New single-isomer chiral selector for capillary electrophoresis: the highly water-soluble *heptakis*(2-*N*,*N*-dimethylcarbamoyl)-β-cyclodextrin

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Abstract

A new single isomer β-cyclodextrin, the *heptakis*(2-*N*,*N*-dimethylcarbamoyl)-β-cyclodextrin (HDMC-β-CD), has been synthesized and spectroscopically characterized. The outstanding feature of the new neutral β-CD derivatives is their high solubility in water (>100 mM) and methanol. The resolution of chiral drugs can take advantage of this property. The resolution power of the new HDMC-β-CD is demonstrated by a selection of acidic and basic compounds using standard conditions. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

In the last decade, cyclodextrins (CDs) became well established as powerful tools for the chiral discrimination of enantiomers by means of capillary electrophoresis (CE) [1–3]. This technique can be used for pharmacopoeia purposes, such as the determination of the enantiomeric purity of single-isomer drugs (e.g., Refs. [4–7]) or for bioanalytical purposes, such as the investigation of the metabolism and pharmacokinetic of chiral drugs (e.g., Refs. [8–11]). Besides the native CDs a wide range of CD derivatives was introduced to enhance the resolution power of the CDs.

(2,3-Di-*O*-methyl)- and (2,3,6-tri-*O*-methyl)-β-cyclodextrins were firstly applied in an electromigration method, namely isotachophoresis, to separate the enantiomers of racemic compounds [12]. These neutral CD derivatives are nowadays as widely used in CE [1–3,13] as the hydroxyethyl- and hydroxypropyl substituted CDs [2,14,15]. In addition, negatively and positively charged CDs appeared on the market, e.g., the sulfobutyl ether β-CD, introduced by Stobaugh and co-workers [16–18] and the carboxymethylated β-CD [19] on the one hand and the methylamino substituted β-CD derivatives [20–23] on the other hand. Most of the CDs used so far are complex mixtures with varying degrees and loci of substitution. Since the batch-to-batch reproducibility in chiral analysis cannot always be guaranteed

[24,25] and the elucidation of the chiral recognition is difficult when using such mixtures, the neutral, single-isomer *heptakis*(2,3-di-*O*-acetyl)-β-cyclodextrin was synthesized [26], which surpassed the native β-CD in resolution power [27]. In addition, negatively charged, single-isomer CDs, e.g., *heptakis*(2,3-di-*O*-acetyl-6-sulfato)-β-, *octakis*(2,3-di-*O*-acetyl-6-sulfato)-γ- and *heptakis*(6-sulfato)-β-cyclodextrin, were developed by Vigh and co-workers [28–32], which were found to be superior in the separation of basic and neutral chiral compounds.

As a part of a greater project dealing with the development of neutral, single-isomer cyclodextrin derivatives on the one hand and the elucidation of the mechanism of chiral recognition on the other hand, it was aimed to synthesize *heptakis*(2- or 3-derivatized)β-CD derivatives. Since the synthesis of the corresponding acetyl compounds was not successful [33] due to the isomeric mixtures obtained from the synthesis and the instability of the 2- or 3-acetylated β-CDs, the corresponding single isomeric *heptakis*(2-*N,N*-dimethylcarbamoyl)-β-cyclodextrin (HDMC-β-CD) was synthesized which is similar to the acetylated CD with regard to the ability to form hydrogen bonds. In order to find out whether HDMC-β-CD is principally able to separate the enantiomers of racemates, a series of randomly chosen racemic drugs were subjected to the HDMC-β-CD modified CE.

2. Experimental

2.1. Synthesis of the heptakis(2-N,N-dimethylcarbamoyl)-β-cyclodextrin

2.1.1. Chemicals

Pyridine was dried over and distilled from CaH_2, dichloromethane was distilled from $CaCl_2$. All other solvents were distilled throughout. 4-Dimethylaminopyridine (DMAP) (Merck, Darmstadt, Germany), dimethylcarbamoylchloride (DMCC) (Aldrich, Steinheim, Germany), chlorodimethyl-1,1,2-trimethylpropylsilane (Merck), triethylamine (TEA) (Merck), boron trifluoride diethyletherate complex and argon (Messer-Griesheim, Germany) were used without further purification. β-CD was a gift from

the Consortium für Elektrochemische Industrie (Munich, Germany).

