A New Model for the Presentation of Tumor-associated Antigens and the Quest for an Anticancer Vaccine: A Solution to the Synthesis Challenge via RCM

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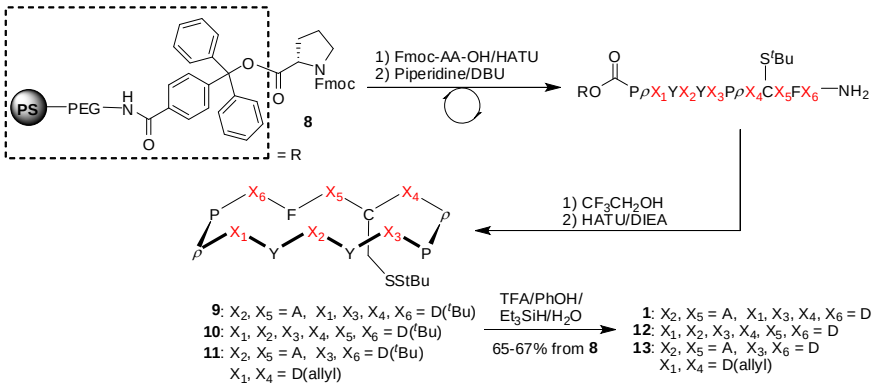
Supporting Information

General Information:

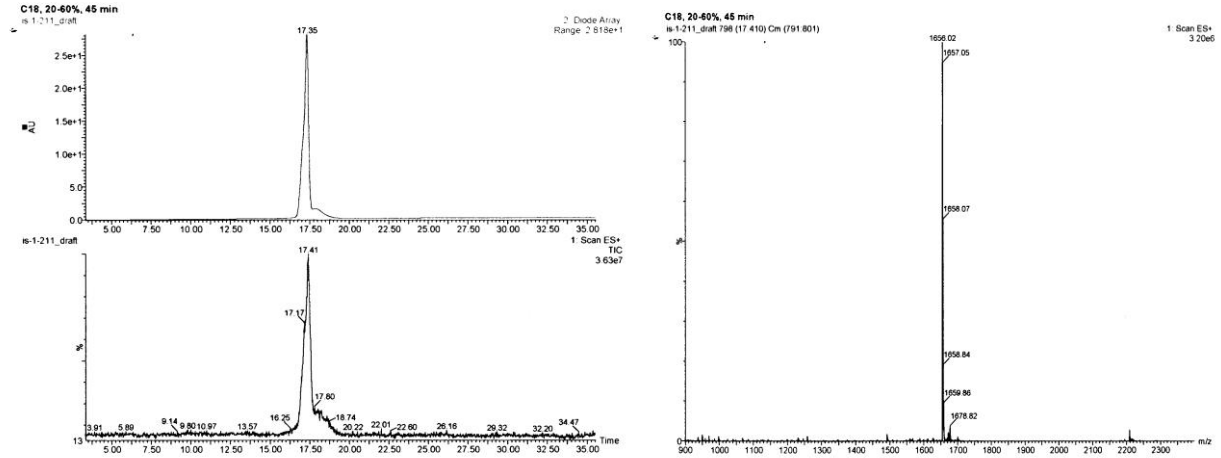
Analytical Equipment: ^{1}H- NMR spectra were recorded on a Bruker AVII+-600 spectrometer in d_6-DMSO or D_2O. Low resolution mass spectra (electrospray ionization) were acquired on a ZQ Micromass spectrometer. Samples were introduced by direct infusion. In the case of LC/MS, analysis was performed with a Waters Alliance analytical LC system in tandem with the Micromass ZQ. The column used was Microsorb C18, 300-5, 2 x 150 mm. All HPLC was run with TFA (trifluoroacetic acid)-buffered eluents: A = 0.05 % v/v TFA/Water, B = 0.04 % v/v TFA/Acetonitrile. The column used was a 21.4 x 250mm Varian Microsorb C18 Dynamax column, 100 Å pore size, 5μM particle size. DMSO was purchased from Aldrich (Anhydrous grade) and used without further purification. DIEA (iPr_2NEt) was freshly distilled from CaH_2. DBU (Diazabicycloundecene) and Piperidine were purchased from Aldrich and used without further purification. HATU (*O*-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate) was purchased from GenScript and used without further purification. Peptide synthesis resins and Fmoc-amino acids were purchased from NovaBiochem.



Cyclic peptide 1: Fmoc-Pro-NovaSyn TGT resin (**8**) (0.5 g, 0.2 mmol/g, purchased from NovaBiochem) was subjected continuous flow automated peptide synthesis. For coupling steps, resin was treated with with a 3-fold excess of HATU and Fmoc amino acids in DIEA/DMF, and for deblocking, a solution of 2% Piperidine/2% DBU in DMF was used. The amino acids used were, in order of synthesis, Fmoc-D-Pro-OH, Fmoc-Asp(^{t}Bu)-OH, Fmoc-Tyr(^{t}Bu)-OH, Fmoc-Ala-OH, Fmoc-Tyr(^{t}Bu)-OH, Fmoc-Asp(^{t}Bu)-OH, Fmoc-Pro-OH, Fmoc-D-Pro-OH, Fmoc-Asp(^{t}Bu)-OH, Fmoc-Cys(S^{t}Bu)-OH, Fmoc-Ala-OH, Fmoc-Phe-OH, Fmoc-Asp(^{t}Bu)-OH. The resin was then transferred to a manual peptide synthesis vessel and treated with a cleavage solution of 5 mL of 20 % trifluoroethanol in dichloromethane for 2 hours. The beads were filtered, rinsed with another 5 mL cleavage solution, filtered again, and then treated for another 2 hours with 5 mL of cleavage solution. This process was repeated for a total of 3 2-hour cleavage cycles, and the combined filtrate was concentrated in vacuo to afford ~160 mg crude linear protected peptide as a colorless glass. This material was redissolved in 50 mL of 1% v/v DIEA in DMF. HOAt (32.5 mg, 0.239 mmol, 3 equiv.) was added, followed by HATU (91 mg, 0.239mmol, 3 equiv.). After 1 hour, the solvent was removed in vacuo (using a rotary evaporator, ~1 mm Hg, 30°C), affording the crude cyclic protected peptide contaminated with HATU/HOAt-derived byproducts. (ESI MS analysis showed predominantly the desired product peak, 1992.1 (M + H)). This material was then redissolved in 10mL of 87.5 %TFA / 5 % water / 5% phenol / 2.5 % triethylsilane (v/v/m/v) solution and stirred

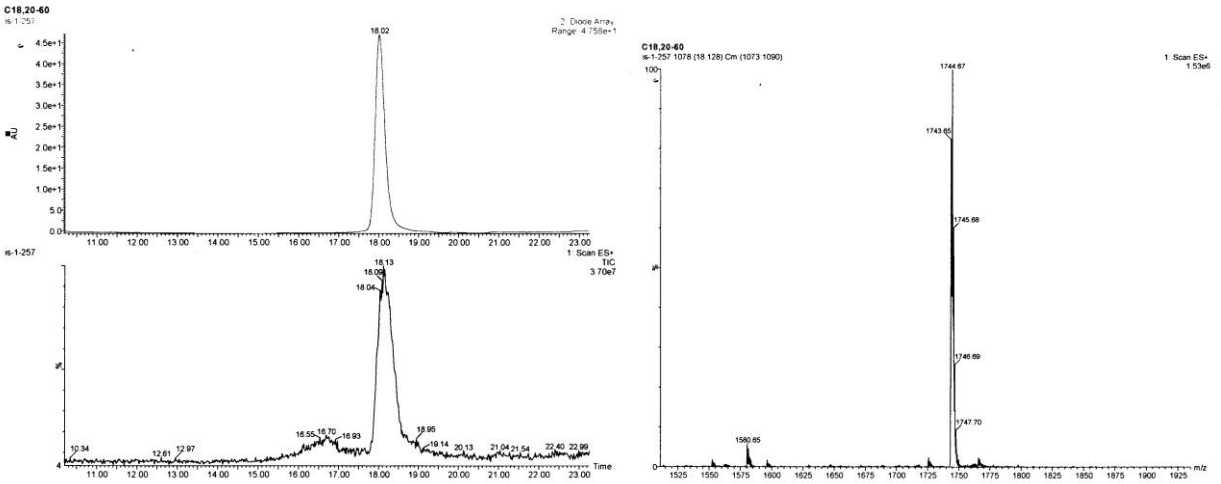
for 30 minutes. Solvent was removed in vacuo then the residue was triturated with 25 mL diethyl ether 4 times to afford crude peptide **1**. This was purified in eight batches by preparative reverse-phase HPLC using a gradient of 20-60% B buffer (see General Info) over 30 min, flow rate 16 mL/min, 235 nm UV detection. The peak with retention time of 19.2 minutes was collected. LC/MS analysis (20-60%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the peptide **1** at 17 min and MS spectrum with base peaks of 1656.0 (M + H, [1655.6 calc]). Lyophilization of these fractions yielded 108 mg of **1** (65% yield based on proline-loaded resin **8**).

Purified peptide **1**: UV and MS detection left, MS spectrum right (single ion)



Cyclic peptide 12: Cyclic peptide **12** was synthesized under the same condition as above. The crude mixture was purified in eight batches by preparative reverse-phase HPLC using a gradient of 25-60% B buffer (see General Info) over 30 min, flow rate 16 mL/min, 235 nm UV detection. The peak with retention time of 16.6 minutes was collected. LC/MS analysis (20-60%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the peptide **12** at 18 min and MS spectrum with base peaks of 1743.7 (M + H, [1743.6 calc]). Lyophilization of these fractions yielded 117 mg of **12** (67% yield based on proline-loaded resin **8**).

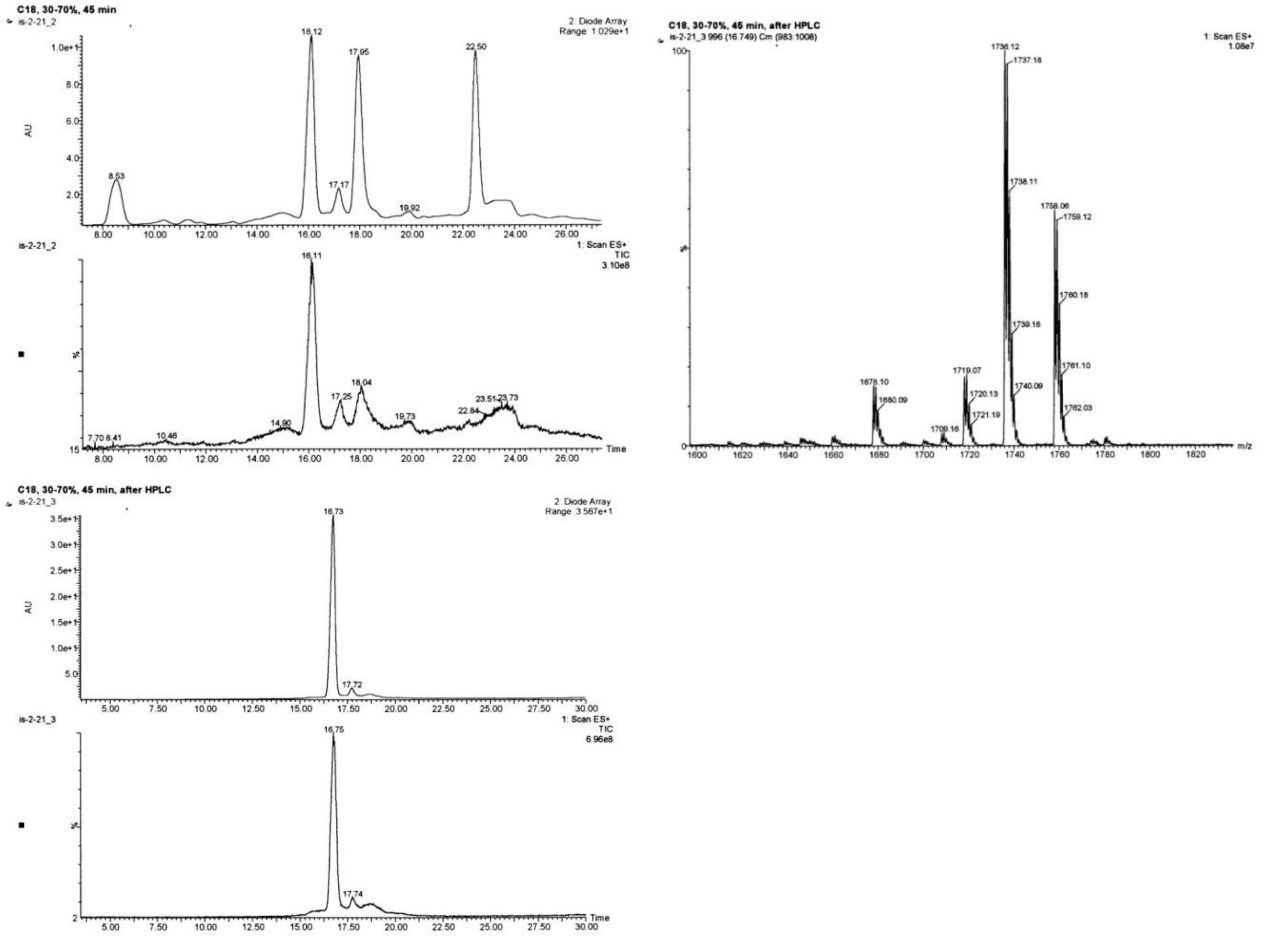
Purified peptide **12**: UV and MS detection left, MS spectrum right (single ion)

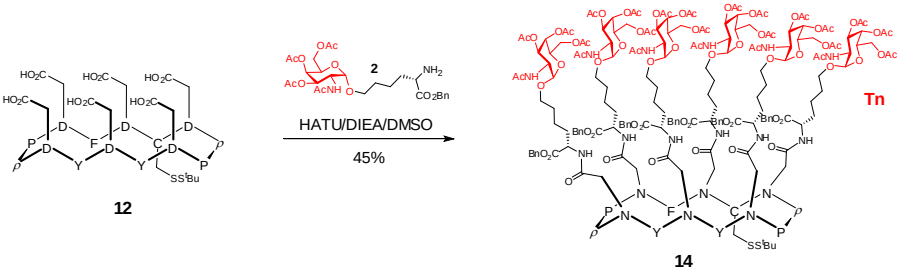


Cyclic peptide 13: Cyclic peptide **13** was synthesized under the same condition as above. The crude mixture was purified in eight batches by preparative reverse-phase HPLC using a gradient of 25-60% B buffer (see

General Info) over 30 min, flow rate 16mL/min, 235 nm UV detection. The peak with retention time of 16.6 minutes was collected. LC/MS analysis (30-70%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the peptide **13** at 16 min and MS spectrum with base peaks of 1736.1 (M + H, [1735.7 calc]). Lyophilization of these fractions yielded 113 mg of **13** (65% yield based on proline-loaded resin **7**).

