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Selective access and full characterization of mono-acidic permethylated β-cyclodextrin derivatives and their methyl esters

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Abstract—Three acidic derivatives of permethylated β-cyclodextrin, 2^{I}-*O*-carboxymethyl-$2^{II\text{-}VII}$,$3^{I\text{-}VII}$,$6^{I\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose, 6^{I}-*O*-carboxymethyl-$2^{I\text{-}VII}$,$3^{I\text{-}VII}$,$6^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose, 6^{I}-desoxy-6^{I}-carboxy-$2^{I\text{-}VII}$,$3^{I\text{-}VII}$,$6^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose and the corresponding methyl esters have been synthesized with good yields starting from mono-hydroxy permethylated β-CD prepared via *tert*-butyldimethylsilyl protection in 6-position and *p*-methoxybenzyl protection at the 2-position. All of these compounds were fully characterized by high field ^{1}H and ^{13}C NMR and HPLC/MS.

1. Introduction

The most commonly used technique to quantify enantiomeric excess of volatile chiral compounds is GC using chiral stationary phases (CSPs) based on CD derivatives, generally modified on all 2, 3 or 6 positions[1,2] or even per-*O*-substituted (as the permethylated β-CD). The permethylated β-CD is the most popular chiral selector due to its thermal stability and ability to recognize a wide range of compounds. CDs are best represented by a truncated cone with the primary hydroxyl groups (6-position) lining the narrower rim and the secondary hydroxyl groups (2- and 3-positions) lining the wider rim. These structures may be considered as symmetrical molecules. On the other hand, the modification of one position causes a loss of the sevenfold pseudo-axis for β-CD; this 'desymmetrisation' may change the conformation and the polarity of the molecule, allowing unique structural and physico-chemical properties with expected modification on chiral recognition.[3]

Carboxymethyl, carboxyethyl and succinyl derivatives are rather popular chiral separation agents in CE.[1,4,5] Furthermore, preparation and evaluation of carboxymethyl-β-CD coated on zirconia as CSP for LC were reported.[6] However, the reports deal with the use of mixtures of compounds with different degrees of substitution. Few mono-derivatives of CD were described in the literature however among them, no publication relates the preparation and the characterization of pure mono-carboxylic derivatives. For example, a mixture of the three isomers of mono-carboxymethyl β-CD was obtained by reaction of sodium chloracetate on native CD,[7] the position of the substitution being dependent on the concentration of sodium hydroxide used for the formation of oxyanion. Závada et al.[8] described a per-substitution at the 6-position of α- and β-CD via reaction with ethyl diazoacetate which was followed by an alkaline hydrolysis. In addition, oxidation of one of primary hydroxyl groups was carried out with N-oxide radical on parent CD, however, no evidence establishing structural identity as well as chemical purity of the product was provided[9].

The regioselective synthesis of mono-functionalized CD, due to the large number of hydroxyl groups (21) with different reactivity and the characterization, remain a difficult task and an interesting challenge. The 2-hydroxyl groups of β-CD are the most acidic (pK_a 12.1 against 15-16 for the 6-position) and the reaction

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with an electrophilic reagent becomes selective after prior formation of nucleophilic oxyanion. The 3-position is the most inaccessible and the most difficult to affect. The 6-hydroxyl group is the most reactive, consequently it could be substituted by using weak base and a moderate to strong electrophile.[10–12] Among the procedures of selective modification of CDs, D'Souza et al.[12] described the 'longest' method consisting in a series of protection and deprotection steps to obtain selectively the desired derivatives. Herein, this strategy is used for the preparation of β-CD derivatives monofunctionalized on each rim of the tore by acidic functionality from $2^{I\text{-}VI},3^{I\text{-}VII},6^{I\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose and $2^{I\text{-}VII},3^{I\text{-}VII},6^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose named, respectively, mono-2 and mono-6-hydroxy permethylated β-CD.[13] The 6^{I}-desoxy-6^{I}-carboxy-$2^{I\text{-}VII},3^{I\text{-}VII},6^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose (mono-6-carboxy permethylated β-CD) was obtained by direct oxidation of the corresponding mono-6-hydroxy permethylated β-CD. Methyl esters were easily prepared from the corresponding acids by diazomethane esterification. Each isomer was analyzed by high performance liquid chromatography/mass spectroscopy (HPLC/MS) and was fully characterized by nuclear magnetic resonance (NMR).