2.1.2. Chromatography

Reversed-phase chromatography was carried out with a medium-pressure LiChroprep RP-18 column (column dimensions 47×5.5 cm, flow-rate 10 ml/min; Merck) and RP-18 plates (Merck), ion-exchange chromatography was performed using Amberlite MB-3 (column dimensions 15×2.5 cm; Merck) and normal-phase chromatography using silica gel (column dimensions 60×2.5 cm; Merck). The CDs were detected on silica gel thin-layer plates by spraying 1% vanillin in ethanolic H_2SO_4 (35%) and heating.

2.1.3. Apparatus

Melting points were determined with a Gallenkamp apparatus and were not corrected. [1]H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL300 (^{1}H-NMR, 299.956 MHz; ^{13}C-NMR, 75 MHz; Darmstadt, Germany) and on a Bruker DRX 500 (Avance 500; Karlsruhe, Germany) equipped with a HP Kajakworkstation (^{1}H-NMR, 500.131 MHz; ^{13}C-NMR, 125.758 MHz) spectrometer. The centers of the peaks of C^2HCl_3 and DMSO-d_6 were used as internal references. Abbreviations for data quoted are d, doublet; t, triplet; q, quartet and m, multiplet. Coupling constants are given in Hertz. IR spectra, recorded as KBr discs, were obtained using a Perkin-Elmer 298 spectrometer. Mass spectrometric analyses were carried out on a laboratory-built linear time of flight (RETOF) instrument using 2,5-dihydroxybenzoic acid as matrix. The main features are summarized as follows: for desorption and ionization of the cyclodextrins, a pulsed nitrogen laser (VSL 337 ND; Laser Sciences, Cambridge, MA, USA) of 337 nm wavelength was used. The formed ions in the linear TOF instrument were accelerated up to a kinetic energy of 18.5 keV by a permanent electrical field. The second instrument was a reflectron-type instrument employing a gridded two-stage reflector. Acceleration voltage in this instrument was 10 keV. Combustion analyses were performed with a VarioEL of Elementar Analysensysteme.

2.1.4. Synthesis procedure

2.1.4.1. Synthesis of heptakis(6-O-dimethylsilyl-1,1,2-trimethylpropyl)-β-cyclodextrin

In the synthesis, β-CD was reacted according to the procedure reported in Ref. [26] with chlorodimethyl-1,1,2-trimethylpropylsilane and purified by means of reversed-phase chromatography (mobile phase: dichloromethane–methanol, v/v, 8:2→7:3→5:5) to obtain *heptakis*(6-*O*-dimethyl-1,1,2-trimethylpropylsilyl)-β-cyclodextrin (**1**) with analytical data identical to that previously reported in Ref. [34].

2.1.4.2. Synthesis of heptakis(2-N,N-dimethylcarbamoyl-6-O-dimethyl-1,1,2-trimethylpropylsilyl)-β-cyclodextrin (**2**)

A solution of **1** (3.0 g; 1.41 mM) in dry dichloromethane (50 ml) was stirred under an argon atmosphere. TEA (16.6 ml; 120 mM) and 4-DMAP (1.83 g; 15 mM) were added and the solution was heated up to 58°C. Afterwards, DMCC (19.3 ml; 210 mM) was added dropwise and the mixture was stirred for 20 h. After cooling to room temperature, the surplus reagent was destroyed by adding HCl (0.1 M; 50 ml), the organic layer separated, washed with HCl (0.1 M; 2×50 ml) and saturated aqueous $NaHCO_3$ (1×50 ml), dried over Na_2SO_4 and evaporated. Reversed-phase chromatography (dichloromethane–methanol, 6:4, v/v) was performed to give 2.9 g (78%) of pure **2**. The process of both the reaction and the purification process was followed by thin-layer chromatography (TLC) using the reversed-phase plates with dichloromethane–methanol (6:4, v/v) (R_F=0.55). The purity was verified by combustion analysis: calculated %: C: 54.37; H: 8.86; N: 3.73, found %: C: 54.17; H: 8.99; N: 3.71. ¹H-NMR (300 MHz, C²HCl₃): δ (ppm)=5.21 (d, J 3.5 Hz, 7H, H₁), 4.59 (dd, J 3.5 Hz, 7H, H₂), 4.02–3.93 (m, 14H, H₄, H₃), 3.70–3.58 (m, 21H, H$_{6a;b}$, H₅), 2.91–2.82 (m, 42H, N–CH₃), 1.59 [q, J 6.8 Hz, 7H, C$\underline{H}$(CH₃)₂], 0.86; 0.85 (2s, 42H, 2CH₃), 0.80 (s, 42H, CH₃), 0.10 (s, 21H, Si–CH₃), 0.06 (s, 21H, Si–CH₃). ¹³C-NMR (75 MHz, C²HCl₃): δ (ppm)= 155.6 (CO), 98.9 (C₁), 80.5 (C₄), 74.8; 72.0; 70.7 (C₂, C₅, C₃), 61.5 (C₆), 36.9 (N–CH₃), 36.2 (N–CH₃), 34.2 [$\underline{C}$H(CH₃)₂], 25.1 (CR₄), 20.4; 20.2;