LCMS of **13**: UV and MS detection left top, MS spectrum right top (single ion), LCMS after purification left bottom

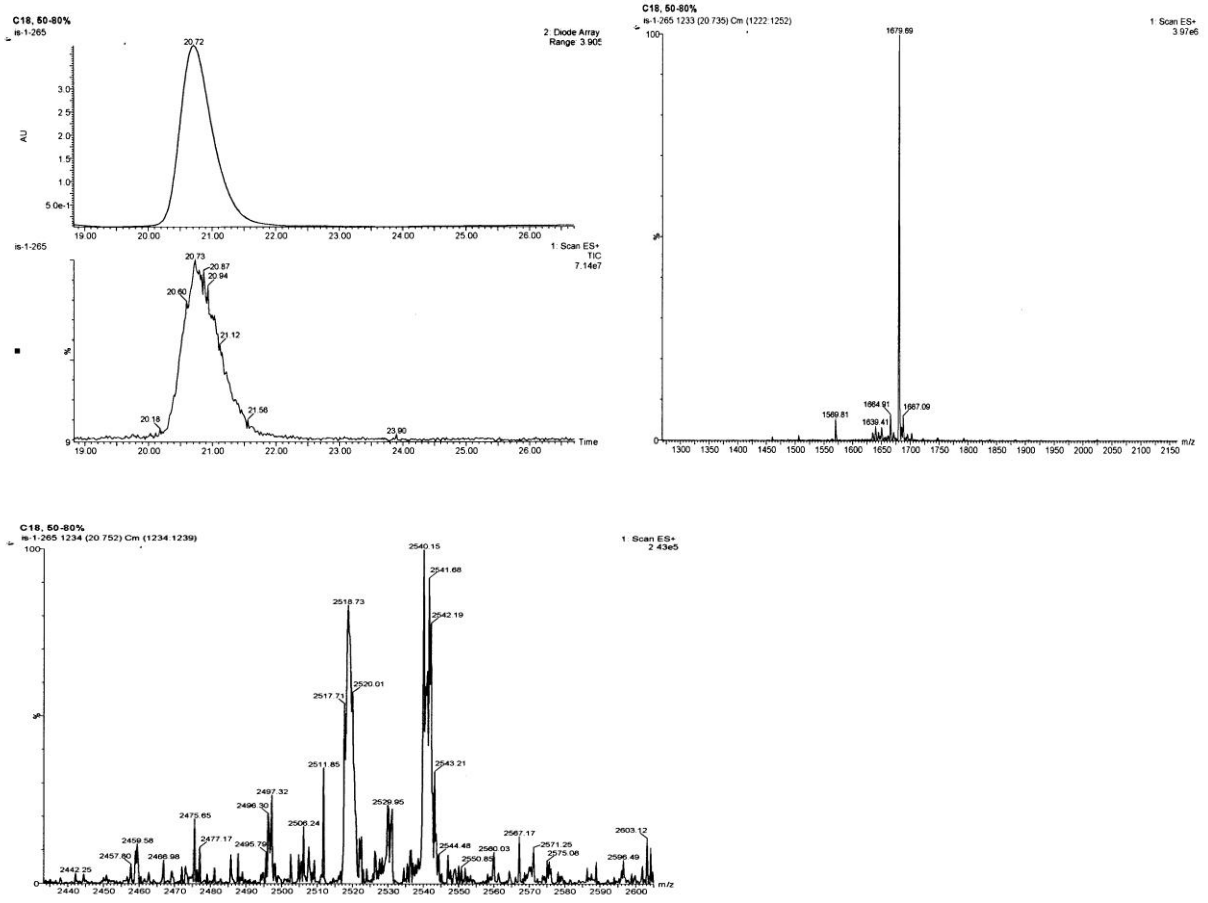


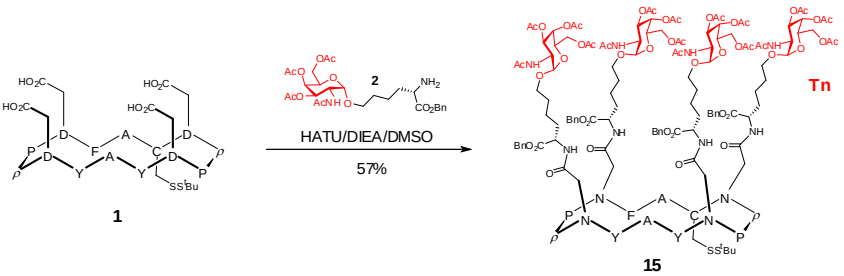


Protected Hexavalent Glycopeptide 14: Solutions of each reaction participant were prepared with a stirring bar in flame-dried vials under argon as follows: Peptide **12** (2 mg, 1.15 µmol), Tn **2** (4.88 mg, 8.62 µmol), were dissolved in 1mL DMSO. To this mixture, HATU (2.9 mg, 7.6 µmol in 100 µL DMSO) was added, followed by dry *i*Pr_2NEt (4.5 µL, 25.74 µmol), producing a strong yellow color. After 4 hours, the crude reaction mixture was diluted with ~ 500 µL of 50%B HPLC buffer and, this was purified in two batches by preparative reverse-phase HPLC using a gradient of 55-80%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection.

Retention time for protected hexavalent glycopeptide **14** was 19 minutes. LC/MS analysis of the crude reaction mixture (50-80%B over 30 min, same solvent system as above, flow rate 0.2mL/min) showed the protected hexavalent glycopeptide **14** at 21 min and MS spectrum with base peaks of 2517.7 (M + 2H, [2517.0 calc]) and 1679.7 (M + 3H [1678.4 calc]). Lyophilization of these fractions yielded 2.6 mg (45 %) of **14**.

Purified glycopeptide **14**: UV and MS detection left top, MS spectrum right top (triple ion) and left bottom (double ion)

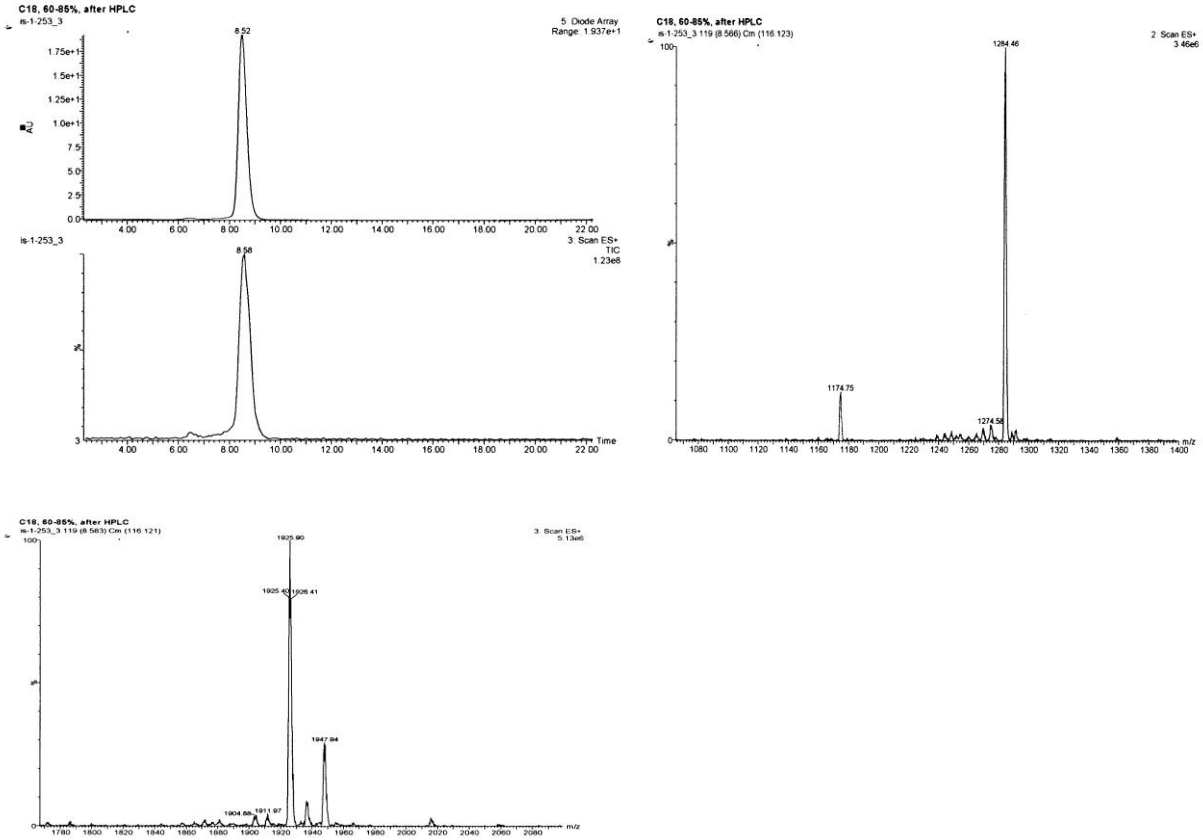


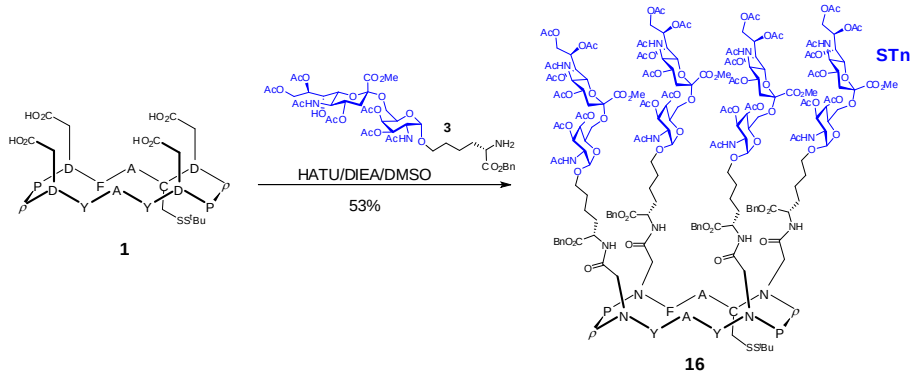


Protected Tetravalent Glycopeptide 15: Solutions of each reaction participant were prepared with a stir bar in flame-dried vials under argon as follows: Peptide **1** (2 mg, 1.21 μmol), Tn **2** (3.43 mg, 6.04 μmol), were dissolved in 0.5mL DMSO. To this mixture, HATU (2.3 mg, 6.04 μmol in 100 μL DMSO) was added, followed by dry *i*Pr_2NEt (5.1 μL, 29 μmol), producing a strong yellow color. After 4 hours, the crude reaction mixture was diluted with ~ 500 μL of 50%B HPLC buffer and, this was purified in two batches by preparative reverse-phase HPLC using a gradient of 50-80%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for protected hexavalent glycopeptide **12** was 9 minutes. LC/MS analysis (60-85%B over 30 min,

same solvent system as above, flow rate 0.2mL/min) showed the protected tetravalent glycopeptide **15** at 8.5 min and MS spectrum with base peaks of 1925.4 (M + 2H, [1924.8 calc]) and 1284.5 (M + 3H [1283.5 calc]). Lyophilization of these fractions yielded 2.7 mg (57 %) of **15**.

Purified glycopeptide **15**: UV and MS detection left top, MS spectrum right top (triple ion) and left bottom (double ion)

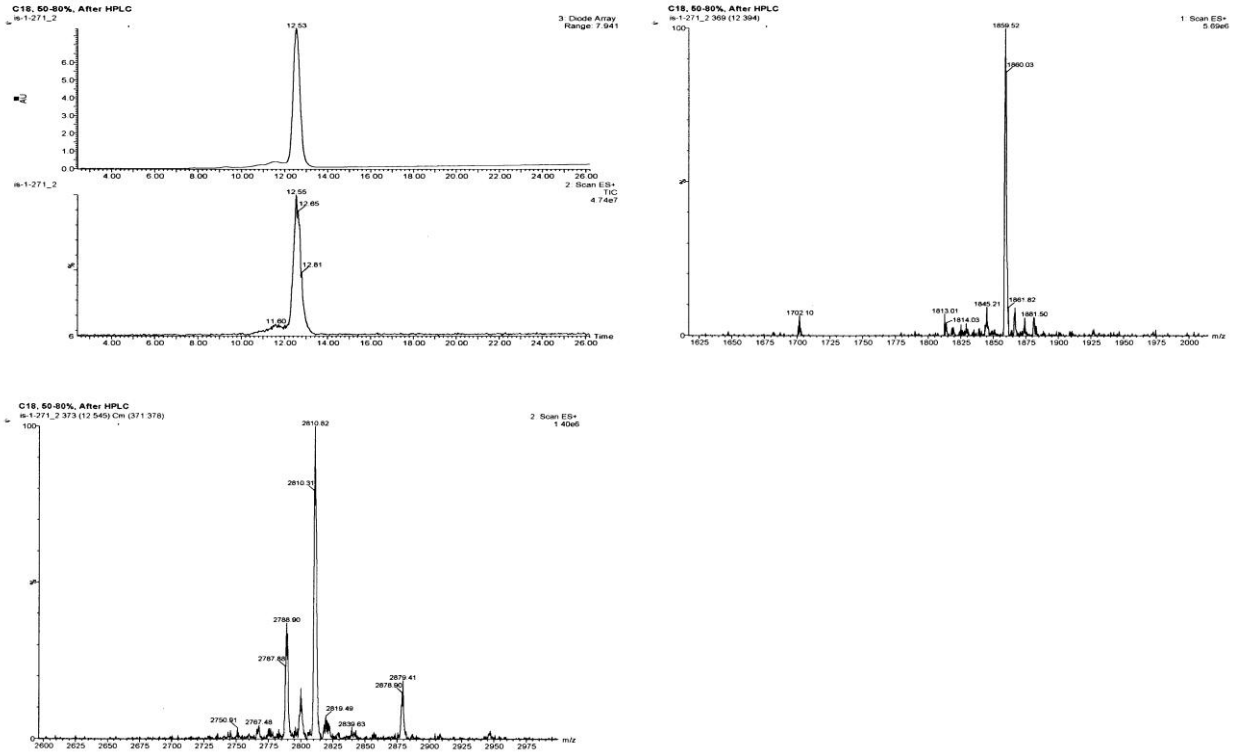




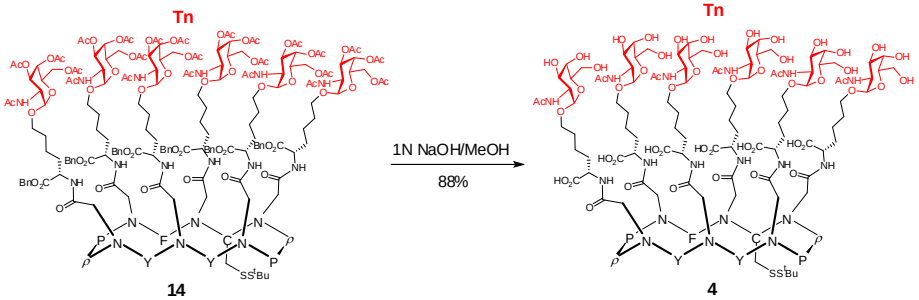
Protected Tetravalent Glycopeptide 16: Solutions of each reaction participant were prepared with a stir bar in flame-dried vials under argon as follows: Peptide **1** (2.3 mg, 1.41 μmol), STn **3** (11.3 mg, 11.31 μmol), were dissolved in 0.9mL DMSO. To this mixture, HATU (2.7 mg, 7.07 μmol in 100 μL DMSO) was added, followed by dry *i*Pr_2NEt (3.7 μL, 21 μmol), producing a strong yellow color. After 4 hours, the crude reaction mixture was diluted with ~ 500 μL of 50%B HPLC buffer and, this was purified in two batches by preparative reverse-phase HPLC using a gradient of 50-80%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for protected tetravalent glycopeptide **16** was 14 minutes. LC/MS analysis of the crude reaction

mixture (50-80%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the protected tetravalent glycopeptide **16** at 13 minutes and MS spectrum with base peaks of 2787.9 (M + 2H, [2787.1 calc]) and 1859.5 (M + 3H, [1858.4 calc]). Lyophilization of these fractions yielded 4.2 mg (53 %) of **16**.