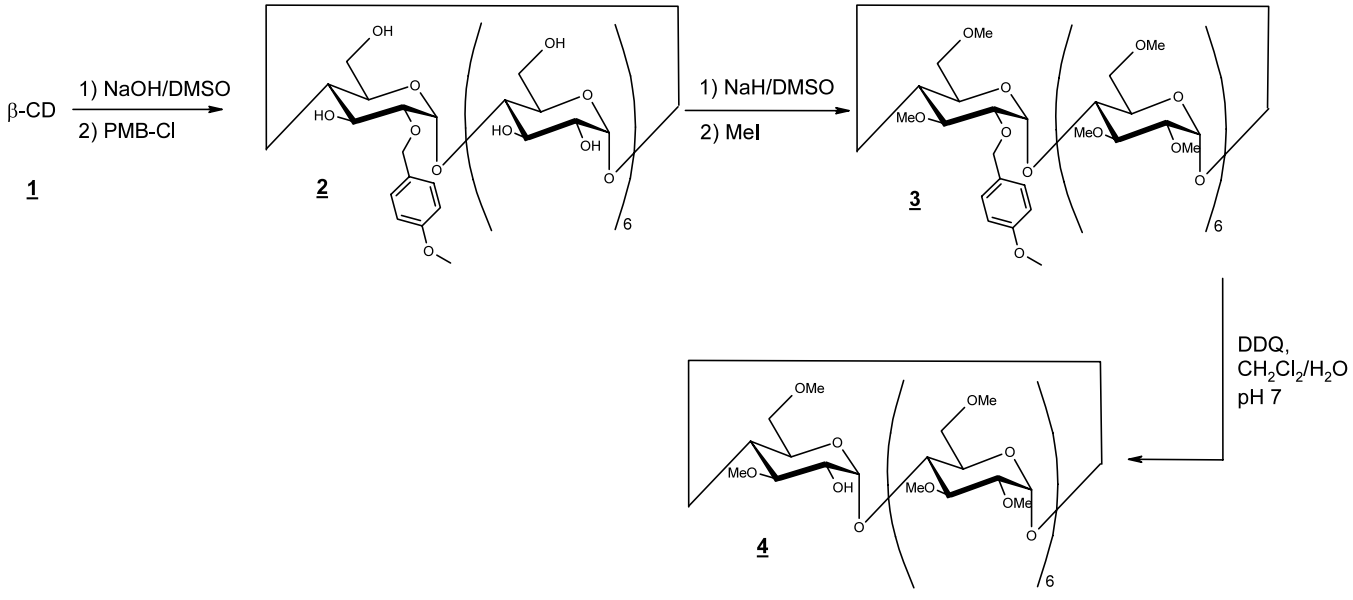
2. Results and discussion

The preparation of the already known mono-2-hydroxy permethylated β-CD was performed by a three steps procedure: protection of one 2-hydroxyl group, permethylation and deprotection.[13,14] In most cases, benzylation was employed for selective protection, with a deprotection step using palladium catalytic hydrogenolysis. Nevertheless, debenzylation remained a tricky task in terms of reproducibility due to the probable inclusion of the benzyl group in the CD cavity. In order to circumvent this problem, a parallel route via the *p*-methoxybenzyl moiety as protecting group and an oxidative cleavage was considered. 2^{I}-*O*-*p*-Methoxybenzyl-$2^{II\text{-}VII},3^{I\text{-}VII},6^{I\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose **3** was isolated from β-CD **1** after prior formation of oxyanion in 2-position using three-fold excess of sodium hydroxide in dimethylsulfoxide, then addition of *p*-methoxybenzyl chloride. The intermediate compound **2**, submitted to the usual permethylation process of the remaining hydroxyl groups was performed to reach the crude product (Scheme 1), which was isolated after purification on silica gel in 48% yield. The *p*-methoxybenzyl group was easily removed by oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone[15] (DDQ) in dichloromethane/water system at room temperature. Neutrality of the reaction medium was maintained with a phosphate buffer, then 2,3-dichloro-5,6-dicyanohydroxybenzoquinone (DDQH) precipitated. The yield of this step reached 82% after purification.

The preparation of $2^{I\text{-}VII},3^{I\text{-}VII},6^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose **5** was realized with a similar protection/deprotection procedure: *tert*-butyldimethylsilyl group was first introduced and the reaction mixture was then subjected to the full methylation without further purification.[16] The silyl protecting group was removed by using tetrabutylammonium fluoride in tetrahydrofuran at room temperature.

These two mono-hydroxy permethylated β-CD **4** and **5** were synthesized with satisfactory global yield, respectively, 39% (30–35% in literature[13,14]) and 45%.

2^{I}-*O*-Carboxymethyl-$2^{II\text{-}VII},3^{I\text{-}VII},6^{I\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose **6** and 6^{I}-*O*-carboxymethyl-$2^{I\text{-}VII},3^{I\text{-}VII},6^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose **7** were prepared by using sodium hydride and three-fold excess

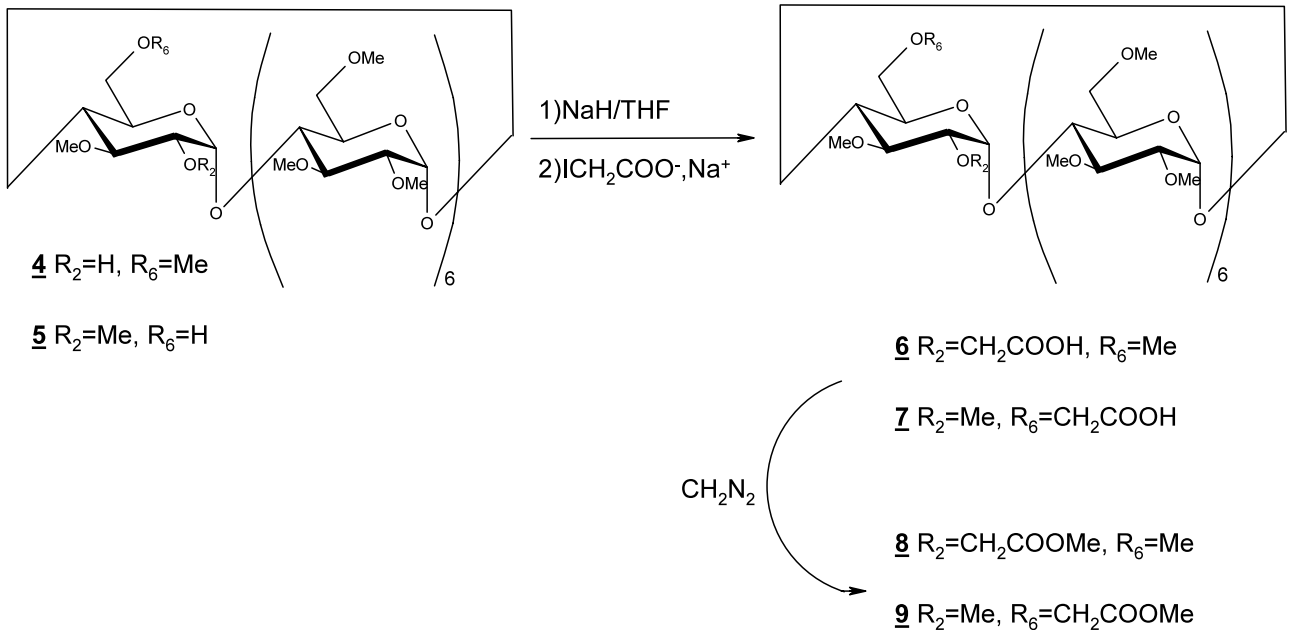


Scheme 1. Synthesis of mono-2-hydroxy permethylated β-CD via *p*-methoxybenzyl protection.

