The design, synthesis and photochemical study of a biomimetic cyclodextrin model of Photoactive Yellow Protein (PYP)

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1.1. 6^{I}-(*O*-*p*-toluenesulfonyl)-cyclomaltoheptaose (1)

Tosyl imidazole

To a stirring solution of imidazole (13.00 g, 0.1910 mol, 2.28 eq) in dry dichloromethane (60 mL) was added at 0 ^{o}C under argon, a solution of *p*-toluenesulfonyl chloride (16.00 g, 83.93 mmol, 1.0 eq) in dry dichloromethane (60 mL), dropwise over a period of 1.5 h. The resulting mixture was allowed to warm to room temperature and was stirred vigorously for a further 1.5 h. The reaction mixture was then filtered through a pad of silica (20 g) and was washed with an ethyl acetate/ cyclohexane mixture (1:1, 200 mL). The filtrate was concentrated under reduced pressure, leaving a residue to which was added ethyl acetate (10 mL) followed by cyclohexane (100 mL) to induce precipitation. Filtration of the resulting suspension yielded **1-(*p*-toluenesulfonyl) imidazole**[2] as a white crystalline solid. The filtrate was concentrated once again and the precipitation procedure was repeated to afford more product (17.00 g, 91% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.00 (s, 1H, CH), 7.81 (d, 2H, *J*=8.2 Hz, 2ArCH), 7.34 (d, 2H, *J*=8.2 Hz, 2ArCH), 7.28 (s, 1H, CH), 7.06 (s, 1H, CH), 2.43 (s, 3H, CH_3).

6^{I}-(*O*-*p*-toluenesulfonyl)-cyclomaltoheptaose (1)

To a stirring suspension of β-cyclodextrin (20.00 g, 17.62 mmol, 1.0 eq), rigorously dried over a week in vacuo under P_2O_5, in water (160 mL) was added in one portion sodium hydroxide (6.871 g, 0.1762 mol, 10 eq). The initial white suspension turned to a pale yellow solution upon stirring. To this mixture was added in one portion tosyl imidazole (3.917 g, 17.62 mmol, 1.0 eq), which remained in suspension. The mixture was allowed to stir at room temperature for 2.5 h, upon which time the mixture had gone into solution. Dilute hydrochloric acid was added slowly to the mixture and the pH was adjusted to 5-6, upon which a white thick precipitate was observed to form. This was filtered in a large sintered funnel (porosity 4) and was subsequently washed with warm water (T ≈ 60 ^{o}C, 40 mL). The filter cake was allowed to dry in the water pump for 1 h, was then collected and was recrystallised twice with hot water (T ≈ 85 ^{o}C) to give **6^{I}-(*O*-*p*-toluenesulfonyl)-cyclomaltoheptaose**[3] (**1**, 8.140 g, 36 % yield) as a white powder. R_f 0.28 (EtOAc:MeOH:H_2O, 3:1:1); $[\alpha]_D^{20}$ = +121.9^{o} (*c*=*1*, DMSO); ^{1}H NMR (250 MHz, d_6-DMSO): δ 7.75 (d, 2H, *J*=8.2 Hz, 2ArCH), 7.43 (d, 2H, *J*=8.2 Hz, 2ArCH), 5.95-5.50 (bs, 14H, 7(OH-2), 7(OH-3)), 4.87-4.80 (m, 5H, $5H_1^{II-VII}$), 4.79-4.73 (m, 2H, H_1^{I}, H_1^{II-VII}), 4.65-4.35 (bs, 6H, 6(OH-6)), 4.33 (d, 1H, $J_{H6^{I}\text{-}H6'^{I}}$=11.0 Hz, H_6-OTs), 4.19 (dd, 1H, $J_{H6^{I}\text{-}H6'^{I}}$=11.0 Hz, $J_{H5^{I}\text{-}H6'^{I}}$=6.5 Hz, $H_{6'}$-OTs), 3.80-3.10 (m, 40H, $6H_6^{II-VII}$, $6H_{6'}^{II-VII}$, $7H_3^{I-VII}$, $7H_4^{I-VII}$, $7H_5^{I-VII}$, $7H_2^{I-VII}$), 2.43 (s, 3H, CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 144.8 (ArC_q), 132.7 (ArC_q), 129.9 (2ArCH), 127.6 (2ArCH), 102.3, 102.0, 101.9, 101.3 ($7C_1^{I-VII}$), 81.7, 81.6, 81.5, 81.2, 80.8 ($7C_4^{I-VII}$), 73.1, 72.2, 72.1, 71.9 ($7C_3^{I-VII}$, $7C_2^{I-VII}$, $6C_5^{II-VII}$), 69.7 (C_6^{I}), 68.9 (C_5^{I}), 59.9, 59.8, 59.6, 59.3 ($6C_6^{II-VII}$), 21.2 (CH_3); LRMS (FAB^-): *m/z* $[M-H]^-$ calculated for $C_{49}H_{75}O_{37}S$: 1287.4, observed: 1287.6.

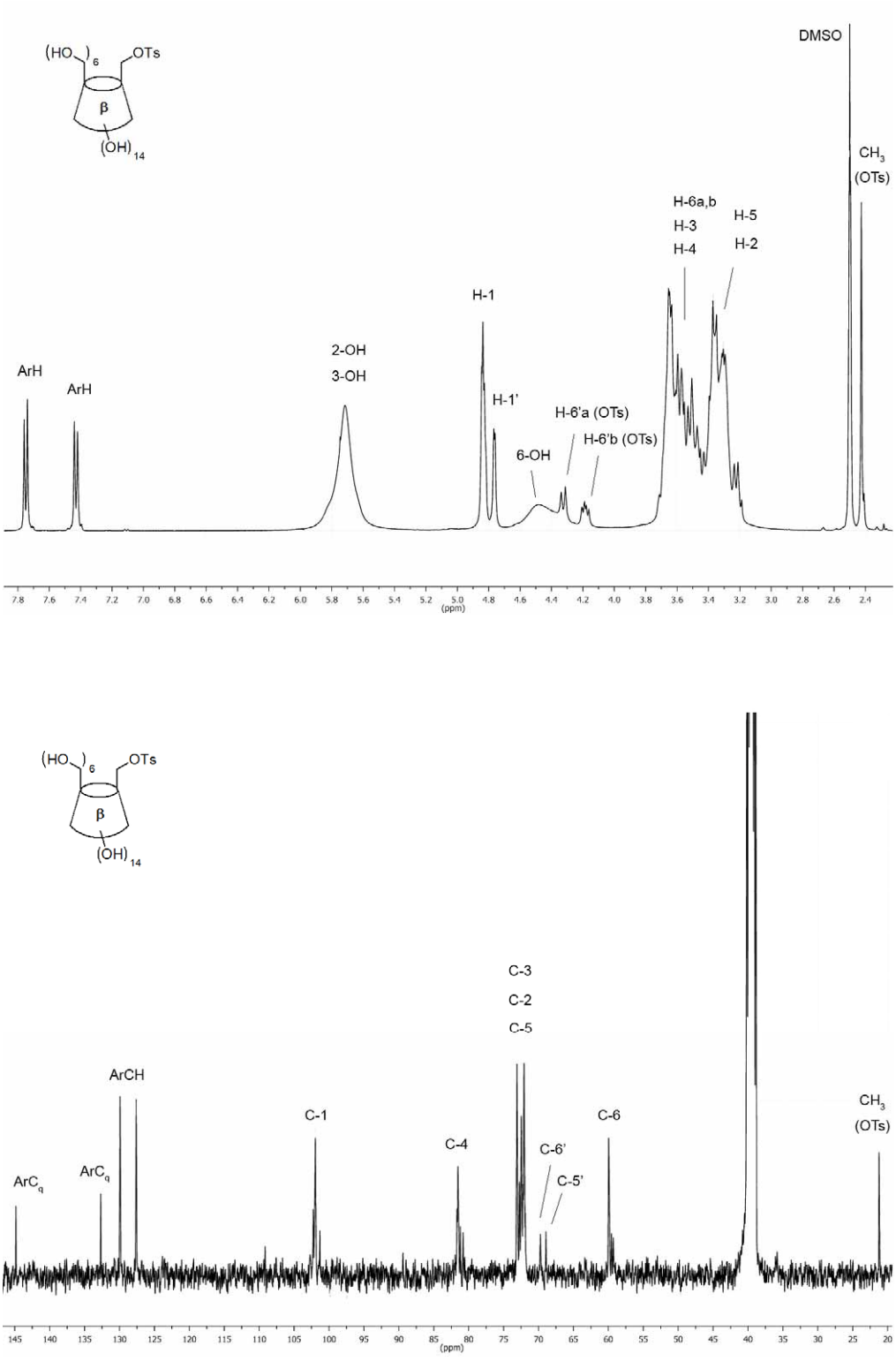

Figure 1: ^{1}H NMR and ^{13}C NMR spectra (250 MHz and 62.5 MHz, $CDCl_3$) of compound (**1**)

1.2. 6^{I}-azido-6^{I}-deoxy-cyclomaltoheptaose (2)

