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Synthesis of the three isomeric mono-2-, 3-, or 6-hydroxy permethylated β-cyclodextrins and unambiguous high field NMR characterisation

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Abstract—The three isomeric mono-2-, 3- or 6-hydroxy permethylated β-cyclodextrins are good precursors for a wide variety of mono functionalised 'permethyl' β-cyclodextrins. In this work, we describe the selective access to mono-6-hydroxy (via the mono-6-tertbutyldimethylsilyl derivative), mono-2-hydroxy (via the mono-2-benzyl derivative) and mono-3-hydroxypermethylated-β-cyclodextrins (by under-methylation of heptakis (2,6-di-*O*-methyl)-β-cyclodextrin). These derivatives were characterised by high field ^{1}H and ^{13}C NMR spectroscopy. The position of the free hydroxyl group was confirmed unambiguously by ^{13}C NMR after methylation with ^{13}C-labelled methyl iodide.

1. Introduction

Cyclodextrin derivatives are commonly used in chiral chromatography, usually as symmetric structures for gas chromatography (GC),[1] as mobile phase additives in liquid chromatography (LC)[2] and in capillary electrophoresis (CE).[3] For use as a component of the stationary phase in LC,[4] supercritical fluid chromatography (SFC),[5] electrochromatography (EC)[6] and sometimes for GC,[7] the chiral selectors need to be immobilised or grafted onto the surface of fused silica capillaries or polymeric material via a spacer arm in order to improve the stability of the stationary phase. Previous work has shown the interest in asymmetric cyclodextrins. For example, Perly et al.[8] observed a strong deformation of the cyclodextrin cavity and noticed that the mono-3,6-anhydro-2,3,6-tri-*O*-methyl-β-cyclodextrin was 330 more times soluble at 90°C than the 2,3,6-tri-methyl-β-cyclodextrin.

Because of the number of hydroxyl groups of β-cyclodextrin that can potentially react with an incoming reagent, modification at only one of the 21 positions is a challenging task. Methods for selective modification of cyclodextrin can be divided into three categories: the shortest route,[9,10] where the chemistry of cyclodextrin is exploited to get the desired product; the longest method,[10,11] where a series of protection and deprotection steps are used to selectively reach the positions which would otherwise not be selectively accessible; and the 'sledgehammer' method, where cyclodextrins are reacted indiscriminately to give mixtures of products from which the desired product is separated by chromatographic methods.[12]

Two primary factors need to be considered in the chemistry of cyclodextrins for their modification. Firstly, the nucleophilicity of the different hydroxyl groups and, secondly, the ability of cyclodextrin to complex with the reagent used. Of the three types of hydroxyl groups, those at the C-6 position are the most reactive in kinetic conditions (and often the most nucleophilic), those at the C-2 position are the most acidic, and those at the C-3 position are the most hindered. These differences in reactivity can easily explain the great number of cyclodextrin derivatives[13] and the possibility of access to asymmetric cyclodextrins.

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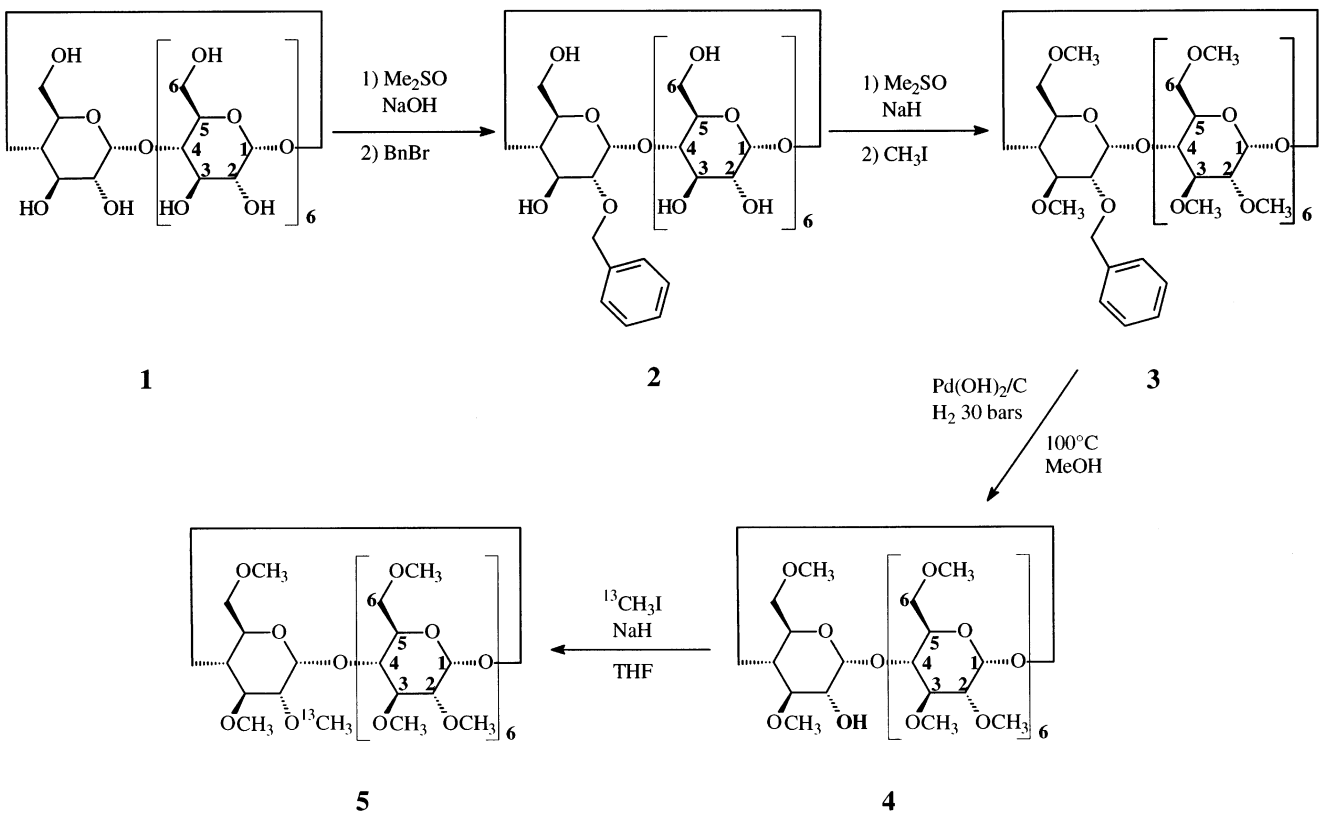
In our laboratory,[14] we have developed a general way to access asymmetric γ-cyclodextrin derivatives by insertion of a selective modified sugar unit in fully methylated β-cyclodextrin. Preliminary results have shown that asymmetric cyclodextrins have a particular behaviour as the stationary phase in chromatography. In that context, it seemed of interest to our group to study the enantioselectivity discrimination of each monohydroxy cyclodextrin derivative. For this, we wish to report herein a simple and novel access to the three isomeric monohydroxy eicosa-*O*-methyl-β-cyclodextrins. The position of the hydroxyl groups in these derivatives was established by ^{1}H and ^{13}C NMR spectroscopy and confirmed by ^{13}C NMR spectroscopy after methylation with ^{13}C-labelled methyl iodide.

