Electronic Supplementary Information

Structural influence of Antibody Recruiting Glycodendrimers (ARGs) on antitumoral cytotoxicity

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NMR and MS of final compounds

Compound **3**

Prepared according already described procedure. Analyses were in agreement with the literature.[2]

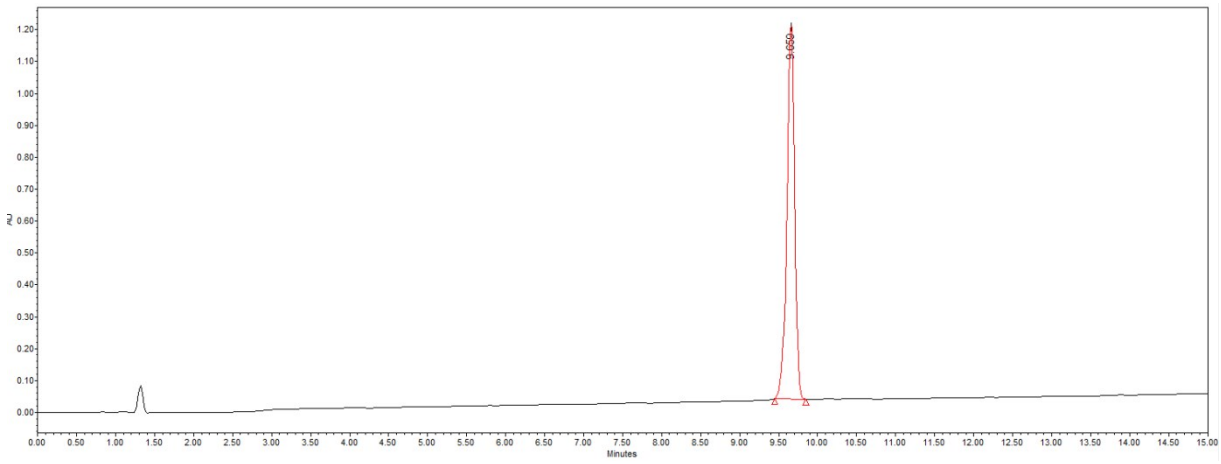

Figure S 1. RP-HPLC Spectrum of compound **3**

Compound **4**

Prepared according already described procedure. Analyses were in agreement with the literature.[2]

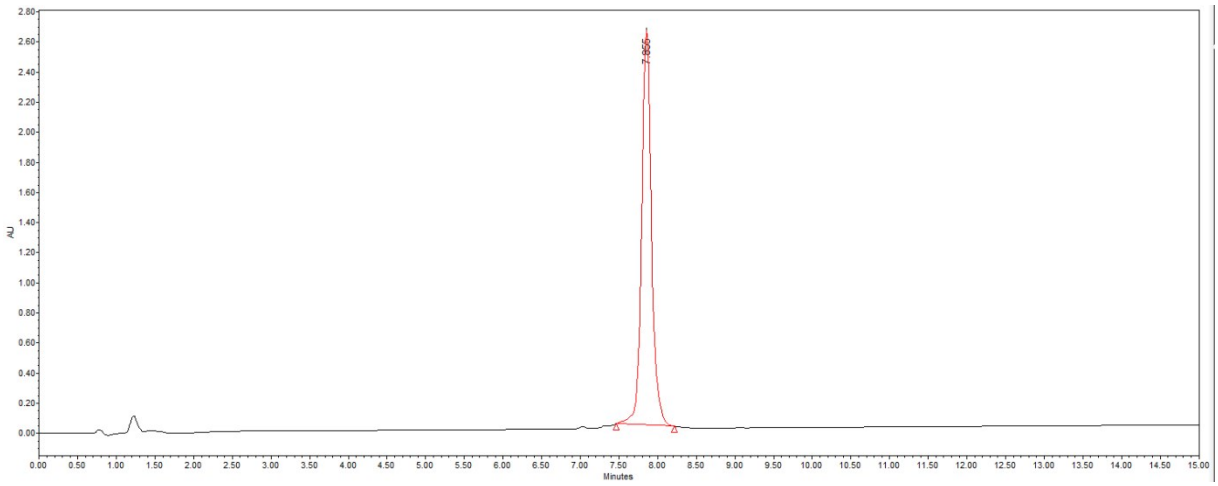

Figure S 2. RP-HPLC Spectrum of compound **4**

Compound **5**

Prepared according general procedure **A** from propargyl α-L-rhamnopyranoside (43.3 mg, 214 µmol) and **2** (39.9 mg, 48.7 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (70.0 mg, 43 µmol, 88%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{67}H_{112}N_{20}O_{27}$ $[M+2H]^{2+}$: 814.3997, found 814.3994; RP-HPLC: R_t = 4.05 min (C18, λ = 214 nm, 5-60% B in 15 min).

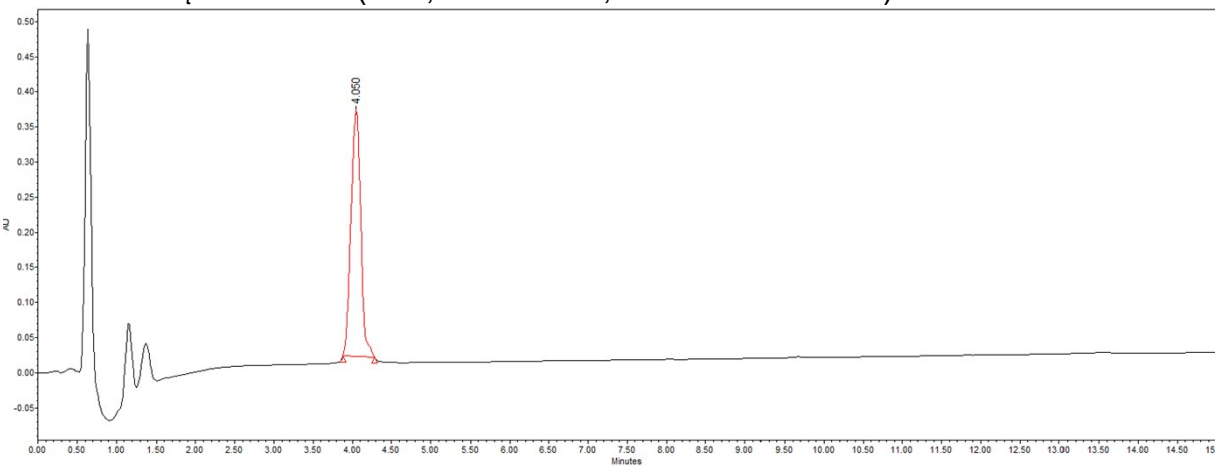

Figure S 3. RP-HPLC Spectrum of compound **5**

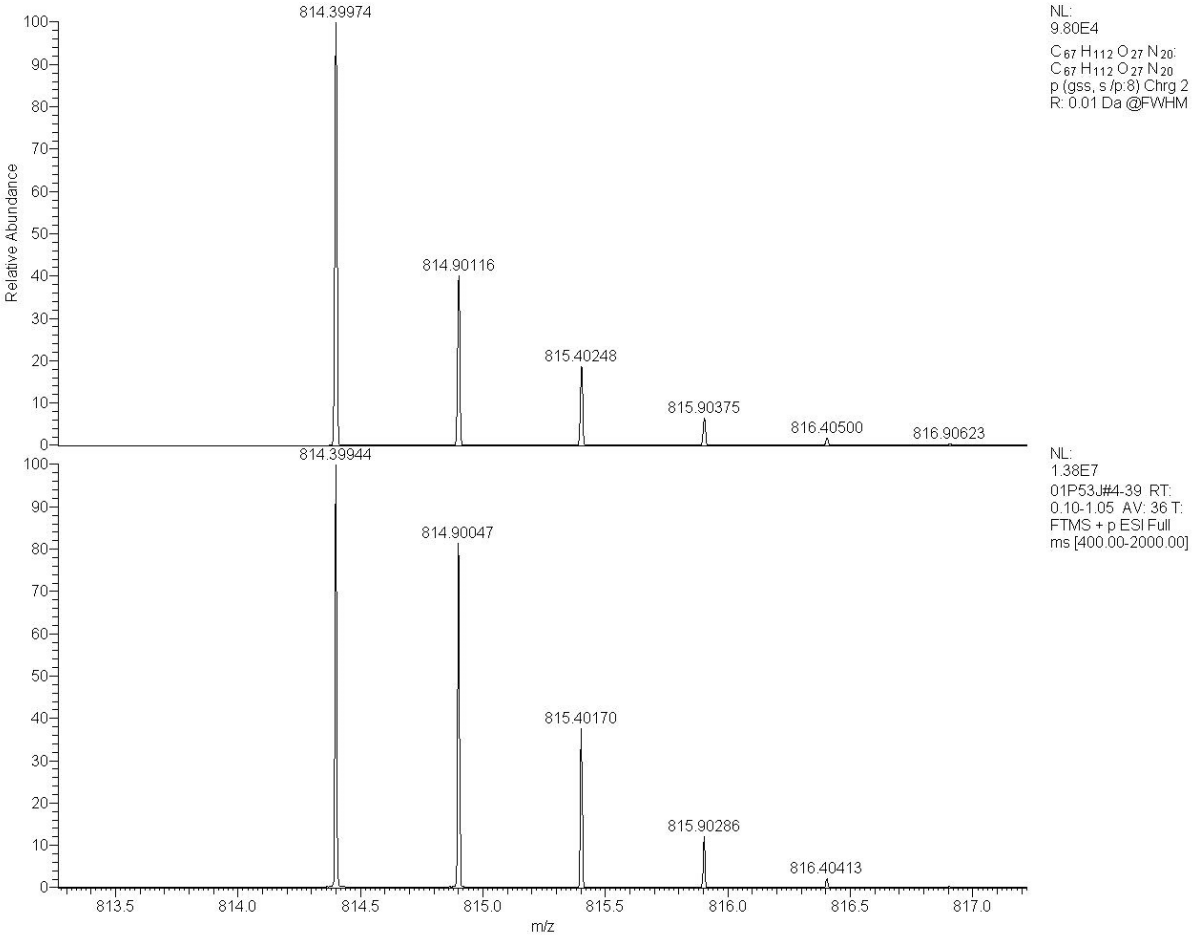

Figure S 4. HRMS spectrum of compound **5**

Compound **6**

Prepared according already described procedure. Analyses were in agreement with the literature.[2]

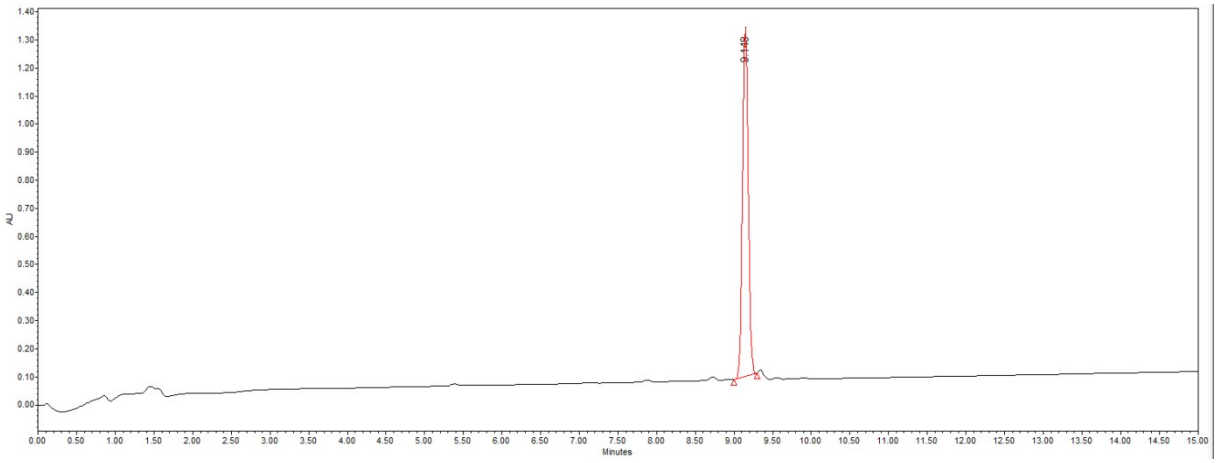

Figure S 5. RP-HPLC Spectrum of compound **6**

Compound **7**

Prepared according general procedure **C** from **5** (24.2 mg, 14.9 µmol) and azidoacetic acid succinimide ester (4.4 mg, 22.3 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (23.0 mg, 13.4 µmol, 90%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{69}H_{113}N_{23}O_{28}$ $[M+2H]^{2+}$: 855.9057, found 855.9055; RP-HPLC: R_t = 4.93 min (C18, λ = 214 nm, 5-60% B in 15 min).