To a stirring suspension of 6^{I}-(*O*-*p*-toluenesulfonyl)-cyclomaltoheptaose (**1**, 8.000 g, 6.205 mmol, 1.0 eq), rigorously dried over a week in vacuo under P_2O_5, in water (120 mL) was added in one portion sodium azide (3.434 g, 59.57 mmol, 9.6 eq) and the mixture was heated at 85 ^{o}C (temperature upon which dissolution was observed) for 16 h. After cooling down to room temperature, acetone (400 mL) was added to the mixture and a white precipitate was observed to form. This was filtered in a large sintered funnel (porosity 4), was subsequently washed with acetone (5 × 20 mL) and was allowed to dry in the water pump for 15 minutes. The product was collected and dried *in vacuo* to afford **6^{I}-azido-6^{I}-deoxy-cyclomaltoheptaose**[4] (**2**, 7.150 g, 99% yield) as a white powder. [The product was of sufficient purity by NMR and was not purified further.] R_f 0.26 (EtOAc:MeOH:H_2O, 3:1:1); $[\alpha]_D^{20}$ = +139.7^{o} (*c=1*, DMSO); ^{1}H NMR (250 MHz, *d*$_6$-DMSO): δ 5.83-5.60 (m, 14H, 7(OH-2), 7(OH-3)), 4.87 (d, 1H, $J_{H1^I\text{-}H2^I}$=3.2 Hz, H_1^I), 4.85-4.78 (m, 6H, $6H_1^{II\text{-}VII}$), 4.57-4.43 (m, 6H, 6(OH-6)), 3.80-3.50 (m, 28H, $7H_3^{I\text{-}VII}$, $7H_5^{I\text{-}VII}$, $7H_6^{I\text{-}VII}$, $7H_{6'}^{I\text{-}VII}$), 3.44-3.23 (m, 14H, $7H_4^{I\text{-}VII}$, $7H_2^{I\text{-}VII}$); ^{13}C NMR (62.5 MHz, *d*$_6$-DMSO): δ 102.3, 102.1, 102.0, 101.6 ($7C_1^{I\text{-}VII}$), 83.0, 81.9, 81.6, 81.6, 81.4 ($7C_4^{I\text{-}VII}$), 73.1, 72.9, 72.8, 72.4, 72.2, 72.1, 70.2 ($7C_5^{I\text{-}VII}$, $7C_3^{I\text{-}VII}$, $7C_2^{I\text{-}VII}$), 60.2, 60.0, 59.8 ($6C_6^{II\text{-}VII}$), 51.1 (C_6^I); LRMS (FAB$^-$): *m/z* [M-H]$^-$ calculated for $C_{42}H_{68}N_3O_{34}$: 1158.4, observed: 1158.5.

1.3. 6^{I}-azido-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2$^{II\text{-}VII}$,3$^{II\text{-}VII}$,6$^{II\text{-}VII}$-tri-*O*-methyl) cyclomaltoheptaose (3)

To a stirring solution of 6^{I}-azido-6^{I}-deoxy-cyclomaltoheptaose (**2**, 7.000 g, 6.034 mmol, 1.0 eq) in anhydrous *N*,*N*-dimethylformamide (300 mL) was added in one portion iodomethane (22.54 mL, 36.21 mmol, 60 eq). Sodium hydride (60% dispersion in mineral oil; 8.690 g, 36.21 mmol, 60 eq) was added in small portions over a period of 3 h and the reaction mixture was left stirring overnight for 20 h. The mixture was then cooled down to 10 ^{o}C and methanol (50 mL) was added dropwise to decompose excess sodium hydride. After gas evolution had ceased, the solvent was evaporated *in vacuo* and the residue was dissolved in ether (300 mL) and water (300 mL) was added. The mixture was extracted, the organic phase was washed with water (4 × 50 mL) and the combined aqueous phases were washed with ether (2 × 50 mL). The organic extracts were collected, dried ($MgSO_4$), filtered and the solvent was evaporated off to yield a pale yellow oil. The crude product was purified by column chromatography on silica gel [eluent: EtOAc:MeOH:H_2O, 70:5:3] to yield **6^{I}-azido-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2$^{II\text{-}VII}$,3$^{II\text{-}VII}$,6$^{II\text{-}VII}$-tri-*O*-methyl) cyclomaltoheptaose**[5] (**3**, 7.280 g, 84% yield) as a white foam. [The product could be further recrystallised by a mixture of DCM:*n*-pentane, 1:50 to obtain a white crystalline solid.] R_f 0.32 (EtOAc:MeOH:H_2O, 70:5:3); $[\alpha]_D^{20}$ +170.0^{o} (*c=1*, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.16-5.07 (m, 6H, $6H_1^{II\text{-}VII}$), 5.06 (d, 1H, $J_{H1^I\text{-}H2^I}$=3.7 Hz, H_1^I), 3.97-3.30 (m, 35H, $7H_5^{I\text{-}VII}$, $7H_6^{I\text{-}VII}$, $7H_4^{I\text{-}VII}$, $7H_{6'}^{I\text{-}VII}$, $7H_3^{I\text{-}VII}$), 3.65, 3.64, 3.63, 3.63 (4×s, 21H, 7(3-OMe)), 3.51, 3.51, 3.50, 3.49 (4×s, 21H, 7(2-OMe)), 3.39, 3.38 (2×s, 18H, 6(6-OMe)), 3.22-3.14 (m, 7H, $7H_2^{I\text{-}VII}$); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 99.5, 99.3, 99.2, 99.0 ($6C_1^{II\text{-}VII}$), 98.6 (C_1^I), 82.2, 82.1, 82.1, 82.0, 81.9, 81.9, 81.5 ($7C_2^{I\text{-}VII}$, $7C_3^{I\text{-}VII}$), 80.6, 80.5, 80.4, 80.3, 80.2 ($7C_4^{I\text{-}VII}$), 71.7, 71.6, 71.5, 71.4, 71.2 ($7C_6^{II\text{-}VII}$), 71.1, 71.0, 71.0 ($7C_5^{I\text{-}VII}$), 61.7, 61.7, 61.6, 61.5 (7(3-OMe)), 52.2 (C_6^I), 59.1, 59.1 (6(6-OMe)), 58.8, 58.7, 58.7, 58.6, 58.6 (7(2-OMe)); LRMS (FAB$^+$): *m/z* [M+Na]$^+$ calculated for $C_{62}H_{109}N_3O_{34}Na$: 1462.7, observed: 1463.0.

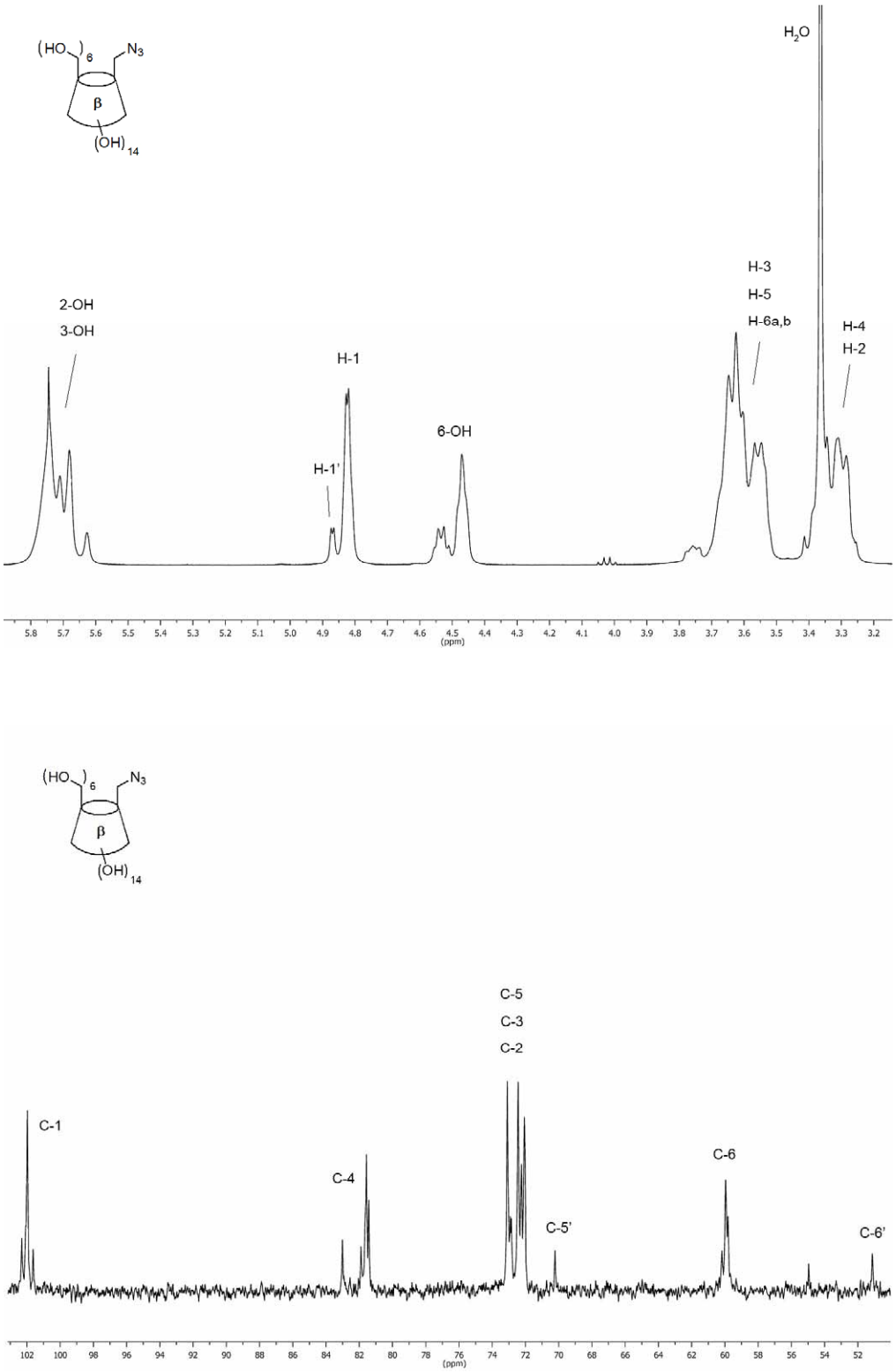

Figure 2: ^{1}H NMR and ^{13}C NMR spectra (250 MHz and 62.5 MHz, $CDCl_3$) of compound (**2**)