2. Results and discussion

Concerning the $2^{I–VI},3^{I–VII},6^{I–VII}$-eicosa-*O*-methyl cyclomaltoheptaose **4**, a three-step reaction procedure from β-cyclodextrin **1** has been used (Scheme 1). The first step involved selective benzylation of only one position with benzyl bromide in the presence of 2.9 equivalents of sodium hydroxide. The principle behind this benzylation method was that the 2-hydroxyl groups of β-cyclodextrin are the most acidic and can be preferentially deprotonated by a strong base. The resulting oxyanion, the most nucleophilic species in the resulting reaction mixture, would then preferentially react with an electrophile. The second step was the methylation of the last 20 hydroxyl groups. The separation of the different components was realised at this step by column chromatography on silica gel. The 2^{I}-*O*-benzyl $2^{II–VII},3^{I–VII},6^{I–VII}$-eicosa-*O*-methyl cyclomaltoheptaose **3** was obtained in 47% yield. The last step involved deprotection by catalytic hydrogenolysis, which was achieved in the presence of $Pd(OH)_2$ at 100°C. The possibility of benzyl inclusion in the cavity of cyclodextrin was considered to explain the difficulty of this deprotection step, but the homonuclear two-dimensional correlation experiments (ROESY) recorded either in $CDCl_3$ or CD_3OD proved unambiguously that the phenyl substituent is not in the cavity. The problematic removal of the protecting group is probably a result of the difficulty in approach of the catalyst to the hindered cyclodextrin. The 2-O position of the last hydroxyl group was identified by using one- and two-dimensional NMR experiments. First, the 2D COSY (Fig. 1) and 2D HSQC (Fig. 2A) experiments allowed the proton (H-2) and carbon (C-2) of the unsubstituted site to be assigned; then the HMBC (Fig. 2B) experiment was used to correlate the unsubstituted carbon to the hydroxyl group. This result was confirmed by ^{13}C NMR after methylation with ^{13}C-labelled methyl iodide. The NMR signals (Fig. 3) of derivative ^{13}C-enriched **5** were compared with the NMR spectrum from fully methylated β-cyclodextrin (prepared using Hakomori's procedure).[15]

For the preparation of $2^{I–VII},3^{I–VI},6^{I–VII}$-eicosa-*O*-methyl cyclomaltoheptaose **7**, the previously employed method with protection and deprotection steps could not be employed. Thus, the 'sledgehammer' method



Scheme 1. Synthesis of mono-2-hydroxy-β-cyclodextrin via monobenzylation.

