

CHEMISTRY

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Supporting Information

Heterovalent Glycodendrimers as Epitope Carriers for Antitumor Synthetic Vaccines

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Compound 1

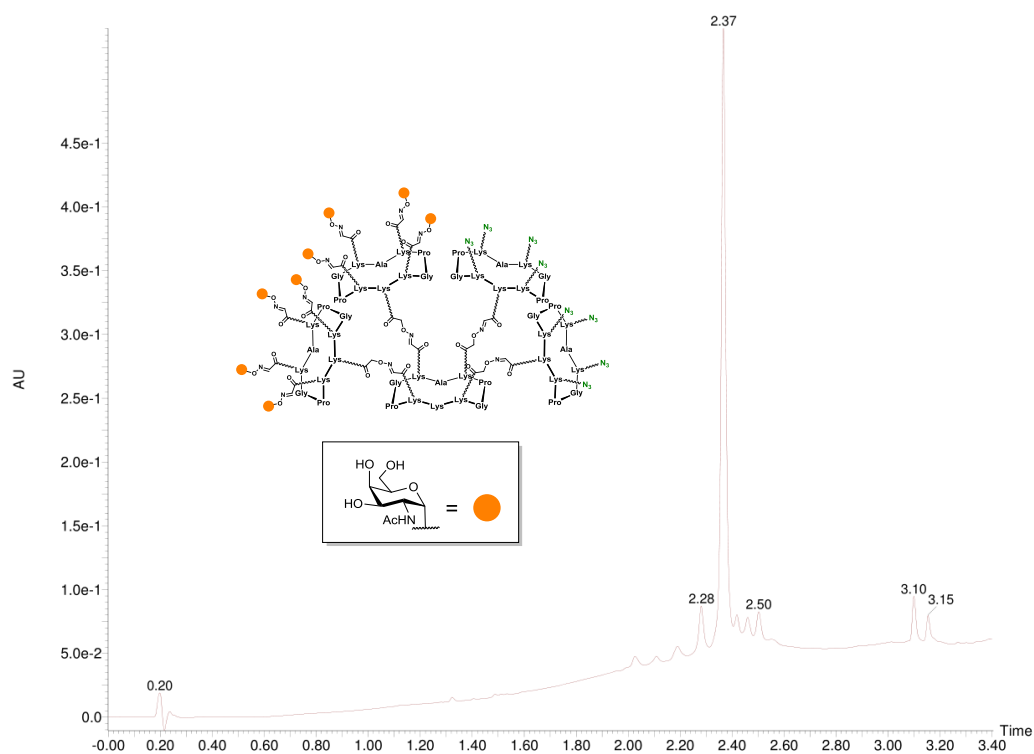


Figure S1. RP-UPLC crude profile of oxime ligation between **27** and **8** at $t=30$ min. Octa-Tn intermediate: $t_R=2.37$ min. (5-60% solv.D in 3.0 min.).

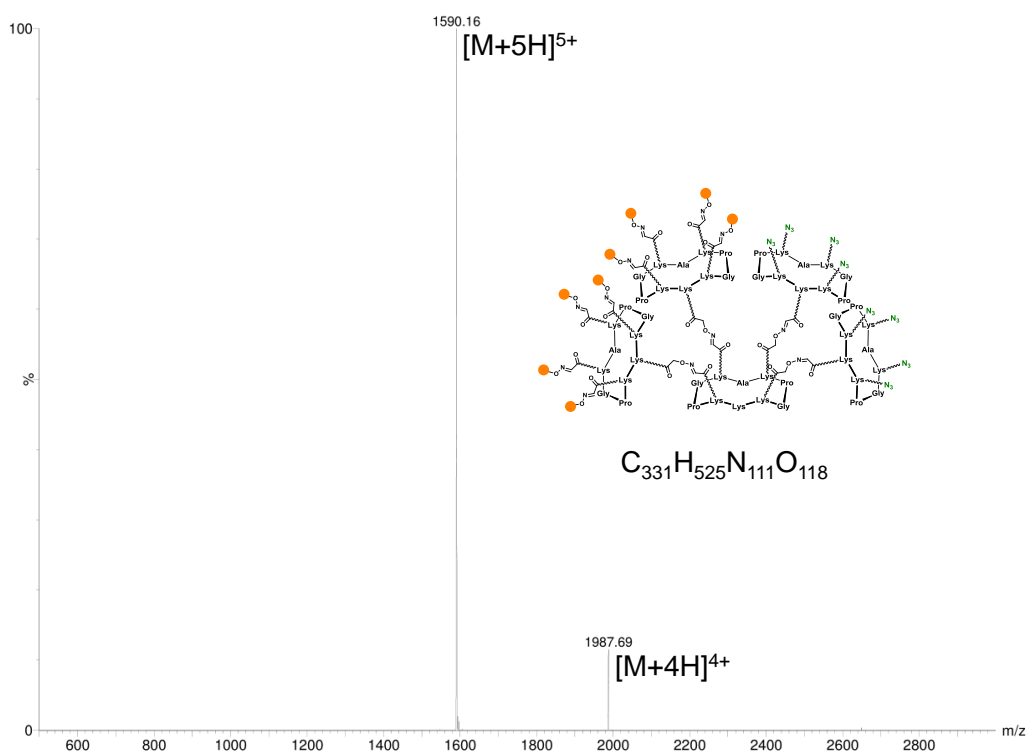


Figure S2. ESI⁺-MS spectrum of Octa-Tn intermediate resulting from oxime ligation between **27** and **8**. m/z (Average Mwt) calcd. for $C_{331}H_{530}N_{111}O_{118}$ $[M+5H]^{5+}$: 1590.5, found: 1590.2; calcd. for $C_{331}H_{529}N_{111}O_{118}$ $[M+4H]^{4+}$: 1987.9, found: 1987.7

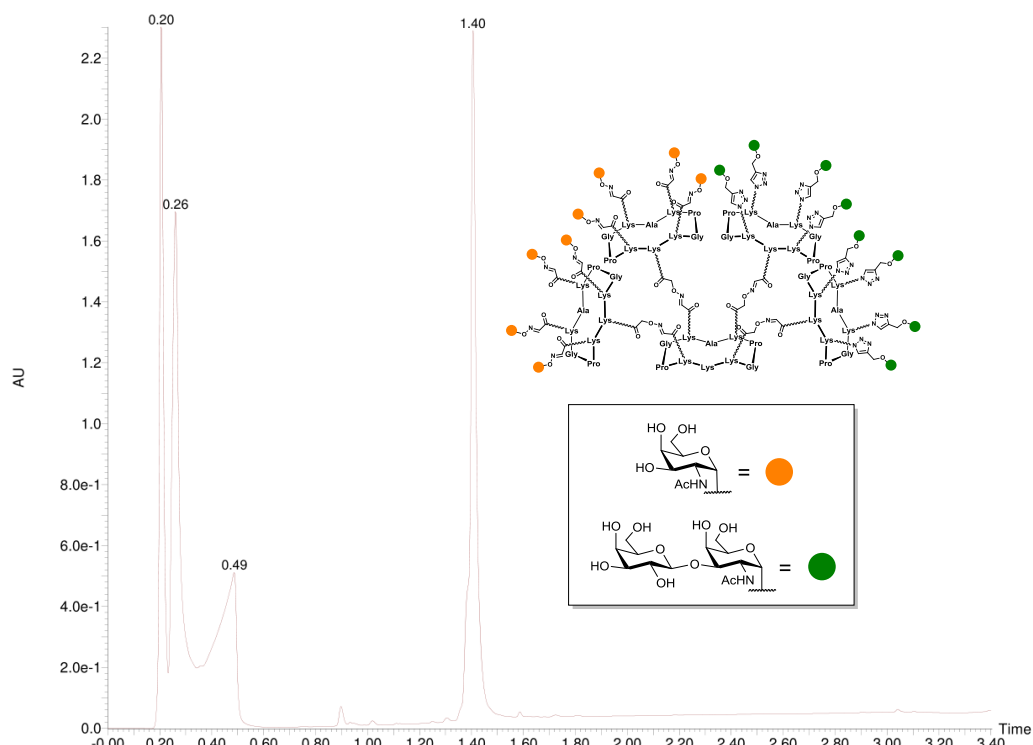


Figure S3. RP-UPLC crude profile of CuAAC ligation at $t=90$ min. Compound **1**: $t_R=1.40$ min. (5-60% solv.D in 3.0 min.).

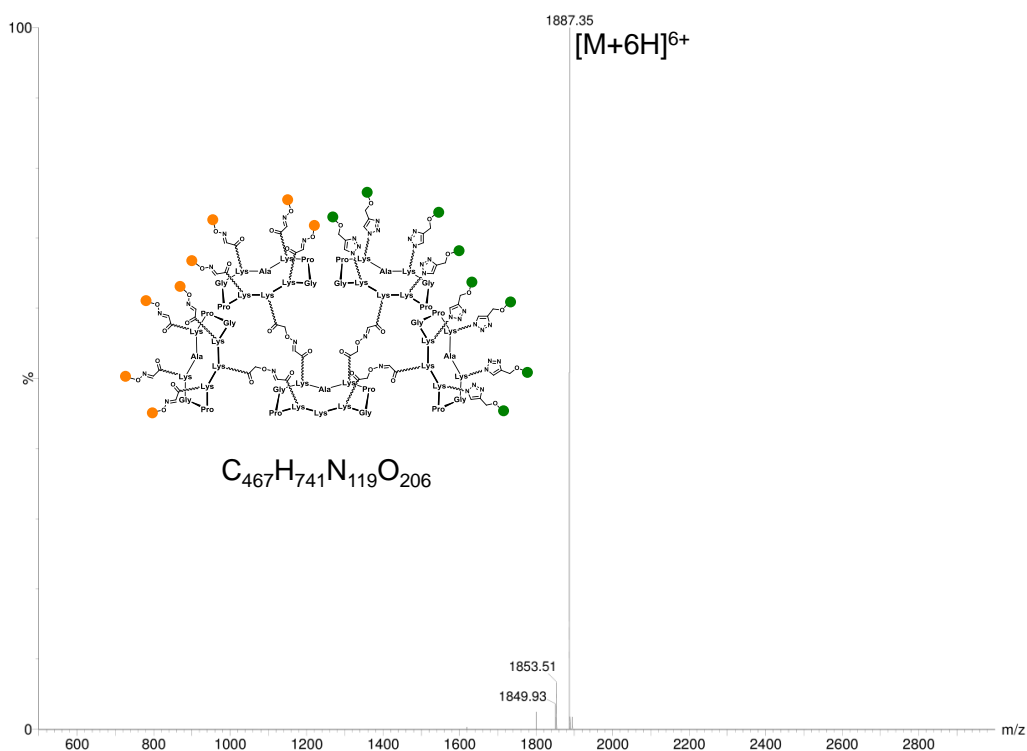


Figure S4. ESI⁺-MS spectrum of **1**. m/z (Average Mwt) calcd. for $C_{467}H_{747}N_{119}O_{206}$ $[M+6H]^{6+}$: 1887.4, found: 1887.4

$^1\text{H-NMR}$ of **1** in D_2O at 400 MHz

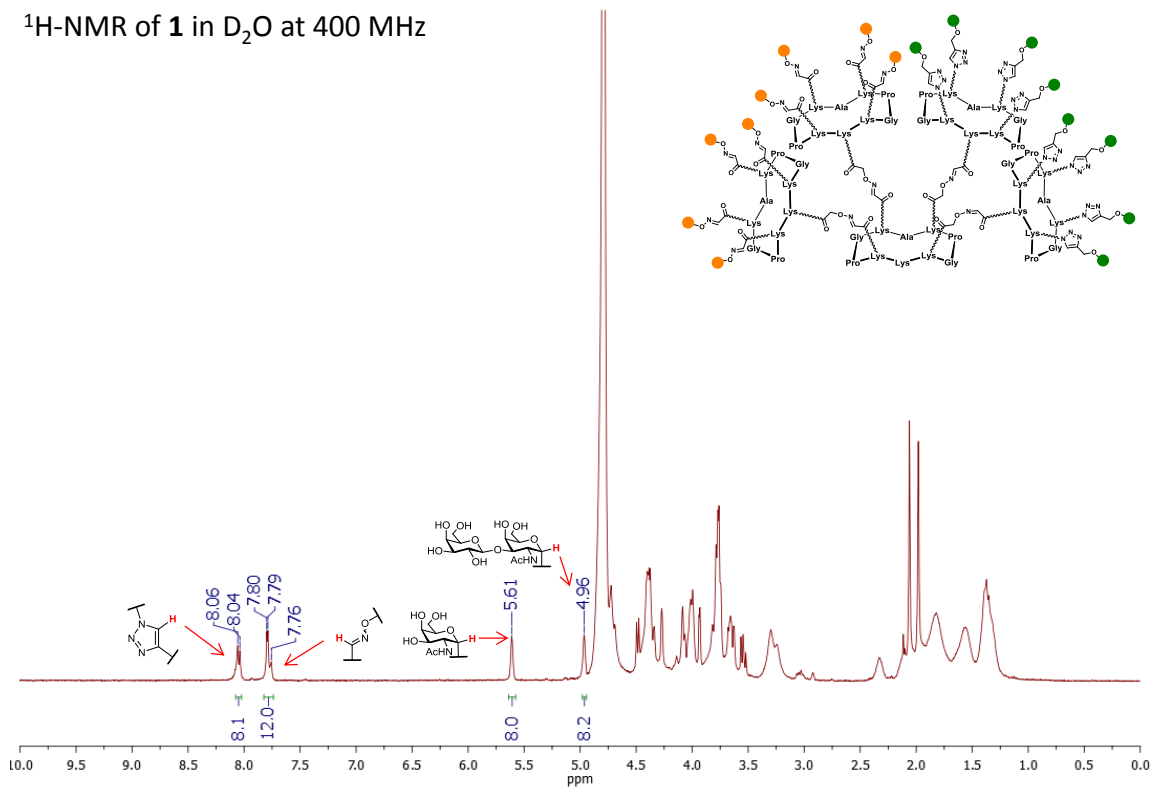


Figure S5. $^1\text{H-NMR}$ spectrum of compound **1** (D_2O , 400 MHz) showing integration of characteristic signals.

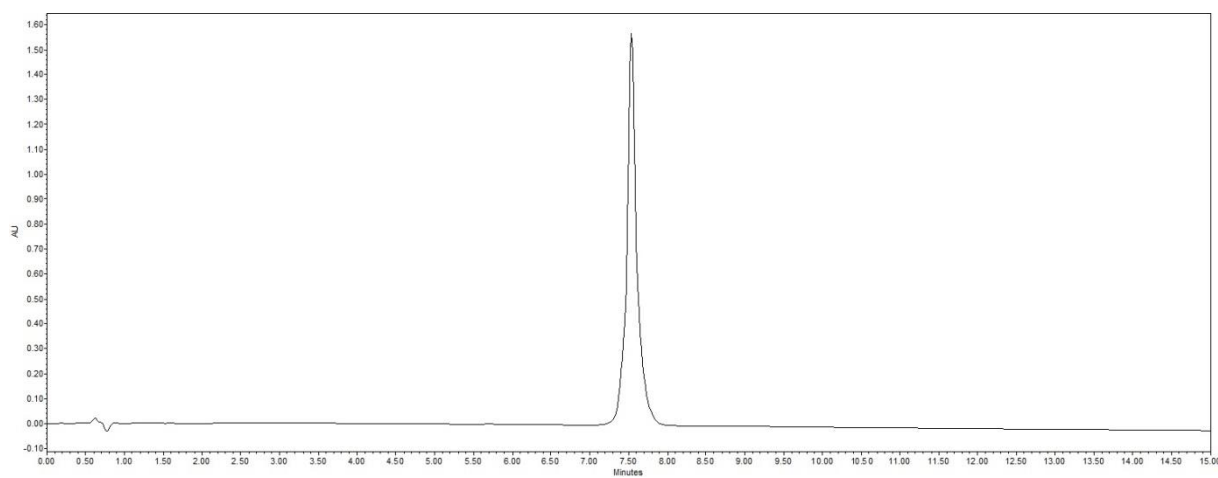


Figure S6. RP-HPLC profile of **1**: $t_R = 7.54$ min. (0-40% solv.B in 15 min.).

Compound 2

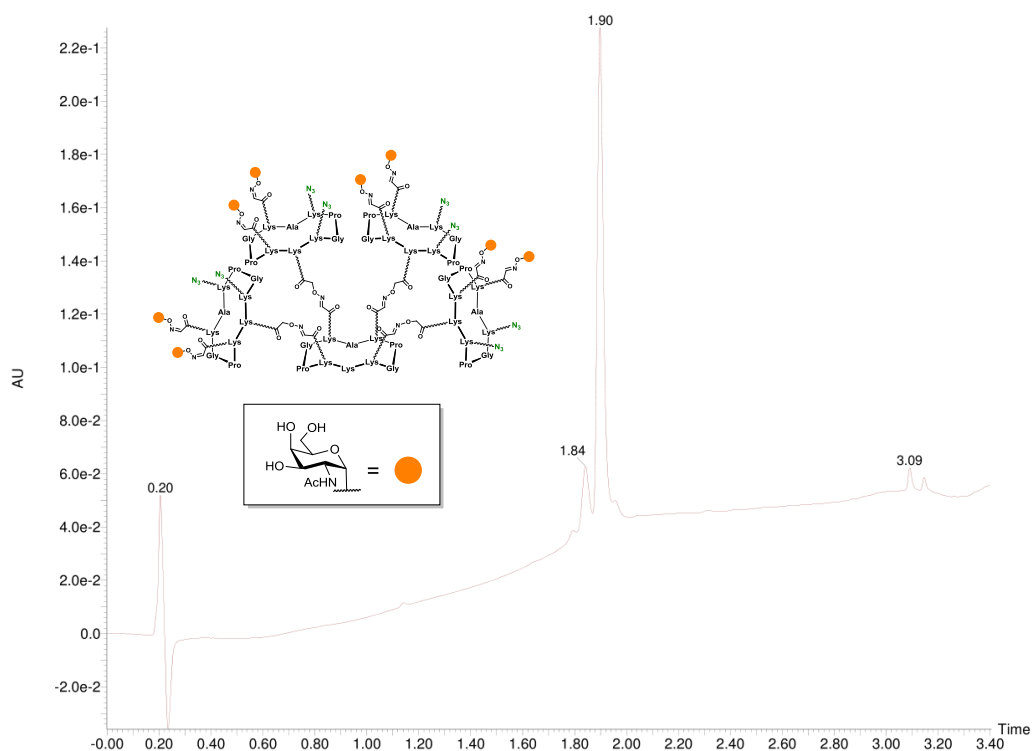


Figure S7. RP-UPLC crude profile of oxime ligation between **29** and **8** at $t=30$ min. Octa-Tn intermediate: $t_R=1.90$ min. (5-60% solv.D in 3.0 min.).

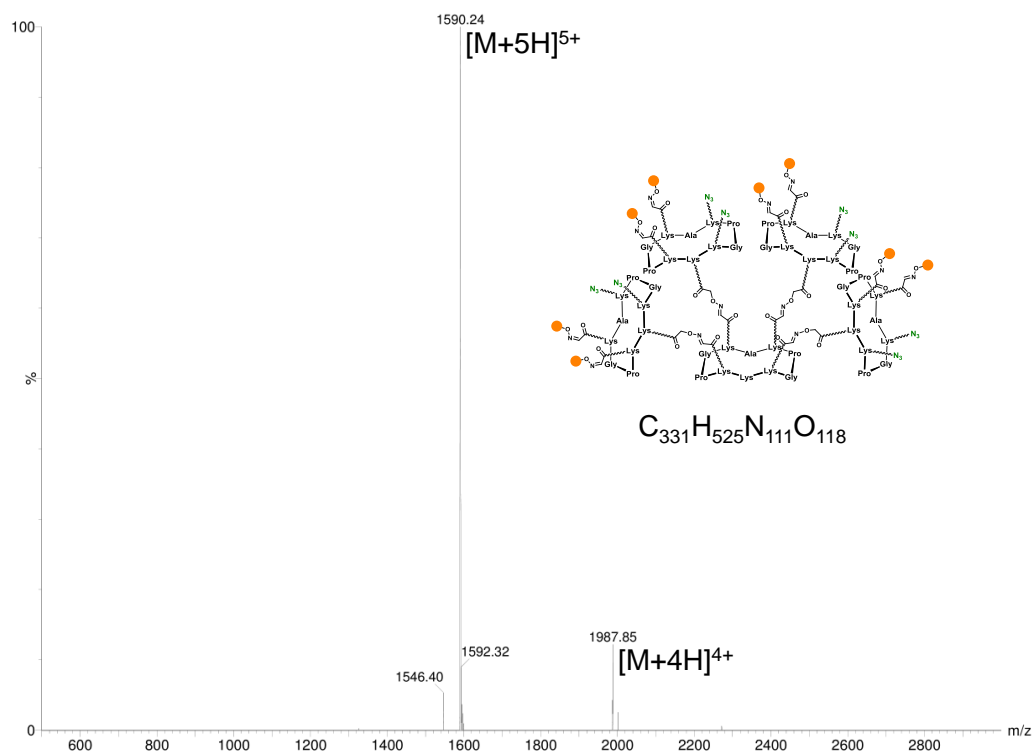


Figure S8. ESI⁺-MS spectrum of Octa-Tn intermediate resulting from oxime ligation between **29** and **8**. m/z (Average Mwt) calcd. for $C_{331}H_{530}N_{111}O_{118}$ $[M+5H]^{5+}$: 1590.5, found: 1590.2; calcd. for $C_{331}H_{529}N_{111}O_{118}$ $[M+4H]^{4+}$: 1987.9, found: 1987.9

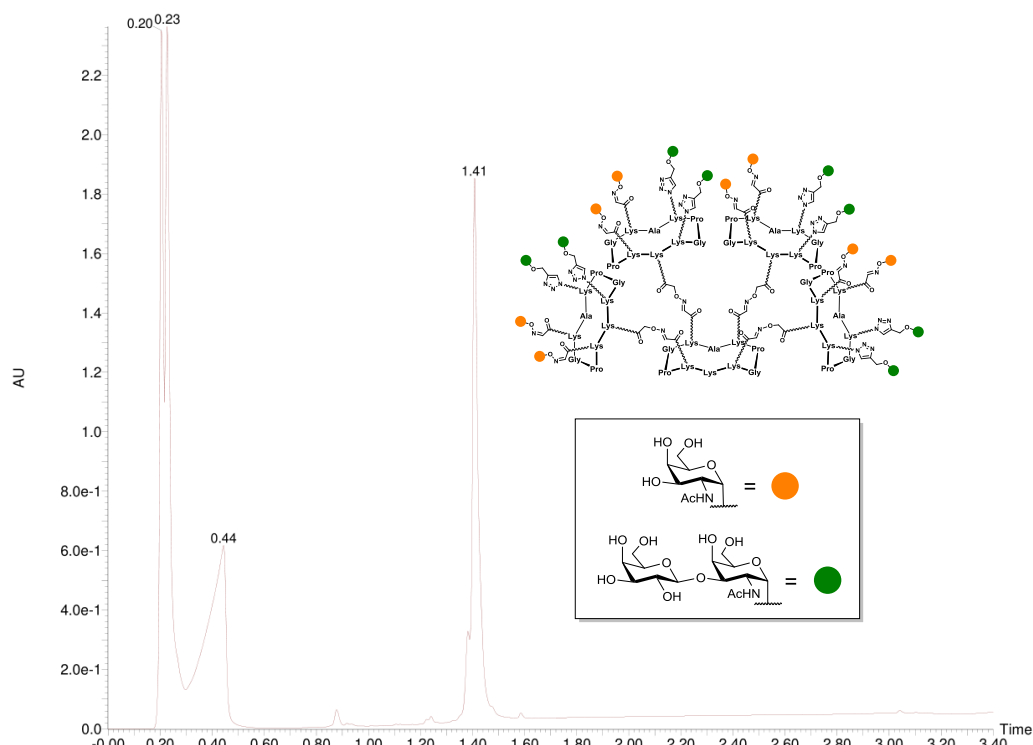


Figure S9. RP-UPLC crude profile of CuAAC ligation at $t=90$ min. Compound **2**: $t_R=1.41$ min. (5-60% solv.D in 3.0 min.).