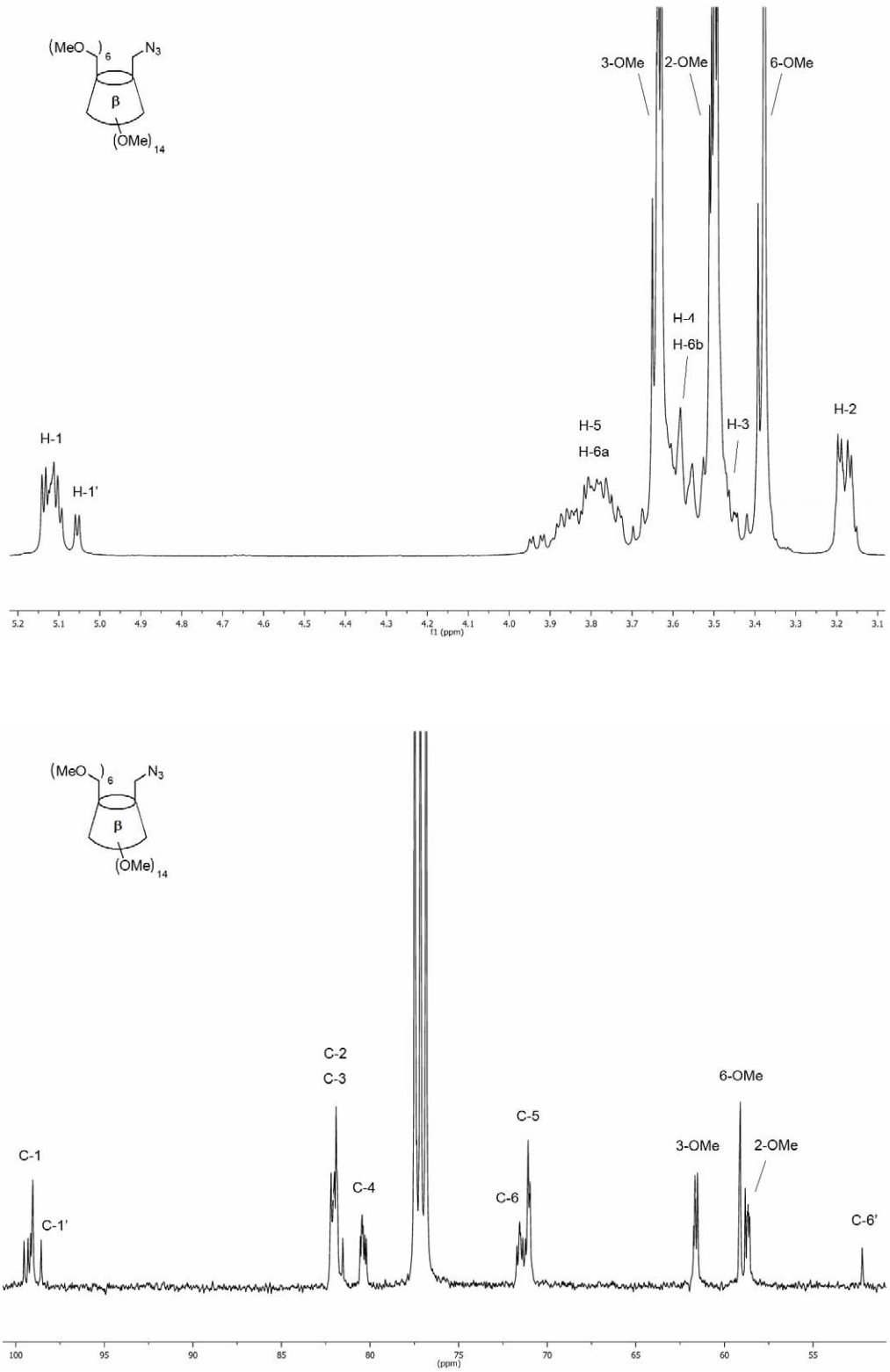

Figure 3: ^{1}H NMR and ^{13}C NMR spectra (250 MHz and 62.5 MHz, $CDCl_3$) of compound (**3**)

1.4. 6^{I}-amino-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose (4)

To a solution of 6^{I}-azido-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis(2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomalto-heptaose (**3**, 5.000 g, 3.471 mmol, 1.0 eq) in anhydrous 1,4-dioxane (100 mL) under argon, was added triphenylphosphine (1.366 g, 5.206 mmol, 1.5 eq) and the mixture was left stirring at room temperature for 4 h. After this time, a solution of ammonia (3M, 5.2 mL) was added dropwise and the reaction mixture was left stirring overnight for 16 h. Water (100 mL) was poured into the reaction mixture and the pH was adjusted to 4 with addition of a dilute hydrochloric acid solution (1M). The mixture was extracted with toluene (5 × 50 mL) and the aqueous phase was made alkaline by addition of a sodium hydroxide solution (2M). The mixture was then extracted with dichloromethane (5 × 50 mL) and the organic phase was washed with water (50 mL) and brine (50 mL). The combined organic extracts were collected, dried ($MgSO_4$), filtered and the solvent was evaporated off to yield a white foam. The crude product was purified by column chromatography on silica gel [gradient elution/ eluent: EtOAc:MeOH:H_2O, 70:5:3, 45:5:3, 3:1:1] to yield **6^{I}-amino-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose**[6,7] (**4**, 4.325 g, 88% yield) as a white foam. R_f 0.20 (EtOAc:MeOH:H_2O, 3:1:1); $[\alpha]_D^{20}$ +135.9^{o} (*c=1*, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.18 (d, 1H, $J_{H1^{II}\text{-}H2^{II}}$=3.9 Hz, H_1^{II}), 5.13 (d, 2H, $J_{H1\text{-}H2}$=3.6 Hz, $2H_1^{III-VII}$), 5.09 (m, 3H, $3H_1^{III-VII}$), 5.06 (d, 1H, $J_{H1^{I}\text{-}H2^{I}}$=3.5 Hz, H_1^{I}), 3.90-3.72 (m, 13H, $7H_5^{I-VII}$, $6H_6^{II-VII}$), 3.68-3.42 (m, 20H, $7H_4^{I-VII}$, $6H_{6'}^{II-VII}$, $7H_3^{I-VII}$), 3.65, 3.64, 3.63, 3.62, 3.62, 3.61 (6×s, 21H, 7(3-OMe)), 3.52, 3.49, 3.49, 3.49, 3.48, 3.47 (6×s, 21H, 7(2-OMe)), 3.37, 3.37, 3.36, 3.36, 3.36 (5×s, 18H, 6(6-OMe)), 3.20-3.14 (m, 6H, $6H_2^{II-VII}$), 3.13 (dd, 1H, $J_{H2^{I}\text{-}H3^{I}}$=9.3 Hz, $J_{H1^{I}\text{-}H2^{I}}$=3.5 Hz, H_2^{I}), 3.05-2.95 (m, 2H, H_6^{I}, $H_{6'}^{I}$); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 99.6, 99.2, 99.0, 99.0, 98.9 ($7C_1^{I-VII}$), 82.7, 82.0, 81.9, 81.9, 81.8 ($7C_2^{I-VII}$, $7C_3^{I-VII}$), 80.8, 80.7, 80.6, 80.3, 80.2, 80.0 ($7C_4^{I-VII}$), 71.7, 71.6, 71.4, 71.4 ($7C_6^{II-VII}$), 71.3, 71.1, 71.1, 71.0, 71.0 ($7C_5^{I-VII}$), 67.2 (C_6^{I}), 61.8, 61.7, 61.5, 61.3 (7(3-OMe)), 59.3, 59.1 (6(6-OMe)), 58.7, 58.6, 58.4, 58.3 (7(2-OMe)); LRMS (FAB^{+}): *m/z* $[M]^{+}$ calculated for $C_{62}H_{111}NO_{34}$: 1413.7, observed: 1414.9.

N-Acetyl-*S*-trityl-L-cysteine

To a solution of *N*-acetyl-L-cysteine (0.5000 g, 3.064 mmol, 1.0 eq) in dry *N*,*N*-dimethylformamide (8 mL) was added triphenylmethyl chloride (1.281 g, 4.596 mmol, 1.5 eq) and the mixture was stirred at room temperature under argon for 20 h. The solvent was evaporated and to the residue were added water (20 mL) and ether (20 mL). The mixture was extracted, the organic phase was washed with water (4 × 20 mL) and the combined aqueous phases were washed with ether (2 × 20 mL). The combined organic extracts were collected, dried ($MgSO_4$), filtered and concentrated to yield an off-white foam. The crude product was purified by column chromatography on silica gel [eluent: EtOAc:MeOH:H_2O, 4:1:1] to yield ***N*-acetyl-*S*-trityl-L-cysteine**[8] as a white foam (1.019 g, 82%). The crude product was then recrystallised with ether to yield the product as a white crystalline solid. R_f 0.38 (EtOAc:MeOH:H_2O, 4:1:1); $[\alpha]_D^{20}$ = +40.7^{o} (*c=1*, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 10.05-9.75 (bs, 1H, COOH), 7.35-7.25 (m, 6H, 6ArCH), 7.25-7.05 (m, 9H, 9ArCH), 6.24 (d, *J*=7.6 Hz, NH), 4.47-4.38 (m, 1H, CH), 2.73-2.51 (m, 2H, CH_2), 1.84 (s, 3H, CH_3).