Purified glycopeptide **16**: UV and MS detection left top, MS spectrum right top (triple ion) and left bottom (double ion)

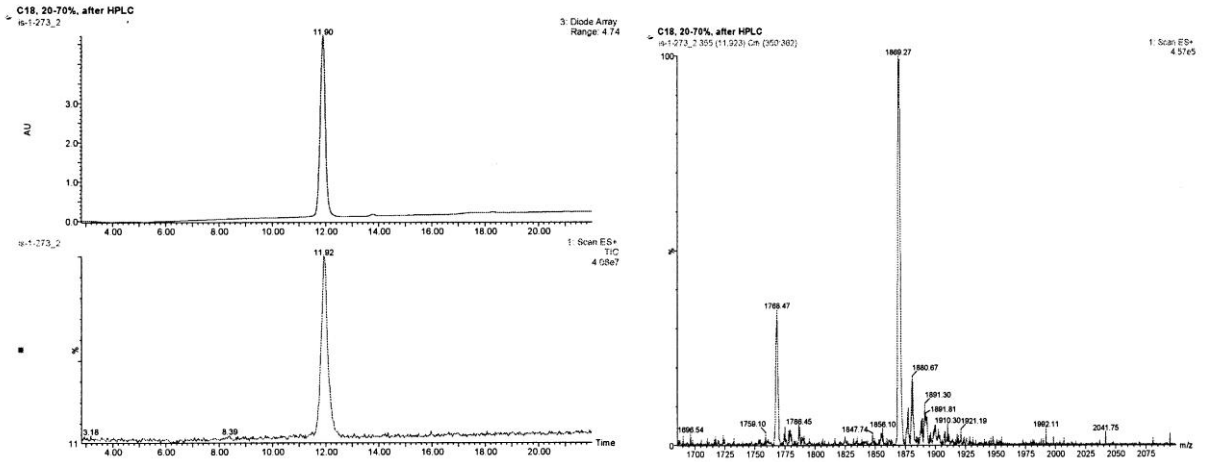


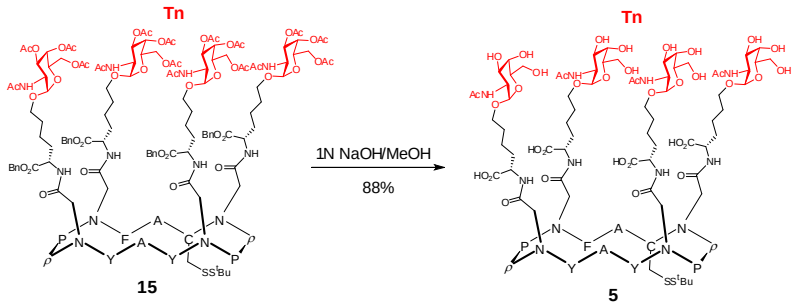
General procedure for global deprotections of 14, 15, and 16: The protected glycopeptide was treated with 1N aq. NaOH (500 µL) and MeOH (750 µL). The resulting mixture was stirred for 14 hours, which was then acidified with 10% aq. HCl (500 µL). This was purified by preparative reverse-phase HPLC.



14 4

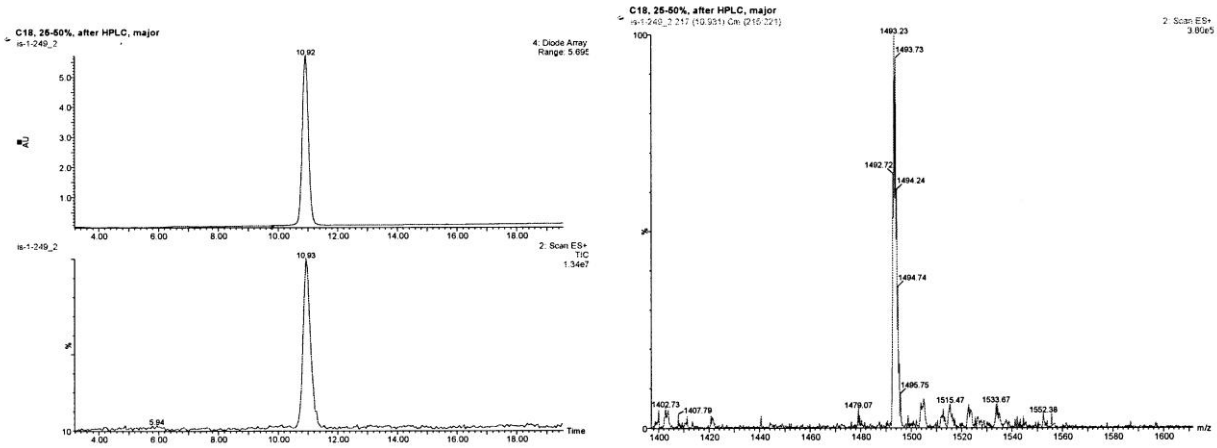
Hexavalent Glycopeptide 4: 2.3 mg of the protected hexavalent glycopeptide **14** was used. Hexavalent glycopeptide **4** was purified in two batches by preparative reverse-phase HPLC using a gradient of 20-70%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for hexavalent Glycopeptide **4** was 13 minutes. LC/MS analysis (20-70%B over 30 min, same solvent system as above, flow rate 0.2mL/min) showed the hexavalent glycopeptide **4** at 12 minutes and MS spectrum with base peaks of 1869.3 (M + 2H, [1868.8 calc]). Lyophilization of these fractions yielded 1.5 mg (88 %) of **4**.
Purified glycopeptide **4**: UV and MS detection left, MS spectrum right (double ion)

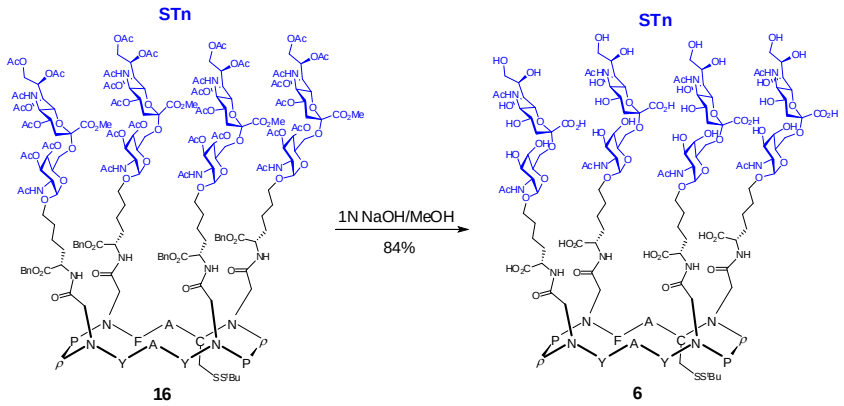




Tetravalent Glycopeptide 5: 5.9 mg of the protected tetravalent glycopeptide **15** was used. Tetravalent glycopeptide **5** was purified in two batches by preparative reverse-phase HPLC using a gradient of 25-50%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for tetravalent glycopeptide **5** was 13 minutes. LC/MS analysis (25-50%B over 30 min, same solvent system as above, flow rate 0.2mL/min) showed the tetravalent glycopeptide **5** at 11 minutes and MS spectrum with base peaks of 1492.7 (M + 2H, [1492.6 calc]) and 996.0 (M + 3H, [995.4 calc]). Lyophilization of these fractions yielded 4 mg (88 %) of **5**.

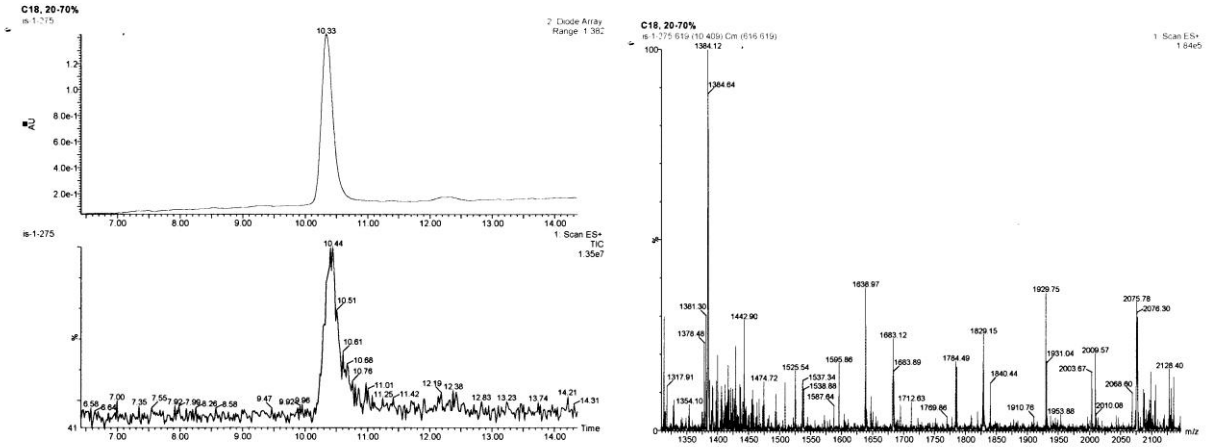
Purified glycopeptide **5**: UV and MS detection left, MS spectrum right (double ion)

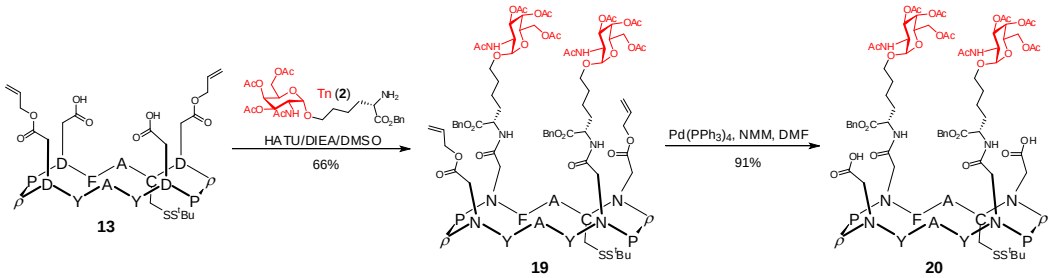




Tetravalent Glycopeptide 6: 2.5 mg of the protected tetravalent glycopeptide **16** was used. Tetravalent glycopeptide **6** was purified in two batches by preparative reverse-phase HPLC using a gradient of 20-70%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for tetravalent glycopeptide **6** was 11 minutes. LC/MS analysis (20-70%B over 30 min, same solvent system as above, flow rate 0.2mL/min) showed the tetravalent glycopeptide **6** at 10 minutes and MS spectrum with base peaks of 2075.8 (M + 2H, [2074.8 calc]) and 1384.1 (M + 3H, [1383.6 calc]). Lyophilization of these fractions yielded 2.5 mg (84 %) of **6**.