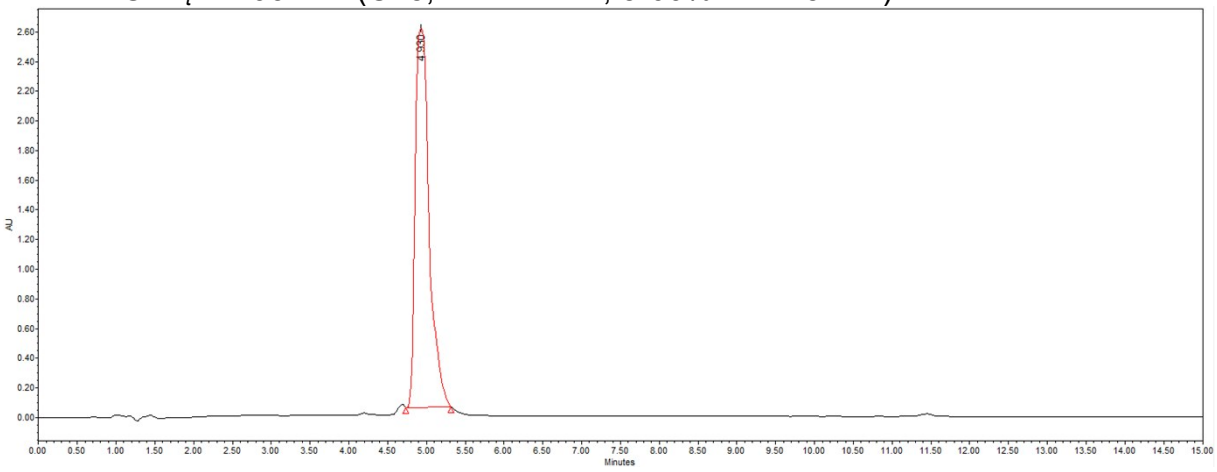

Figure S 6. RP-HPLC Spectrum of compound **7**

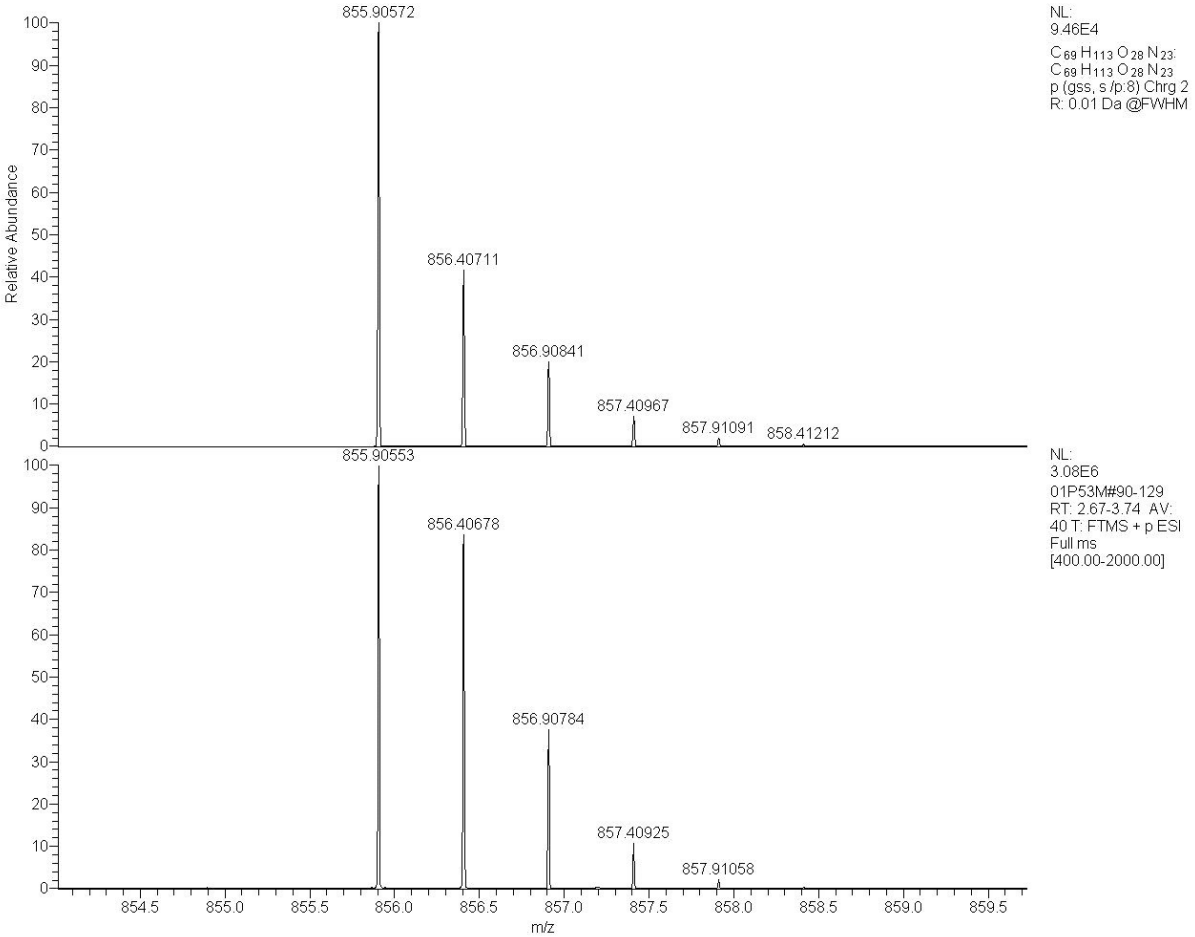

Figure S 7. HRMS spectrum of compound **7**

Compound **8**

Prepared according already described procedure. Analyses were in agreement with the literature.[2]

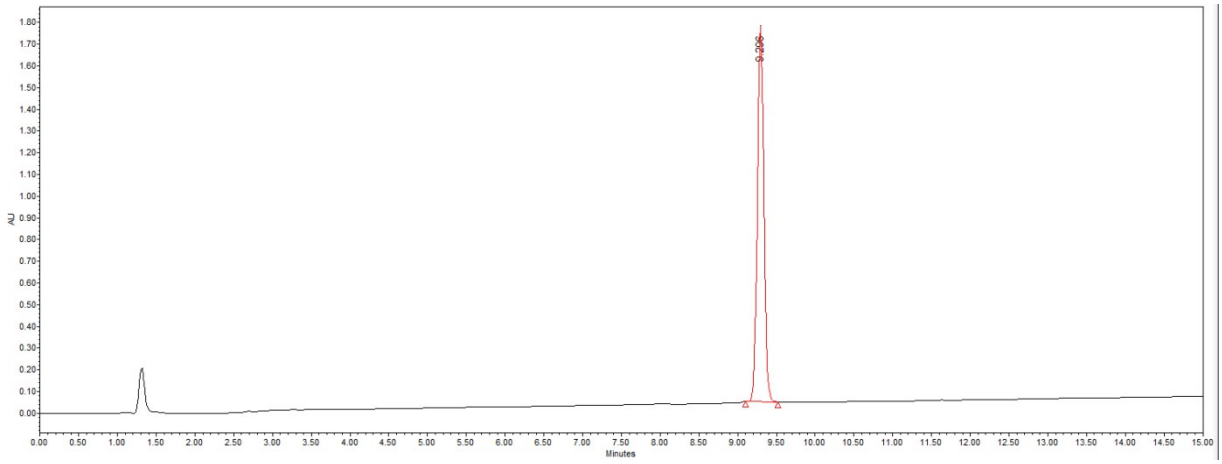

Figure S 8. RP-HPLC Spectrum of compound **8**

Compound **9**

Prepared according already described procedure. Analyses were in agreement with the literature.[2]

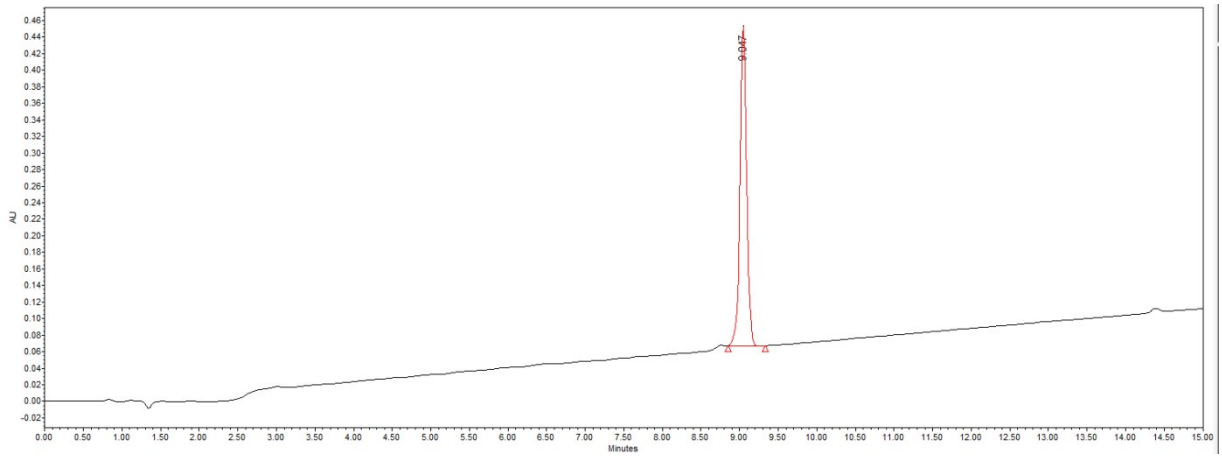

Figure S 9. RP-HPLC Spectrum of compound **9**

Compound **10**

Prepared according general procedure **C** from **5** (79.3 mg, 48.7 µmol) and pentynoic acid succinimide ester (14.3 mg, 73.0 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (79.9 mg, 46.8 µmol, 96%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{72}H_{116}N_{20}O_{28}$ $[M+2H]^{2+}$: 854.4128, found 854.4125; RP-HPLC: R_t = 4.92 min (C18, λ = 214 nm, 5-60% B in 15 min).