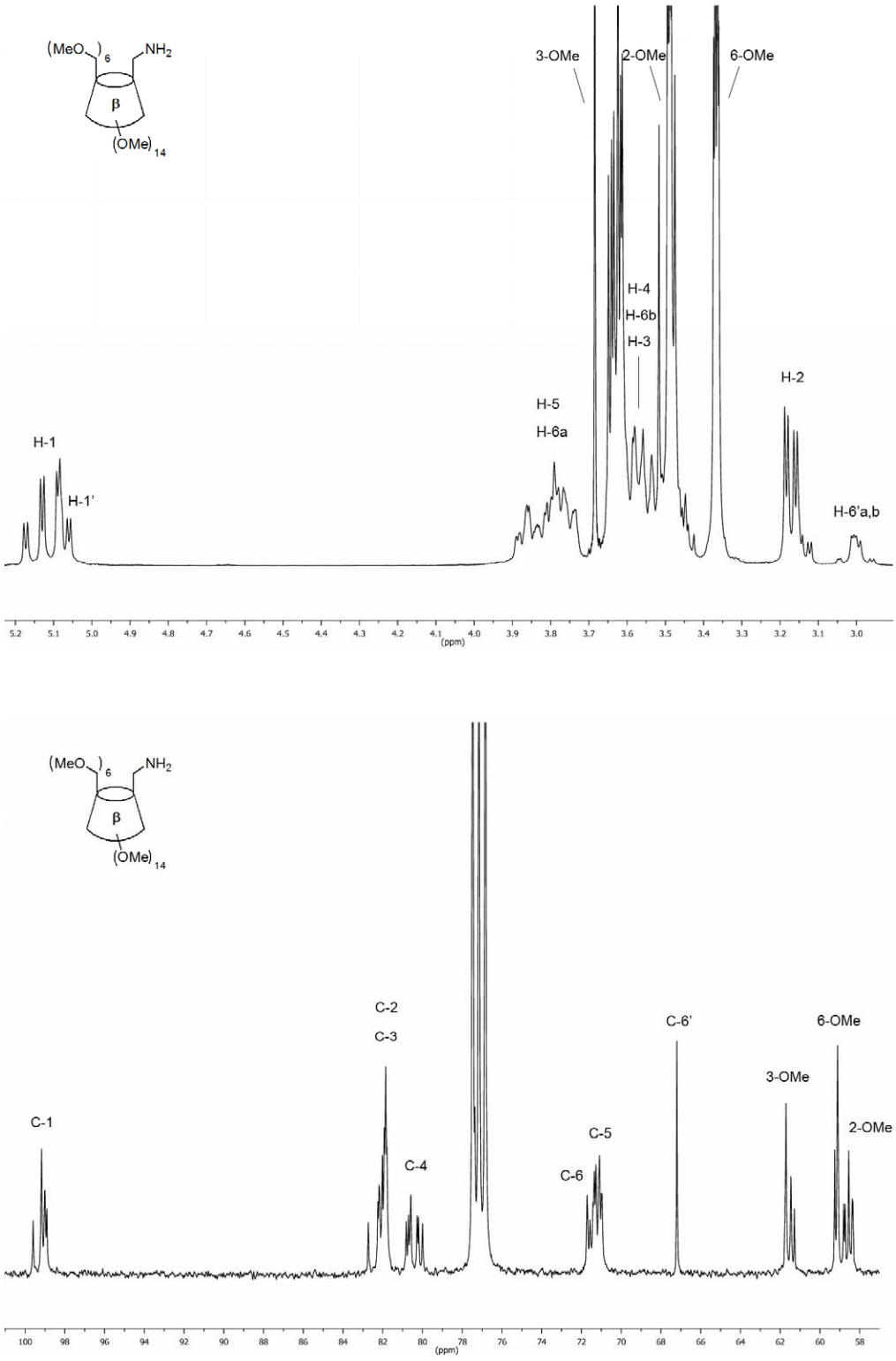

Figure 4: ^{1}H NMR and ^{13}C NMR spectra (250 MHz and 62.5 MHz, $CDCl_3$) of compound (**4**)

1.5. 6^{I}-((*N*-acetyl-*S*-trityl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose (5)

To a stirring solution of 6^{I}-amino-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose (**4**, 2.000 g, 1.414 mmol, 1.0 eq) and *N*-acetyl-*S*-trityl-L-cysteine (0.5272 g, 1.300 mmol, 0.92 eq) in dry dichloromethane (60 mL), under argon, were added successively 1-hydroxybenzotriazole (0.1757 g, 1.300 mmol, 0.92 eq), 4-dimethylaminopyridine (0.1588 g, 1.300 mmol, 0.92 eq) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.2492 g, 1.300 mmol, 0.92 eq) and the reaction mixture was allowed to stir at room temperature for 20 h. The mixture was quenched with water (60 mL) and was extracted with dichloromethane (5 × 40 mL). The combined organic extracts were washed with water (40 mL) and brine (40 mL) and were then collected, dried ($MgSO_4$), filtered and concentrated to yield a white foam. The crude product was purified by column chromatography on silica gel [eluent: EtOAc then EtOAc:MeOH:H_2O, 20:1:1] to yield **6^{I}-((*N*-acetyl-*S*-trityl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose** (**5**, 2.490 g, 98% yield) as a white foam. R*f* 0.20 (EtOAc:MeOH:H_2O, 20:1:1); $[\alpha]_D^{20}$ +117.8^{o} (*c=1*, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.37 (m, 7H, 7ArCH), 7.31-7.25 (m, 5H, 5ArCH), 7.24-7.18 (m, 3H, 3ArCH), 6.42-6.35 (m, 1H, CH_2NH), 5.94 (d, 1H, $J_{NH,CH}$=7.5 Hz, N*H*Ac), 5.15-5.08 (m, 7H, $7H_1^{I-VII}$), 4.19 (m, 1H, AcNHC*H*), 3.90-3.72 (m, 14H, $7H_5^{I-VII}$, $7H_6^{I-VII}$), 3.70-3.29 (m, 21H, $7H_4^{I-VII}$, $7H_{6'}^{I-VII}$, $7H_3^{I-VII}$), 3.64, 3.63, 3.62 (3×s, 21H, 7(3-OMe)), 3.50, 3.49, 3.49 (3×s, 21H, 7(2-OMe)), 3.40, 3.39, 3.37, 3.37, 3.32 (5×s, 18H, 6(6-OMe)), 3.22-3.13 (m, 6H, $6H_2^{II-VII}$), 3.07 (dd, 1H, $J_{H2^{I}-H3^{I}}$=9.3 Hz, $J_{H1^{I}-H2^{I}}$=3.5 Hz, H_2^{I}), 2.66 (dd, 1H, ^{2}J=12.6 Hz, ^{3}J=5.8 Hz, C*H*$_2$STr), 2.52 (dd, 1H, ^{2}J=12.6 Hz, ^{3}J=6.5 Hz, C*H*$_2$STr), 1.88 (s, 3H, NHCOC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0 (NH*C*OCH$_3$), 169.7 (CH$_2$NH*C*O), 144.5 (3ArC_q), 129.7, 128.2, 127.0 (18ArCH), 99.2, 99.1, 99.1, 99.0, 99.0, 98.9, 98.9 ($7C_1^{I-VII}$), 82.3, 82.2, 82.2, 82.1, 82.0, 81.9,81.9, 81.8, 81.8, 81.5, 81.4 ($7C_2^{I-VII}$, $7C_3^{I-VII}$), 80.6, 80.5, 80.4, 80.4, 80.3, 80.2, 79.7 ($7C_4^{I-VII}$), 77.4 (Ph$_3$*C*), 71.7, 71.5, 71.3 ($7C_6^{I-VII}$), 71.5, 71.1, 70.1 ($7C_5^{I-VII}$), 61.7, 61.6, 61.6, 61.5, 61.5 (7(3-OMe)), 59.5, 59.1 (6(6-OMe)), 58.7, 58.6, 58.5 (7(2-OMe)), 52.3 (AcNH*C*H), 33.9 (*C*H$_2$STr), 23.2 (NHCO*C*H$_3$); LRMS (FAB^{+}): *m/z* [M+Na]$^{+}$ = 1823.8; HRMS (FAB^{+}): *m/z* [M+Na]$^{+}$ calculated for $C_{86}H_{132}N_2O_{36}SNa$ 1823.8178, observed 1823.8217.

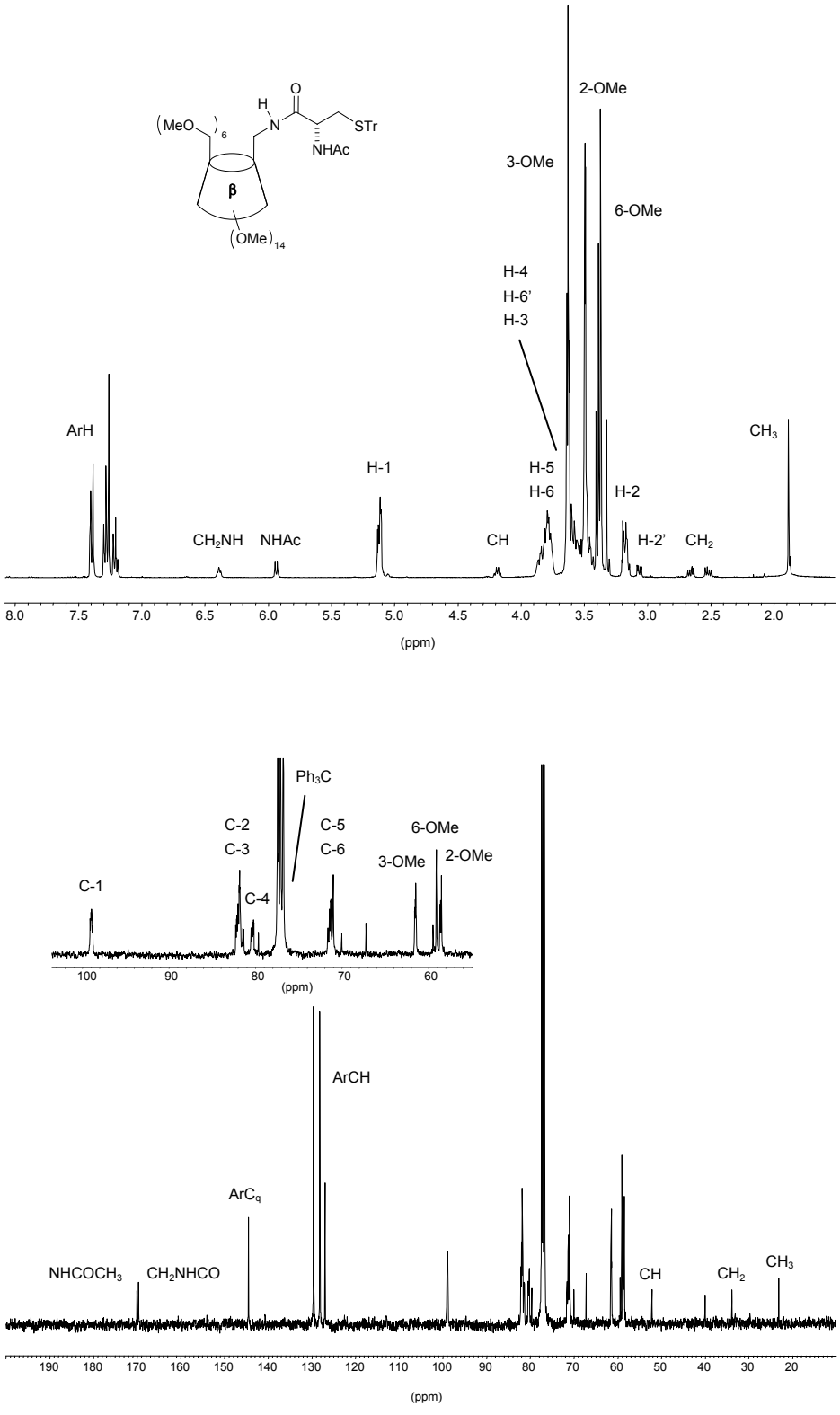

Figure 5: ^{1}H NMR and ^{13}C NMR spectra (400 MHz and 100 MHz, $CDCl_3$) of compound (**5**)

