

Novel synthetic strategy for monosubstituted cyclodextrin derivatives

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ELECTRONIC SUPPLEMENTARY INFORMATION:

Experimental details

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Materials and Methods

TLC analyses were carried out on silica gel plates from Merck (60, F254). Reaction products were visualized on TLC plates by UV light and then by treatment with a H₂SO₄/AcOH aqueous solution. For column chromatography, silica gel from Merck (Kieselgel 40, 0.063-0.200 mm) was used. Novagel[®]-NH₂ resin was purchased from Novabiochem (0.77 meq/g). The functionalizations of the solid support were carried out in a short glass column (5 cm length, 1 cm i.d.), equipped with a sintered glass filter, a stopcock and a cap.

The activator solution (0.45 M tetrazole in CH₃CN) and the oxidizer solution (0.1 M iodine/THF/H₂O/pyridine) were all purchased from Applied Biosystems. 5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-O-(2-cyanoethyl)-N,N-diisopropylphosphoramidite-thymidine (**D**) was purchased from Sigma-Aldrich.

The HPβCD (Molar Substitution, MS = 0.60) and the MeβCD (Degree of Substitution, DS = 1.7) were purchased from Sigma-Aldrich. The Cyclodextrins were dried under vacuum (70 °C) for 4 h.

Unless otherwise indicated, all chemicals were obtained from Sigma – Aldrich.

HPLC analyses and purifications were performed on a Agilent Technologies 1200 series instrument equipped with a UV detector. The crude materials were analyzed and purified by HPLC on a RP18 column [Phenomenex LUNA, 5μm C18, 10.0 × 250 mm] eluted with a linear gradient from 0 to 100 % in 30 min of CH₃CN in H₂O, flow = 1.5 mL/min, detection at λ = 255 nm.

ESI MS analyses were performed on a Waters Micromass ZQ instrument – equipped with an Electrospray source. MALDI TOF mass spectrometric analyses were performed on a PerSeptive Biosystems Voyager – De Pro MALDI mass spectrometer in the linear mode, using DHB as matrix.

NMR spectra for labels (**A** - **D**) were recorded in CDCl₃ with a Bruker WM 400 spectrometer. NMR spectra of new CD modified (**4** - **9**) were carried out at 500 MHz by using a Varian UNITY 500 spectrometer. Deuterated D₂O (99.9% relative isotopic abundance) was purchased from Cambridge Isotope Laboratories. The proton chemical shifts were collected at 298 K and referenced to external TMS (δ=0 ppm). Two-dimensional phase-sensitive TOCSY, NOESY, ROESY, DQFCOSY, ¹H-¹³C HSQC spectra¹ were collected using the States and Haberkorn method. A spectral width of 6000 Hz was used in both dimensions; typically, 4096 was the number of complex points collected in the ω₂ dimension and 512 in the ω₁ dimension. The data were zero-filled to 2000 in ω₁. Squared-shifted sine-bell functions were applied in both dimensions before Fourier transformation and baseline correction. TOCSY, NOESY and ROESY experiments were recorded with mixing times of 70, 200 and 150 ms, respectively. Water suppression, when necessary, was achieved utilizing the DPGSE sequence². The data were processed and analyzed using the VNMRJ and XEASY software.³

³¹P NMR spectra were recorded at 161.98 MHz on a Bruker WM-400 spectrometer using 85% H₃PO₄ as external standard.

¹ Cavanagh, J., Fairbrother, W., Palmer, A. G., and Skelton, N. J. in *Protein NMR Spectroscopy, Principles, and Practice*, Academic Press, Inc., San Diego, CA, 1996.

² T. L. Hwang, and A. J. Shaka, *J. Magn. Reson. A*, 1995, **112**, 275.

³ C. Bartels, T. Xia, M. Billeter, P. Guntert and K. J. Wüthrich, *J. Biomol. NMR*, 1995, **6**, 1.

Synthesis of A

A was obtained as reported by G. Di Fabio et al. *Chem. Commun.* 2011, **47**, 2363.

Synthesis of B

Phenyl- β -D-glucopyranoside (500.0 mg, 1.95 mmol) was dissolved in anhydrous pyridine (5 mL), was reacted with DMTrCl (860.0 mg, 2.53 mmol). The reaction mixture, left at room temperature overnight under stirring, was then diluted with CH₃OH and concentrated under reduced pressure. The crude was next purified on a silica gel column, eluted with DCM containing growing amounts of CH₃OH (from 1 to 5%) in the presence of a few drops of triethylamine, affording pure phenyl-(6-ODMT)- β -D-glucopyranoside. The compound thus obtained, dissolved in anhydrous pyridine (5 mL), was reacted with Ac₂O (1.5 mL). The mixture was left under stirring at room temperature 5 h and then quenched by addition of a few drops of CH₃OH. The mixture, concentrated under reduced pressure, was diluted with CHCl₃, transferred into a separatory funnel, and washed three times with a saturated NaHCO₃ aqueous solution and then twice with water. The organic phase, dried over anhydrous Na₂SO₄ and filtered, was then concentrated under reduced pressure, furnishing the desired product 2,3,4-three-O-acetyl-phenyl-6-ODMT- β -D-glucopyranoside, in an almost quantitative yield.

A solution of iodine (0.30g, 1.1 mmol) in 20 mL of anhydrous methanol was added to the above product (1.0g, 3.52 mmol). The mixture was stirred vigorously at room temperature for 2h, then, sodium thiosulfate solution was added to remove excess iodine. The reaction mixture was extracted with DCM. The organic phase was combined, washed with water, dried over anhydrous Na₂SO₄, and the solvent was removed under reduced pressure. The crude product was purified by chromatography on silica gel column (eluent CHCl₃/n-hexane, 9:1, v/v) to give a light yellow syrup. The residue afforded 447.0 mg (1.17 mmol, 80% yield) of desired product.

R_f = 0.5 [CHCl₃/CH₃OH 99:1, (v/v)]. δ_{H} (CDCl₃, 400 MHz, room temperature) 7.29 (t, 2H, J = 11.0 and 11.0 Hz, H *ortho*-Ph), 7.07 (m, 1H, H *para*-Ph), 6.99 (t, 2H, J = 5.5 and 5.5 Hz, H *meta*-Ph), 5.21 (apparent t, 1H, J = 8.0 and 8.0 Hz, H-2), 5.12 (m, 1H, H-3), 5.06 (d, 1H, J = 8.0 Hz, H-1), 4.49 (d, 1H, J = 12.0 Hz, H-4), 4.35 (d, 1H, J = 12.0 Hz, H-5), 3.68 (m, 2H, H-6), 2.11, 2.10, 2.07 (3s, 9H, OCOCH₃). MS (ESI) m/z calcd for C₁₈H₂₃O₉ = 382.13, found 383.10 [MH]⁺.