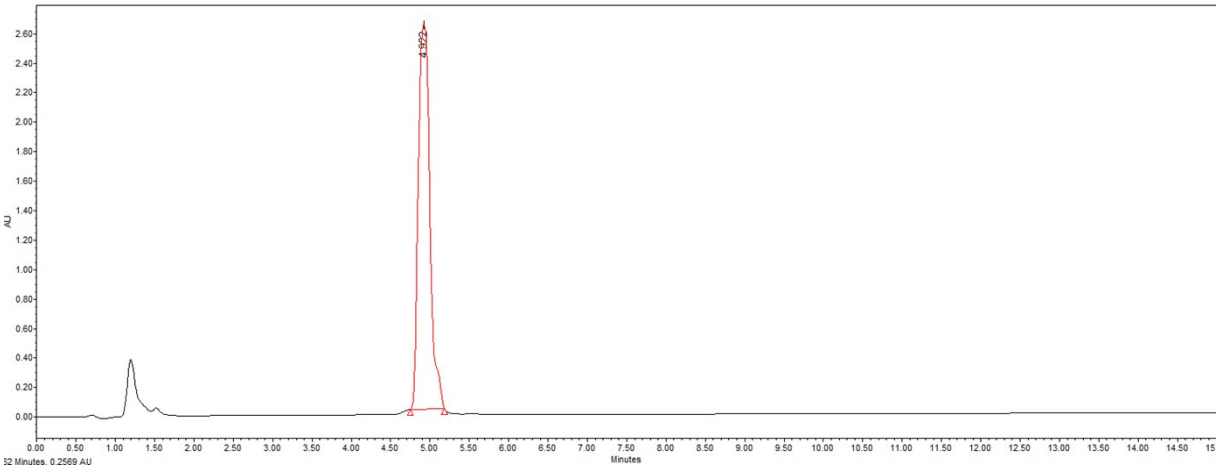

Figure S 10. RP-HPLC Spectrum of compound **10**

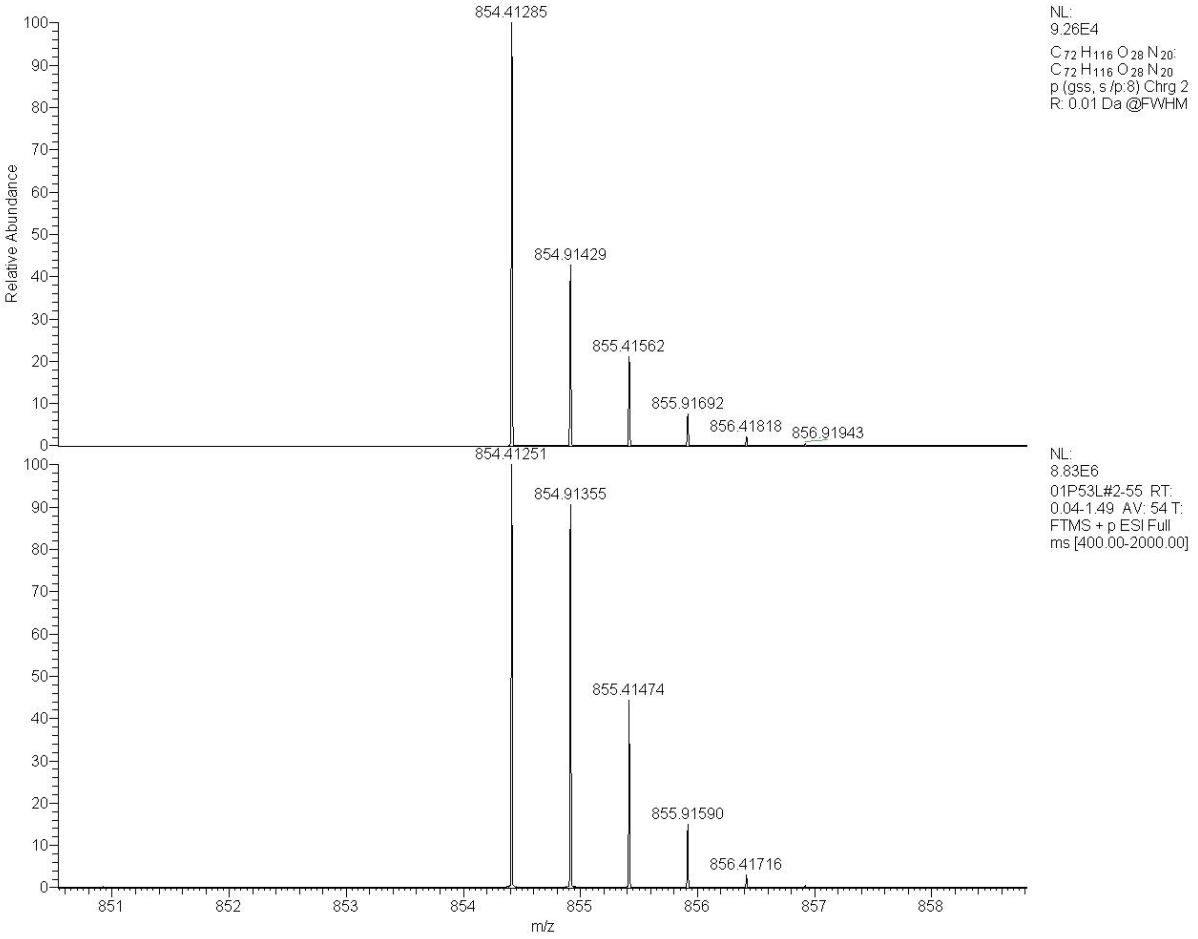

Figure S 11. HRMS spectrum of compound **10**

Compound **11**

Prepared according general procedure **D** from **4** (27.9 mg, 14.4 µmol) and 2-[2-(2-Azidoethoxy)ethoxy]-acetic acid potassium salt (6.6 mg, 28.8 µmol) and PyBoP (15.3 mg, 28.8 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (22.2 mg, 10.5 µmol, 73%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{89}H_{144}N_{26}O_{33}$ $[M+2H]^{2+}$: 1052.5189, found 1052.5207; RP-HPLC: R_t = 6.71 min (C18, λ = 214 nm, 5-60% B in 15 min).

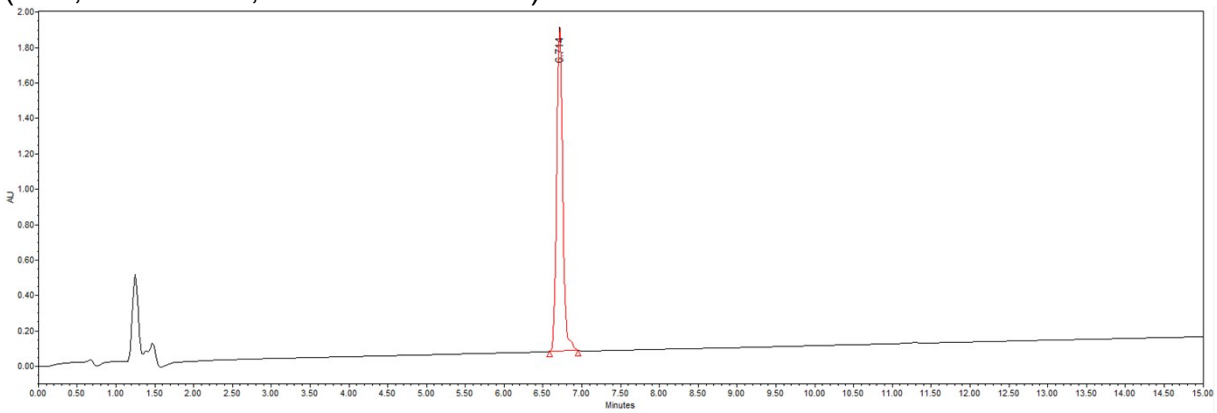

Figure S 12. RP-HPLC Spectrum of compound **11**

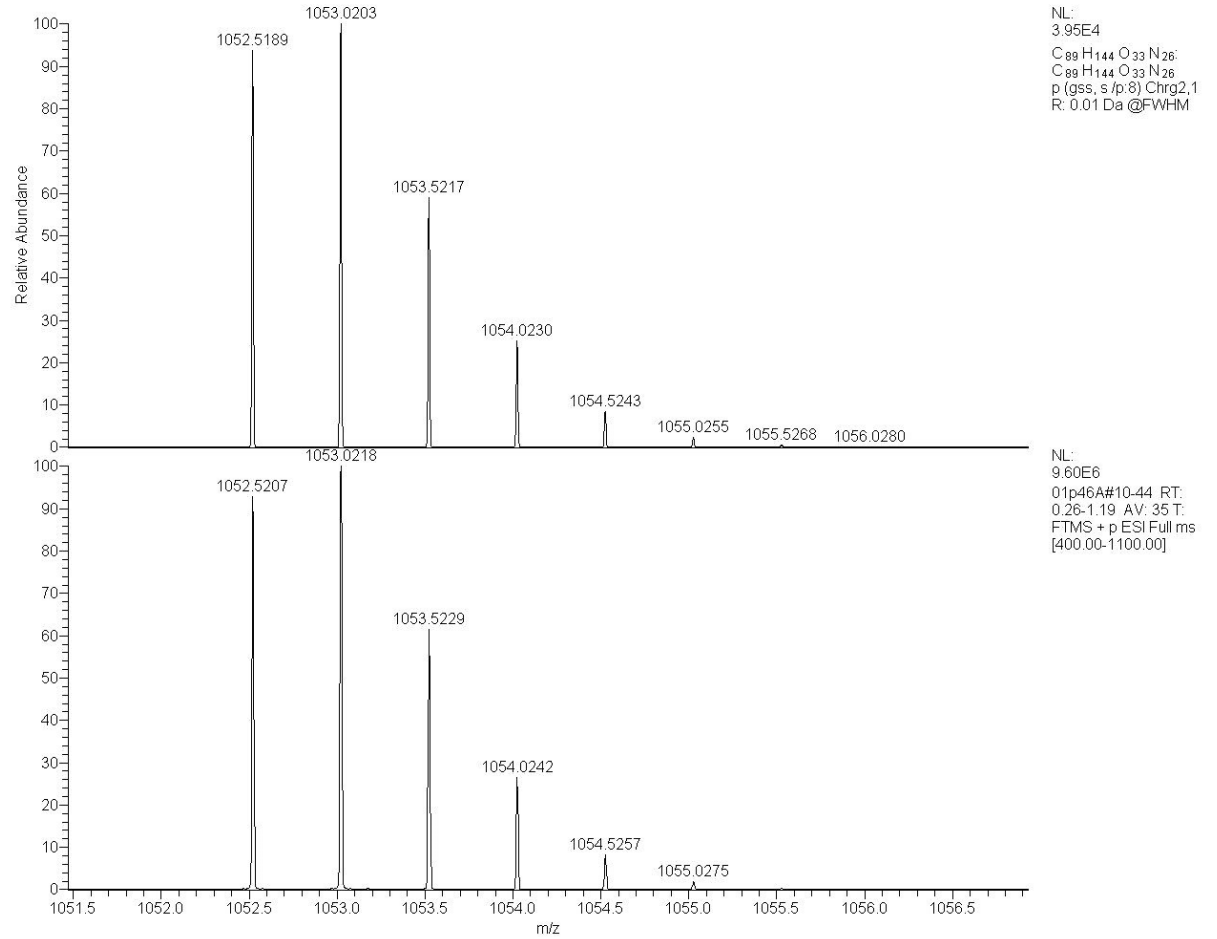

Figure S 13. HRMS spectrum of compound **11**

Compound **12**

Prepared according already described procedure. Analyses were in agreement with the literature.[2]

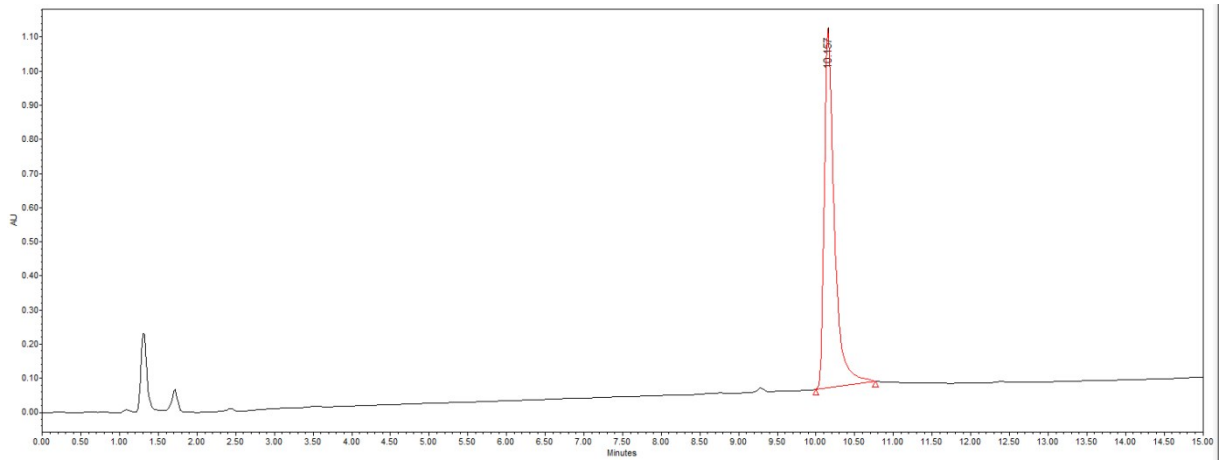

Figure S 14. RP-HPLC Spectrum of compound **12**

Compound **13**

Prepared according general procedure **A** from **8** (36.9 mg, 18.0 µmol) and **2** (3.4 mg, 4.0 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (29.0 mg, 3.3 µmol, 82%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{383}H_{602}N_{112}O_{131}$ $[M+5H]^{5+}$: 1775.0872, found 1775.0887; RP-HPLC: R_t = 8.76 min (C18, λ = 214 nm, 5-40% B in 15 min).

