings: positive ions; desolvation gas (N_2) 220 L/h; cone gas (skimmer): 32 L/h; desolvation temp. 120 °C; capillary voltage: 2.8 kV; cone voltage: 45 V; hexapole extractor: 3 V.

Materials. β-CD, DM-β-CD, TM-β-CD, D,L-amino acids, (*R*) and (*S*)-2-amino-1-butanol, (*R*) and (*S*)-α-methylbenzylamine, and (*R*) and (*S*)-*N*-benzyl-α-methylbenzylamine are commercially available (Sigma-Aldrich) and were used as received.

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Supporting Information Available: Preparation of amines **1a**−**1i** and of their deuterated analogues, some representative EPR spectra of nitroxides **2a**, **2c**−**2g**, ESI−MS spectrum of **1g** in the presence of DM-β-CD and TM-β-CD, and a plot of the decrease of $a(N)_{water}-a(N)_{CD}$ versus the distance between the guest chiral carbon and the H3 plane in DM-β-CD/amine complexes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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