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Effect of β-cyclodextrin acetylation on the resolution of phenethylamines with capillary electrophoresis and nuclear magnetic resonance spectroscopy

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Abstract

The effect of acetylation of β-cyclodextrin (CD) on the chiral discrimination of a series of phenethylamines has been investigated using capillary electrophoresis and nuclear magnetic resonance (NMR) spectroscopy. Therefore, pure, fully derivatized CDs were synthesized. Capillary electrophoresis measurements revealed that the 2,3-diacetylated CD was a better discriminator than the 6-acetylated and the native CD. NMR investigations of the complexes suggest that the phenyl ring of the phenethylamines is inserted in the cavity and the side chain interacts with the hydroxyl rim of the macrocycle. The structures of the complexes indicated by the NMR results were supported by molecular models of the derivatised CDs.

Keywords: Enantiomer separation; Cyclodextrins; Phenethylamines; Acetylated cyclodextrins

1. Introduction

Cyclodextrins are widely accepted as discriminatory tools for the resolution of racemic drugs [1]. We have previously reported on the chiral discrimination of phenethylamines with β-cyclodextrin (β-CD) and *heptakis*(2,3-di-O-acetyl)-β-cyclodextrin (Diac-CD, Fig. 1) by capillary electrophoresis and nuclear magnetic resonance (NMR) spectroscopy [2]. Acetylation of the secondary hydroxyl groups apparently weakened ligand binding but enhanced the resolution of these racemic drugs. As part of a systematic study into the structural requirements for chiral discrimination, we have now compared the effect of acetylating the primary hydroxyl groups [to give *heptakis*(6-O-acetyl)-β-CD; 6Ac-CD] with the effect of secondary hydroxyl acetylation. A wider range of ligands has been studied and some preliminary molecular modelling studies undertaken to give some insight into the behaviour of the three cyclodextrins.

The structures of the compounds studied are presented in Table 1. It is convenient to divide the fifteen compounds into two sets. Group I (comp. **1–7**) are related to ephedrine and are methylated at C2 (R_2) giving two chiral centres, except **5** and **7**, which do not have the side-chain OH (R_1). Otherwise, the variations in this group are due to the presence or absence of a *para*-hydroxyl group in the phenyl ring (X) and the degree of alkylation of the

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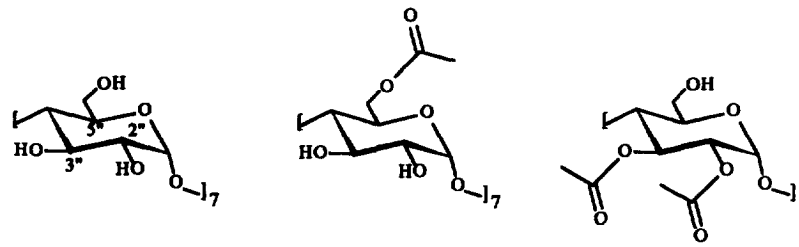


Fig. 1. Structures of β-cyclodextrin (β-CD), *heptakis*(6-O-acetyl)-β-cyclodextrin (6Ac-CD) and *heptakis*(2,3-di-O-acetyl)-β-cyclodextrin (Diac-CD).

amine (R_3, R_4). One racemic diastereoisomer has been examined in each case. Those in group II (comp. **8–15**) lack a second chiral centre through the absence of the 2-methyl group.

2. Experimental

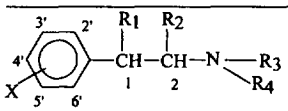
2.1. Chemicals

Pyridine was dried over and distilled from CaH_2, tetrahydrofuran was distilled throughout and stored over Na, dichloromethane was distilled from $CaCl_2$. All other solvents were distilled throughout. Benzylbromide (Merck 801815), tetrabutylammonium fluoride (Janssen 22.108.89), Pd–C 10% (Merck 807104), NaH (Merck 6863), acetic anhydride (Merck 42) and P_4O_{10} (Fluka 1741) were used without further purification.

2.2. Synthesis

Diac-CD was synthesised according to Branch et al. [2].

Table 1
Structures of the racemic phenethylamines



Number	Compound	X	R_1	R_2	R_3	R_4
1	Norephedrine	H	OH	CH_3	CH_3	H
2	Ephedrine	H	OH	CH_3	CH_3	H
3	Methylephedrine	H	OH	CH_3	CH_3	CH_3
4	4-Hydroxynorephedrine	4-OH	OH	CH_3	H	H
5	4-Hydroxyamphetamine	4-OH	H	CH_3	H	H
6	Oxilofrine (Oxyephedrine)	4-OH	OH	CH_3	CH_3	H
7	Pholedrine	4-OH	H	CH_3	CH_3	H
8	Oxedrine (Synephrine)	4-OH	OH	H	CH_3	H
9	Norfenefrine (Norphenylephrine)	3-OH	OH	H	H	H
10	Etilefrine	3-OH	OH	H	CH_2CH_3	H
11	Orciprenaline (Metaproterenol)	3,5-diOH	OH	H	$CH(CH_3)_2$	H
12	Terbutaline	3,5-diOH	OH	H	$C(CH_3)_3$	H
13	Noradrenaline (Norepinephrine)	3,4-diOH	OH	H	H	H
14	Isoprenaline	3,4-diOH	OH	H	$CH(CH_3)_2$	H
15	Salbutamol (Albuterol)	3-CH_2OH,4-OH	OH	H	$C(CH_3)_3$	H

2.2.1. Synthesis of heptakis(2,3-di-O-benzyl-6-O-(dimethylsilyl-1,1,2-trimethylpropyl))-cyclodextrin (17)

To a solution of **16** (2.0 g; 0.94 m*M*) in tetrahydrofuran (THF; 50 ml) was added NaH (1.6 g; 65.7 m*M*) and benzylbromide under N_2 at room temperature. After refluxing for 24 h, methanol was added (20 ml) and the solution was poured into ice-water (100 ml). The aqueous phase was extracted with dichloromethane (3×30 ml), which then was washed with water (3×30 ml), dried over Na_2SO_4 and evaporated in vacuo. Column chromatography on silica gel [petroleum ether–ethyl acetate, 40:1 (v/v); R_F 0.2] of the residue gave **17** (2.4 g; 75%). ^{1}H NMR 7.15 (m, 70H, Arom.), 5.39 (d, ^{3}J 3.2 Hz, 7H, H_1), 4.92 (ABq, ^{2}J 10.9 Hz, 14H, Phe-CH_{2a}), 4.55 (ABq, ^{2}J 11.9 Hz, Phe-CH_{2b}), 4.08 (m, 14H, H_3; H_4), 4.02 (ABq, ^{2}J 10.2 Hz, 14H, H_6), 3.70 (m, 7H, H_5,), 3.42 (dd, ^{3}J 3.2; 9.4 Hz, 7H, H_2), 1.65 (sep, ^{3}J 7.0 Hz, 7H, C*H*$(CH_3)_2$), 0.90 (dd, ^{3}J 7.0; 1.6 Hz, 42H, $2CH_3$), 0.88 (s, 21H, CH_3), 0.81 (s, 21H, CH_3), 0.114 (s, 21H, Si-CH_3), 0.109 (s, 21H, Si-CH_3); ^{13}C NMR 139.20 (C_{1arom}), 138.19 (C_{1arom}), 127.93–126.71 ($C_{2-6arom}$), 97.83 (C_1), 80.82 (C_4), 79.24 (C_2), 77.80 (C_3), 75.44 (Phe-CH_2), 72.58 (C_5), 72.55 (Phe-CH_2), 62.10 (C_6), 34.24 (*C*H$(CH_3)_2$), 25.21 (CR_4), 20.50, 20.40, 18.75, 18.65 (4 CH_3),–2.73,–3.01 (2 Si-CH_3).

