Selective immobilization on silica gel of permethylated β-cyclodextrin for liquid chromatography

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Abstract

Permethylated 6-mono-O-alkenyl-β-cyclodextrin was synthesized with a yield of 16.5–24% using an indirect method with six reaction steps. The number and position of the monoalkenyl groups in β-cyclodextrin were verified by fast atom bombardment, ^{1}H NMR and GC–MS analysis. β-Cyclodextrin derivatives were immobilized on silica gel after hydrosylation with dimethoxymethylhydrosilane of the pentenyl or octenyl group. The effects of immobilization position, length of spacer arm, the bulky enantiomers and the amount of immobilized β-cyclodextrin on the enantiomeric separation were critically assessed. The efficiency of enantiomeric separation by HPLC was found to be better for the 6-OH position of the spacer arm in β-cyclodextrin than for the 2-OH position for seven racemic compounds.

Keywords: Immobilized cyclodextrins; Chiral stationary phases, LC; Enantiomer separation; Cyclodextrins

1. Introduction

Cyclodextrins (CDs) chemically bonded on silica gel via several spacer arms are used extensively as stationary phases in high-performance liquid chromatography (HPLC) for enantiomeric separations [1–9]. CDs have a large number of hydroxyl groups which can participate in the immobilization. The number of immobilization bonds between CD and silica gel and the position of the hydroxyl group in the D-glucopyranosyl residue of CD used for immobilization can be determined only when the CD molecule is monofunctionalized at a well known position with a

spacer arm with a reactive group for grafting on silica gel. This is the only way to produce repeatable immobilization results. Many workers [1–8] have indicated in their papers the positions of immobilization without an exact determination.

Recently, the possibility of obtaining functionalization in a well characterized position of β-CD by reaction with 5-bromo-1-pentene in dimethyl sulfoxide in the presence of sodium hydroxide, which gave 95.9% substitution at the 2-OH position, has been shown [9]. The selectivity for the 6-OH position was 41.5% when the reaction medium was dimethylformamide, but the final yield was not higher than 2% since the separation of positional isomers by liquid chromatography on silica gel was difficult.

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This paper reports a method for the preparation of (6-O-alkenyl-2,3-di-O-methyl)hexakis-(2,3,6-tri-O-methyl)-β-CD in six reaction steps for immobilization at the 6-OH position of β-CD on silica gel and compares the results of enantiomeric separation on immobilized permethylated-β-CD at the 6-OH position with those immobilized at the 2-OH position.

2. Experimental

2.1. Reagents and materials

β-Cyclodextrin, dimethoxymethylhydrosilane (DMMHS), 3-bromo-1-propene, 5-bromo-1-pentene, 8-bromo-1-octene, hexachloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$) and *tert.*-butyldimethylsilyl chloride (TBDMS-Cl) were obtained from Fluka (Buchs, Switzerland). Microporous spherical silica gel (LiChrosorb Si 100) with a median particle diameter of 5 μm, a mean pore size of 110 nm and a specific surface area of 320 m^2/g, dimethyl sulfoxide (DMSO), dimethylacetamide (DMAA), dimethylformamide (DMFA) dioxane, iodomethane, and thin-layer chromatography (TLC) aluminium roll precoated silica gel 60F$_{254}$ were purchased from Merck (Darmstadt, Germany). All solvents were of HPLC grade and water was purified by means of a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Apparatus

All HPLC separations were carried out at 20°C using a Spectra-Physics (Santa Clara, CA, USA) Model 8500 liquid chromatograph equipped with UV detector and a Merck Model L-6200 liquid chromatograph equipped with a Model L-4200 UV–Vis detector and a 20-μl loop sample injector. HPLC columns (250 × 4.6 mm I.D.) were packed by the usual slurry method.

Gas chromatography (GC) was carried out on a Fractovap 2101 gas chromatograph (Carlo Erba, Milan, Italy) equipped with split injection, a flame ionization detector and a Hewlett-Packard Model 3390A integrator.

Electron impact mass spectrometry (EI-MS) was performed with a Finnigam MAT Model 311A mass spectrometer (MAT, Bremen, Germany) coupled to a Hewlett-Packard Model HP-5840 gas chromatograph.