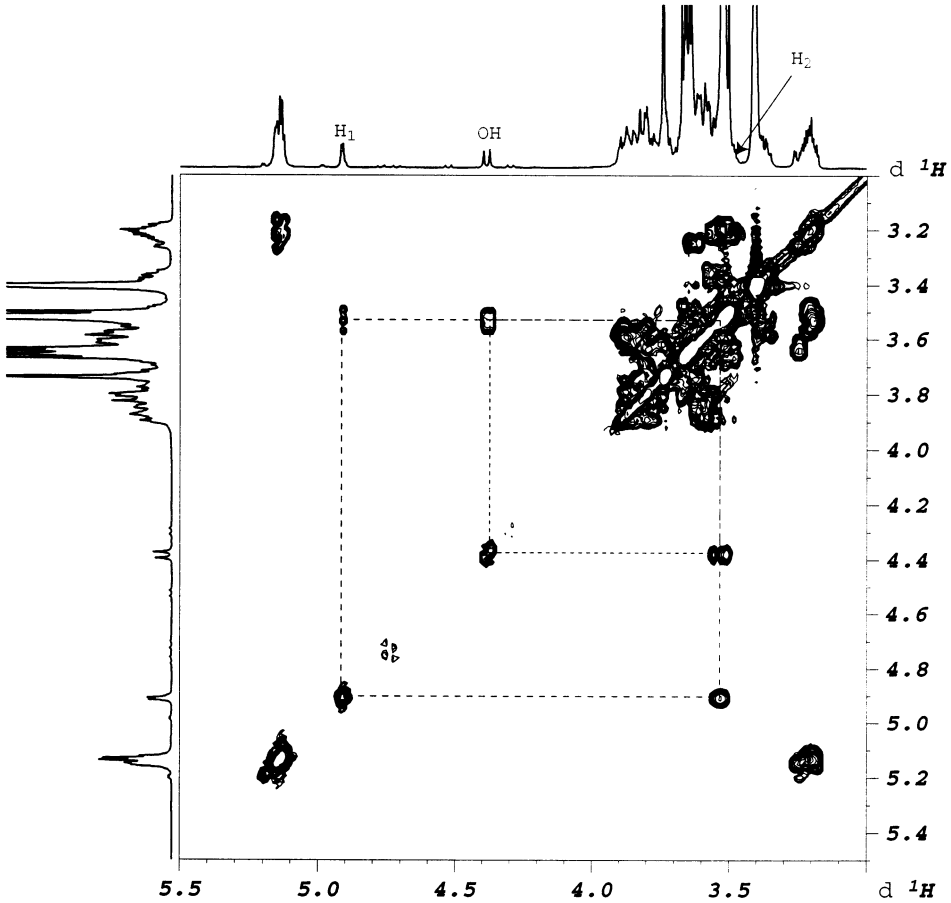


Figure 1. ^{1}H NMR COSY ($CDCl_3$, 25°C) spectrum of compound **4** showing cross-peaks corresponding to the correlation between H_1, H_2 and 2-OH.

was used. A mixture of methylated cyclodextrins was prepared and the desired product was separated out by chromatographic purification. Two strategies were investigated. The first was the synthesis of **7** by reaction of cyclodextrin **1** with 20 equivalents of sodium hydroxide and 20 equivalents of CH_3I. However, analysis of the resulting mixture by HPLC/MS showed two varieties of eicosa-*O*-methyl β-cyclodextrin (**4** and **7**). The separation of these products was very difficult, so another strategy was considered to avoid this. In this alternative approach, compound **7** was prepared starting from heptakis(2,6-di-*O*-methyl)-β-cyclodextrin **6**, which was purchased from Cyclolab Product (Budapest, Hungary) or prepared and purified as described by Lehn et al.[16] The cyclodextrin derivative **6** was methylated (Scheme 2) with 10 equivalents of sodium hydride and only 6 equivalents of CH_3I to give a mixture of undermethylated β-cyclodextrin derivatives. This mixture contained only one monohydroxy variety, eicosa-*O*-methyl β-cyclodextrin **7**, which was obtained after purification in 22% yield. The 3-O position of the last hydroxyl group was identified as previously described for **4** (data not shown) and confirmed by ^{13}C NMR (Fig. 3).

In the preparation of $2^{I–VII},3^{I–VII},6^{I–VI}$-eicosa-*O*-methyl cyclomaltoheptaose **11**, a strategy similar to that of Bradshaw and co-workers[17] was chosen (Scheme 3). Compound **11** was obtained from β-cyclodextrin **1** in three steps by selective *tert*-butyldimethylsilylation at only the less hindered O-6 position to give **9**; subsequent methylation of the last 20 hydroxyl groups and finally removal of the silyl protecting group[18] gave the desired product in 45% yield. The 6-O position of the free hydroxyl group was identified as previously described for **4** (data not shown) and confirmed by ^{13}C NMR experiments (Fig. 3). Moreover, the three isomers were easily separated by high performance liquid chromatography (Fig. 4) and could easily be located even in complex chromatograms.

In conclusion, we were able to prepare the three monohydroxy isomers **4**, **7** and **11** in satisfactory yields and without complex purification steps. Their use as chiral selectors in GC, pure or diluted in polysiloxane, is presently under investigation. These three monohydroxy permethylated β-cyclodextrins are good precursors for a wide variety of monofunctionalised permethyl β-cyclodextrins, in particular monoalkenyl β-cyclodextrins. It seemed of interest to our group to investigate the link between the position of cyclodextrin attachment and the related enantioselectivity observed with various racemic compounds, which has been partially studied by Bradshaw et al.[19] Nevertheless, we will propose a systematic investigation of the influence of the nature, the length and the position (2, 3 or 6) of the