2.2.2. Synthesis of heptakis(2,3-di-O-benzyl)-β-cyclodextrin (18)

A solution of **17** (4.7 g; 1.39 m*M*) and tetrabutylammonium fluoride (6.7 g; 11.65 m*M*) in THF was refluxed for 2 h and then concentrated in vacuo. The residue was dissolved in dichloromethane (100 ml), which was then washed with water (3×30 ml), dried over Na_2SO_4 and evaporated under reduced pressure. Column chromatography on silica gel [ethyl acetate–acetone 7:3 (v/v), R_F 0.3] gave **18** (2.96 g; 89%). ^{1}H NMR 7.15 (m, 70H, Arom.), 5.02 (d, ^{3}J 3.4 Hz, 7H, H_1), 4.78 (ABq, ^{2}J 11.3 Hz, 14H, Phe-CH_{2a}), 4.49 (ABq, ^{2}J 12.3 Hz, 14H, Phe-CH_{2b}), 3.94 (m, 21H, H_3; H_6), 3.80 (dd, ^{3}J 4.4; 11.7 Hz, 7H, H_5), 3.65 (t, ^{3}J 7H, H_4), 3.49 (dd, ^{3}J 3.4; 8.7 Hz, 7H, H_2); ^{13}C NMR 138.90 (C_{1arom}), 138.17 (C_{1arom}), 128.10–126.95 ($C_{2-6arom}$), 98.26 (C_1), 80.38 (C_4), 78.67 (C_2), 78.29 (C_3), 74.88 (Phe-CH_2), 72.90 (C_5), 72.76 (Phe-CH_2), 61.53 (C_6).

2.2.3. Synthesis of heptakis(2,3-di-O-benzyl-6-O-acetyl)-β-cyclodextrin (19)

A solution of **18** (1.8 g; 0.75 m*M*) in pyridine (40 ml) and acetic anhydride (10 ml) was stirred at 80°C for 4 h and then evaporated under reduced pressure. Column chromatography on silica gel [ethyl acetate–petroleum ether 1:1 (v/v), R_F 0.35] on a 10-cm column yielded **19** (1.8 g; 89%). ^{1}H NMR 7.20 (m, 70H, Arom.), 4.95 (d, ^{3}J 3.3 Hz, 7H, H_1), 4.89 (ABq, ^{2}J 10.9 Hz, 14H, Phe-CH_2), 4.49 (ABq, ^{2}J 12.4 Hz, 14H, Phe-CH_2), 4.41 (m, 14H, H_3; H_{6a}), 4.07 (m, 14H, H_5; H_{6b}), 3.70 (t, ^{3}J 8.8 Hz, 7H, H_4), 3.48 (dd, ^{3}J 3.3; 9.4 Hz, 7H, H_2), 2.05 (s, 21H, CH_3); ^{13}C NMR 170.28 (CO), 138.91 (C_{1arom}), 138.16 (C_{1arom}), 128.13–126.99 (C_{arom}), 98.91 (C_1), 80.38 (C_4), 79.72 (C_2), 78.59 (C_3), 75.35 (Phe-CH_2), 73.03 (Phe-CH_2), 69.88 (C_5), 63.48 (C_6), 20.90 (CH_3CO).

2.2.4. Synthesis of heptakis(6-O-acetyl)-β-cyclodextrin (6Ac-CD)

A solution of **19** (2.0 g; 0.74 m*M*) in methanol–acetic acid (300 ml; 20:1, v/v) was hydrogenated in the presence of 10% Pd–C (3.95g) with 1 atm H_2 for 12 h at room temperature. Then it was filtered through a bed of Kieselgur. The solvents were removed in the presence of silica gel (3 g). Column chromatography of the residue on silica gel [ethyl acetate–methanol, 7:3 (v/v), R_F 0.2] gave 6Ac-CD (0.58 g; 55%). $C_{56}H_{84}O_{42}$ fast atom bombardment–mass spectrometry (FAB–MS) 1428.3; ^{1}H NMR 4.83 (d, ^{3}J 3.7 Hz, 7H, H_1), 4.20 (AB, ^{2}J 11.2 Hz, 14H, H_6), 3.81 (dd, ^{3}J 6.3 10.0 Hz, 7H, H_5), 3.61 (t, ^{3}J 9.3 Hz, 7H, H_3), 3.38 (dd, ^{3}J 3.7; 8.5 Hz, 7H, H_2), 3.33 (t, ^{3}J 9.3 Hz, 7H, H_4), 1.94 (s, 21H, CH_3); ^{13}C NMR 169.83 (CO), 102.02 (C_1), 82.16 (C_4), 72.84 (C_2), 72.11 (C_3), 69.11 (C_5), 63.07 (C_6), 20.28 (CH_3CO).

2.3. Capillary electrophoresis

Work was carried out on a Bio-Rad HPE CE system, using a Bio-Rad coated capillary with an internal diameter of 25 μm and a total length of 20 cm. Samples were loaded by electromigration and separated at room temperature using a constant current of 12 μA and 0.1 *M* potassium dihydrogen

phosphate buffer. Higher concentrations of buffer caused the formation of crystals at the end of the capillary and 0.025 *M* buffer gave poorer resolution. This is in good agreement with Altria et al. [3] who found that high concentrations of buffer salt favoured resolution of clenbuterol enantiomers. In order to avoid electromigration dispersive effects, which cause peak distortion, it is important to work with a buffer of an ionic concentration at least 100 times greater than that of the analyte. Data were recorded at the analyte λ_{max} value with the Bio-Rad 800 HRLC system, version 2.30. Samples of compounds **1–15** were prepared by dissolution in acetonitrile–potassium dihydrogen phosphate (0.1 *M*, pH 3.0) (10:90, v/v) at about 0.5 mg/ml. Buffers at pH values of 3 to 6 for CE were all prepared from 0.1 *M* potassium dihydrogen phosphate using freshly distilled and filtered water and the pH was adjusted with orthophosphoric acid or 1 *M* sodium hydroxide. The buffer at pH 7.5 was a 50:50 (v/v) mixture of 0.1 *M* potassium dihydrogen phosphate and 0.1 *M* dipotassium hydrogen phosphate, adjusted with 1 *M* sodium hydroxide. All samples and buffers were filtered through a 0.2-μm cellulose nitrate filter (Whatman, Maidstone, UK) and centrifuged for 5 min before use.

2.4. NMR spectroscopy

For the measurement of cyclodextrin-induced shifts, sufficient quantities of compounds **1–15**, with and without the appropriate cyclodextrin, were dissolved in deuterated 0.1 *M* phosphate buffer equivalent to pH 4.5 to give 12 m*M* concentrations of each. Spectra were obtained on a Jeol EX400 FT NMR spectrometer operating at 399.05 MHz for ^{1}H. 64 scans with a frequency range of 5000 Hz were collected into 32K data points, giving a digital resolution of 0.31 Hz/point. An appropriate Gaussian function was applied before Fourier transformation to enhance spectral resolution. The temperature was controlled at 30(±1)°C and the residual protonated water signal was suppressed using homo-gated secondary irradiation (decoupler off during data acquisition). β-CD and Diac-CD were dried throughout in vacuo over P_4O_{10} before use and all chemical shifts were referenced to the HDO signal at 4.65 ppm.

2.5. Molecular modelling

The cyclodextrin structures were minimized on a Silicon Graphics Indigo Workstation using the force field program VA09A of Insight and Discover (Biosym, San Diego, CA, USA). The starting geometry was an X-ray structure of β-CD without any ligand [4]. The acetylated CDs were built using the same X-ray structure. One thousand iterations were run until the gradient of the obtained derivative was less than 0.1 kcal/Å.