Fast atom bombardment mass spectrometry (FAB-MS) was carried out on a VG mass spectrometer (VG Analytical, Manchester, UK) using nitrobenzyl alcohol as matrix.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WM 400 spectrometer operating at 400.13 MHz for proton (^{1}H). All measurements were carried out on solutions in deuterochloroform and tetramethylsilane was used as the internal standard.

2.3. Preparation of stationary phases

To a solution of 0.01 mol of β-CD (dried overnight under vacuum) in dry DMFA was added dropwise 0.02 mol of TBDMS-Cl at 0°C with stirring and then dropwise 0.02 mol of triethylamine at 0°C. The reaction mixture was stirred at room temperature and was followed by TLC on silica gel using 25% ammonia solution-n-pentanol–ethanol (2:1:1) as eluent. After 10 h, the free hydroxyl groups were acetylated by addition of 0.3 mol of acetic anhydride and 0.3 mol of triethylamine. Liquid chromatography on silica gel using as eluent pentane–acetone (1:1) furnished crystalline (2,3-di-O-acetyl-6-O-TBDMS)hexakis(2,3,6-tri-O-acetyl)-β-CD in 40.5% yield. Deacetylation with an excess of sodium hydroxide in 90% methanol gave mono(6-O-TBDMS)-β-CD in quantitative yield after purification by gel chromatography on Sephadex G-15, in 30 min at room temperature. The free hydroxyl groups of the mono-β-CD derivative were O-methylated with methyl iodide in DMSO and in the presence of powdered sodium hydroxide at room temperature. The reaction was almost quantitative in one step [10]. Extraction and purification by silica gel liquid chromatography afforded (2,3-di-O-methyl-6-O-TBDMS)hexakis(2,3,6-tri-O-methyl)-β-CD in 87% yield. Desilylation of this product in dioxane with tetrabutylammonium fluoride for 2 h

under reflux afforded (2,3-di-O-methyl)hexakis-(2,3,6-tri-O-methyl)-β-CD in 89.2% yield.

The 1-alkenyl group was introduced by reaction of a bromo-1-alkene with the free hydroxyl group of CD in DMFA in the presence of sodium hydride at 45°C for 24 h. The peralkylated product was extracted three times with chloroform. The combined extracts were washed with water and dried over anhydrous magnesium sulfate. The filtered chloroform solution was concentrated under reduced pressure and the residue was purified by liquid chromatography over silica gel using light petroleum and light petroleum–acetone (1:1) as eluents, affording (2,3-di-O-methyl-6-O-pentenyl)hexakis(2,3,6-tri-O-methyl)-β-CD in 76.4% yield and (2,3-di-O-methyl-6-O-octenyl) hexakis (2,3,6-tri-O-methyl)-β-CD in 52.5% yield.

Hydrosilylation was performed with 0.01 mol of the appropriate permethylated mono-O-alk-1-enyl-β-CD dissolved in 150 ml of anhydrous toluene by addition of 0.012 mol of DMMHS in a 250-ml three-necked, round-bottomed flask equipped with a nitrogen inlet and reflux condenser. A few drops of a 4% solution of hexachlorplatinic acid hexahydrate in anhydrous tetrahydrofuran were added at intervals of 2 h and the mixture was heated 50–90°C with stirring. The reaction was monitored by IR spectrometry, measuring the decrease in the absorption band at 2060–2080 cm^{-1} (Si–H), usually requiring more than 36 h. The excess of hydrosilane was removed under reduced pressure. The products were purified by liquid chromatography on silica gel using hexane–toluene (5:1) as eluent.

2.4. Preparation of O-alkylated alditol acetates

Permethylated mono-O-1-alkenyl-β-CD (10 mg) was hydrolysed in 1 ml of trifluoroacetic acid–80% formic acid (1:1) at 80°C for 20 h. The solution containing the hydrolysate was evaporated to dryness under reduced pressure. The reduction was carried out in water (10 ml) with sodium tetrahydroborate (100 mg) for 6 h at room temperature. After acidification with few drops of 80% formic acid and concentration to

dryness in the presence of methanol, the product, dried under vacuum, was acetylated with acetic anhydride (0.2 ml) in pyridine (0.2 ml) at 100°C for 2 h.