Purified glycopeptide **6**: UV and MS detection left, MS spectrum right (triple ion and double ion)

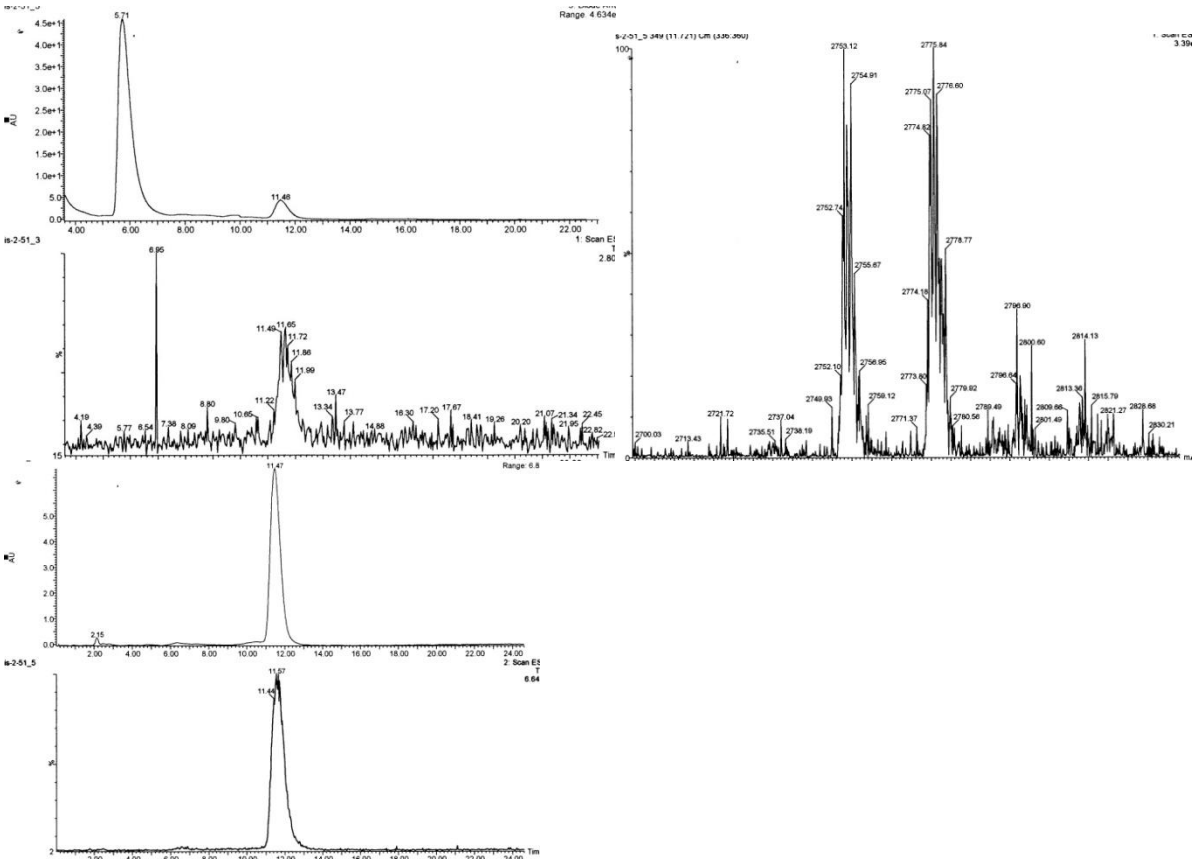


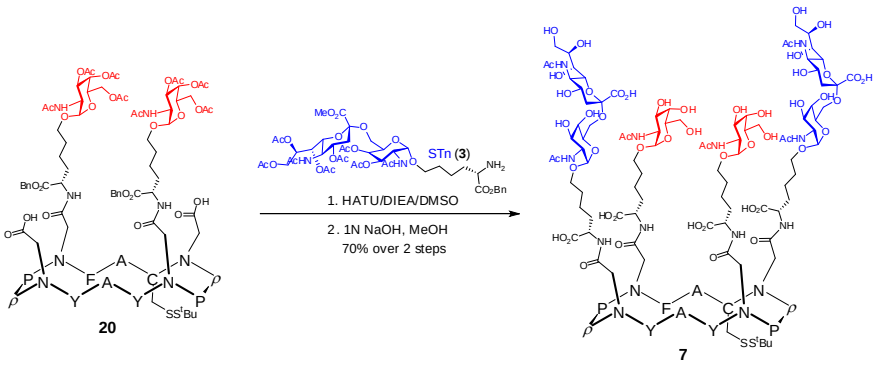


Protected Divalent Glycopeptide 20: Solutions of each reaction participant were prepared with a stir bar in flame-dried vials under argon as follows: Peptide **13** (8.8 mg, 5.07 µmol), Tn **2** (8.6 mg, 15.21 µmol), were dissolved in 1.0mL DMSO. To this mixture, HATU (5.8 mg, 15.21 µmol in 100 µL DMSO) was added, followed by dry *i*Pr_2NEt (8 µL, 45.6 µmol), producing a strong yellow color. After 2 hours, the crude reaction mixture was diluted with ~ 500 µL of 50%B HPLC buffer and, this was purified in two batches by preparative reverse-phase HPLC using a gradient of 5-55%B over 3 minutes then 55-90%B over 30 minutes, flow rate 16

mL/min, 235 nm UV detection. Retention time for diallyl divalent glycopeptide **19** was 14 minutes. LC/MS analysis of the crude reaction mixture (55-75%B over 30 min, same solvent system as above, Microsorb C18, 300-5, 2 x 150 mm, flow rate 0.2 mL/min) showed the diallyl divalent glycopeptides **19** at 9.5 minutes and MS spectrum with base peaks of 2833.14 (M + H, [2832.18 calc]) and 2855.23 (M + Na, [2854.15 calc]). Lyophilization of these fractions yielded 9.5 mg (66 %) of diallyl divalent glycopeptide. This construct was then treated with 4-methlylmorpholine (2.7 μL, 24 μmol) and tetrakis(triphenylphosphine)palladium (8.1 mg, 7 μmol) in 1 mL DMF. After 2 hours, this was purified in two batches by preparative reverse-phase HPLC using a gradient of 45-70%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for protected divalent glycopeptides **20** was 14 minutes. LC/MS analysis of the crude reaction mixture (45-70%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the protected divalent glycopeptides **20** at 11.5 minutes and MS spectrum with base peaks of 2752.74 (M + H, [2752.11 calc]) and 1376.74 (M + 2H, [1376.56 calc]). Lyophilization of these fractions yielded 8.4 mg (91 %) of **20**.

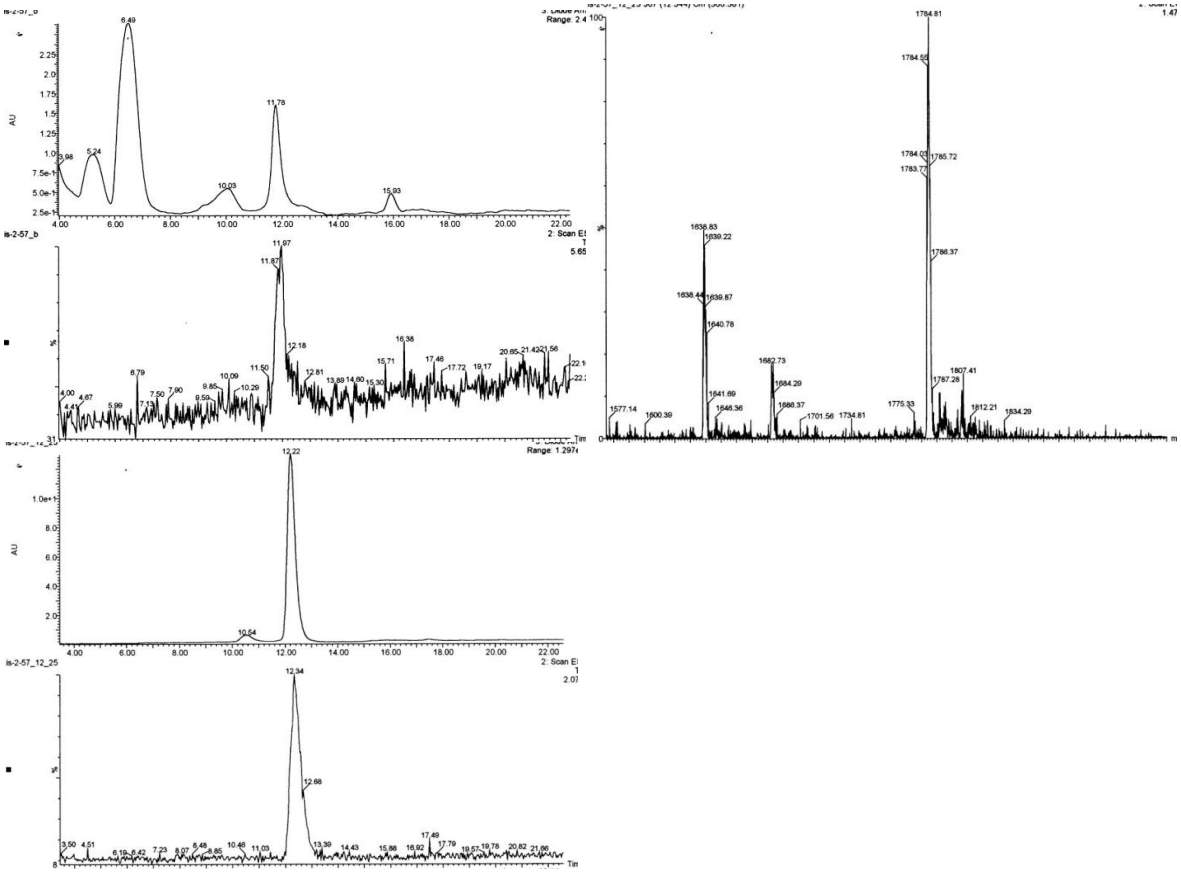
LCMS of **20**: UV and MS detection left top, MS spectrum right top (single ion), LCMS after purification left bottom

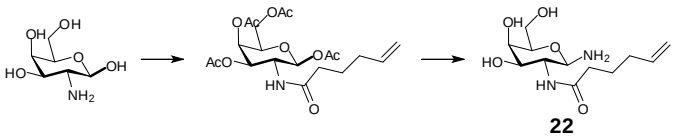




Tetravalent Glycopeptide 7: Solutions of each reaction participant were prepared with a stir bar in flame-dried vials under argon as follows: Glycopeptide **20** (2.1 mg, 0.76 μmol), STn **3** (2.3 mg, 2.3 μmol), were dissolved in 1.0mL DMSO. To this mixture, HATU (0.87 mg, 2.3 μmol in 100 μL DMSO) was added, followed by dry *i*Pr_2NEt (1.6 μL, 9.2 μmol), producing a strong yellow color. After 3 hours, LC/MS analysis of the crude reaction mixture (50-80%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the protected tetravalent glycopeptides **21** at 15 minutes and MS spectrum with base peaks of 2356.75 (M + 2H, [2355.94 calc]) and 1571.99 (M + 3H, [1570.96 calc]). After the removal of solvent by vigorous stream of air, this was subjected to global deprotection under the condition described above. This was then purified by preparative reverse-phase HPLC using a gradient of 20-70%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for tetravalent glycopeptides **7** was 12 minutes. LC/MS analysis of the crude reaction mixture (20-70%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the tetravalent glycopeptides **7** at 12 minutes and MS spectrum with base peaks of 1784.55 (M + 2H, [1783.74 calc]). Lyophilization of these fractions yielded 1.9 mg (70 %) of **7**.

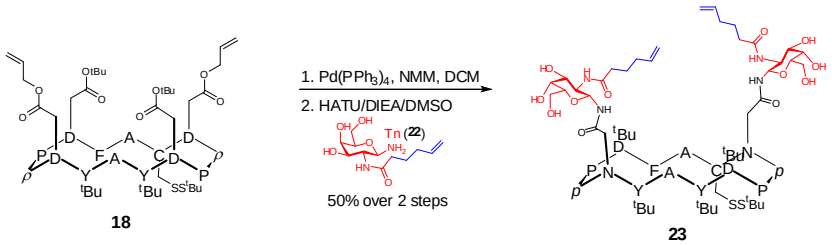
LCMS of **7**: UV and MS detection left top, MS spectrum right top (double ion), LCMS after purification left bottom





22

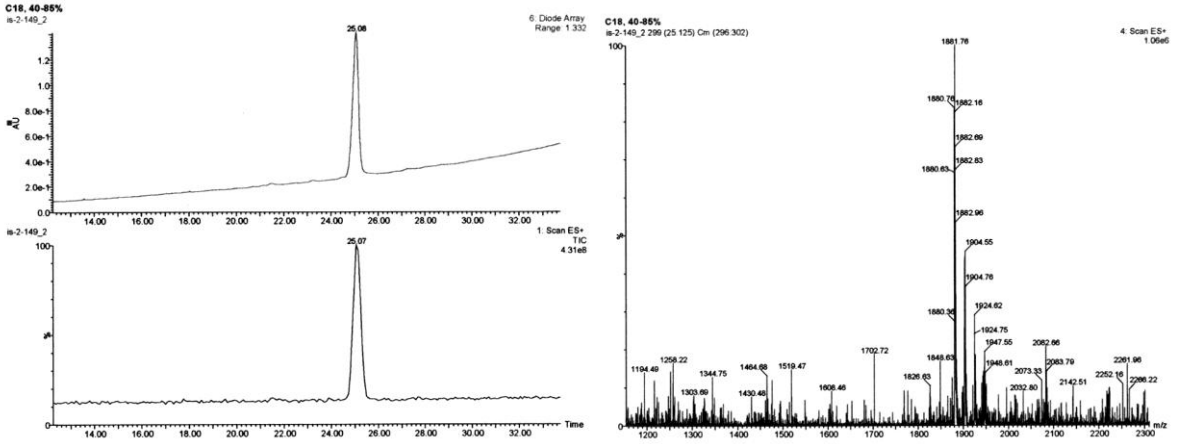
Tn 22: To a solution of 5-hexenoic acid (558 μL, 4.6 mmol) and D-(+)-galactosamine hydrochloride (992 mg, 4.6 mmol) in DMF was added HATU (1.75 g, 4.6 mmol) followed by dry *i*Pr_2NEt (4 mL, 23 mmol). After the resulting mixture was stirred overnight, pyridine (8.2 mL, 101.2 mmol) and acetic anhydride (8.7 mL, 92 mmol) were added. The mixture was stirred for 20 h at r.t. then diluted with cold water and EtOAc, washed with 10% aqueous HCl, water, $NaHCO_3$ saturated aqueous solution, water, brine, dried over anhydrous $MgSO_4$ and filtered. The organic layer was concentrated and residue purified by silica gel column chromatography (40-50% ethyl acetate/hexane) to yield 1.16 g (57% over two steps) of the fully protected Tn as α and β mixtures.). ^{1}H NMR (500 MHz, $CDCl_3$) selected signals: δ 5.72-5.63 (m, 1H), 5.30-5.24 (m, 1H), 5.14-5.11 (m, 1H), 4.95-4.89 (m, 2H), 4.17-4.14 (m, 2H), 4.06-4.02 (m, 2H), 2.09-1.92 (m, 14H). LRMS (ESI) calcd for $C_{20}H_{29}NO_{10}Na^+$ $[M + Na]^+$ 466.2, found 466.7. This protected Tn was dissolved in MeOH (10 mL) then treated with sodium methoxide in MeOH (25%, 50 μL) and stirred for 12 h. The residue was neutralized with Dowex 50WX8-400 ion exchange resin, filtered on celite pad, and concentrated. The crude product (720 mg) in saturated aqueous H_4HCO_3 (20 mL) was heated to 40 ^{o}C for 2 days and Mass spectrum indicated that reaction is complete. The reaction mixture was frozen and lyophilized. The residue was dissolved in 10 mL water, frozen and lyophilized again. This process was repeated until the weight of the residue is constant (700 mg, 98% over two steps). ^{1}H NMR (600 MHz, DMSO): δ 5.82-5.76 (m, 1H), 5.03-4.93 (m, 2H), 4.53-4.45 (m, 1H), 3.65-3.62 (m, 1H), 3.50-3.32 (m, 5H), 2.10-2.00 (m, 4H), 1.63-1.54 (m, 2H). ^{13}C NMR (150 MHz, DMSO) δ 172.79, 138.49, 114.95, 86.00, 75.76, 72.15, 67.66, 60.60, 53.29, 35.12, 32.65, 24.54. LRMS (ESI) calcd for $C_{12}H_{22}N_2O_5Na^+$ $[M + Na]^+$ 297.1, found 297.8.