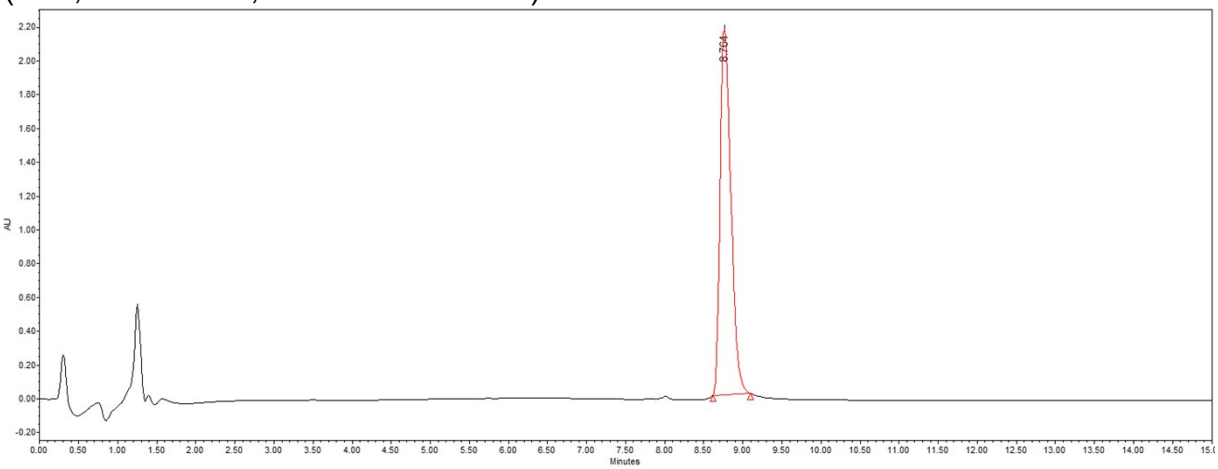

Figure S 15. RP-HPLC Spectrum of compound **13**

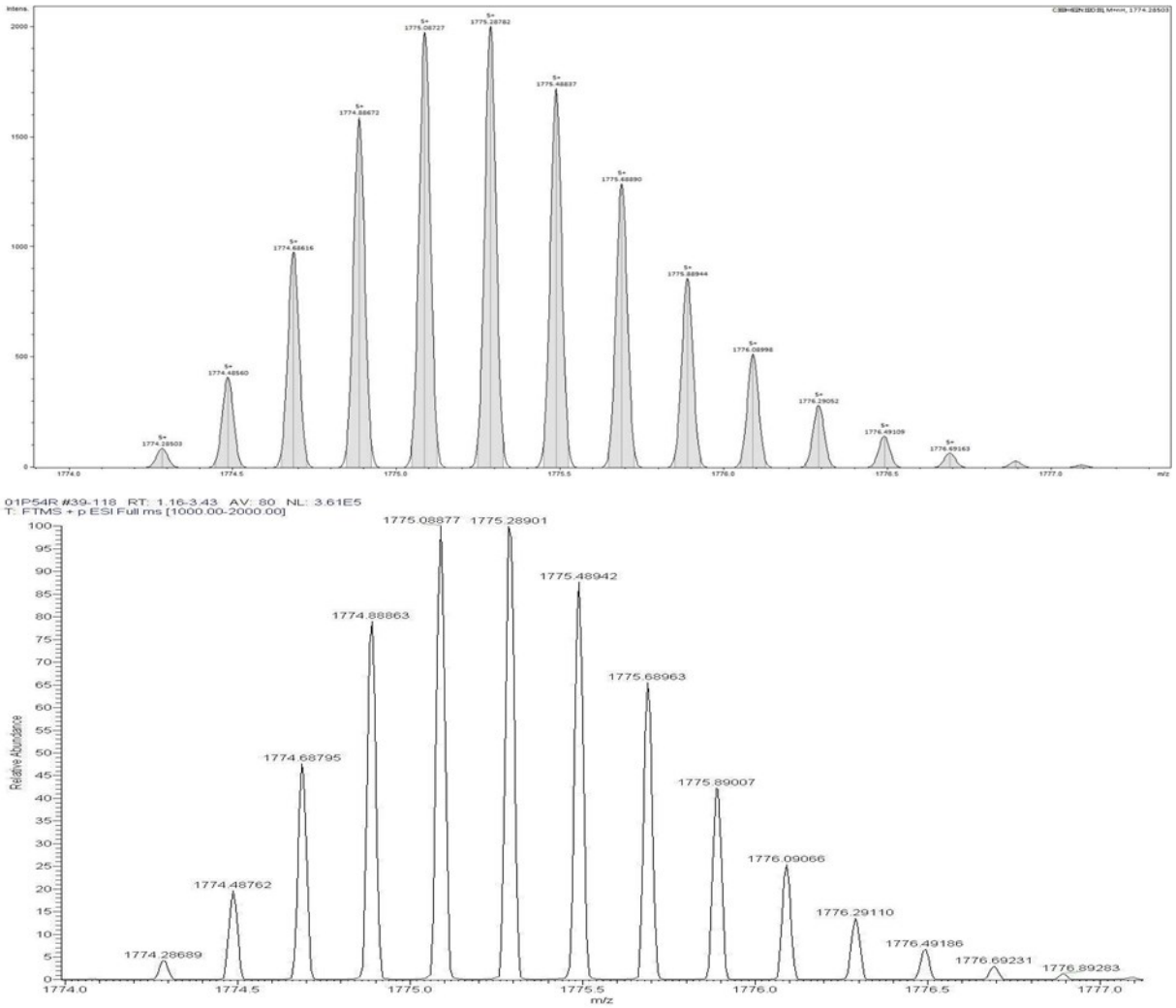

Figure S 16. HRMS spectrum of compound **13**

Compound **14**

Prepared according general procedure **A** from **10** (7.9 mg, 4.6 µmol) and **1** (1.2 mg, 1.0 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (6.6 mg, 0.8 µmol, 83%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{335}H_{537}N_{103}O_{122}$ $[M+4H]^{4+}$: 1988.7240, found 1988.7281; RP-HPLC: R_t = 5.62 min (C18, λ = 214 nm, 5-60% B in 15 min).

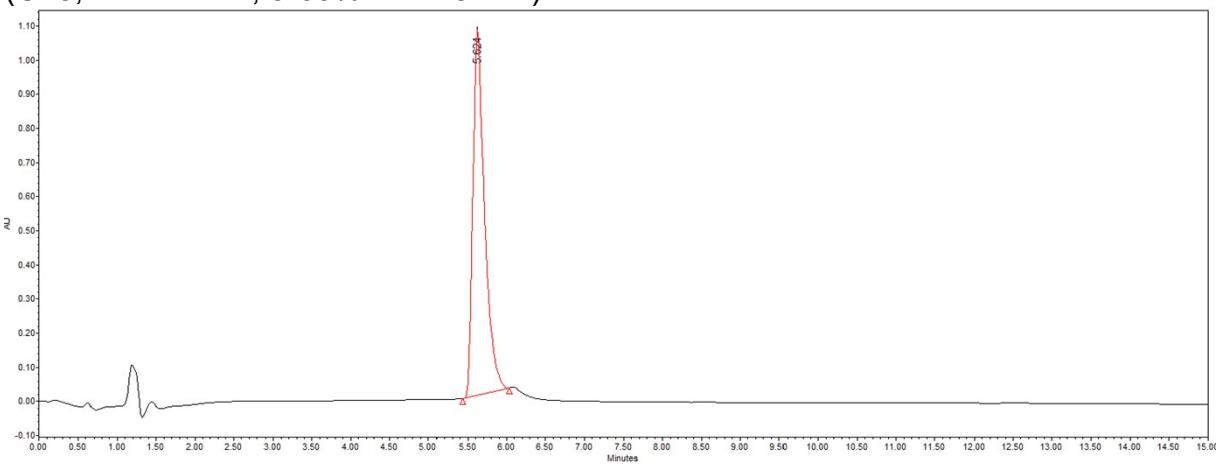

Figure S 17. RP-HPLC Spectrum of compound **14**

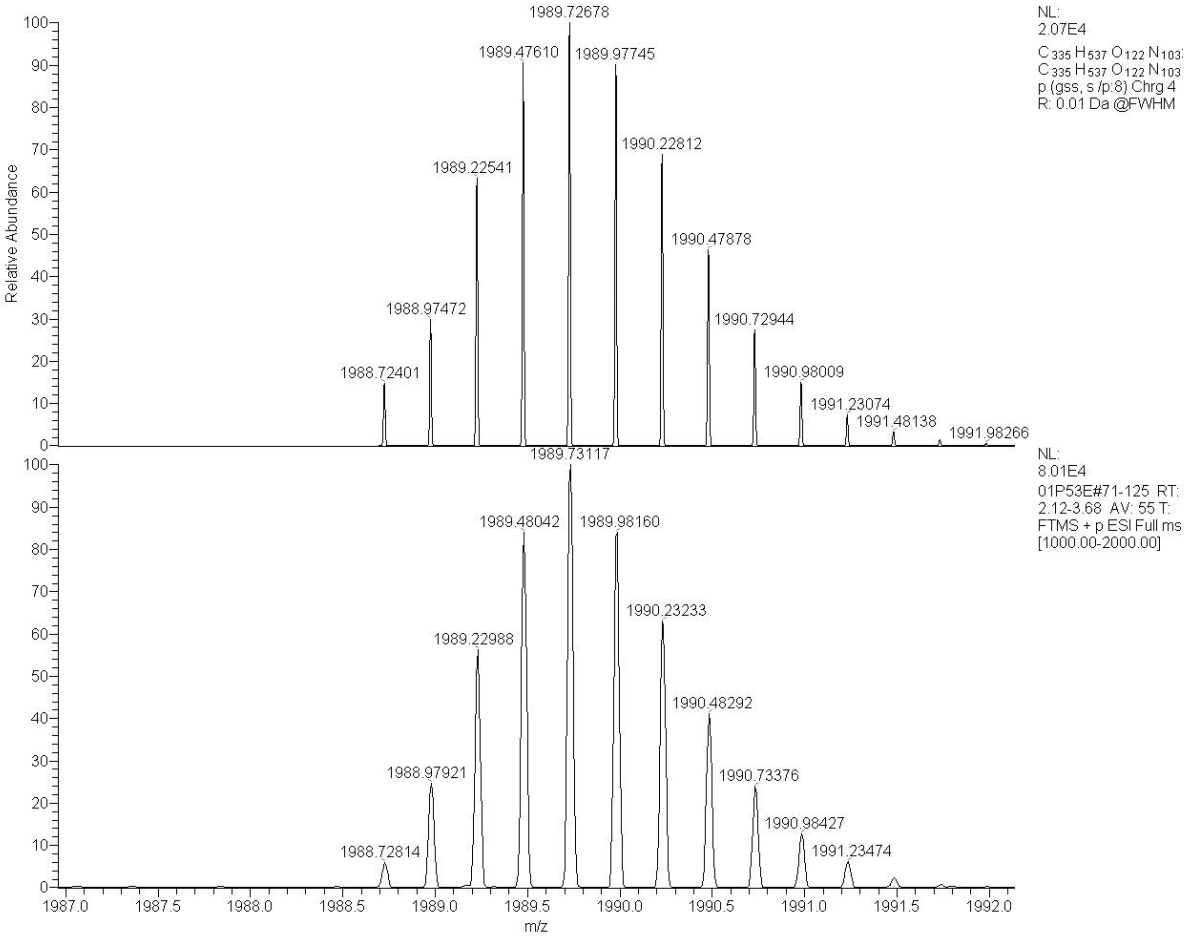

Figure S 18. HRMS spectrum of compound **14**

Compound **15**

Prepared according general procedure **A** from **10** (38.7 mg, 22.7 µmol) and **2** (4.2 mg, 5.1 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (29.4 mg, 3.8 µmol, 75%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{319}H_{514}N_{100}O_{119}$ $[M+4H]^{4+}$: 1912.4305, found 1912.4374; RP-HPLC: R_t = 5.41 min (C18, λ = 214 nm, 5-60% B in 15 min).