To 320.0 mg (0.84 mmol) of 2,3,4-three-O-acetyl-phenyl- β -D-glucopyranoside, dissolved in anhydrous dichloromethane (7 mL), DIPEA (880 μ L, 5.05 mmol) and 2-cyanoethyl-N,N-diisopropylamino-chlorophosphoramidite (245 μ L, 1.10 mmol) were added under argon. After 30 minutes the solution was diluted with ethyl acetate and the organic phase was washed twice with brine and then concentrated. Silica gel chromatography of the residue (eluent n-hexane/ethyl acetate, 6:4, v/v; in the presence of a few drops of triethylamine), afforded desired compound **2** (420.0 mg, 0.72 mmol) in a 86% yield.

B. R_f = 0.5 (hexane/ ethyl acetate, 7:3, v/v); δ_{H} (CDCl₃, 400 MHz, room temperature as a diastereomeric mixture) 7.31 – 7.02 (overlapped signals, aromatic protons, 10H), 5.35 (overlapped signals, 4H, H-2 and H-3), 5.10 (complex signal, 2H, H-1), 4.51 – 4.40 (overlapped signals, 4H, H-4 and H-5), 3.80-3.65 (overlapped signals, 8H, OCH₂CH₂CN, N[CH(CH₃)₂]₂), 3.47 (complex signals, 4H, H-6), 2.70 (complex signals, 4H, OCH₂CH₂CN), 2.11, 2.10, 2.07 (complex signals, 18H, OCOCH₃), 1.19 – 1.16 (complex signals, 24H, N[CH(CH₃)₂]₂). ³¹P NMR (CDCl₃, 161.98 MHz) δ : 147.7. MS (ESI) m/z calcd for C₂₇H₃₉N₂O₁₀P = 582.23, found 583.21 [MH]⁺.

Synthesis of C

To a solution of 2-aminoethanol (125 μ L, 2.07 mmol) in THF were added DIEA (420 μ L, 2.41 mmol) and dansyl chloride (500 mg, 1.86 mmol). After stirring for 2 h at rt, all the volatile components were evaporated and the residue was partitioned between ethyl acetate and water. The organic phase was washed with water (\times 3), then dried with Na_2SO_4 . The crude product was purified by chromatography on silica gel column (hexane/ethyl acetate, 6:4, v/v) afforded 507 mg of desired product (1.72 mmol, 92% yield).⁴

Rf = 0.55 (hexane/ ethyl acetate, 7:3, v/v). δ_{H} (CDCl_3 , 400 MHz, room temperature) 8.57 (d, 1H, $J = 5.6$ Hz); 8.30 – 8.26 (m, 2H); 7.60 – 7.50 (m, 2H); 7.20 (d, 1H, $J = 7.5$ Hz); 5.18 (br s, 1H, CH_2OH); 3.61 (m, 2H, CH_2OH); 3.05 (m, 2H, $\text{SO}_2\text{NHCH}_2\text{CH}_2$); 2.90 (s, 6H, $\text{N}(\text{CH}_3)_2$); MS (ESI) m/z calcd for $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_3\text{S} = 294.10$, found 295.15 $[\text{MH}]^+$.

372.0 mg (1.26 mmol) of 5-(dimethylamino)-N-(2-hydroxyethyl)naphthalene-1-sulfonamide, dissolved in anhydrous dichloromethane (10 mL), DIPEA (580 μ L, 5.05 mmol) and 2-cyanoethyl-N,N-diisopropylamino-chlorophosphoramidite (370 μ L, 1.64 mmol) were added under argon. After 30 minutes the solution was diluted with ethyl acetate and the organic phase was washed twice with brine and then concentrated. Silica gel chromatography of the residue (eluent n-hexane/ethyl acetate, 7:3, v/v; in the presence of a few drops of triethylamine), afforded desired compound C (548.0 mg, 1.11 mmol) in a 88% yield.

C. Rf = 0.65 (hexane/ ethyl acetate, 7:3, v/v); δ_{H} (CDCl_3 , 400 MHz, room temperature, as an enantiomeric mixture) 8.51 (d, 1H, $J = 5.6$ Hz); 8.30 – 8.25 (m, 2H); 7.65 – 7.50 (m, 2H); 7.22 (d, 1H, $J = 7.5$ Hz); 3.81-3.55 (overlapped signals, 6H, $\text{OCH}_2\text{CH}_2\text{CN}$, $\text{SO}_2\text{NHCH}_2\text{CH}_2\text{OP}$, $\text{N}[\text{CH}(\text{CH}_3)_2]_2$); 3.03 (m, 2H, $\text{SO}_2\text{NHCH}_2\text{CH}_2$); 2.88 (s, 6H, $\text{N}(\text{CH}_3)_2$); 2.58 (t, 2H, $J = 6.5$ and 6.5 Hz, $\text{OCH}_2\text{CH}_2\text{CN}$), 1.18 (d, 6H, $J = 6.7$ Hz, $\text{N}[\text{CH}(\text{CH}_3)_2]_2$), 1.14 (d, 6H, $J = 6.7$ Hz, $\text{N}[\text{CH}(\text{CH}_3)_2]_2$). ^{31}P NMR (CDCl_3 , 161.98 MHz): 147.6. MS (ESI) m/z calcd for $\text{C}_{23}\text{H}_{35}\text{N}_4\text{O}_4\text{PS} = 494.21$, found 495.27 $[\text{MH}]^+$.

⁴ T. W. Kim, J. Park, J. Hong, *J. Chem. Soc., Perkin Trans. 2*, 2002, 923.

General procedure for the preparation of the support 1

300 mg of Novagel[®]-NH₂ (0.77 meq/g, 0.23 mmol) swelled in anhydrous pyridine, were reacted, at r.t. overnight, with a mixture of 228.0 mg (1.1 mmol) of 4-hydroxy-3-nitrophenylacetic acid, 190.0 mg (0.9 mmol) of DICCl, 290 μL (1.7 mmol) of DIEA dissolved in 5 mL of anhydrous pyridine. After exhaustive washings with pyridine, DCM and Et₂O, the support was dried under reduced pressure. After capping of the unreacted amino functions with 10 mL of Ac₂O/Py (1:1, v/v) for 1 h at r.t., the support were treated with conc. aq. ammonia (28%) at 50 °C for 1 h. By Kaiser test, the incorporation of the linker was almost quantitative. After exhaustive washings with CH₃OH, DCM and Et₂O, resulting support **5** was dried under reduced pressure.