3. Results and discussion

3.1. Synthesis of acetylated cyclodextrins

Since the goal of these investigations was to determine the influence of acetylation in different positions of β-CD or, in turn, the importance of each hydroxyl group for the chiral recognition, it was indispensable to have uniform material. Diac-CD was synthesised and purified as described previously. The synthesis of 6Ac-CD has been reported before but no analytical data was given [5,6]. In order to obtain a highly selective and full derivatisation, a modified synthesis pathway, similar to the one given in the patent [6], was chosen. Firstly, the 6-silylated compound **16** was benzylated in positions 2 and 3 using NaH and benzylbromide in THF to yield **17**. After purification of the dibenzylated derivative by means of column chromatography on silica gel, the blocking group in 6-position was removed with tetrabutylammonium fluoride in THF. Again, the product **18** had to be purified by column chromatography on silica gel. Subsequent acetylation of the 6 position was performed in acetic anhydride–pyridine. The working up was followed by column chromatography of the residue on silica gel which gave pure **19**. Finally, the benzyl groups in positions 2 and 3 were removed by catalytic hydrogenation with Pd–C (10%) in methanol–acetic acid. Column chromatography on silica gel using ethyl acetate–methanol (7:3, v/v) as the mobile phase yielded the

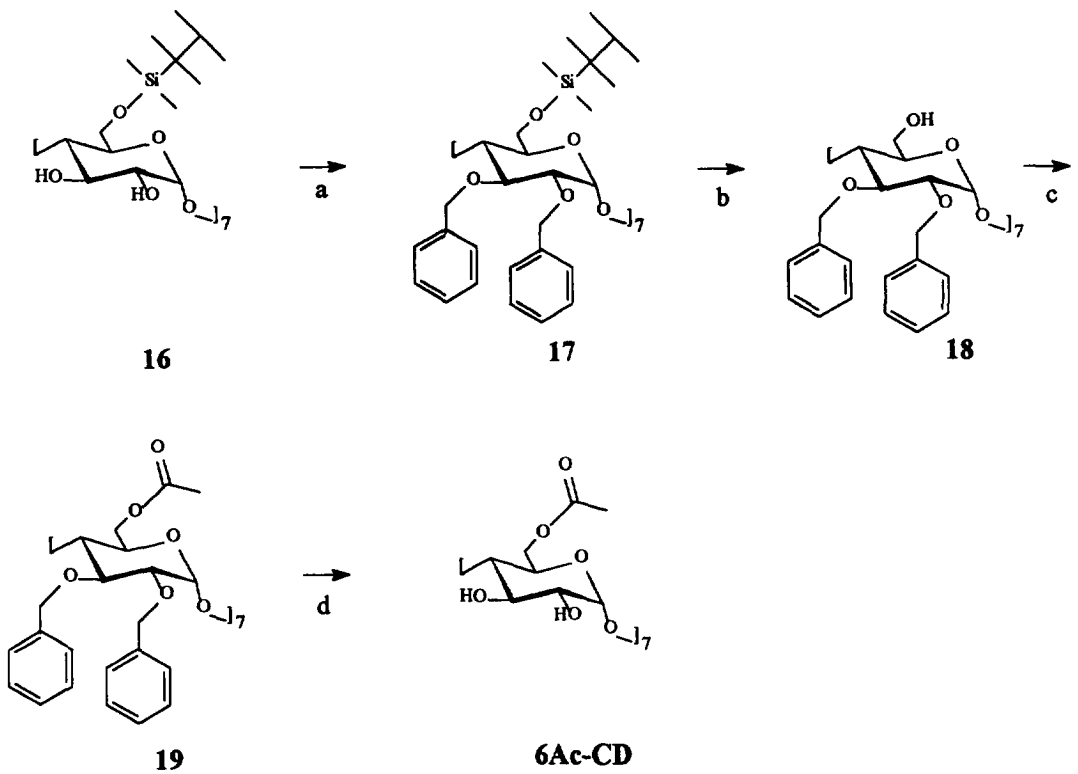
desired pure 6-acetylated CD (Fig. 2). The uniformity of 6Ac-CD was proven by ^{13}C NMR spectroscopy and FAB–MS.

3.2. CE studies

Within the framework of this study, the ability of each CD derivative to chirally discriminate using each phenethylamine was tested. For different CDs and derivatised CDs, maximum resolution was found to be sensitive to the pH value [7], CD concentration [8,9], buffer concentration [10] and the concentration of organic modifier [11]. Since no information on the behaviour of pure acetylated CDs was known, each parameter was systematically varied.

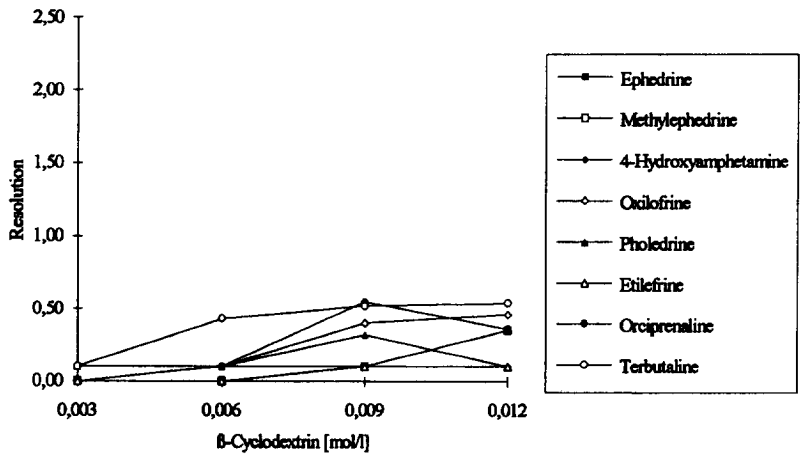
3.2.1. Variation of the molar concentration of the CDs

For β-CD, small increases in resolution were observed for some compounds over the molar concentration range of 0.003 to 0.012 *M* (Fig. 3a). Resolution values were, in general, low (0.3 to 0.5). Due to the low solubility of the 6-acetylated CD, only the 3 m*M* concentration was examined, and

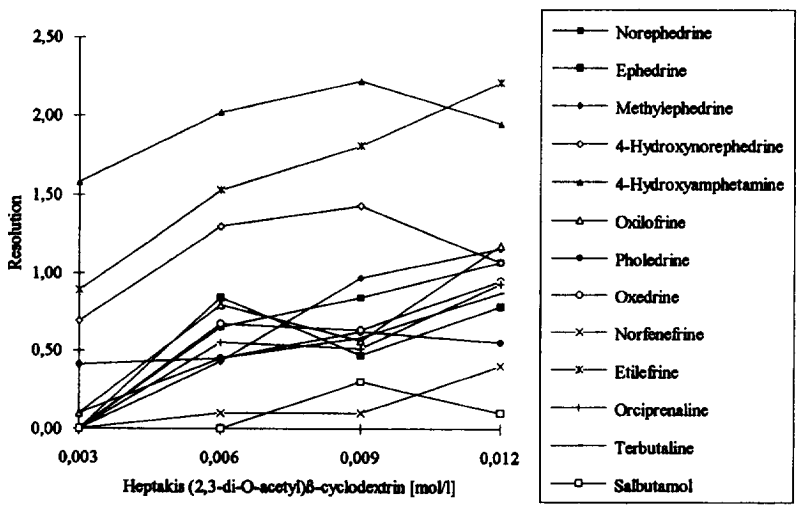


a: benzylbromide, NaH, THF
b: N(C4H9)4F, THF
c: acetic anhydride, pyridine
d: Pd/C 10%, MeOH, acetic acid

Fig. 2. Synthesis pathway for *heptakis*(6-O-acetyl)-β-cyclodextrin.