1.6.6^{I}-((*N*-acetyl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose (6)

To a stirring solution of 6^{I}-((*N*-acetyl-*S*-trityl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose (**5**, 1.000 g, 0.5549 mmol, 1.0 eq) in dry dichloromethane (15 mL) was added triethylsilane (0.36 mL, 2.220 mmol, 4.0 eq).[9] Trifluoroacetic acid (1.5 mL) was added dropwise turning the solution into a bright yellow colour, which disappeared after 15 minutes. The reaction mixture was left stirring for 3 h, it was diluted with dichloromethane (50 mL) and water (50 mL) was added. The mixture was extracted from dichloromethane (5 × 20 mL), the organic phase was washed with a saturated sodium bicarbonate solution (2 × 20 mL), water (20 mL) and brine (20 mL). The combined organic extracts were collected**,** dried ($MgSO_4$), filtered and concentrated to yield a white foam. The crude product was purified by column chromatography on silica gel [eluent: EtOAc then EtOAc:MeOH:H_2O, 10:1:1] to yield **6^{I}-((*N*-acetyl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose** (**6**, 0.8326 g, 96% yield) as a white foam. R*f* 0.16 (EtOAc:MeOH:H_2O, 10:1:1); $[\alpha]_D^{20}$ +144.0^{o} (*c*=*1*, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.67 (dd, 1H, *J*=5.5 Hz, CH_2NH), 6.53 (d, 1H, $J_{NH,CH}$=7.7 Hz, N*H*Ac), 5.16-5.07 (m, 7H, 7H$_1^{I-VII}$), 4.58 (ddd, 1H, J_{CH-CH_2}=6.7, 4.3 Hz, AcNHC*H*), 3.89-3.73 (m, 14H, 7H$_5^{I-VII}$, 7H$_6^{I-VII}$), 3.67-3.32 (m, 21H, 7H$_4^{I-VII}$, 7H$_6$,$^{I-VII}$, 7H$_3^{I-VII}$), 3.64, 3.63, 3.62, 3.61 (4×s, 21H, 7(3-OMe)), 3.50, 3.50, 3.50, 3.49, 3.48 (5×s, 21H, 7(2-OMe)), 3.44, 3.38, 3.37 (3×s, 18H, 6(6-OMe)), 3.24-3.15 (m, 6H, 6H$_2^{II-VII}$), 3.12 (dd, 1H, $J_{H2^I-H3^I}$=9.6 Hz, $J_{H1^I-H2^I}$=3.5 Hz, H$_2^{I}$), 2.97 (ddd, 1H, ^{2}J=13.7 Hz, J_{CH2-SH}=7.9 Hz, J_{CH2-CH}=4.3 Hz, C*H*$_2$SH), 2.77 (ddd, 1H, ^{2}J=13.7 Hz, J_{CH2-SH}=9.8 Hz, J_{CH2-CH}=6.7 Hz, C*H*$_2$SH), 2.03 (s, 3H, NHCOC*H*$_3$), 1.61 (dd, 1H, J_{CH2-SH} =9.8, 7.9 Hz, CH_2SH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0 (NH*C*OCH$_3$), 169.7 (CH_2NHCO), 99.4, 99.2, 99.2, 99.1, 98.9, 98.9, 98.7 (7C$_1^{I-VII}$), 82.3, 82.3, 82.2, 82.1, 82.0, 81.9, 81.9, 81.8, 81.6, 81.5, 81.5 (7C$_2^{I-VII}$, 7C$_3^{I-VII}$), 80.7, 80.6, 80.4, 80.2, 80.1, 79.6 (7C$_4^{I-VII}$), 71.8, 71.5, 71.4, 71.4, 71.4 (7C$_6^{I-VII}$), 71.7, 71.3,71.2, 71.1, 71.1, 71.0, 70.0 (7C$_5^{I-VII}$), 61.7, 61.6, 61.5, 61.5, 61.4 (7(3-OMe)), 59.7, 59.2, 59.2, 59.2, 59.1 (6(6-OMe)), 59.0, 58.8, 58.7, 58.7, 58.6, 58.5, 58.5 (7(2-OMe)), 54.5 (AcNH*C*H), 27.2 (CH_2SH), 23.3 (NHCO*C*H$_3$); LRMS (FAB^{+}): *m/z* [M+Na]$^{+}$ = 1581.6; HRMS (FAB^{+}): *m/z* [M+Na]$^{+}$ calculated for $C_{67}H_{118}N_2O_{36}SNa$ 1581.7083, observed 1581.7102.

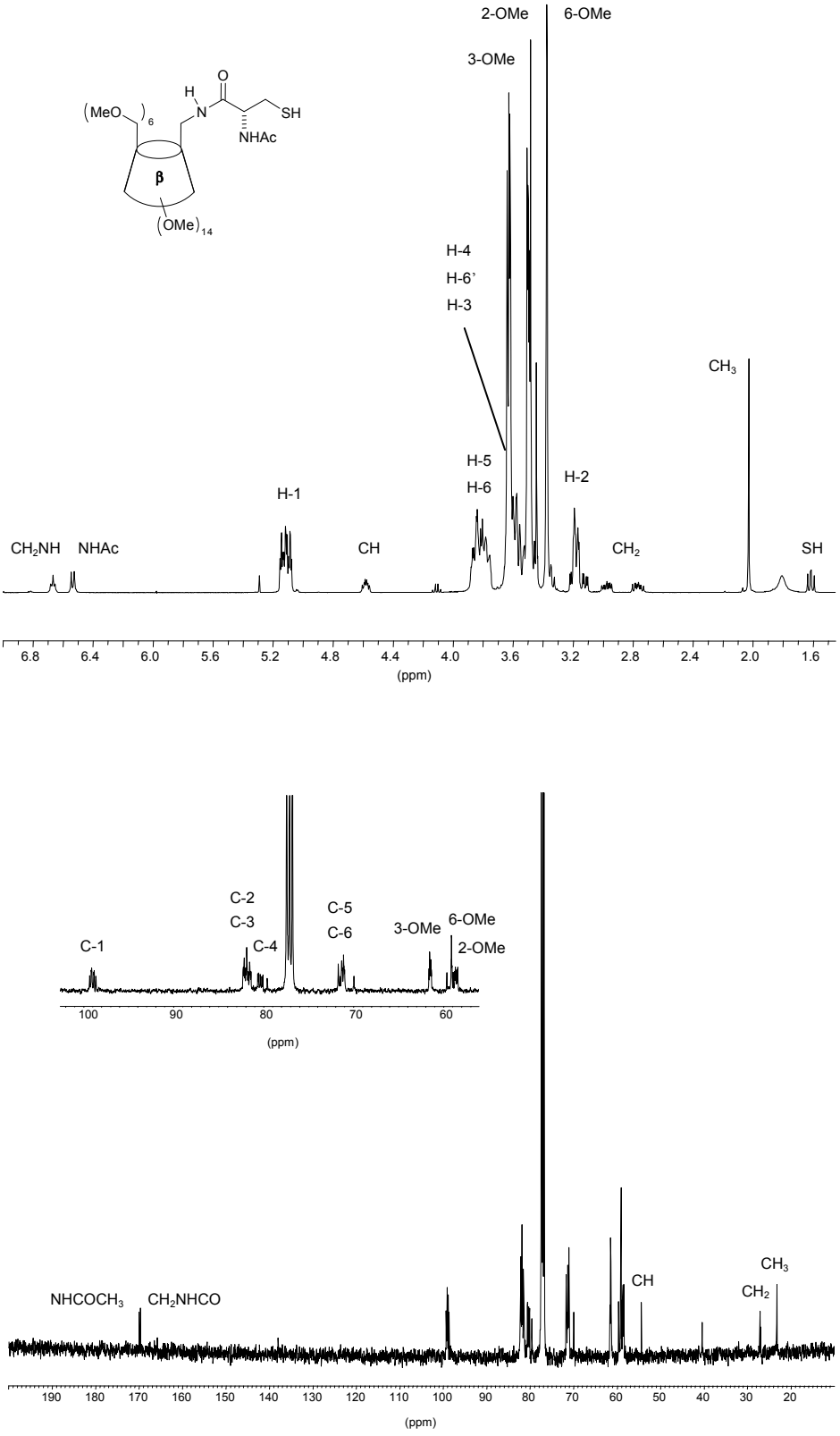

Figure 6: ^{1}H NMR and ^{13}C NMR spectra (400 MHz and 100 MHz, $CDCl_3$) of compound (**6**)

1.7. CD-PYP1:6^{I}-[((*N*-acetyl-*S*-(*p*-hydroxycinnamoyl)-L-cysteinyl)amino]-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose (7)