General procedure for the preparation of supports 3(A–D)

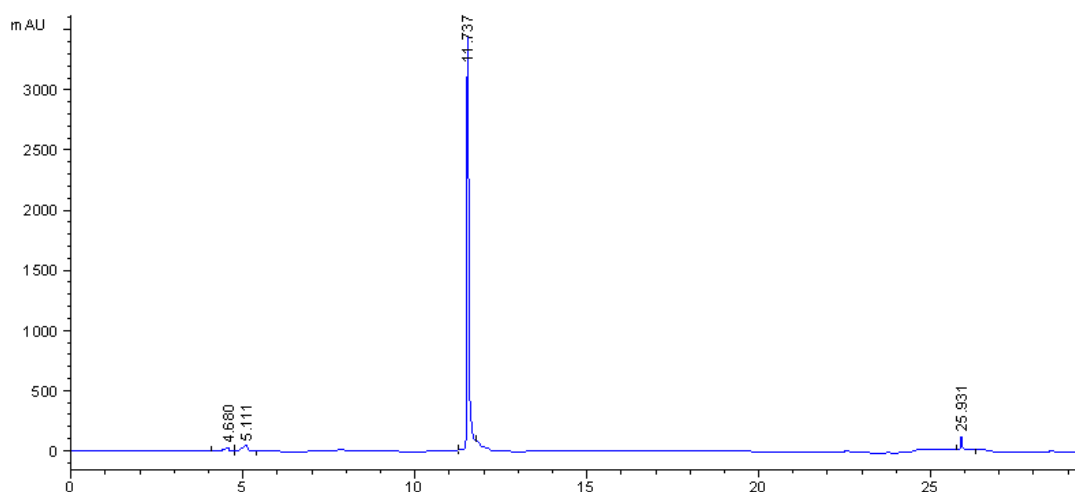
1.5 mL (0.67 mmol) of a commonly used ‘activator solution’ (0.45 M tetrazole in CH₃CN) were added to a mixture of 0.15 mmol of the chosen 2-cyanoethyl-N,N-diisopropyl-phosphoramidite derivative (**A – D**) and 100.0 mg (0.077 mmol) of support **1** swelled in 500 μL of dry DCM. After 30’ the support was exhaustively washed with CH₃CN and treated with 5 mL of a commonly used ‘oxidizer’ solution (0.02M I₂/pyridine/H₂O/THF) for 5 min. After exhaustive washings with CH₃CN, DCM and Et₂O, resulting supports **2(A–D)** were dried under reduced pressure. A typical loading of the label, determined by quantitation of the DMT cation released from weighed amounts of support **2D** upon acidic treatment, was always above 60% (0.46 mmol/g). After standard capping procedure with Ac₂O/pyridine 1:1 (v/v) 1h at r.t., the phosphate deprotection from 2-cyanoethyl group was then achieved by treatment with 20% piperidine solution (v/v) for 5’ at r.t. (3 times), giving supports **3(A–D)**. The total deprotection were confirmed by ³¹P-NMR of the resin suspended in CDCl₃ and in all cases the diagnostic upfield shift in the ³¹P NMR signals was observed.

support	³¹ P NMR (ppm) ^a	support	³¹ P NMR (ppm) ^a
2A	– 8.2	3A	– 6.0
2B	– 8.5	3B	– 6.2
2C	– 8.7	3C	– 6.3
2D	– 9.5	3D	– 6.8

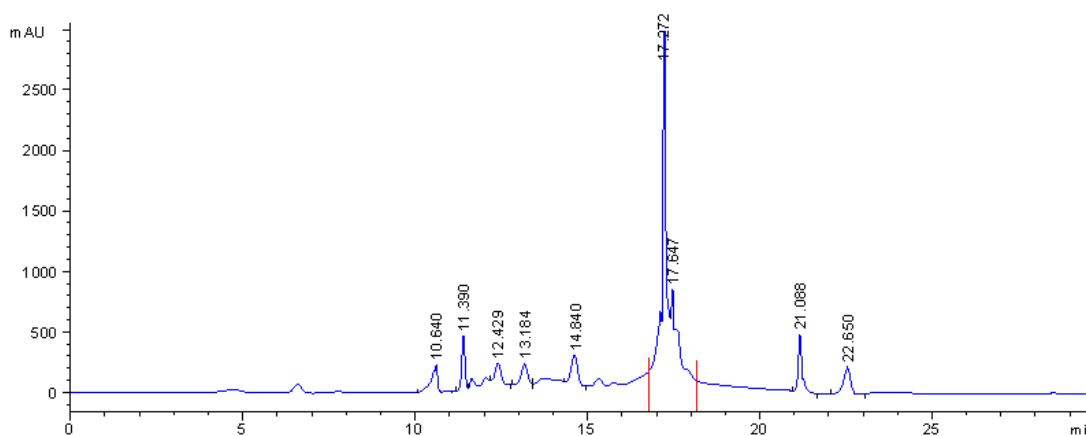
^aIn the ³¹P NMR spectra was observed one broad signal centred on the reported value, giving sharp and better resolved signals in the support with loading in the range 0.10 – 0.18 mmol/g.

A general procedure for the synthesis of modifieds CDs 4 – 9

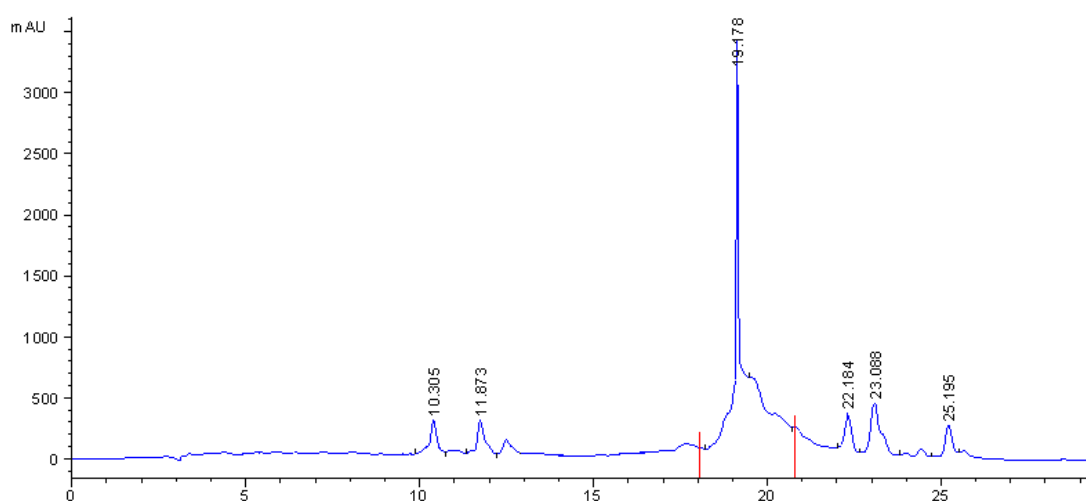
30 mg of dried supports **3(A–D)** were swelled in anhydrous pyridine and then reacted with 35.0 mg (0.11 mmol) of MSNT in 500 μL of anhydrous pyridine. After 30’ at r. t., 0.035 mmol of the chosen CDs (see Table), were added to mixture and left for 12 h at r. t. After exhaustive washings with pyridine, DMF, DCM and Et₂O, the target analogues were detached from the support by conc. aq. ammonia treatment at 50 °C for 1 h.



HPLC profile of the crude released on the compound 7.



HPLC profile of the crude released on the compound 8. Red lines indicate the borders of the fraction collected by semi-preparative RP-HPLC chromatography.



HPLC profile of the crude released on the compound 9. Red lines indicate the borders of the fraction collected by semi-preparative RP-HPLC chromatography.

Compound **4**: is obtained in 38% yield starting from 30 mg of support **3A**; HPLC retention time 15.8 min.