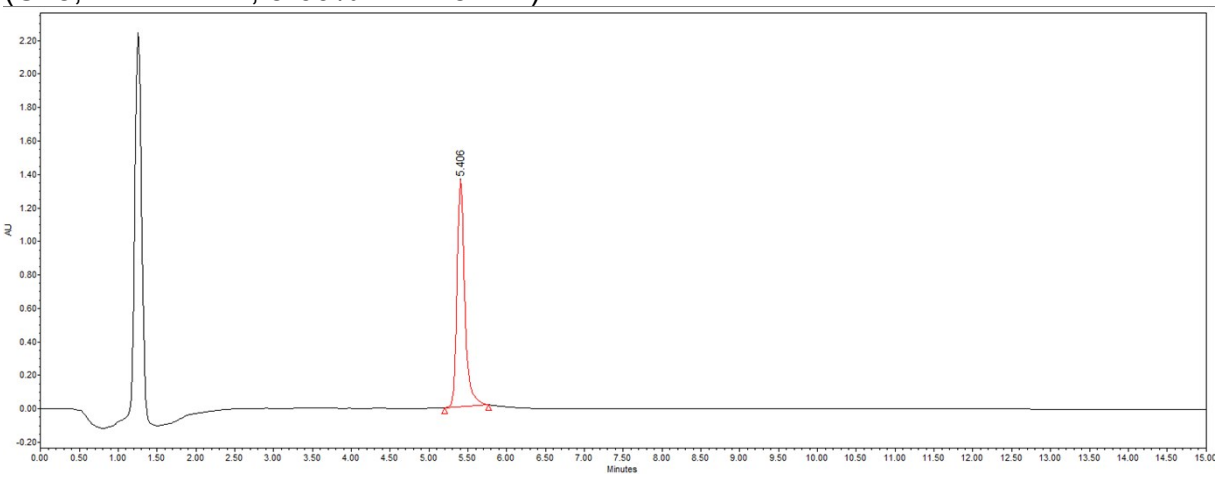

Figure S 19. RP-HPLC Spectrum of compound **15**

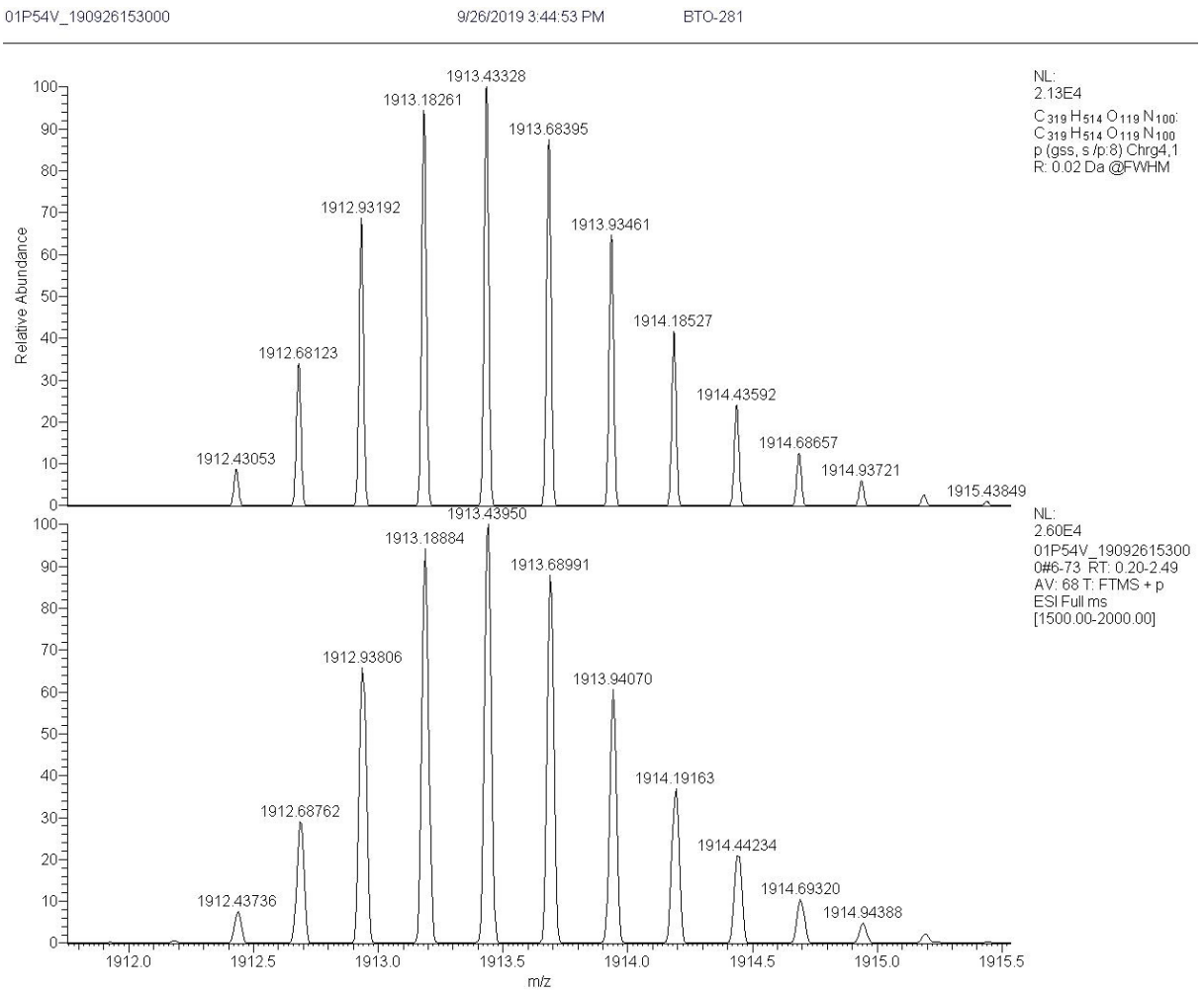

Figure S 20. HRMS spectrum of compound **15**

Compound **16**

Prepared according already described procedure. Analyses were in agreement with the literature.[2]

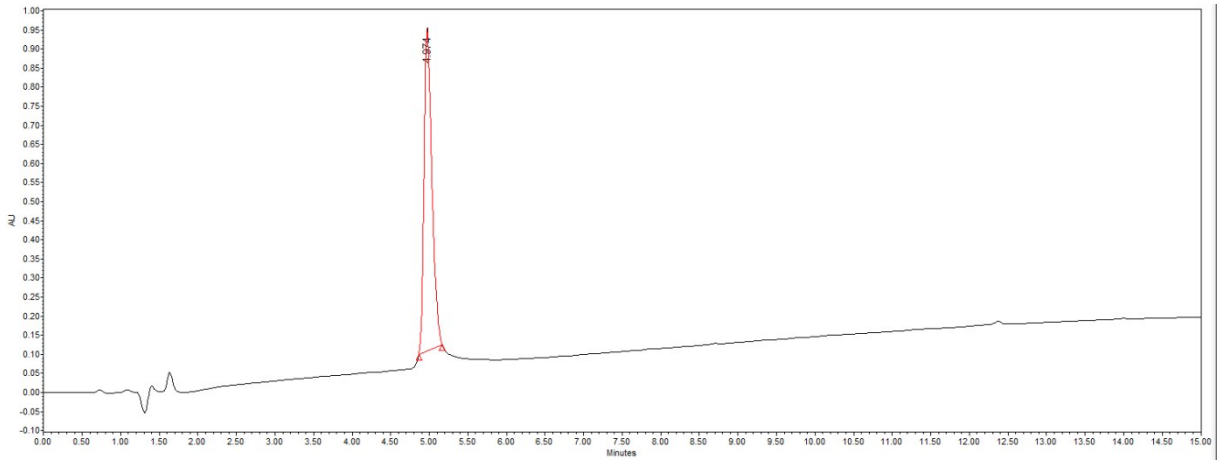

Figure S 21. RP-HPLC Spectrum of compound **16**

Compound **17**

Prepared according general procedure **C** from **13** (21.5 mg, 2.4 µmol) and azidoacetic acid succinimide ester (1.0 mg, 4.8 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (19.4 mg, 2.1 µmol, 88%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{385}H_{603}N_{115}O_{132}$ $[M+5H]^{5+}$: 1791.6896, found 1791.6916; RP-HPLC: R_t = 7.57 min (C18, λ = 214 nm, 5-60% B in 15 min).