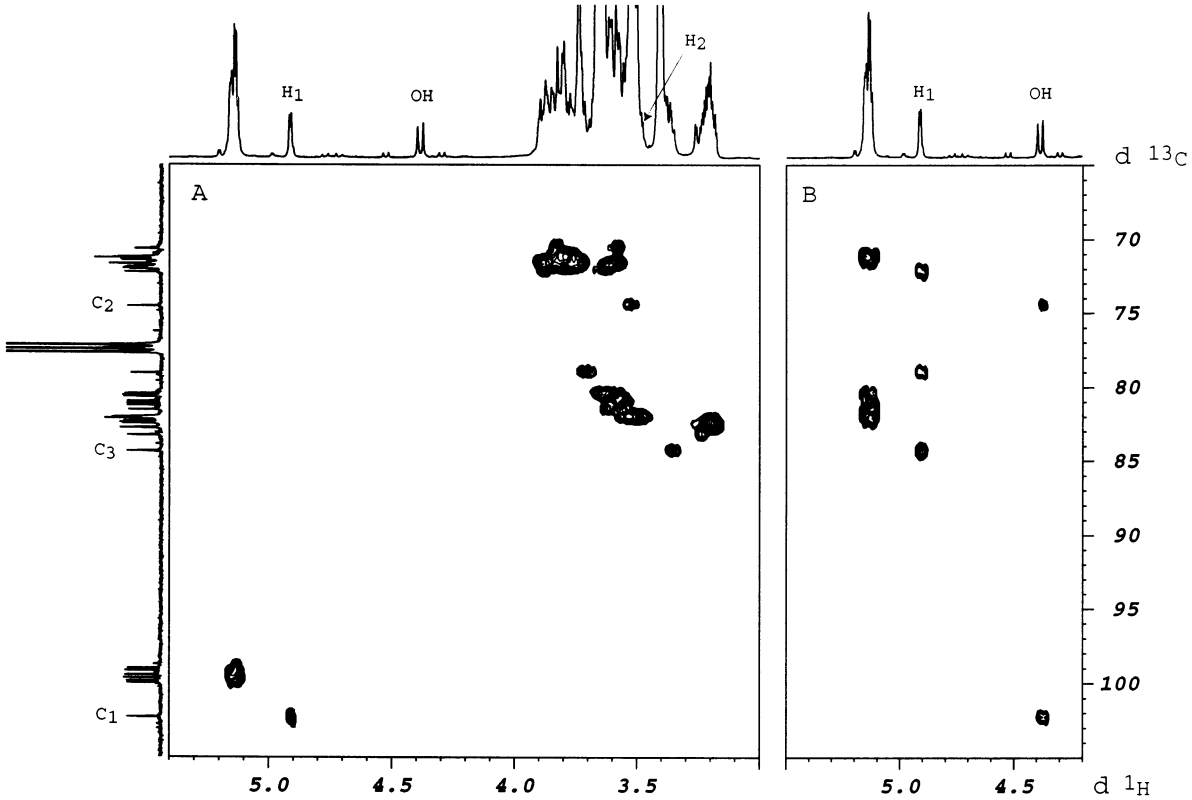


Figure 2. Sections of the 500 MHz HSQC and HMBC ($CDCl_3$, 25°C) spectra of compound **4**, with the 1D ^1H and ^{13}C spectra along the side and the top. (A) HSQC; the cross-peaks corresponding to C_1, C_2 and C_3 of the cycle with hydroxyl group are indicated. (B) HMBC shows the correlations between 2-OH, C_2 and C_3.

arm attachment with various chromatographic techniques.

3. Experimental

3.1. Instrumentation and general methods

NMR spectra (^1H, 500.13 MHz; ^{13}C, 125.75 MHz) were recorded on a Bruker Avance 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, but to improve peak resolution DMSO-d_6 was used for compound **7**. Approximately 30 mg of sample was directly dissolved into the NMR tube in 0.6 mL of solvent.

The purity of synthetic products was established by NMR spectroscopy, HPLC/MS and HPLC/ELSD (HPLC: ThermoQuest P1500, column Macherey–Nagel Nucleosil C18, 25 cm×4.6, 5 μm, MP: water/methanol, 1 mL/min) (MS: Finnigan Navigator, APCI, Source Heater 150°C and APCI Heater 550°C, cone voltage 25 V) (ELSD: Evaporative Light Scattering Detectors, Eurosep instrument DDL31, $T=45$°C) and thin layer chromatography (TLC) on silica gel (Merck, Darmstadt, Germany) with H_2SO_4 (10% in EtOH) revelation. All reactions were carried out in nitrogen atmosphere. Purification steps were carried out using flash column chromatography with silica gel (63–200 mesh; Normasil Prolabo, Fontenay-sous-bois, France), or preparative TLC using 2 mm Merck glass backed precoated silica gel plates.

Elemental analyses were carried out on an EA 1110 (CE instruments). Optical rotations were measured on a Perkin–Elmer 241 polarimeter (265 nm) with a path length of 1 dm at 20°C in $CHCl_3$.

3.2. 2^{I}-*O*-Benzyl,$2^{II–VII}$,$3^{I–VII}$,$6^{I–VII}$-eicosa-*O*-methyl cyclomaltoheptaose, 3

Under an atmosphere of nitrogen, anhydrous β-cyclodextrin (2.00 g, 1.76 mmol, 1 equiv.), dried under vacuum at 100°C for 48 h, was added to dry DMSO (30 mL) in a 125 mL three-necked flask equipped with a dropping funnel and a nitrogen inlet. Sodium hydroxide (220 mg, 5.17 mmol, 2.9 equiv.) was added and magnetic stirring was continued for 48 h at room temperature (rt). Benzyl bromide (750 mg, 5.17 mmol, 2.5 equiv.) dissolved in DMSO (6 mL) was added dropwise. The mixture was stirred at rt for 24 h. The reaction mixture was monitored by TLC on silica gel (1-BuOH–EtOH–H_2O: 5/4/3 v/v/v), which showed three major spots having R_f values of 0.35 (β-cyclodextrin), 0.60 (monobenzyl β-cyclodextrin), and 0.72–0.77 (dibenzyl β-cyclodextrin). In another flask sodium hydride, washed three times with diethyl ether (1.69 g, 70 mmol, 40 equiv.), was added to DMSO (20 mL). The mixture was stirred for 45 min at 50°C. A green–blue colouration was seen at this point. After cooling to