2.5. Immobilization of stationary phase

A 6-g amount of silica gel (dried under vacuum at 150°C for 10 h) was added to 150 ml of a 10% (w/v) solution of permethylated-mono-O-(dimethoxymethylsilyl)alkenyl-β-CD in dry toluene with gentle shaking and then the suspension was refluxed for 30 h under nitrogen without stirring. After cooling, the resulting silica gel was filtered through a membrane filter, washed successively with toluene, methanol and diethyl ether and then dried under vacuum at 40°C for 10 h. The amounts of bonded permethylated-β-CD on silica gel were calculated [11] from the results of elemental analysis.

3. Results and discussion

Immobilization of CDs is normally possible in one position (2-, 3- or 6-OH) or more positions since β-CD has 21 hydroxyl groups. The reactivity of the hydroxyl groups of the D-glucopyranosyl residue is clearly dependent on the class and specific nature of the attacking reagent, steric hindrance and the reaction conditions.

Regioselective substitution of all primary hydroxyl groups of β-CD can be easily achieved by reaction with TBDMS-Cl in DMFA with imidazole as base [12], pyridine [13] or DMFA in the presence of triethylamine. Monofunctionalization of β-CD with TBDMS-Cl was judged to be optimum in DMFA with triethylamine. Other reagents such as mesitylsulfonyl chloride [14] or p-toluensulfonyl chloride [15] instead of TBDMS-Cl gave a poor yield of monofunctionalized-β-CD.

The final yield of (2,3-di-O-methyl-6-O-1-pentenyl)hexakis(2,3,6-tri-O-methyl)-β-CD after six reaction steps using as intermediate TBDMS derivative was 24% and the yield of (2,3-di-O-methyl-6-O-1-octenyl)hexakis (2,3,6-tri-O-methyl)-β-CD was 16.5%. The reaction with 3-

bromo-1-propene gave a β-CD derivative with low stability in the immobilization step and in the structure analysis, making repeatable results difficult.

The presence of the 1-alkenyl group in permethylated β-CD gave a slight modification of the proton chemical shift by comparison with the ^{1}H NMR spectrum of permethylated β-CD [16]. As observed previously [9], the proton absorption at $\delta = 5.68$–5.83 gave a multiplet that may be ascribed to vinyl CH from the 1-alkenyl group. The NMR spectrum gives the first indication of the degree of substitution of β-CD with 1-alkenyl groups. This direct method used the ratio between the integrated peak area from the vinyl group and the peak area of the proton from H–C_1 in the D-glucopyranosyl residue. The ratio was 1:7 in both cases and indicated monosubstitution of β-CD with the 1-alkenyl group. The number of 1-alkenyl groups in the β-CD derivative can also be easily recognized from the FAB mass spectra on the basis of the molecular ion. FAB-MS is a powerful tool for the determination of molecular mass. The best relative sensitivity of the molecular ion was obtained with nitrobenzyl alcohol that generated $[M + H]^+$ ion over $[M + Na]^+$ and $[M + K]^+$ ions. The positive-ion FAB mass spectrum of permethylated mono-O-1-pentenyl-β-CD gave the expected $[M + H]^+$ ion at m/z 1484.4 with relative intensity higher than $[M + Na]^+$ and $[M + K]^+$ ions at m/z 1506.4 and 1522.4, respectively. The positive-ion spectrum of permethylated mono-O-1-octenyl-β-CD gave $[M + H]^+$ at m/z 1526.5, $[M + Na]^+$ ion at m/z 1548.5 and $[M + K]^+$ ion at m/z 1564.6. Both of the spectra showed no trace of the ion from di- or trisubstituted β-CD with 1-alkenyl groups. These results indicated the presence only of a one 1-alkenyl group in β-CD derivatives.

The position of the 1-alkenyl group in the D-glucopyranosyl residue of β-CD was verified for permethylated mono-O-1-pentenyl-β-CD and permethylated mono-O-1-octenyl-β-CD by GC–MS. These derivatives of β-CD were successively hydrolysed, reduced, acetylated and analysed by GC. In both cases there were obtained two major peaks, which were identified by EI-MS (Table 1). Based on the fragment ions, the compound of

Table 1
Selected EI mass spectra of derivatives obtained by GC–MS of permethylated 6-O-1-pentenyl-β-CD and permethylated 6-O-1-octenyl-β-CD after hydrolysis, reduction and acetylation

