Dendritic upconverting nanoparticles enable *in vivo* multiphoton microscopy with low power continuous wave sources

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SUPPORTING INFORMATION

Appendix

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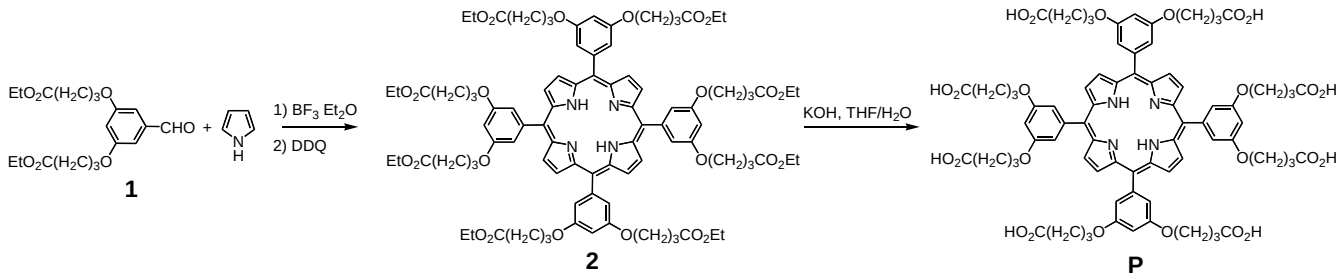
I. Synthesis of dendrimers

A) Dendrimer cores

1) Core **P**

Scheme 1



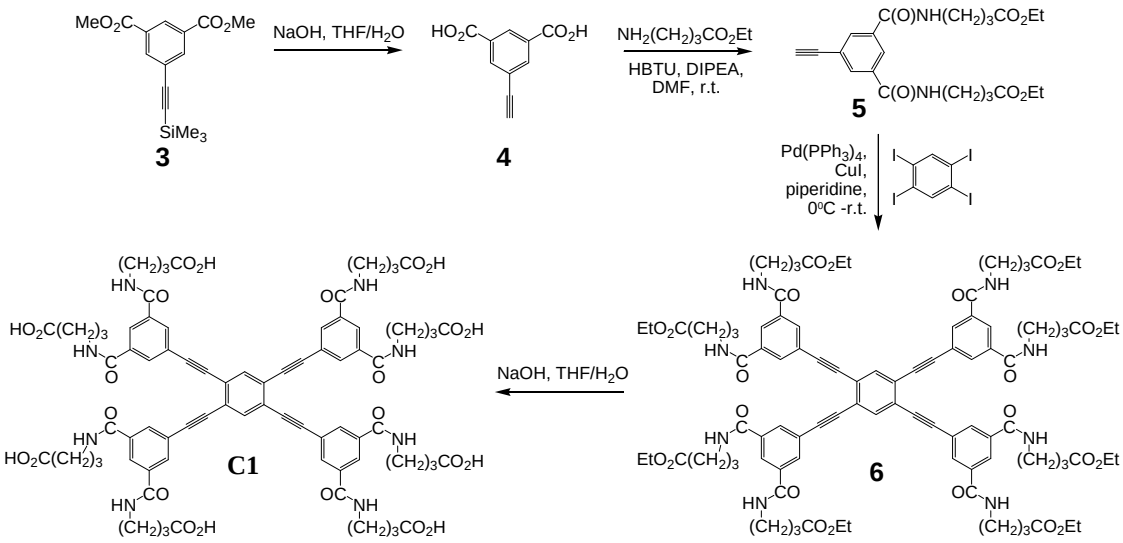
***tetra-meso*-[3,5-Bis(3-carboxypropyloxy)phenyl]-porphyrin (P).** To a solution of 3,5-bis(3-ethoxycarbonylpropyloxy)benzaldehyde (**1**) (1) (0.366 g, 1 mmol) and pyrrole (0.07 ml, 1 mmol) in CH_2Cl_2 (100 ml) $BF_3{\cdot}Et_2O$ (0.025 ml, 0.2 mmol) was added, and the reaction mixture was stirred in the dark under Ar for 2 h. DDQ (0.227 g, 1 mmol) was added, and the mixture stirred overnight at r.t. in the dark. The solvent was evaporated to dryness, and the product was purified by column chromatography (SiO_2, eluent CH_2Cl_2/THF, 20:1) to give porphyrin octaethyl ester **2**. Yield: 90 mg, 22%.

Octaethyl ester **2** (90 mg, 0.054 mmol) was dissolved in THF (15 ml), KOH (0.152 g, 2.7 mmol) and H_2O (0.15 ml) were added, and the reaction mixture was stirred overnight at r.t. The formed precipitate was isolated by centrifugation, dissolved in aq. KOH (0.1 M, 20 ml) and stirred for additional 2 h at r. t. The solution was cooled to 0°C on an ice bath, acidified with 10% HCl to pH 4-5. The formed precipitate was isolated by centrifugation, washed with water 3 times and dried in vacuum, giving the target product **P** as a green powder. Yield: 0.065 g (83%). MALDI-TOF (m/z): calc. for $C_{76}H_{78}N_4O_{24}$: 1430.5, found 1431.8 $[M+H]^+$. NMR ^1H (DMSO-d_6), δ, ppm: 2.14 (16H, dddd, $^3J_1$=$^3J_2$=6.7 $^3J_3$=$^3J_4$=6.5 Hz, -CH_2-), 2.53 (16H, t, ^3J=7.1 Hz, -$CH_2C(O)$-), 4.34 (16H, t, broad, ^3J=5.8 Hz, -OCH_2-), 7.22 (4H, s, Ar) 7.86 (8H, s, Pyr), 8.85 (8H, s, Ar).

2) Core **C1**

Dimethyl-5-(trimethylsilylethynyl)isophthaloate (**3**) was synthesized as described previously (2).

Scheme 2



5-Ethynylisophthalic acid (4). 5-Ethynylisophthalic acid was prepared in one step by reacting dimethyl-5-(trimethylsilylethynyl)isophthaloate (**3**) (0.7 g, 3.2 mmol) with NaOH (1.412 g, 35.3 mmol) in THF (20 ml) at r. t. for ~4 h. The precipitate formed was centrifuged down, dissolved in aq. NaOH (0.1 M, 50 ml) and stirred for 2 h at room temperature. The reaction mixture was cooled to 0°C on an ice bath, acidified with 10% HCl to pH~1-2. The precipitate formed was isolated by centrifugation, washed twice with water and dried, giving the target compound as a powder. Yield: 0.47 g, 77%. The analytical data match those reported previously.(3)

Diethyl 4,4'-[(5-ethynyl-1,3-phenylene)bis(carbonylimino)]dibutanoate (5). To a solution of 5-ethynylisophthalic acid (**4**) (0.37 g, 1.95 mmol) in dry DMF (20 ml), HBTU (2 g, 5.28 mmol) was added in one portion. After stirring for 10 min at r. t., the mixture of ethyl 4-aminobutyrate hydrochloride (1 g, 5.97 mmol) and DIPEA (2 ml, 11.5 mmol) in dry DMF (10 ml) was added, and the reaction mixture was stirred overnight at r. t. The reaction mixture was poured into ice-cold water (200 ml), and a few drops of conc. HCl were added. The formed precipitate was centrifuged, washed 3 times with water and dried in vacuum. The product was purified by column chromatography (SiO_2, eluent CH_2Cl_2/THF, gradient from 100:0 to 80:20) to yield the target compound as a yellowish solid. Yield: 0.6 g, 74%. NMR ^{1}H (DMSO-d_6), δ, ppm: 1.16 (6H, t, ^3J=7.0 Hz, -OCH$_2$C<u>H</u>$_3$), 1.77 (4H, dddd, $^3J_1$=$^3J_2$=7.0, $^3J_3$=$^3J_4$=7.3 Hz, -C<u>H</u>$_2$-), 2.35 (4H, t,

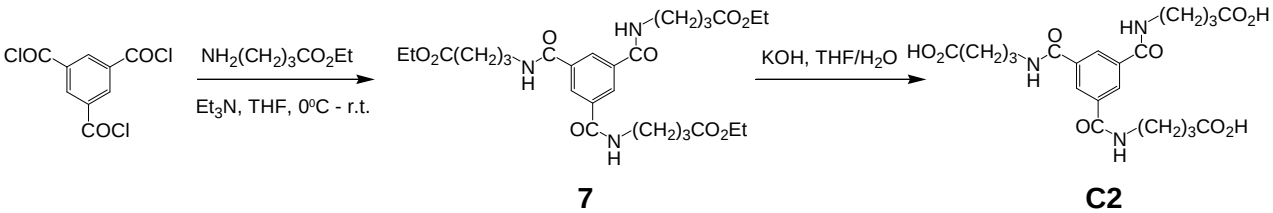
^3J=7.3 Hz, -C$\underline{H}_2$C(O)-), 3.24-3.31 (4H, m, broad, -NHC$\underline{H}_2$-), 4.03 (4H, q, ^3J=7.0 Hz, -OC$\underline{H}_2$CH$_3$), 4.37 (1H, s, -C≡$\underline{H}$), 8.05 (2H, d, ^4J=1.7 Hz, Ar), 8.30 (1H, t, ^4J=1.7 Hz, Ar), 8.60 (2H, t, ^3J=5.5 Hz, -N$\underline{H}$-).

4,4',4'',4''',4'''',4''''',4'''''',4'''''''((4-tetrayltetrakis[ethyn-1,2-diylbenzene-5,1,3-triylbis(carbonylimino)]))octabutanoic acid (C1). 1,2,4,5-Tetraiodobenzene (0.165 g, 0.284 mmol) was dissolved in piperidine (3 ml), and the solution was bubbled with Ar under stirring over 30 min period. The catalyst, Pd(PPh$_3$)$_4$ (0.131 g, 0.114 mmol, 40 mol%), was added, and the reaction mixture was bubbled with Ar for additional 15 min. **5** (0.568 g, 1.36 mmol) was dissolved in piperidine (10 ml) under Ar, and the solution was cooled to 0°C on an ice bath. The mixture of tetraiodobenzene and the catalyst was rapidly added to the solution of **5**, followed by addition of CuI (0.011 g, 0.057 mmol, 20 mol%). The reaction mixture was stirred at r. t. overnight under Ar, after which it was quenched with saturated aqueous solution of NH$_4$Cl (30 ml). The products were extracted with CH$_2$Cl$_2$ (3×30 ml), and the combined organic layers were dried over Na$_2$SO$_4$. The solvent was evaporated, and the product was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$ then CH$_2$Cl$_2$/THF, 3:1, and finally, CH$_2$Cl$_2$/MeOH, 30:1) to give **6** as a light-yellow solid. Yield: 0.150 g (30%).

6 (0.15 g, 0.086 mmol) was dissolved in THF (15 ml), NaOH (0.275 g, 6.9 mmol) and water (0.15 ml) were added, and the reaction mixture was stirred overnight at r. t. The formed precipitate was isolated by centrifugation, dissolved in aq. NaOH (0.1 M, 20 ml) and stirred for additional 2 h at r. t. The solution was cooled to 0°C on an ice bath, acidified with 10% aq. HCl to pH 4-5. The precipitate formed was collected by centrifugation and washed with water 3 times, yielding bright yellow powder. Yield: 0.12 g, 92%. MALDI-TOF (m/z): calc. for C$_{78}$H$_{78}$N$_8$O$_{24}$: 1510.5, found 1533.7 [M+Na]$^+$. NMR ^{1}H (DMSO-d$_6$), δ, ppm: 1.73 (16H, dddd, $^3J_1$=$^3J_2$=7.0, $^3J_3$=$^3J_4$=7.3 Hz, -CH$_2$-), 2.26 (16H, t, ^3J=7.3 Hz, -CH$_2$C(O)-), 3.22-3.31 (16H, m, broad, -NHCH$_2$-), 4.03 (16H, q, ^3J=7.0 Hz, -OCH$_2$CH$_3$), 8.09 (2H, s, Ar), 8.15-8.19 (8H, d, ^4J=1.5 Hz, Ar), 8.32-8.36 (4H, t, ^4J=1.5 Hz, Ar), 8.67 (2H, t, ^3J=5.5 Hz, -NH-), 12.06 (8H, broad, -C(O)O$\underline{H}$).

3) Core **C2**

Scheme 3



7 **C2**

Triethyl 4,4',4''-[benzene-1,3,5-triyltris(carbonylimino)]tributanoate (7). To a solution of ethyl 4-aminobutyrate hydrochloride (1.983 g, 11.3 mmol) in THF (40 ml), a mixture of 1,3,5-benzenetricarbonyl trichloride (1.0 g, 3.8 mmol) and triethylamine (3.5 ml, 25 mmol) was added dropwise at 0°C. The reaction mixture was stirred at r. t. overnight, then poured into water (120 ml), extracted with Et_2O (4×40 ml) and dried over Na_2SO_4. The solvent was evaporated, and the product was purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc/MeOH, 45:45:10) to give the title compound as a white solid. Yield: 1.65 g, 79%. MALDI-TOF (m/z): calc. for $C_{27}H_{39}N_3O_9$: 549.6, found 550.5 $[M+H]^+$, 572.5 $[M+Na]^+$, 588.5 $[M+K]^+$. NMR ^{1}H (DMSO-d_6), δ, ppm: 1.16 (9H, t, ^3J=7.0, Hz, -OCH$_2$C<u>H</u>$_3$), 1.79 (6H, dddd, $^3J_1$=$^3J_2$=$^3J_3$=$^3J_4$=7.3 Hz, -C<u>H</u>$_2$-), 2.35 (6H, t, ^3J=7.3 Hz, -C<u>H</u>$_2$C(O)-), 3.27-3.33 (6H, m, broad, -NHC<u>H</u>$_2$-), 4.04 (6H, q, ^3J=7.0 Hz, -OC<u>H</u>$_2$CH$_3$), 8.37 (3H, s, Ar), 8.71 (3H, t, ^3J=5.5 Hz, -N<u>H</u>-).

4,4',4''-[Benzene-1,3,5-triyltris(carbonylimino)]tributanoic acid (C2). **7** (1.5 g, 2.73 mmol) was dissolved in THF (50 ml). KOH (2.3 g, 41 mmol) and water (0.5 ml) were added, and the mixture was stirred overnight at r. t. The formed precipitate was isolated by centrifugation, dissolved in KOH aq. (0.1M, 50 ml) and stirred for additional 2 h at r. t. The reaction mixture was cooled to 0°C on an ice bath, acidified with 10% HCl to pH 4-5. The formed precipitate was collected by centrifugation, washed with water 3 times and dried in vacuum. Yield: 0.6 g, 50%. The obtained compound was used in the following coupling reactions without further purification.