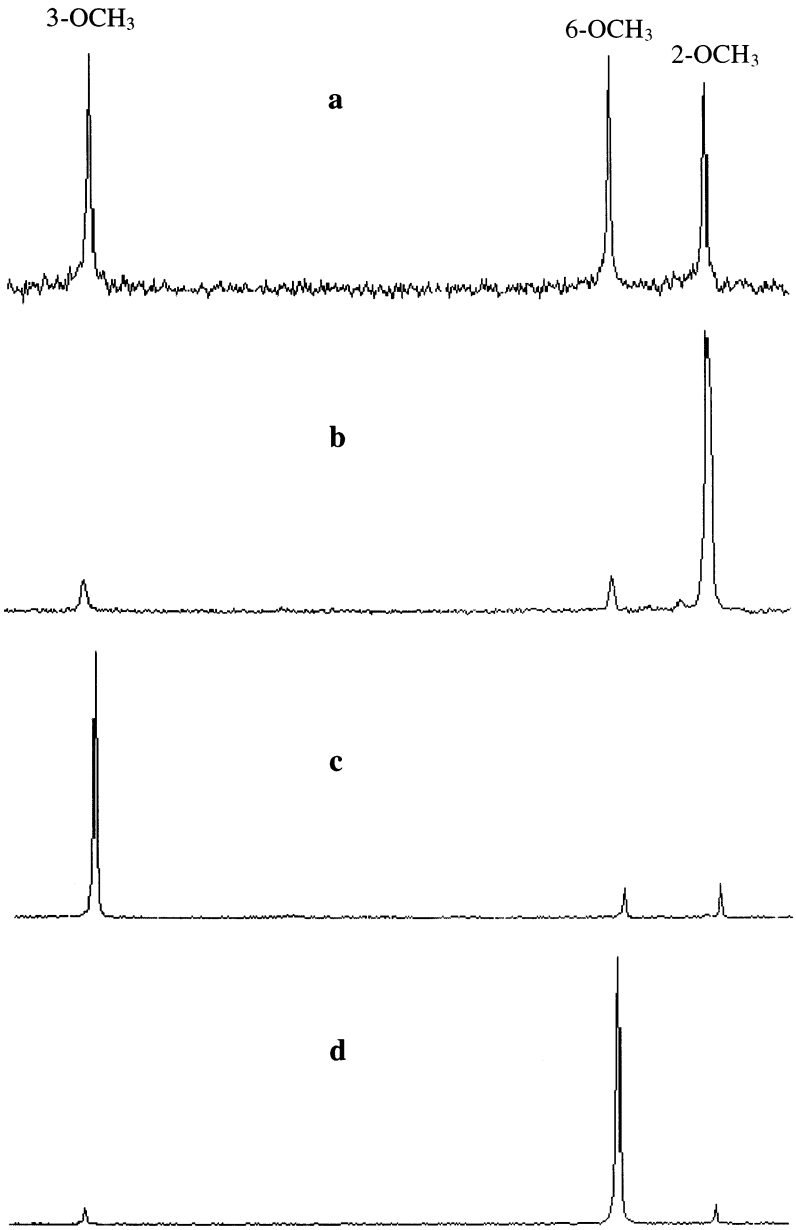
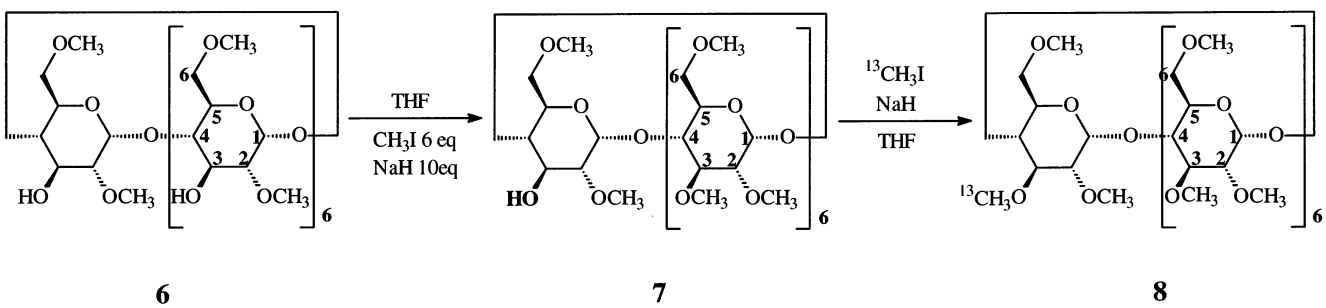


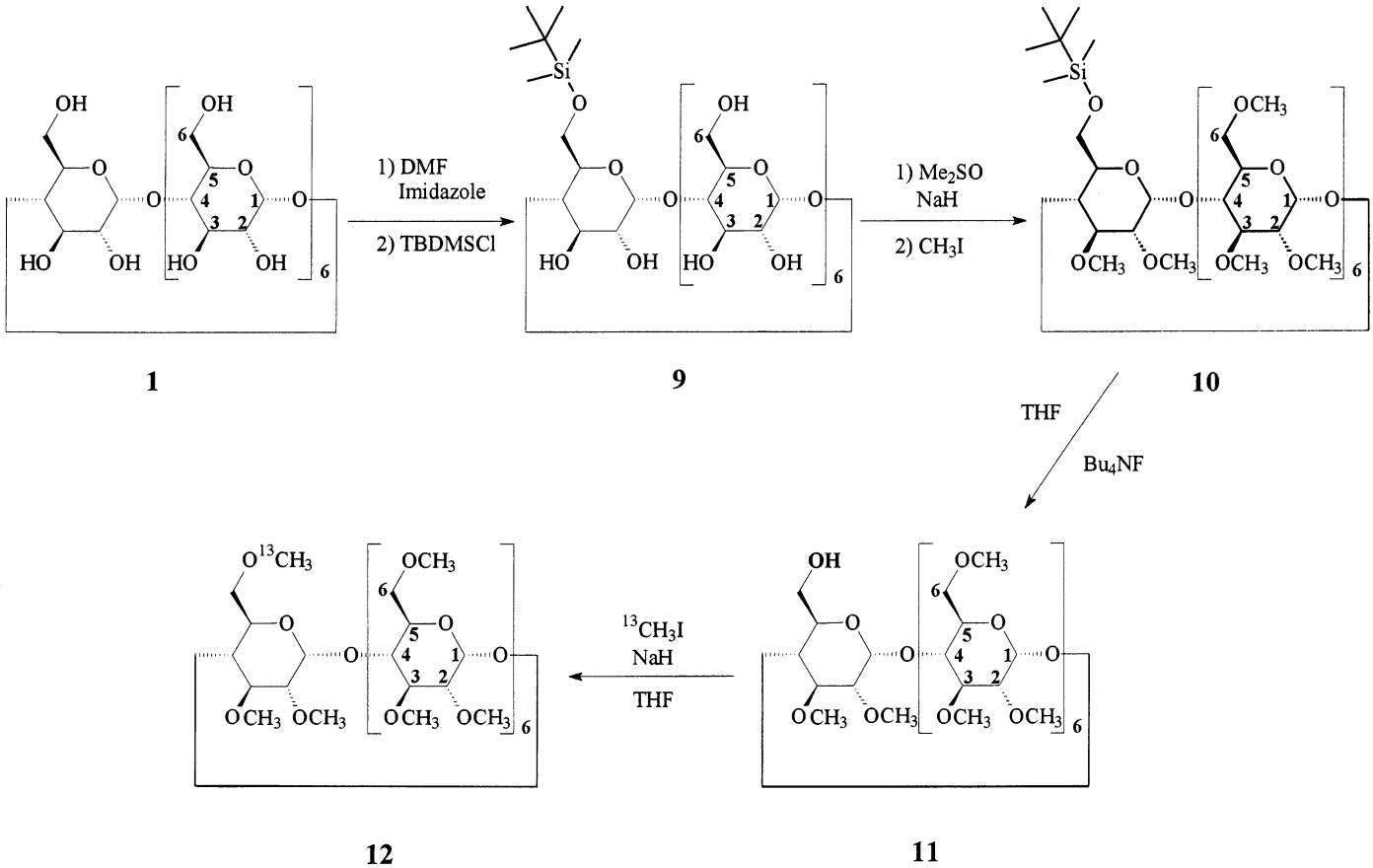
Figure 3. Portion of the ^{13}C NMR ($CDCl_3$, 25°C) spectra. (a) 2,3,6-Tri-*O*-methyl-β-cyclodextrin. (b) Mono-2-hydroxy-β-cyclodextrin methylated with $^{13}CH_3I$ **5**. (c) Mono-3-hydroxy-β-cyclodextrin methylated with $^{13}CH_3I$ **8**. (d) Mono-6-hydroxy-β-cyclodextrin methylated with $^{13}CH_3I$ **12**.