(a)



(b)

Fig. 3. (a) Resolution of various phenethylamines using different concentrations of β-CD. Only the compounds that showed resolution are shown here. (b) Resolution of various phenethylamines using different concentrations of Diac-CD.

only orciprenaline (**11**), terbutaline (**12**), pholedrine (**7**) and 4-hydroxyamphetamine (**5**) displayed small values of resolution (data not shown). At this concentration, β-CD provided slightly lower values whilst Diac-CD generally gave better values for the same compounds. Resolution for most compounds increased using the 2,3-diacetylated CD up to a concentration of 0.012 *M*, with the notable exceptions of 4-hydroxynorephedrine (**4**), 4-hydroxyamphetamine (**5**) and salbutamol (**15**), which gave maximum resolution at 0.09 *M* (Fig. 3b).

3.2.2. Addition of an organic modifier

For most of the compounds that displayed some resolution with β-CD, the addition of 5% methanol produced little change, whilst 10% methanol reduced

or removed the resolution. Pholedrine (**7**) was the only compound whose resolution (0.35) remained unaffected, even at 20% methanol (Fig. 4a). For 6Ac-CD the addition of 5% methanol removed all resolution. With Diac-CD, small significant losses of resolution were observed for most compounds over the methanol range tested (Fig. 4b). However, 4-hydroxyamphetamine (**5**), pholedrine (**7**) and salbutamol (**15**) showed improved resolutions upon the addition of up to 10% methanol, followed by a decrease at 20% methanol.

The addition of acetonitrile either removed or considerably reduced resolution for all compounds with β-CD, 6Ac-CD and Diac-CD, even at the 5% level. With THF, all resolution was lost at the 5% level using β-CD and 6Ac-CD and for most compounds with the Diac-CD. Interestingly, etilefrine (**10**) showed the strongest loss of resolution, whereas

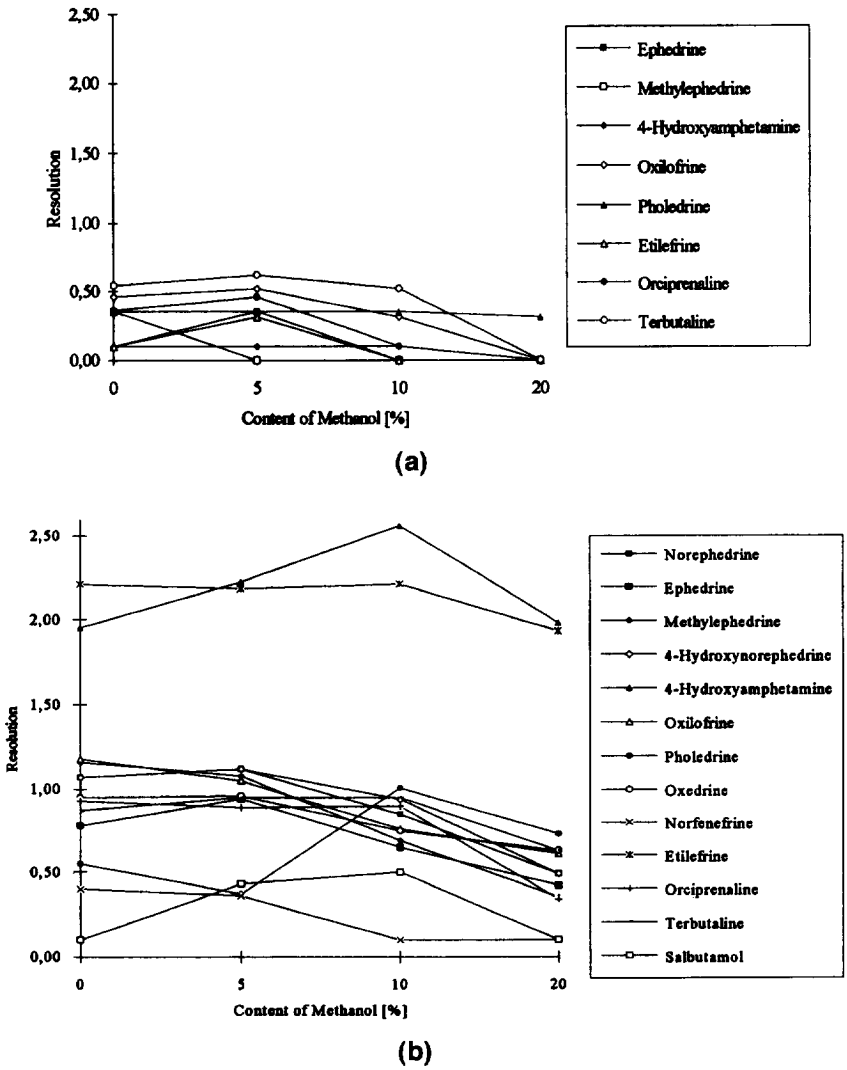


Fig. 4. (a) Resolution of various phenethylamines using 12 m*M* β-CD with different contents of methanol. (b) Resolution of various phenethylamines using 12 m*M* Diac-CD with different contents of methanol.

pholedrine (**7**) remained almost unaffected at 5% (data not shown).

3.2.3. Variation of the pH value

The compounds studied have pK_a values between 8.6 and 9.5 and, using a coated capillary that has no electroendosmotic flow (eof), a positive charge on the amine is essential for mobility. Thus, acidic pH values were necessary. Resolutions were found to vary little between pH 3 and 6, as reported previously [2], and so a pH of 4.5 was selected.

Taking all the variations in the parameters studied above into consideration, the optimised CE conditions were found to be a CD concentration of 12 m*M*, no organic modifier, a buffer strength of 0.1 *M* and a pH of 4.5. The observed resolution values using these optimised CE conditions are given in Table 2. Since the 6-acetylated CD could only be applied at a concentration of 3 m*M*, due to its limited solubility, no exact comparison with the other two CDs was possible.

3.2.4. Contributions of functional groups to the resolution using optimised CE conditions

Pairwise comparison of compounds within the Groups I (comp. **1–7**) and II (**8–15**) reveals which functional groups of the phenethylamines are important for chiral recognition.

3.3. Compounds possessing a C2-methyl group (Group I, 1–7)

3.3.1. Influence of para-hydroxy function on the phenyl ring

The introduction of the *para*-OH group significantly improved the resolution using Diac-CD; for example, ephedrine (**2**; 0.78) and oxilofrine (**6**; 1.18); norephedrine (**1**; 1.07) and 4-hydroxynorephedrine (**4**; 1.80). For the same examples, β-CD displayed little or no change.

3.3.2. Influence of N-methylation

The introduction of a single N-methyl group reduced the resolution using Diac-CD; for example, norephedrine (**1**; 1.07) and ephedrine (**2**; 0.78); 4-hydroxynorephedrine (**4**; 1.80) and oxilofrine (**6**; 1.18); 4-hydroxyamphetamine (**5**; 1.95) and pholedrine (**7**; 0.55). However, further methyl substitution of ephedrine (**2**) to give the dimethylamino compound methylephedrine (**3**) increased the resolution (0.78/1.16). In contrast, with β-CD, N-methylation produced little or no effect.