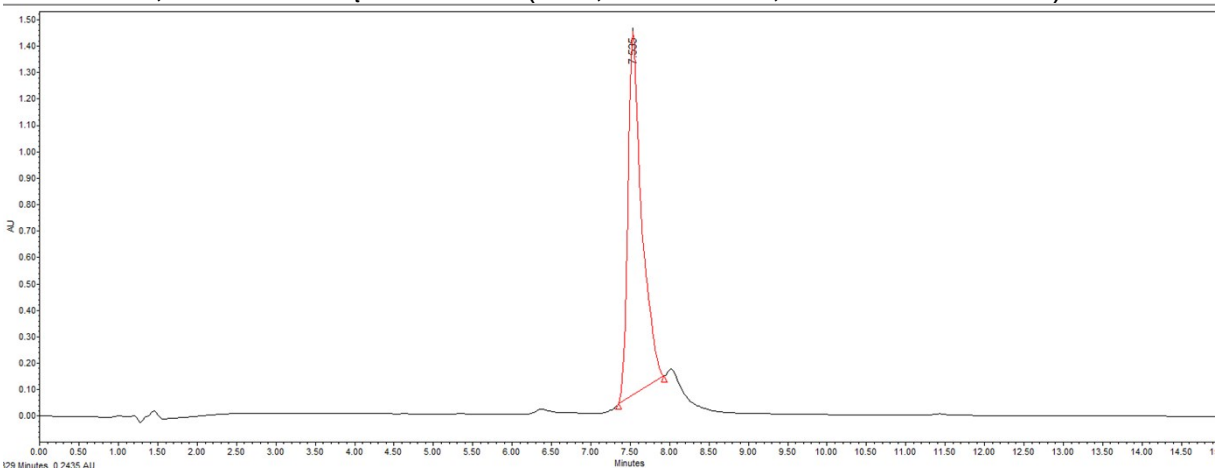

Figure S 22. RP-HPLC Spectrum of compound **17**

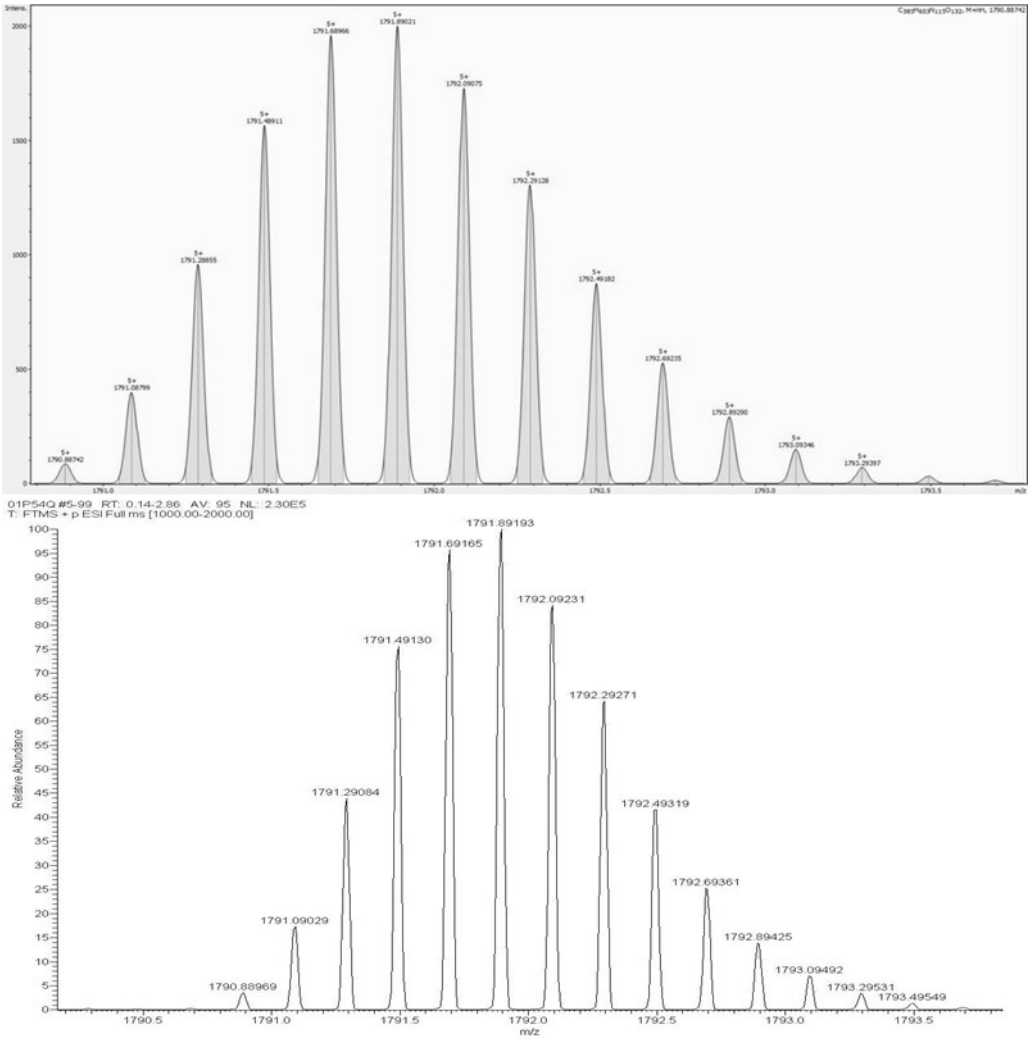

Figure S 23. HRMS spectrum of compound **17**

Compound **18**

Prepared according general procedure **C** from **14** (21.5 mg, 2.7 µmol) and azidoacetic acid succinimide ester (0.8 mg, 4.0 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (19.1 mg, 2.4 µmol, 88%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{337}H_{534}N_{106}O_{123}$ $[M+5H]^{5+}$: 1608.5852, found 1608.5903; RP-HPLC: R_t = 4.35 min (C18, λ = 214 nm, 5-100% B in 15 min).

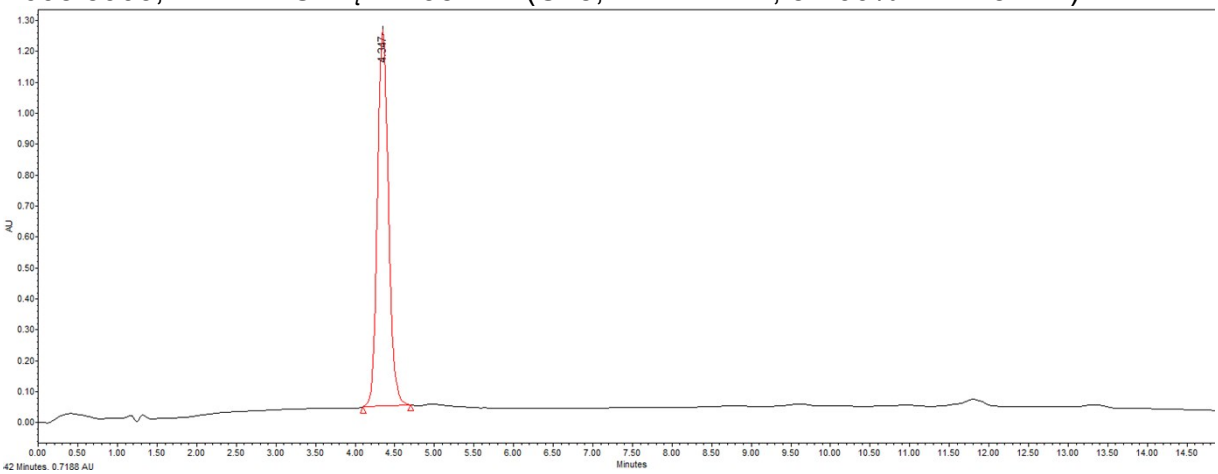

Figure S 24. RP-HPLC Spectrum of compound **18**

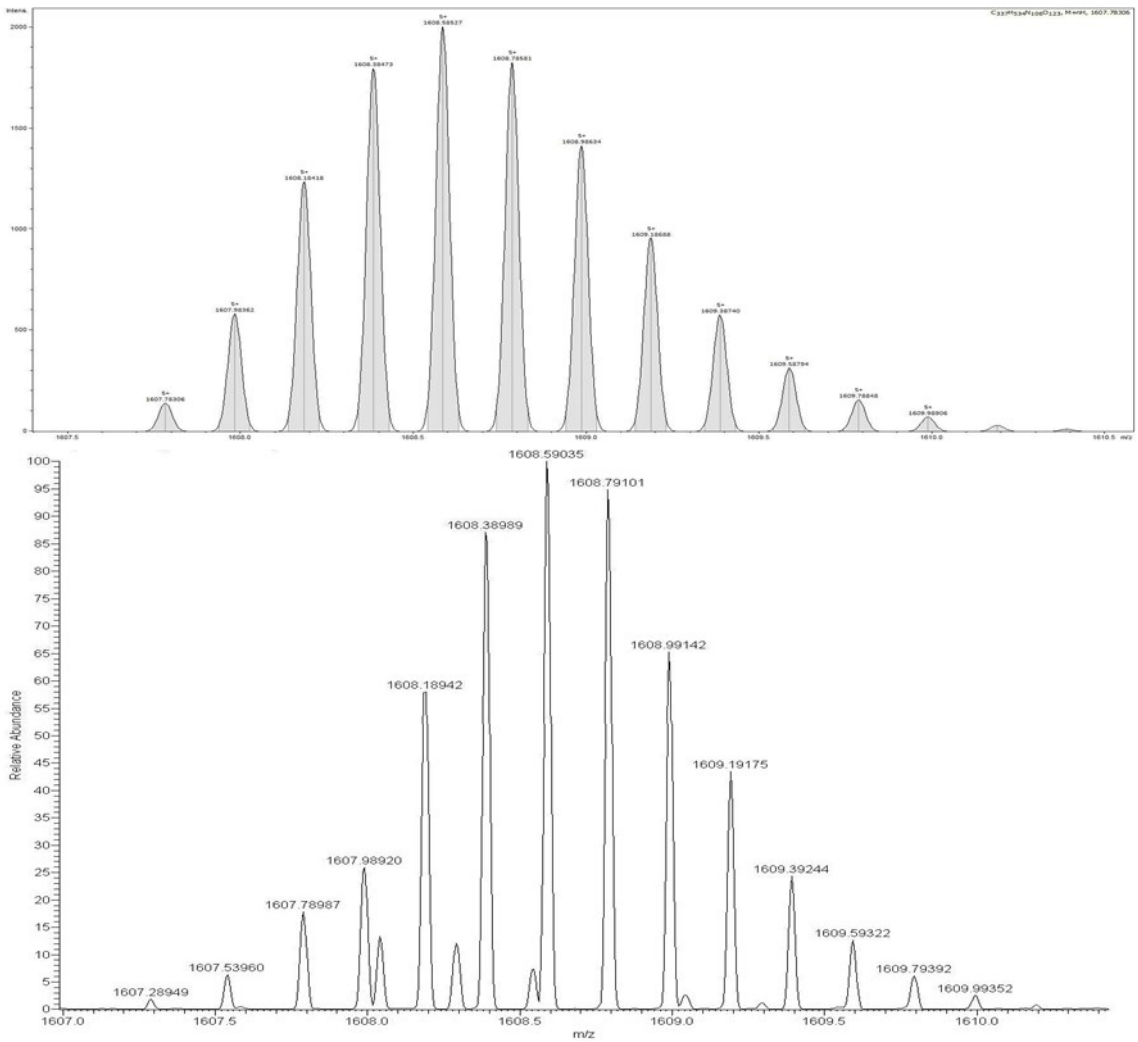

Figure S 25. HRMS spectrum of compound **18**

Compound **19**

Prepared according general procedure **C** from **15** (39.2 mg, 5.1 µmol) and azidoacetic acid succinimide ester (1.5 mg, 7.7 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (38.0 mg, 4.9 µmol, 96%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{321}H_{515}N_{103}O_{120}$ $[M+4H]^{4+}$: 1933.1835, found 1933.1879; RP-HPLC: R_t = 4.11 min (C18, λ = 214 nm, 5-100% B in 15 min).