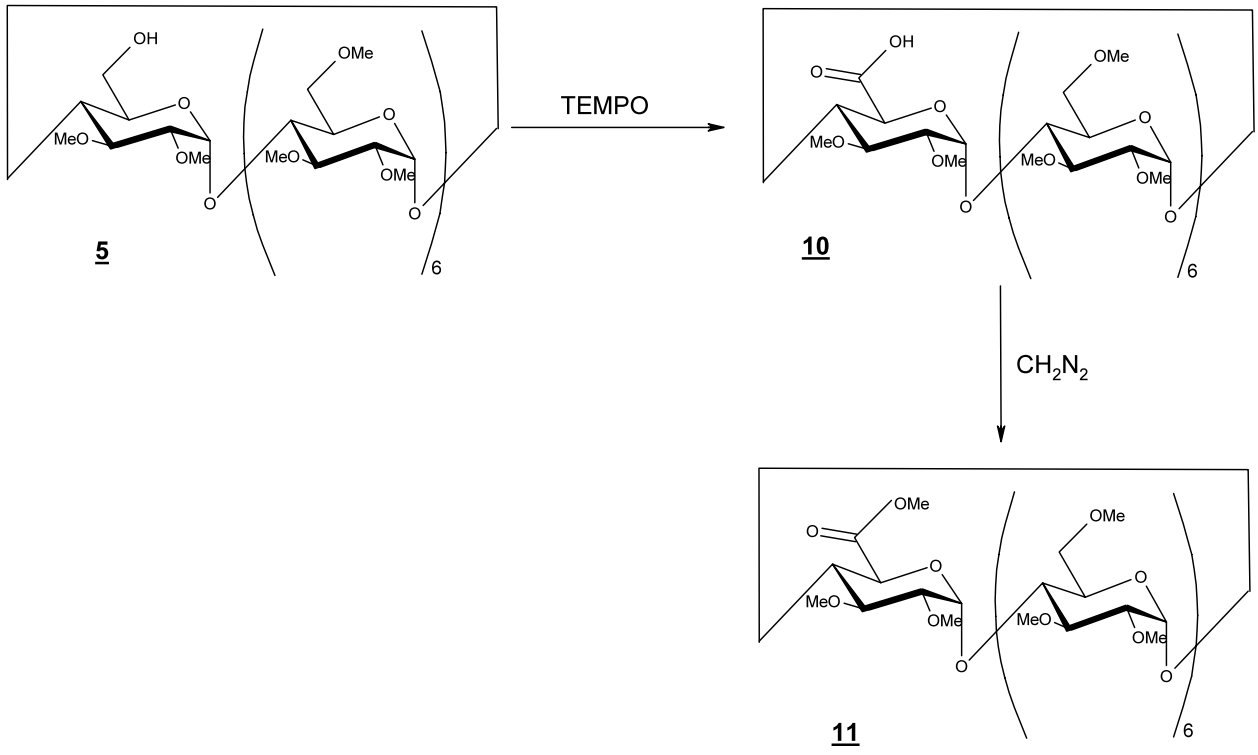
of the sodium iodoacetate in refluxing tetrahydrofuran (Scheme 2) and the expected products **6** and **7** were obtained with 73 and 80% yield, respectively, after purification.

The corresponding methyl esters **8**, **9** of **6** and **7** were formed quantitatively by esterification with an excess of an ether solution of diazomethane.

Another possibility to obtain acidic derivatives of permethylated CDs lies in the direct and full oxidation of one 6-position. Recently Vignon et al.[9] have described oxidation of the primary face β-CD with 2,2,6,6-tetramethylpiperidin-1-oxyl radical (TEMPO) (Scheme 3). The authors claimed that they obtained a mixture from which the 6^I-desoxy-6^I-carboxycyclomaltoheptaose was isolated. In the same way, we developed a selective



Scheme 2. Synthesis of mono-2 and mono-6-carboxymethyl permethylated β-CD and their corresponding esters.



Scheme 3. Direct oxidation of mono-6-hydroxy-β-CD and esterification.

oxidative method starting from $2^{I\text{-}VII},3^{I\text{-}VII},6^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose **5** and TEMPO that led satisfactorily to acid **10**.

An HPLC method was developed (see Section 4) allowing the control of various reaction media and purity of corresponding compounds. Mass spectra data (Table 1) were collected with HPLC coupling with MS using ESI as ionization technique: The molecular ions $[M+NH_4]^+$ and $[M+Na]^+$ were observed and, some times, the $[M+K]^+$. The mass data were in very good correlation with the isotopic calculated mass. The presence of $[M+2Na-H]^+$ and $[M+Na+K-H]^+$ was also noticed on the acidic derivatives **6**, **7** and **10** spectra according to the formation of corresponding acetate. A doubly charged ions peak was also detected in good agreement with proposed structure.