Superscript ‘ indicates the nuclei of the 6-O modified glucopyranosil unit.

δ_{H} (D_2O , 500 MHz, room temperature) 7.33-7.28 (m, 4H, *ortho*-H phenyl residue), 7.11-7.09 (6H, m, *meta* and *para*-H phenyl residue), 5.05-4.96 (m, 7H, H-1), 4.28 (m, 2H, H-6’), 3.87 (2H, m, OCH_2CH_2), 3.75-3.54 (complex signals, 40H, H-2, H-3, H-4, H-5, H-6), 3.57 (2H, m, $\text{OCH}_2\text{CH}_2\text{CH}$), 2.18 (1H, m, $\text{OCH}_2\text{CH}_2\text{CH}$). δ_{C} (D_2O , 125 MHz, room temperature) 131.9, 129.9, 129.7 (C-phenyl residue), 104.8 (C-1), 82.7-83.8 (C-4), 74.9-75.8 (C-2), 74.6-75.9 (C-3), 74.7-75.7 (C-5), 69.9 (C-6’), 66.8 (OCH_2CH_2), 62.1-62.9 (C-6), 50.2 ($\text{OCH}_2\text{CH}_2\text{CH}$), 40.3 ($\text{OCH}_2\text{CH}_2\text{CH}$). δ_{P} (D_2O , 161.98 MHz) 0.78. MALDI-TOF m/z calculated for $\text{C}_{57}\text{H}_{85}\text{O}_{38}\text{P}$ = 1408.4; found = 1431.6 $[\text{MNa}]^+$. FT-IR (CaF_2) ν/cm^{-1} 3401, 2948, 2920, 1630, 1612, 1460, 1229, 1130, 1074, 997, 843.

Compound **5**: is obtained in 36% yield starting from 30 mg of support **3B**; HPLC retention time 16.4 min.

Superscript ‘‘ indicates the nuclei of the β -1-O-phenyl-glucopyranose residue

δ_{H} (D_2O , 500 MHz, room temperature) 7.29 (t, 2H, $J = 7.40$ Hz, *meta* of phenyl residue), 7.04 (m, 3H, *ortho* and *para* of phenyl residue), 5.12 (d, 1H, $J = 8.0$ Hz, H-1’), 5.04 (br s, 1H, H-1’), 4.96 (d, 6H, $J = 4.0$ Hz, H-1), 4.14 (m, 2H, H-6’), 3.99 (m, 2H, H-6’), 3.86 (m, 6H, H-3), 3.84 (m, 1H, H-3’), 3.81 (m, 1H, H-3’), 3.77 (m, 12H, H-6), 3.74 (m, 6H, H-5), 3.72 (m, 1H, H-5’), 3.70 (m, 1H, H-4’), 3.64 (m, 1H, H-5’), 3.56 (m, 1H, H-2’), 3.53 (m, 7H, H-2 and H-2’), 3.48 (m, 6H, H-4), 3.47 (m, 1H, H-4’). δ_{C} (D_2O , 125 MHz, room temperature) 132.5 (C *para* of phenyl residue), 125.9 (C *meta* of phenyl residue), 119.2 (C *ortho* of phenyl residue), 104.4 (C-1), 102.7 (C-1’), 102.4 (C-1’), 83.8 (C-4), 78.3 (C-4’), 77.9 (C-5’), 77.5 (C-5’), 75.6 (C-2’), 75.7 (C-3 and C-2’), 74.7 (C-2 and C-4’), 74.5 (C-3’ and C-3’), 74.4 (C-5), 67.3 (C-6’), 62.9 (C-6). δ_{P} (D_2O , 161.98 MHz) 4.33. MALDI-TOF m/z calculated for $\text{C}_{54}\text{H}_{85}\text{O}_{43}\text{P}$ = 1452.4; found = 1475.3 $[\text{MNa}]^+$. FT-IR (CaF_2) ν/cm^{-1} 3425, 2975, 2905, 1626, 1601, 1490, 1250, 1172, 1044, 1021.

Compound **6**: is obtained in 43% yield starting from 30 mg of support **3C**; HPLC retention time 16.0 min.

δ_{H} (D_2O , 500 MHz, room temperature) 8.49 (d, 1H, $J = 9.6$ Hz, H-8 of dansyl residue), 8.26 (d, 1H, $J = 9.5$ Hz, H-4 of dansyl residue), 8.18 (d, 1H, $J = 7.9$ Hz, H-2 of dansyl residue), 7.61 (t, 1H, $J = 8.7$ Hz, H-6 of dansyl residue), 7.56 (t, 1H, $J = 8.7$ Hz, H-3 of dansyl residue), 7.28 (d, 1H, $J = 8.3$ Hz, H-5 of dansyl residue), 5.01-4.86 (m, 7H, H-1), 3.90-3.60 (m, 7H, H-3), 3.87 (m, 2H, H-6’), 3.84-3.66 (m, 12H, H-6), 3.60-3.20 (m, 21H, H-2, H-4 and H-5), 3.50-3.30 (m, 2H, OCH_2CH_2), 3.13-3.06 (m, 2H, $\text{OCH}_2\text{CH}_2\text{NH}$), 2.84 (s, 6H, $\text{N}(\text{CH}_3)_2$). δ_{C} (D_2O , 125 MHz, room temperature) 132.5 (C-4 of dansyl residue), 131.4 (C-7 of dansyl residue), 126.8 (C-3 of dansyl residue), 121.6 (C-2 of dansyl residue), 118.6 (C-6 of dansyl residue), 104.0-105.0 (C-1), 84.1-82.7 (C-4), 63.1 (C-6’), 76.1-74.5 (C-3), 73.5-76.2 (C-2, C-5), 67.1 (OCH_2CH_2), 62.8-61.7 (C-6), 48.2 ($\text{N}(\text{CH}_3)_2$), 46.3 ($\text{OCH}_2\text{CH}_2\text{NH}$). δ_{P} (D_2O , 161.98 MHz) 0.49. MALDI-TOF m/z calculated for $\text{C}_{56}\text{H}_{87}\text{O}_{40}\text{PS}$ = 1490.4; found = 1513.4 $[\text{MNa}]^+$. FT-IR (CaF_2) ν/cm^{-1} 3413, 2955, 2920, 2778, 1645, 1453, 1335, 1295, 1250, 1163, 1145, 1102, 1040, 1030.

Compound **7**: is obtained in 40% yield starting from 30 mg of support **3D**; HPLC retention time 11.7 min.