18.7; 18.5 (4×CH₃), −3.2 (2×Si–CH₃). IR 3540, 3440, 2950, 1710, 1455, 1390, 1250, 1155, 1040, 820, 770 cm^{-1}.

2.1.4.3. Synthesis of heptakis(2-N,N-dimethylcarbamoyl)-β-cyclodextrin

2 (2.8 g; 1.07 mM) was dissolved in dry dichloromethane (50 ml). Boron trifluoride diethyletherate complex (2.9 ml; 24.6 mM) was added and the mixture was stirred for 24 h at room temperature. Afterwards, ice (5.0 g; 0.28 M) was added and the solution was stirred for 4 h. The mixture was evaporated (60°C; in vacuo) to dryness and dissolved in deionized water. The solution was washed with diethyl ether (2×50 ml) and evaporated. Diethyl ether (10 ml) was added to the oily crude residue to receive a sticky precipitate, which was separated by filtration. Reversed-phase chromatography (methanol), column chromatography on silica gel (methanol) and ion-exchange chromatography (deionized water) was performed to give 1.3 g (74%) of the pure HDMC-β-CD **3**. Combustion analysis: calculated %: C: 46.33; H: 6.49; N: 6.01, found %: C: 44.79; H: 6.81; N: 5.81. Positive ion matrix-assisted laser desorption ionization (MALDI) TOF-MS spectra were measured for every batch. To obtain the mass spectra, HDMC-β-CD was dissolved at a concentration of 1 pM (mixing ratio: matrix–CD= 1000:1). See Fig. 2. ¹H-NMR (500 MHz, ²D₂O): δ (ppm)=5.19 (d, J 3.7 Hz, 7H, H₁), 4.44 (dd, J 3.7 Hz, 7H, H₂), 3.95 (t, J 9.29 Hz, 7H, H₃), 3.86–3.75 (m, 21H, H$_{6a;b}$, H₅), 3.56 (t, J 9.13 Hz, 7H, H₄), 2.88 (s, 21H, N–CH₃), 2.80 (s, 21H, N–CH₃). ¹³C-NMR (125 MHz, ²H₂O): δ (ppm)=158.9 (CO), 100.3 (C₁), 81.8 (C₄), 75.8(C₂), 73.7 (C₅), 71.8 (C₃), 62.2 (C₆), 37.9 (N–CH₃), 37.5 (N–CH₃). IR: 3400, 2930, 1680, 1490, 1390, 1200, 1155, 1040, 840, 765 cm^{-1}.

2.2. Capillary electrophoresis

All experiments were performed on a Beckman P/ACE 5500 system (Fullerton, CA, USA) using a fused-silica capillary of 0.47 m (detection length 0.40 m)×75 μm I.D. Samples were injected by pressure (5 s) and separated at a temperature of 25°C (liquid cooling system) using constant voltages of 8

and 10 kV. A diode array detector was used at 220 nm. The capillary was conditioned for 20 min with 0.1 *M* NaOH and 10 min with water. Additionally, the capillary was washed for 2 min with 0.1 *M* NaOH or 0.1 *M* HCl, 1 min with water and 2 min with the running buffer before each run.