The structural determination of all compounds was achieved by using one or two-dimensional NMR experiments. In the first step, the two dimensional NMR experiments COSY (^1H–^1H) and HSQC (^1H–^{13}C) allowed the proton and carbon NMR spectra to be assigned and substituted carbon to be identified. In a second step, the analysis of the HMBC experiment showed the correlation between the substituted carbon and the methylenic protons of substituent carboxymethyl group. Some of the spectral data of compound **8** are illustrated in Figure 1, showing regions of HSQC (Fig. 1A) and HMBC (Fig. 1B) containing the $^1J_{CH}$ connectivity between substituted carbon C2′/H2′ and $^3J_{CH}$ carbon C2′/methylenic protons of substituent carboxymethyl group. Moreover, Figure 2 presents HSQC experiment related to compound **10** with an amazing set of different values for each correlation $^1J_{CH}$

Table 1. ESI mass spectra of acidic and ester derivatives of permethylated β-CD

Compounds	$[M+NH_4]^+$ *m/z*		$[M+Na]^+$ *m/z*		$[M+K]^+$ *m/z*	
	Calcd[a]	Measured (%)[b]	Calcd	Measured (%)	Calcd	Measured (%)
2-OPMB **3**	1552.8	1552.7	1557.7	1557.6	1573.7	1573.6
2-OCH_2COOH **6**	1490.7	1490.6	1495.7	1495.6	1511.6	1511.6
6-OCH_2COOH **7**	1490.7	1490.6	1495.7	1495.6	1511.6	1511.5
6-COOH **10**	1446.7	1446.7	1451.6	1451.6	1467.6	1467.6
2-OCH_2COOMe **8**	1504.7	1504.8	1509.7	1509.7	1525.7	–
6-OCH_2COOMe **9**	1504.7	1504.7	1509.7	1509.7	1525.7	1525.8
6-COOMe **11**	1460.7	1460.8	1465.7	1465.7	1481.6	–

[a] Isotopic mass.
[b] Relative intensity of peaks.

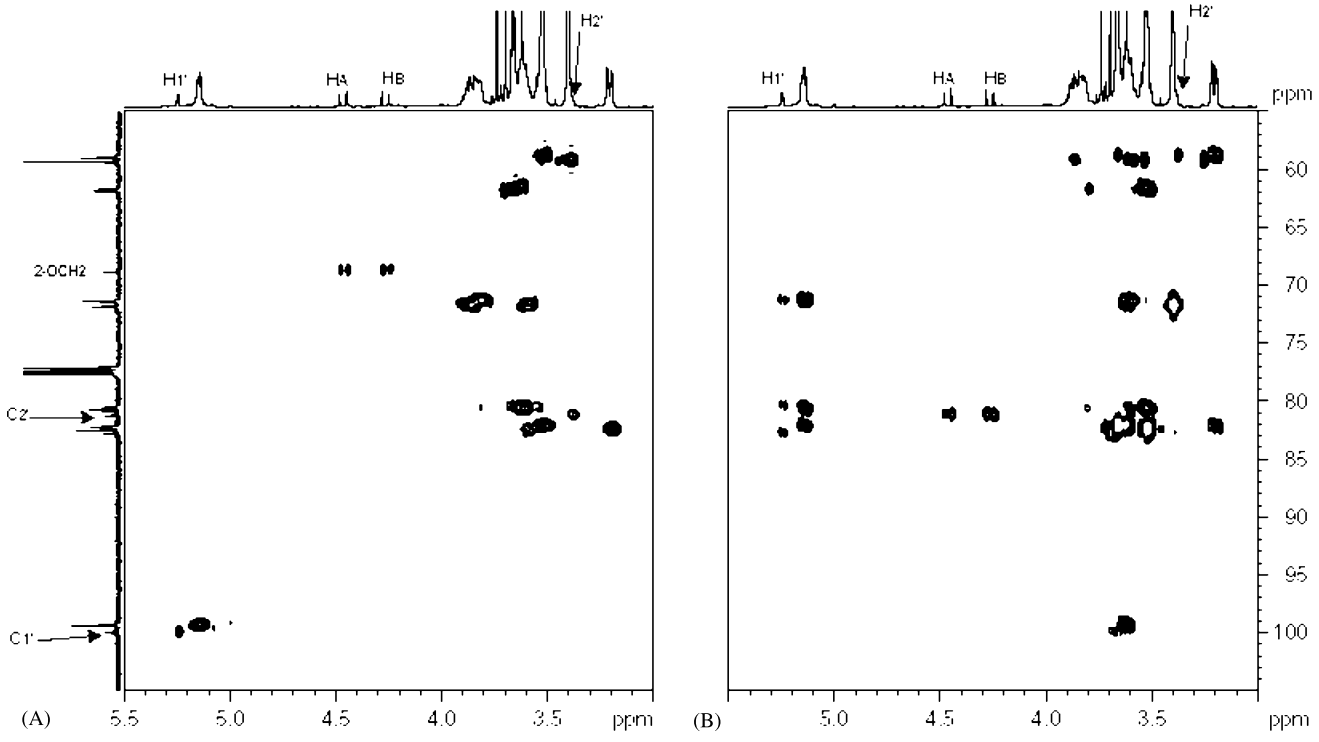


Figure 1. Section of the 500 MHz HSQC and HMBC ($CDCl_3$, 25°C) spectra of compound **8**, with the 1D ^1H and ^{13}C spectra along the side and the top. (A) HSQC; the cross-peak corresponding to C1′, C2′ and 2′-OCH_2COOH of the substituted cycle are indicated. (B) HMBC shows the correlation between C2′ and methylenic proton of the substituent carboxymethyl group.

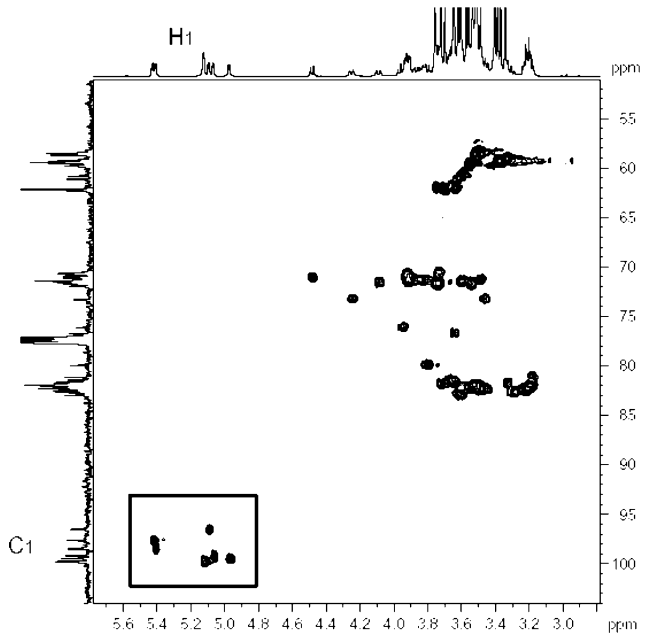


Figure 2. Section of the 500 MHz HSQC ($CDCl_3$, 25°C) spectra of compound **10**, with the 1D ^1H and ^{13}C spectra along the side and the top. The frame corresponds to correlation $^1J_{CH}$ C1 and H1.