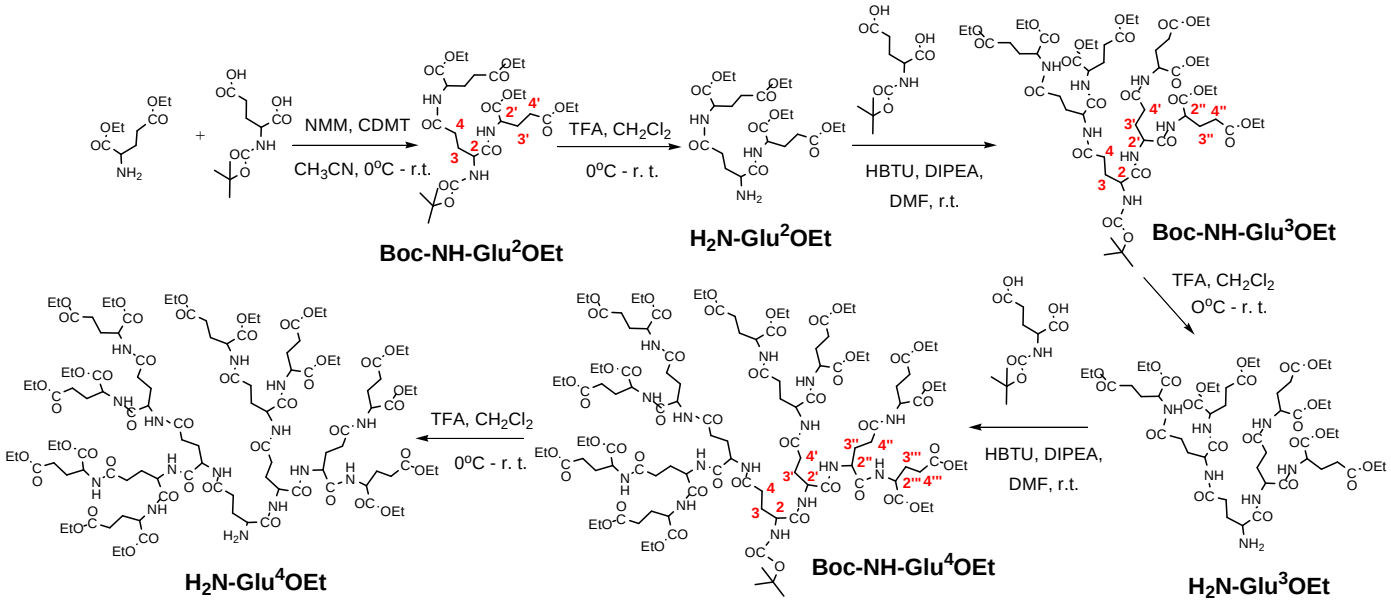
<u>B) Polyglutamic dendrons (Scheme 4)</u>

In Scheme 4 and description below, dendrons and dendrimers are designated using abbreviations developed previously.(5, 6) The convergent synthesis of polyglutamic dendrons generally followed procedures developed previously.(5, 7, 8)

Boc-HN-Glu^{2}OEt. To a mixture of N-(*tert*-butoxycarbonyl)-L-glutamic acid (6 g, 24.3 mmol), L-glutamic acid diethyl ester hydrochloride (11.64 g, 48.5 mmol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), (9.38 g, 53.4 mmol) in dry acetonitrile (420 ml), kept on an ice-cold bath, N-methylmorpholine (NMM), (10.67 ml, 97 mmol) was added drop-wise over 10 min period. The mixture was stirred overnight at r. t. The obtained mixture was concentrated in vacuum to ~80 ml, 300 ml of water was added, and the resulting mixture was stirred for 2-3 h at r. t. until a white precipitate formed. The precipitate was collected by filtration, washed with water (4×100 ml) and dried in vacuum to give pure title compound. Yield: 11.6 g, 77%. NMR ^{1}H (DMSO-d_6, 80°C), δ, ppm: 1.15-1.20 (12H, m, -C(O)OCH$_2$C<u>H</u>$_3$), 1.38 (9H, s, -C(O)OC(C<u>H</u>$_3$)$_3$), 1.66-1.77 (1H, m, (3)-C<u>H</u>$_2$-), 1.79-1.93 (3H, m, (3,3',3')-C<u>H</u>$_2$-), 1.93-2.07 (2H, m, (3',3')-

C$\underline{H}_2$-), 2.14-2.23 (2H, m, (4)-C$\underline{H}_2$-), 2.30-2.39 (4H, m, (4',4')-C$\underline{H}_2$-), 3.88-3.97 (1H, m, (2)-C$\underline{H}$(NH)-), 4.01-4.12 (8H, m, -C(O)OC$\underline{H}_2$CH$_3$), 4.21-4.33 (2H, m, (2',2')-C$\underline{H}$(NH)-), 6.60-6.70 (1H, m, broad, -N$\underline{H}$-), 7.96-8.04 (2H, m, -N$\underline{H}$-).

Scheme 4



H_2N-Glu^{2}OEt. Boc-N-Glu^{2}OEt (10 g, 16.2 mmol) was dissolved in CH_2Cl_2 (50 ml). The solution was cooled to 0°C on an ice bath, trifluoroacetic acid (50 ml) was added slowly, and the mixture was allowed to react overnight under stirring, after which the solution was concentrated and dried under vacuum. The residue was dissolved in CH_2Cl_2 (350 ml), washed with water (250 ml), then with aq. $NaHCO_3$ (10%, 2×200 ml), finally with brine (250 ml) and dried over Na_2SO_4. The solvent was evaporated to give the title compound as a light-yellow solid (6.95 g, 83%). H_2N-Glu^{2}OEt was used in the following coupling reactions without further purification.

Boc-NH-Glu^{3}OEt. To a solution of N-(*tert*-butoxycarbonyl)-L-glutamic acid (0.86 g, 3.48 mmol) in dry DMF (20 ml), HBTU (2.9 g, 7.66 mmol) was added, and the mixture was stirred for 10 min at r. t. A solution of H_2N-Glu^{2}OEt (3.6 g, 7.0 mmol) in dry DMF (25 ml) was rapidly added to the mixture, followed by addition of DIPEA (2.5 ml, 13.9 mmol). The reaction mixture was stirred overnight at r. t. and poured into water (250 ml). The white precipitate formed was collected by centrifugation, washed with water (2×100 ml) and dried in vacuum to the title compound as a white solid. Yield: 4.2 g, 97%. MALDI-TOF (m/z): calc. for $C_{57}H_{93}N_7O_{23}$: 1244.37, found 1268.159 $[M+Na]^+$. NMR ^{1}H (DMSO-d$_6$, 80°C), δ, ppm: 1.14-1.20 (24H, m, -C(O)OCH$_2$C$\underline{H}_3$), 1.37 (9H, s, -C(O)OC(C$\underline{H}_3$)$_3$), 1.69-2.05 (14H, m, (3,3',3'')-C$\underline{H}_2$-), 2.14-

2.23 (6H, m, (4,4')-C$\underline{H}_2$-), 2.31-2.38 (8H, m, (4")-C$\underline{H}_2$-), 3.85-3.92 (1H, m, (2)-C$\underline{H}$(NH)-), 4.01-4.12 (16H, m, -C(O)OC$\underline{H}_2$CH$_3$), 4.22-4.34 (6H, m, (2',2")-C$\underline{H}$(NH)-), 6.66-6.82 (1H, m, broad, -N$\underline{H}$-), 7.74-7.86 (2H, m, -N$\underline{H}$-), 7.98-8.02 (2H, m, -N$\underline{H}$-), 8.15 (1H, d, ^3J=7.8 Hz, -N$\underline{H}$-), 8.19-8.25 (1H, m, broad -N$\underline{H}$-).

H_2N-Glu^{3}OEt. The synthesis of H_2N-Glu^{3}OEt from Boc-NH-Glu^{3}OEt (4.2 g, 3.38 mmol) followed the procedure described above for Gen 2 dendron H_2N-Glu^{2}OEt. The title compound was isolated as a light-yellow solid. Yield: 3.79 g, 98%. It was used in the following coupling reactions without further purification.

Boc-NH-Glu^{4}OEt. To a solution of N-(*tert*-butoxycarbonyl)-L-glutamic acid (0.37 g, 1.5 mmol) in dry DMF (10 ml), HBTU (1.26 g, 3.3 mmol) was added, and the mixture stirred for 10 min at r. t. A solution of NH_2Glu^{3}OEt (3.79 g, 3.3 mmol) in dry DMF (25 ml) was rapidly added to the mixture, followed by addition of DIPEA (1.1 ml, 6.0 mmol). The reaction mixture was stirred overnight at r. t. and poured into water (150 ml). The white precipitate formed was collected by centrifugation, washed with water (2×80 ml) and dried in vacuum to give the title compound as a white solid. Yield: 2.9 g, 77%. MALDI-TOF (m/z): calc. for $C_{112}H_{179}N_{15}O_{48}$: 2503.7, found 2526.1 $[M+Na]^+$, 2404.1 $[M-Boc]^+$. NMR ^{1}H (DMSO-d_6, 80°C), δ, ppm: 1.13-1.21 (48H, m, -C(O)OCH$_2$C$\underline{H}_3$), 1.38 (9H, s, -C(O)OC(C$\underline{H}_3$)$_3$), 1.71-2.05 (30H, m, (3,3',3",3'")-C$\underline{H}_2$-), 2.15-2.25 (14H, m, (4,4',4")-C$\underline{H}_2$-), 2.31-2.39 (16H, m, (4'")-C$\underline{H}_2$-), 3.87-3.94 (1H, m, (2)-C$\underline{H}$(NH)-), 4.02-4.13 (32H, m, -C(O)OC$\underline{H}_2$CH$_3$), 4.21-4.34 (14H, m, (2',2",2'")-C$\underline{H}$(NH)-), 6.58-6.66 (1H, m, broad, -N$\underline{H}$-), 7.64-7.78 (4H, m, -N$\underline{H}$-), 7.86-7.94 (5H, m, -N$\underline{H}$-), 8.04 (1H, d, ^3J=7.8 Hz, -N$\underline{H}$-), 8.06-8.10 (4H, m, broad -N$\underline{H}$-).

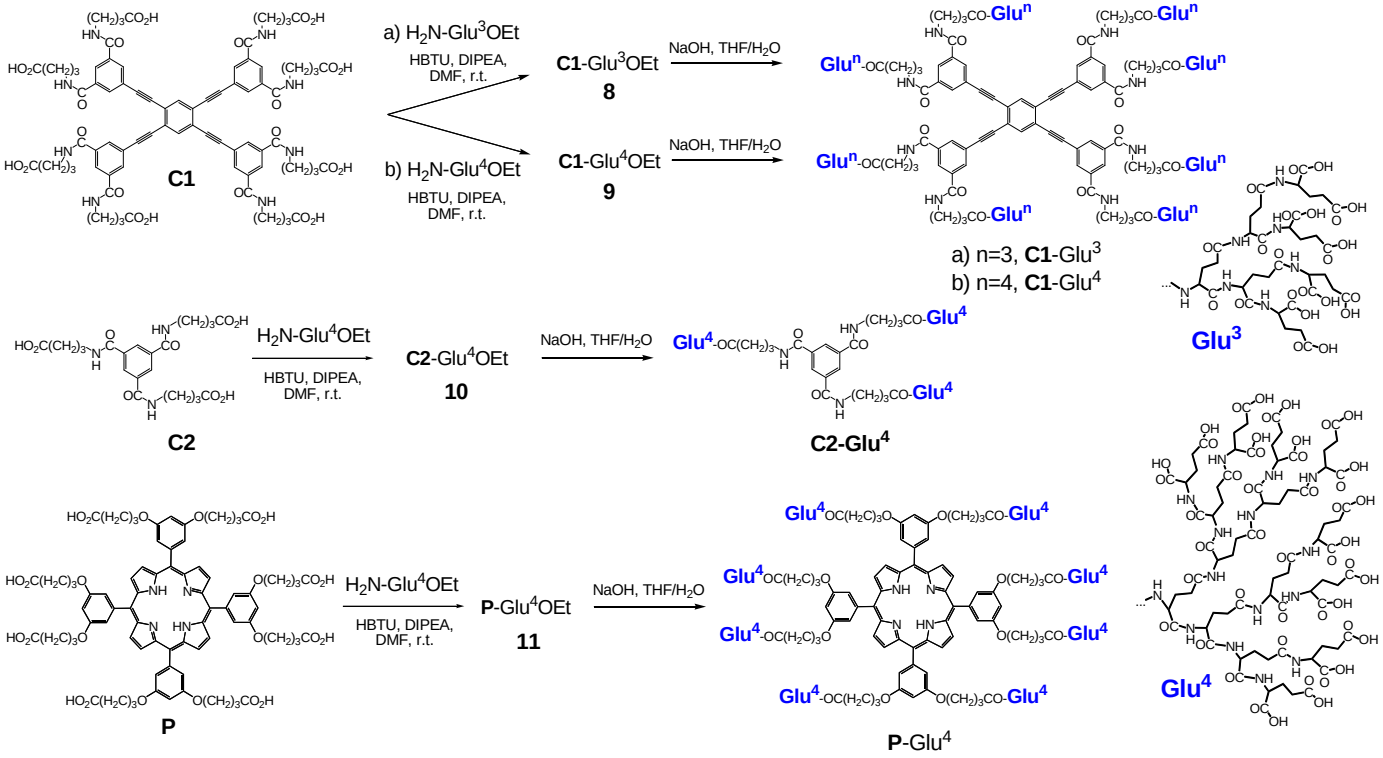
H_2N-Glu^{4}OEt. The synthesis of H_2N-Glu^{4}OEt from Boc-NH-Glu^{4}OEt (2.9 g, 1.16 mmol) followed the procedure described for the Gen 2 and Gen 3 dendrons above. The title compound was isolated as a light-yellow solid. Yield: 2.4 g, 86%. H_2N-Glu^{4}OEt was used in the following coupling reactions without further purification.

C) Dendrimers (Scheme 5)

C1-Glu^{3}OEt (8). HBTU (0.082 g, 0.22 mmol) was added to a solution of **C1** (0.0325 g, 0.022 mmol) in dry DMF (5 ml), and the resulted mixture was stirred for 10 min at r. t. A solution of H_2N-Glu^{3}OEt (0.1254 g, 0.175 mmol, 8.1 eq) in dry DMF (10 ml) was rapidly added to the mixture, followed by addition of DIPEA (0.15 ml, 0.86 mmol). The mixture was stirred at r. t. for 48 h, while monitored by MALDI-TOF analysis. After the reaction completion, the mixture was poured into water (150 ml). The while precipitate formed was collected by centrifugation, washed with water (3×100 ml) and dried in

vacuum to give the title compound as a light-yellow solid (0.174 g, 87%). Yield: 0.174 g, 87%. MALDI-TOF (m/z): calc. for $C_{486}H_{726}N_{64}O_{192}$: 10526.0, found 10565.0 $[M+K]^+$. NMR ^1H (DMSO-d_6, 80°C), δ, ppm: 1.12-1.20 (192H, m, -C(O)OCH$_2$C$\underline{H}_3$), 1.69-2.05 (128H, m, -C$\underline{H}_2$-, (3,3',3")-C$\underline{H}_2$-), 2.15-2.25 (64H, m, -C$\underline{H}_2$C(O)-, (4,4')-C$\underline{H}_2$-), 2.29-2.38 (64H, m, (4")-C$\underline{H}_2$-), 3.23-3.32 (16H, m, broad, -NHC$\underline{H}_2$-), 3.99-4.11 (128H, m, -C(O)OC$\underline{H}_2$CH$_3$), 4.16-4.34 (56H, m, (2,2',2")-C$\underline{H}$(NH)-), 7.76-7.84 (16H, m, broad, -N$\underline{H}$-), 7.87-7.97 (24H, m, broad, -N$\underline{H}$-), 8.00 (2H, s, Ar), 8.06-8.12 (16H, m, broad, -N$\underline{H}$-), 8.12-8.15 (8H, m, broad, Ar), 8.31-8.36 (4H, m, broad, Ar), 8.40-8.49 (8H, m, broad Ar-N$\underline{H}$-).

Scheme 5



C1-Glu^3. **8** (0.15 g, 0.014 mmol) was dissolved in THF (50 ml). NaOH (0.37 g, 9.3 mmol) and water (0.2 ml) were added to the mixture, and it was let under stirring overnight at r. t. The precipitate formed was collected by centrifugation, re-dissolved in aq. NaOH (0.1 M, 15 ml) and stirred for additional 6 h at r. t. The solution was cooled to 0°C on an ice bath, acidified with 10% HCl to pH 7, and the residual THF was removed under vacuum. The resulting solution was passed through a Millipore filter (0.45μm av. pore diameter), the pH was adjusted to 7.0, and the solution was purified by dialysis for 3 days (12 kDa cut-off membrane). The purified solution was lyophilized to give the title dendrimer (sodium salt) as a bright-yellow solid. Yield: 0.12 g, 83%.

C1-Glu^{4}OEt (9). To a solution of **C1** (0.045 g, 0.03 mmol) in dry DMF (5 ml), HBTU (0.113 g, 0.298 mmol) was added, and the resulted mixture was stirred for 10 min at r. t. A solution of H_2NGlu^{4}OEt (0.584 g, 0.24 mmol, 8.1 eq) in dry DMF (25 ml) was rapidly added to the mixture, followed by addition of DIPEA (0.21 ml, 1.19 mmol). The reaction mixture was stirred at r. t. for 3 days, while being monitored by MALDI-TOF analysis. After reaction completion, the mixture was poured into water (300 ml). The precipitate formed was collected by centrifugation, washed with water (3×100 ml), and dried in vacuum to give the title compound as light-yellow solid. Yield: 0.537 g, 87%. MALDI-TOF (m/z): calc. for $C_{934}H_{1430}N_{128}O_{384}$: 20574.0, found range ~13500-~21000 with maxima at 18228.5 [**C1**-Glu^{4}OEt+K]$^+$, and 15912.1. NMR ^{1}H (DMSO-d_6, 80°C), δ, ppm: 1.12-1.20 (384H, m, -C(O)OCH$_2$C<u>H</u>$_3$), 1.68-2.06 (256H, m, -C<u>H</u>$_2$-, (3,3',3",3'")-C<u>H</u>$_2$-), 2.14-2.27 (128H, m, -C<u>H</u>$_2$C(O)-, (4,4',4")-C<u>H</u>$_2$-), 2.29-2.38 (128H, m, (4'")-C<u>H</u>$_2$-), 3.23-3.31 (16H, m, broad, -NHC<u>H</u>$_2$-), 4.00-4.12 (256H, m, -C(O)OC<u>H</u>$_2$CH$_3$), 4.17-4.34 (120H, m, (2,2',2",2'")-C<u>H</u>(NH)-), 7.71-8.19 (130H, broad, Ar, -N<u>H</u>-), 8.29-8.37 (4H, m, broad, Ar), 8.38-8.53 (8H, m, broad Ar-N<u>H</u>-).