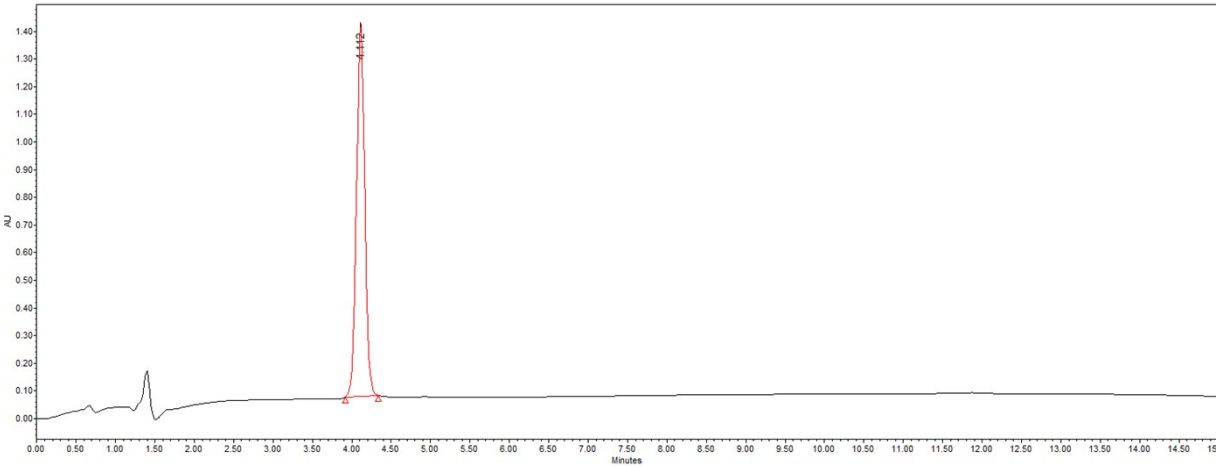

Figure S 26. RP-HPLC Spectrum of compound **19**

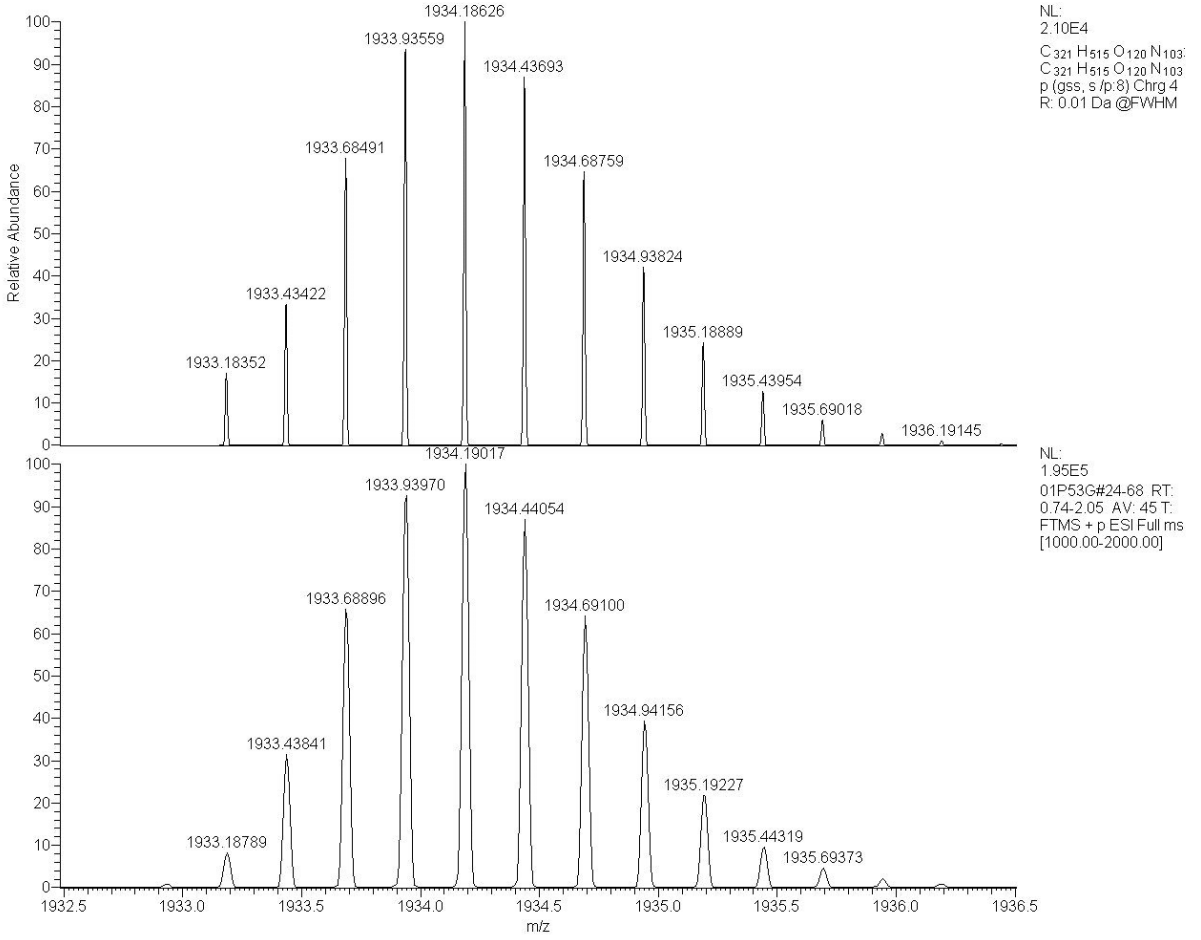

Figure S 27. HRMS spectrum of compound **19**

Compound **20**

Prepared according general procedure **D** from **12** (7.0 mg, 0.7 µmol) and 2-[2-(2-Azidoethoxy)ethoxy]-acetic acid potassium salt (0.3 mg, 9.5 µmol) and PyBoP (0.8 mg, 1.5 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (6.1 mg, 0.6 µmol, 92%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{405}H_{639}N_{118}O_{137}$ $[M+5H]^{5+}$: 1869.5327, found 1869.5446; RP-HPLC: R_t = 7.10 min (C18, λ = 214 nm, 5-60% B in 15 min).

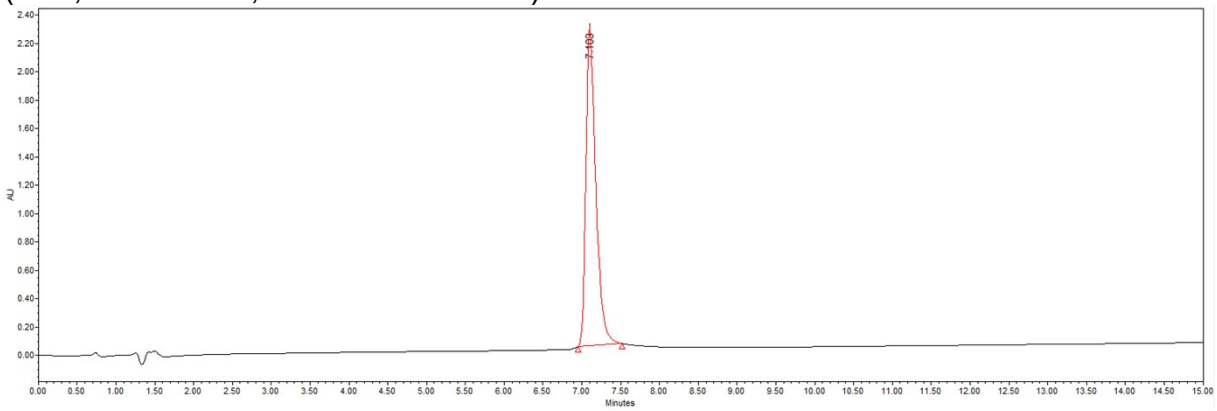

Figure S 28. RP-HPLC Spectrum of compound **20**

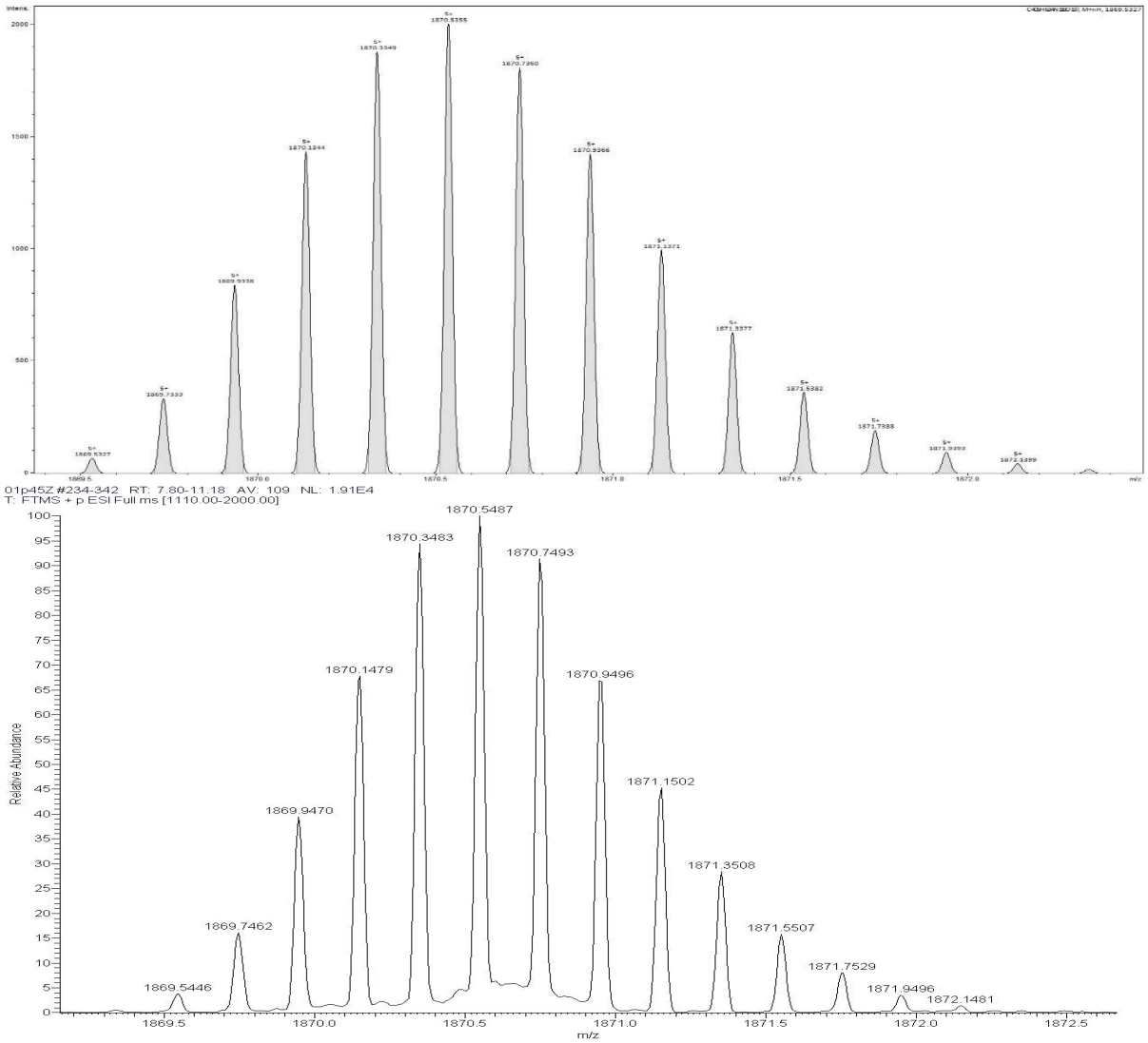

Figure S 29. HRMS spectrum of compound **20**

Compound **4C**

Prepared according already described procedure. Analyses were in agreement with the literature.[2]

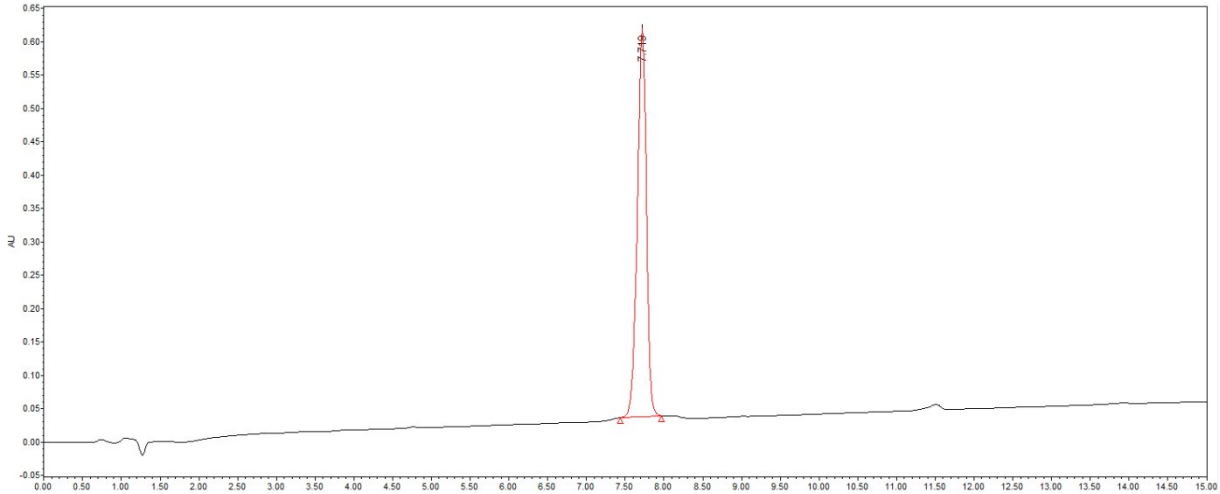

Figure S 30. RP-HPLC Spectrum of compound **4C**

Compound **16CC**

Prepared according already described procedure. Analyses were in agreement with the literature.[2]