Scheme 2. Synthesis of mono-3-hydroxy-β-cyclodextrin via methylation of 2,6-di-*O*-methyl-β-cyclodextrin.

rt the benzylated cyclodextrin solution was then slowly introduced and the resulting suspension vigorously stirred for 2 h. Methyl iodide (10.00 g, 70 mmol, 40 equiv.) was then added dropwise to the suspension. The mixture was stirred at rt overnight and dichloromethane (150 mL) was added and the organic phase 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, the crude product was analysed by TLC (R_f 0.33 full methylated β-cyclodextrin; R_f 0.43 monobenzyl eicosa-*O*-methyl β-cyclodextrin; R_f 0.50, 0.55, 0.61 dibenzyl nonadeca-*O*-methyl β-cyclodextrins) and by HPLC/MS (MeOH–H_2O 90/10 tr: 7.4 min, m/z 1451.6 [M+Na$^+$], 1467.6



Scheme 3. Synthesis of mono-6-hydroxy-β-cyclodextrin via mono *tert*-butyl dimethyl silylation.

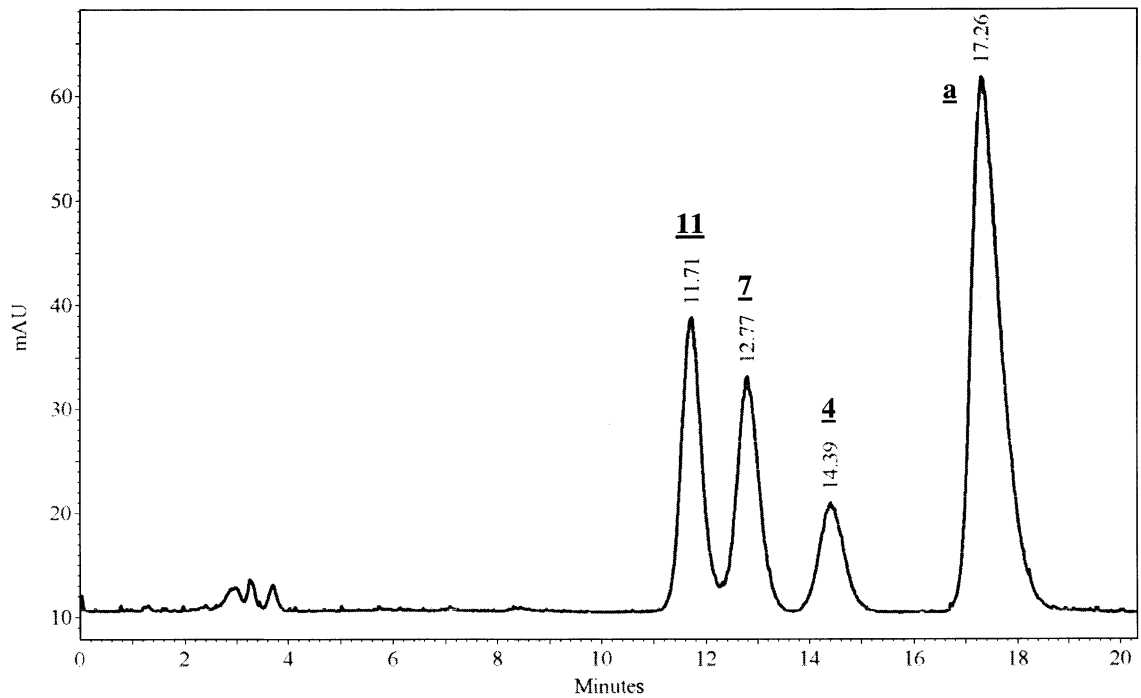


Figure 4. Separation of mono-2 **4**, mono-3 **7**, mono-6 **11** and 2,3,6-tri-*O*-methyl-β-cyclodextrin **a** by liquid chromatography.