Peak	m/z (% of base peak)
1 + 1'	43 (100), 101 (20), 117 (61), 129 (10), 161 (16), 173 (5), 189 (1), 233 (20), 234 (4).
2	43 (100), 69 (59), 101 (26), 117 (81), 129 (43), 161 (11), 171 (2), 233 (3), 243 (3), 245 (4), 319 (5), 348 (5).
2'	43 (100), 101 (15), 111 (54), 117 (65), 127 (11), 141 (16), 161 (8), 231 (3), 233 (3), 245 (5), 285 (3), 319 (6), 348 (4).

the first peak (1 and 1') was consistent with 1,4,5-tri-O-acetyl-2,3,4-tri-O-methylglucitol, having a similar mass spectrum to those reported [17]. The compound of peak 2 was identified on the basis of the mass spectrum as 1,4,5-tri-O-acetyl-2,3-di-O-methyl-6-O-pentenylglucitol. The mass spectrum had characteristic fragment ions at m/z 69 $[CH_2CH_2CH_2CH=CH_2]^+$, 319 $[M - 85]^+$, 348 $[M - CH_3CH_2CH=CH_2]$ and 243 from fission between C-3 and C-4. These data and absence of fragment ions at m/z 171, which are characteristic of 2-OH substitution, indicated that the 1-pentenyl group is in the 6-OH position. The compound of peak 2' was identified from the EI mass spectrum as 1,4,5-tri-O-acetyl-2,3-di-O-methyl-6-O-octenylglucitol owing to the presence of characteristic fragment ions at m/z 111 $[(CH_2)_6CH=CH_2]$, 319 $[M - 127]^+$, 348 $[M - 98]^+$ and 285.

The chromatograms in Fig. 1 show the effect of the immobilization position of permethylated-β-CD (PM-β-CD) on the resolution of enantiomers of α-phenylsuccinimide using a pentenyl group as the spacer arm. As by changing the mobile phase the separation factor and resolution can be substantially modified, the separations of a racemic mixture were performed with the same mobile phase composition for all columns with different stationary phases. The influence of the concentration of the injected sample on the retention time was eliminated using the same injected amount for each com-
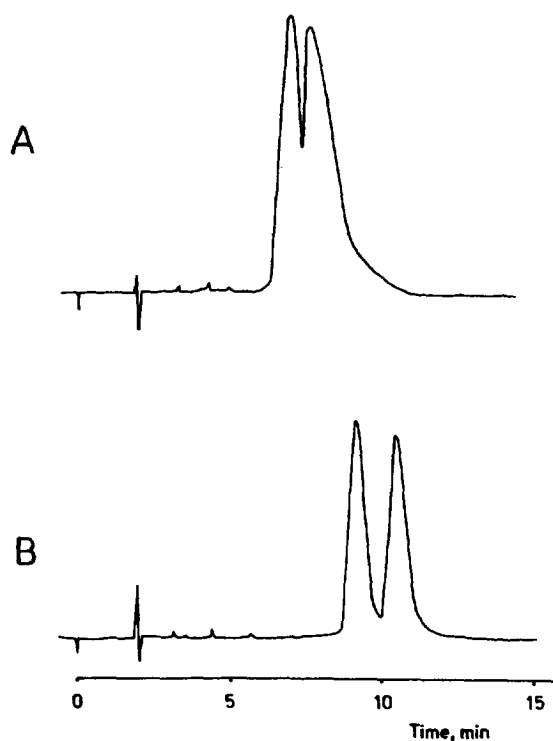
Fig. 1. Liquid chromatographic separation of the enantiomers of α-phenylsuccinimide as a function of immobilization position of PM-β-CD on silica gel: (A) 2-OH position; (B) 6-OH position. For the separation conditions, see Table 2.

pound. The optimum resolution, peak shape and detector sensitivity were achieved for 0.2–0.3 μg of racemic compound in the injected sample.

As can be seen from Table 2, the separation factors and resolution of the studied racemic mixture were not appreciably changed on increasing from pentyl (C_5) to octyl (C_8) the length of the spacer arm of PM-β-CD in the 6-OH position. No significant modification of the loading with PM-β-CD on silica gel was noticed when the same amount of PM-β-CD derivative was used for immobilization in the 2-OH and 6-OH positions. However, the most remarkable observation was a dramatic improvement in the separation factor and resolution when the immobilization of PM-β-CD on silica gel was changed from the 2-OH to the 6-OH position (Tables 2 and 3). An explanation of this result cold be an improvement in the access of enantiomers to the cavity of CD when PM-β-CD was immobilized in the 6-OH position since the most obvious requirement for enantiomeric separation is generally accepted to be the formation of an inclusion complex. This presumption was verified by following the influence of the density of PM-β-CD on silica gel and the size of the molecule of the racemic compound.