C1-Glu^4. **9** (0.5 g, 0.024 mmol) was dissolved in THF (100 ml). NaOH (1.2 g, 30 mmol) and water (0.5 ml) were added to the solution, and the mixture was allowed to react overnight under stirring at r. t. The precipitate formed was collected by centrifugation, dissolved in aq. NaOH (0.1 M, 20 ml) and stirred for additional 6 h at r. t. The following workup and purification followed those for **C1**-Glu^3. **C1**-Glu^4 was isolated as a bright-yellow solid (sodium salt). Yield: 0.42 g, 87%.

C2-Glu^{4}OEt (10). To a solution of **C2** (0.03 g, 0.065 mmol) in dry DMF (2 ml), HBTU (0.082 g, 0.216 mmol) was added, and the reaction mixture was stirred for 10 min at r. t. A solution of H_2NGlu^{4}OEt (0.5 g, 0.208 mmol, 3.2 eq) in DMF (15 ml) was rapidly added to the mixture, followed by addition of DIPEA (0.2 ml, 1.25 mmol). The mixture was stirred for 48 h at r. t., monitored by MALDI-TOF analysis. After reaction completion, the mixture was poured into water (200 ml). The precipitate formed was collected by centrifugation, washed with water 3 times and dried in vacuum to give the title compound as a white solid. Yield: 0.428 g, 87%. MALDI-TOF (m/z): calc. for $C_{342}H_{534}N_{48}O_{114}$: 7614.0, found 7652.9 [M+K]$^+$. NMR ^{1}H (DMSO-d_6, 80°C), δ, ppm: 1.13-1.21 (144H, m, -C(O)OCH$_2$C<u>H</u>$_3$), 1.68-2.06 (96H, m, -C<u>H</u>$_2$-, (3,3',3",3'")-C<u>H</u>$_2$-), 2.13-2.30 (48H, m, -C<u>H</u>$_2$C(O)-, (4,4',4")-C<u>H</u>$_2$-), 2.30-2.39 (48H, m, (4'")-C<u>H</u>$_2$-), 3.24-3.36 (6H, m, broad, -NHC<u>H</u>$_2$-), 4.00-4.12 (96H, m, -C(O)OC<u>H</u>$_2$CH$_3$), 4.18-4.34 (45H, m, (2,2',2",2'")-C<u>H</u>(NH)-), 7.73-8.17 (45H, m, broad, -N<u>H</u>-), 8.37 (3H, s, Ar), 8.42-8.49 (3H, m, broad Ar-N<u>H</u>-).

C2-Glu^4. **10** (0.425 g, 0.056 mmol) was dissolved in THF (100 ml), NaOH (1.1 g, 27.5 mmol) and water (0.5 ml) were added to the solution, and it was left to react overnight under stirring at r. t. The precipitate formed was collected by centrifugation, dissolved in NaOH aq. (0.1 M, 20 ml) and stirred for

additional 6 h at r. t. The following work up and purification followed that for **C1**-Glu^3 and **C1**-Glu^4. The title dendrimer (sodium salt) was isolated as an off-white solid. Yield: 0.37 g, 91%.

P-Glu^{4}OEt (11). To a solution of **P** (0.0273 g, 0.019 mmol) in dry DMF (5 ml) HBTU (0.075 g, 0.19 mmol) was added, and the reaction mixture was stirred for 10 min at r. t. A solution of NH_2Glu^{4}OEt (0.3718 g, 0.155 mmol, 8.1 eq) in DMF (15 ml) was rapidly added to the mixture, followed by the addition of DIPEA (0.15 ml, 0.76 mmol). The mixture was stirred at r. t. for 3 days, while being monitored by MALDI-TOF analysis. After reaction completion, the mixture was poured into water (300 ml). The precipitate formed was collected by centrifugation, washed with water (3×100 ml), and dried in vacuum to give the title compound as green solid. Yield: 0.429 g, 87%. MALDI-TOF (m/z): calc. for $C_{932}H_{1430}N_{124}O_{384}$: 20494.0, found 20532.6 $[M+K]^+$, fragment ions: 19468.5, 18212, 17110, 15835.5, 14778. NMR ^{1}H (DMSO-d_6, 80°C), δ, ppm: 1.09-1.20 (384H, m, -C(O)OCH$_2$C<u>H</u>$_3$), 1.71-2.05 (256H, m, -C<u>H</u>$_2$-, (3,3',3",3'")-C<u>H</u>$_2$-), 2.15-2.25 (112H, m, (4,4',4")-C<u>H</u>$_2$-), 2.25-2.38 (144H, m, -C<u>H</u>$_2$C(O)-, (4'")-C<u>H</u>$_2$-), 3.97-4.11 (256H, m, -C(O)OC<u>H</u>$_2$CH$_3$), 4.15-4.34 (136H, m, Ar-O-C<u>H</u>$_2$-, (2,2',2",2'")-C<u>H</u>(NH)-), 6.82-7.09 (4H, broad, Ar), 7.71-8.23 (128H, broad, Ar, -N<u>H</u>-), 8.72-8.94 (8H, m, broad, Pyr).

P-Glu^4. **11** (0.4 g, 0.02 mmol) was dissolved in THF (100 ml), NaOH (1.0 g, 25.6 mmol) and water (0.5 ml) were added to the solution, and the mixture was allowed to react overnight under stirring at r. t. The precipitate formed was collected by centrifugation, dissolved in aq. NaOH (0.1M, 20 ml) and stirred for additional 6 h at r. t. The following work up and purification followed that for **C1**-Glu^3, **C1**-Glu^4 and **C2**-Glu^4. The title dendrimer (sodium salt) was isolated as a green solid. Yield: 0.305 g, 90%.

Synthesis of UCNP-dendrimers

In a typical procedure for modification of UCNP's with organic ligands (9), a solution of UCNP's (~20 mg/ml) pre-treated with $NOBF_4$ in DMF was added to a stirred aqueous solution of a dendrimer (or PAA) (~60 mg/ml), so that the total ratio (by mass) of UCNP/organic ligand was ~1:2. The mixture was stirred overnight at r. t., then centrifuged at 10,000 g for 1-3 h. To remove the excess of the ligand and traces of DMF, the obtained precipitate was re-dissolved (or re-dispersed) in distilled water and precipitated again by centrifugation. This procedure was repeated 3 times.

II. Calculations

To evaluate the geometries of the dendrimer cores, calculations were performed using the DFT method, as implemented in Gaussian 03 (Rev. D.01, Intel EM64T/AMD, Gaussian, Inc).(10) The structures of cores **C1'** (**C1** without aminobutyrate extension arms), *meso*-tetra-3,5-dimethoxyphenylporphyrin (**P'**) (a prototype of porphyrin **P**), and **C2** were optimized using B3LYP/6-31G(d) model chemistry, beginning with AM1-optimized structures. A structure of core **C1'**, in which the peripheral aryl rings are fixed at 90^{o} with respect to the central benzene ring, was also computed using the same model chemistry. Frequency calculations were ran on the equilibrium structures to confirm the stationary points.

To facilitate comparison between equilibrium and non-equilibrium (90^{o}) structures of **C1'** pure electronic energies were used. These were found to be:

C1' (equilibrium) - E=-2969.67360279 Ha

C1' (90^{o}) – E= -2969.66534351 Ha

Simulations involving dendritic macromolecules were performed using AMBER force field as implemented in HyperChem 7.0 (HyperCube Inc.). Water was modeled as a distance-dependent dielectric continuum (scale factor 4). Molecular dynamics simulations were ran typically at 1000^{o}C, a conformation was selected and the energy was minimized. 8-10 conformations were sampled for each scenario. The surface was modeled as a single-sheet LiF. Simulations at this level of course cannot reproduce the actual surface effects (e.g. surface long-range potential was not explicitly taken into account), but rather serve as an illustration of the geometrical considerations underpinning our design. Nevertheless, even at this level it is apparent that there are significant differences between the conformations that the molecules studied can adopt near surfaces.

Molecular cross-sections of the dendrimers were calculated by taking several measurements across their molecular skeletons, averaging them and finding the areas of the corresponding circles.

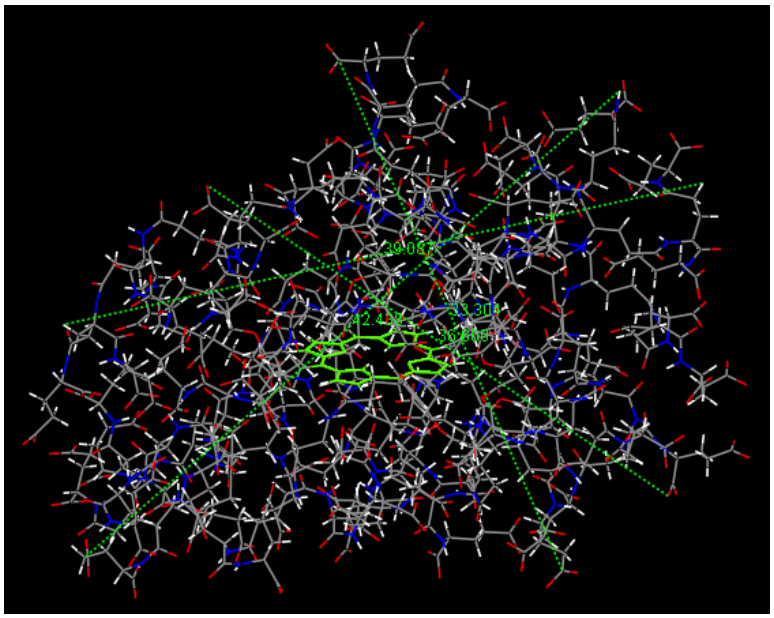


Figure S1. A structure of **P**-Glu[4]. The average molecular diameter, determined by taking several measurements across the molecular skeleton, is 3.9 nm.

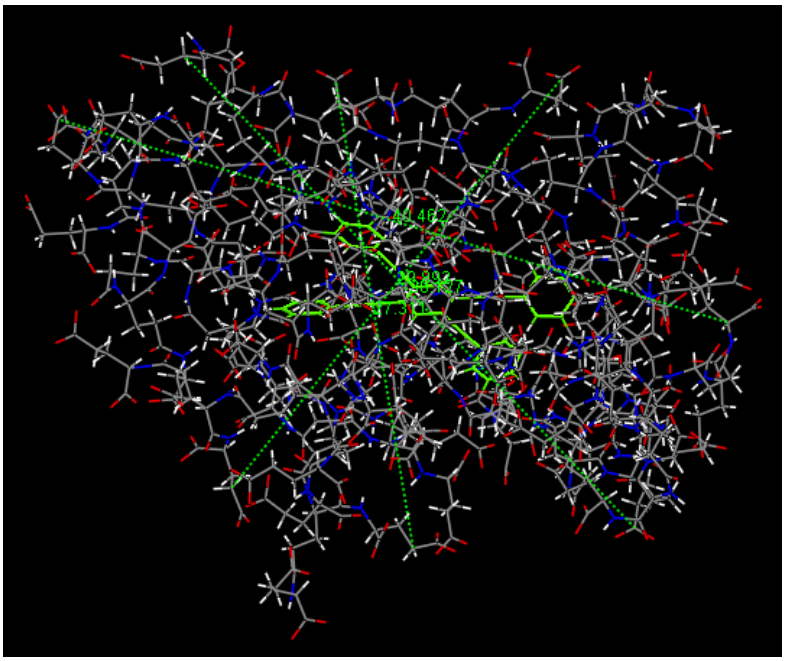


Figure S2. A structure of **C1**-Glu[4]. The average molecular diameter, determined by taking several measurements across the molecular skeleton, is 3.8 nm.

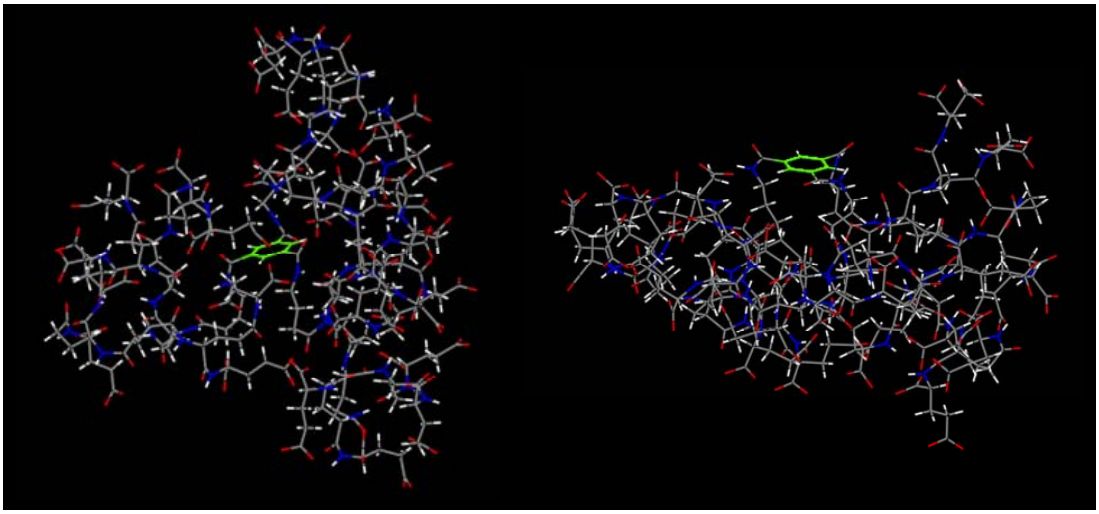


Figure S3. Two conformations of **C2**-Glu^4. The three flexible dendritic arms can easily adopt a conformation (right) where many of their termini occur "on the same side" of the molecule.

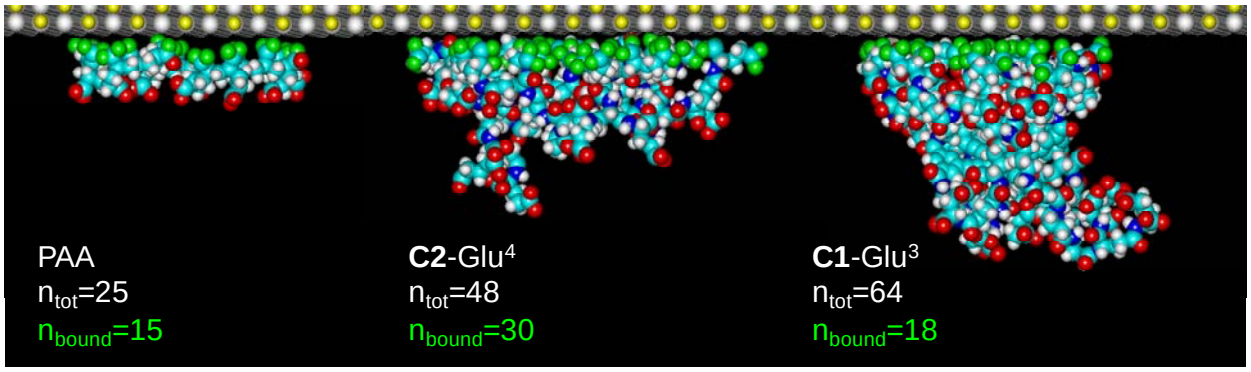


Figure 3a. Simulations of surface bound PAA and polyglutamic dendrimers. Fully ionized molecules of PAA (25 carboxylates), **C2**-Glu^4 and **C1**-Glu^3 were placed near a model surface (LiF, single sheet) and subjected to molecular dynamics simulations, followed by energy minimization (see S15 for details). Out of 25, 15 carboxylates (~60%) in PAA became engaged with the surface. (Bound carboxylates are shown in green). In the case of **C2**-Glu^4, 30 out of 48 carboxylates (~62%) turned to the surface; while only 18 out of 64 carboxylates (28%) of **C1**-Glu^3 adhered to the surface, leaving 46 carboxylates (72%) free in contact with solution.

III. Elemental analysis

The elemental analysis (C, H, N) by combustion was performed in the Micro-Analysis Inc. laboratory (Delaware, USA). UCNP-dendrimers and UCNP-PAA were obtained by reacting UCNP's with aqueous solutions of the organic ligands. The dendrimers were purified by dialysis and assumed to be retained in solutions as sodium salts, i.e. all carboxylate groups in the dendrimer are complexed with Na^+, i.e. CO_2Na. All samples were extensively dried in vacuum prior to the analysis.

Table 1. Results of elemental analysis.

Sample	%C	% H	%N
C2-Glu[4]	35.24	4.54	7.65
C1-Glu[4]	38.00	4.74	8.30
UCNP/**C2**-Glu[4]	4.66	0.64	0.74
UCNP/**C1**-Glu[4]	6.04	0.76	1.04
UCNP/PAA	4.04	0.44	0.00

Estimation of number of organic ligands (dendrimers and PAA) adhered to UCNP surface

Abbreviations:

m - mass of the ligand as a sodium salt.

Δm - mass of Na^+ ions in the sodium salt.

M - mass of UCNP.

k - number of ligand molecules per UCNP.