C1/H1 thus supporting the loss of symmetry for these monosubstituted CDs.

3. Conclusion

In this work, the synthesis and full characterization of mono-carboxymethyl permethylated β-CD isomers **6**, **7** as well as mono-6-carboxy permethylated β-CD **10** are achieved with a satisfying overall yield. The corresponding esters **8**, **9** and **11** were isolated via a quantitative reaction using diazomethane.

Nowadays, the complete and unambiguous characterization of cyclodextrin derivatives is required for studying the connection between the perfectly determined substituent position and the enantioselectivity of these molecules as CSP in various chromatographic techniques. Having thus a set of fully characterized monofunctionalized CDs corresponding to desymmetrised structures and their expected unique and attractive properties, we are currently investigating chiral recognition abilities (chiral complexes and new CSPs for HPLC and GC) of these new products. This study will be extended to the preparation of same acidic and ester derivatives in 3-position.

4. Experimental

4.1. General methods

Cyclomaltoheptaose β-CD (generously supplied by Roquette-Frères 62136 Lestrem, FRANCE) was dried at 80°C under vacuum 48 h. Dimethylsulfoxide (DMSO), dimethylformamide (DMF) and diethyl ether (ether) were dried over calcium hydride (CaH_2) and fractionally distilled. Tetrahydrofuran (THF) was distilled over sodium. Dichloromethane was dried under calcium chloride ($CaCl_2$) and distilled. Other commercially available reagents were used without further purification. Reactions were followed by thin layer chromatography (TLC) on silica gel (Merck, Darmstadt, Germany) with H_2SO_4 (10% in ethanol) revelation. Purifications were carried out using flash column chromatography with silica gel (63–200 mesh; Normasil Prolabo, Fontenay-sous-bois, France).

The purity of synthetic products was established by HPLC/ESLD and confirmed by NMR spectroscopy data, and HPLC/MS analysis. HPLC: ThermoQuest P1500, column Macherey-Nagel Nucleosil C18, 25 cm× 4.6 mm, 5 μm, Mobile Phase: methanol/water, 1 mL min^{-1}. ELSD: Evaporative Light Scattering Detectors, Eurosep instrument DDL31, PM = 600 mV, T = 55°C. MS: Finnigan Navigator, Electrospray Ionisation, Source Heater T = 130°C, cone voltage = 60V. NMR spectra ^1H 500.13 MHz; ^{13}C 125.75 MHz were recorded on a Bucker Advance DMX 500 instrument. The assignment of ^1H and ^{13}C signals was supported by one- and two-dimensional ^1H–^1H COSY, DEPT, ^1H–^{13}C HMBC and HSQC experiments. All the experiments were recorded using $CDCl_3$ as solvent. Approximately 20 mg of sample were dissolved with 0.5 mL of solvent. Elemental analyses were carried out on an EA 1110 (CE instruments).

4.2. 2^{I}-*O*-*p*-Methoxybenzyl-$2^{II\text{-}VII}$,$3^{I\text{-}VII}$,$6^{I\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose 3

Under an atmosphere of nitrogen, anhydrous β-CD (2.00 g, 1.76 mmol, 1 equiv.), dried under vacuum at 80°C for 48 h, was added to anhydrous DMSO (30 mL) in a 125-mL three-necked flask equipped with a dropping funnel and nitrogen inlet. Sodium hydroxide (211 mg, 5.29 mmol, 3 equiv.) was added and magnetic stirring was continued for 48 h at room temperature (rt). *p*-Methoxybenzyl chloride (689 mg, 4.40 mmol, 2.5 equiv.) dissolved in DMSO (6 mL) was added dropwise. The mixture was stirred at rt for 24 h. In another flask sodium hydride 60% in mineral oil, washed three times with diethyl ether (1.69 g, 70.42 mmol, 40 equiv.), was added to DMSO (20 mL). The mixture was stirred for 45 min at 50°C. A green-blue coloration was observed at this point. After cooling to rt the benzylated CD solution was then slowly introduced and the resulting suspension was vigorously stirred for 2 h. Methyl iodide (10 g, 70.42 mmol, 40 equiv.) was then added dropwise to the suspension. The mixture was stirred at rt overnight. The excess of NaH was quenched by methanol (5 mL) and water (50 mL). The reaction mixture was extracted by chloroform (3×50 mL). The organic phase was washed with 10% aqueous HCl solution (3×30 mL) and saturated aqueous $NaHCO_3$ (2×30 mL). After drying over $MgSO_4$, filtration and evaporation under vacuum, the crude product was purified by column chromatography on silica gel