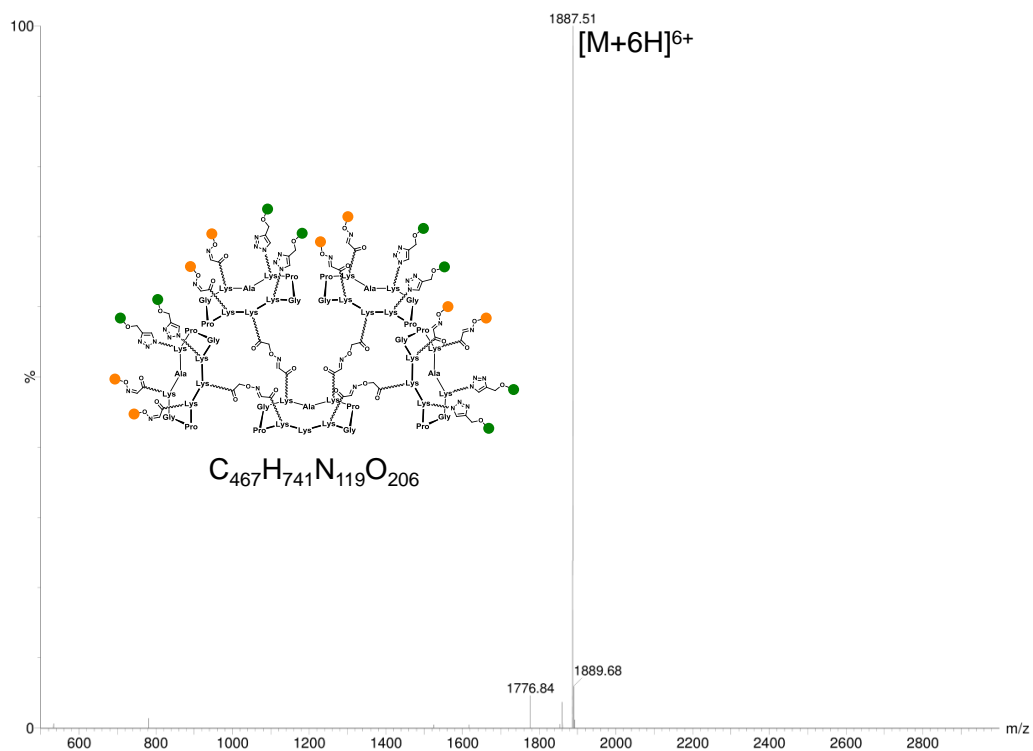


Figure S10. ESI⁺-MS spectrum of **2**. m/z (Average Mwt) calcd. for $C_{467}H_{741}N_{119}O_{206}$ $[M+6H]^{6+}$: 1887.4, found: 1887.5

$^1\text{H-NMR}$ of **2** in D_2O at 400 MHz

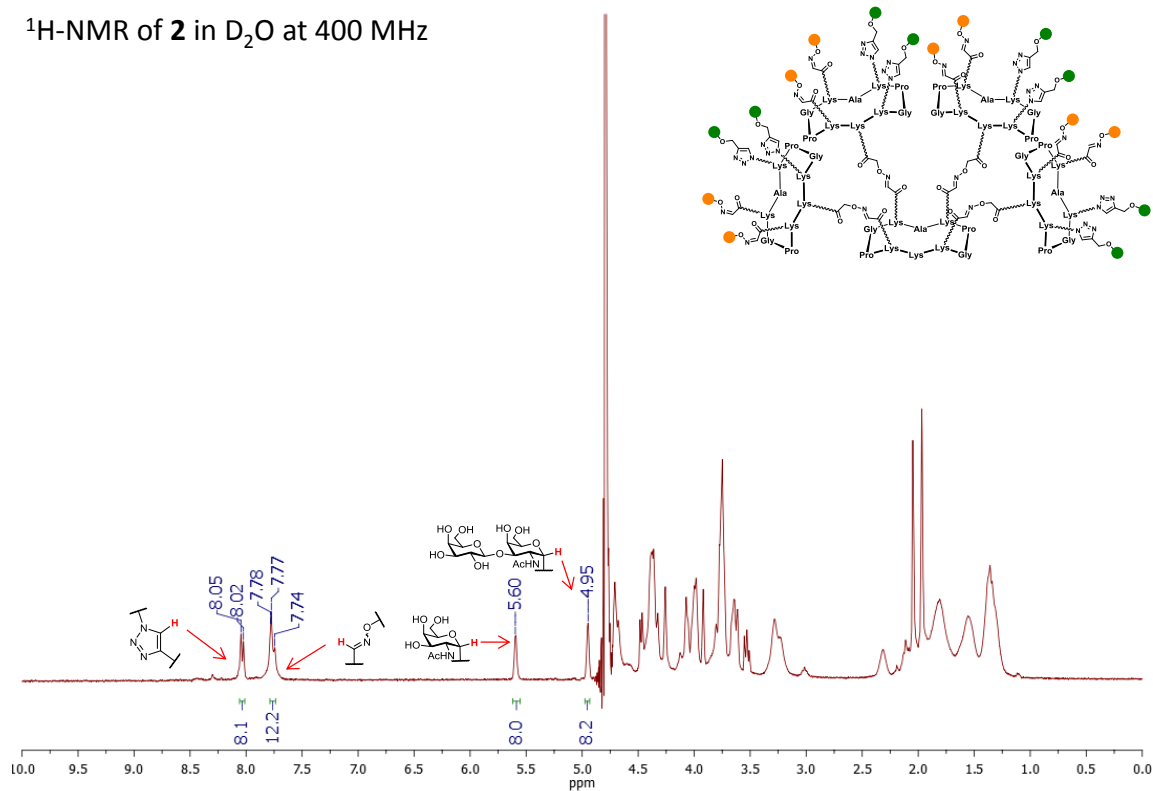


Figure S11. $^1\text{H-NMR}$ spectrum of compound **2** (D_2O , 400 MHz) showing integration of characteristic signals.

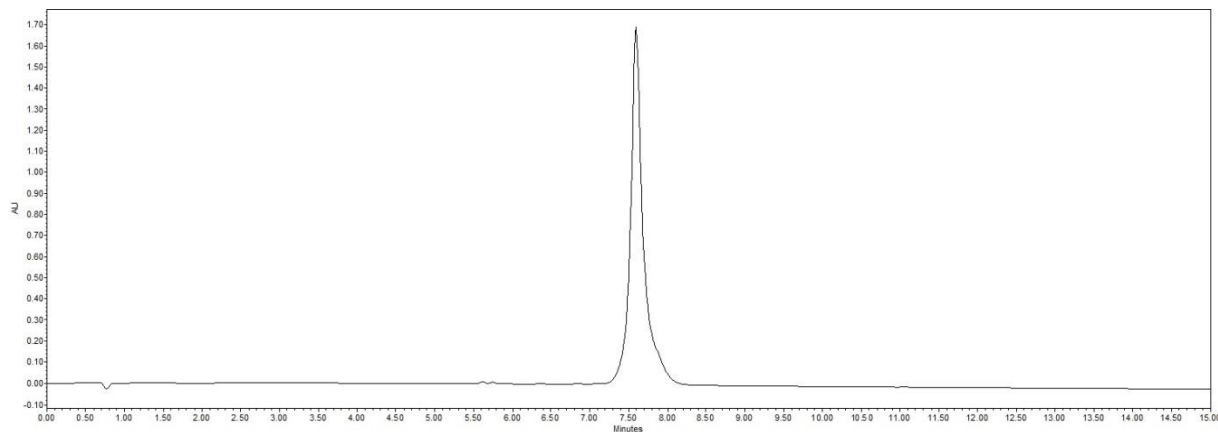


Figure S12. RP-HPLC profile of **2**: $t_R = 7.60$ min. (0-40% solv.B in 15 min.).

Compound 3.

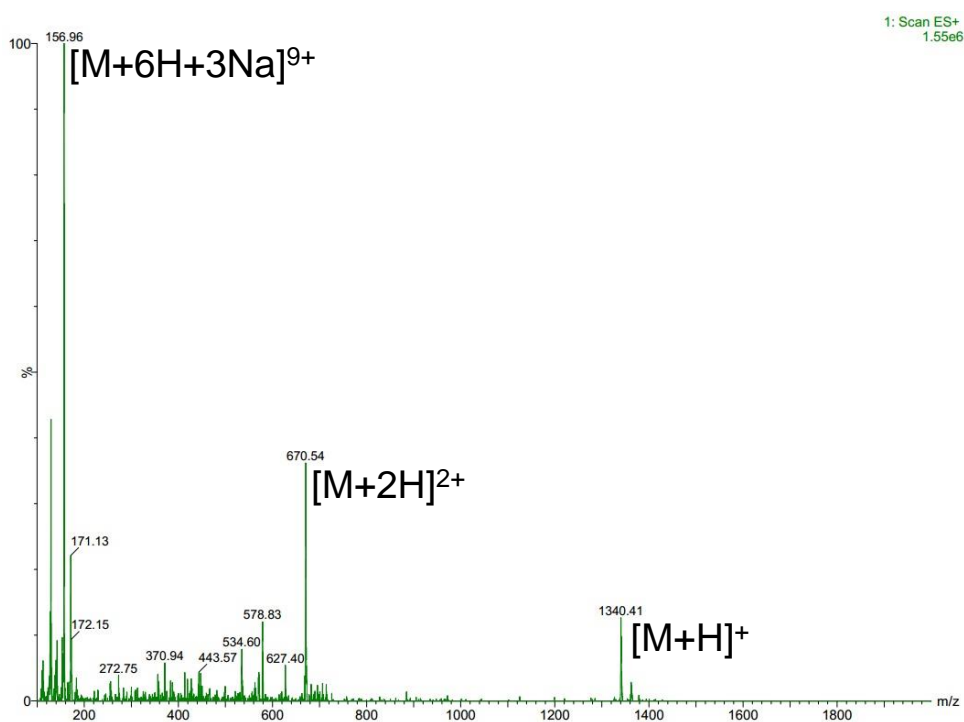


Figure S13. ESI⁺-MS spectrum of **3**. *m/z* (Monoisotopic Mwt) calcd. for C₅₇H₁₀₂N₁₉O₁₈ [M+H]⁺: 1340.8, found: 1340.4 ; calcd. for C₅₇H₁₀₃N₁₉O₁₈ [M+2H]²⁺: 670.9, found: 670.5 ; C₅₇H₁₀₇N₁₉O₁₈ Na₃ [M+6H+3Na]⁹⁺: 157.2, found: 157.0

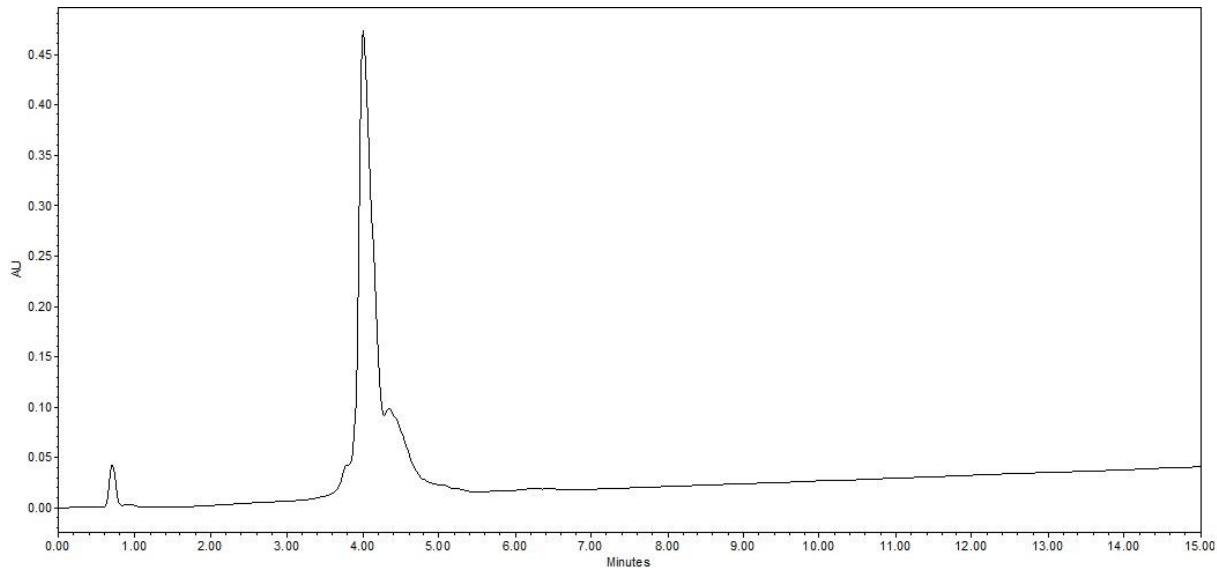


Figure S14. RP-HPLC profile of **3**: *t_R* = 4.00 min. (0-30% solv.B in 15 min.).

Compound 4.

1: (Time: 0.11) Combine (2:8-8:11)

1: TOF MS ES+
1.5e+006

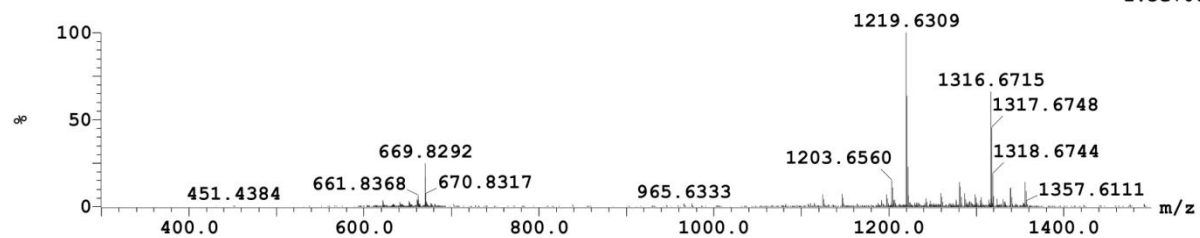


Figure S15. HRMS (ESI⁺-TOF) of **4**: *m/z* (Monoisotopic Mwt) calcd. for C₅₅H₉₄N₁₅O₂₂ [M+H₂O+H]⁺: 1316.6698, found: 1316.6715

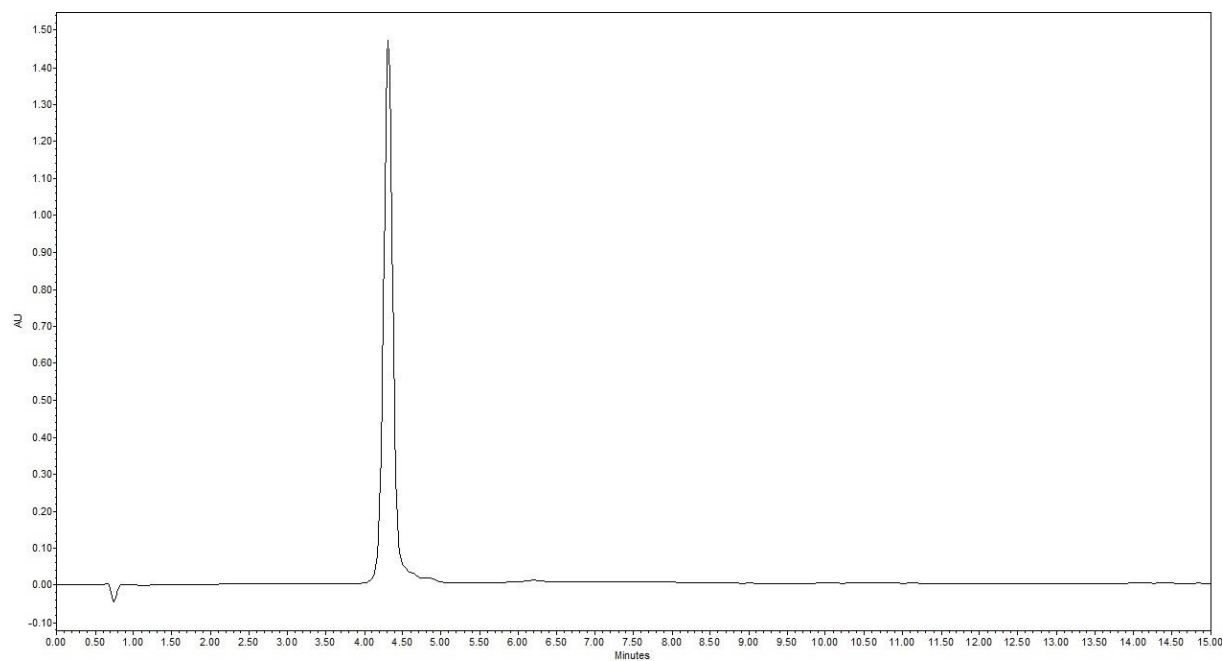


Figure S16. RP-HPLC profile of **4**: *t_R* = 4.31 min. (0-40% solv.B in 15 min.).

Compound 5.

1: (Time: 0.13) Combine (3:9-15:18)

1: TOF MS ES+
3.4e+004

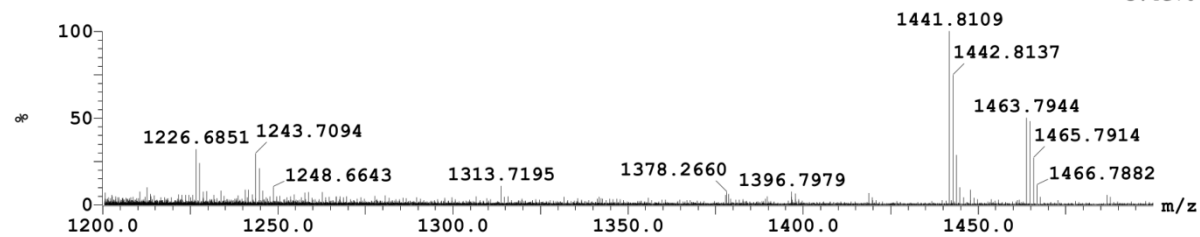


Figure S17. HRMS (ESI⁺-TOF) of **5**: *m/z* (Monoisotopic Mwt) calcd. for C₆₁H₁₀₉N₂₀O₂₀ [M+H]⁺: 1441.8127, found: 1441.8109 ; calcd. for C₆₁H₁₀₈N₂₀O₂₀Na [M+Na]⁺: 1463.7946, found: 1463.7944

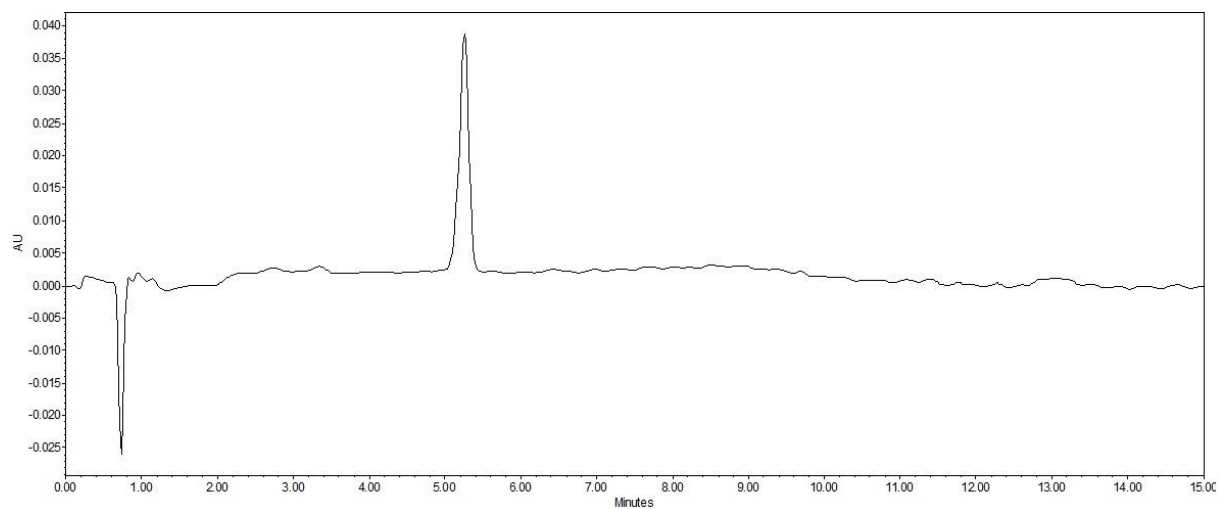


Figure S18. RP-HPLC profile of **5**: *t_R* = 5.25 min. (0-30% solv.B in 15 min.).