Analytical-grade NaH_2PO_4, Na_2HPO_4, orthophosphoric acid and methanol were purchased from Merck. Phosphate buffer, pH 3 was prepared by mixing appropriate amounts of H_3PO_4 and NaH_2PO_4 solution. Phosphate buffers, pH 7.0 were prepared by mixing appropriate amounts of NaH_2PO_4 and Na_2HPO_4 solutions. The HDMC-β-CD was weighed directly into the background electrolyte (BGE). It was applied in concentrations of 18, 36 and 72 m*M*. The samples subjected to the CE were dissolved in methanol and diluted with deionized water and/or methanol (concentration≈50 μg/ml). All solutions were prepared with deionized water and filtered through a 0.45-μm filter before use (Schleicher and Schüll, Germany). The sample concentration was 50 μg/ml. The separations were carried out in 50 m*M* phosphate buffer, pH 3 for basic compounds and pH 7 for acidic compounds.

3. Results and discussion

3.1. Synthesis of HDMC-β-CD

The synthesis of the monocarbamoylated β-CD displayed in Fig. 1 was carried out in analogy to the *heptakis*(2,3-di-*O*-acetyl)-β-cyclodextrin [26]. The first aim was to block the hydroxyl groups in the 6 position in order to protect them from carbamoylation. The chlorodimethyl-1,1,2-trimethylpropylsilane

turned out to be advantageous to the most frequently used tertiary butyl(dimethyl) group. In order to remove the side products obtained from this reaction a medium-pressure reversed-phase chromatography was performed giving the pure silyl ether **1**. The carbamoylation was performed in presence of triethylamine and dimethylaminopyridine and a huge excess of dimethylcarbamoylchloride. Interestingly, whereas the corresponding acetylation of compound **1** occurred in positions 2 <u>and</u> 3, the dimethylcarbamoylchloride reacted almost selectively with the OH-group in position 2. The subsequent reversed-phase chromatography gave pure **2**. In the last step the silyl protecting group was removed by means of boron trifluoride diethyletherate complex and again the crude product was purified by reversed-phase column chromatography to give HDMC-β-CD. It has to be stressed at this point, that pure HDMC-β-CD can only be obtained when every intermediate on the synthesis pathway was carefully purified.

The composition of HDMC-β-CD was checked by the positive ion MALDI-TOF-MS spectra for every batch. Very low amounts of monodemethylated product and 6- and 8-times carbamoylated derivatives, respectively, were detected in the mass spectrum shown in Fig. 2. However, the composition of the by-products could be reproduced from batch to batch. The 500 MHz ^{1}H and 125 MHz ^{13}C spectra recorded in ^{2}H$_2$O at 298 K (see Fig. 3) are consistent with the postulated structure (for numbering of the H and C atoms see Fig. 1). The assignment was confirmed by running a H, H-correlation spectroscopy (COSY) experiment with Z-gradient and a C, H-COSY experiment (Fig. 3).

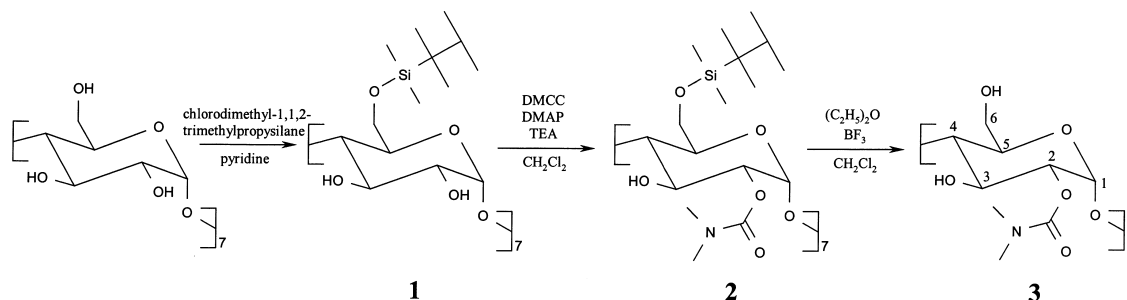The outstanding feature of the new CD derivative HDMC-β-CD is the high solubility in water (>100