(toluene–acetone, 75/25 v/v) yielded (1.30 g, 0.84 mmol, 48%) as white powder. TLC (1/1 toluene–acetone) R_f 0.42; ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 7.36 (d, 2H, $^3J_{ortho,meta}$ 8.55 Hz, H-*ortho*); 6.87 (d, 2H, $^3J_{ortho,meta}$ 8.55 Hz, H-*meta*); 5.20–5.13 (m, 6H, H-1); 4.95 (m, 6H, H-1′); 4.73–4.62 (m, 2H, CH_2-Ph); 3.85 (m, 7H, H-6b); 3.82 (m, 7H, H-5); 3.82 (s, 3H, Ph-OCH_3); 3.58 (m, 7H, H-6a); 3.67–3.65 (m, 21H, 3-OCH_3); 3.53 (m, 6H, H-4); 3.49 (m, 1H, H-3); 3.54–3.52 (m, 15H, 2-OCH_3); 3.39 (s, 21H, 6-OCH_3); 3.38 (m, 1H, H-2′); 3.25–3.19 (m, 6H, H-2′); ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 159.50 (C*para*-OCH_3); 131.12 (O-CH_2-PH); 129.85 (C-*ortho*); 113.91 (C-*meta*); 99.62 (C1′); 99.15 (C-1); 82.57–82.00 (C-2,3); 79.91 (C2′); 80.86–80.15 (C-4); 72.65 (Ph-CH_2-); 71.89–71.16 (C-5,6); 62.09–62.07 (3-OCH_3); 59.04 (6-OCH_3); 59.09–58.82 (2-OCH_3).HPLC/MS (MeOH–H_2O 85/15) tr: 24.9 min m/z 1552.7 [M+NH_4+]; 1557.6 [M+Na^+]; 1573.61 [M+K^+]. Anal. calcd for $C_{70}H_{118}O_{36}$: C, 54.75; H, 7.74. Found: C, 54.62; H, 7.80.

4.3. $2^{I\text{-}VI}$,$3^{I\text{-}VII}$,$6^{I\text{-}VII}$-Eicosa-*O*-methyl-cyclomaltoheptaose 4

To a stirred solution of **3** (1.3 g, 0.847 mmol, 1 equiv.) and CH_2Cl_2 (35 mL), H_2O (3.5 mL), phosphate buffer pH 7.01 (2.7 mL), DDQ (577 mg, 2.54 mmol, 3 equiv.) was added in one step. The reaction mixture was monitored by TLC on silica gel (toluene–acetone, 1/1 v/v) and after 2.5 h, the reaction mixture was quenched in addition of H_2O (3 mL) and extracted by CH_2Cl_2 (3×20 mL). The organic phase was washed with saturated solutions of $Na_2S_2O_4$ (2×10 mL), $NaHCO_3$ (3×10 mL) and NaCl (3×10 mL). After drying over $MgSO_4$, filtration and evaporation under vacuum, the crude product was purified by column chromatography on silica gel (4/1–3/1 toluene–acetone, stepwise) yielding (983 mg, 0.694 mmol, 82%) in white powder: TLC (1/1 toluene–acetone) R_f 0.37; ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 5.15–5.12 (m, 6H, H-1); 4.90 (d, 1H, $^3J_{2,1}$ 3.3 Hz, H-1); 4.38 (d, 1H, $J_{OH,2}$ 11.2 Hz, 2-OH); 3.89–3.87 (m, 7H, H-6_a); 3.82–3.79 (m, 7H, H-5); 3.74 (s, 3H, 3-OCH_3); 3.73 (s, 3H, 3-OCH_3); 3.67 (s, 3H, 3-OCH_3); 3.66 (s, 3H, 3-OCH_3); 3.65 (s, 3H, 3-OCH_3); 3.64 (s, 3H, 3-OCH_3); 3.63 (s, 3H, 3-OCH_3); 3.72–3.55 (m, 14H, H-4, H-6_b); 3.61–3.43 (m, 6H, H-3); 3.52 (m, 18H, 2-OCH_3); 3.50 (m, 1H, H-2); 3.41–3.40 (m, 21H, 6-OCH_3); 3.35 (m, 1H, H-3); 3.27–3.18 (m, 6H, H-2). ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 102.30–99.04 (C-1); 84.40 (C-3); 83.30–79.05 (C-2, 3, 4); 74.57 (C-2-OH); 72.23 (C-5); 72.04–71.61 (C-6); 71.43–70.91 (C-5); 70.66 (C-6); 62.37–61.27 (3-OCH_3); 59.51–58.65 (6-OCH_3, 2-OCH_3); HPLC/MS (MeOH–H_2O 80/20) tr: 14.3 min m/z 1437.7 [M+Na^+], 1453.7 [M+K^+]. Anal. calcd for $C_{62}H_{110}O_{35}$: C. 52.61; H. 7.83. Found: C. 52.42; H. 7.90.

4.4. 2^{I}-*O*-Carboxymethyl-$2^{II\text{-}VII}$,$3^{I\text{-}VII}$,$6^{I\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose 6

To a stirred mixture of sodium hydride (68 mg, 2.82 mmol, 5 equiv., washed three times with diethyl ether) in anhydrous THF (10 mL) a solution of $2^{I\text{-}VI}$,$3^{I\text{-}VII}$,6^{I}-VII-eicosa-*O*-methyl-cyclomaltoheptaose (800 mg, 0.565 mmol, 1 equiv.) in THF (12 mL) was added dropwise. The resulting mixture was stirred and heated under reflux during 2 h. After cooling to 0°C, the sodium salt of iodoacetic acid (353 mg, 1.70 mmol, 3 equiv.) was added step by step and stirring was continued at 0°C for 1 h, and then refluxed overnight. The reaction mixture was quenched by addition of methanol (3 mL). The solvent was evaporated under reduced pressure; the residue was dissolved in CH_2Cl_2 (10 mL) and washed with HCl 3% (3×5 mL) and brine (5×5 mL). After drying over $MgSO_4$, and filtration, the solvent was removed under vacuum. The crude product was purified by column chromatography on silica gel (20/1 chloroform–methanol) yielding (583 mg, 0.396 mmol, 73%) in white powder. TLC (9/1 chloroform–methanol) R_f 0.34; ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 5.21–5.07 (m, 6H, H-1); 5.01 (d, 1H, $^3J_{1',3'}$ 3.67 Hz, H-1′); 4.46 (d, 1H, $^2J_{A,B}$ 16.32 Hz, H-A); 4.21 (d, 1H, $^2J_{A,B}$ 16.42 Hz, H-B); 3.91–3.76 (m, 7H, H-6_a); 3.87–3.84 (m, 7H, H-5); 3.73–3.62 (m, 21H, 3-OCH_3); 3.65–3.55 (m, 7H, H-4); 3.65–3.53 (m, 7H, H-6_b); 3.62 (m, 1H, H-3′); 3.57–3.48 (m, 6H, H-3); 3.54–3.49 (m, 18H, 2-OCH_3); 3.41–3.39 (m, 21H, 6-OCH_3); 3.37 (m, 1H, H-2′); 3.24–3.18 (m, 6H, H-2). ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 171.28 (COOH); 99.83–99.16 (C-1); 82.90 (C-3′); 82.67–81.93 (C-3, 2); 81.66 (C-2′); 80.99–79.88 (C-4); 72.36–70.94 (C-5, 6); 70.02 (CH_2-COOH); 62.35–61.41 (3-OCH_3); 59.56–59.13 (6-OCH_3); 58.72–58.61 (2-OCH_3); HPLC/MS (MeOH–H_2O with 5% acetic acid 75/25) tr: 17.9 min m/z 1490.6 [M+NH_4]$^+$, 1495.6 [M+Na]$^+$, 1511.6 [M+K]$^+$, 1517.6 [M+2Na−H]$^+$, 1533.5 [M+Na+K-H]$^+$. Anal. calcd for $C_{64}H_{112}O_{37}$: C, 52.17; H, 7.66. Found: C, 51.90; H, 7.77.