Compound 6.

1: (Time: 0.13) Combine (5:7-10:13)

1: TOF MS ES+
1.5e+006

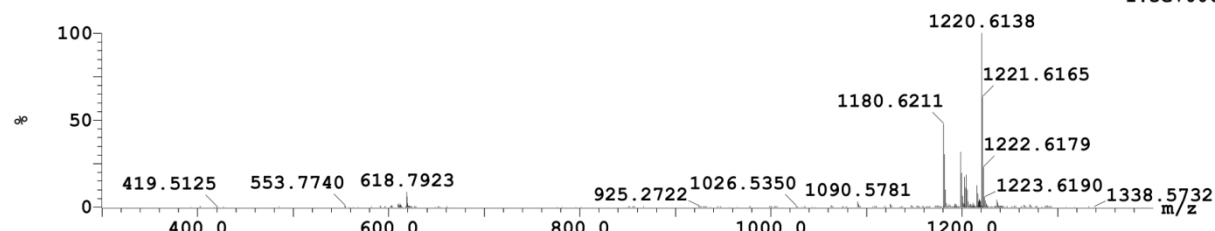


Figure S19. HRMS (ESI⁺-TOF) of **6**: m/z (Monoisotopic Mwt) calcd. for $C_{49}H_{78}N_{23}O_{12}$ $[M+H]^+$: 1180.6200, found: 1180.6211 ; calcd. for $C_{49}H_{79}N_{23}O_{13}Na$ $[M+H_2O+Na]^+$: 1220.6125, found: 1220.6138

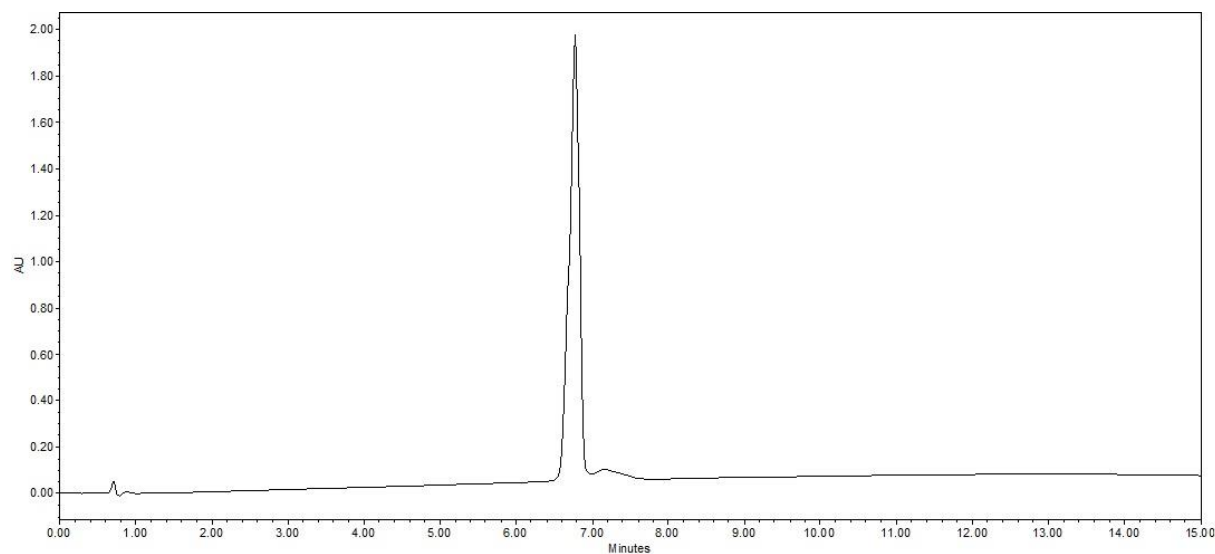


Figure S20. RP-HPLC profile of **6**: t_R = 6.78 min. (5-100% solv.B in 15 min.).

Compound 7.

1: (Time: 0.13) Combine (3:7-14:16)

1: TOF MS ES+
2.0e+005

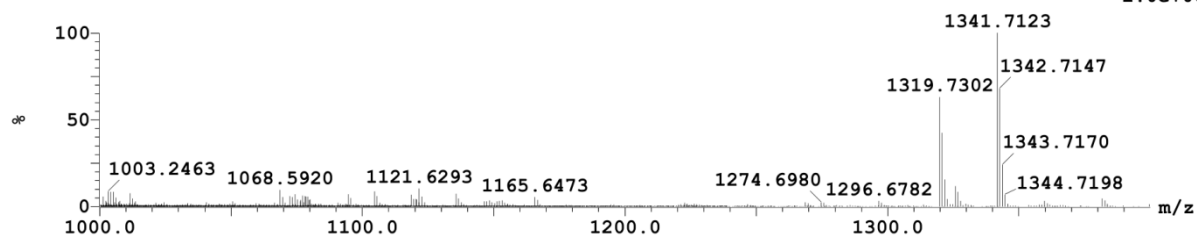


Figure S21. HRMS (ESI⁺-TOF) of **7**: m/z (Monoisotopic Mwt) calcd. for $C_{55}H_{95}N_{22}O_{16}$ $[M+H]^+$: 1319.7296, found: 1319.7302 ; calcd. for $C_{55}H_{94}N_{22}O_{16}Na$ $[M+Na]^+$: 1341.7116, found: 1341.7123

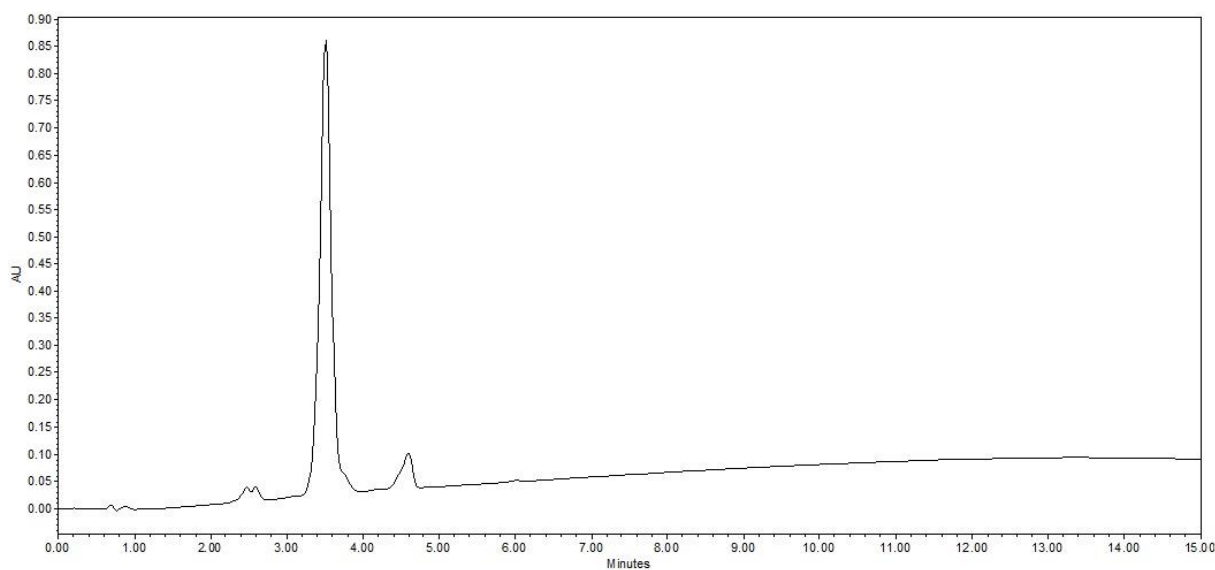


Figure S22. RP-HPLC profile of **7**: t_R = 3.52 min. (5-100% solv.B in 15 min.).

Compound 9 .

Propargyl (β -D-galactopyranosyl)-(1-3)-2-acetamido-2-deoxy- α -D-galactopyranoside.

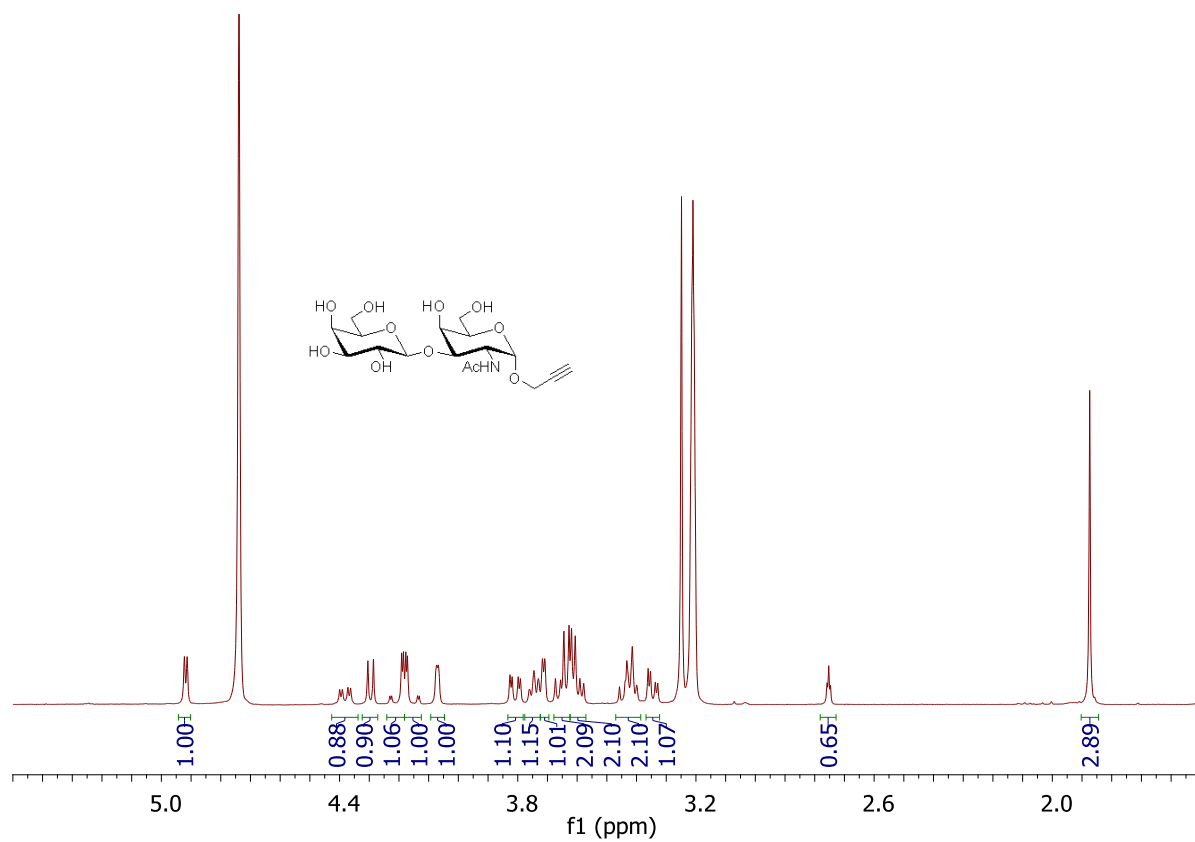


Figure S23. ^1H spectrum of compound 9 (D_2O , 400 MHz).

Compound 13.

Propargyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy- α -D-galactopyranoside.

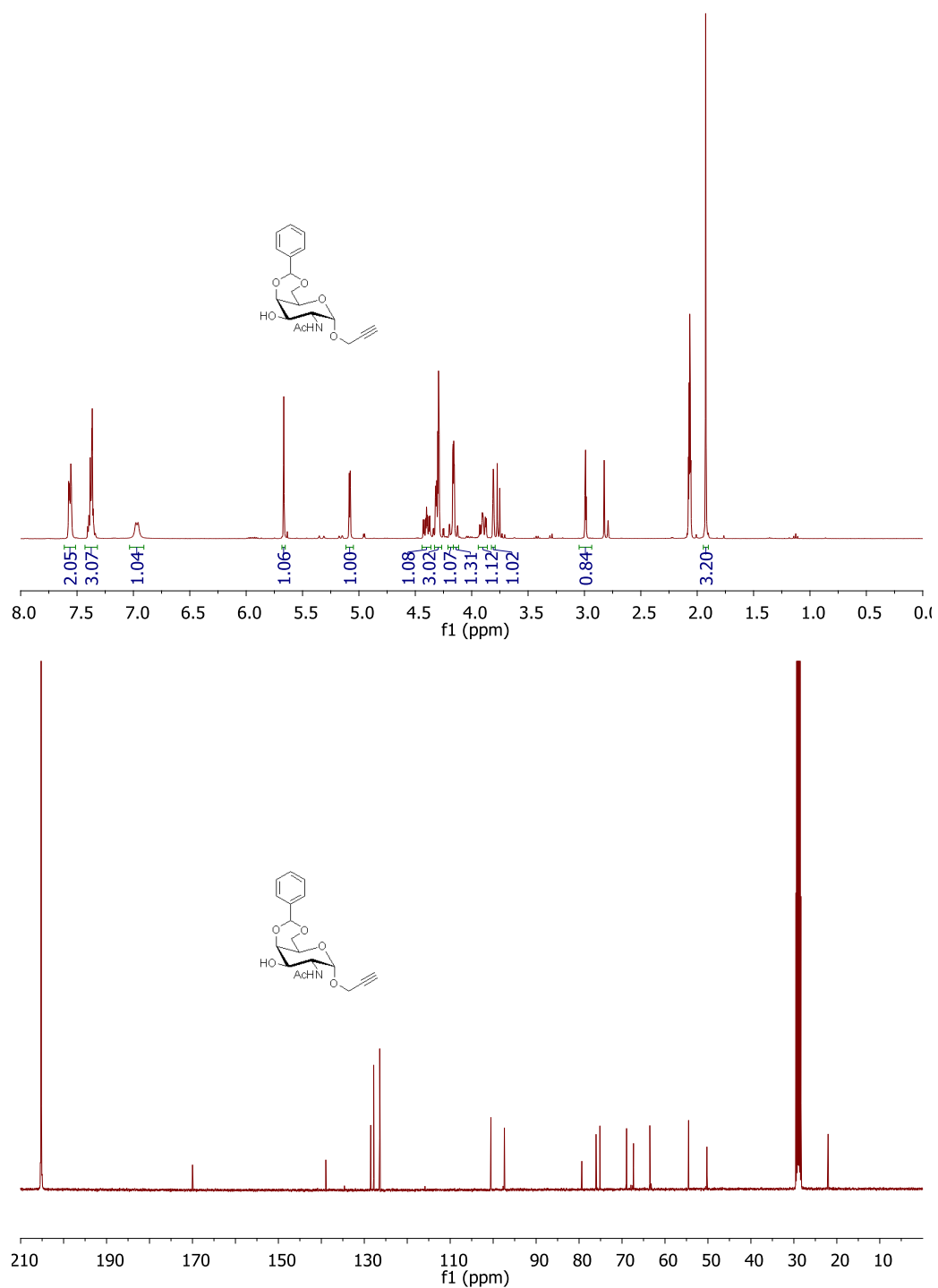


Figure S24. ^1H (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) NMR spectra of compound 13.

Compound 15.

Propargyl (2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1-3)-2-acetamido-4,6-*O*-benzilidene-2-deoxy- α -D-galactopyranoside.

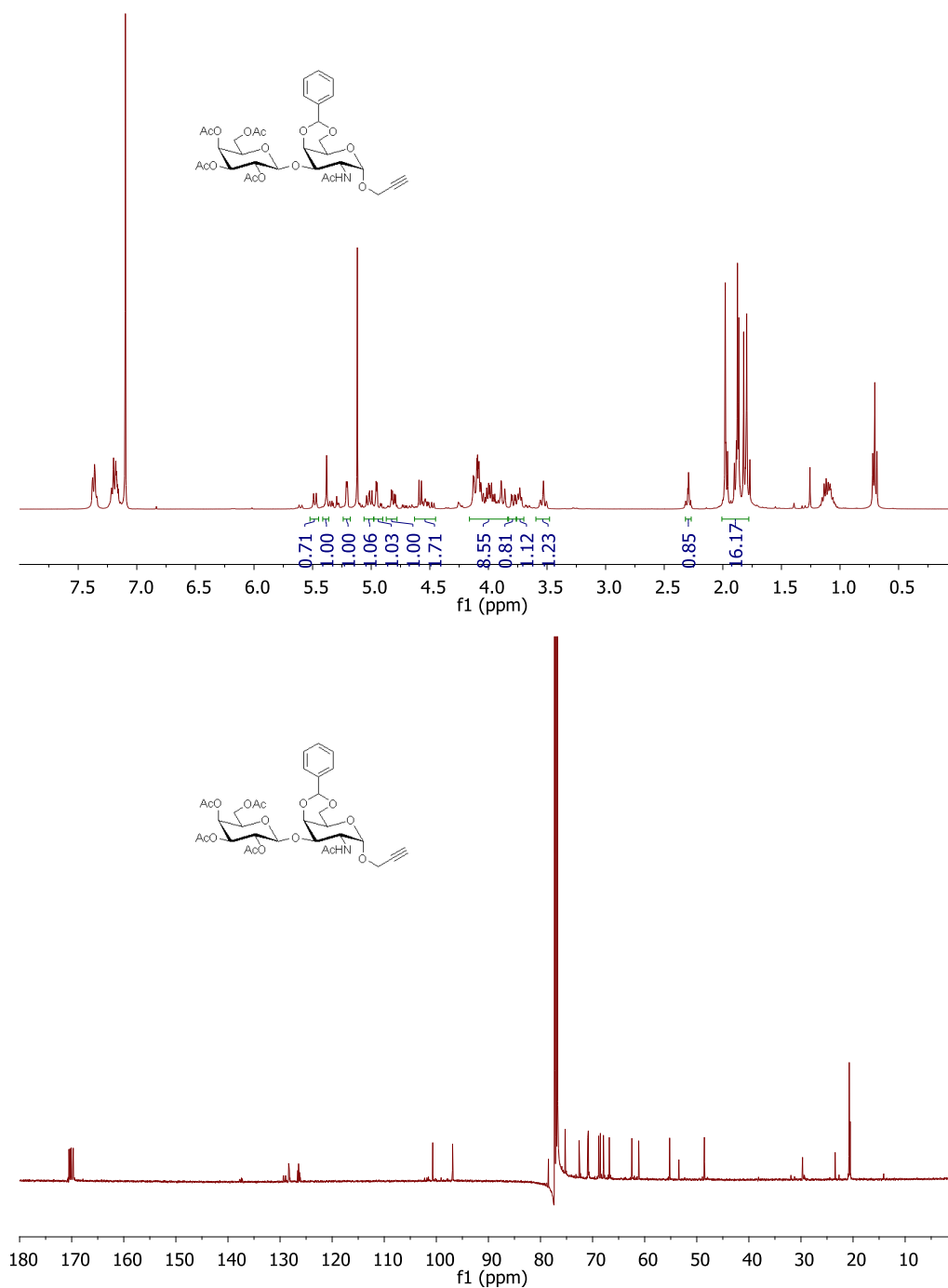


Figure S25. ^1H (400 MHz, CDCl_3) and ^{13}C (100 MHz, CDCl_3) NMR spectra of compound 15.

Compound 16.