3.3.3. Influence of the hydroxyl group at the C1 position of the side chain

The effect of the presence or absence of the secondary alcohol group on C1 of the side chain is inconclusive with the small number of examples available; 4-hydroxynorephedrine (**4**; 1.80) and 4-

Table 2
Resolution of various phenethylamines using different cyclodextrins under optimised CE conditions

Number	Compound	β-CD	6Ac-β-CD	2,3-Diac-β-CD
1	Norephedrine	–	–	1.07
2	Ephedrine	0.35	–	0.78
3	Methylephedrine	0.35	–	1.16
4	4-Hydroxynorephedrine	<0.3	–	1.80
5	4-Hydroxyamphetamine	<0.3	<0.3	1.95
6	Oxilofrine	0.46	–	1.18
7	Pholedrine	<0.3	<0.3	0.55
8	Oxedrine	–	–	0.95
9	Norfenefrine	–	–	0.40
10	Etilefrine	<0.3	–	2.21
11	Orciprenaline	0.33	0.31	0.93
12	Terbutaline	0.54	0.36	0.87
13	Noradrenaline	–	–	–
14	Isoprenaline	–	–	–
15	Salbutamol	–	–	<0.3

hydroxyamphetamine (**5**; 1.95); oxilofrine (**6**; 1.18) and pholedrine (**7**; 0.55). With β-CD, the lower resolution values were almost unaffected.

*3.4. Compounds without a C2-methyl group (Group II, **8–15**)*

These compounds only differ in the pattern of the hydroxyl groups on the phenyl ring and in the alkyl residues on the nitrogen ranging from hydrogen to tertiary butyl. Thus, the influence of the N-alkyl group on the resolution will be discussed with respect to the phenyl substitution.

3.4.1. Meta-phenyl-substituted compounds

For 3-hydroxy compounds, resolution is dramatically increased by N-ethylation using Diac-CD; for example, norfenefrine (**9**; 0.40) and etilefrine (**10**; 2.21). With β-CD the improvement is marginal (nil to less than 0.3).

3.4.2. 3′,4′-Dihydroxy-substituted compounds

The presence of 3,4-dihydroxy substitution caused a complete loss of resolution using Diac-CD; for example, norfenefrine (**9**; 0.4) and noradrenaline (**13**; nil); this is also demonstrated by a comparison between etilefrine (**10**; 2.21) and isoprenaline (**14**; nil), in which the difference in N-substitution (N-ethyl to N-isopropyl) should have improved the resolution. The same pattern is observed with β-CD.

3.4.3. 3′,5′-Dihydroxy-substituted compounds

In contrast, resolution can be restored when the hydroxyl pattern of substitution is changed from 3,4-dihydroxy to 3,5-dihydroxy using Diac-CD; for example, isoprenaline (**14**; nil) and orciprenaline (**11**; 0.93). The effect with β-CD is less dramatic but showed a small improvement for the same examples (nil to 0.33). Terbutaline (**12**; 3,5-dihydroxy, N-tertiary butyl) was also well resolved with Diac-CD (0.87) and β-CD (0.54). On the other hand, salbutamol (**15**; 3-hydroxymethyl, 4-hydroxy, N-tertiary butyl) gave poor resolution with Diac-CD (<0.3) and no resolution with β-CD.

Taken together, single hydroxyl substitution on the aromatic ring (3 or 4 position) is more beneficial for resolution than double- or no substitution. For the analytes with a *para*-hydroxy group on the aromatic ring, maximum resolution was obtained in the presence of a C2 methyl substituent and the absence of a benzylic hydroxy group. In the case of the *meta*-hydroxy-substituted analytes, a bulky group on the nitrogen is necessary to achieve high resolution.

3.5. NMR spectroscopy studies

The use of cyclodextrins as chiral resolution agents in NMR spectroscopy has been well established in the last decade [12]. The changes in chemical shifts of both ligands and hosts upon complexation, as well as signal splitting of the racemic ligands, can provide information about the geometry of the complexes. In order to be able to compare the NMR results with the CE findings, similar conditions were chosen. All spectra were measured in 0.1 *M* dihydrogen phosphate buffer in ^2H_2O equivalent to pH 4.5.

3.5.1. Induced chemical shifts in cyclodextrins

The chemical shift difference between free and complexed cyclodextrins induced by phenethylamines show that the signals for the β-CD protons H3″ and H5″, which are both located inside the cavity (Fig. 5), are shifted significantly more than the other proton signals. In the case of β-CD, the $\Delta\delta$ values of the H5″ proton signals are mostly larger than the $\Delta\delta$ values of the H3″ proton signals. This indicates a deep penetration of the analyte into the cavity. Salbutamol (**15**) is the only compound that did not show significant interaction with H5″, indicating that the molecule does not enter the cavity completely, due to the large CH_2-OH group in the *meta*-position of the aromatic ring. The analytes that cause the highest $\Delta\delta$ values are the ephedrine derivatives and the compounds lacking the benzylic

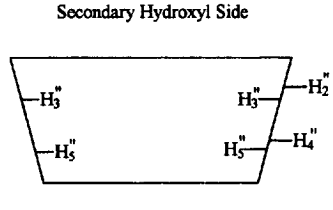


Fig. 5. Display of the location of the cyclodextrin protons H3″ and H5″ in the macrocycle.

hydroxy group, 4-hydroxyamphetamine (**5**) and pholedrine (**7**). This may indicate that these compounds are included more deeply in the cavity than the other racemates (see Fig. 6a). A possible reason may be that they are not able to form hydrogen bonds with the substituents on the wider rim of the CD, which could hold other ligands nearer the cavity entrance.

A very similar pattern is visible for 6Ac-CD, however, in contrast to β-CD, the compounds noradrenaline (**13**) and isoprenaline (**14**) do not show any significant shift of any cyclodextrin resonance, indicating a loss of interaction (see Fig. 6b). Salbutamol (**15**) only causes a substantial chemical shift change for H5″ of 6Ac-CD. Furthermore, terbutaline (**12**) induces a larger shift in the H6a″ than in the H5″ resonance. The most impressive changes in chemical shifts are induced by the same compounds as with β-CD.

The $\Delta\delta$ values for the proton signals of the Diac-CD are much smaller in comparison to β-CD and 6Ac-CD (see Fig. 6c). Significant changes of the chemical shift are only visible for the H5″ proton signal induced by 4-hydroxyamphetamine (**5**) and pholedrine (**7**), both of which lack a benzylic hydroxy group. The absence of significant chemical