4.5. 2^{I}-*O*-Methoxycarbonylmethyl-$2^{II\text{-}VII}$,$3^{I\text{-}VII}$,$6^{I\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose 8

A cold solution (−5°C) of 2^{I}-*O*-carboxymethyl-$2^{II\text{-}VII}$, $3^{I\text{-}VII}$,$6^{I\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose **6** (50 mg, 0.034 mmol, 1 equiv.) in ether (4.5 mL) was esterified by addition of slight excess of diazomethane (CH_2N_2) in ether generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald, Aldrich) according to the Aldrich Bulletin Al-180. After 30 min, reaction was considered to be complete, solvent and unreacted CH_2N_2 were removed by evaporation under atmospheric pressure. Purification of the residue recrystallisation in pentane afforded methyl ester 2^{I}-*O*-methoxycarbonylmethyl-$2^{II\text{-}VII}$,$3^{I\text{-}VII}$,$6^{I\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose (48.0 mg, 0.034 mmol, 95%) as white powder. ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 5.25 (d, 1H, $^3J_{1',3'}$ 3.67 Hz, H-1′); 5.15 (m, 6H, H-1); 4.46 (d, 1H, $^2J_{A,B}$ 16.50 Hz, H-A); 4.26 (d, 1H, $^2J_{A,B}$ 16.50 Hz, H-B); 3.87 (m, 7H, H-6_a); 3.82 (m, 7H, H-5); 3.74 (OCH_3 ester); 3.60–3.70 (m, 21H, 3-OCH_3); 3.67–3.56 (m, 7H, H-4); 3.59 (m, 7H, H-6_b); 3.60 (m, 1H, H-3′); 3.55–3.49 (m, 6H, H-3); 3.52 (m, 18H, 2-OCH_3); 3.39 (m, 21H, 6-OCH_3); 3.38 (m, 1H, H-2′); 3.20 (m, 6H, H-2). ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 171.28 ($COOCH_3$); 99.99 (C-1′); 99.38 (C-1); 82.82 (C-3′); 82.51, 82.34, 82.23, 82.12 (C-3, 2); 81.26 (C-2′); 80.80, 80.65, 80.61, 80.61, 80.55 (C-4); 80.47 (C-4′); 71.86, 71.77, 71.40, 71.37 (C-5, 6); 68.80 (CH_2-COOH); 61.85, 61.82, 61.79, 61.77, 61.65 (3-OCH_3); 59.33 (6-OCH_3);

58.94, 58.86 (2-OCH_3); HPLC/MS (MeOH–H_2O, 85/15) tr: 10.5 min m/z 763.6 $[M+NH_4+Na]^{2+}$, 766.5 $[M+Na+Na]^{2+}$, 1504.7 $[M+NH4]^+$, 1509.7 $[M+Na]^+$. Anal. calcd for $C_{65}H_{114}O_{37}$: C, 52.48; H., 7.72. Found: C, 52.37; H, 7.77.

4.6. 6^{I}-*O*-Carboxymethyl-2$^{I\text{-}VII}$,3$^{I\text{-}VII}$,6$^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose 7

Using the same procedure as for the preparation of **6**, the crude product was obtained after purification on column chromatography on silica gel (20/1 chloroform–methanol) yielding (666 mg, 0.452 mmol, 80%) in white powder. TLC (10/1 chloroform–methanol) R_f 0.29; ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 5.23–5.01 (m, 7H, H-1); 4.13 (d, 1H, $^2J_{A,B}$ 16.33 Hz, H-A); 4.04 (d, 1H, $^2J_{A,B}$ 16.33 Hz, H-B); 4.01 (m, 7H, H-6_a); 3.92–3.76 (m, 7H, H-5); 3.90 (m, 7H, H-6_b); 3.68–3.59 (m, 21H, 3-OCH_3); 3.67–3.54 (m, 7H, H-4); 3.54–3.40 (m, 7H, H-3); 3.54–3.48 (m, 21H, 2-OCH_3); 3.44 (s, 3H, 6′-OCH_3); 3.41–3.38 (m, 18H, 6-OCH_3); 3.15–3.21 (m, 7H, H-2). ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 171.55 (COOH); 99.80–98.30 (C-1); 82.50–81.40 (C-3, 2); 80.90 (C-4); 71.71–71.36 (C-5, 6); 72.14 (C-6′); 68.42 (CH_2-COOH); 62.45–61.07 (3-OCH_3); 60.05–58.16 (6-OCH_3/2-OCH_3); HPLC/MS (MeOH–acetic acid 5% 75/25) tr: 23.9 min m/z 1490.6 $[M+NH_4]^+$, 1495.6 $[M+Na]^+$, 1511.5 $[M+K]^+$, 1517.6 $[M+2Na-H]^+$, 1533.5 $[M+Na+K-H]^+$. Anal. calcd for $C_{64}H_{112}O_{37}$: C, 52.17; H, 7.66. Found: C, 52.32; H, 7.82.