1: (Time: 0.10) Combine (3:5-7:9)

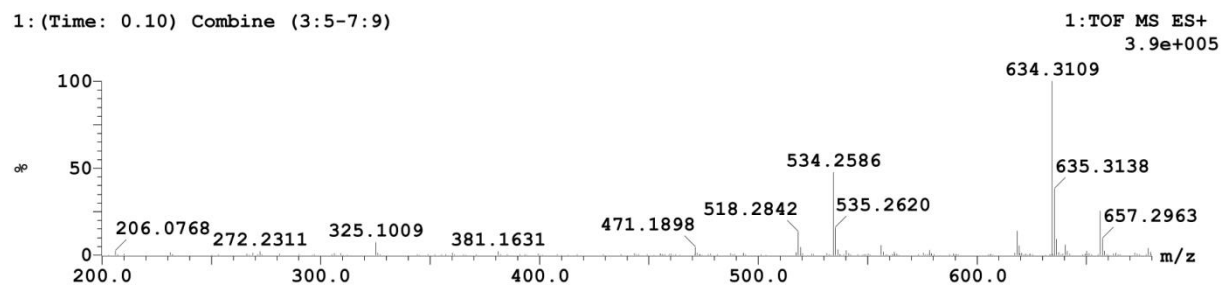


Figure S26. HRMS (ESI⁺-TOF) of **16**: *m/z* (Monoisotopic Mwt) calcd. for C₃₃H₄₅N₃O₈Na [M+Na]⁺: 634.3104, found: 634.3109

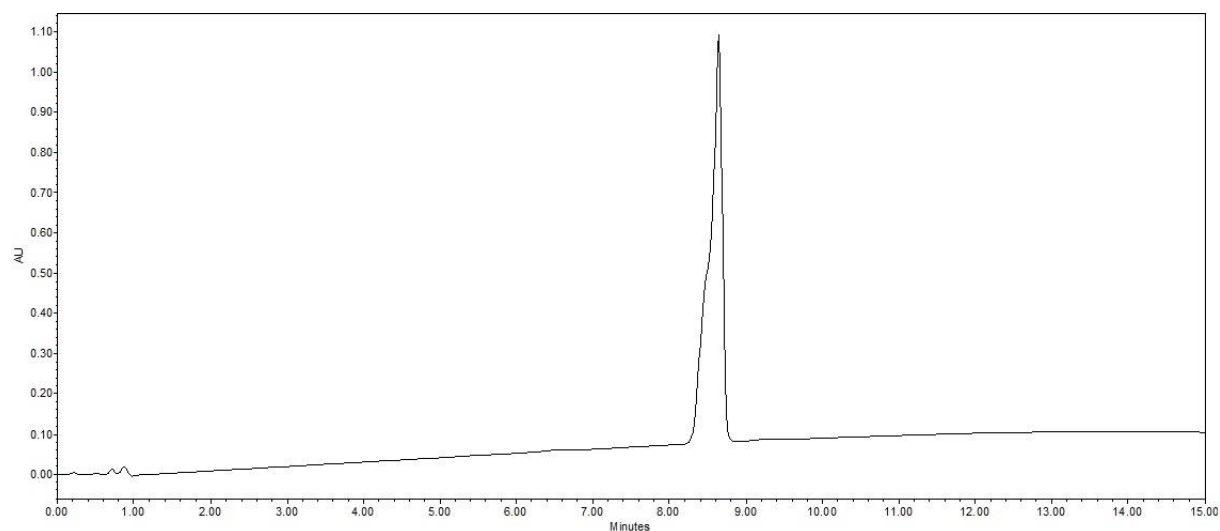


Figure S27. RP-HPLC profile of **16**: *t_R* = 8.64 min. (5-100% solv.B in 15 min.).

Compound 17.

1: (Time: 0.13) Combine (3:9-21)

1: TOF MS ES+
5.8e+004

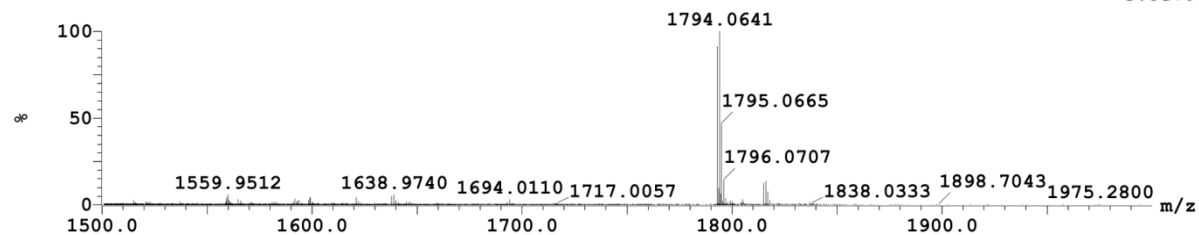


Figure S28. HRMS (ESI⁺-TOF) of the linear form of compound **17** resulting from cleavage of sequence **A**: m/z (Monoisotopic Mwt) calcd. for $C_{84}H_{146}N_{17}O_{25} [M+H]^+$: 1794.0706, found: 1794.0641

1: (Time: 0.13) Combine (3:9-21)

1: TOF MS ES+
3.5e+005

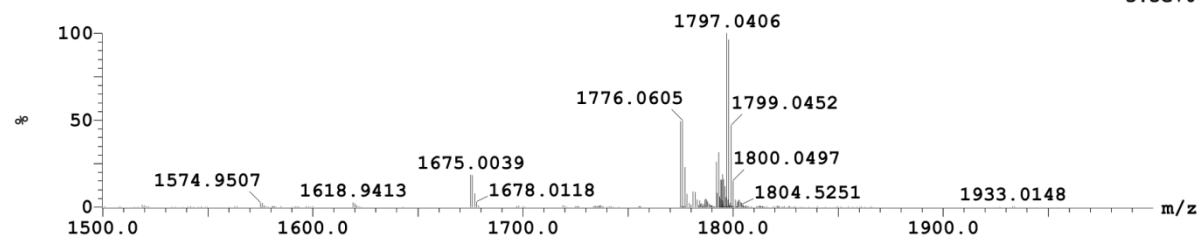


Figure S29. HRMS (ESI⁺-TOF) of **17**: m/z (Monoisotopic Mwt) calcd. for $C_{84}H_{144}N_{17}O_{24} [M+H]^+$: 1776.0600, found: 1776.0605 ; calcd. for $C_{84}H_{143}N_{17}O_{24}Na [M+Na]^+$: 1797.0390, 1797.0406

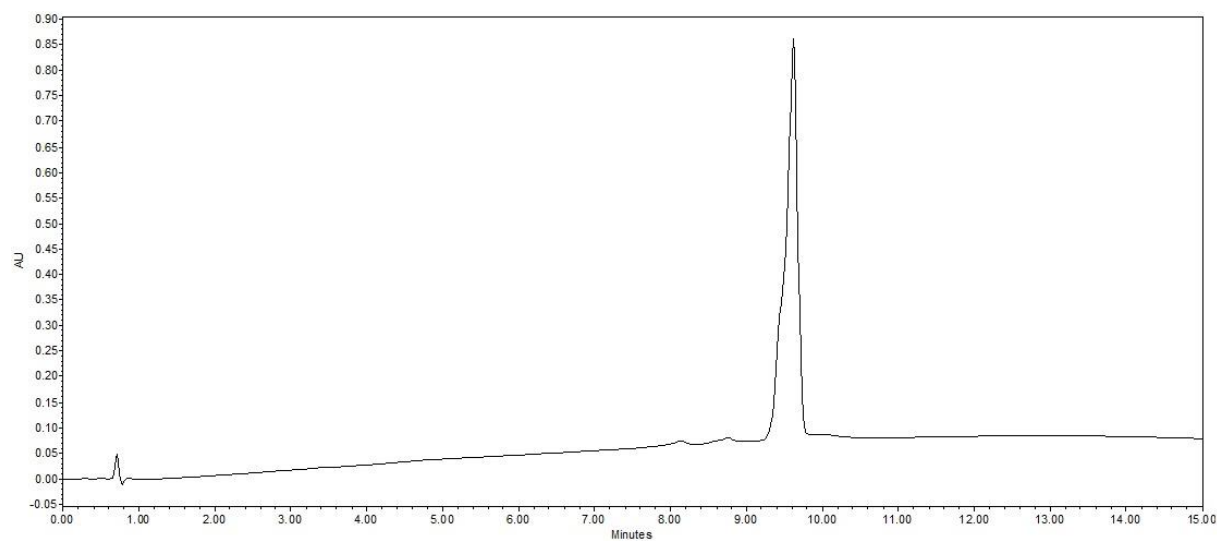


Figure S30. RP-HPLC profile of **17**: $t_R = 9.62$ min. (5-100% solv.B in 15 min.).

Compound 18.

CPIF_152 4 (0.104) Cm (4:5)

1: TOF MS ES+
9.39e+002

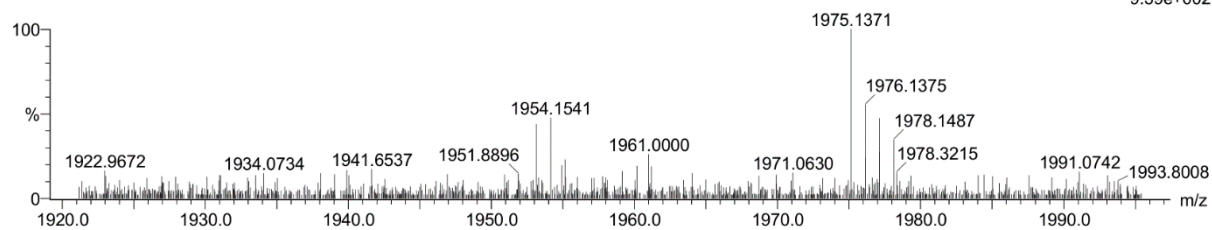


Figure S31. HRMS (ESI⁺-TOF) of **18**: *m/z* (Monoisotopic Mwt) calcd. for C₉₀H₁₅₇N₁₉O₂₈Na [M+ Na]⁺: 1975.1343, found: 1975.1371

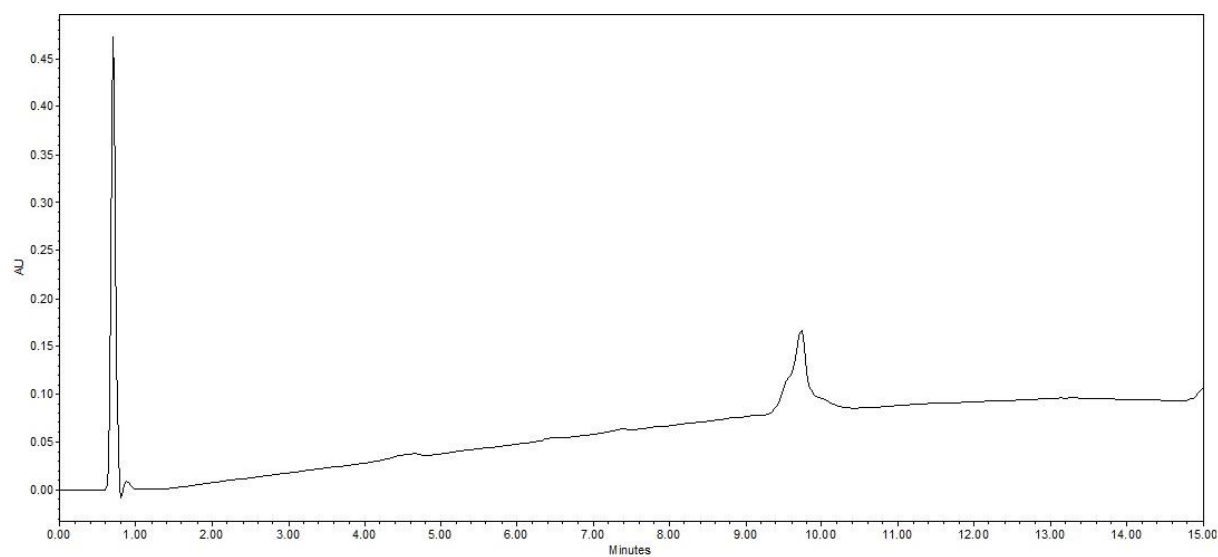


Figure S32. RP-HPLC profile of **18**: *t_R*= 9.72 min. (5-100% solv.B in 15 min.).

Compound 19.

1: (Time: 0.10) Combine (3:5-7:9)

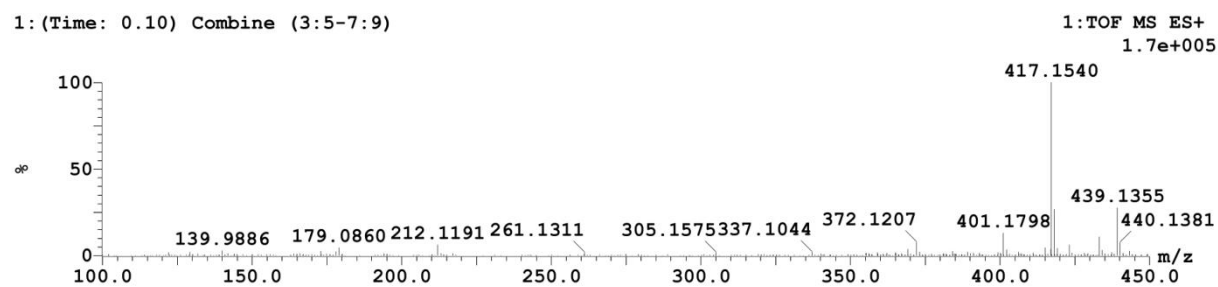


Figure S33. HRMS (ESI⁺-TOF) of **19**: *m/z* (Monoisotopic Mwt) calcd. for C₂₁H₂₂N₄O₄Na [M+ Na]⁺:
417.1539 found: 417.1540

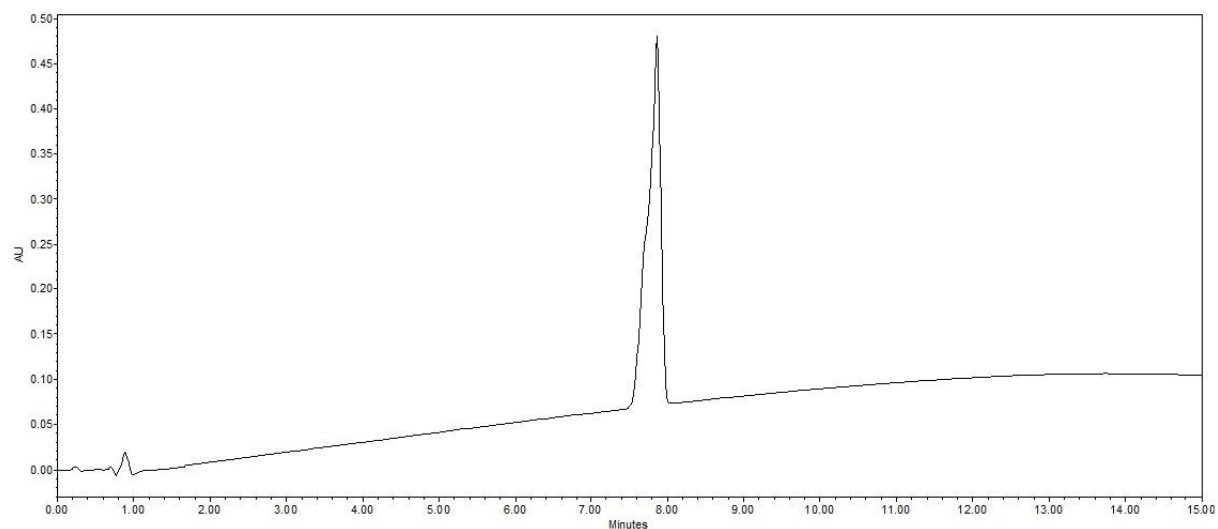


Figure S34. RP-HPLC profile of **19**: *t_R*= 7.86 min. (5-100% solv.B in 15 min.).

Compound 20.

1: (Time: 0.13) Combine (3:9-21)

1: TOF MS ES+
6.5e+005

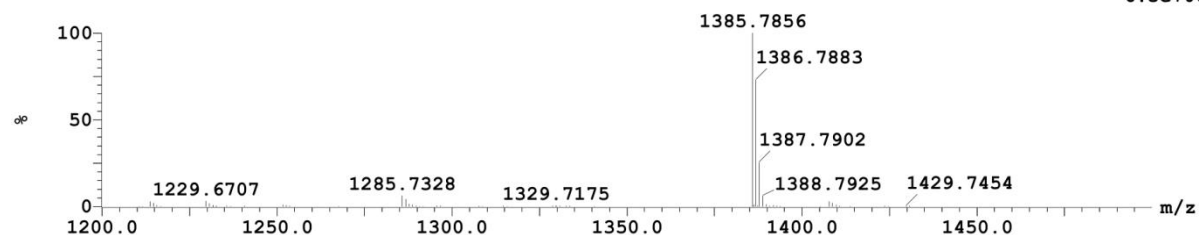


Figure S35. HRMS (ESI⁺-TOF) of the linear form of compound **20** resulting from cleavage of sequence **B**: *m/z* (Monoisotopic Mwt) calcd. for C₅₉H₁₀₁N₂₄O₁₅ [M+H]⁺: 1385.7878, found: 1385.7856

1: (Time: 0.13) Combine (3:9-21)

1: TOF MS ES+
1.0e+005

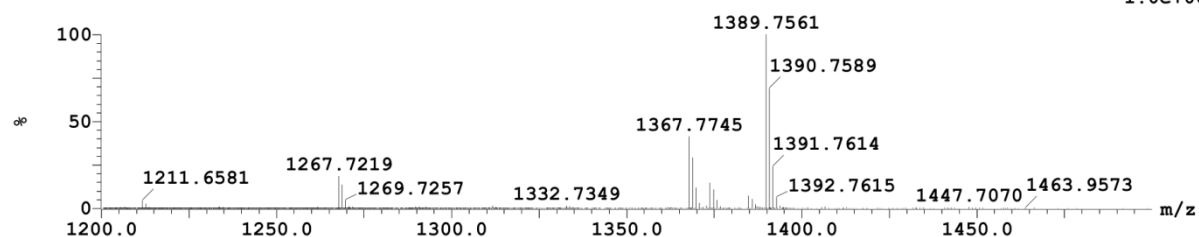


Figure S36. HRMS (ESI⁺-TOF) of **20**: *m/z* (Monoisotopic Mwt) calcd. for C₅₉H₉₉N₂₄O₁₄ [M+H]⁺: 1367.7773, found: 1367.7745 ; calcd. for C₅₉H₉₈N₂₄O₁₄Na [M+Na]⁺: 1389.7592, found: 1389.7561

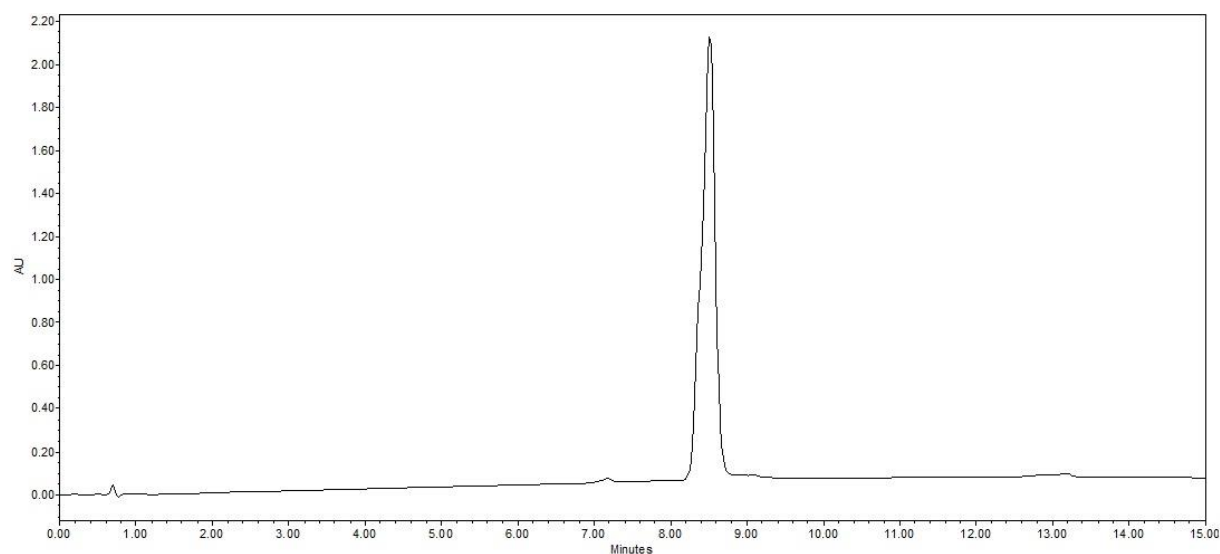


Figure S37. RP-HPLC profile of **20**: *t_R* = 8.52 min. (5-100% solv.B in 15 min.).

Compound 21.

1: (Time: 0.13) Combine (3:9-21)

1: TOF MS ES+
3.3e+05

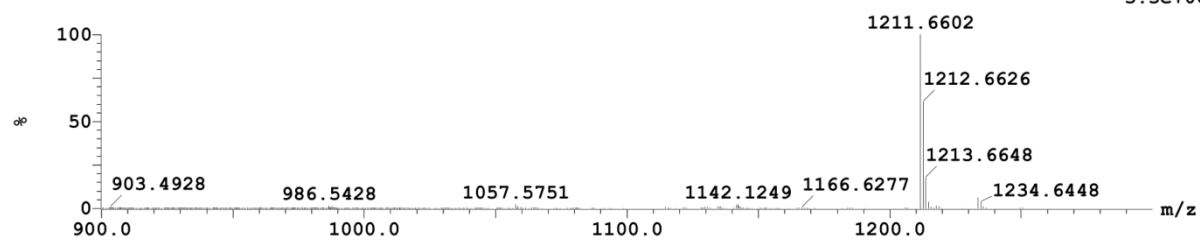


Figure S38. HRMS (ESI⁺-TOF) of **21**: *m/z* (Monoisotopic Mwt) calcd. for C₅₀H₈₃N₂₄O₁₂ [M+H]⁺: 1211.6622, found: 1211.6602

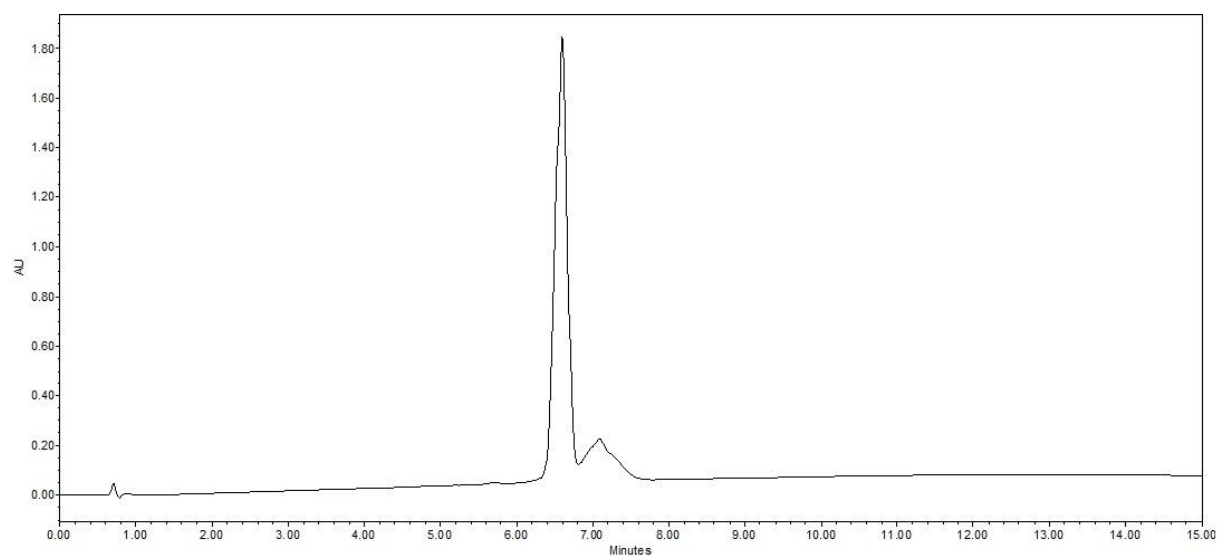


Figure S39. RP-HPLC profile of **21**: *t_R* = 6.59 min. (5-100% solv.B in 15 min.).