(The nanoparticle mass (*M*) was calculated assuming ideal spherical geometry, r=10 nm radius. The density was calculated for undoped $Na_{1.5}Y_{1.5}F_6$ hexagonal bipyramidal crystals: a=5.973, c=3.529 Å.(11) M=281.83 g/mol; ρ=4.5 g/cm^3. The mass of a single UCNP was found to be 18.84×10^{-18} g. The surface area of the sphere is 1256 nm^2.)

Using these abbreviations, the mass of UCNP modified with *k* organic ligands is: $k\times(m-\Delta m)+M$.

Here for simplicity we assume that upon interaction with the surface, all Na^+ ions are replaced by the ligand-UCNP contacts. The error introduced by this assumption is rather small, since the fractional mass of

Na^+ ions ($k\Delta m$) in the overall modified UCNP is not large (e.g. <2% error for UCNP/**C1**-Glu^4, even assuming all of its sodium atoms are retained).

Thus, the fractional mass of organic material in modified UCNP is expressed as:

$$f_{org}=k\times(m-\Delta m)/[k\times(m-\Delta m)+M] \quad (1)$$

The following calculations are concerned specifically with UCNP/**C1**-Glu^4 and UCNP/**C2**-Glu^4.

C1-Glu^4 sodium salt: $C_{678}H_{790}N_{128}O_{384}Na_{128}$, MW 19806
C2-Glu^4 sodium salt: $C_{246}H_{294}N_{48}O_{144}Na_{48}$, MW 7326

%Na (fractional mass) of Na atoms in these dendrimers (as sodium salts) can be expressed as: %Na=(23/14)×%N (the numbers of Na and N atoms are equal). Thus:

$$(m-\Delta m)=m\times(1-(23/14)\times\%N) \quad (2)$$

Assuming the number of C atoms in the dendrimer is x, %C in the dendrimer is found as:

$$\%C=12x/m, \text{ or } 12x=\%Cm. \quad (3)$$

In UCNP/dendrimers fraction mass of C atoms is found as:

$$\%C'=k\times12x/(k\times(m-\Delta m)+M), \text{ or}$$
$$\%C'=k\times\%Cm/[k\times(m-\Delta m)+M], \text{ and}$$
$$[k\times(m-\Delta m)+M]=(km\%C)/\%C'. \quad (4)$$

Substituting (2) and (4) into (1) we find that:

$$f_{org}=(1-(23/14)\times\%N)\times\%C'/\%C \quad (5)$$

UCNP/**C2**-Glu^4: f_{org}=11.6
UCNP/**C1**-Glu^4: f_{org}=13.7

Similar calculations give:
UCNP/PAA: f_{org}=8.1

Based on the above results molecules adhere UCNP surfaces with the following surface densities:

1) PAA - ~550 molecules per nanoparticle (1 molecule per ~2.3 nm^2);

2) UCNP/**C1**-Glu^4 - 106 molecules/particle (1 molecule per 11.8 nm^2);

3) UCNP/**C2**-Glu^4 - 237 molecules/particle (1 molecule per 5.3 nm^2).

From calculations, average cross-sections of **C1**-Glu^4 and **C2**-Glu^4 were found to be ~11 nm^2 and 4.7 nm^2, respectively (see IV).

IV. Optical properties

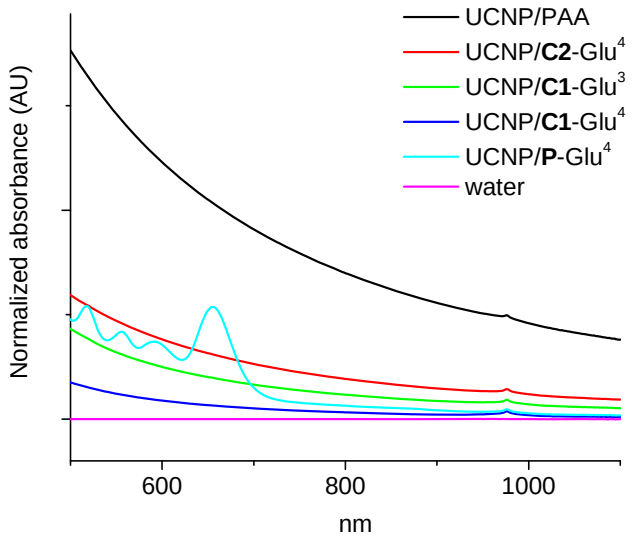


Figure S4. Absorption spectra of UCNPs modified with dendritic ligands and PAA, normalized by the integrated intensity of the Yb^{3+} absorption band (λ_{max}=977 nm).

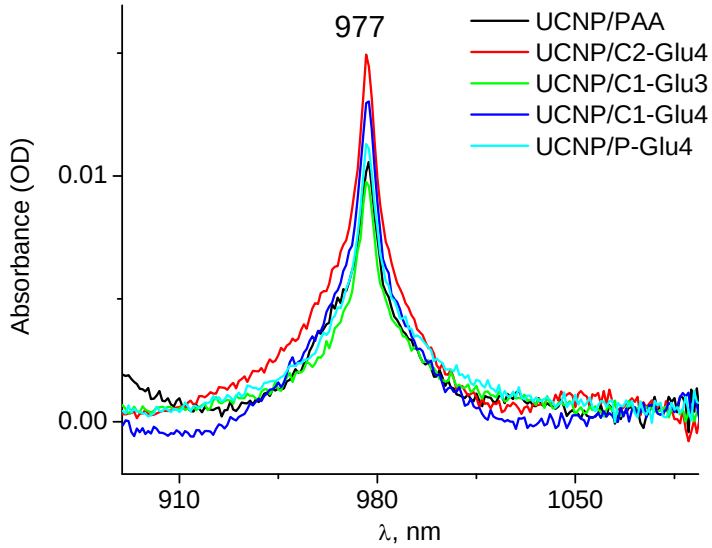


Figure S5. Absorption spectra of UCNPs modified with dendritic ligands and PAA near 980 nm. The scattering baseline was subtracted.

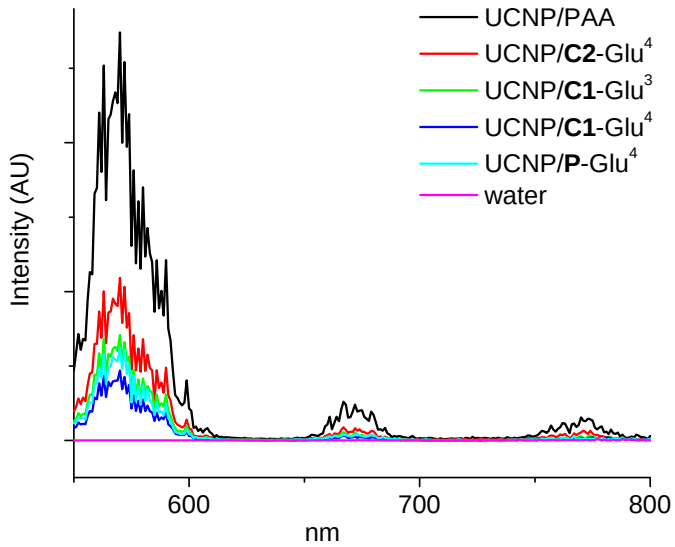


Figure S6. Scattering spectra of UCNPs and dendritic ligands, normalized by the integrated intensity of the Yb^{3+} absorption band (λ_{max}=977 nm).

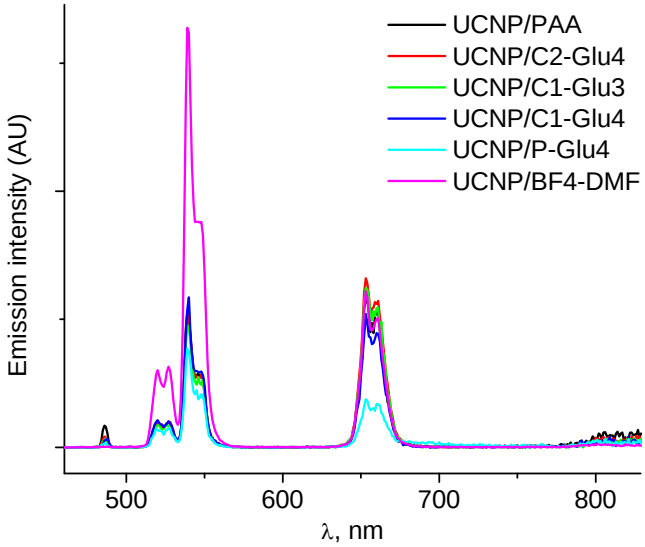
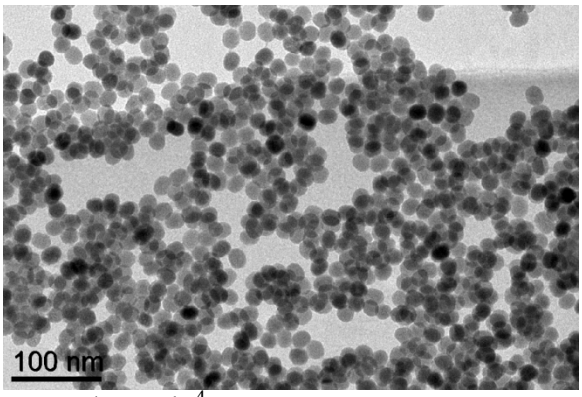
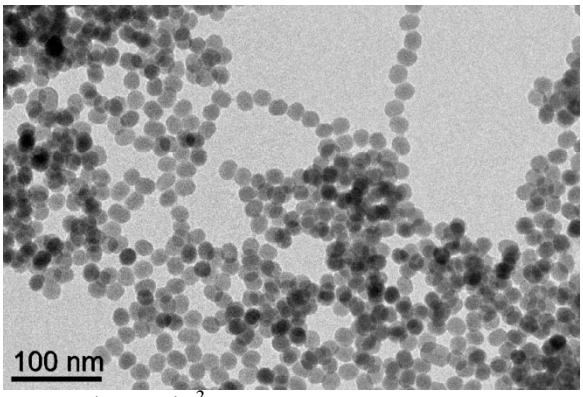


Figure S7. Emission spectra of UCNPs and dendritic ligands (λ_{ex}=980 nm), normalized by the integrated intensity of the Yb^{3+} absorption band (λ_{max}=977 nm).

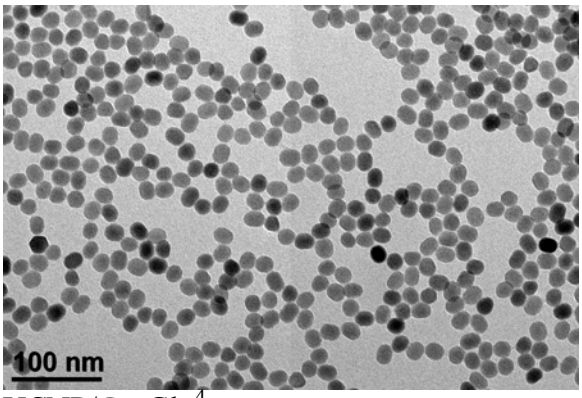
V. Transmission Electron Microscopy



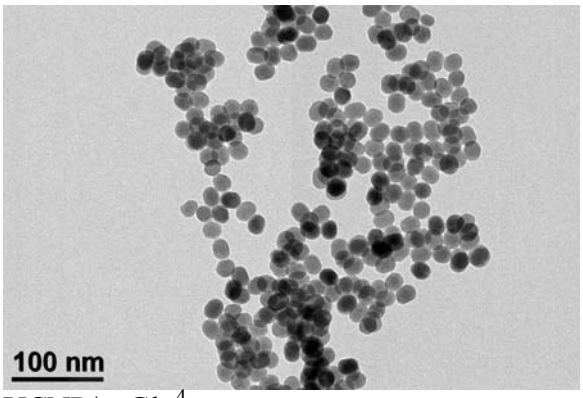
UCNP/**C2**-Glu[4]



UCNP/**C1**-Glu[3]



UCNP/**C1**-Glu[4]



UCNP/**P**-Glu[4]

VI. pH titrations of UCNP/P-Glu[4]

pH titrations were performed at 22°C using solutions of **P**-Glu[4] or UCNP/**P**-Glu[4] HEPES buffer (15 mM) or in distilled water. pH was adjusted by addition of HCl or KOH. For measurements by absorption, absorbances of solutions at Soret peak maxima were kept below 1.0 OD. For measurements by emission and two-photon excitation, solutions containing ~10 mg/ml of UCNPs were used.

The titration curves were constructed by plotting absorbances at selected wavelengths, or, in the case of fluorescence, integrated emission bands *vs* pH. In ratiometric titrations, ratios of the integrated emission bands upon excitation at 980 nm were plotted *vs* pH. The titration data were fitted to standard sigmoidal curve with an extra parameter *n* to account for heterogeneity of the sample and multiple interfering protonation reactions of porphyrin-dendrimers on the nanoparticle surface:

$$S_{\lambda 1}(\mathrm{pH}) = P_1 \frac{10^{n(\mathrm{pH}-\mathrm{pK})}}{1+10^{n(\mathrm{pH}-\mathrm{pK})}} + P_2$$

Fitting was performed using Microcal Origin software (Origin 7.0).

VII. References

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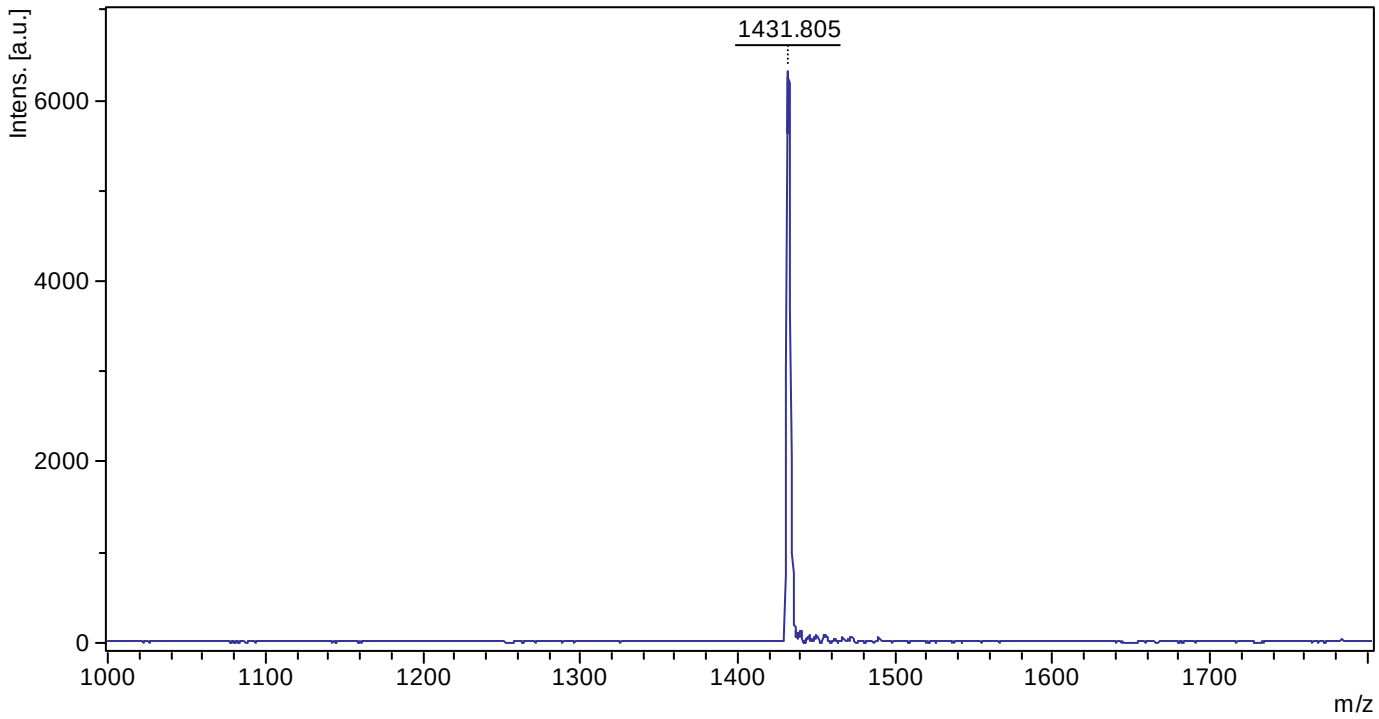
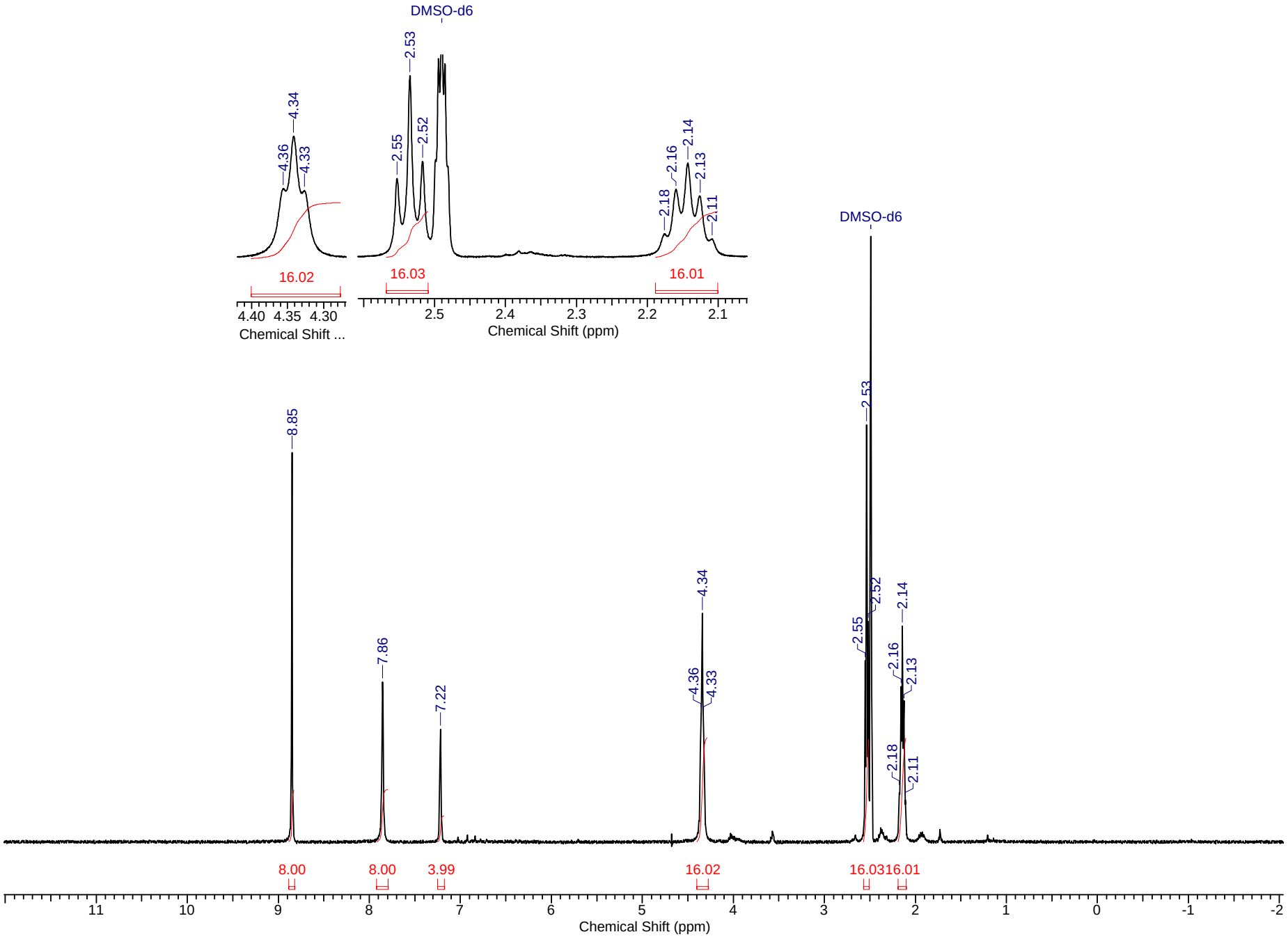
NMR and MALDI-TOF spectra