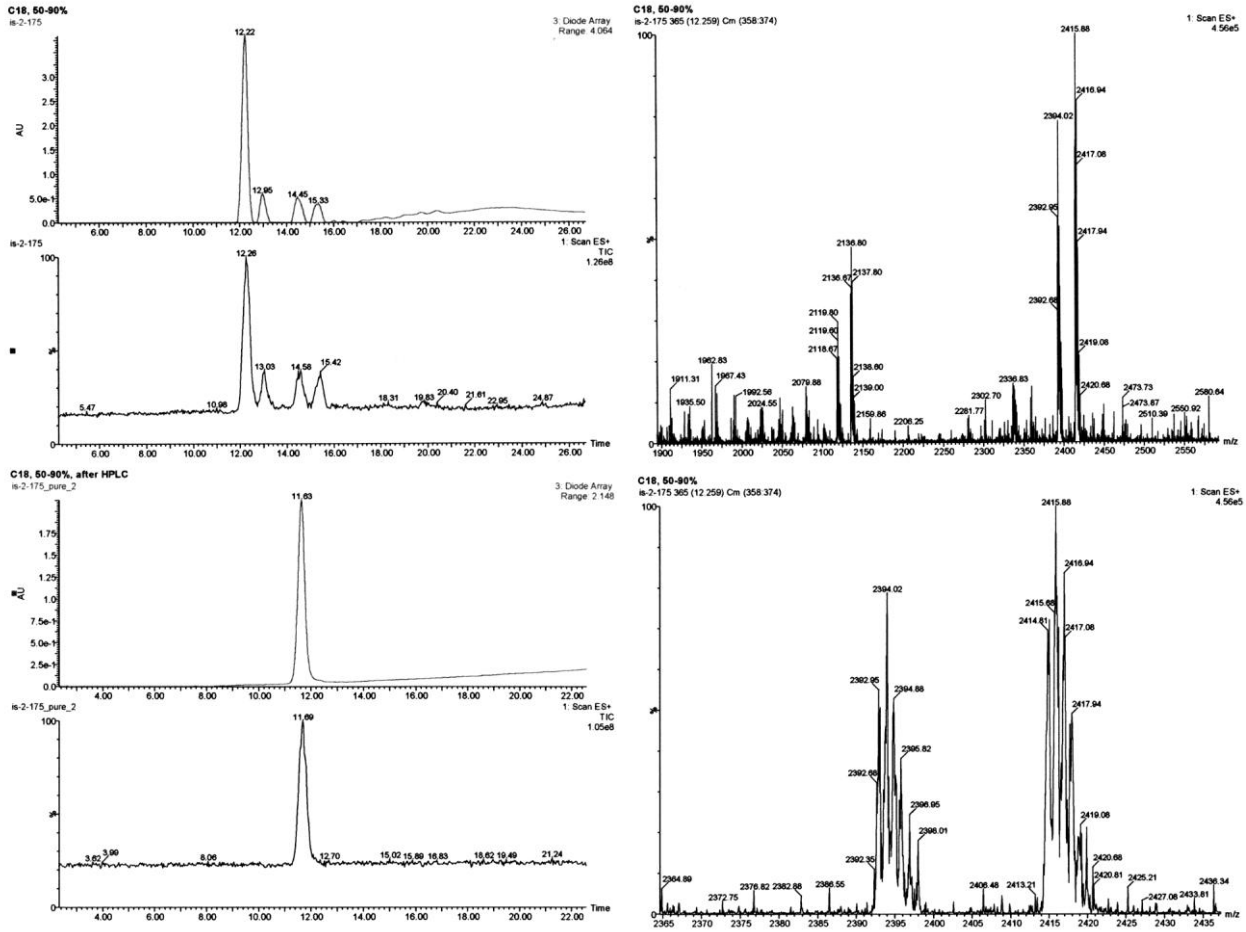
18 23

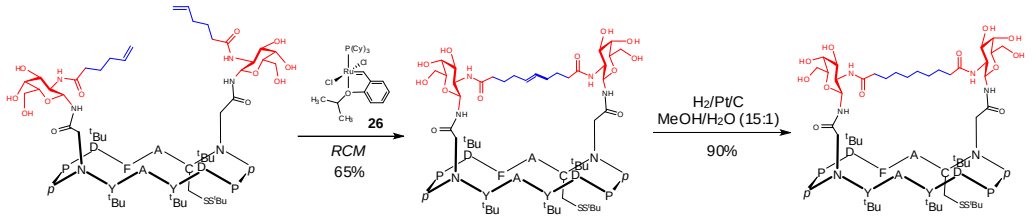
Divalent Glycopeptide 23: Cyclic peptide **18** (169.6 mg, 86.5 μmol) was then treated with 4-methylmorpholine (29 μL, 43.3 μmol) and tetrakis(triphenylphosphine)palladium (50 mg, 43.3 μmol) in 10 mL CH_2Cl_2 and stirred overnight. This was purified by silica gel column chromatography (1% acetic acid/10% MeOH/89%CH_2Cl_2) to yield 154.5 mg (95% yield) of the deprotected cyclic peptide. A small aliquot (5 mg) of the deprotected cyclic peptide was further purified using preparative reverse-phase HPLC using a gradient of 50-90%B over 20 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for the deprotected cyclic peptide was 19.9 minutes. LC/MS analysis (40-85%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the deprotected cyclic peptide at 25.08 minutes and MS spectrum with base peaks of 1902.7 (M + Na, [1901.9 calc]). To a stirred solution of deprotected peptide (6.7 mg, 3.56 μmol) in 0.9 mL DMSO was added HATU (3.4 mg, 8.91 μmol in 100 μL DMSO) followed by dry *i*Pr_2NEt (7.5 μL, 42.76 μmol), producing a yellow color. Within 30 seconds Tn **22** (2.93 mg, 10.7 μmol) was added, then the mixture was further stirred for 3h. The crude reaction mixture was diluted with ~ 500 μL of 50%B HPLC buffer, and this was purified in two batches by preparative reverse-phase HPLC using a gradient of 50-90%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for the divalent glycopeptide **23** was 15.5 minutes. LC/MS analysis of the crude reaction mixture (50-90%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the divalent glycopeptide **23** at 11.6 minutes and MS spectrum with base peaks of 2392.7 (M + H, [2392.2 calc]). Lyophilization of these fractions yielded 4.5 mg (53 %) of **23**.

Purified deprotected cyclic peptide: UV and MS detection left, MS spectrum right



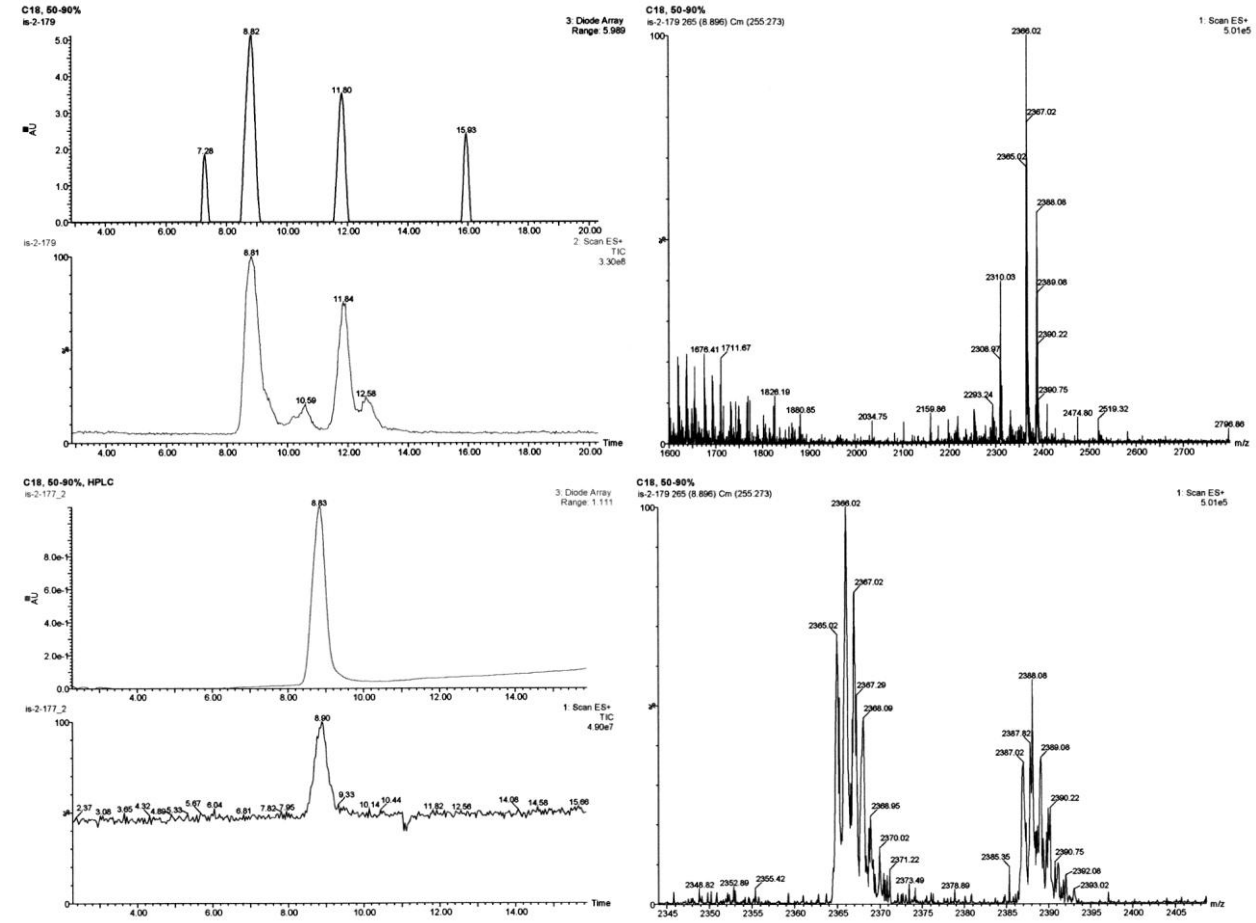
Purified Divalent Glycopeptide **23**: UV and MS detection of the crude left top, MS spectrum right top, UV and MS detection of pure **23** left bottom, MS spectrum expansion right bottom



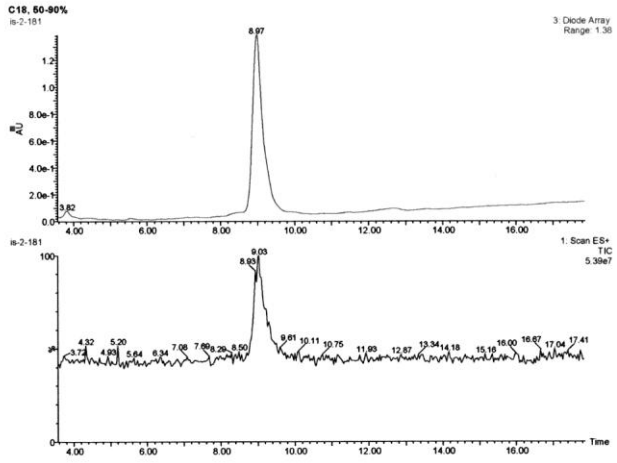


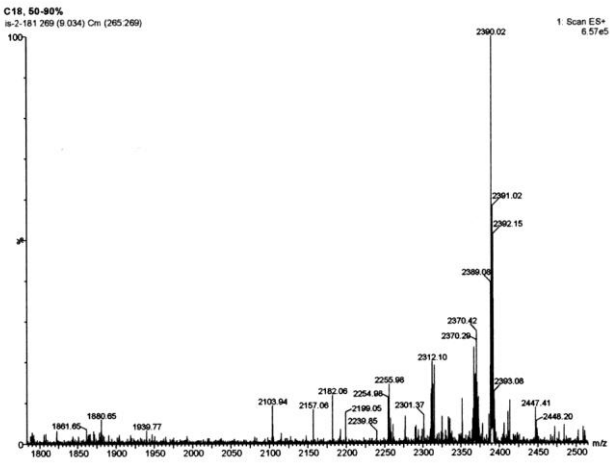
Cross-linked Glycopeptide 28: Divalent glycopeptide **23** (8 mg, 3.34 μmol) in 5 mL CH_2Cl_2/MeOH(4:1) was treated with Hoveyda-Grubbs catalyst **26** (2 mg, 3.34 μmol) and refluxed for 24 h. The resulting mixture was cooled to r.t. and quenched with addition of few drops of DMSO. After evaporation of solvent in vaccuo, the crude reaction mixture was diluted with ~ 1 mL of 50%B HPLC buffer, and this was purified in two batches by preparative reverse-phase HPLC using a gradient of 50-90%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for the RCM product **27** was 12.4 minutes. LC/MS analysis of the crude reaction mixture (50-90%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the RCM product **27** at 8.8 minutes and MS spectrum with base peaks of 2365.0 (M + H, [2364.1 calc]). Lyophilization of these fractions yielded 5.14 mg (65 %) of **27** as the mixture of *E* and *Z* olefins. The catalytic hydrogenation of *E* and *Z* olefins was realized in presence of 10% Pt/C in MeOH/H_2O (15/1) under H_2 for 24 h. After filtration on celite pad, the crude mixture was purified by preparative reverse-phase HPLC using a gradient of 50-90%B over 30 minutes, flow rate 16 mL/min, 235 nm UV detection. Retention time for the cross-linked glycopeptide **28** was 12.5 minutes. LC/MS analysis of the crude reaction mixture (50-90%B over 30 min, same solvent system as above, flow rate 0.2 mL/min) showed the cross-linked glycopeptide **28** at 9.0 minutes and MS spectrum with base peaks of 2388.6 (M + Na, [2388.1 calc]). Lyophilization of these fractions yielded 1.17 mg (90 %) of **28**.