Compound 22.

1: (Time: 0.13) Combine (3:9-20:21)

1: TOF MS ES+
3.7e+005

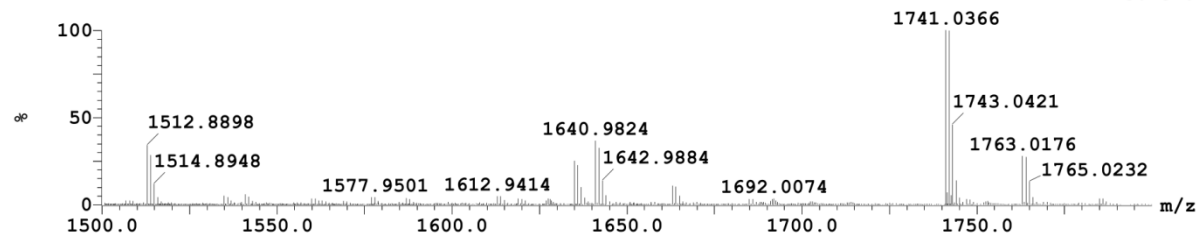


Figure S40. HRMS (ESI⁺-TOF) of the linear form of compound **22** resulting from cleavage of sequence **C**: m/z (Monoisotopic Mwt) calcd. for C₈₁H₁₃₈N₂₁O₂₁ [M+H]⁺: 1741.0376, found: 1741.0366 ; calcd. for C₈₁H₁₃₇N₂₁O₂₁Na [M+Na]⁺: 1763.0196, found: 1763.0176

1: (Time: 0.13) Combine (3:9-12:15)

1: TOF MS ES+
2.8e+004

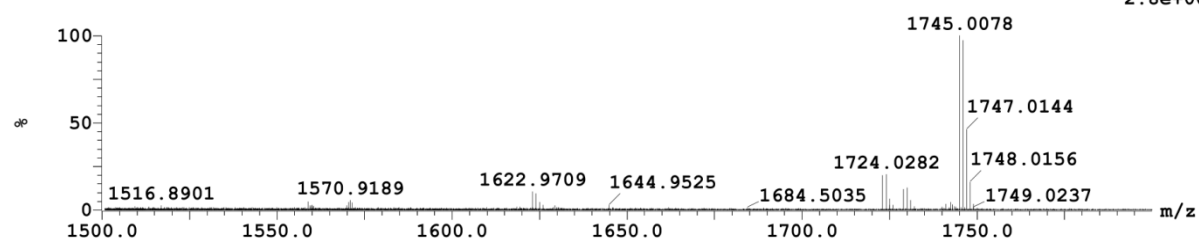


Figure S41. HRMS (ESI⁺-TOF) of **22**: m/z (Monoisotopic Mwt) calcd. for C₈₁H₁₃₆N₂₁O₂₀ [M+H]⁺: 1724.0300, found: 1724.0282 ; calcd. for C₈₁H₁₃₅N₂₁O₂₀Na [M+Na]⁺: 1745.0090, found: 1745.0078

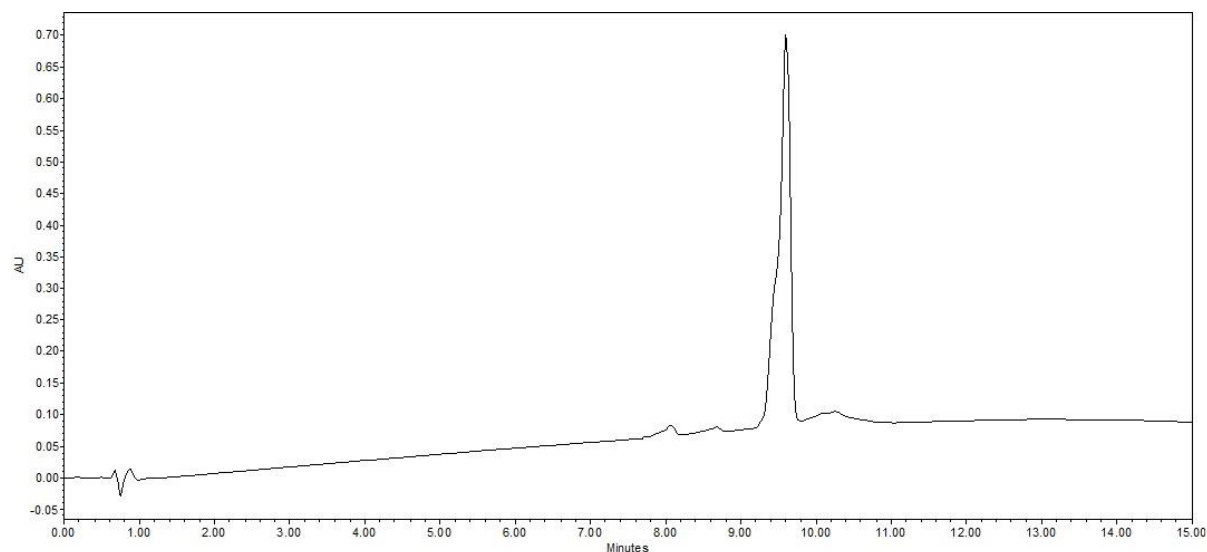


Figure S42. RP-HPLC profile of **22**: t_R = 9.60 min. (5-100% solv.B in 15 min.).

Compound 23.

1: (Time: 0.13) Combine (3:9-21)

1: TOF MS ES+
8.6e+005

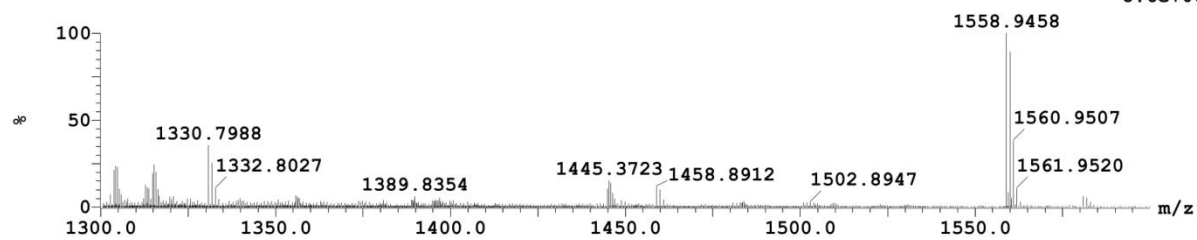


Figure S43. HRMS (ESI⁺-TOF) of **23**: m/z (Monoisotopic Mwt) calcd. for $C_{71}H_{124}N_{21}O_{18}$ $[M+H]^+$: 1558.9433, found: 1558.9458

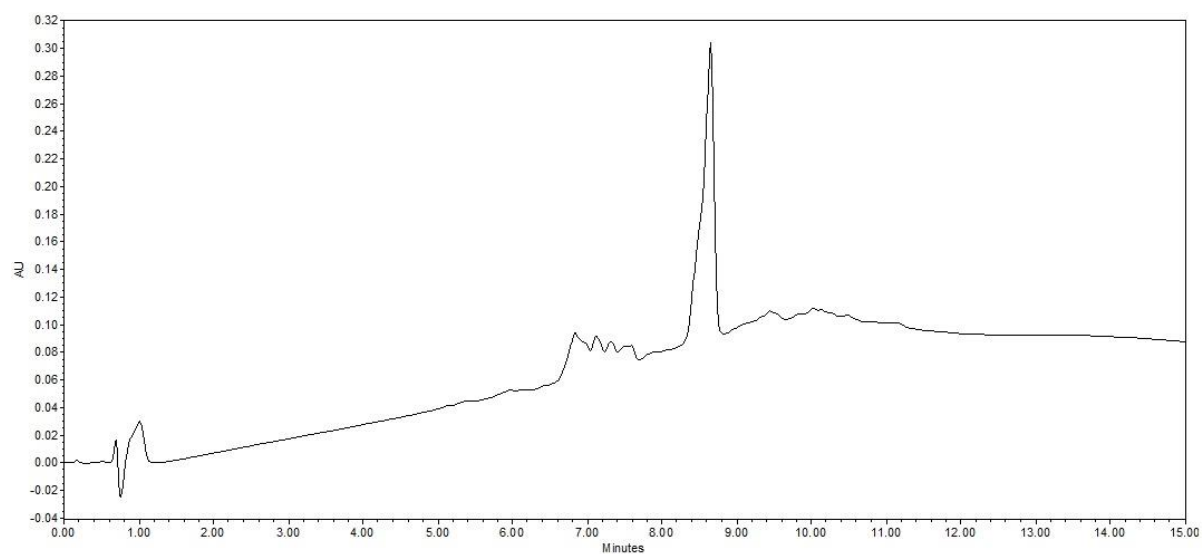


Figure S44. RP-HPLC profile of **23**: $t_R = 8.65$ min. (5-100% solv.B in 15 min.).

Compound 24.

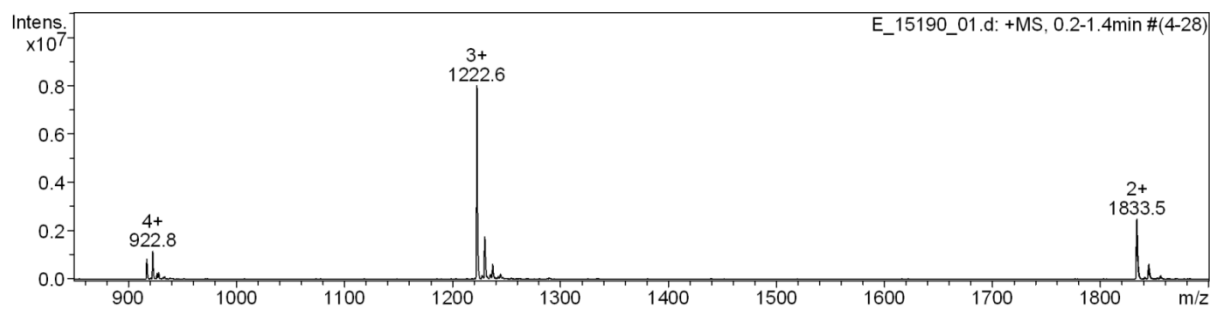


Figure S45. ESI⁺-MS spectrum of **24**. *m/z* (Average Mwt) calcd. for C₁₅₅H₂₅₃N₆₅O₄₀ [M+2H]²⁺: 1833.5, found: 1833.5 ; calcd. for C₁₅₅H₂₅₄N₆₅O₄₀ [M+3H]³⁺: 1222.7, found: 1222.6 ; calcd. for C₁₅₅H₂₅₄N₆₅O₄₀ [M+3H+Na]⁴⁺: 922.8, found: 922.8

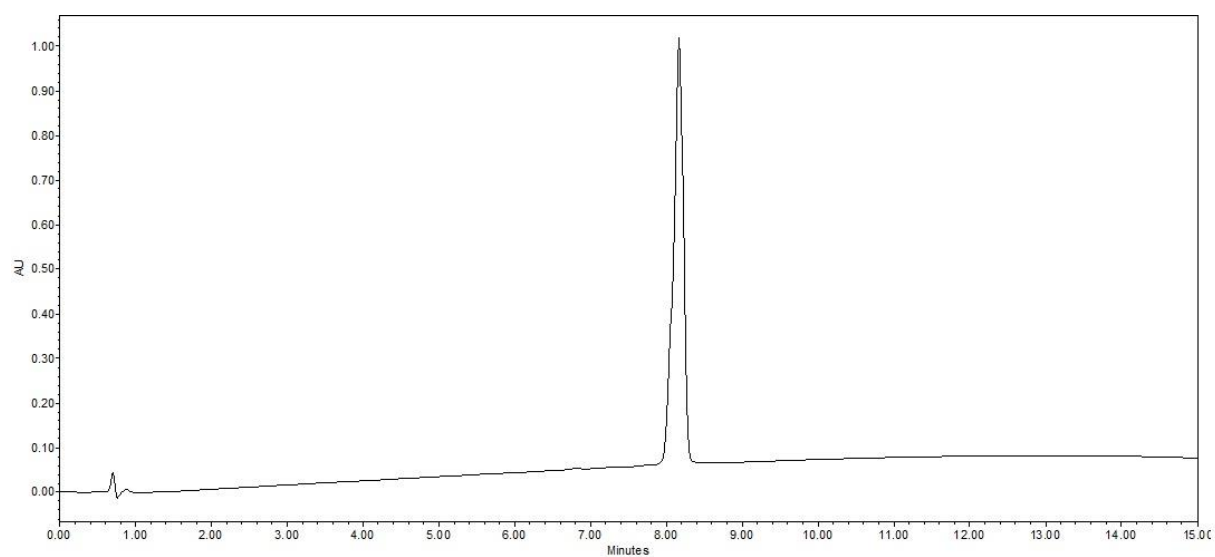


Figure S46. RP-HPLC profile of **24**: *t_R*= 8.17 min. (5-100% solv.B in 15 min.).

Compound 25.

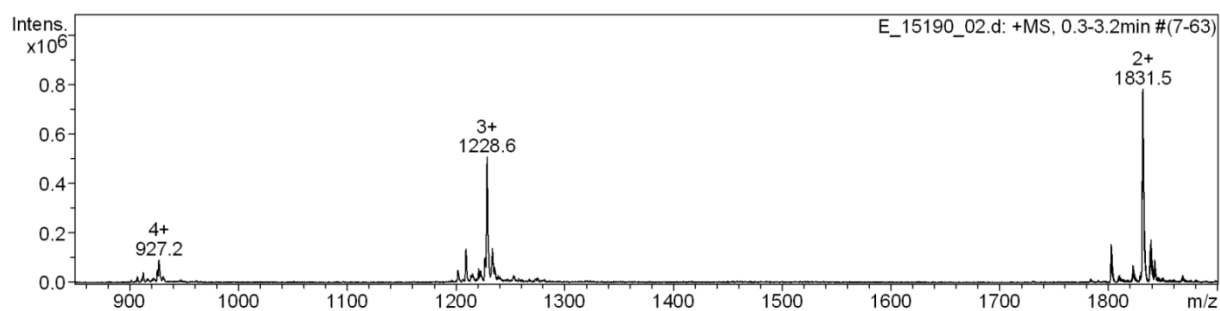


Figure S47. ESI⁺-MS spectrum of **25**. *m/z* (Average Mwt) calcd. for C₁₅₃H₂₄₆N₆₃O₄₂Na [M+H₂O+H+Na]²⁺: 1831.5, found: 1831.5 ; calcd. for C₁₅₃H₂₄₆N₆₃O₄₂Na₂ [M+H₂O+H+2Na]³⁺: 1228.7, found: 1228.6 ; calcd. for C₁₅₃H₂₄₆N₆₃O₄₂Na₃ [M+H₂O+H+3Na]⁴⁺: 927.2, found: 927.2

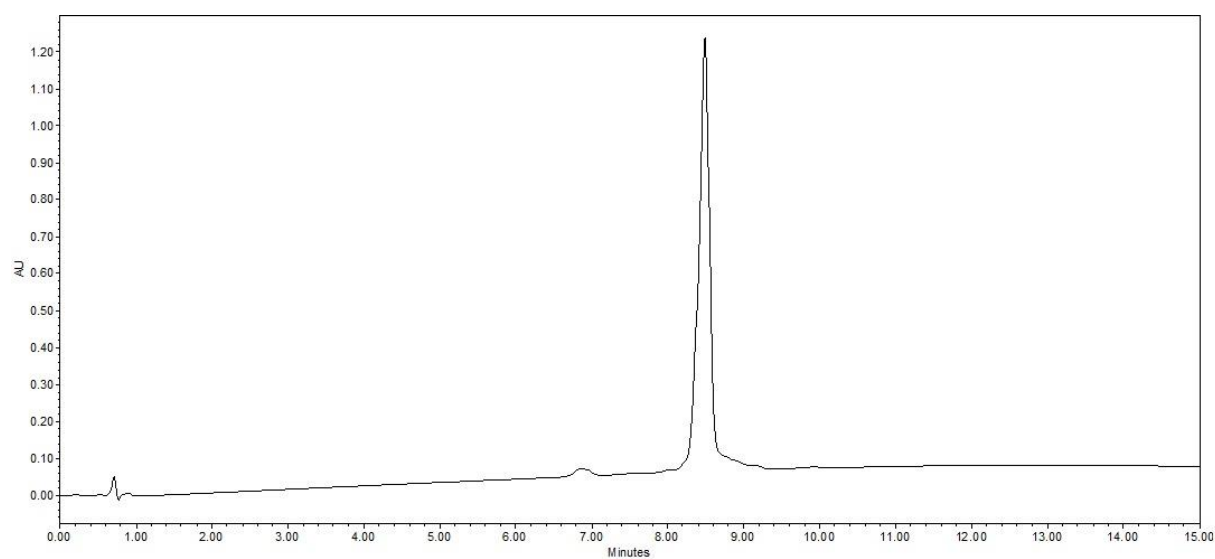


Figure S48. RP-HPLC profile of **25**: *t_R*= 8.49 min. (5-100% solv.B in 15 min.).

Compound 26.

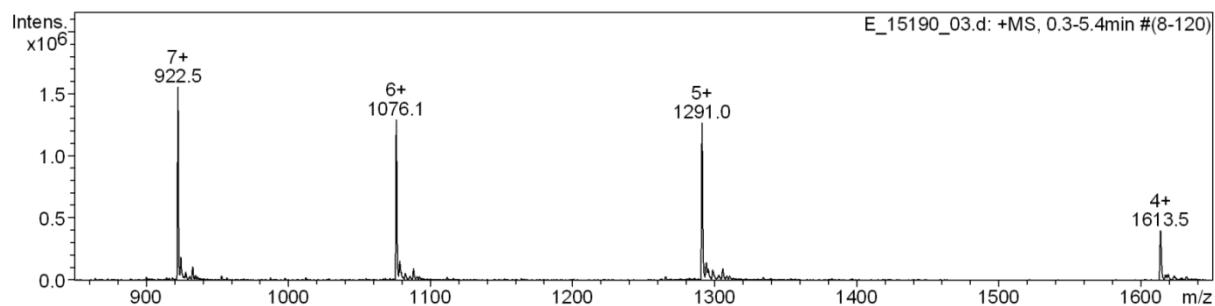


Figure S49. ESI⁺-MS spectrum of **26**. *m/z* (Average Mwt) calcd. for C₂₇₅H₄₅₇N₁₀₃O₇₈ [M+4H]⁴⁺:1613.6, found: 1613.5 ; calcd. for C₂₇₅H₄₅₈N₁₀₃O₇₈ [M+5H]⁵⁺: 1291.0, found: 1291.0 ; calcd. for C₂₇₅H₄₅₉N₁₀₃O₇₈ [M+6H]⁶⁺: 1076.0, found: 1076.1 ; calcd. for C₂₇₅H₄₆₀N₁₀₃O₇₈ [M+7H]⁷⁺: 922.5, found: 922.5

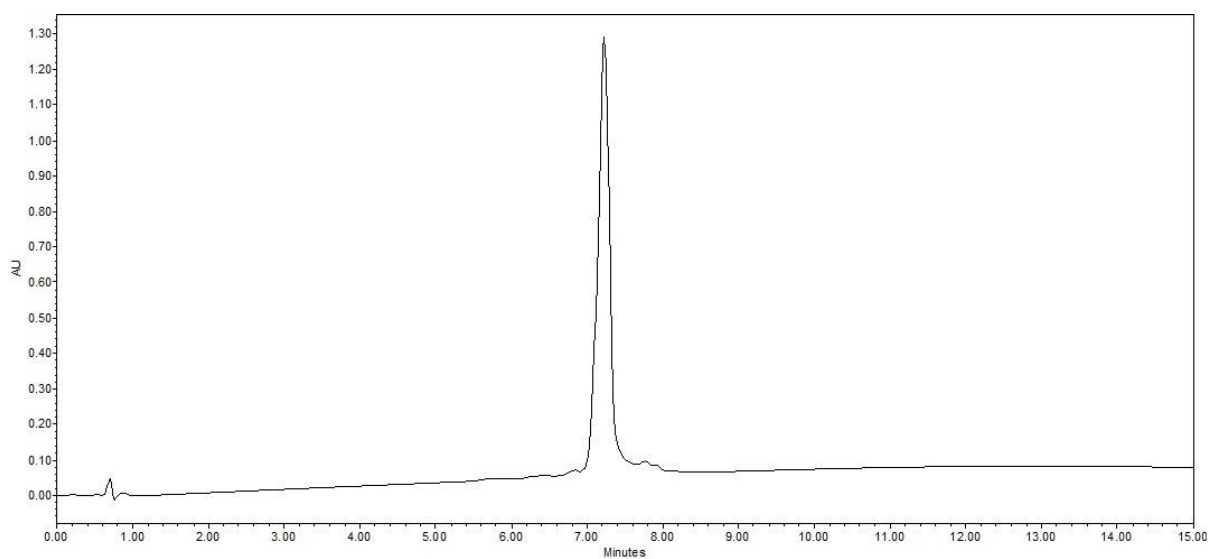


Figure S50. RP-HPLC profile of **26**: *t_R*= 7.23 min. (5-100% solv.B in 15 min.).