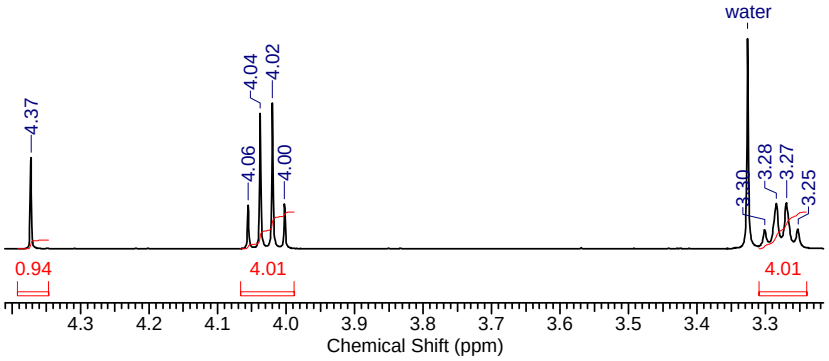
tetra-meso-[3,5-*bis*(3-carboxypropyloxy)phenyl]-porphyrin (**P**): MALDI-TOF

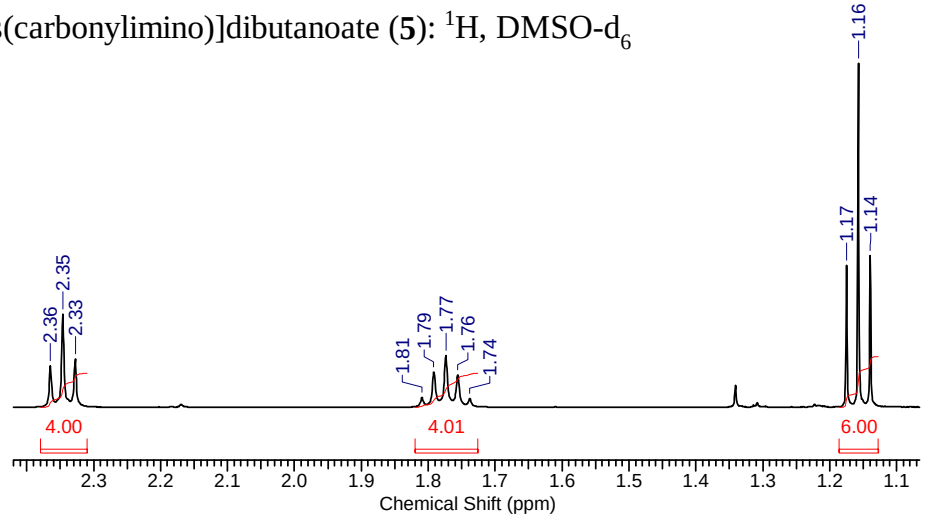


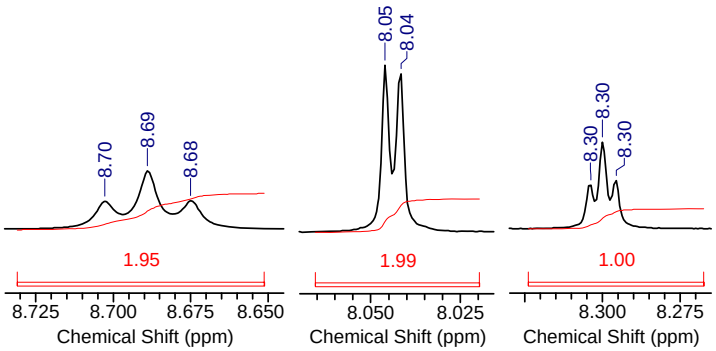
tetra-meso-[3,5-*bis*(3-carboxypropyloxy)phenyl]-porphyrin (**P**): ^{1}H DMSO-d$_6$



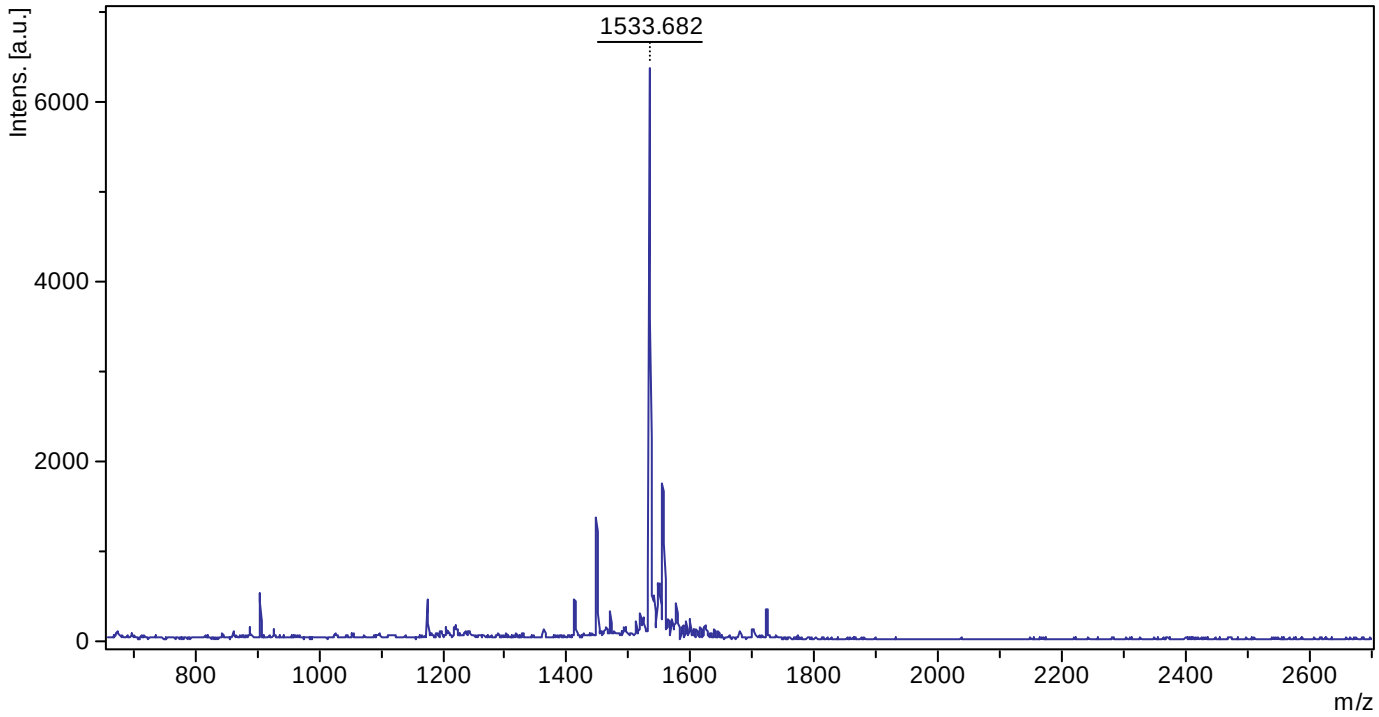
diethyl 4,4'-[(5-ethynyl-1,3-phenylene)bis(carbonylimino)]dibutanoate (**5**): ^{1}H, DMSO-d$_6$