To a stirring solution of 6^{I}-((*N*-acetyl-L-cysteinyl)amino)-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose (**6**, 0.6850 g, 0.4392 mmol, 1.0 eq) and *trans*-*p*-hydroxycinnamic acid (0.0721 g, 0.4392 mmol, 1.0 eq) in dry *N*,*N*-dimethylformamide (30 mL), under argon, were added successively 1-hydroxybenzotriazole (0.0712 g, 0.5270 mmol, 1.2 eq), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.1010 g, 0.5270 mmol, 1.2 eq) and the reaction mixture was allowed to stir at room temperature for 20 h. The solvent was evaporated *in vacuo* (T ≈ 40°C), the residue was dissolved in dichloromethane (30 mL) and water was added (30 mL). The mixture was extracted from dichloromethane (5 × 20 mL) and the organic phase was washed with water (20 mL) and brine (20 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the concentrated down to yield a pale yellow foam. The crude product was purified by column chromatography on silica gel [gradient elution/ eluent: EtOAc then EtOAc:MeOH:H_2O, 20:1:1, 10:1:1] to yield **6^{I}-[((*N*-acetyl-*S*-(*p*-hydroxycinnamoyl)-L-cysteinyl)amino]-6^{I}-deoxy-2^{I},3^{I}-di-*O*-methyl-hexakis (2^{II-VII},3^{II-VII},6^{II-VII}-tri-*O*-methyl) cyclomaltoheptaose** (**CD-PYP1**: **7**, 0.5225 g, 70% yield) as a white foam. R*f* 0.18 (EtOAc:MeOH:H_2O, 10:1:1); $[\alpha]_D^{20}$ +116.0° (*c=1*, $CHCl_3$); ***Protonated form-CDCl$_3$:*** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, 1H, J_{Ha-Hb}=15.8 Hz, H_b), 7.36 (d, 2H, ^3J=8.5 Hz, H_c, H_d), 6.94-6.88 (m, 1H, CH_2NH), 6.84 (d, 2H, ^3J=8.5 Hz, H_e, H_f), 6.72 (d, 1H, $J_{NH,CH}$=7.5 Hz, NHAc), 6.49 (d, 1H, J_{Ha-Hb} =15.8 Hz, H_a), 5.17-5.08 (m, 7H, 7H$_1^{I-VII}$), 4.68-4.63 (m, 1H, AcNHC*H*), 3.90-3.73 (m, 14H, 7H$_5^{I-VII}$, 7H$_6^{I-VII}$), 3.73-3.25 (m, 23H, 7H$_4^{I-VII}$, 7H$_{6'}^{I-VII}$, 7H$_3^{I-VII}$, CH_2S), 3.64, 3.62 (2×s, 21H, 7(3-OMe)), 3.50, 3.49, 3.48, 3.47 (4×s, 21H, 7(2-OMe)), 3.44, 3.38, 3.37 (3×s, 18H, 6(6-OMe)), 3.22-3.14 (m, 6H, 6H$_2^{II-VII}$), 3.14 (dd, 1H, $J_{H3^I-H2^I}$=9.7 Hz, $J_{H1^I-H2^I}$=3.4 Hz, H$_2^I$), 2.02 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.0 (CH_2SCO), 171.1 ($NHCOCH_3$), 170.1 (CH_2NHCO), 159.9 (ArC_q phenol), 142.2 (C_b), 130.8 (C_c, C_d), 125.6 (ArC_q), 121.2 (C_a), 116.4 (C_e, C_f), 99.2, 99.2, 99.1, 99.1, 98.9, 98.8, 98.7 (7C$_1^{I-VII}$), 82.3, 82.3, 82.2, 82.2, 82.0, 81.9, 81.9, 81.8, 81.7, 81.7, 81.6, 81.5 (7C$_2^{I-VII}$, 7C$_3^{I-VII}$), 80.6, 80.3, 80.1, 80.1, 79.6 (7C$_4^{I-VII}$), 71.7, 71.5, 71.4, 71.3, 71.3 (7C$_6^{I-VII}$), 71.6, 71.1, 71.0, 70.0 (7C$_5^{I-VII}$), 61.7, 61.6, 61.6, 61.5, 61.5, 61.4 (7(3-OMe)), 59.6, 59.2, 58.8 (6(6-OMe)), 59.1, 58.6, 58.5, 58.5 (7(2-OMe)), 53.9 (AcNHCH), 31.6 (CH_2SCO), 23.2 ($NHCOCH_3$); ***Protonated form-D$_2$O:*** ^{1}H NMR (400 MHz, D_2O): δ 7.60 (d, 2H, ^3J=8.3 Hz, H_c, H_d), 7.59 (d, 1H, J_{Ha-Hb} =15.0 Hz, H_b), 6.91 (d, 2H, ^3J=8.3 Hz, H_e, H_f), 6.58 (bd, 1H, J_{Ha-Hb}=15.0 Hz, H_a), 5.28-5.11 (m, 7H, 7H$_1^{I-VII}$), 4.52-4.43 (m, 1H, AcNHC*H*), 4.17-3.07 (m, 44H, 7H$_5^{I-VII}$, 7H$_6^{I-VII}$, 7H$_4^{I-VII}$, 7H$_{6'}^{I-VII}$, 7H$_3^{I-VII}$, CH_2S, 7H$_2^{I-VII}$), 3.57, 3.56, 3.53, 3.51 (4×s, 21H, 7(3-OMe)), 3.50, 3.48, 3.47, 3.45 (4×s, 21H, 7(2-OMe)), 3.42, 3.40, 3.38, 3.38 (4×s, 18H, 6(6-OMe)), 2.08 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, D_2O): δ 187.8 (CH_2SCO), 174.3 ($NHCOCH_3$), 173.6 (CH_2NHCO), 159.7 (ArC_q phenol), 142.6 (C_b), 130.9 (C_c, C_d), 125.4 (ArC_q), 120.9 (C_a), 116.5 (C_e, C_f), 99.3, 99.2, 99.0, 98.7, 98.6, 98.4, 97.6 (7C$_1^{I-VII}$), 81.6, 81.5, 81.4, 81.3, 81.0, 80.9, 80.8, 80.6, 80.5 (7C$_2^{I-VII}$, 7C$_3^{I-VII}$), 80.3, 80.2, 80.0, 79.9, 79.8, 79.6, 78.3 (7C$_4^{I-VII}$), 71.4, 71.2, 71.0, 70.9, 70.7 (7C$_6^{I-VII}$, 7C$_5^{I-VII}$), 61.3, 61.1, 60.9, 60.9, 60.8, 60.6, 60.5 (7(3-OMe)), 59.0, 59.0, 58.8, 58.8, 58.7, 58.6, 58.5, 58.3, 58.3, 58.2 (6(6-OMe), 7(2-OMe)), 54.3 (AcNHCH), 29.2 (CH_2SCO), 22.2 ($NHCOCH_3$); ***Deprotonated form:*** ^{1}H NMR (400 MHz, D_2O, pH=10.1): δ 7.60 (d, 1H, J_{Ha-Hb}=15.2 Hz, H_b), 7.53 (d, 2H, ^3J=8.0 Hz, H_c, H_d), 6.61 (d, 2H, ^3J=8.0 Hz, H_e, H_f), 6.37 (bd, 1H, J_{Ha-Hb}=15.2 Hz, H_a), 5.30-5.12 (m, 7H, 7H$_1^{I-VII}$), 4.51-4.43 (m, 1H, AcNHC*H*), 4.13-3.15 (m, 44H, 7H$_5^{I-VII}$, 7H$_6^{I-VII}$, 7H$_4^{I-VII}$, 7H$_{6'}^{I-VII}$, 7H$_3^{I-VII}$,CH_2S, 7H$_2^{I-VII}$), 3.60, 3.58, 3.56, 3.50 (4×s, 21H, 7(3-OMe)), 3.49, 3.48, 3.47 (3×s, 21H, 7(2-OMe)), 3.43, 3.42, 3.39, 3.38 (4×s, 18H, 6(6-OMe)), 2.09 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, D_2O, pH=10.1): δ 188.0 (CH_2SCO), 174.6 ($NHCOCH_3$), 173.8 (CH_2NHCO), 172.3 (ArC_q phenolate), 145.2 (C_b), 131.9 (C_c, C_d), 120.1 (C_e, C_f), 119.4 (ArC_q), 115.7 (C_a), 99.8, 99.3, 99.1, 98.8, 98.6, 97.7 (7C$_1^{I-VII}$), 81.7, 81.5, 81.3, 81.1, 81.1, 80.8, 80.5, 80.4, 80.4, 80.1, 79.9, 79.7, 79.6, 79.4, 79.3, 79.2 (7C$_3^{I-VII}$, 7C$_2^{I-VII}$, 7C$_4^{I-VII}$), 71.7, 71.6, 71.4, 71.2, 71.0, 70.9, 70.7 (7C$_6^{I-VII}$, 7C$_5^{I-VII}$), 61.3, 61.1, 60.9, 60.8, 60.6, 60.5 (7(3-OMe)), 59.0, 59.0, 58.8, 58.8, 58.7, 58.6, 58.6, 58.3, 58.3, 58.2 (6(6-OMe), 7(2-OMe)), 54.7 (AcNHCH), 28.8 (CH_2SCO), 21.9 ($NHCOCH_3$); LRMS (FAB$^+$): *m/z* [M+Na]$^+$ = 1727.8; HRMS (FAB$^+$): *m/z* [M+Na]$^+$ calculated for $C_{76}H_{124}N_2O_{38}SNa$ 1727.7451, observed 1727.7384.