[M+K$^+$] fully methylated β-cyclodextrin; tr: 12.2 min, m/z 1527.7 [M+Na$^+$], 1543.7 [M+K$^+$] mono-*O*-benzyl-eicosa-*O*-methyl β-cyclodextrin; tr: 22.0 min m/z 1603.7 [M+Na$^+$], 1619.7 [M+K$^+$] dibenzyl-nonadeca-*O*-methyl β-cyclodextrin). Column chromatography (75/25 toluene–acetone) yielded **3** (1.25 g, 0.83 mmol, 47%) as a white powder: $[\alpha]_{365}^{20}$ +397 (c 1.0, $CHCl_3$); ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 7.43 (d, 2H, $^3J_{ortho,meta}$ 7.33 Hz, H-*ortho*), 7.32 (dd, 2H, $^3J_{para,meta}$ 7.41 Hz, H-*meta*), 7.25 (t, 1H, H-*para*), 5.15 (m, 6H, H-1), 4.98 (d, 1H, $^3J_{1,2}$ 3.46 Hz, H-1), 4.75 (d, 1H, $^2J_{a,b}$ 12.15 Hz, -CH_b-Ph), 4.71 (d, 1H, -CH_a-Ph), 3.85 (m, 7H, H-6b), 3.80 (m, 7H, H-5), 3.70 (m, 1H, H-4), 3.65–3.66 (m, 21H, 3-OCH_3), 3.64 (m, 6H, H-4), 3.62 (m, 1H, H-3), 3.59 (m, 7H, H-6a), 3.52–3.53 (m, 18H, 2-OCH_3), 3.51 (m, 6H, H-3), 3.40 (s, 21H, 6-OCH_3), 3.40 (m, 1H, H-2), 3,20 (dd, 6H, $^3J_{2,3}$ 9.73 Hz, H-2); ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 128.61 (C-*meta*), 128.15 (C-*ortho*), 127.81 (C-*para*), 99.69 (C_1-C-O-CH_2-PH), 99.33, 99.39, 99.45 (C-1), 82.06, 82.16, 82.27, 82.42, 82.45, 82.50, 82.57 (C-2,3), 80.47, 80.51, 80.58, 80.63, 80.83 (C-4), 80.25 (C-2-O-CH_2-Ph), 73.05 (Ph-CH_2-), 71.65, 71.76, 71.89 (C-6), 71.25, 71.32, 71.37, 71.48 (C-5), 61.77, 61.93 (3-OCH_3), 59.34, 59.38 (6-OCH_3), 58.82, 58.88, 58.94, 59.05, 59.11 (2-OCH_3). Anal. calcd for $C_{69}H_{116}O_{35}$: C, 55.03; H, 7.77. Found: C, 55.15; H, 7.65.

3.3. 2$^{I–VI}$,3$^{I–VII}$,6$^{I–VII}$-Eicosa-*O*-methyl cyclomaltoheptaose, 4

A solution of **3** (500 mg, 0.33 mmol, 1 equiv.) and $Pd(OH)_2$ (47 mg, 0.33 mmol, 1 equiv.) in MeOH (15 mL) was heated to 100°C under H_2 at a pressure of 30 bar and vigorously stirred overnight. After filtration through Celite, the filtrate was concentrated under vacuum. The residue was dissolved in $CHCl_3$ (30 mL) and washed with water (2×10 mL) and saturated aqueous $NaHCO_3$ (10 mL). After drying over $MgSO_4$, filtration and evaporation the crude product was purified by column chromatography (4/1–3/1 toluene–acetone, stepwise) yielding **4** (350 mg, 0.24 mmol, 75%) as a white powder: $[\alpha]_{365}^{20}$ +415 (c 1.0, $CHCl_3$); 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, $J_{2,1}$ 3.3 Hz, H-1), 4.38 (d, 1H, $J_{2\text{-OH,H-2}}$ 11.2, 2-OH), 3.89–3.87 (m, 7H, H-6a), 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-6b), 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, 100.01, 99.87, 99.69, 99.49, 99.20, 99.04 (C-1), 84.40 (C-3), 83.30, 82.80, 82.50, 82.42, 82.32, 82.15, 82.06, 81.56, 81.39, 81.26, 81.12, 80.99, 80.89, 80.65, 80.59, 80.46, 79.05 (C-2, 3, 4), 74.57 (C-2-OH), 72.23 (C-5), 72.04, 72.00, 71.94, 71.89, 71.68, 71.61 (C-6), 71.43, 71.38, 71.28, 71.21, 70.99, 70.91 (C-5), 70.66 (C-6), 62.37, 61.88, 61.85, 61.76, 61.65, 61.60, 61.27 (3-OCH_3), 59.51, 59.45, 59.43, 59.41, 59.40, 59.38, 59.34, 59.10, 58.98, 58.88, 58.67, 58.74, 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.