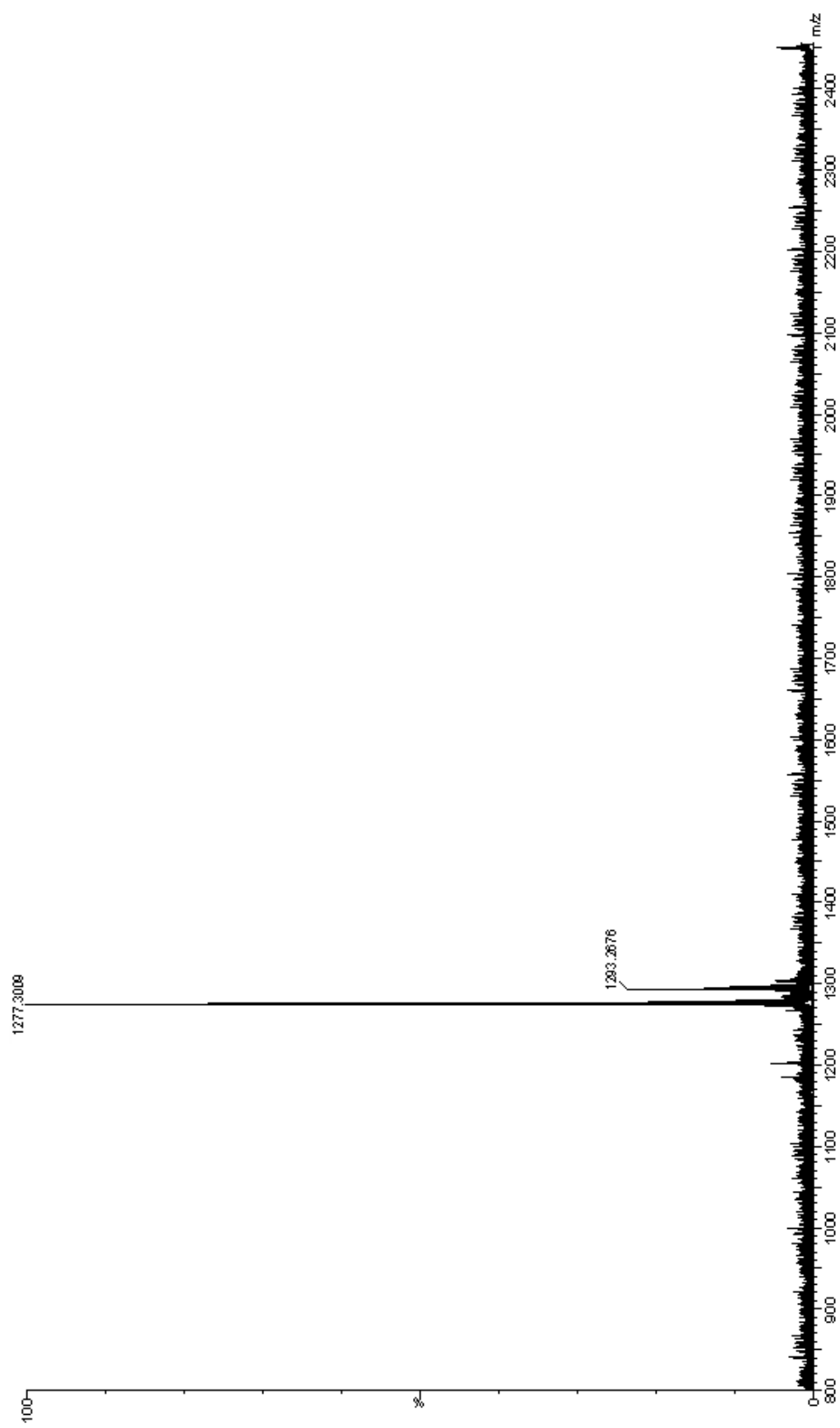
δ_{H} (D_2O , 500 MHz, room temperature) 7.56 (s, 1H, H-6 of thymidine residue), 6.14 (br s, 1H, H-1' of thymidine residue), 4.90 (br s, 6H, H-1), 4.68 (br s, 1H, H-3' of thymidine residue), 4.09 (br s, 1H, H-4' of thymidine residue), 4.06 (br s, 2H, H-6'), 3.73 (br s, 2H, H-5' of thymidine residue), 3.88 (m, 6H, H-3), 3.87 (m, 6H, H-5), 3.71 (m, 10H, H-6), 3.56 (m, 6H, H-2), 3.46 (m, 6H, H-4), 2.29 and 2.45 (br s, 2H, H-2' of thymidine residue), 1.78 (s, 3H, CH_3 of thymidine residue). δ_{C} (D_2O , 125 MHz, room temperature) 140.3 (C-6 of thymidine residue), 104.8 (C-1), 87.9 (C-1' of thymidine residue), 88.5 (C-4' of thymidine residue), 84.3 (C-4), 76.1 (C-3), 74.5 (C-2), 73.6 (C-5), 67.5 (C-6'), 64.2 (C-6 and C-5' of thymidine residue), 78.4 (C-3' of thymidine residue), 40.4 (C-2' of thymidine residue), 14.5 (CH_3 of thymidine residue). δ_{p} (D_2O , 161.98 MHz) 2.55. MALDI-TOF m/z calculated for $\text{C}_{46}\text{H}_{73}\text{N}_2\text{O}_{37}\text{P}$ = 1276.4; found = 1277.4 $[\text{MH}]^+$. FT-IR (CaF_2) ν/cm^{-1} 3415, 3401, 2965, 2339, 1655, 1477, 1322, 1280, 1223, 1205, 1110, 1070, 1001.

Compound **8**: is obtained in 38% yield starting from 30 mg of support **3D**; HPLC retention time 17.3 min.