Table 2
Retention, selectivity and resolution parameters for the separation of the enantiomers of some chiral compounds on permethylated β-CD as a function of position of immobilization and the length of the spacer arm

Compound	Position of immobilization	μmol PM-β-CD/g silica gel	Spacer arm	Capacity factor[b] (k)	Separation factor (α)	Resolution (R)	Mobile phase[c]
Ibuprofen	6-OH	183	C_5	11.05	1.13	1.29	60:40
	6-OH	190	C_8	11.21	1.13	1.32	60:40
	2-OH	187[a]	C_5	7.51	1.07	0.52	60:40
Ketoprofen	6-OH	183	C_5	8.91	1.12	1.14	40:60
	6-OH	190	C_8	9.02	1.12	1.15	40:60
	2-OH	187[a]	C_5	5.63	1.06	0.38	40:60
α-Phenyl-succinimide	6-OH	183	C_5	4.22	1.16	1.27	20:80
	6-OH	190	C_8	4.35	1.16	1.26	20:80
	2-OH	187[a]	C_5	2.83	1.09	0.45	20:80

[a] From Ref. [9].
[b] For the first-eluted enantiomer.
[c] Volume ratio of methanol to 0.5% triethylamine acetate buffer (pH 4.2) in an isocratic separation at a flow-rate of 0.6 ml/min.

Table 3
Chromatographic data for some racemates as a function of the amount of permethylated-β-CD immobilized on silica gel and the position of immobilization using pentyl as spacer arm

Compound	μmol PM-β-CD/g silica gel	Position of immobilization	Capacity factor[b] (k)	Separation factor (α)	Resolution (R)	Mobile phase[c]
Mephobarbital	183	6-OH	3.80	1.51	2.75	70:30
	88	6-OH	1.95	1.21	0.68	70:30
	187[a]	2-OH	1.82	1.16	0.47	70:30
	91	2-OH	2.17	1.23	0.80	70:30
α-Methyl-α- phenylsuccinimide	183	6-OH	5.27	1.14	1.31	45:55
	88	6-OH	2.98	1.09	0.49	45:55
	187[a]	2-OH	2.56	1.08	0.37	45:55
	91	2-OH	3.28	1.12	0.70	45:55
Norgestrel	183	6-OH	5.02	1.14	1.26	60:40
	88	6-OH	2.77	1.08	0.59	60:40
	187[a]	2-OH	0.36	1.00	0.00	60:40
	91	2-OH	0.28	1.00	0.00	60:40
Ethinylestradiol	183	6-OH	3.52	1.42	1.55	65:35
	88	6-OH	1.82	1.19	0.75	65:35
	187[a]	2-OH	0.35	1.00	0.00	65:35
	91	2-OH	0.25	1.00	0.00	65:35

[a] From Ref. [9].
[b] For the first-eluted enantiomer.
[c] Volume ratio of methanol to 0.5% triethylamine acetate buffer (pH 4.2) in an isocratic separation at a flow-rate of 0.6 ml/min.

Table 3 shows the effect of the amount of immobilized PM-β-CD on the enantiomeric separation using a pentyl group as the spacer arm. A smaller amount of immobilized PM-β-CD in the 6-OH position on silica gel gave a decrease in the peak retention parameter, the separation factor and resolution, which is in agreement with the normal variation of the column loading with stationary phase. However, a very similar reduction in the amount of immobilized PM-β-CD in the 2-OH position enhanced the separation. The results emphasize that the inclusion complex is sterically favourable on the side of the PM-β-CD torus with secondary hydroxyl groups. A large density of PM-β-CD molecules immobilized in the 2-OH position could hinder the chiral interaction. A similar conclusion may be drawn from an attempt to separate the bulky enantiomers of norgestrel and ethinylestradiol. The enantiomeric separation was only possible on immobilized PM-β-CD in the 6-OH position and no separation was observed for immobilized PM-β-CD in the 2-OH position.

All these preliminary results show the importance of the immobilization position in the separation mechanism on chemically bonded CD in liquid chromatography. The immobilization of CD and CD derivatives on silica gel over one spacer arm in a defined position of the D-glucopyranosyl residue of CD seems to be the only way to produce repeatable results.

Acknowledgements

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