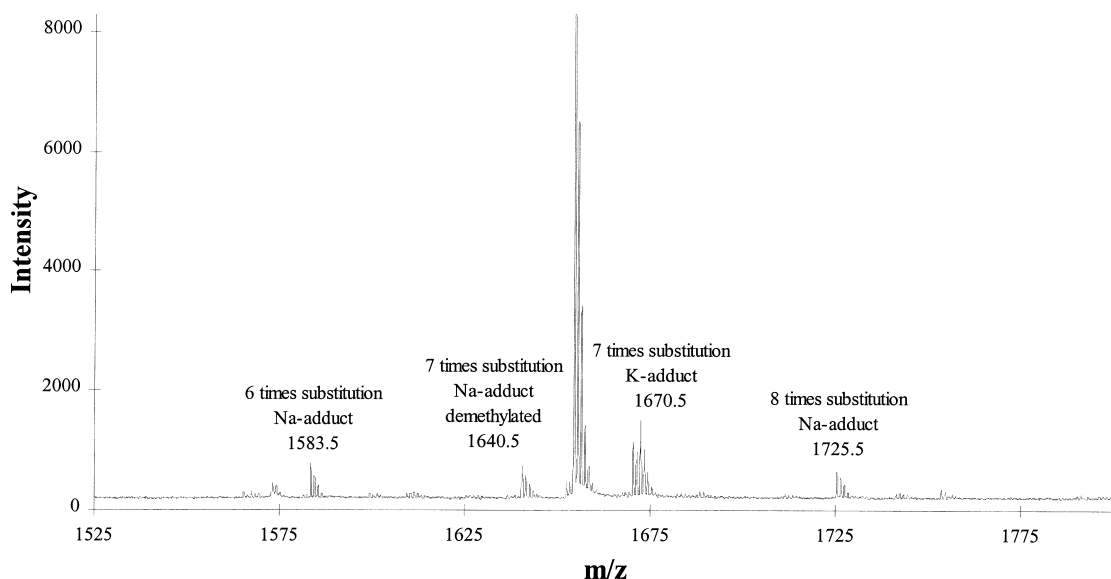Fig. 1. Synthesis pathway of *heptakis*(2-*N,N*-dimethylcarbamoyl)-β-cyclodextrin.

Fig. 2. MALDI-TOF mass spectrum of *heptakis*(2-*N*,*N*-dimethylcarbamoyl)-β-cyclodextrin.

m*M*) and methanol which enhances the resolution possibilities tremendously.

3.2. Separation of enantiomers with HDMC-β-CD

A series of basic and acidic compounds were checked to demonstrate the resolution power of HDMC-β-CD. Tables 1 and 2 (see Fig. 6 for further structural formula) displays the migration times of the enantiomers and the resolution of the racemates studied in dependence of the HDMC-β-CD concentration. A few typical separations obtained with HDMC-β-CD are depicted in Fig. 4 (acidic compounds) and Fig. 5 (basic compounds). The numbers next to the structural formula indicate the actual HDMC-β-CD concentration. From this data, it can be said, that HDMC-β-CD has a high resolution power. Interestingly, in the case of the basic compounds the resolution increased with increasing CD concentration, whereas in case of the acidic compounds the resolution often decreased on addition of increasing CD concentration. However, in order to assess the resolution power of the new CD the resolution of a series of racemic dihydropyridine derivatives were evaluated whose separation were previously studied on the same instrument under the comparable conditions [35,36]. With exception of

compounds **3** and **7** (Fig. 6) all dihydropyridines could be resolved using α-CD. The resolution (R_s) values ranged between 1.5 and 5.0. In almost all cases the resolution is comparable to the one found with HDMC-β-CD. In contrast, the resolution found for **3** with β-CD is much higher (R_s=2.2) than the resolution found with HDMC-β-CD (R_s=0.44). Ketoprofen and the basic drugs studied here were also compared with β-CD. The racemates of ketoprofen, fluoxetine, aminoglutethimide, hydroxyzine and the compound **8** could not be resolved with β-CD but show baseline separation with HDMC-β-CD. The other drugs could be resolved with both CDs in a similar manner.