Compound 27.

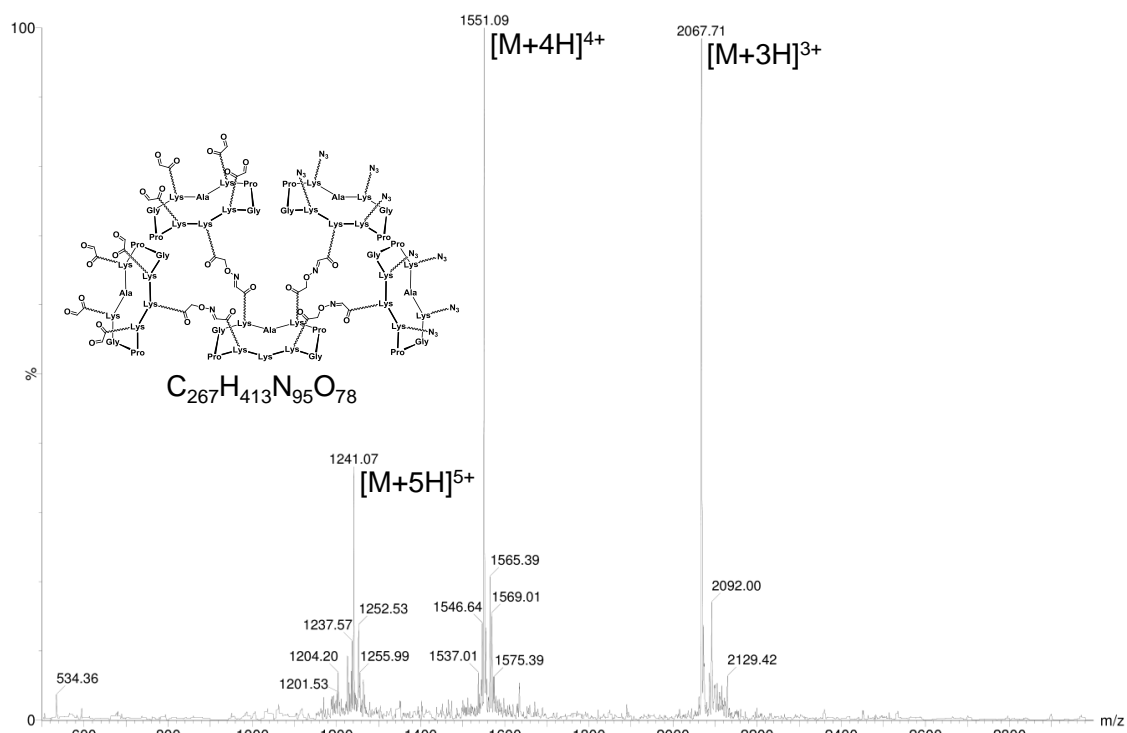


Figure S51. ESI-MS spectrum of **27**. m/z (Average Mwt) calcd. for $C_{267}H_{416}N_{95}O_{78}$ $[M+3H]^{3+}$: 2068.2, found: 2067.7; calcd. for $C_{267}H_{417}N_{95}O_{78}$ $[M+4H]^{4+}$: 1551.4, found: 1551.1; calcd. for $C_{267}H_{418}N_{95}O_{78}$ $[M+5H]^{5+}$: 1241.4, found: 1241.1

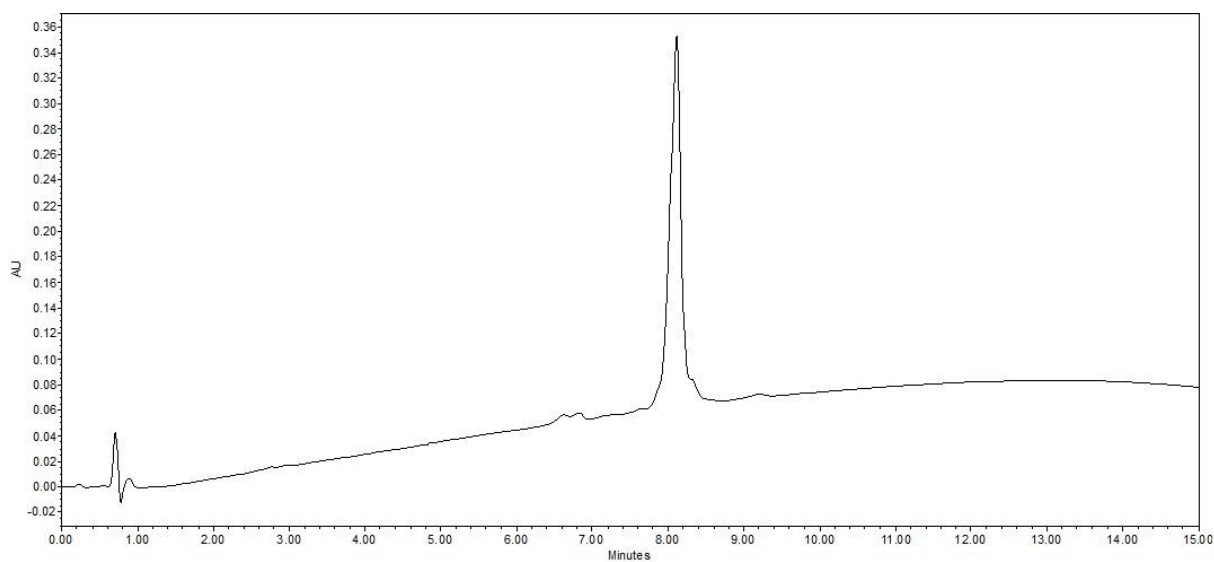


Figure S52. RP-HPLC profile of **27**: $t_R = 8.11$ min. (5-100% solv.B in 15 min.).

Compound 28.

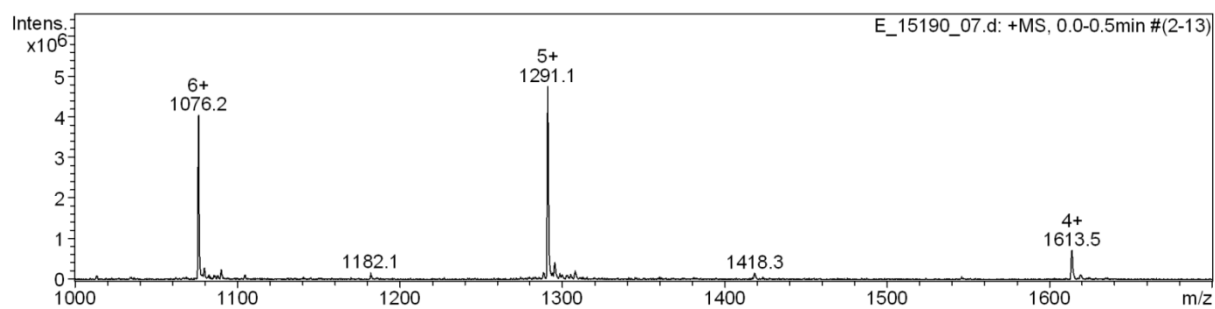


Figure S53. ESI⁺-MS spectrum of **28**. *m/z* (Average Mwt) calcd. for C₂₇₅H₄₅₇N₁₀₃O₇₈ [M+4H]⁴⁺:1613.6, found: 1613.5 ; calcd. for C₂₇₅H₄₅₈N₁₀₃O₇₈ [M+5H]⁵⁺: 1291.0, found: 1291.1 ; calcd. for C₂₇₅H₄₅₉N₁₀₃O₇₈ [M+6H]⁶⁺: 1076.0, found: 1076.2

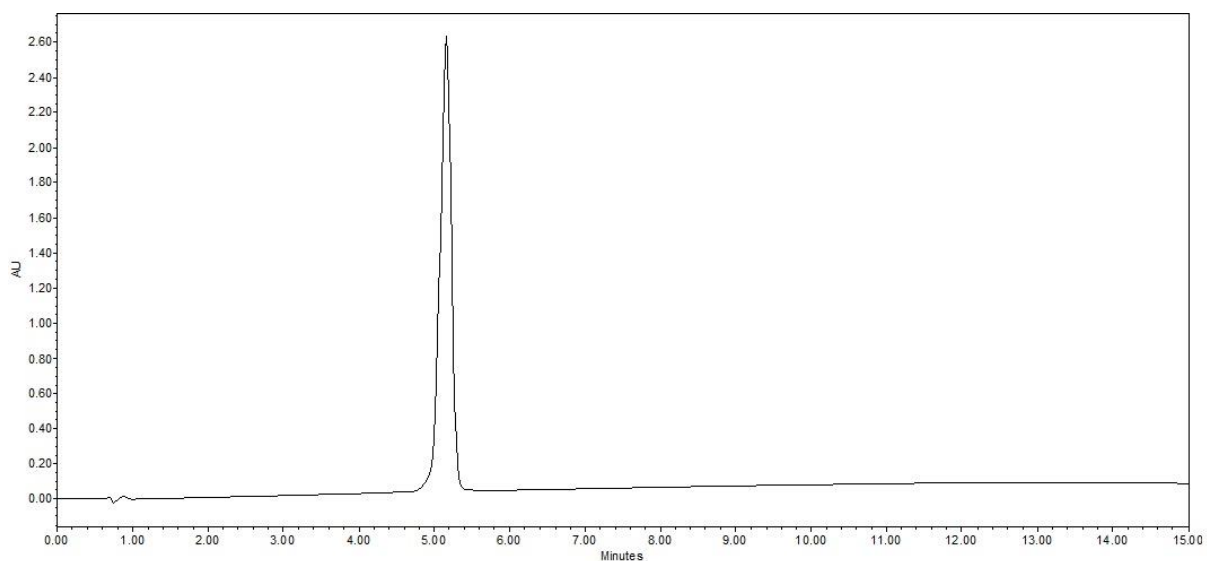


Figure S54. RP-HPLC profile of **28**: *t_R*= 4.31 min. (5-100% solv.B in 15 min.).

Compound 29.

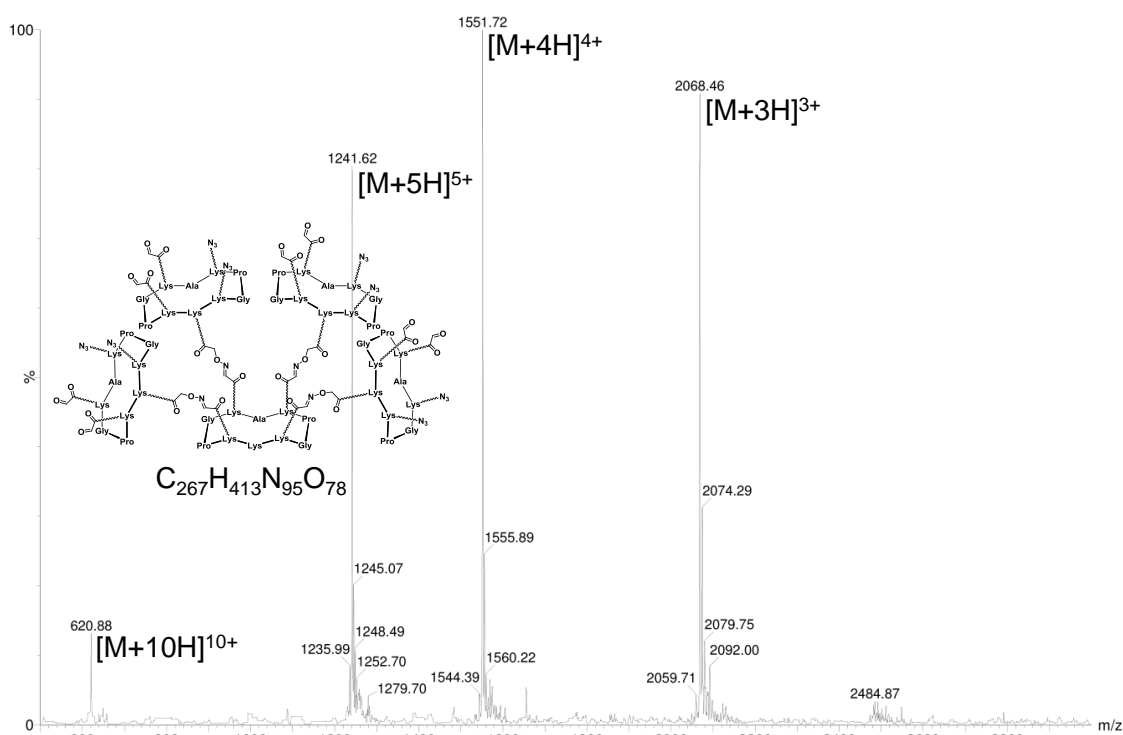


Figure S55. ESI+-MS spectrum of **29**. m/z (Average Mwt) calcd. for $C_{267}H_{416}N_{95}O_{78}$ $[M+3H]^{3+}$: 2068.3, found: 2068.5 ; calcd. for $C_{267}H_{417}N_{95}O_{78}$ $[M+4H]^{4+}$: 1551.4, found: 1551.7 ; calcd. for $C_{267}H_{418}N_{95}O_{78}$ $[M+5H]^{5+}$: 1241.4, found: 1241.6 ; $C_{267}H_{423}N_{95}O_{78}$ $[M+10H]^{10+}$: 621.2, found: 620.9

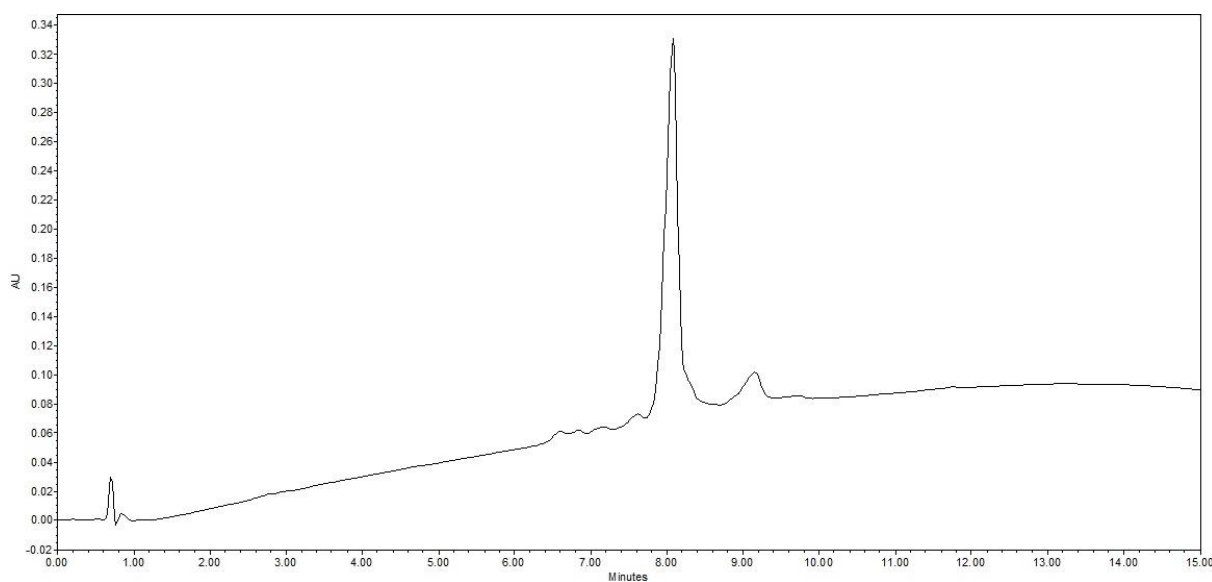
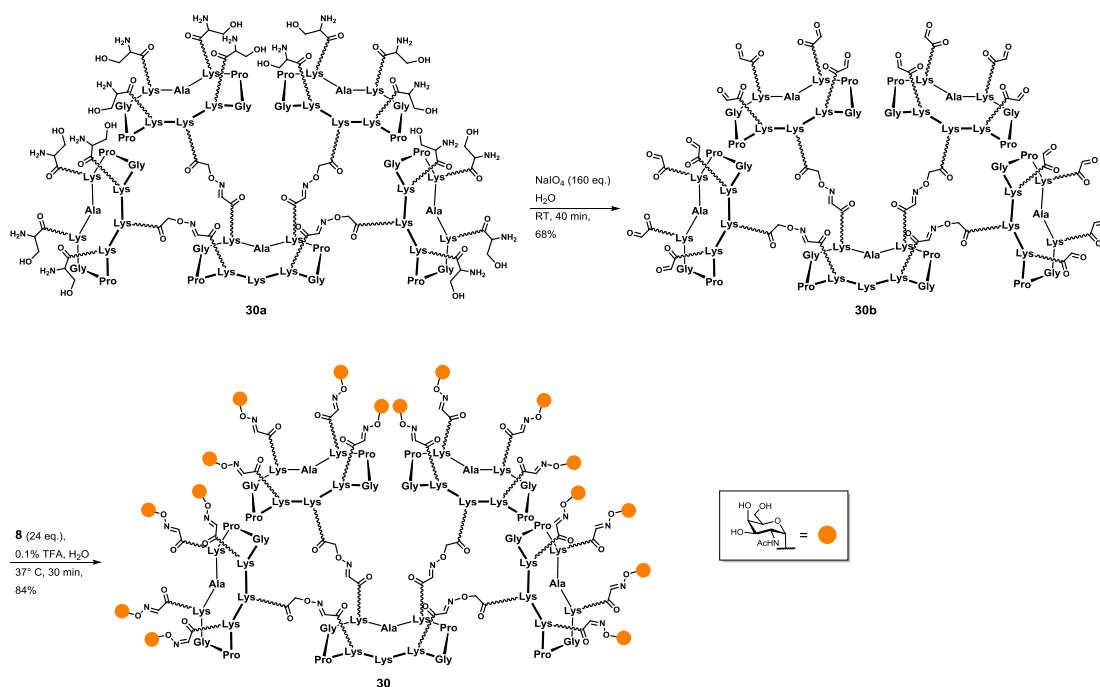


Figure S56. RP-HPLC profile of **29**: t_R = 8.08 min. (5-100% solv.B in 15 min.).

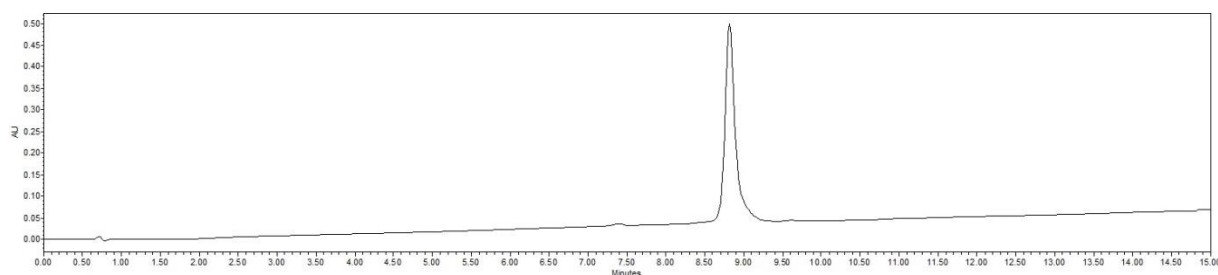
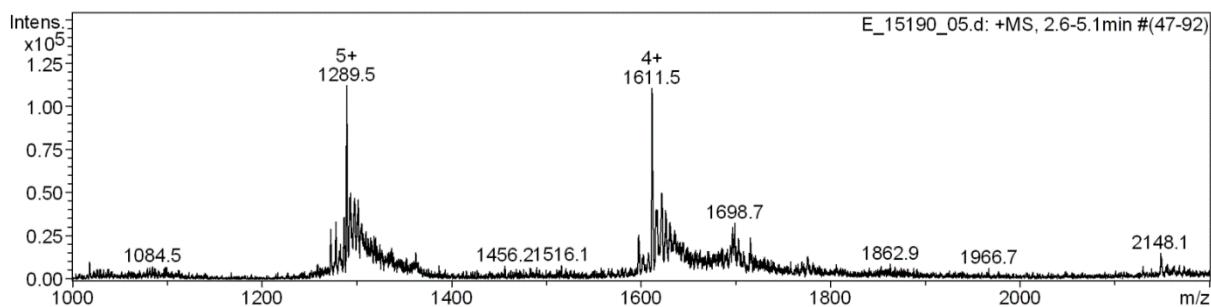
Compound 30.



Scheme S1. Synthesis of compound **30**.

Synthesis of compound **30b**.

To a solution of compound **30a**¹ (9.7 mg, 1.4 μmol) in H_2O (1.0 mL), sodium periodate (47.9 mg, 224 μmol) was added and the reaction mixture stirred at room temperature for 40 minutes. Direct RP-HPLC purification, followed by lyophilization afforded compound **30b** (6.1 mg) in 68% yield.



Synthesis of compound **30**.