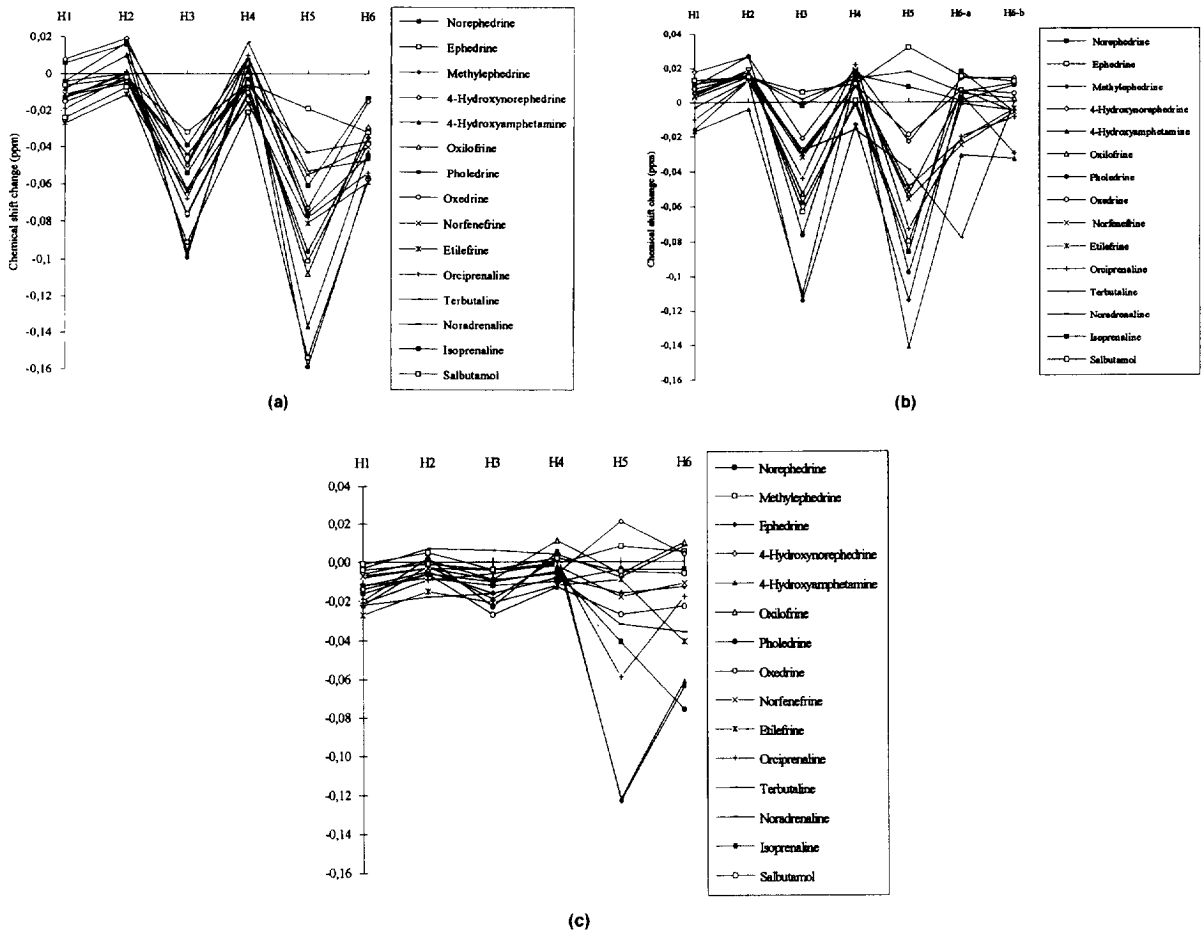


Fig. 6. (a) Chemical shift changes of the β-CD proton signals (ppm) induced by various phenethylamines. (b) Chemical shift changes of the 6Ac-CD proton signals (ppm) induced by various phenethylamines. (c) Chemical shift changes of the Diac-CD proton signals (ppm) induced by various phenethylamines.

shift changes for the H3″ proton signals is difficult to explain. Additionally, orciprenaline (**11**) causes changes in the H5″ resonance. The other phenethylamines do not cause any significant chemical shift change of the Diac-CD proton signals. A possible reason for this observation may be a generally weak interaction between Diac-CD and the phenethylamines caused by a different arrangement of the phenethylamines in the cavity of the Diac-CD (in comparison with native and 6-acetylated CD). Yamashoji et al. [13] made a similar observation with the same CD but different ligands. They tried to explain this behaviour with a penetration of the cavity of the CD by the aromatic moiety of the ligand from the primary hydroxyl side. This contrasts with spectroscopic evidence we have found using the ROESY experiment [14]. Thus, it seems that weak binding between CD and ligand or a higher flexibility of the secondary side through acetylation are more appropriate to explain this observation.

3.5.2. Induced chemical shifts in phenethylamines

The changes in phenethylamine chemical shifts induced by complexation with the three cyclodextrins are reported in Tables 3–5. Inclusion of the phenyl ring in the cyclodextrin cavity is demonstrated by changes in the chemical shifts of the aromatic signals. In the spectra of the β-CD complexes, the aromatic signals are shifted upfield by up to 0.08 ppm, except for H6″ in some compounds that have non-symmetrical substitution of the phenyl ring, eg., norfenefrine, isoprenaline and salbutamol (**9**, **14** and **15**). In these cases, H6′ is shifted downfield by up to 0.02 ppm and this is consistent with the proposal by Li and Purdy [15] and Mularz et al. [16] that the phenyl ring is tilted within the cyclodextrin cavity. In symmetrically substituted phenyl rings, magnetic equivalence is maintained on inclusion, showing that the ring must maintain some free rotation inside the cavity. Signals from the side chain show greater shifts of up to 0.18 ppm indicating interaction with the rim of the macrocycle.

In complexes with the 6-acetylated CD, smaller shifts are induced in the phenethylamine with the aromatic signals moving by up to 0.06 ppm (a mixture of upfield and downfield shifts) and the side-chain resonances by up to 0.11 ppm.

Again, in the Diac-CD complexes, the changes in position of the aromatic signals are generally less (up to 0.04 ppm in the aromatic region with varying direction and up to 0.09 ppm for the aliphatic signals). However, two compounds, 4-hydroxyamphetamine and pholedrine (**5** and **7**), show extraordinarily large shifts upon complexation with this derivatised cyclodextrin. In **5**, the resonance for the *ortho* aromatic protons moves upfield by 0.15 ppm and the side chain CH_2 signals display upfield shifts of 0.17 and 0.13 ppm, respectively. The *meta*-phenyl proton in pholedrine (**7**) moves upfield by 0.12 ppm, while the methylene resonance shifts are 0.19 and −0.03, respectively. These compounds lack the hydroxyl group at C1 and its removal appears to allow a stronger interaction with the macrocycle, thus creating larger induced shifts. The free amine in **5** is methylated in **7** and this additional functional group is apparently sufficient to cause a reorientation of the phenyl ring in the cavity such that the *meta* protons experience the greatest change in chemical shift rather than the *ortho* protons, as found in **5**.

3.5.3. Resolution of signals in phenethylamines

NMR resolution is represented by the difference between the chemical shifts for the same proton in each enantiomer ($|\delta_R - \delta_S|$). These resolution parameters are shown in parentheses in Tables 3–5. The pattern and size of splitting in the side-chain signals varies from compound to compound and presumably depends on how well this part of the molecule interacts with the cyclodextrin rim. For Group I in the presence of β-CD, an *N*-methyl group is required for duplication of the aromatic signals. A *para*-hydroxyl group causes larger splittings in this region, which are further increased by removal of the side-chain hydroxyl group. In complexes with 6Ac-CD, a 4′-OH group abolishes aromatic splitting in Group I and removal of either the side-chain hydroxy or the N-methyl group allows duplication of H1. In 6Ac-CD the primary hydroxyl group (at C6″) is acetylated, thus preventing any hydrogen bond donation from the CD to the ligand *para*-hydroxyl group and this may account for the loss of resolution in the aromatic region. Complexation with Diac-CD generally causes larger splittings in both the aromatic and side-chain signals than found in the other CD complexes.