4. Conclusion

The new, single isomer, neutral β-CD derivative, the *heptakis*(2-*N*,*N*-dimethylcarbamoyl)-β-cyclodextrin, has been synthesized and spectroscopically characterized. It has been found to have a very high water solubility which is advantageous for the chiral resolution. The HDMC-β-CD modified capillary electrophoretic studies of the series of randomly chosen racemates clearly demonstrates that the en-
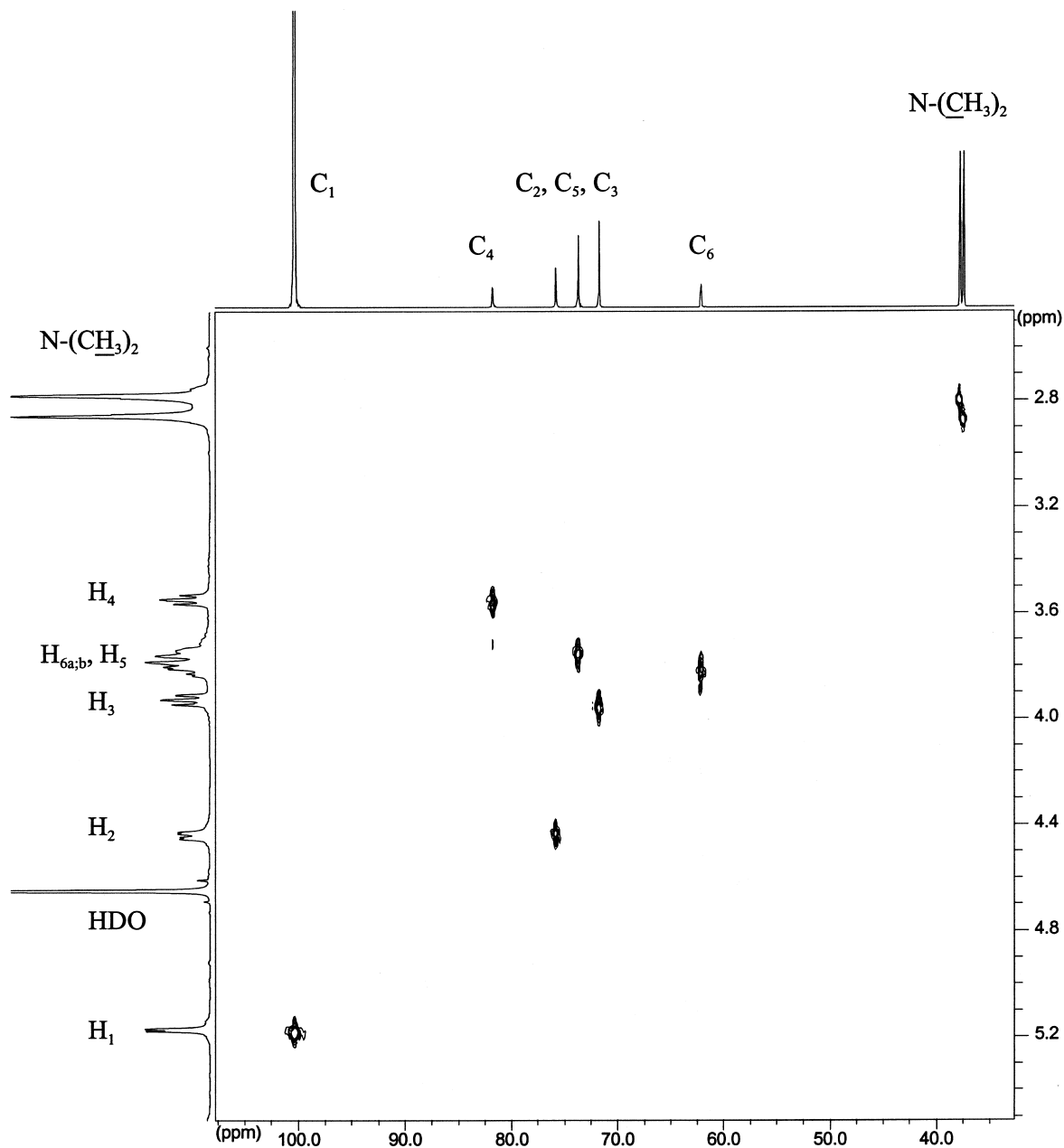
Fig. 3. C, H-Correlation spectroscopy (COSY) experiment for *heptakis*(2-*N*,*N*-dimethylcarbamoyl)-β-cyclodextrin.

antiomers of both acidic and basic compounds can be separated.