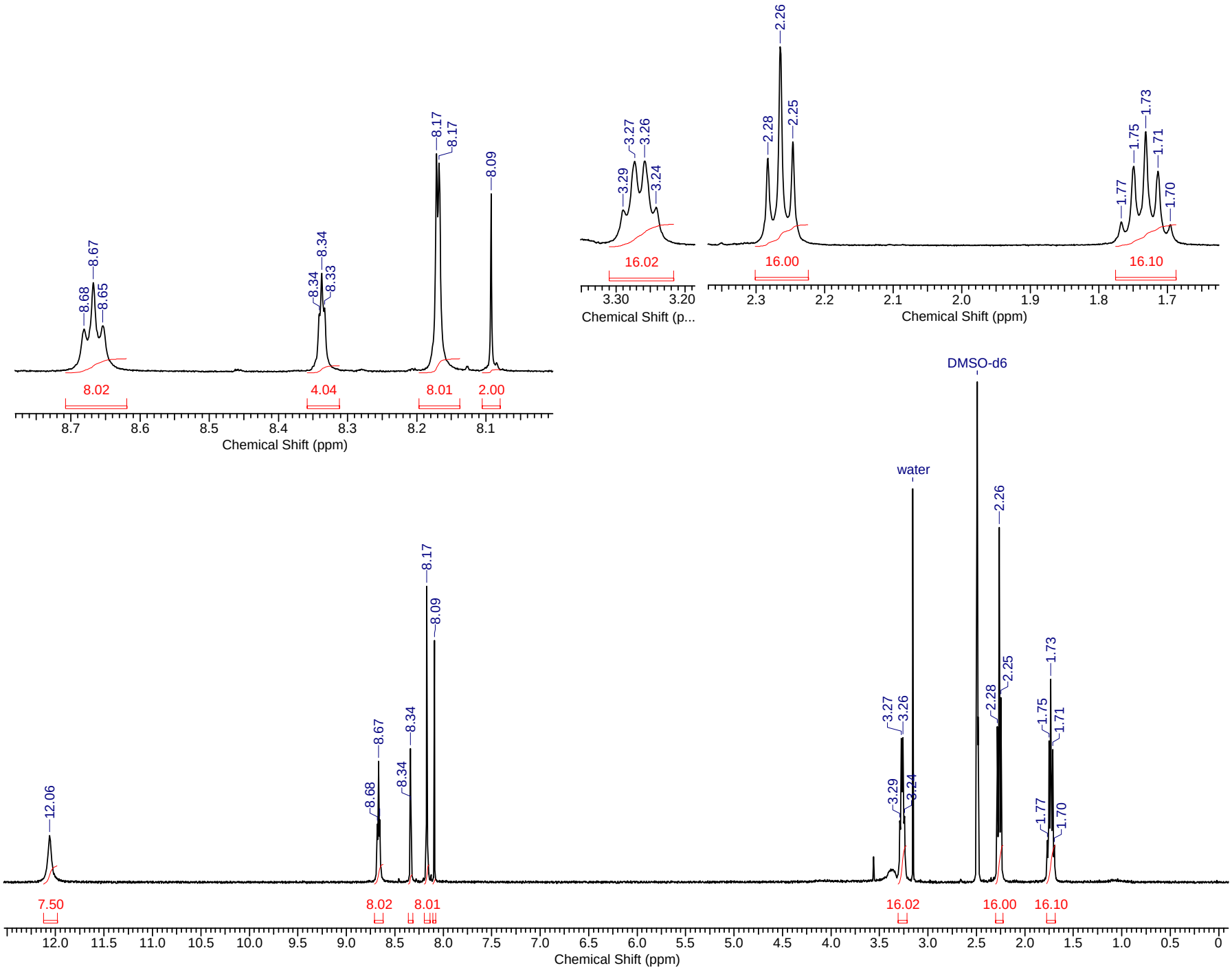




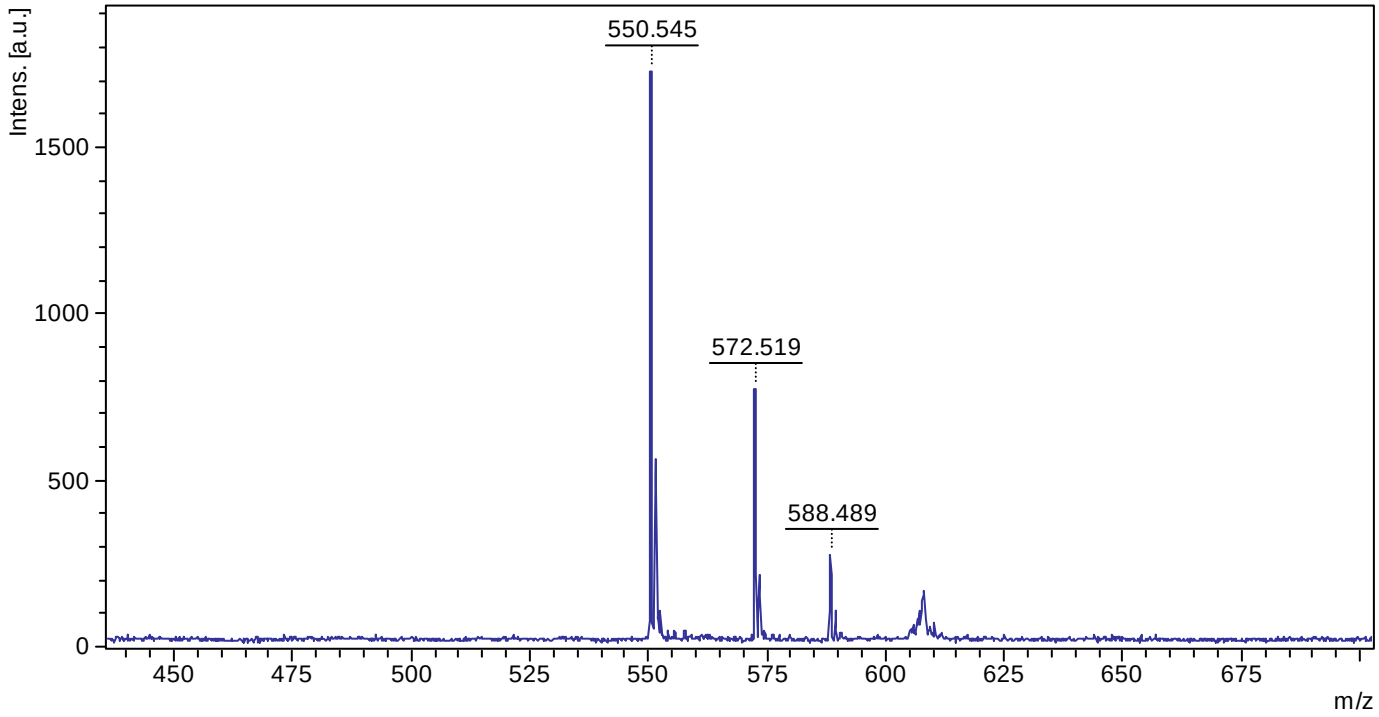
C1: MALDI-TOF


1533.682
Intens. [a.u.]
6000
4000
2000
0
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
m/z

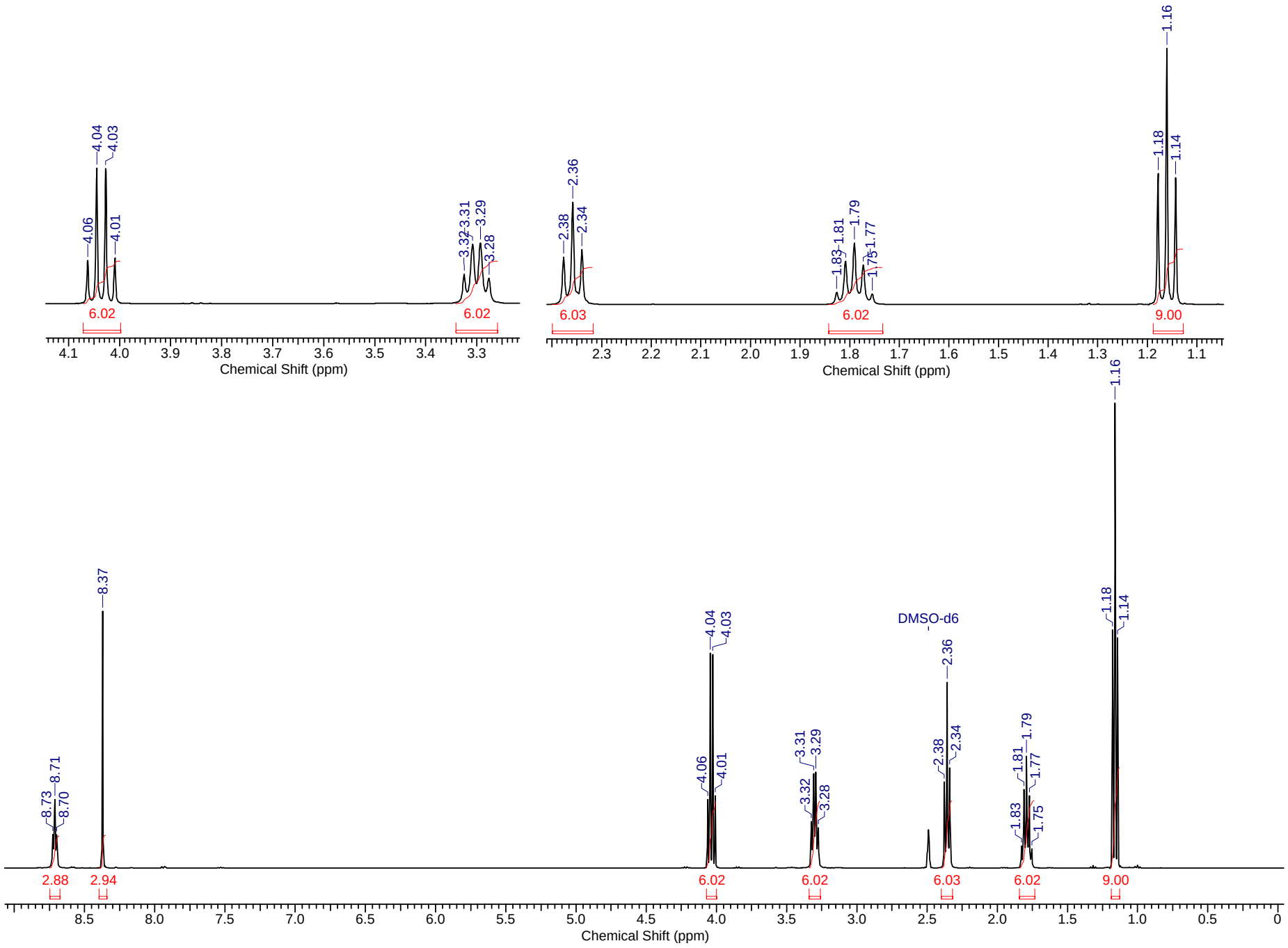
C1: ^{1}H, DMSO-d$_6$


8.68
8.67
8.65
8.34
8.34
8.33
8.17
8.17
8.09
8.02
4.04
8.01
2.00
Chemical Shift (ppm)
3.29
3.27
3.26
3.24
16.02
Chemical Shift (p...
2.28
2.26
2.25
1.77
1.75
1.73
1.71
1.70
16.00
16.10
Chemical Shift (ppm)
DMSO-d6
water
12.06
7.50
8.02
8.01
16.02
16.00
16.10
Chemical Shift (ppm)

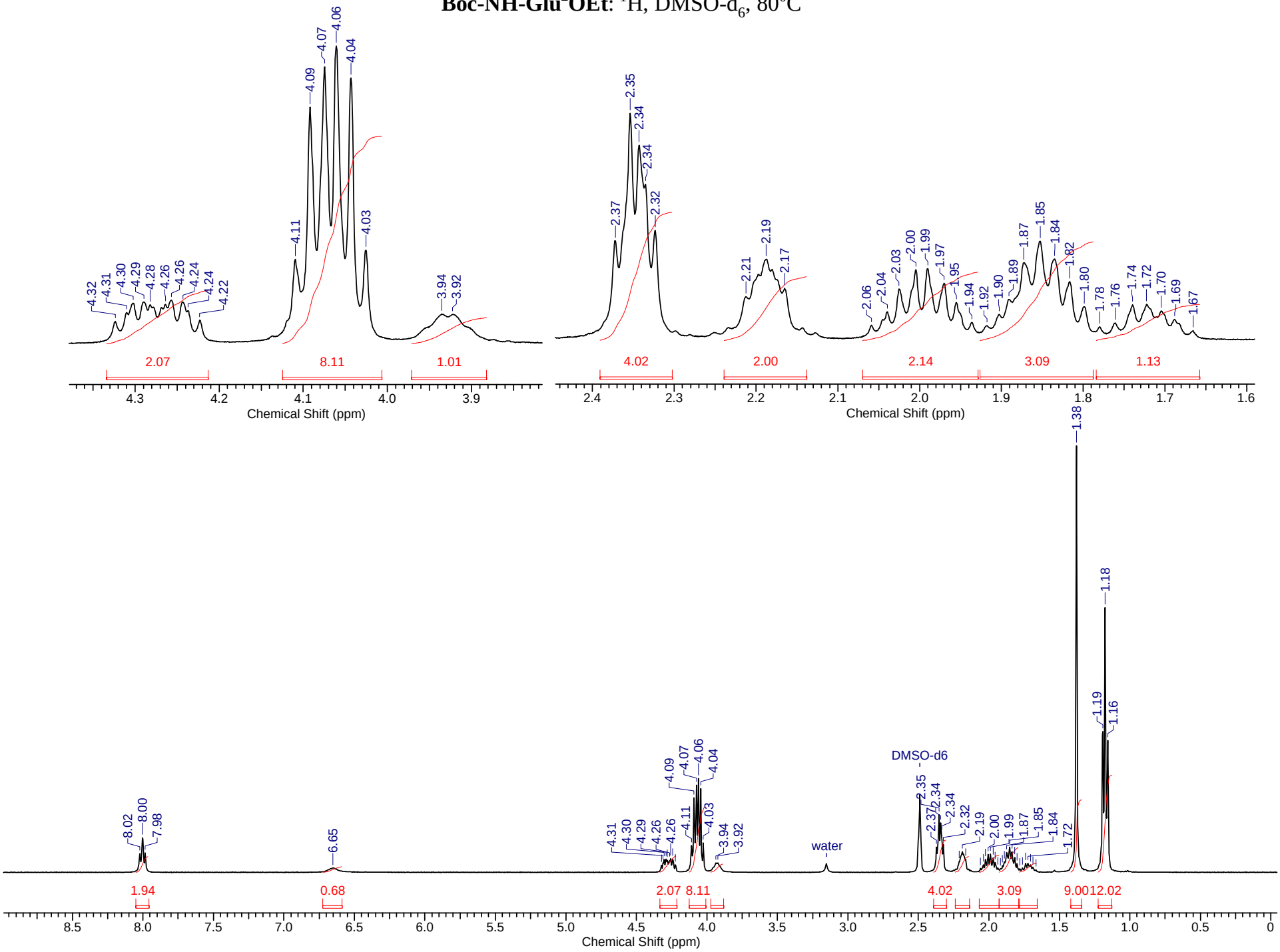
triethyl 4,4’,4’’-[benzene-1,3,5-triyltris(carbonylimino)]tributanoate (**7**): MALDI-TOF



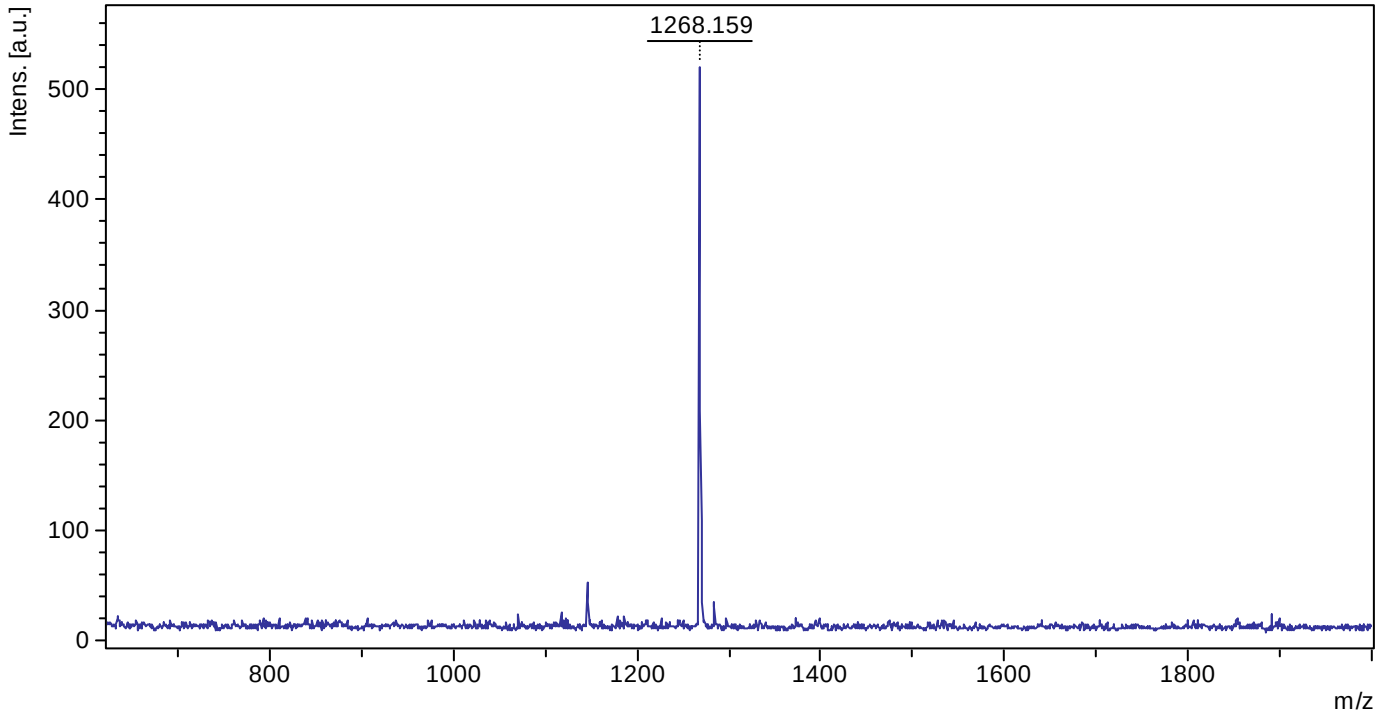
triethyl 4,4',4"-[benzene-1,3,5-triyltris(carbonylimino)]tributanoate (**7**): ^{1}H, DMSO-d_6



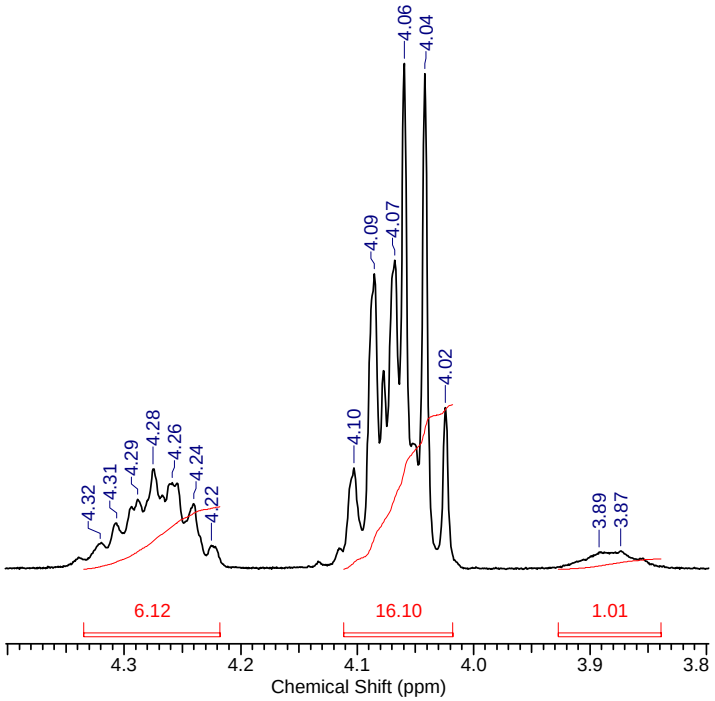
Boc-NH-Glu^{2}OEt: ^{1}H, DMSO-d$_6$, 80^{0}C