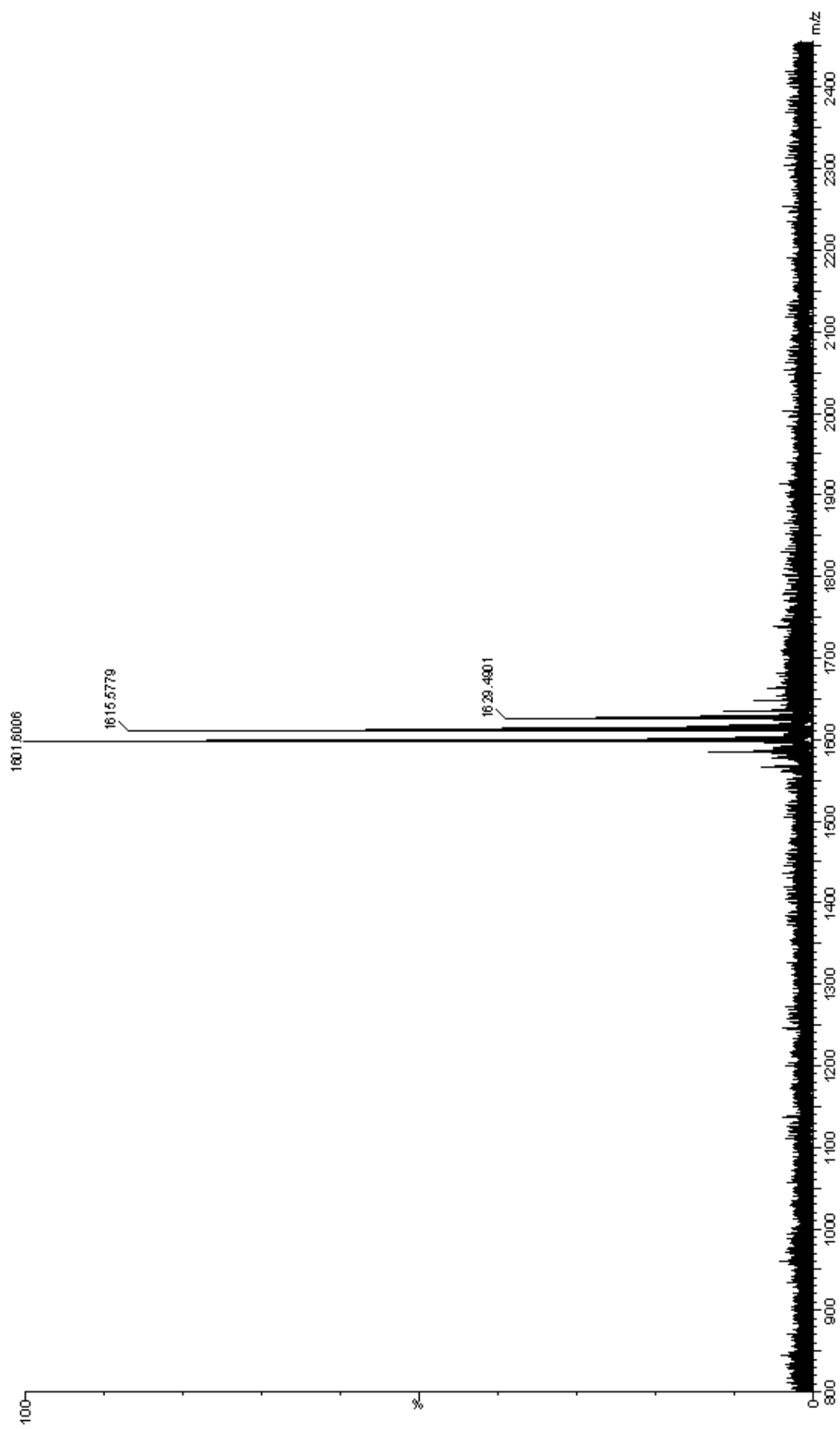
δ_{H} (D_2O , 500 MHz, room temperature) 7.52 (br s, 1H, H-6 of thymidine residue), 6.22 (br s, 1H, H-1' of thymidine residue), 5.20-4.91 (H-1), 4.67 (br s, 1H, H-3' of thymidine residue), 4.11 (br s, 1H, H-4' of thymidine residue), 4.00-3.30 (H-2, H-3, H-4, H-5, H-6), 3.90 ($\text{OCH}_2\text{CHOHCH}_3$), 3.72 (br s, 2H, H-5' of thymidine residue), 3.66 and 3.48 ($\text{OCH}_2\text{CHOHCH}_3$), 2.49 and 2.26 (br s, 2H, H-2' of thymidine residue), 1.77 (s, 3H, CH_3 of thymidine residue), 1.04 ($\text{OCH}_2\text{CHOHCH}_3$). δ_{p} (D_2O , 161.98 MHz) – 0.60. MALDI-TOF m/z calculated for **8** with four 2-hydroxypropyl substituents on the βCD $\text{C}_{64}\text{H}_{107}\text{N}_2\text{O}_{46}\text{P}$ = 1670.6; found = 1693.8, 1751.6 (this values corresponds to the Na^+ adducts of **8** with four and five 2-hydroxypropyl substituents on the βCD , respectively). FT-IR (CaF_2) ν/cm^{-1} 3422, 3395, 2945, 2341, 1653, 1469, 1318, 1278, 1203, 1200, 1031.

Compound **9**: is obtained in 36% yield starting from 30 mg of support **3D**; HPLC retention time 19.2 min.

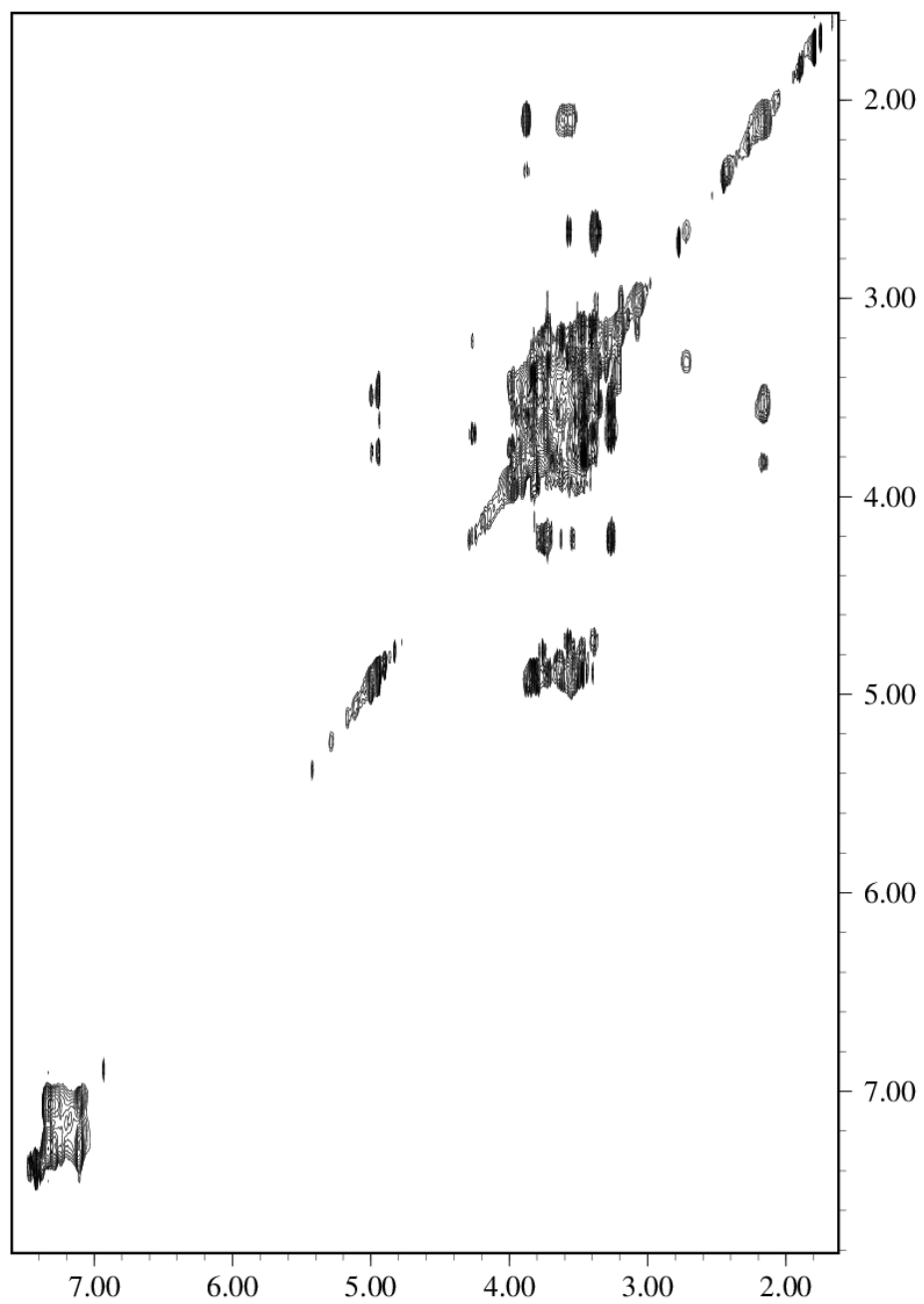
δ_{H} (D_2O , 500 MHz, room temperature) 7.55 (br s, 1H, H-6 of thymidine residue), 6.16 (br s, 1H, H-1' of thymidine residue), 5.15 (br s, H-1' protons of glucopyranose units methylated at position 2), 4.93 (br s, H-1'), 4.71 (br s, 1H, H-3' of thymidine residue), 4.15-3.20 (H-2, H-3, H-4, H-5, H-6), 4.08 (br s, 1H, H-4' of thymidine residue), 3.71 (br s, 2H, H-5' of thymidine residue), 3.20 (OCH_3), 2.49 and 2.30 (br s, 2H, H-2' of thymidine residue), 1.81 (s, 3H, CH_3 of thymidine residue). δ_{p} (D_2O , 161.98 MHz) – 0.54. MALDI-TOF m/z calculated for **9** with ten methyl substituents on the βCD $\text{C}_{62}\text{H}_{103}\text{N}_2\text{O}_{42}\text{P}$ = 1578.6; found 1601.6, 1615.5, 1629.4 (this values corresponds to the Na^+ adducts of **9** with ten, eleven and twelve methyl substituents on the βCD , respectively). FT-IR (CaF_2) ν/cm^{-1} 3422, 2985, 2930, 2343, 1635, 1474, 1277, 1211, 1109, 1072, 1038.