To a solution of compound **30b** (5.8 mg, 0.9 μmol) in H_2O (1.0 mL) containing 0.09% $\text{CF}_3\text{CO}_2\text{H}$, **8** (5.1 mg, 21.6 μmol) was added and the reaction heated at 37°C without stirring. After 30 minutes the reaction mixture was directly purified by preparative RP-HPLC and lyophilized to afford pure **30** (7.5 mg) in 84% yield. ESI⁺-MS m/z (Average Mwt) calcd. for $\text{C}_{411}\text{H}_{657}\text{N}_{111}\text{O}_{174}$ $[\text{M}+4\text{H}]^{4+}$: 2484.3, found: 2484.9 ; calcd. for $\text{C}_{411}\text{H}_{658}\text{N}_{111}\text{O}_{174}$ $[\text{M}+5\text{H}]^{5+}$: 1987.7, found: 1987.3 ; calcd. for $\text{C}_{411}\text{H}_{659}\text{N}_{111}\text{O}_{174}$ $[\text{M}+6\text{H}]^{6+}$: 1656.5, found: 1656.2 ; Analytical RP-HPLC: t_{R} = 8.78 min. (0-40% solv.B in 15 min.).

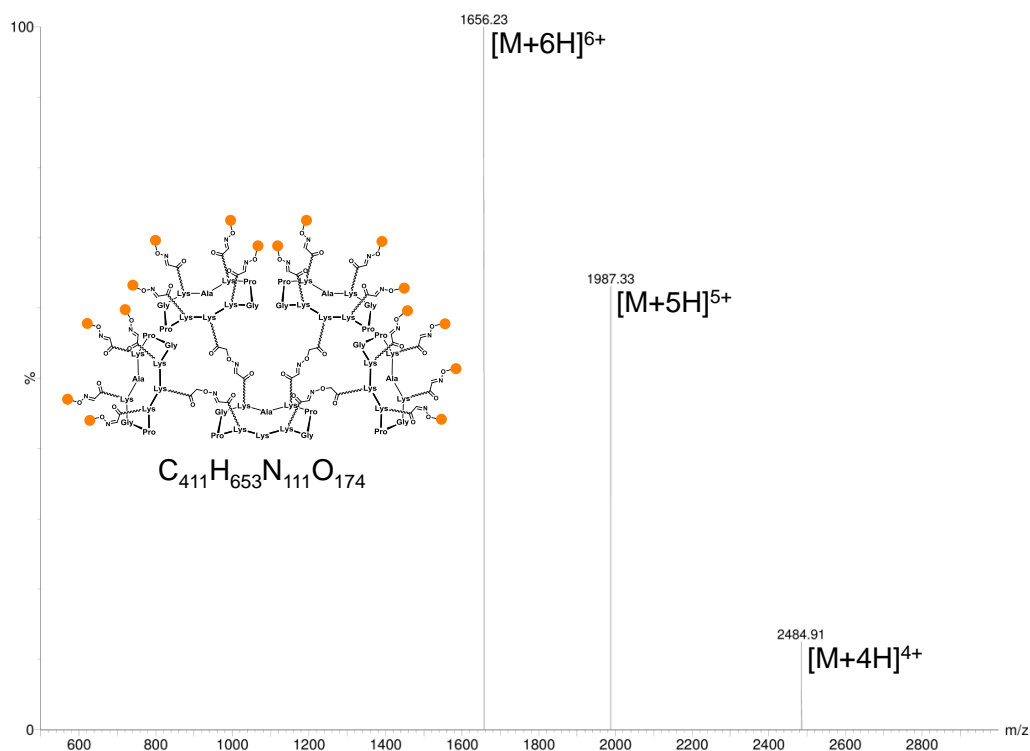


Figure S59. ESI⁺-MS spectrum of **30**. m/z (Average Mwt) calcd. for $\text{C}_{411}\text{H}_{657}\text{N}_{111}\text{O}_{174}$ $[\text{M}+4\text{H}]^{4+}$: 2484.3, found: 2484.9 ; calcd. for $\text{C}_{411}\text{H}_{658}\text{N}_{111}\text{O}_{174}$ $[\text{M}+5\text{H}]^{5+}$: 1987.7, found: 1987.3 ; calcd. for $\text{C}_{411}\text{H}_{659}\text{N}_{111}\text{O}_{174}$ $[\text{M}+6\text{H}]^{6+}$: 1656.5, found: 1656.2

$^1\text{H-NMR}$ of **30** in D_2O at 400 MHz

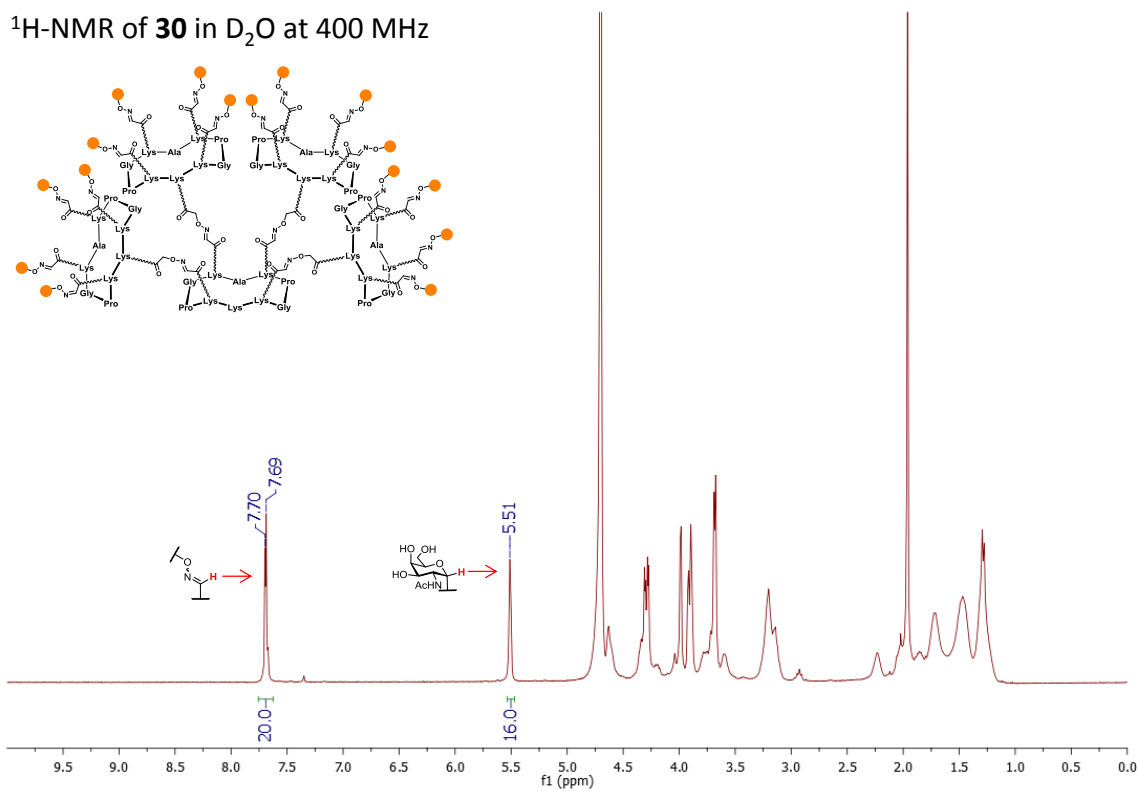


Figure S60. $^1\text{H-NMR}$ spectrum of compound **30** (D_2O , 400 MHz) showing integration of characteristic signals.

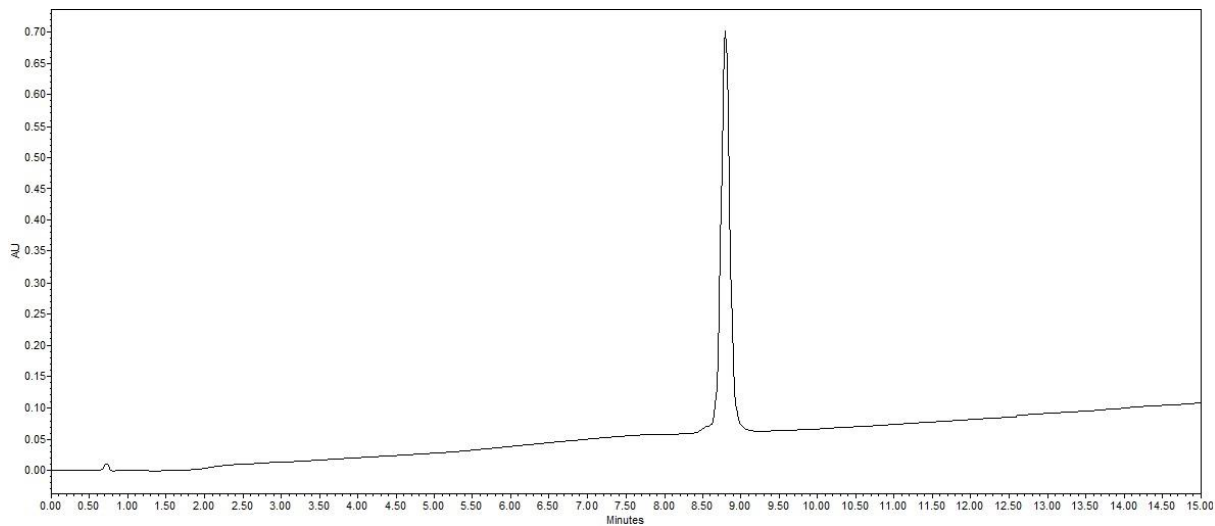
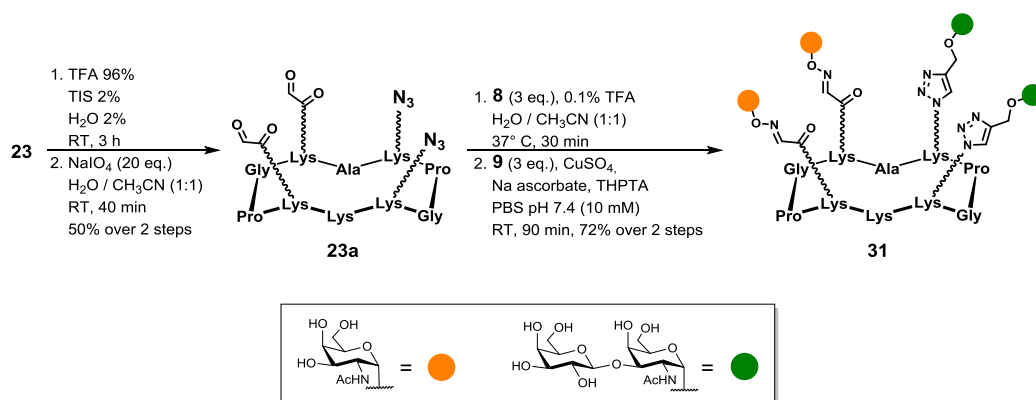


Figure S61. RP-HPLC profile of **30**: $t_R = 8.78$ min. (0-40% solv.B in 15 min.).

Compound 31.



Scheme S2. Synthesis of compound **31**.

Synthesis of compound **23a**.

A solution containing trifluoroacetic acid (TFA), triisopropylsilane (TIS) and water (1.0 mL, 96:2:2) was added to **23** (6.8 mg, 4.4 μmol). After 3 hours stirring at room temperature the reaction mixture was added to ice-cold Et₂O (10 mL) and the resulting precipitate was filtrated and dried to give a powder which was solubilized in H₂O (1.0 mL). To this solution, sodium periodate (18.8 mg, 87.9 μmol) was added and the reaction mixture stirred at room temperature for 40 minutes. Direct RP-HPLC purification, followed by lyophilization afforded compound **23a** (2.6 mg) in 50% yield over two steps. HRMS (ESI⁺-TOF) *m/z* (Monoisotopic Mwt) calcd. for C₅₁H₈₂N₁₉O₁₄ [M+H]⁺: 1184.6289, found: 1184.6309; calcd. for C₁₀₁H₈₆N₁₉O₁₆Na [M+H₂O+H]⁺: 1220.6500, found: 1220.6527; Analytical RP-HPLC: *t_R* = 3.78 min. (5-100% solv.B in 15 min.).

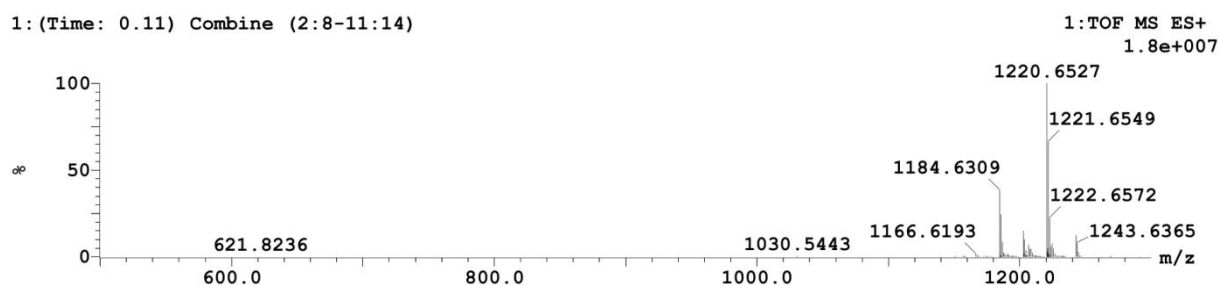


Figure S62. HRMS (ESI⁺-TOF) of **23a**: *m/z* (Monoisotopic Mwt) calcd. for C₅₁H₈₂N₁₉O₁₄ [M+H]⁺: 1184.6289, found: 1184.6309; calcd. for C₁₀₁H₈₆N₁₉O₁₆Na [M+H₂O+H]⁺: 1220.6500, found: 1220.6527

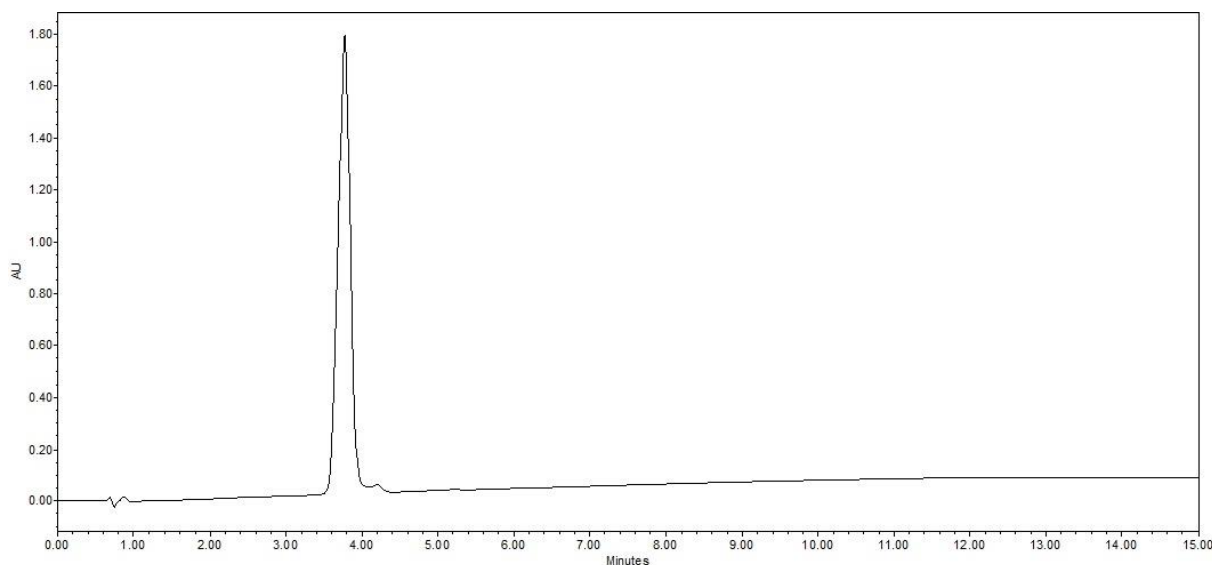


Figure S63. RP-HPLC profile of **23a**: $t_R = 3.78$ min. (5-100% solv.B in 15 min.).

Synthesis of compound **31**.

To a solution of **23a** (2.2 mg, 1.9 μmol) in a $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (1:1, 0.5 mL) mixture containing 0.09% $\text{CF}_3\text{CO}_2\text{H}$, **8** (1.4 mg, 5.9 μmol) was added and the reaction heated at 37°C without stirring. After 30 minutes, **9** (2.4 mg, 5.7 μmol) was added to this mixture, then 1.0 mL of PBS buffer (pH 7.4, 10mM) was added and the solution degassed by argon bubbling for 15 minutes. A separate solution, containing CuSO_4 (0.2 mg, 0.8 μmol), THPTA (1.7 mg, 3.9 μmol) and sodium ascorbate (1.5 mg, 7.6 μmol) in previously degassed PBS buffer (1.0 mL, pH 7.4, 10mM) was added to the reaction mixture. After 90 minutes stirring at room temperature, Chelex[®] resin was added to the reaction mixture and stirred for 30 minutes at room temperature in order to remove residues of copper. The crude was then purified by preparative RP-HPLC and lyophilized to afford 3.4 mg of pure **31** (72% yield over two steps). HRMS (ESI⁺-TOF) m/z (Monoisotopic Mwt) calcd. for $\text{C}_{101}\text{H}_{164}\text{N}_{25}\text{O}_{46}$ $[\text{M}+\text{H}]^+$: 2463.1262, found: 2463.1223 ; calcd. for $\text{C}_{101}\text{H}_{163}\text{N}_{25}\text{O}_{46}\text{Na}$ $[\text{M}+\text{Na}]^+$: 2485.1081, found: 2485.1047 ; Analytical RP-HPLC: $t_R = 5.40$ min. (0-40% solv.B in 15 min.).

R2(TRZ)TF2(Ox)Tn_4 (0.104) Cm (4:7)

1: TOF MS ES+
8.19e+003

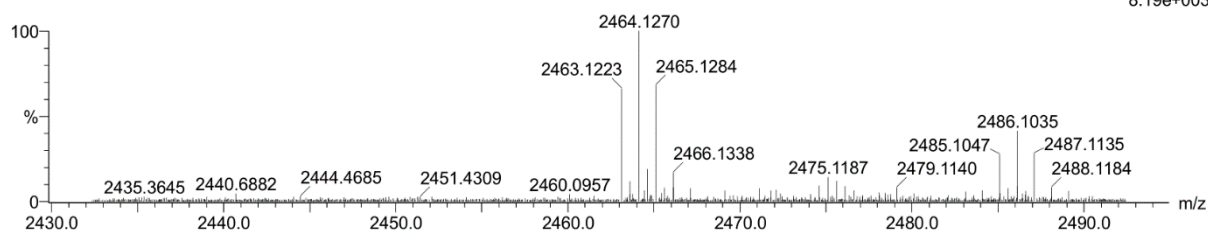


Figure S64. HRMS (ESI⁺-TOF) of **31**: m/z (Monoisotopic Mwt) calcd. for $\text{C}_{101}\text{H}_{164}\text{N}_{25}\text{O}_{46}$ $[\text{M}+\text{H}]^+$: 2463.1262, found: 2463.1223 ; calcd. for $\text{C}_{101}\text{H}_{163}\text{N}_{25}\text{O}_{46}\text{Na}$ $[\text{M}+\text{Na}]^+$: 2485.1081, found: 2485.1047

$^1\text{H-NMR}$ of **31** in D_2O at 400 MHz

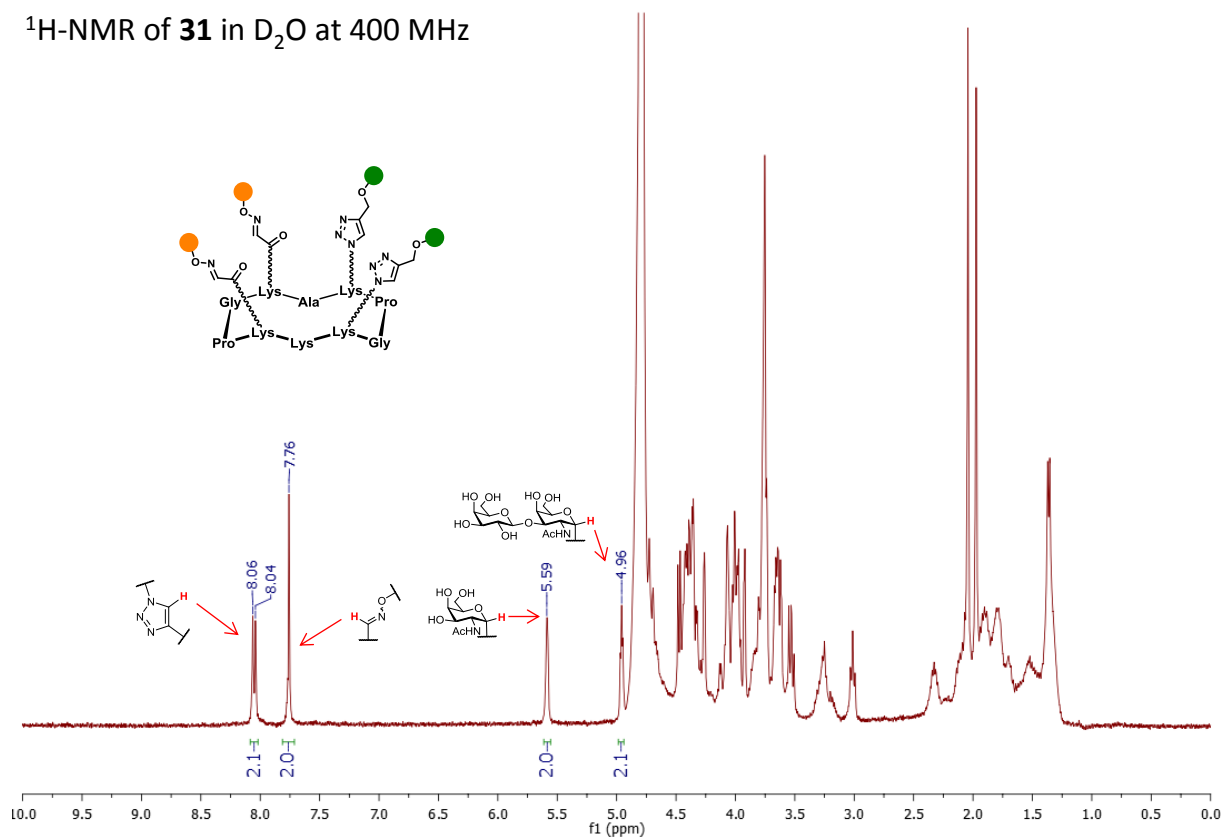


Figure S65. $^1\text{H-NMR}$ spectrum of compound **31** (D_2O , 400 MHz) showing integration of characteristic signals.