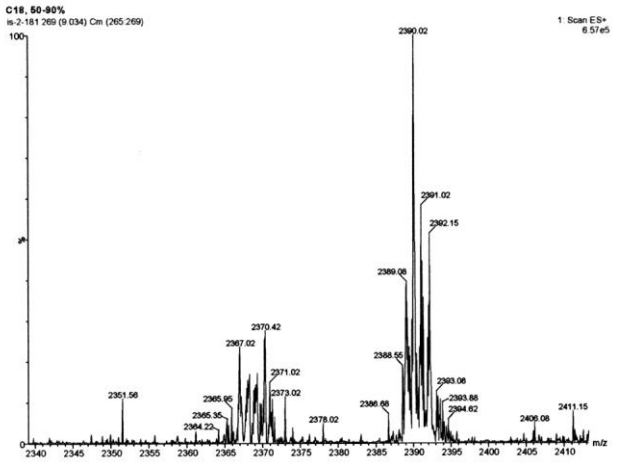
Purified glycopeptide **27**: UV and MS detection of the crude left top, MS spectrum right top, UV and MS detection of pure **23** left bottom, MS spectrum expansion right bottom



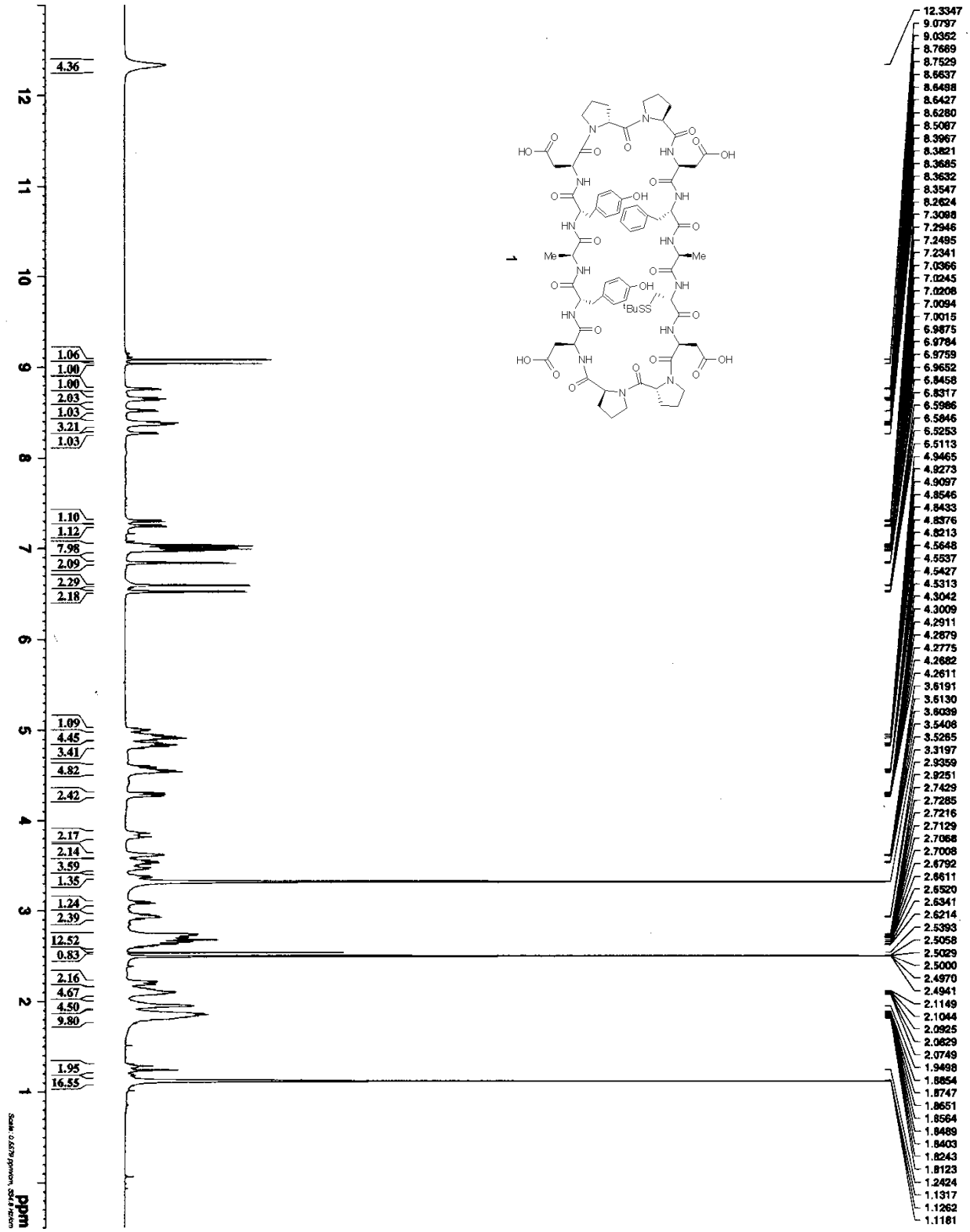
Purified glycopeptide **28**: UV and MS detection of pure **28** left top, MS spectrum right top, MS spectrum expansion left bottom



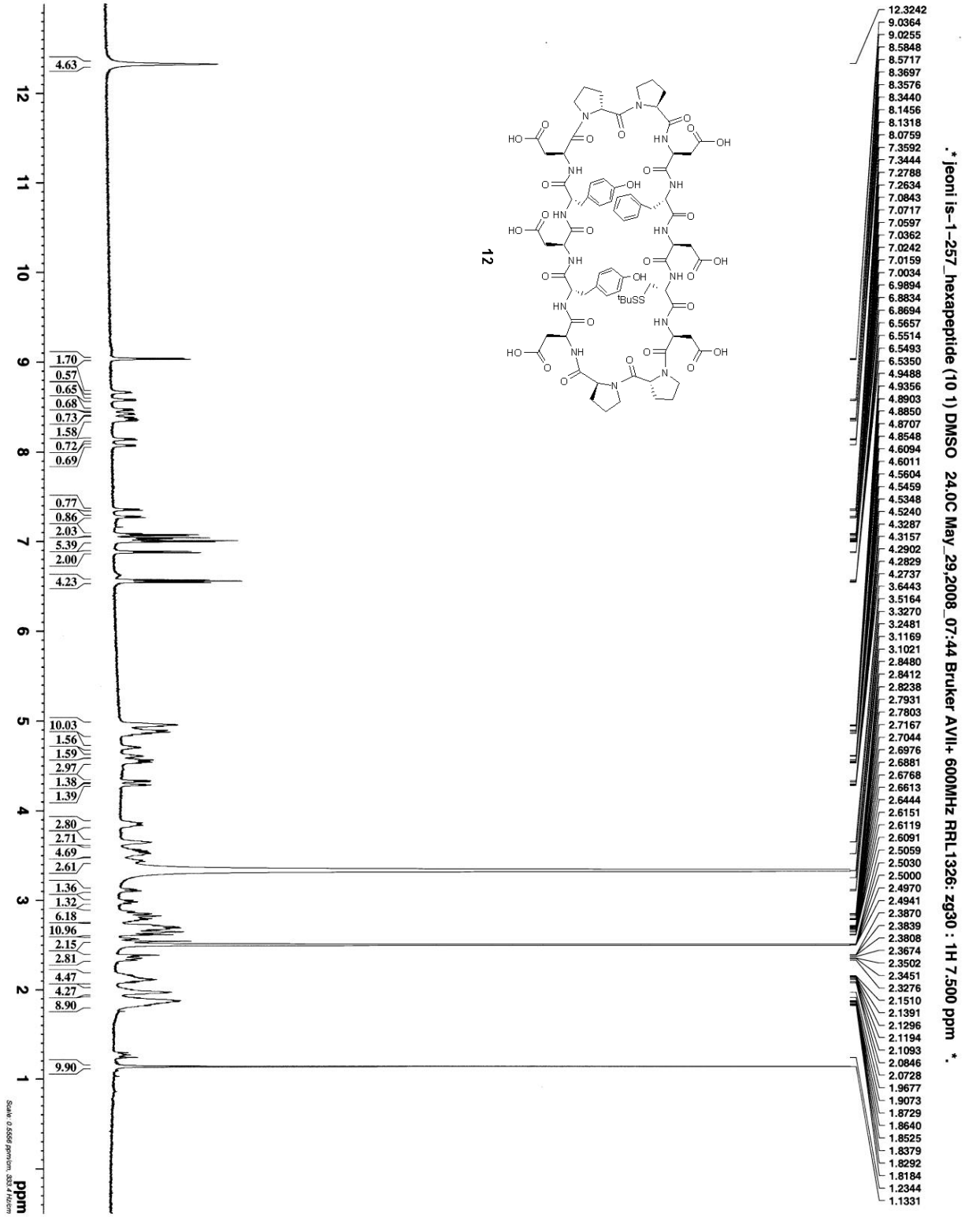




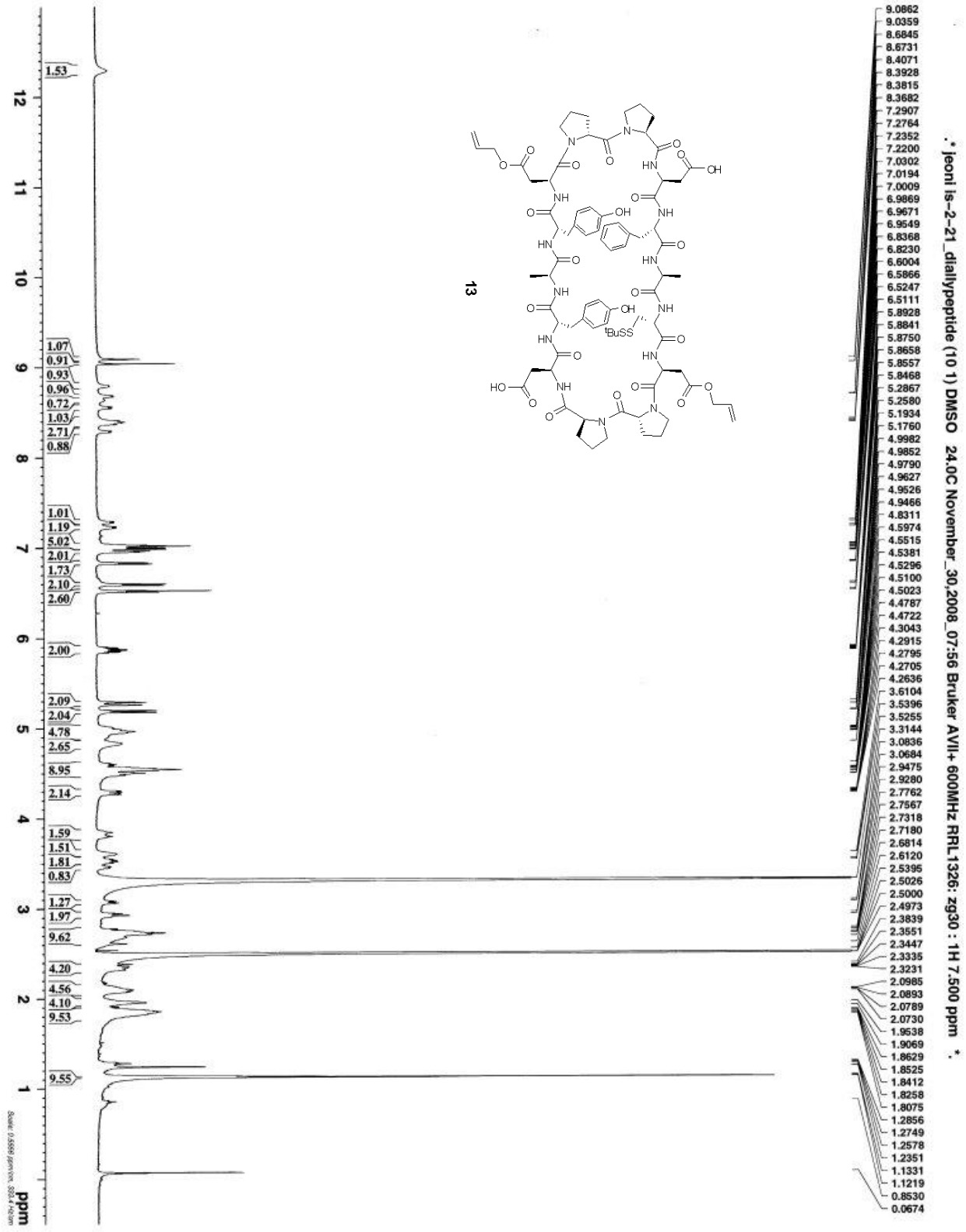
600 MHz ^{1}H NMR spectrum of **1** in d_6-DMSO



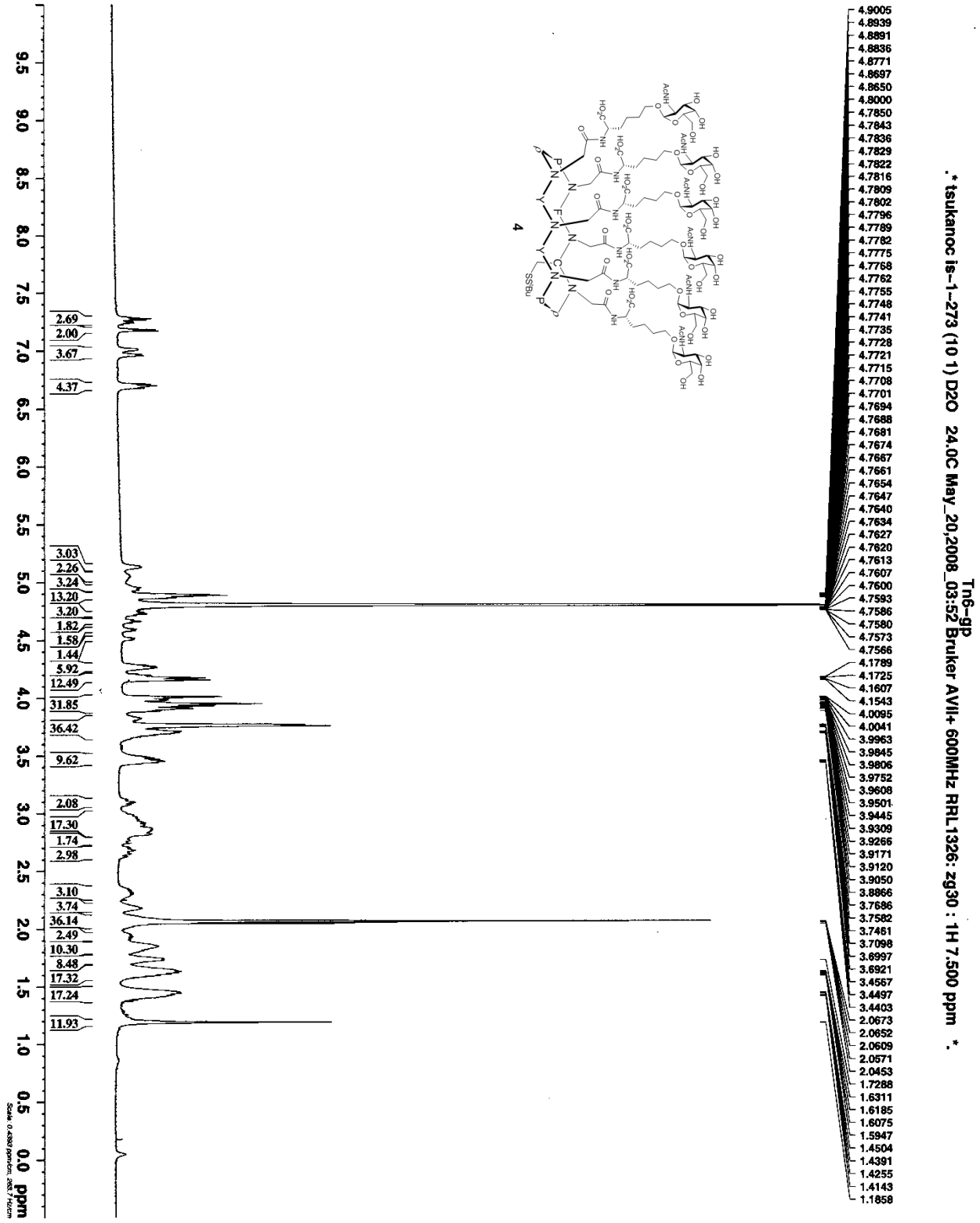
600 MHz ^{1}H NMR spectrum of **12** in d_6-DMSO