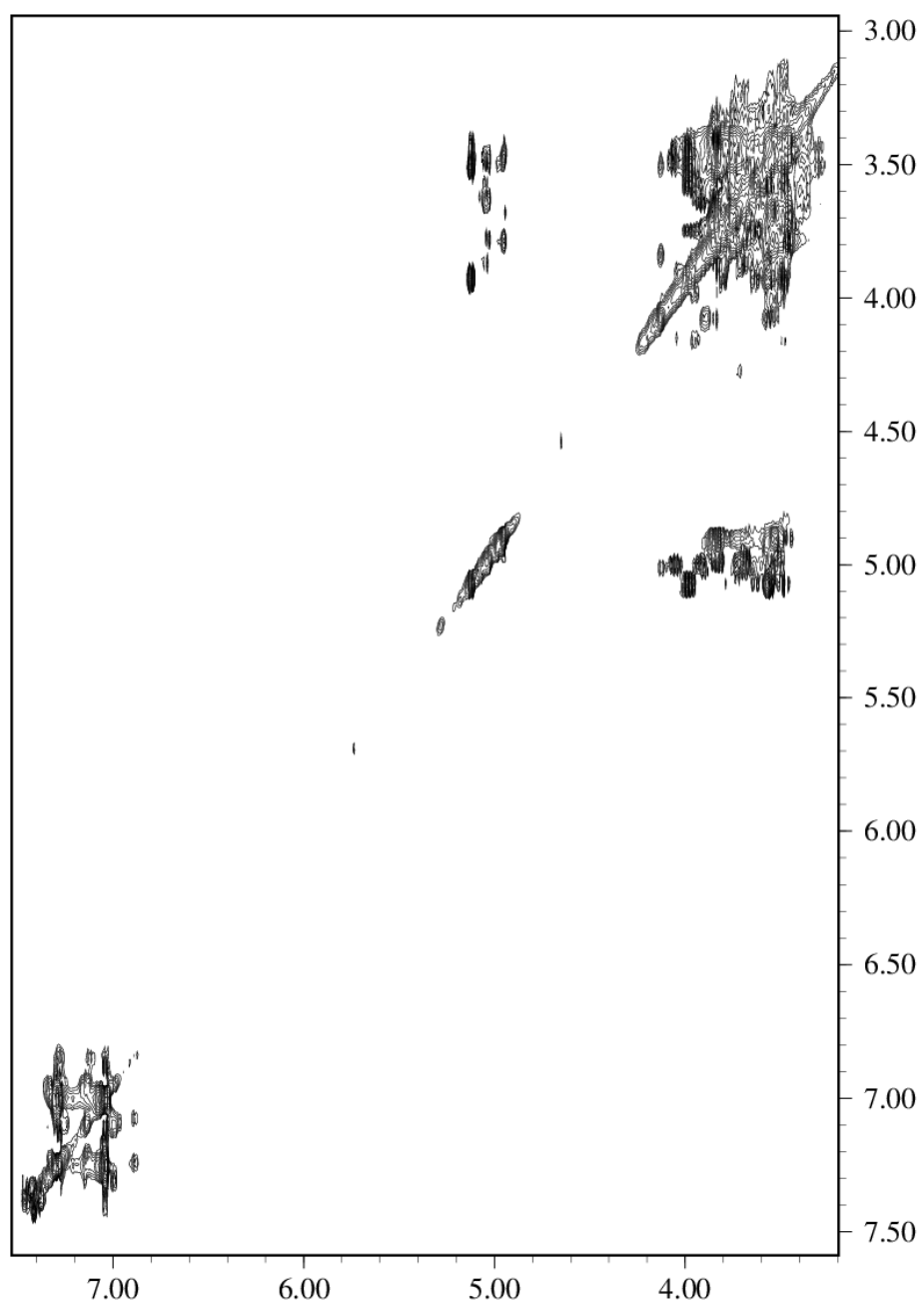
Mass spectrum of compound 7; the peak at $m/z = 1277.3$ and 1293.2 corresponds to the H^+ and NH_4^+ adducts, respectively.