For complexes of Group II with β-CD or 6Ac-CD,

Table 3
Chemical shift changes ($\Delta\delta$ values in ppm) of the phenethylamines induced by β-cyclodextrin

Number	H2′	H3′	H4′	H5′	H6′	H1	H2	R_3	2-CH_3(R_2)
1	−0.025 [a] for H2′ to H6′					0.062 (–)	[a]	–	−0.041 (–)
2	−0.002 ([a]) for H2′ to H6′					0.072 (–)	−0.096 (0.018)	CH_3 0.046 (–)	−0.027 (–)
3	−0.032 ([a]) for H2′ to H6′					−0.032 (–)	0.015 (–)	CH_3 0.000 (–)	−0.026 (–)
4	−0.012 (–)	−0.057 (–)	–	−0.057 (–)	−0.012 (–)	0.012 (–)	[a]	–	−0.053 (–)
5	−0.044 (–)	−0.065 (–)	–	−0.065 (–)	−0.044 (–)	0.178 (–)	−0.054 ([a])	–	−0.036 (–)
						−0.103 (–)			
6	−0.010 (0.005)	−0.058 (0.005)	–	−0.058 (0.005)	−0.010 (0.005)	0.086 (–)	−0.100 (0.017)	CH_3 0.052 (0.002)	−0.015 (0.003)
7	−0.032 (0.021)	−0.043 (0.021)	–	−0.043 (0.021)	−0.032 (0.021)	0.141 (–)	[a]	0.074 (0.004)	0.000 (0.024)
						−0.078 (–)			
8	0.000 (–)	−0.024 (–)	–	−0.024 (–)	0.000(–)	0.008 (–)	−0.037 ([a])	CH_3 0.010 (–)	–
9	−0.013 (–)	–	−0.027 (–)	−0.040 (0.003)	0.023 (–)	0.000 (–)	−0.024 (0.013)	–	–
							−0.044 (0.009)		
10	−0.015 (0.005)	–	−0.032 ([a])	−0.053 (0.005)	−0.027 (–)	0.005 (–)	[a]	CH_2 [a]	–
								CH_3 0.016 (0.010)	
11	−0.051 (0.006)	–	−0.045 (0.005)	–	−0.051 (0.006)	−0.003 (–)	−0.071 (–)	CH 0.014 (–)	–
							0.068 (–)	CH_3 0.021 (–)	
								CH_3 0.023 (0.008)	
12	−0.080 (0.006)	–	−0.068 (0.005)	–	−0.080 (0.006)	−0.009 (–)	−0.114 (0.032)	Bu^t 0.024 (–)	–
							−0.119 (0.032)		
13	−0.018 (–)	–	–	−0.041 ([a])	−0.012 (–)	−0.002 (–)	−0.034 (–)	–	–
							−0.041 (–)		
14	0.001 (–)	–	–	−0.041 (–)	0.019 (–)	0.007 (–)	−0.051 (–)	CH 0.007 (–)	–
							0.024 (–)	CH_3 0.012 (–)	
								CH_3 0.011 (–)	
15	0.000 (–)	CH_2OH 0.000(–)	–	−0.007 (–)	0.001 (–)	0.008 (–)	−0.033 (–)	Bu^t 0.006 (–)	–
							−0.005 (–)		

A dash in parentheses indicates no signal splitting and [a] indicates that full analysis was not possible due to signal overlap.
The actual chemical shift of each enantiomer can be obtained by adding or subtracting half ($|\delta_R - \delta_S|$) to the chemical shift of the uncomplexed drug.

Table 4
Chemical shift changes ($\Delta\delta$ values in ppm) of the phenethylamines induced by 6Ac-CD

	H2′	H3′	H4′	H5′	H6′	H1	H2	R_3	2-CH_3 (R_2)
1	−0.035 [a] for H2′ to H6′					0.037 (−)	[a]	−	−0.032
2	0.008 [a] for H2′ to H6′					0.041 (−)	−0.019 (0.009)	0.032 (−)	0.003 (−)
3	−0.031 [a] for H2′ to H6′					−0.001 (−)	[a]	−0.005 (−)	−0.029 (−)
4	0.000 (−)	−0.020 (−)	−	−0.020 (−)	0.000 (−)	0.045 (−)	[a]	−	0.059 (0.002)
5	−0.031 (−)	−0.043 (−)	−	−0.043 (−)	−0.031 (−)	−0.042 (0.034) 0.049 (0.034)	−0.016 (−)	−	−0.025 (−)
6	0.009 (−)	−0.010 (−)	−	−0.010 (−)	0.009 (−)	−0.049 (−)	−0.018 ([a])	0.029 (0.004)	0.006 (−)
7	−0.008 (−)	0.023 (−)	−	0.023 (−)	−0.008 (−)	0.087 (0.034) −0.027 (0.033)	−0.014 (−)	0.050 (−)	0.013 (−)
8	0.013 (−)	−0.001 (−)	−	−0.001 (−)	0.013 (−)	0.014 (−)	[a]	0.018 (−)	−
9	0.008 (−)	−	0.002 (−)	−0.015 (−)	0.002 (−)	−0.014 (−)	0.001 (0.032) −0.012 (0.032)	−	−
10	0.019 (−)	−	0.008 (0.002)	−0.007 (0.004)	0.015 (0.002)	0.029 (−)	−0.016 (0.006) −0.028 (−)	CH_2 0.035 (−) CH_3 0.034 (−)	− −
11	−0.029 (−)	−	0.014 (−)	−	−0.029 (−)	0.015 (−)	−0.047 (0.010) −0.039 (0.029)	CH 0.028 (−) CH_{3a} 0.038 (0.003) CH_{3b} 0.029 (0.003)	−
12	−0.063 (0.010)	−	0.003 (−)	−	−0.063 (0.010)	0.005 (−)	−0.096 (0.031) −0.108 (0.032)	Bu^t 0.028 (−)	−
13	0.009 (−)	−	−	0.006 (−)	−0.009 (−)	0.013 (−)	0.009 (−) 0.002 (−)	−	−
14	0.008 (−)	−	−	0.004 (−)	0.009 (−)	0.044 (−)	−0.006 (−) 0.004 (−)	CH 0.025 (−) CH_{3a} 0.020 (−) CH_{3b} 0.019 (−)	−
15	0.011 (−) CH_2OH 0.014 (−)	−	−	0.009 (−)	0.011 (−)	0.010 (−)	−0.009 (−) 0.030 (−)	Bu^t 0.013 (−)	

A dash in parentheses indicates no signal splitting and [a] indicates that full analysis was not possible due to signal overlap.
The actual chemical shift of each enantiomer can be obtained by adding or subtracting half ($|\delta_R - \delta_S|$) to the chemical shift of the uncomplexed drug

Table 5
Chemical shift changes ($\Delta\delta$ values in ppm) of the phenethylamines induced by Diac-CD

	H2	H3	H4	H5	H6	H1	H2	R_3	2-CH_3 (R_2)
1	−0.025 [a] for H2′ to H6′					0.015 (0.082)	−0.001 (0.019)	–	0.021 (–)
2	0.026 [a] for H2′ to H6′					0.063 (0.040)	0.050 (0.036)	CH_3 0.041 (0.026)	0.016 (–)
3	0.05 [a] for H2′ to H6′					[a]	0.090 (0.031)	CH_3 0.072 (0.009)	0.063 (–)
4	0.007 (0.025)	−0.027 (0.017)	–	−0.027 (0.017)	0.007 (0.025)	0.045 (0.066)	0.018 ([a])	–	−0.010 (–)
5	0.148 (0.021)	0.025 (0.038)	–	0.025 (0.038)	0.148 (0.021)	0.170 (–)	[a]	–	0.139 (0.016)
						0.134 (–)			
6	0.039 (0.021)	−0.010 (0.018)	–	−0.010 (0.018)	0.039 (0.021)	0.062 (0.046)	−0.042 (0.037)	CH_3 −0.043 (0.031)	0.019 (–)
7	0.028 (0.037)	−0.123 (0.021)	–	−0.123 (0.021)	0.028 (0.037)	0.191 (0.039)	[a]	0.136 (0.034)	0.000 (0.024)
						−0.028 (0.049)			
8	0.015 (0.006)	−0.007 (0.003)	–	−0.007 (0.003)	0.015 (0.006)	0.030 (0.018)	0.001 (–)	CH_3 0.022 (0.007)	
							−0.003 (–)	–	
9	0.009 (–)	–	−0.001 (0.006)	−0.004 (–)	0.023 (0.016)	0.038 (0.030)	0.043 (0.013)	–	–
							−0.008 (0.015)		
10	0.011 (0.006)		0.006 (0.006)	−0.019 (0.016)	0.029 (0.018)	0.081 (0.067)	[a]	CH_2 [a]	–
								CH_3 0.034 (0.033)	
11	−0.005 (–)	–	−0.011 (0.008)	–	−0.005 (–)	~0.025 ([a])	0.010 (0.004)	CH 0.039 (0.016)	–
							0.005 (0.032)	CH_3 0.034 (0.005)	
								CH_3 0.029 (–)	
12	0.002 (–)	–	−0.015 (–)	–	0.002 (–)	[a]	0.031 (0.032)	Bu^t 0.046 (–)	–
							0.031 (0.032)		
13	−0.005 (–)	–	–	−0.026 (–)	−0.004 (–)	~0.022 ([a])	0.033 (–)	–	–
							−0.018 (–)		
14	−0.025 (0.003)	–	–	−0.015 (–)	0.006 (0.005)	[a]	0.003 (–)	CH 0.030 (0.016)	–
							0.019 (–)	CH_3 0.025 (0.008)	
								CH_3 0.025 (0.008)	
15	0.011 (–)	CH_2OH 0.004(–)	–	0.002 (–)	0.010 (–)	~0.014 ([a])	~0.032 ([a])	Bu^t 0.036 (0.015)	–