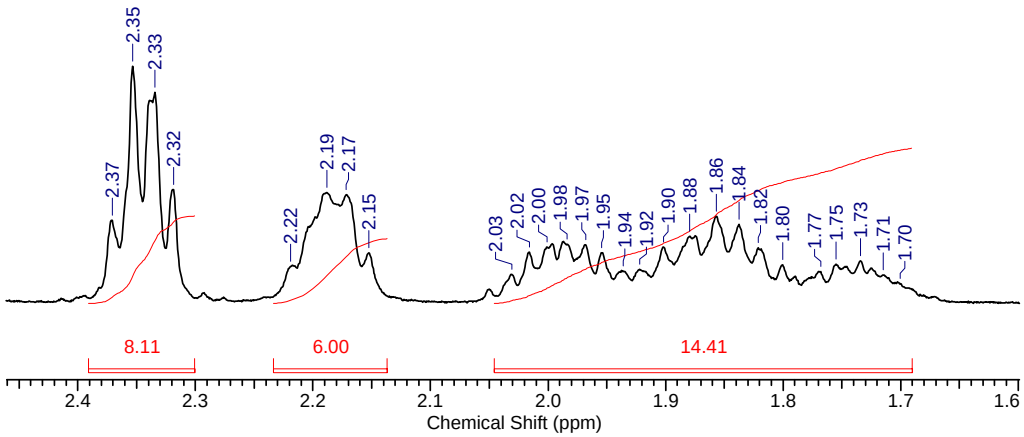


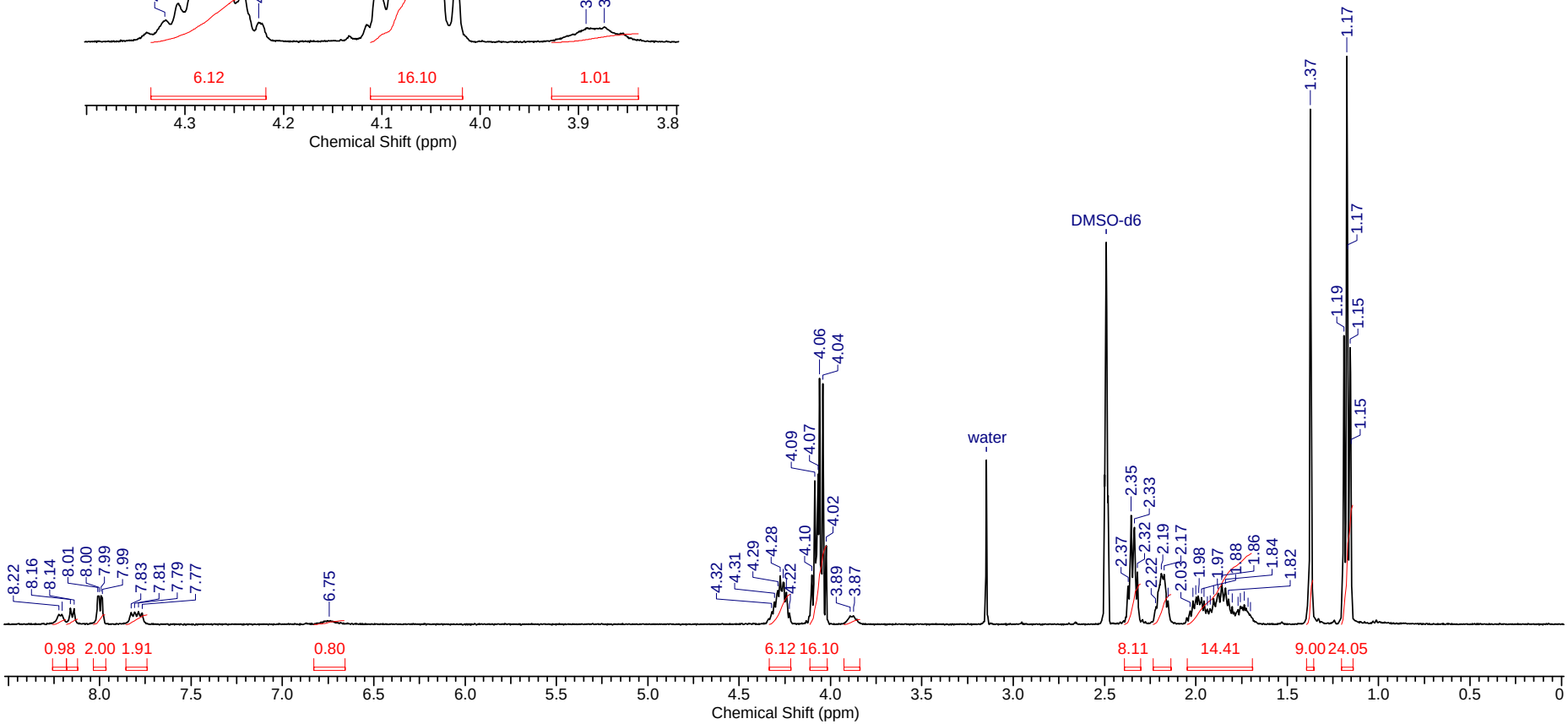
Boc-NH-Glu^{3}OEt: MALDI-TOF



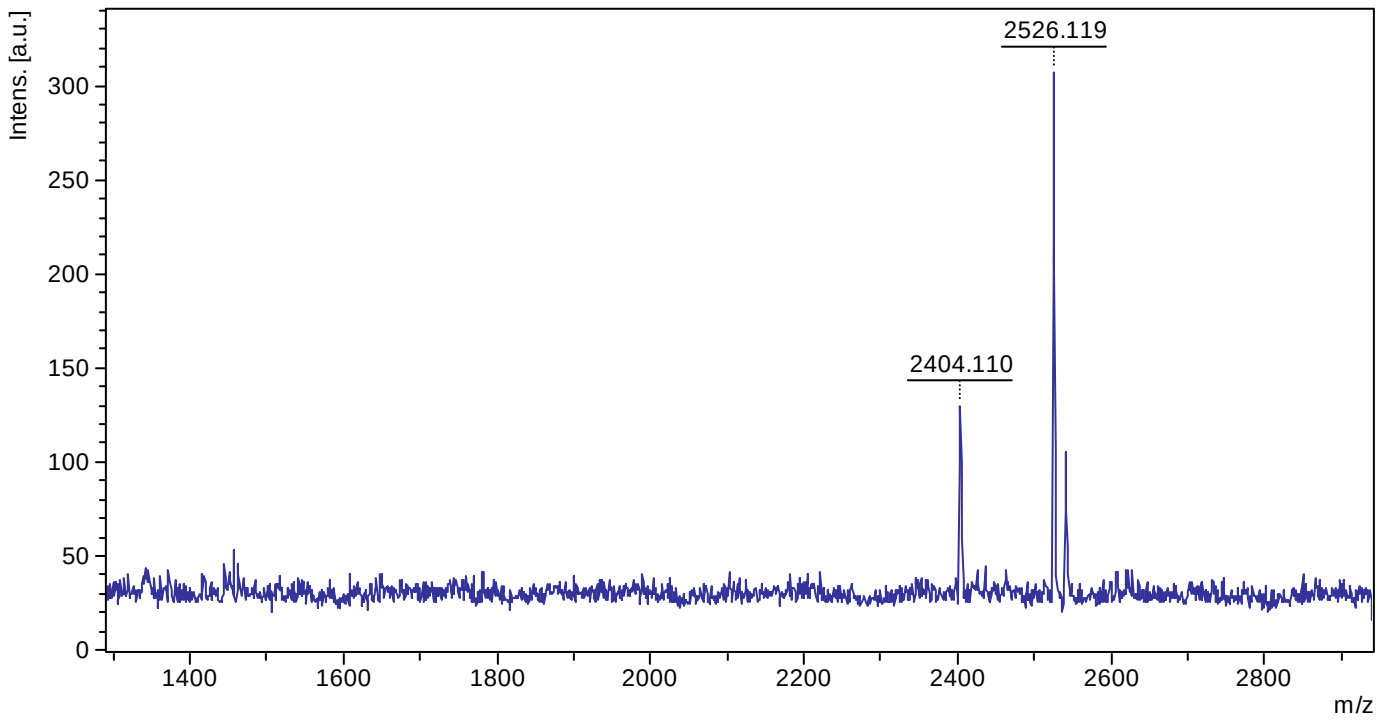
Boc-NH-Glu^{3}OEt: ^{1}H, DMSO-d$_6$, 80^{0}C



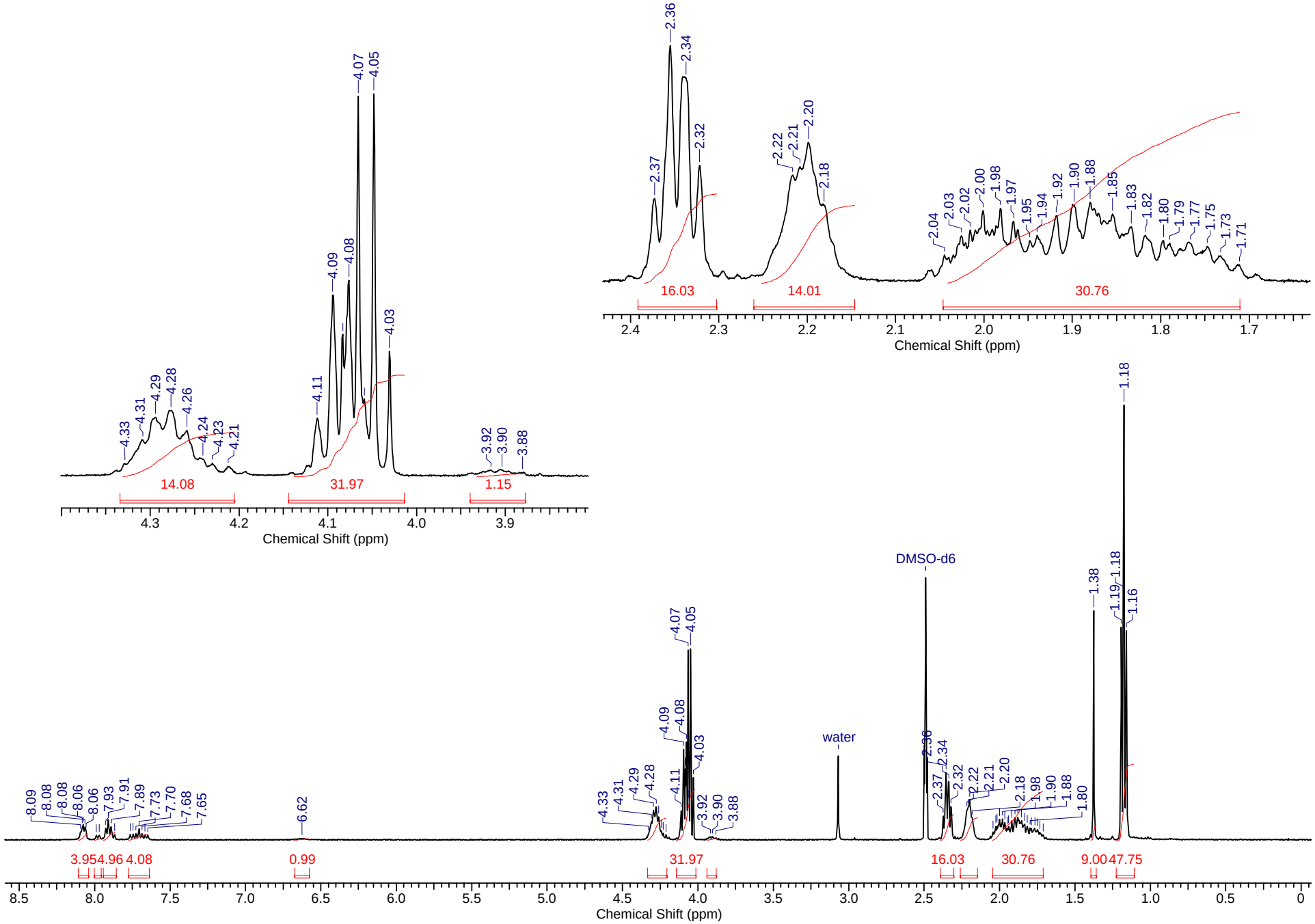




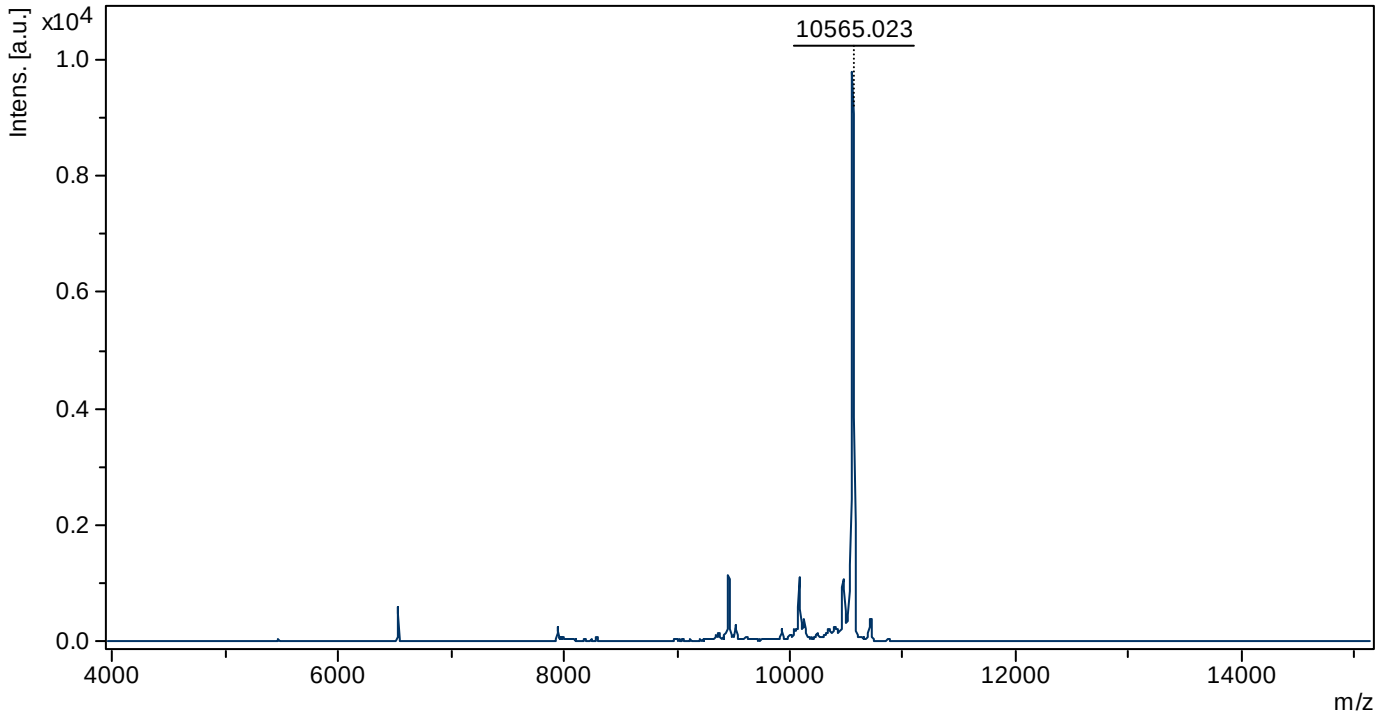
Boc-NH-Glu^{4}OEt: MALDI-TOF



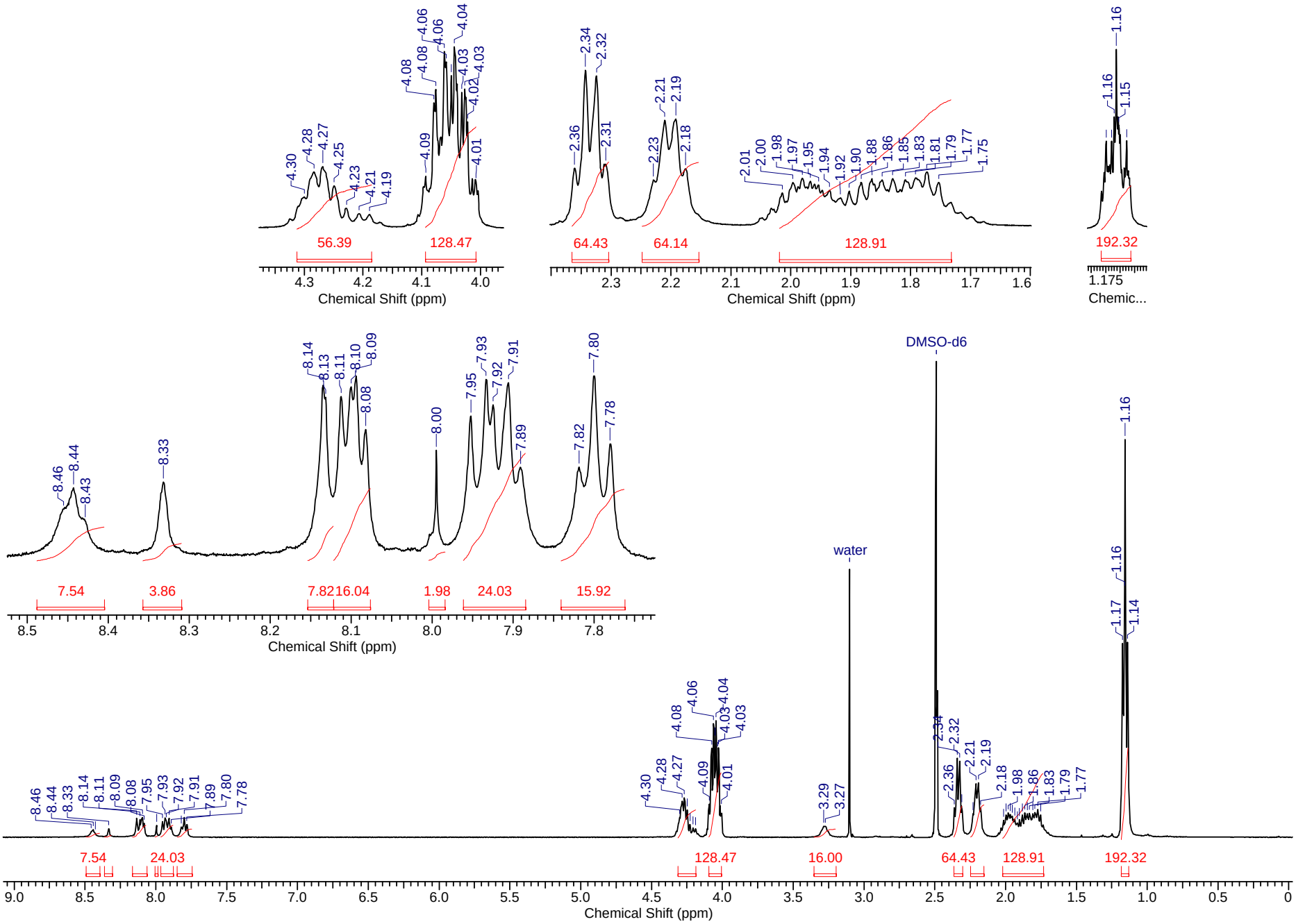
Boc-NH-Glu^{4}OEt: ^{1}H, DMSO-d$_6$, 80^{0}C