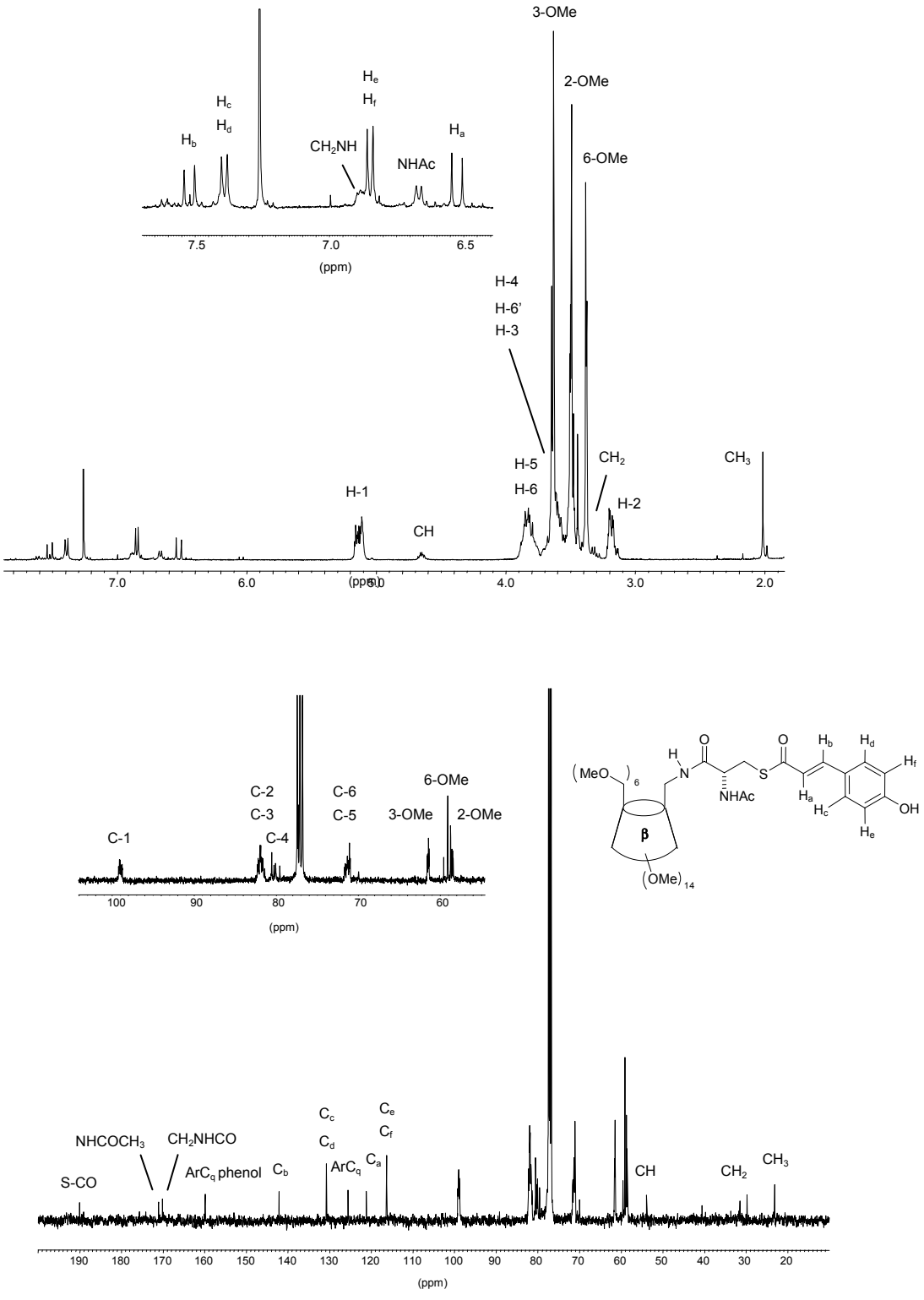

Figure 7: ^{1}H NMR and ^{13}C NMR spectra (400 MHz and 100 MHz, $CDCl_3$) of compound (**7**) [CD-PYP1 - protonated form]

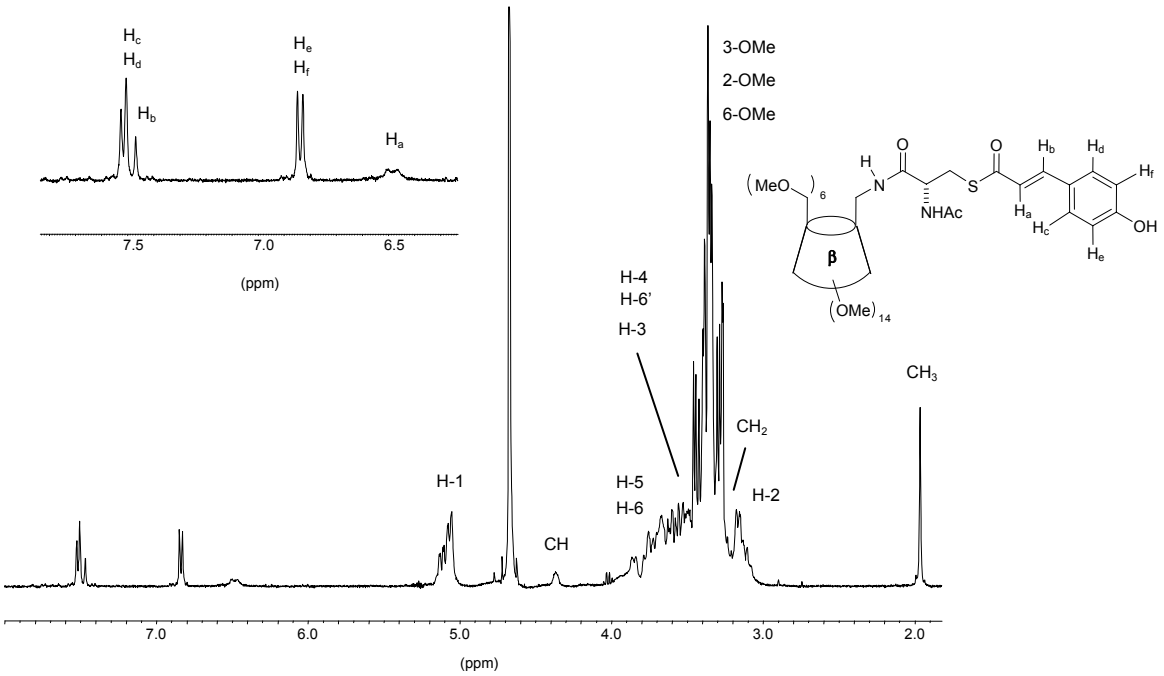

Figure 8: ^{1}H NMR spectrum (400 MHz, D_2O) of compound (**7**) [CD-PYP1 - protonated form]

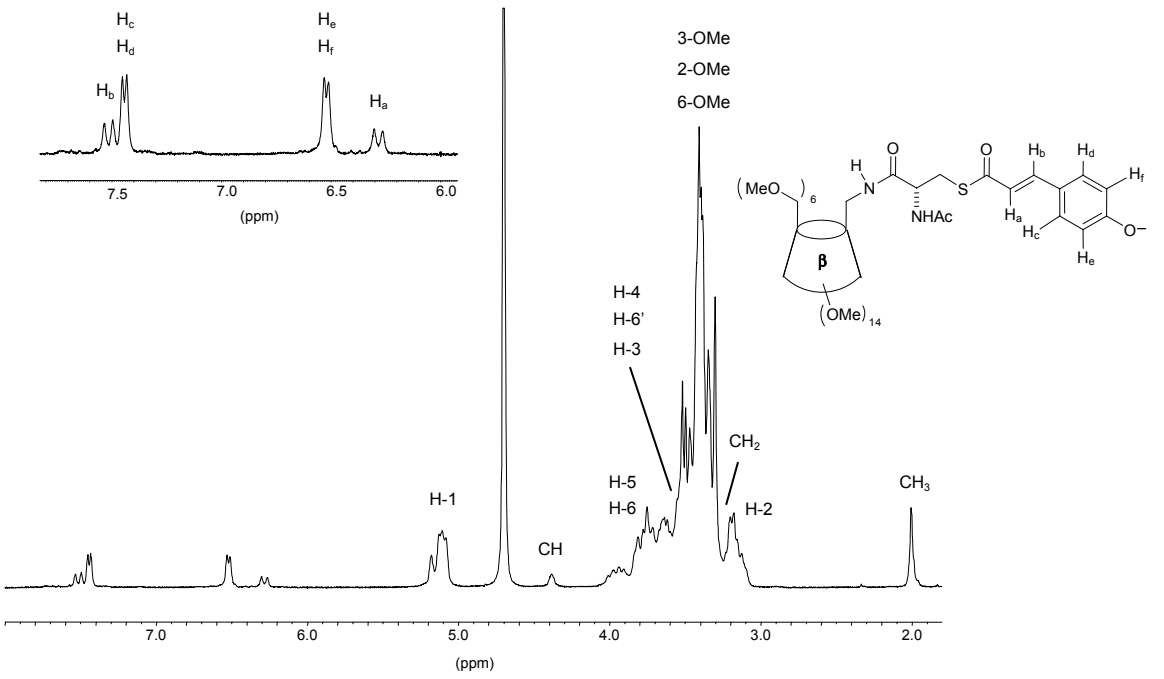

Figure 9: ^{1}H NMR spectrum (400 MHz, D_2O/ pH=10.1) of compound (**7**) [CD-PYP1 - deprotonated form]

2. Chromophore inclusion studies of protonated CD-PYP1

2.1. 2D ROESY NMR spectra of protonated form

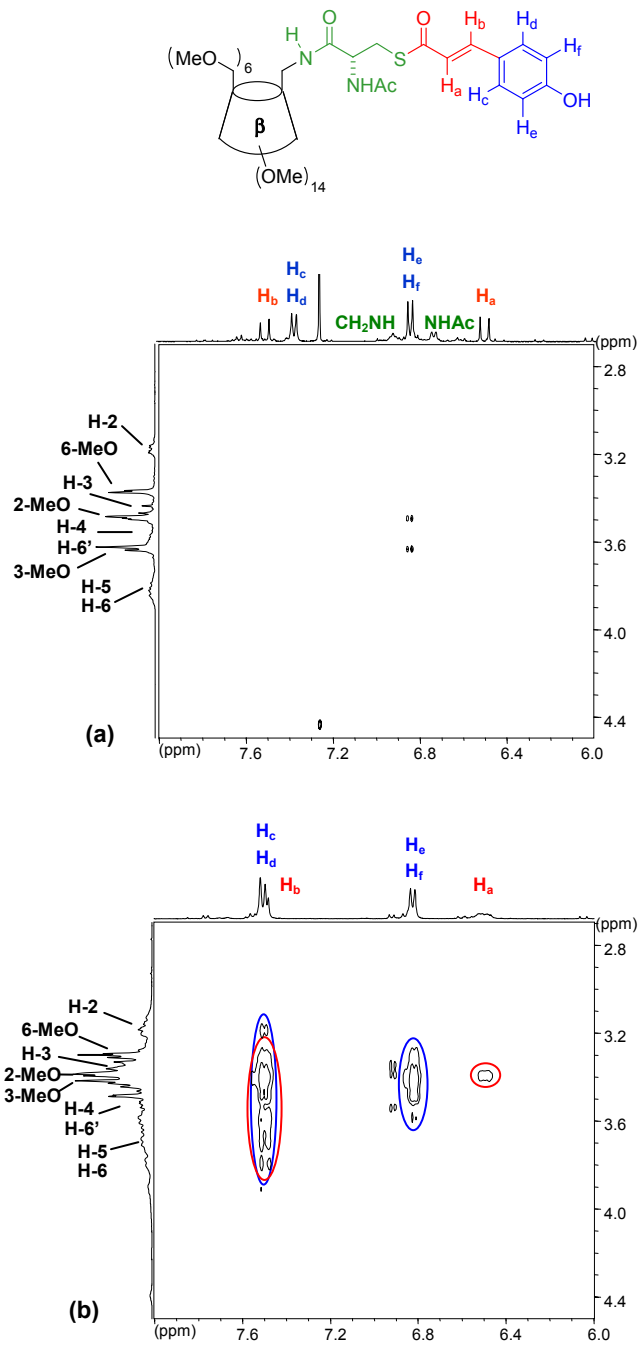

Figure 10: Partial contour plots of 2D ROESY NMR spectra (400 MHz, *T* 25 °C, mixing time: 200 ms) of protonated CD-PYP1 (**7**) (*c* 10 mM) in: **(a)** $CDCl_3$, **(b)** D_2O at pH 7.