4.7. 6^{I}-*O*-Methoxycarbonylmethyl-2$^{I\text{-}VII}$,3$^{I\text{-}VII}$,6$^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose 9

The same method as for preparation of **8** was carried out. **9** was recrystallized with pentane (49.0 mg, 0.033 mmol, 97%) to obtain a white powder. ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 5.17–5.15 (m, 7H, H-1); 4.22 (d, 1H, $^2J_{A,B}$ 16.35 Hz, H-A); 4.17 (d, 1H, $^2J_{A,B}$ 16.35 Hz, H-B); 3.87 (m, 7H, H-6_a); 3.82 (m, 7H, H-5); 3.75 (s, 3H, OCH_3 ester); 3.69 (m, 7H, H-6_b); 3.66 (m, 21H, 3-OCH_3); 3.64–3.60 (m, 7H, H-4); 3.53–3.50 (m, 7H, H-3); 3.52 (m, 21H, 2-OCH_3); 3.40 (m, 18H, 6-OCH_3); 3.22–3.17 (m, 7H, H-2). ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 171.08 (CO); 99.58 (C-1); 82.60–81.7 (C-3, 2); 81.50–81.00 (C-4); 73.00–71.00 (C-5, 6); 72.14 (C-6′); 69.20 (CH_2-COOH); 61.81 (3-OCH_3); 59.40 (6-OCH_3); 58.94 (2-OCH_3); HPLC/MS (MeOH–H_2O 85/15) tr: 10.7 min m/z 763.59 $[M+NH_4+Na]^{2+}$, 766.51 $[M+Na+Na]^{2+}$, 1504.70 $[M+NH4]^+$, 1509.74 $[M+Na]^+$, 1525.83 $[M+K]^+$. Anal. calcd for $C_{65}H_{114}O_{37}$: C, 52.48; H, 7.72. Found: C, 52.64; H, 7.77.

4.8. 6^{I}-Desoxy-6^{I}-carboxy-2$^{I\text{-}VII}$,3$^{I\text{-}VII}$,6$^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose 10

Compound **10** was prepared as previously described from compound **5**.[9]

^{1}H NMR (500.13 MHz, $CDCl_3$): δ 5.12 (d, 1H, H-1′); 5.12–4.96 (m, 6H, H-1); 4.45 (m, 1H, H-5′), 4.25–3.46 (m, 7H, H-6); 4.00–3.80 (m, 7H, H-5); 3.95 (m, 1H, H-4′); 3.80–3.58 (m, 7H, H-4); 3.76–3.12 (m, 60H, 3-OCH_3, 2-OCH_3, 6-OCH_3); 3.73 (m, 1H, H-3′); 3.62–3.42 (m, 6H, H-3); 3.33 (m, 1H, H-2′); 3.24–3.17 (m, 6H, H-2). ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 171.25 (COOH); 99.77 (C-1′); 99.70–96.01 (C-1); 81.80 (C-3′); 82.89–81.93 (C-3); 82.89–81.25 (C-2); 81.73 (C-2′); 79.99–79.98 (C-4); 75.99 (C-4′); 73.39–70.59 (C-5, 6); 62.36–58.47 (3-OCH_3, 6-OCH_3, 2-OCH_3); HPLC/MS (MeOH–H_2O with 5% acetic acid 75/25) tr: 23.9 min m/z 741.66 $[M+NH_4+NH_4]^{2+}$, 1446.66 $[M+NH_4]^+$, 1451.62 $[M+Na]^+$, 1467.59 $[M+K]^+$, 1473.64 $[M+2Na-1]^+$. Anal. calcd for $C_{62}H_{108}O_{36}$: C, 52.09; H, 7.61. Found: C, 52.11; H, 7.90.

4.9. 6^{I}-Methoxycarbonyl-6^{I}-desoxy-2$^{I\text{-}VII}$,3$^{I\text{-}VII}$,6$^{II\text{-}VII}$-eicosa-*O*-methyl-cyclomaltoheptaose 11

The same method as for preparation of **8** was carried out. **11** was recrystallised in pentane (47.8 mg, 0.032 mmol, 95%) to obtain a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.19–5.01 (m, 7H, H-1); 4.23 (d, 1H, H-5′); 3.83 (d, 1H, H-4′); 3.73–3.49 (m, 7H, H-6); 3.71 (s, 3H, OCH_3 ester); 3.70–3.90 (m, 6H, H-5); 3.61–3.53 (m, 21H, 3-OCH_3); 3.52–3.68 (m, 6H, H-4); 3.40–3.50 (m, 7H, H-3); 3.48–3.40 (m, 21H, 2-OCH_3); 3.33–3.27 (m, 18H, 6-OCH_3); 3.20 (d, 1H, H-2′); 3.17–3.05 (m, 6H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.97 (CO); 99.25–98.22 (C-1); 82.01–81.53 (C-3, 2); 80.06 (C-2′); 80.07–79.97 (C-4); 81.17 (C-4′); 72.12 (C-5′); 71.18–70.16 (C-5, 6); 61.04–61.02 (3-OCH_3); 58.84–58.84 (6-OCH_3); 58.35–58.06 (2-OCH_3); 52.25 (OCH_3 ester); HPLC/MS (MeOH–H_2O 85/15) tr: 10.7 min m/z 741.66 $[M+NH_4+Na]^{2+}$, 745.70 $[M+Na+Na]^{2+}$, 1460.79 $[M+NH4]^+$, 1465.72 $[M+Na]^+$, 1525.83 $[M+K]^+$. Anal. calcd for $C_{63}H_{110}O_{36}$: C, 52.42; H. 7.68. Found: C, 52.50; H, 7.80.

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