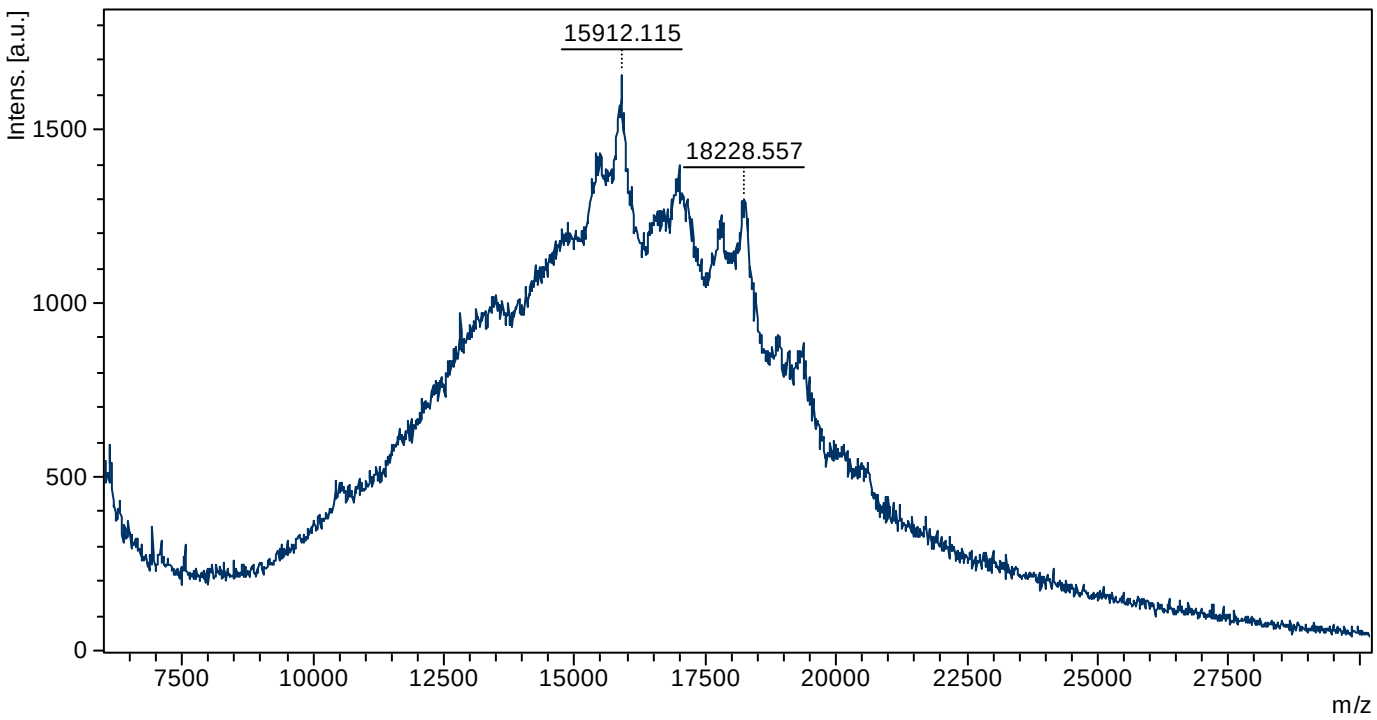
C1-Glu^{3}OEt (8): MALDI-TOF



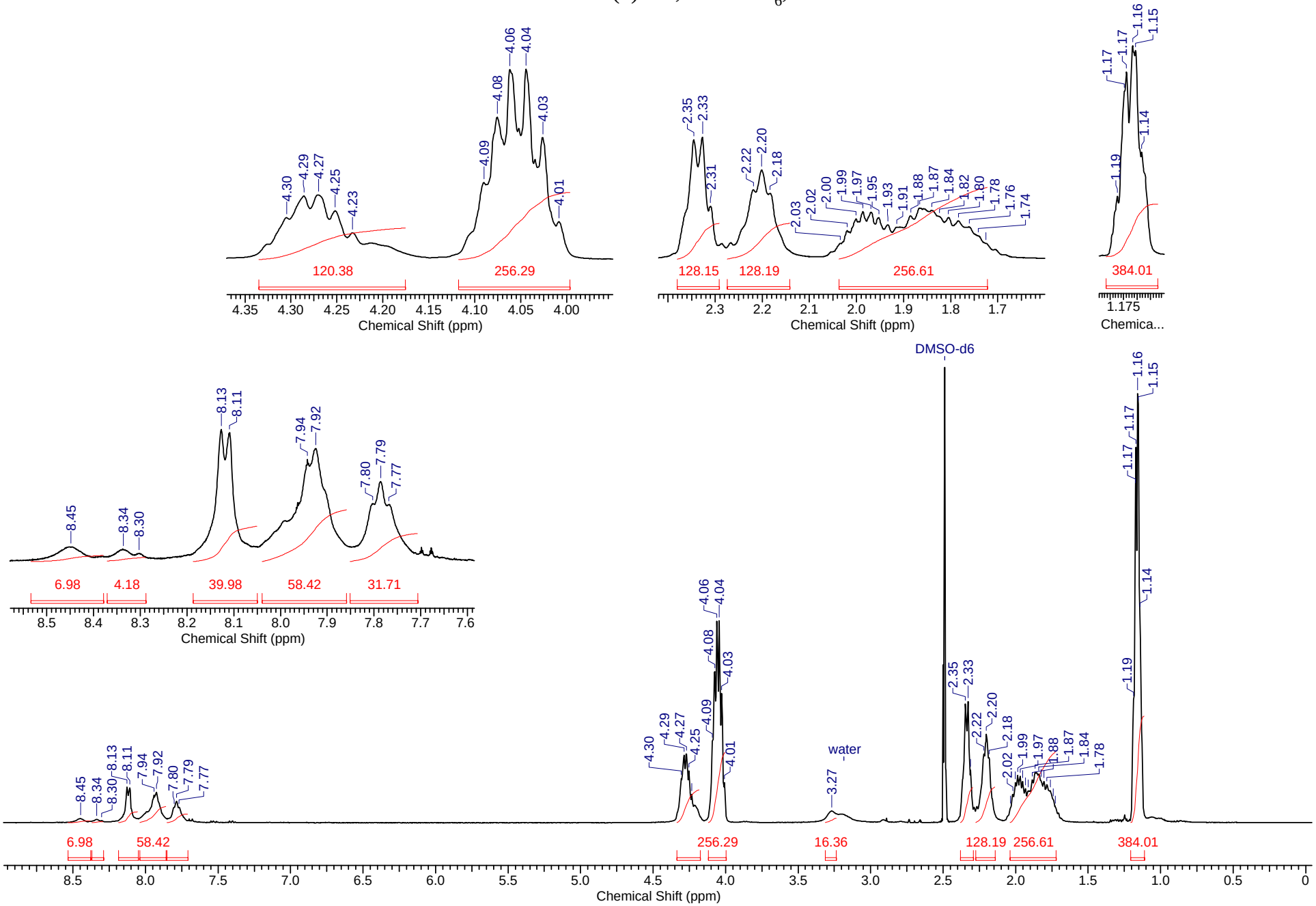
C1-Glu^{3}OEt (8): ^{1}H, DMSO-d$_{6}$, 80^{0}C



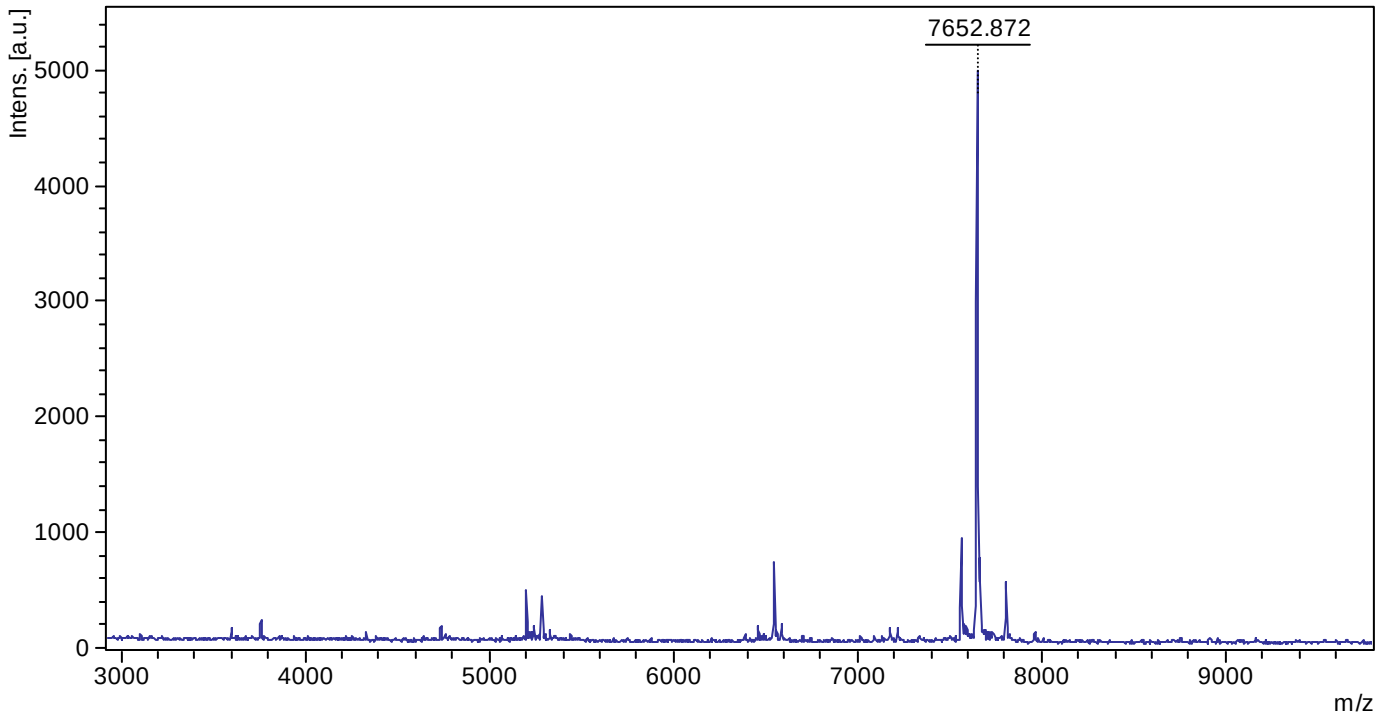
C1-Glu^{4}OEt (9): MALDI-TOF



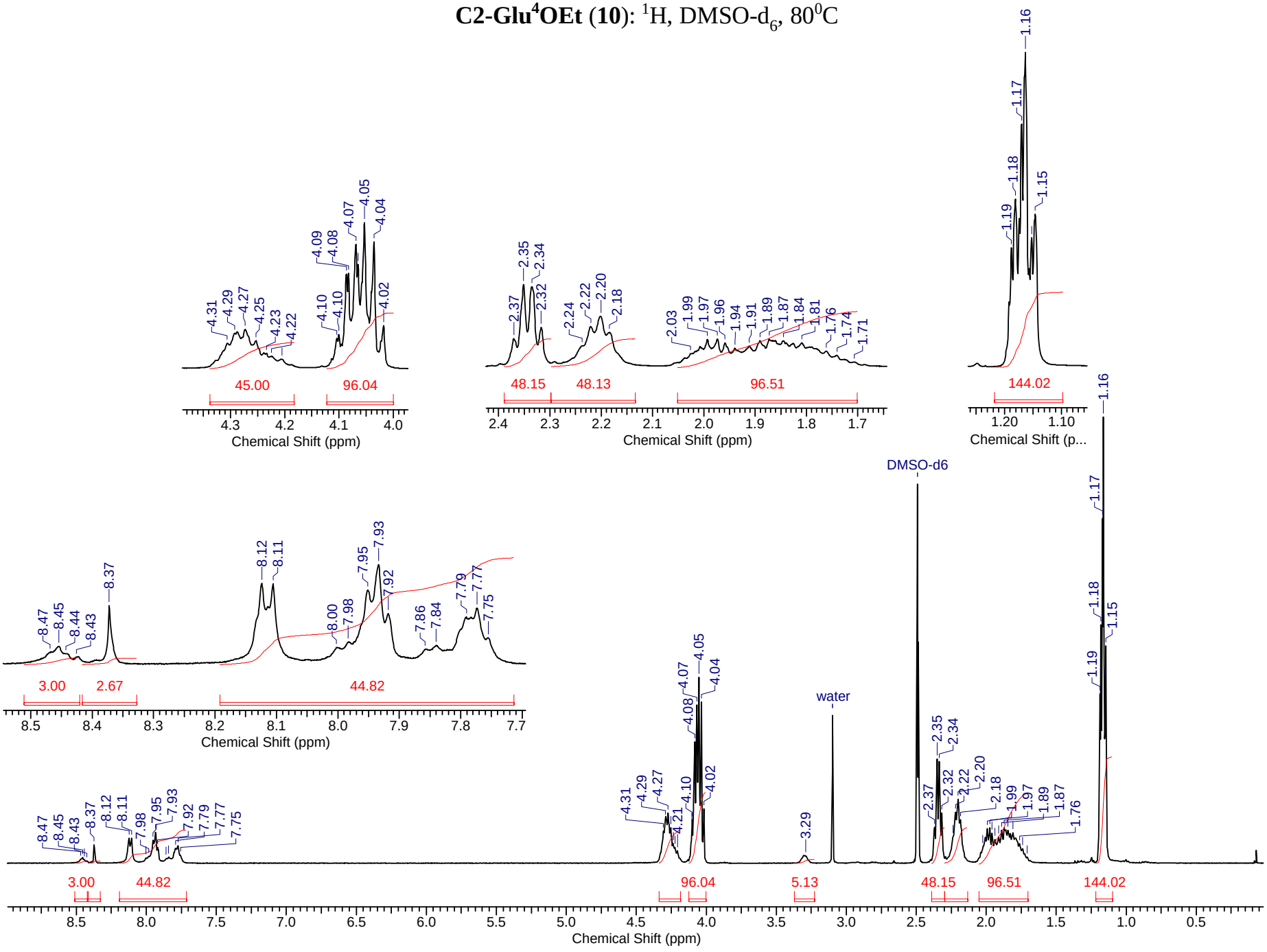
C1-Glu^{4}OEt (9): ^{1}H, DMSO-d$_6$, 80^{0}C



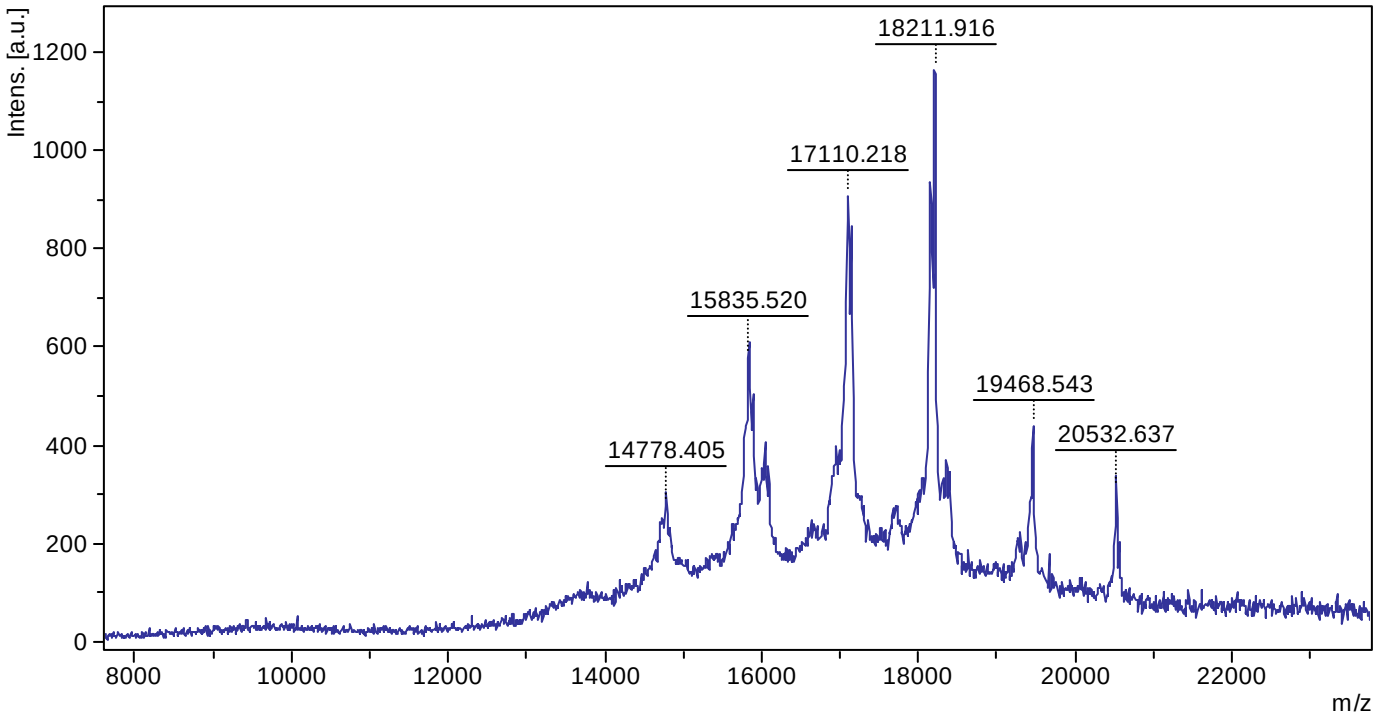
C2-Glu^{4}OEt (10): MALDI-TOF



C2-Glu^{4}OEt (10): ^{1}H, DMSO-d$_{6}$, 80^{0}C



P-Glu^{4}OEt (11): MALDI-TOF



P-Glu^{4}OEt (11): ^{1}H, DMSO-d$_6$, 80^{0}C