3.4. 2$^{I–VII}$,3$^{I–VI}$,6$^{I–VII}$-Eicosa-*O*-methyl cyclomaltoheptaose, 7

Heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (1.00 g, 0.75 mmol, 1 equiv.) in dry THF (50 mL) was added to a suspension of sodium hydride (0.21 g, 9.01 mmol, 10 equiv., washed three times with THF) in dry THF (10 mL). The mixture was stirred at rt for 30 min and then stirred under reflux for 2 h. After cooling to 0°C, methyl iodide (0.64 g, 4.50 mmol, 6 equiv.) was added dropwise and the reaction mixture stirred at 0°C for 10 min, then warmed to rt and stirred for 1 h. The mixture was then heated under reflux overnight. The reaction mixture was cooled to 0°C and then quenched by addition of MeOH, and the solvent was evaporated under vacuum. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with saturated aqueous $Na_2S_2O_3$ (2× 15 mL) and water (15 mL). After drying over $MgSO_4$, filtration and evaporation, the crude product was purified by column chromatography (4/1–3/1 toluene–acetone, stepwise) yielding **7** (240 mg, 0.17 mmol, 22%) as a white powder: $[\alpha]_{365}^{20}$ +444 (c 1.0, $CHCl_3$); TLC (1/1 toluene–acetone) R_f 0.32; ^{1}H NMR (500.13 MHz, DMSO): δ 5.04 (m, 7H, H-1), 4.87 (s, 1H, 3-O-H), 3.80 (m, 1H, H-3), 3.79–3.69 (m, 14H, H-5, 6a), 3.62–3.45 (m, 7H, H-6b), 3.52–3.46 (m, 18H, 3-OCH_3), 3.46–3.41 (m, 7H, H-4), 3.39 (s, 21H, 2-OCH_3), 3.41–3.25 (m, 6H, H-3), 3.25 (s, 21H, 6-OCH_3), 3.20 (dd, 1H, H-2), 3.09–3.04 (m, 6H, H-2); ^{13}C NMR (125.75 MHz, DMSO): δ 99.62, 99.34, 98.85, 98.60, 98.51, 98.48 (C-1), 82.99, 82.89, 82.73, 82.47, 82.39, 82.27, 82.20, 82.12, 82.08, 81.68 (C-2,3), 80.99, 80.92, 80.78, 80.36, 80.10 (C-4), 72.55 (C-3-OH), 72.04, 71.93, 71.88, 71.78 (C-6), 71.50, 71.31, 71.28, 71.24, 71.17, 71.41 (C-5), 61.77, 61.63, 61.57, 61.51 (3-OCH_3), 60.01 (2-OCH_3), 59.03, 59.01, 58.99, 58.96, 58.86, 58.74, 58.68, 58.58 (6-OCH_3, 2-OCH_3). HPLC/MS (MeOH–H_2O 80/20) tr: 12.7 min m/z 1437.8 [M+Na$^+$], 1453.6 [M+K$^+$]. Anal. calcd for $C_{62}H_{110}O_{35}$: C, 52.61; H, 7.83. Found: C, 52.52; H, 7.90.

3.5. 2$^{I–VII}$,3$^{I–VII}$,6$^{I–VI}$-Eicosa-*O*-methyl cyclomaltoheptaose, 11

This derivative was prepared as previously described.[17] The crude product was purified by column chromatography (75/25 toluene–acetone) yielding **11** (45%) as a white powder: $[\alpha]_{365}^{20}$ +421 (c 1.0, $CHCl_3$); TLC (1/1 toluene–acetone) R_f 0.25; ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 5.20 (d, 1H, $J_{2,1}$ 3.8 Hz, H-1), 5.15 (t, 2H, $J_{2,1}$ 3.9 Hz, H-1), 5.08 (d, 3H, $J_{2,1}$ 3.2 Hz, H-1) 5.01 (d, 1H, $J_{2,1}$ 3.4 Hz, H-1), 3.95 (d, 1H, $J_{6a,6b}$ 11.2, H-6a (OH)), 3.90–3.65 (m, 13H, H-5, 6a), 3.81 (m, 1H, H-6b (OH)) 3.65–3.61 (m, 21H, 3-OCH_3), 3.62–3.57 (m, 7H, H-4), 3.63–3.58 (m, 6H, H-6b), 3.42–3.59 (m, 7H, H-3), 3.50–3.46 (m, 21H, 2-OCH_3), 3.37–3.36 (m, 18H, 6-OCH_3), 3.19–3.16 (m, 7H, H-2), 2.97 (s, 1H, 6-OH); ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 33.43, 99.40, 99.37, 99.35, 99.19, 99.17 (C-1), 82.75, 82.49, 82.42, 82.36,

82.29, 82.21, 82.19, 82.12, 82.03, 81.99, 81.82, 81.52, 81.08, 81.03, 80.95, 80.35, 80.22, 78.89 (C-3, 2, 4), 72.11, 71.70, 71.55, 71.42, 71.40, 71.21 (C-5), 72.03, 71.95, 71.87, 71.73, 71.64, 71.56 (C-6), 62.05 (HO-C-6), 62.00, 61.97, 61.89, 61.70, 61.66, 61.42 (3-O-CH_3), 59.52, 59.51, 59.42, 59.36, 59.32, 59.10, 59.03, 58.87, 58.74, 58.66, 58.59 (6-O-CH_3, 2-O-CH_3); HPLC/MS (MeOH–H_2O 80/20) tr: 11.7 min m/z 1437.6 [M+Na^+], 1453.9 [M+K^+]. Anal. calcd for $C_{62}H_{110}O_{35}$: C, 52.61; H, 7.83. Found: C, 52.45; H, 7.95.

3.6. General procedure for ^{13}C labelling of the last free hydroxyl group of compounds 4, 7 and 11

To a suspension of sodium hydride (5 mg, 0.21 mmol) in THF (2 mL), eicosa-*O*-methyl cyclodextrin **4**, **7** or **11** (100 mg, 0.07 mmol) in THF (4 mL) was added. The resulting mixture was stirred under reflux for 1 h, and then cooled to rt. ^{13}C-Labelled methyl iodide (30 mg, 0.21 mmol) was added and the mixture was refluxed overnight. After cooling to rt, excess hydride was decomposed by the addition of MeOH. The solvent was then evaporated under vacuum, the residue dissolved in CH_2Cl_2 (5 mL) and washed with saturated aqueous $Na_2S_2O_3$ (2×2 mL) and water (2 mL). After drying over $MgSO_4$, filtration and evaporation, the crude product was purified by preparative TLC (1/1 toluene–acetone) to give pure **5**, **8** or **12**. TLC (1/1 toluene–acetone) R_f 0.43; HPLC/MS (MeOH–H_2O 80/20) tr: 17.2 min m/z 1452.5 [M+Na^+], 1468.6 [M+K^+].

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