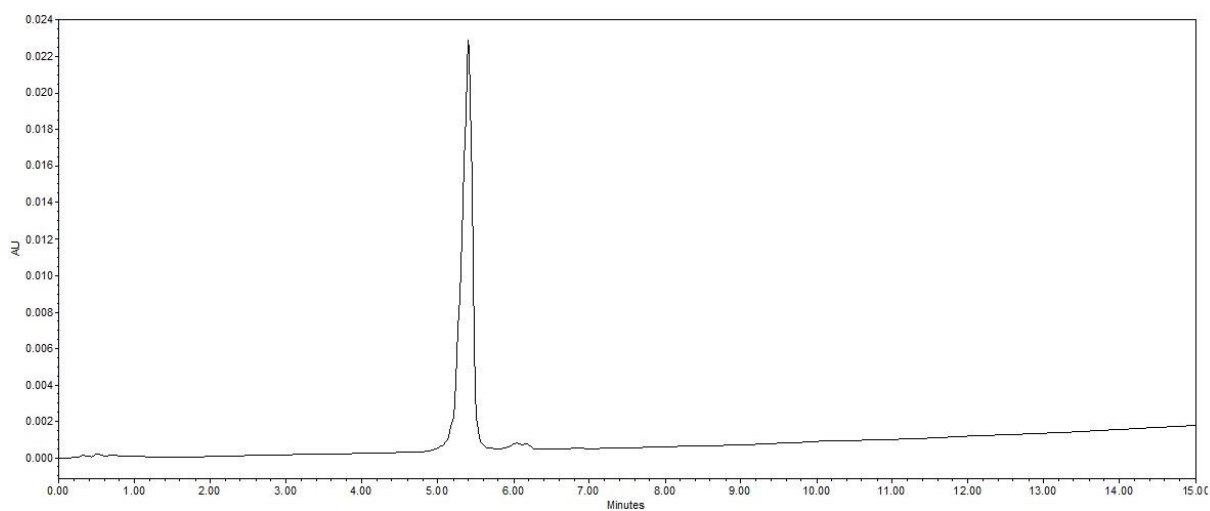
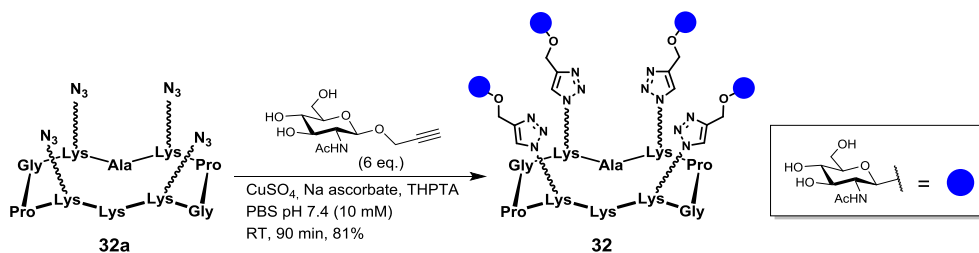


Figure S66. RP-HPLC profile of **31**: $t_R = 5.40$ min. (0-40% solv.B in 15 min.).

Compound 32.



Scheme S3. Synthesis of compound **32**.

Synthesis of compound **32**.

To a solution of **32a** (5.4 mg, 4.8 μmol) in DMF (0.6 mL), prop-2-ynyl 2-acetamido-2-deoxy- β -D-glucopyranoside² (7.5 mg, 28.9 μmol) was added and the mixture degassed by argon bubbling for 15 minutes. A separate solution, containing CuSO_4 (0.2 mg, 0.8 μmol), THPTA (8.3 mg, 19.1 μmol) and sodium ascorbate (7.6 mg, 38.4 μmol) in previously degassed PBS buffer (1.2 mL, pH 7.4, 10mM) was added to the reaction mixture. After 90 minutes stirring at room temperature, Chelex[®] resin was added to the reaction mixture and stirred for 30 minutes at room temperature in order to remove residues of copper. The crude was then purified by preparative RP-HPLC and lyophilized to afford pure **32** (8.4 mg) in 81% yield. HRMS (ESI⁺-TOF) m/z (Monoisotopic Mwt) calcd. for $\text{C}_{91}\text{H}_{146}\text{N}_{27}\text{O}_{34}$ $[\text{M}+\text{H}]^+$: 2161.0526, found: 2161.0530 ; calcd. for $\text{C}_{91}\text{H}_{145}\text{N}_{27}\text{O}_{34}\text{Na}$ $[\text{M}+\text{Na}]^+$: 2184.0373, found: 2184.0386 ; Analytical RP-HPLC: t_{R} = 5.48 min. (0-40% solv.B in 15 min.).

1: (Time: 0.13) Combine (5:7-9:12)

1:TOF MS ES+
1.1e+04

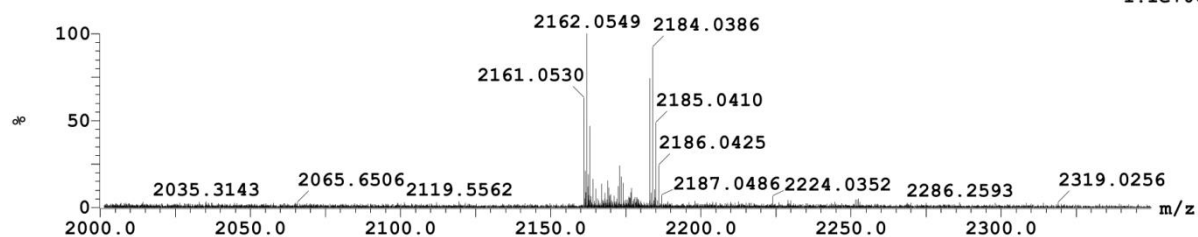


Figure S67. HRMS (ESI⁺-TOF) of **32**: m/z (Monoisotopic Mwt) calcd. for $\text{C}_{91}\text{H}_{146}\text{N}_{27}\text{O}_{34}$ $[\text{M}+\text{H}]^+$: 2161.0526, found: 2161.0530 ; calcd. for $\text{C}_{91}\text{H}_{145}\text{N}_{27}\text{O}_{34}\text{Na}$ $[\text{M}+\text{Na}]^+$: 2184.0373, found: 2184.0386

$^1\text{H-NMR}$ of **32** in D_2O at 400 MHz

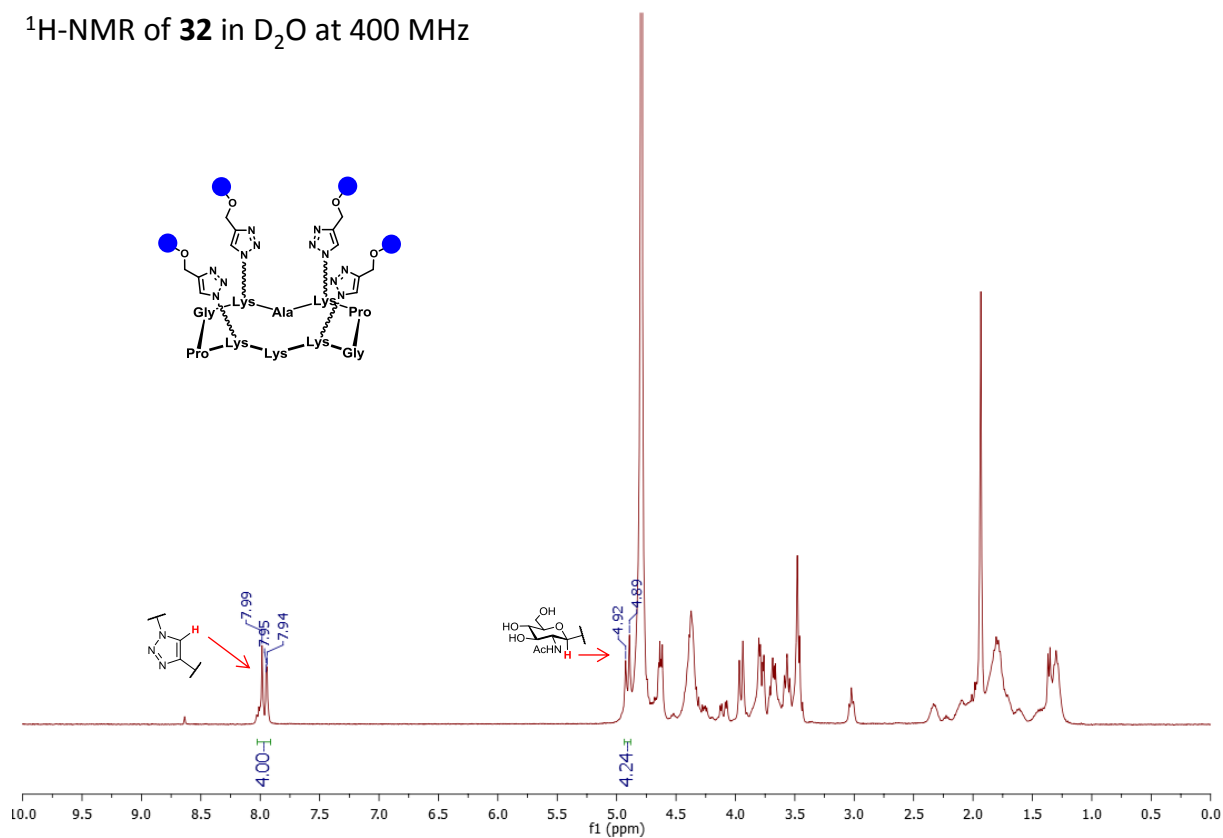


Figure S68. $^1\text{H-NMR}$ spectrum of compound **32** (D_2O , 400 MHz) showing integration of characteristic signals.

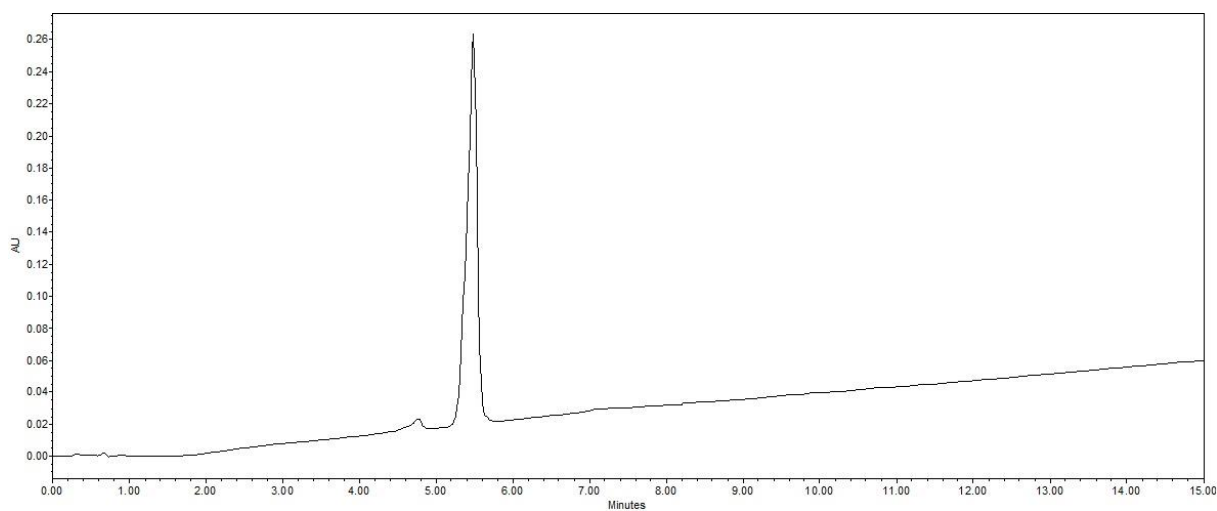
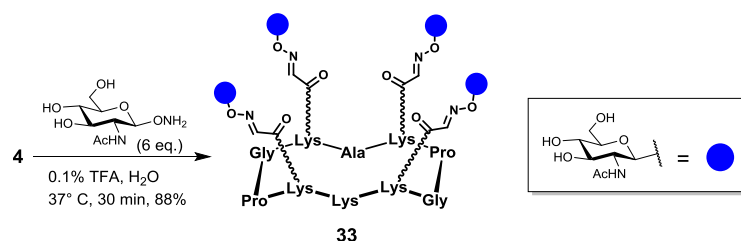


Figure S69. RP-HPLC profile of **32**: $t_R = 5.48$ min. (0-40% solv.B in 15 min.).

Compound 33.



Scheme S4. Synthesis of compound **33**.

Synthesis of compound 33.

To a solution of **4**³ (4.9 mg, 3.9 μmol) in H_2O (1.0 mL) containing 0.09% $\text{CF}_3\text{CO}_2\text{H}$, 2-acetamido-2-deoxy- β -D-glucopyranosyl hydroxylamine² (5.5 mg, 23.3 μmol) was added and the reaction heated at 37°C without stirring. After 30 minutes the reaction mixture was directly purified by preparative RP-HPLC and lyophilized to afford pure **33** (7.3 mg) in 88% yield. HRMS (ESI⁺-TOF) m/z (Monoisotopic Mwt) calcd. for $\text{C}_{87}\text{H}_{142}\text{N}_{23}\text{O}_{38}$ [M+H]⁺: 2116.9886, found: 2116.9878 ; calcd. for $\text{C}_{87}\text{H}_{141}\text{N}_{23}\text{O}_{38}\text{Na}$ [M +Na]⁺: 2138.9706, found: 2138.9624 ; Analytical RP-HPLC: t_R = 5.39 min. (0-40% solv.B in 15 min.).

R4(Ox)GlcNAc 4 (0.104) Cm (4:6)

1: TOF MS ES+
4.56e+002

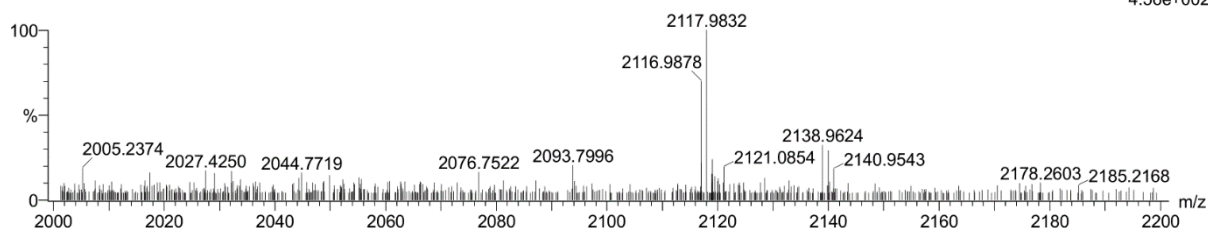


Figure S70. HRMS (ESI⁺-TOF) of **33**: m/z (Monoisotopic Mwt) calcd. for $\text{C}_{87}\text{H}_{142}\text{N}_{23}\text{O}_{38}$ [M+H]⁺: 2116.9886, found: 2116.9878 ; calcd. for $\text{C}_{87}\text{H}_{141}\text{N}_{23}\text{O}_{38}\text{Na}$ [M +Na]⁺: 2138.9706, found: 2138.9624

$^1\text{H-NMR}$ of **33** in D_2O at 400 MHz

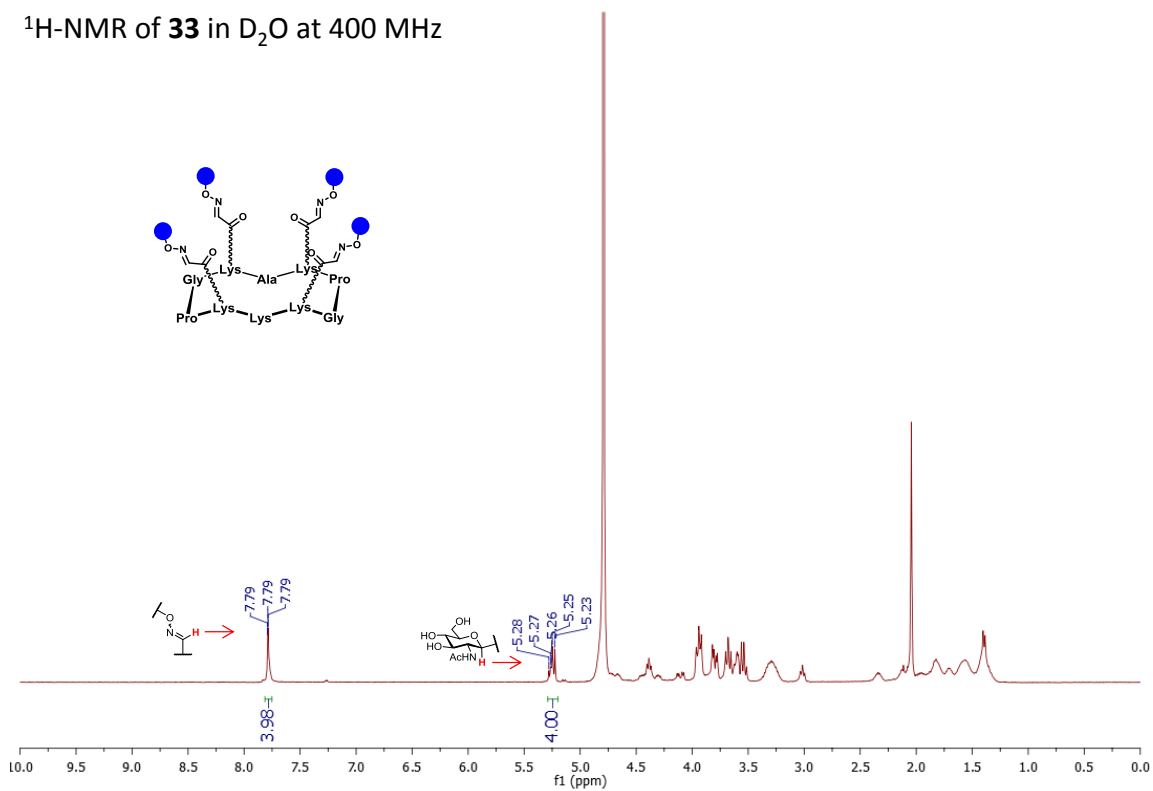


Figure S71. $^1\text{H-NMR}$ spectrum of compound **32** (D_2O , 400 MHz) showing integration of characteristic signals.

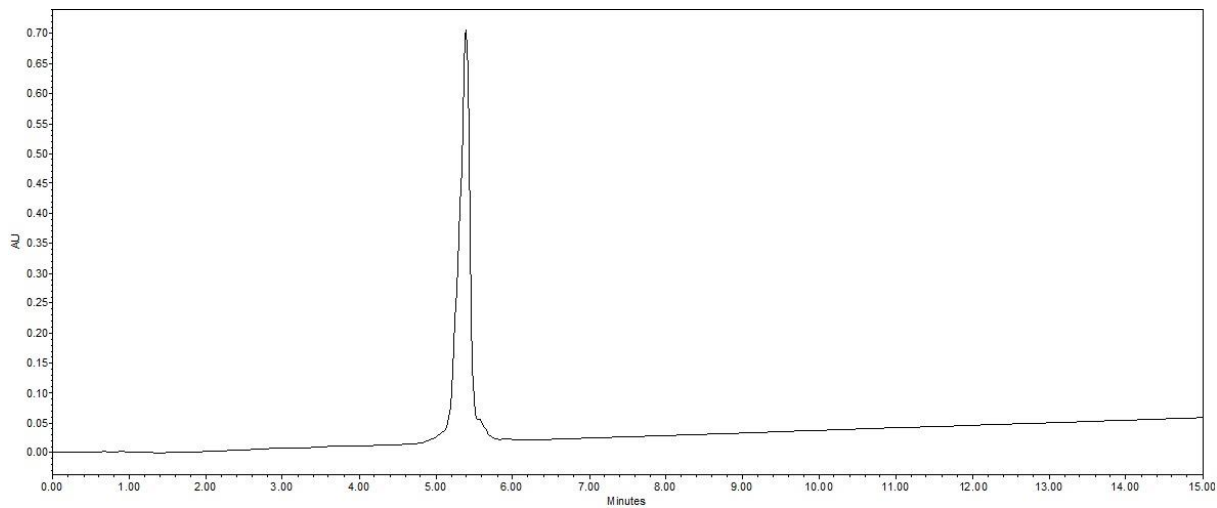


Figure S72. RP-HPLC profile of **33**: $t_R = 5.39$ min. (0-40% solv.B in 15 min.).

References

- [1] B. Thomas, N. Berthet, J. Garcia, P. Dumy, O. Renaudet, *Chem. Commun.* **2013**, 49, 10796-10798.
- [2] M. Fiore, N. Berthet, A. Marra, E. Gillon, P. Dumy, A. Dondoni, A. Imberty, O. Renaudet, *Org. Biomol. Chem.* **2013**, 11, 7113-7122.
- [3] Y. Singh, O. Renaudet, E. Defrancq, P. Dumy, *Org. Lett.* **2005**, 7, 1359-1362.