In contrast to the diacetylated CD derivative we have previously synthesized and studied with respect to chiral recognition [26,27,37–39] the wider rim of

the HDMC-β-CD cavity provides H-donor and H-acceptor groups for the recognition of chiral drugs. Thus, it will be interesting to see whether the mechanism of chiral recognition of HDMC-β-CD is different from the diacetylated cyclodextrin.

Table 1
Results of the acidic compounds in 50 mM phosphate buffer, pH 7 using a constant voltage of 8 kV

	0.0 mM HDMC-β-CD, t_M	18 mM HDMC-β-CD			36 mM HDMC-β-CD			72 mM HDMC-β-CD		
		t_{M1}	t_{M2}	R_s	t_{M1}	t_{M2}	R_s	t_{M1}	t_{M2}	R_s
Ketoprofen	12.5	13.6	13.8	1.23	14.0	14.1	0.42	19.1	19.2	0.10
1	14.4	12.1	12.3	1.40	14.5	14.6	0.96	17.3	17.4	0.28
2	17.1	17.6	–	–	20.6	20.8	0.21	25.6	25.9	0.83
3	16.5	16.6	–	–	18.5	18.7	0.26	23.2	23.4	0.44
4	15.4	15.2	15.6	2.11	16.7	17.1	2.32	21.2	21.7	2.16
5	22.8	23.2	24.1	2.92	23.1	24.3	3.69	22.8	23.8	3.27
6	16.1	15.5	15.7	0.84	16.6	16.7	0.11	21.7	–	–
7	16.5	18.5	–	–	21.0	–	–	28.8	29.1	0.52

Table 2
Results of the basic compounds in 50 mM phosphate buffer, pH 3 using a constant voltage of 10 kV

|	0.0 mM HDMC-β-CD, t_M	18 mM HDMC-β-CD			36 mM HDMC-β-CD			72 mM HDMC-β-CD		
		t_{M1}	t_{M2}	R_s	t_{M1}	t_{M2}	R_s	t_{M1}	t_{M2}	R_s
Mianserin	10.7	15.4	15.6	0.77	18.1	18.5	1.32	27.4	28.1	1.79
Cyclopentolat	15.0	17.4	–	–	20.1	20.3	0.47	27.1	27.4	0.49
Fluoxetine	16.1	21.1	22.4	3.80	28.0	30.0	4.24	38.1	40.5	4.10
Nisoxetine	14.2	17.5	18.1	2.07	20.6	21.9	3.01	*		
Verapamil	21.1	22.5	–	–	26.6	26.9	0.70	29.1	29.6	1.27
Aminoglutethimide	15.5	20.2	25.9	14.70	27.0	38.0	17.31	*		
Dimethindene	9.6	10.6	–	–	11.7	11.9	0.69	13.8	14.0	1.18
Doxapram	18.8	21.9	–	–	24.3	24.6	0.86	28.1	28.6	1.42
Hydroxyzine	18.3	24.9	25.6	1.79	31.2	32.3	2.19	*		
Meclozine	19.1	28.4	30.1	3.55	35.6	37.7	3.53	*		
8	21.5	24.5	24.9	1.18	28.7	29.4	1.90	*		