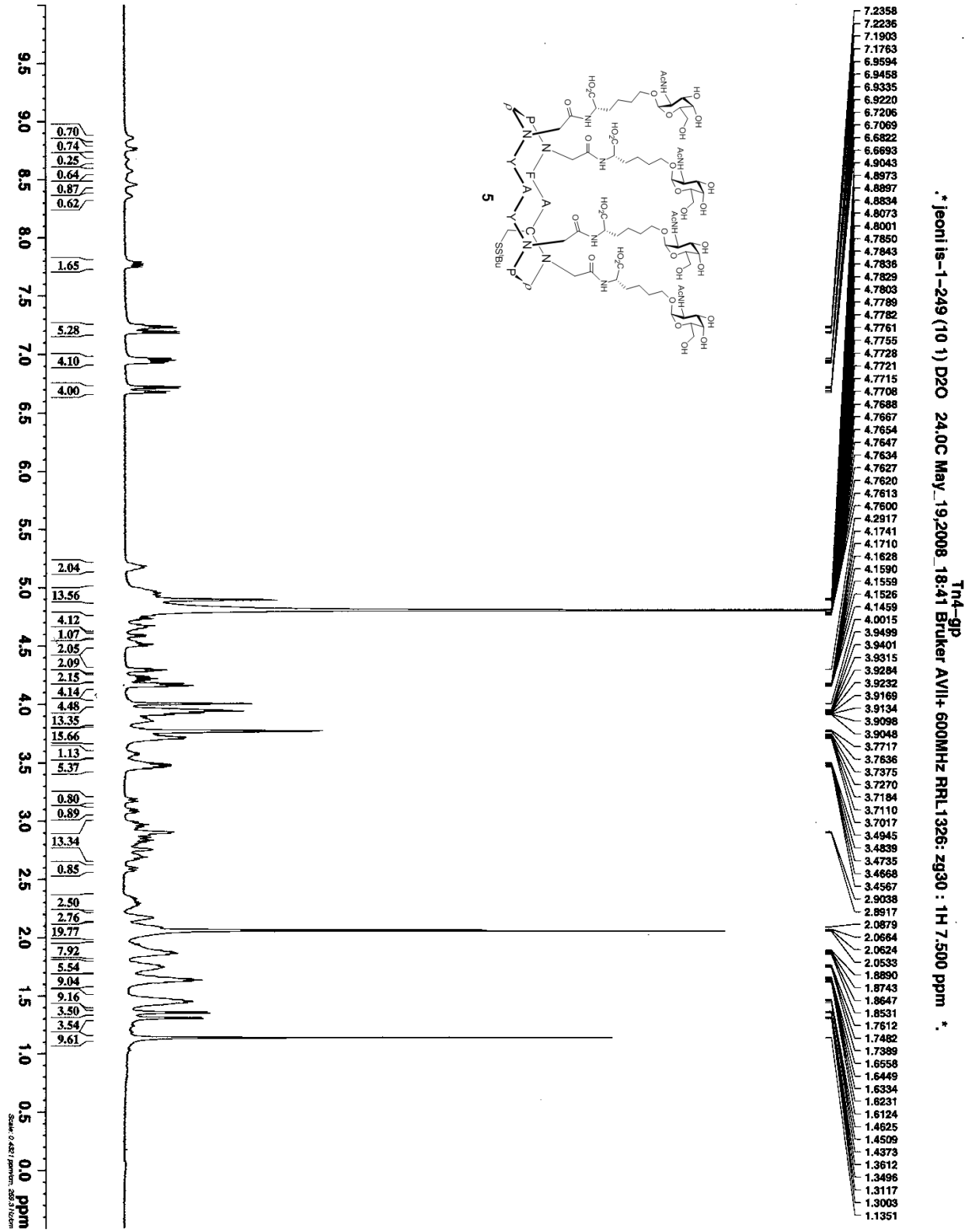
600 MHz ^{1}H NMR spectrum of **13** in d_6-DMSO



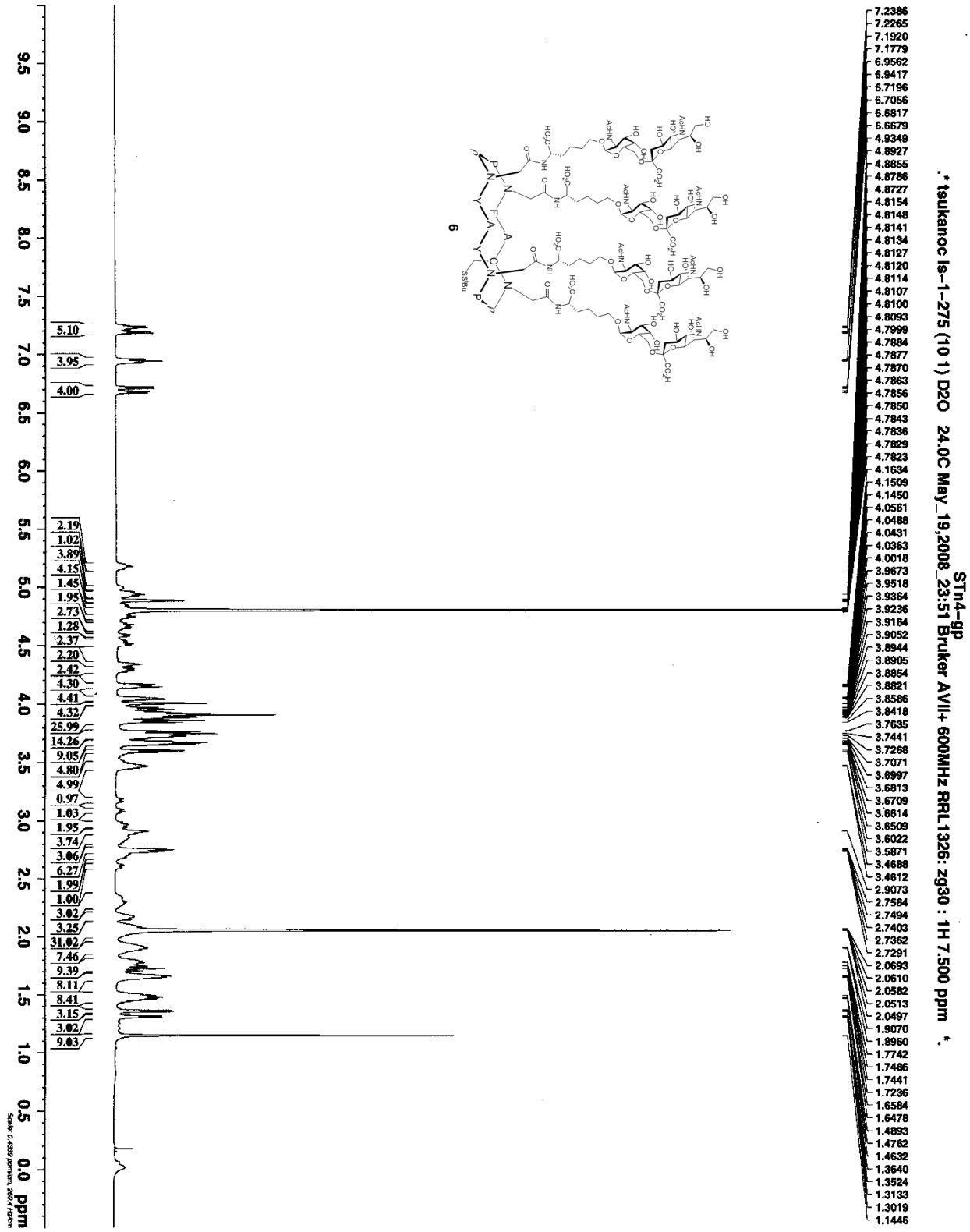
600 MHz ^{1}H NMR spectrum of **4** in D_2O



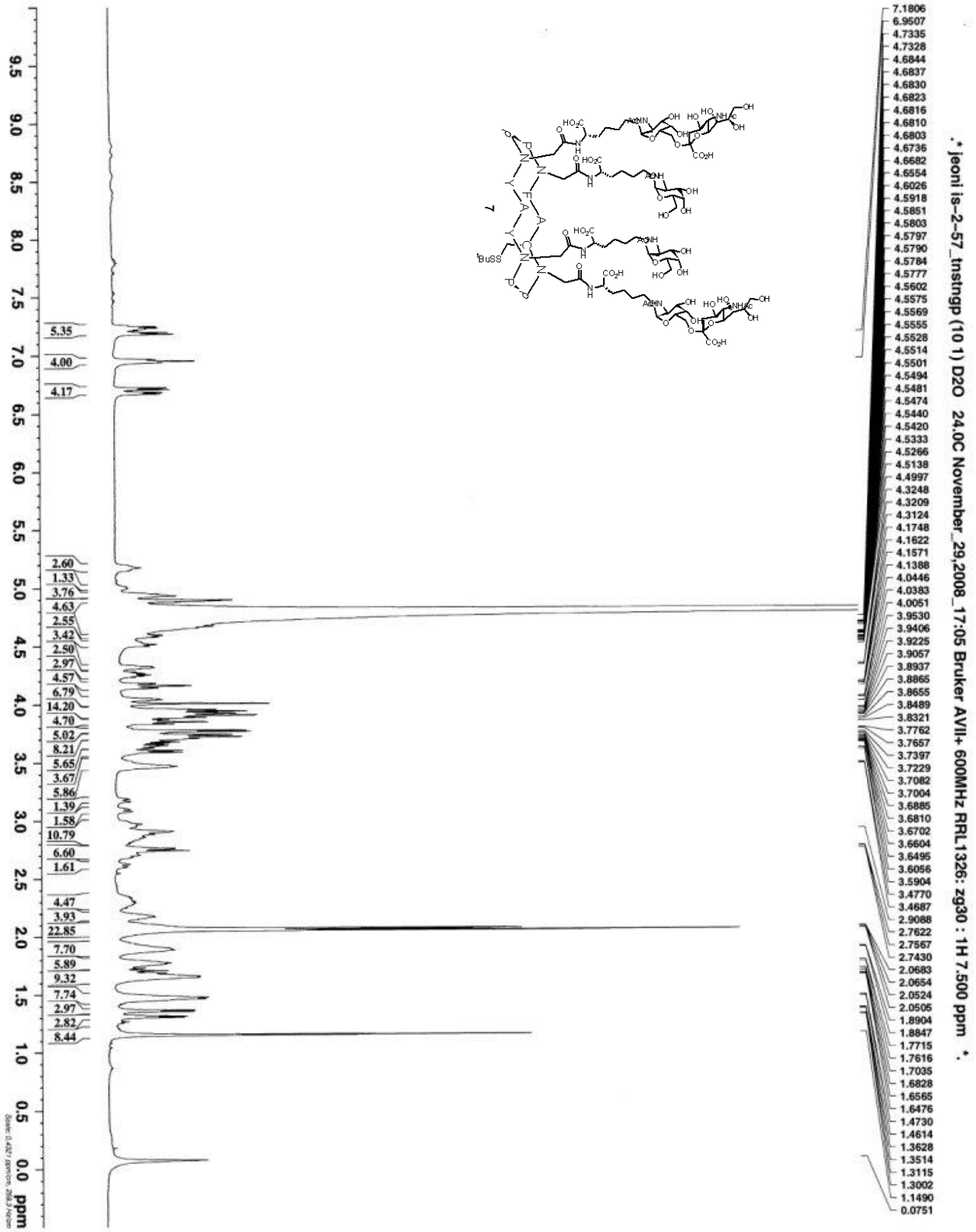
600 MHz ^{1}H NMR spectrum of **5** in D_2O