2.2. Concentration dependence of protonated form

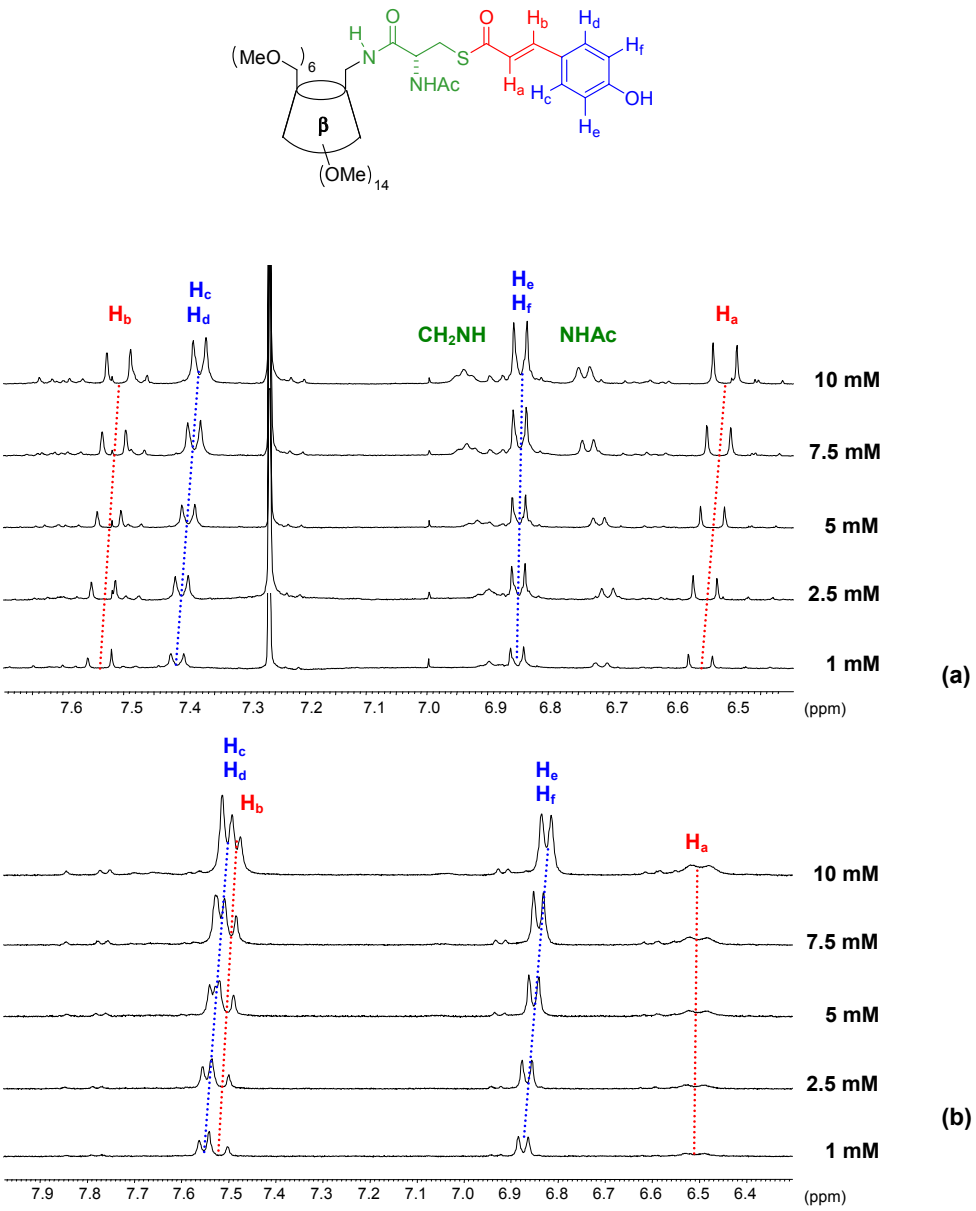

Figure 11: Concentration dependence (*c* 1-10 mM) of the aromatic and vinylic ^{1}H NMR (400 MHz) chemical shifts of protonated CD-PYP1 (**7**) in: **(a)** $CDCl_3$, **(b)** D_2O at pH 7.

3. Host-guest competition studies of protonated CD-PYP1

3.1. ^{1}H NMR spectra of protonated form with 1-adamantanol

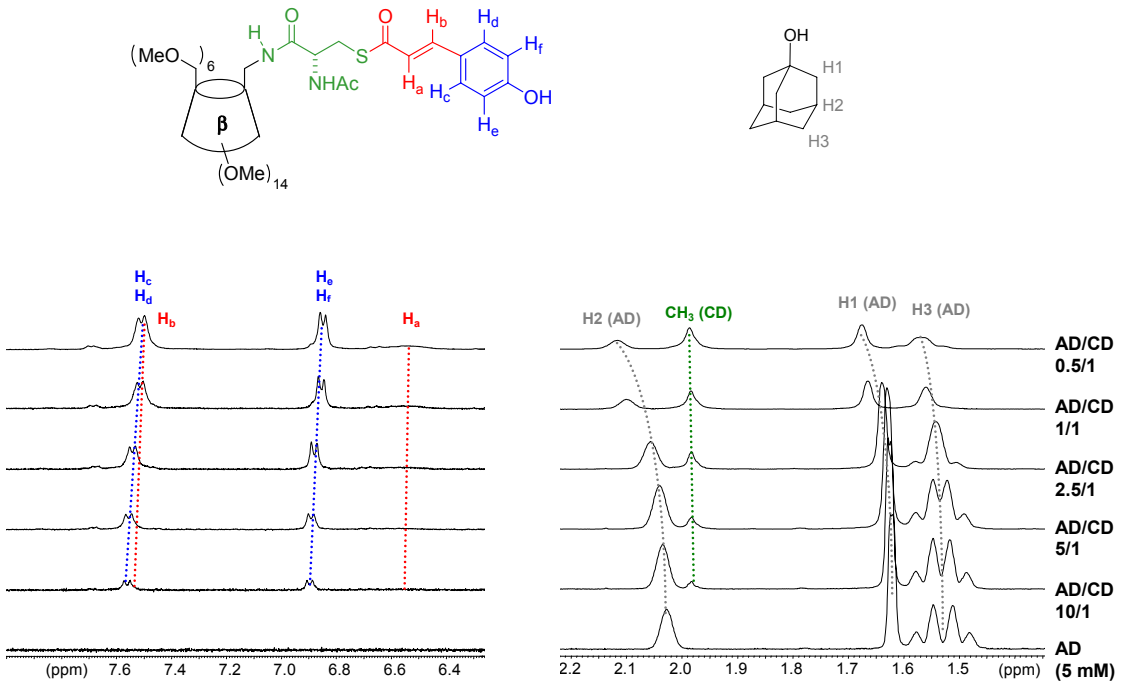

Figure 12: ^{1}H NMR (400 MHz) spectra upon addition of protonated CD-PYP1 (**7**) (*c* 5×10^{-2} M) to competitive guest 1-adamantanol (AD) (*c* 5 mM) in D_2O at pH 7.

3.2. 2D ROESY NMR spectrum of protonated form with 1-adamantanol

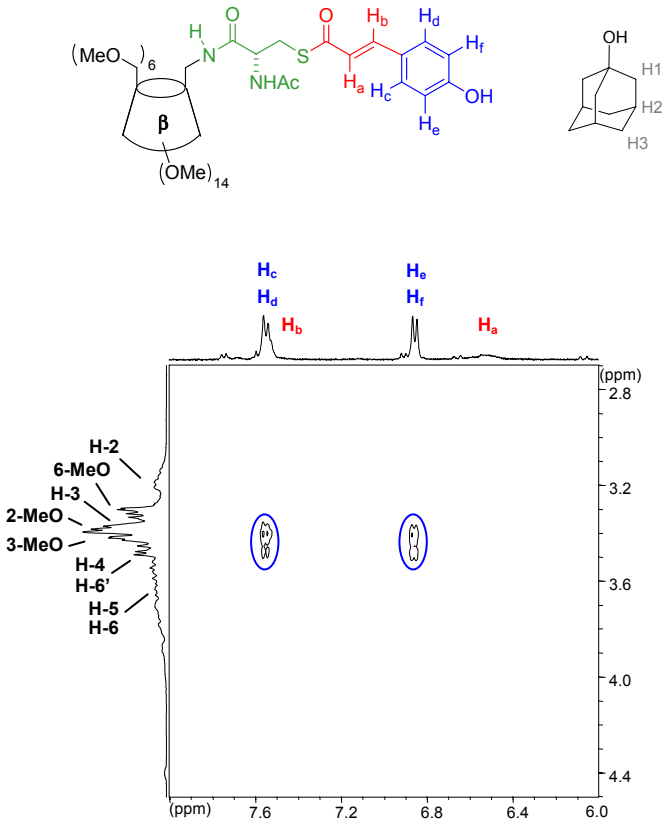

Figure 13: Partial contour plots of 2D ROESY NMR spectrum (400 MHz, *T* 25 °C, mixing time: 200 ms) of 1:1 solution of 1-adamantanol and protonated CD-PYP1 (**7**) in D_2O at pH 7.

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