*, Not measured.
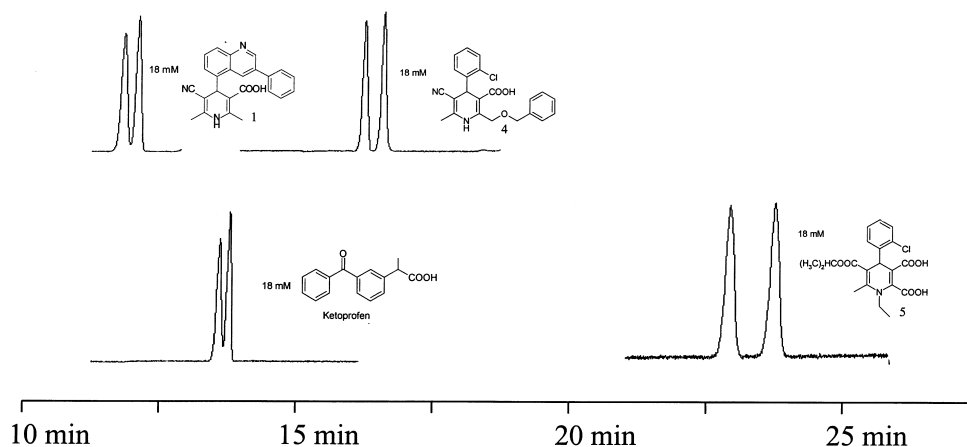
Fig. 4. Electropherograms of acidic compounds in 50 mM phosphate buffer, pH 7.
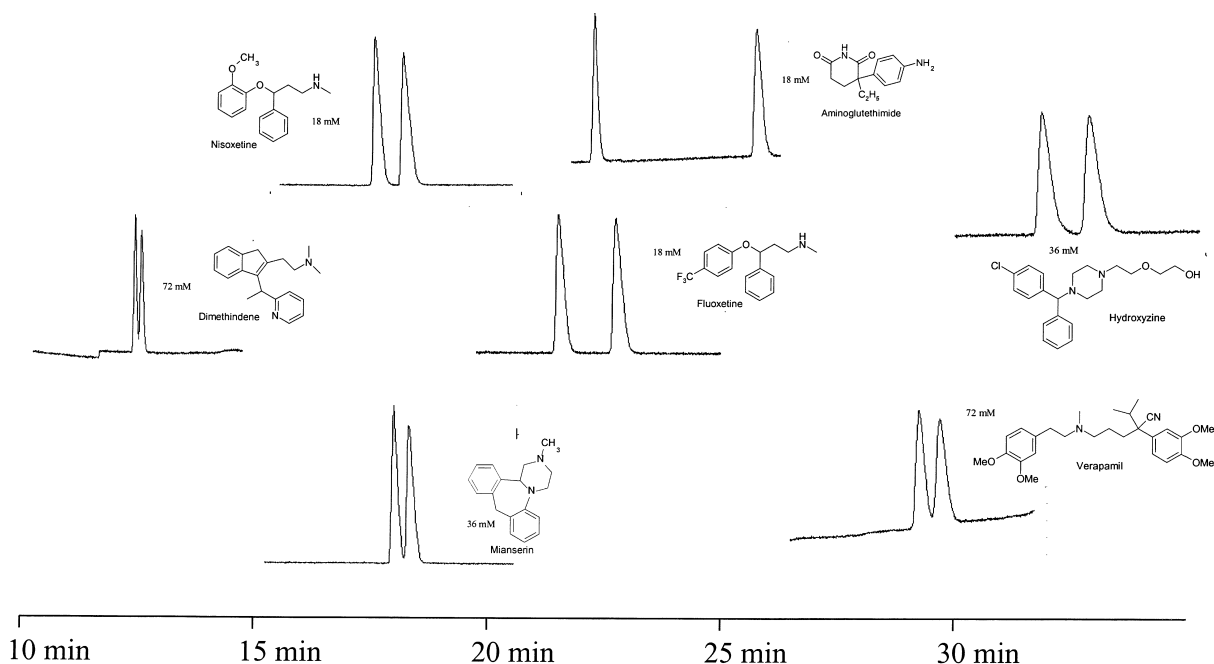
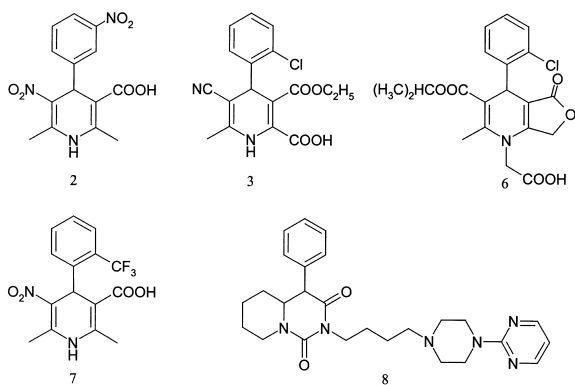Fig. 5. Electropherograms of basic compounds in 50 mM phosphate buffer, pH 3.

Fig. 6. Structural formulae of further compounds studied: **2**, **3** and **6**–**8** (see Table 1).

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Acknowledgements

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