A dash in parentheses indicates no signal splitting and [a] indicates that full analysis was not possible due to signal overlap.
The actual chemical shift of each enantiomer can be obtained by adding or subtracting half ($|\delta_R - \delta_S|$) to the chemical shift of the uncomplexed drug.

no splitting of any signal is observed in compounds **13**, **14** and **15** with the 3′,4′-substitution pattern in the phenyl ring, which compares with the lack of resolution obtained with CE in these cases. In the second group of compounds, no duplication of the H1 signal is observed in the presence of β-CD or 6Ac-CD and splitting of H2 and the N-alkyl group is variable. In complexes of Group II with Diac-CD, the aromatic signals of the ligands are more widely split in correlation with the CE results, but duplication is less likely to occur with 3′,4′-substitution of the phenyl ring. Splitting of the H1 signals may be

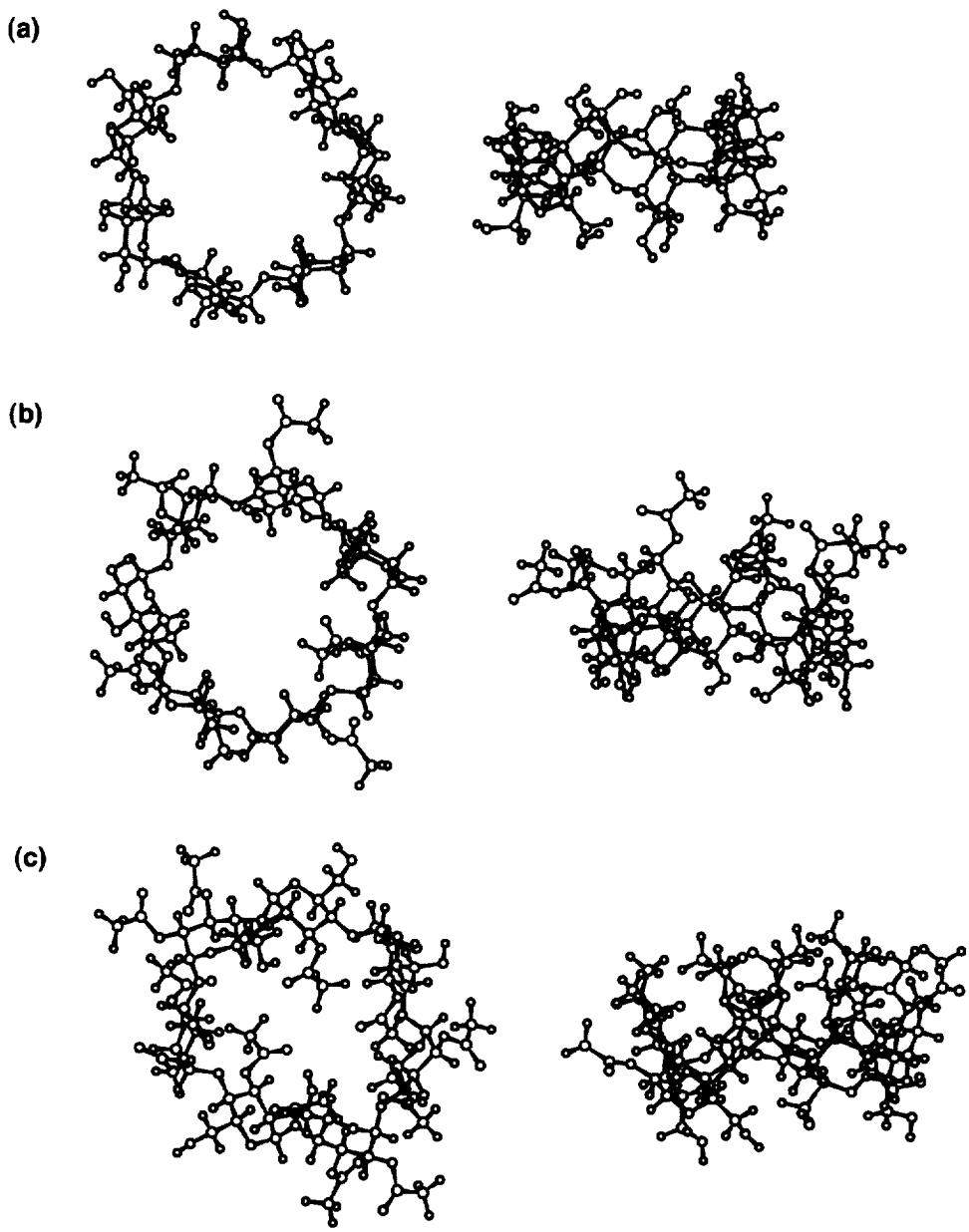


Fig. 7. Structures of the geometry-optimised cyclodextrins. (a) β-CD, (b) Diac-CD, (c) 6Ac-CD.

seen when the signal is not obscured by cyclodextrin peaks and duplication of the other side-chain signals is again variable.

Some of the NMR results obtained may be explained by simple molecular models of each cyclodextrin. An X-ray structure of β-CD was minimised using Discover software and displayed with Insight. The acetylated cyclodextrins were built manually from the β-CD structure and also were minimised. Fig. 7 shows views of the minimised structures through the cavity of each cyclodextrin and from the side. Comparison of the figures shows that acetylation of either the primary or the secondary hydroxyl groups can give conformations with distortions of the torus. Steric interactions apparently expand the substituted rim and in the case of the 2′,3′-disubstituted compound in which the effect would be expected to be more severe, distortion of the circular shape of the cavity occurs as well. This distortion could prevent a good fit of the ligand in the cavity and the generally weaker NMR shifts observed on complexation with Diac-CD may reflect this less effective binding. On the other hand, the distorted cavity may cause the binding of a ligand to depend more critically on the correct molecular geometry and thus better discrimination of enantiomers would be expected, as observed by NMR and CE. In contrast, the primary rim is wider in 6Ac-CD, giving a looser fit than in β-CD and weaker interactions (smaller shifts than β-CD) and also offering less discrimination between enantiomers.

4. Conclusion

The NMR evidence presented here suggests that the phenyl ring of the phenethylamine ligand is inserted in the cavity and that the side chain interacts substantially with the secondary hydroxyl rim of the macrocycle. Overall, Diac-CD is a better chiral discriminator than the other two cyclodextrins, but it interacts less strongly with the ligands. Preliminary measurements show that Diac-CD has significantly lower binding constants than the other two CDs [14]. This is in good agreement with Penn et al. [17] who also found that the CD selector with the weakest binding constant gave the largest chiral selectivity.

Acknowledgments

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