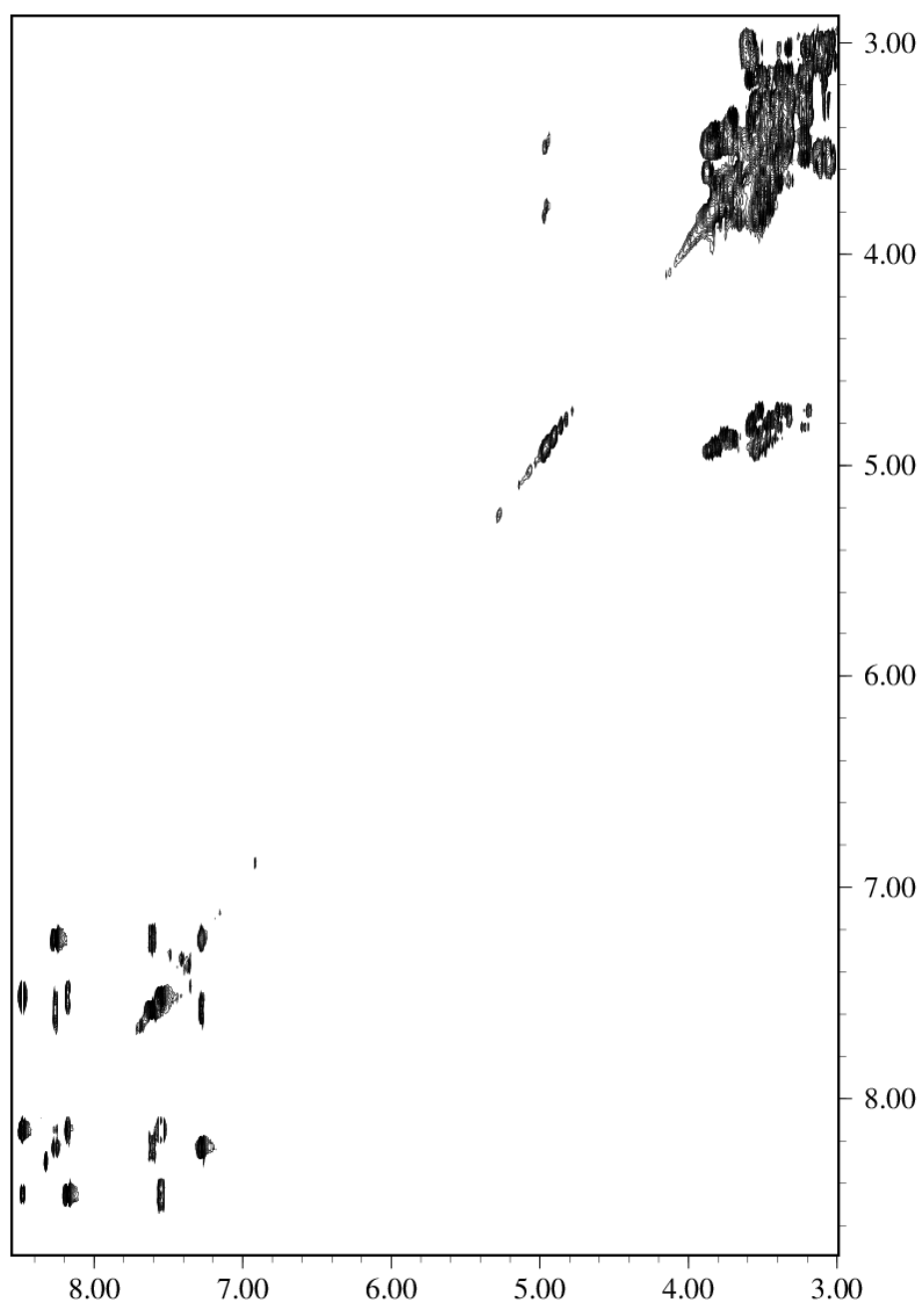
Mass spectrum of compound **9**; the peak at $m/z = 1601.6$, 1615.5 , 1629.4 corresponds to the Na^+ adducts of **9** with ten, eleven and twelve methyl substituents on the βCD , respectively.



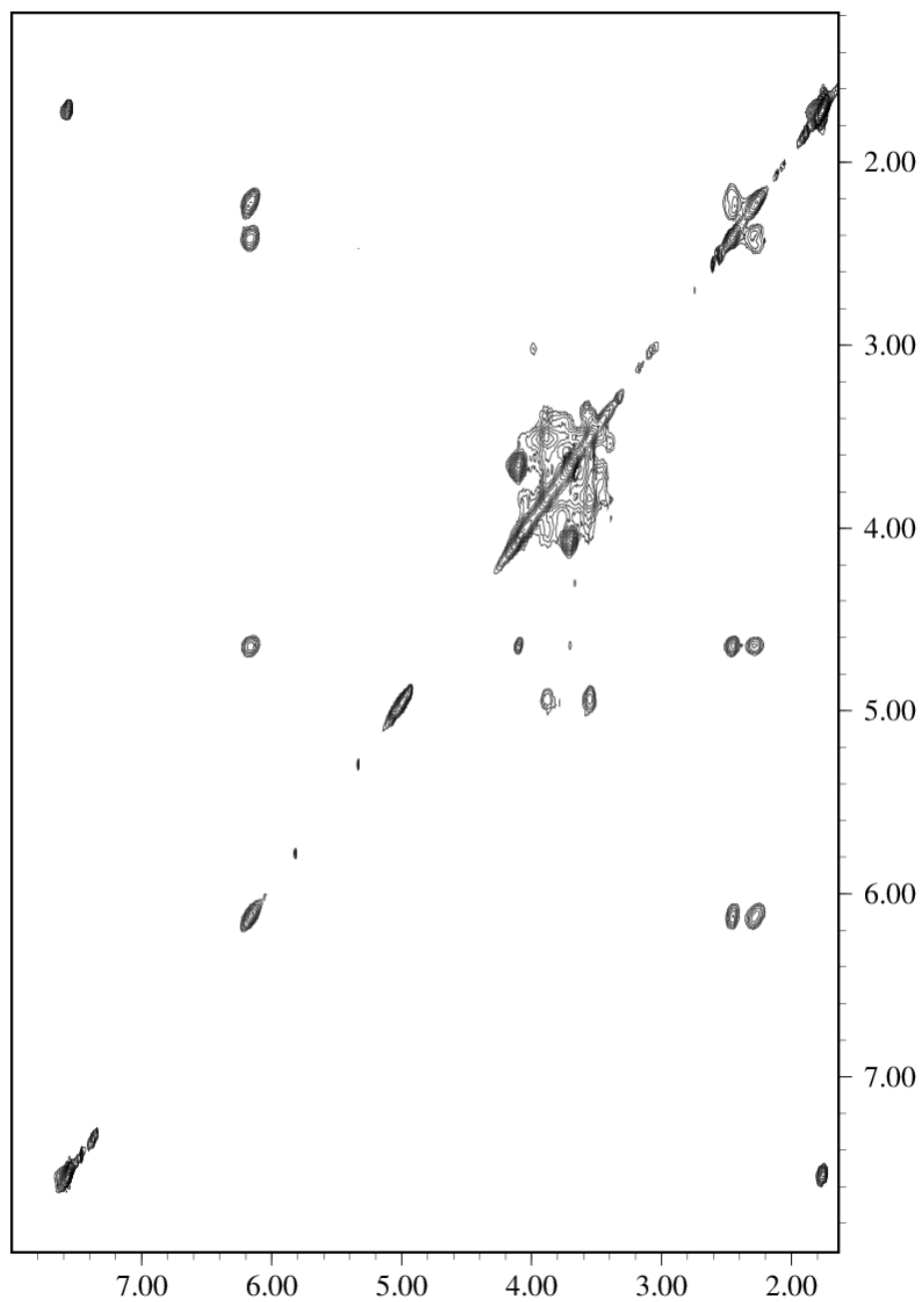
2D-TOCSY spectrum of compound **4** in $^2\text{H}_2\text{O}$ at 298 K



2D-TOCSY spectrum of compound **5** in ²H₂O at 298 K



2D-TOCSY spectrum of compound **6** in ²H₂O at 298 K.



2D-TOCSY spectrum of compound **7** in $^2\text{H}_2\text{O}$ at 298 K