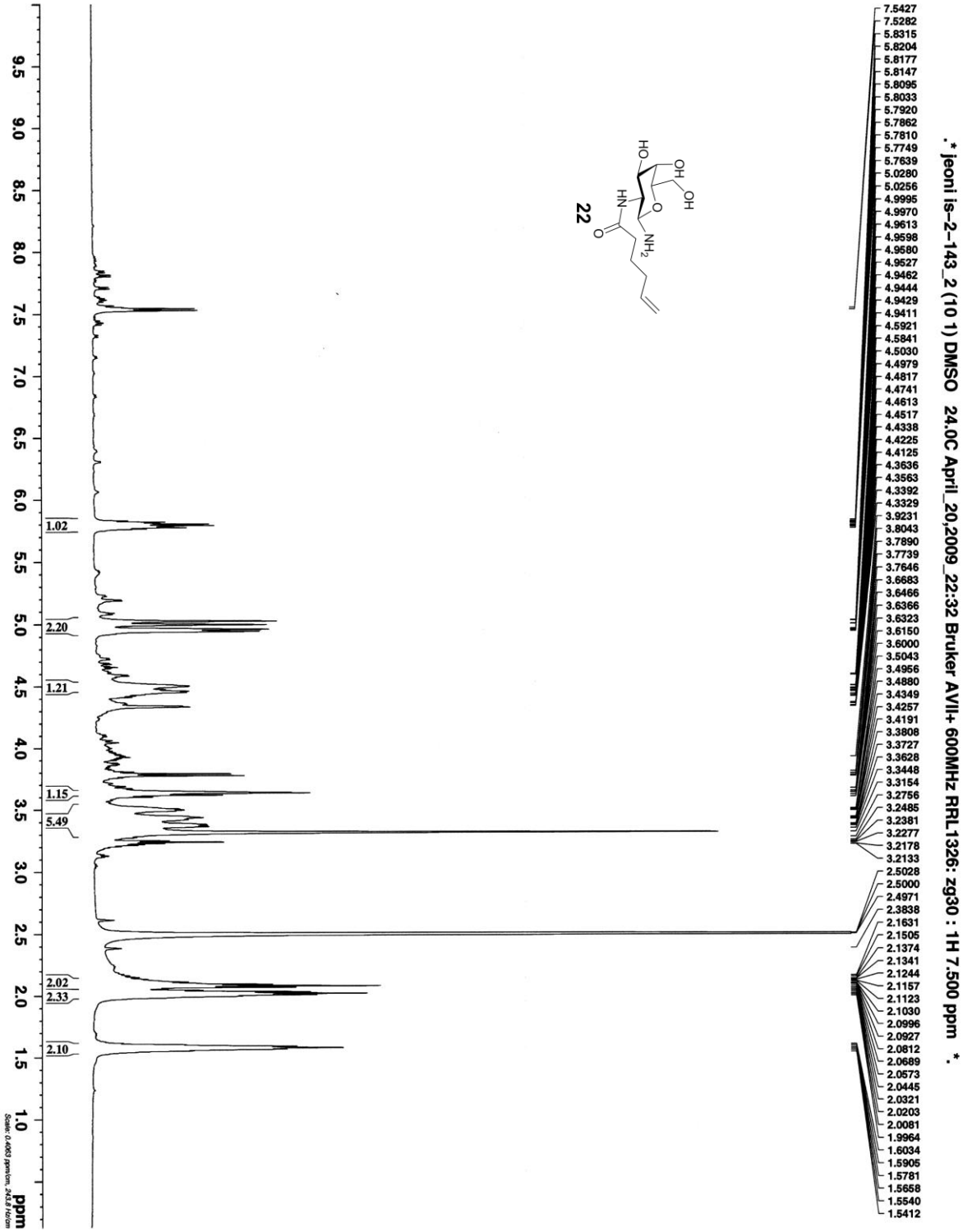
600 MHz ^{1}H NMR spectrum of **6** in D_2O



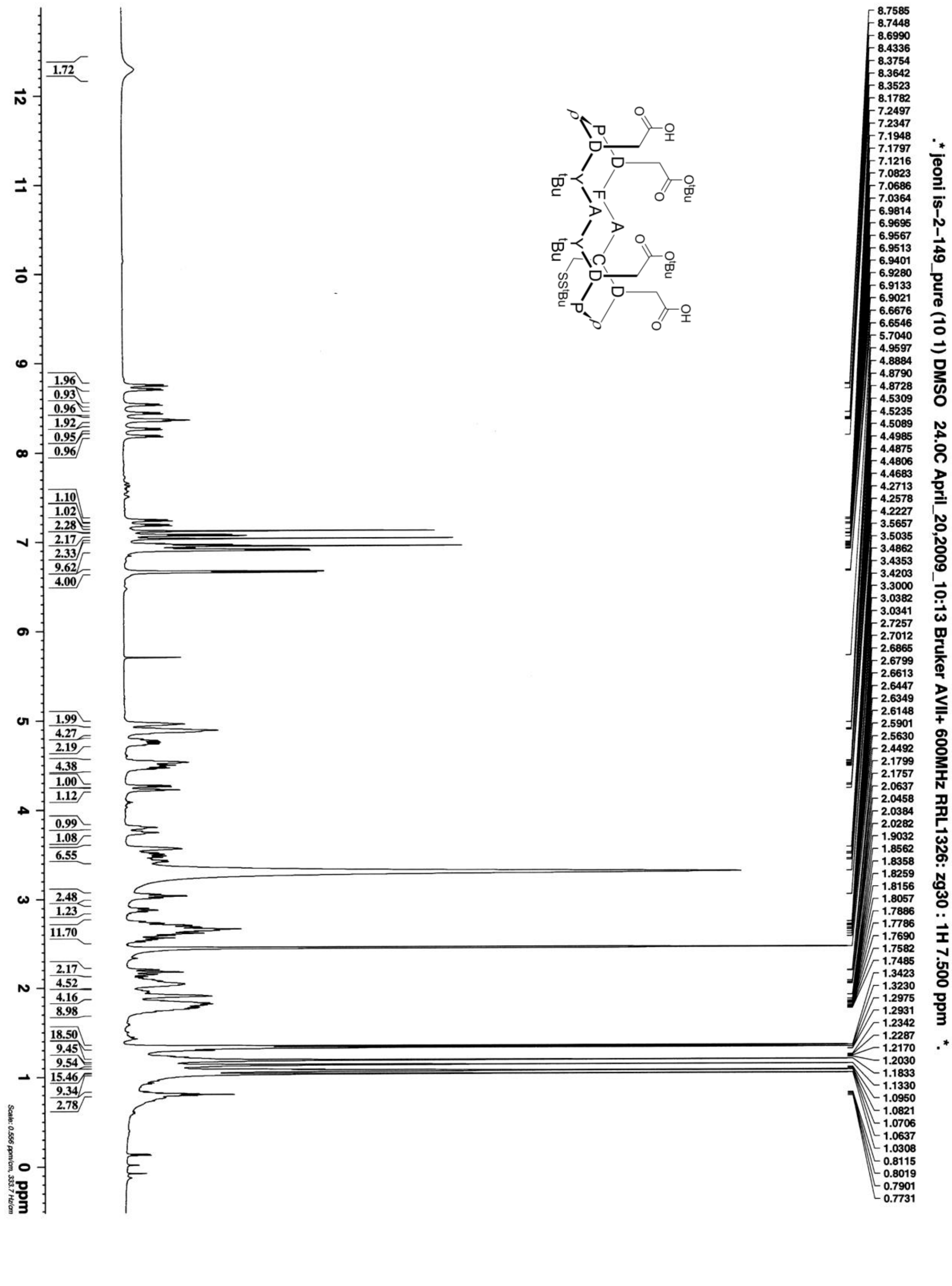
600 MHz ^{1}H NMR spectrum of **7** in D_2O



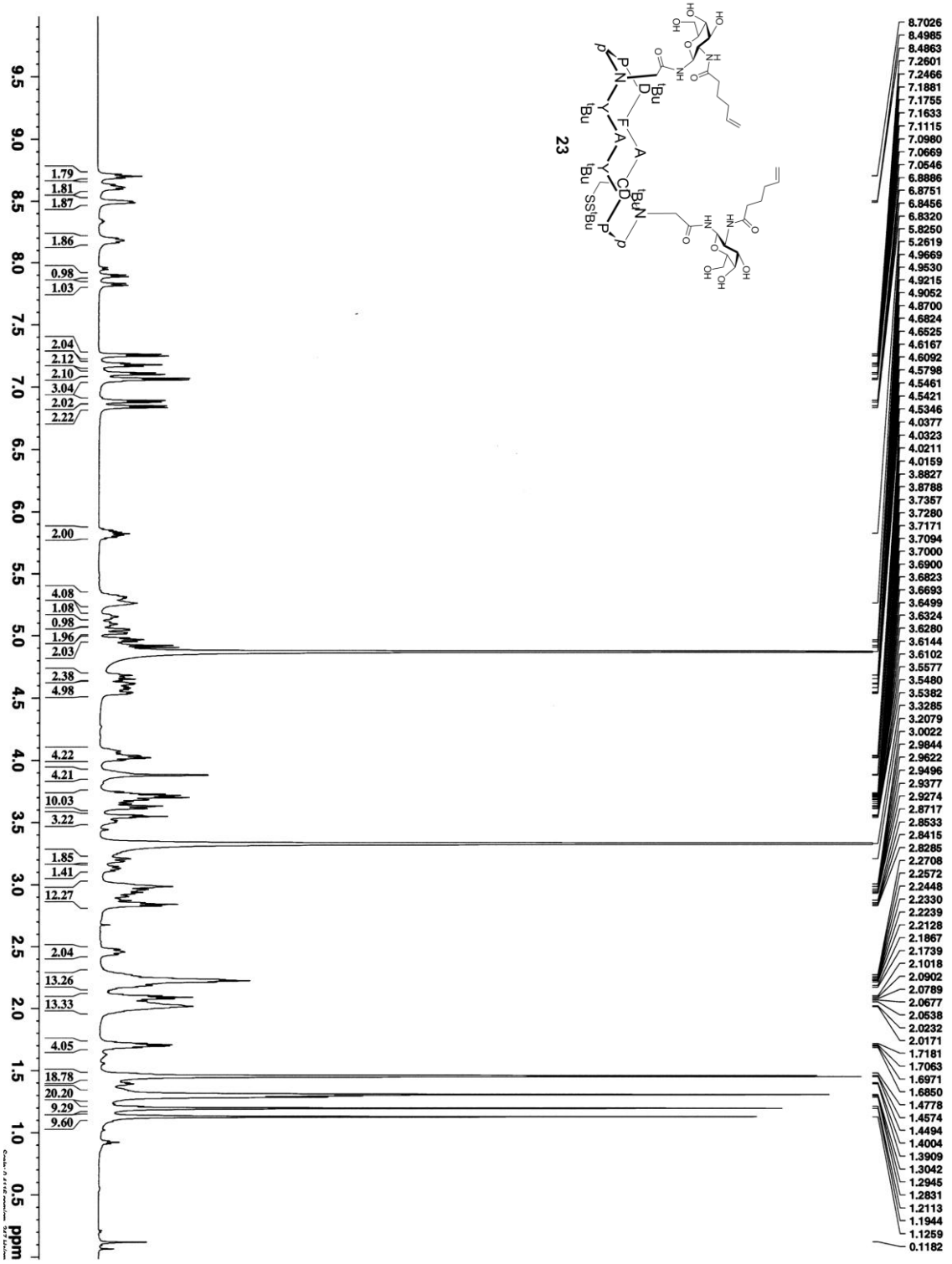
600 MHz ^{1}H NMR spectrum of **22** in d_6-DMSO



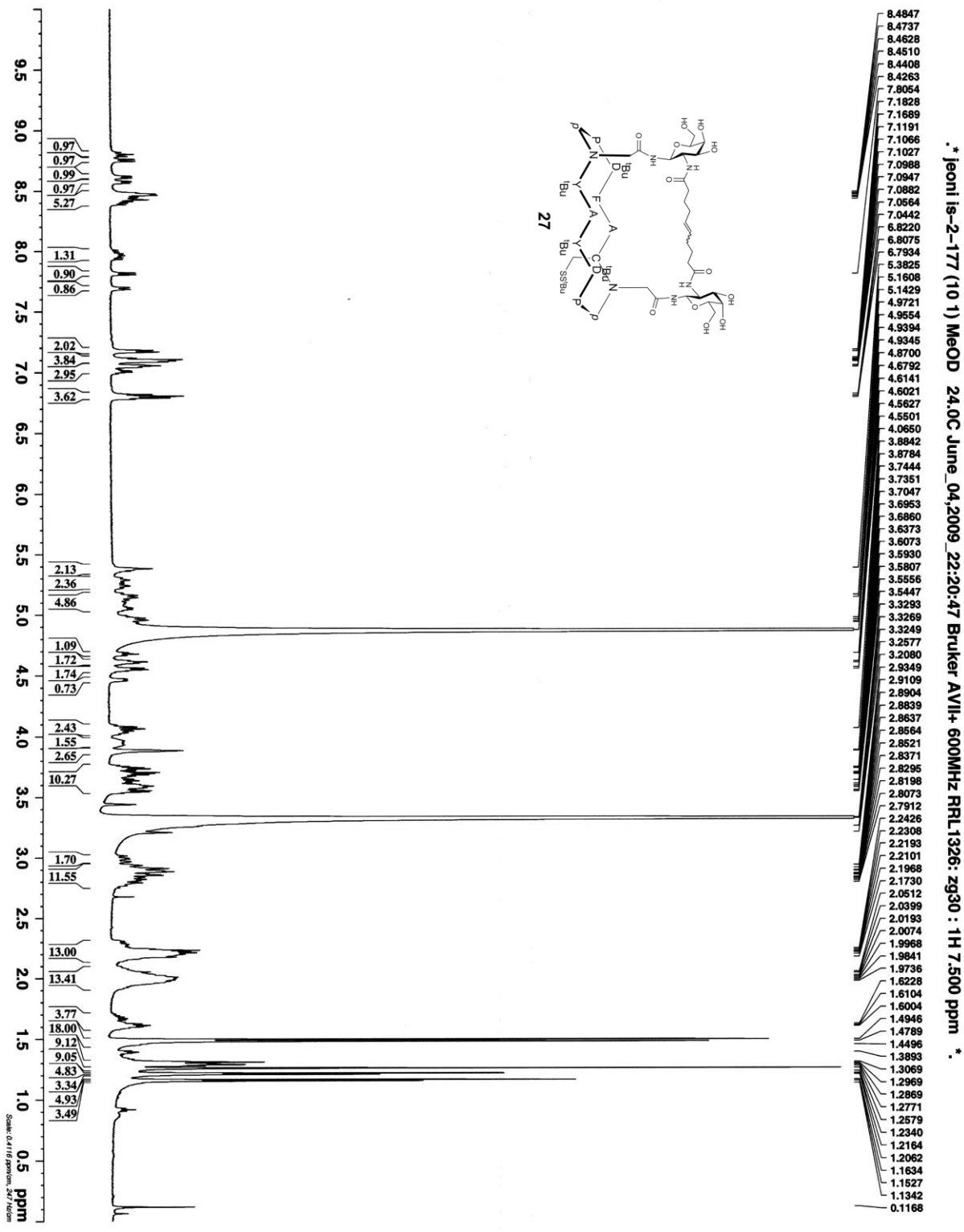
600 MHz ^{1}H NMR spectrum of deprotected cyclic peptide in d_6-DMSO



600 MHz ^{1}H NMR spectrum of **23** in d_4-MeOD



600 MHz ^{1}H NMR spectrum of **27** in d_4-MeOD



600 MHz ^{1}H NMR spectrum of **27** in d_4-MeOD