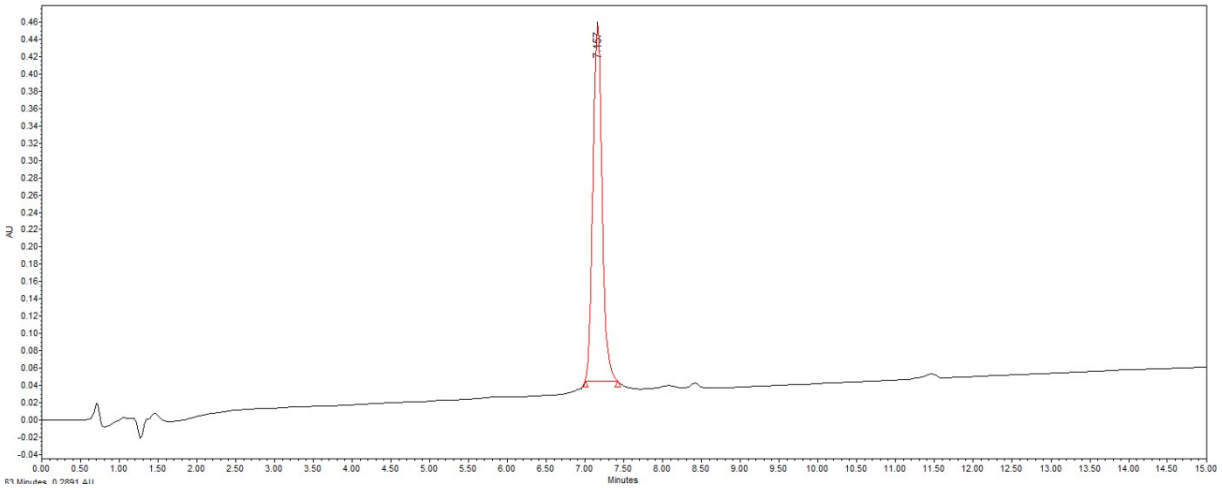

Figure S 31. RP-HPLC Spectrum of compound **16CC**

Compound **16DC**

Prepared according general procedure **E** from **20** (8.1 mg, 0.9 µmol) and **10** (3.6 mg, 0.9 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (8.0 mg, 0.6 µmol, 69%). MALDI-ToF *m/z*: calcd for $C_{565}H_{865}N_{174}O_{175}$ $[M+H]^+$: 12887.4057, found 12887.3170; RP-HPLC: R_t = 7.53 min (C18, λ = 214 nm, 5-60% B in 15 min).

Figure S 32. RP-HPLC Spectrum of compound **16DC**

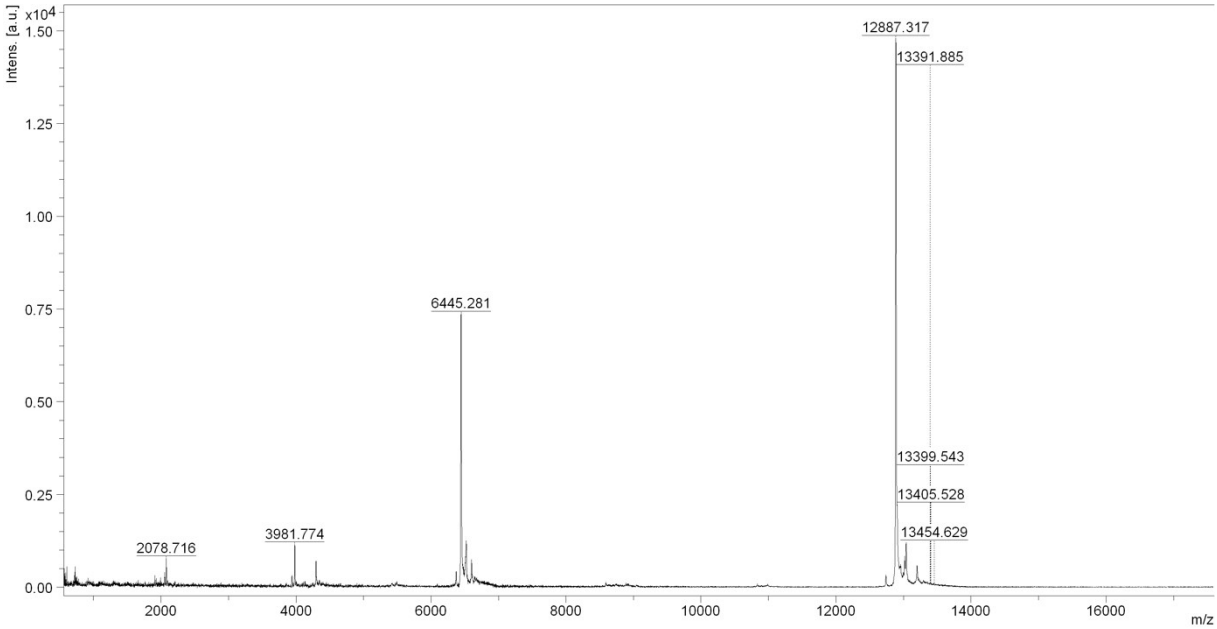

Figure S 33. Maldi-ToF spectrum of compound **16DC**

Compound **16CD**

Prepared according general procedure **E** from **22** (2.4 mg, 0.3 μmol) and **10** (1.2 mg, 0.3 μmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (2.0 mg, 0.16 μmol, 56%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{517}H_{795}N_{165}O_{166}$ $[M+6H]^{6+}$: 1996.9902, found 1996.9913; RP-HPLC: R_t = 5.30 min (C18, λ = 214 nm, 5-100% B in 15 min).

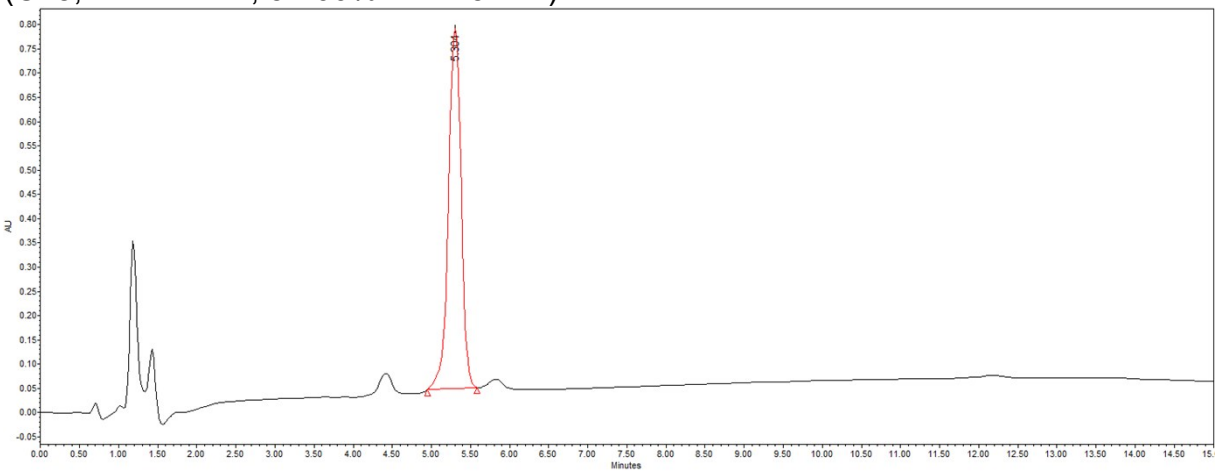

Figure S 34. RP-HPLC Spectrum of compound **16CD**

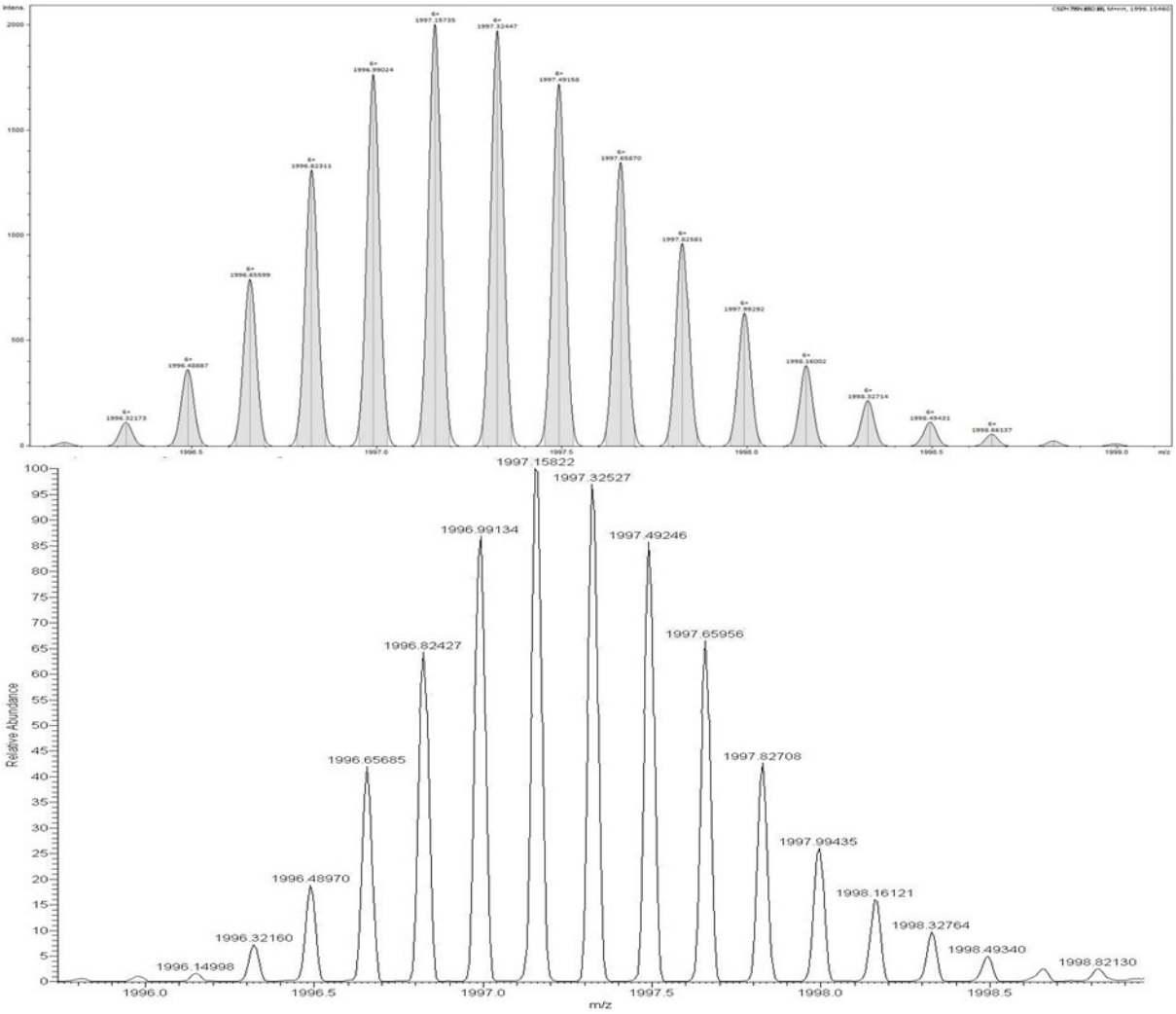

Figure S 35. HRMS spectrum of compound **16CD**

Compound **16DD**

Prepared according general procedure **E** from **24** (3.5 mg, 0.45 µmol) and **10** (1.8 mg, 0.45 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (4.1 mg, 0.35 µmol, 78%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{501}H_{772}N_{162}O_{163}$ $[M+6H]^{6+}$: 1946.1279, found 1946.1286; RP-HPLC: R_t = 5.31 min (C18, λ = 214 nm, 5-100% B in 15 min).

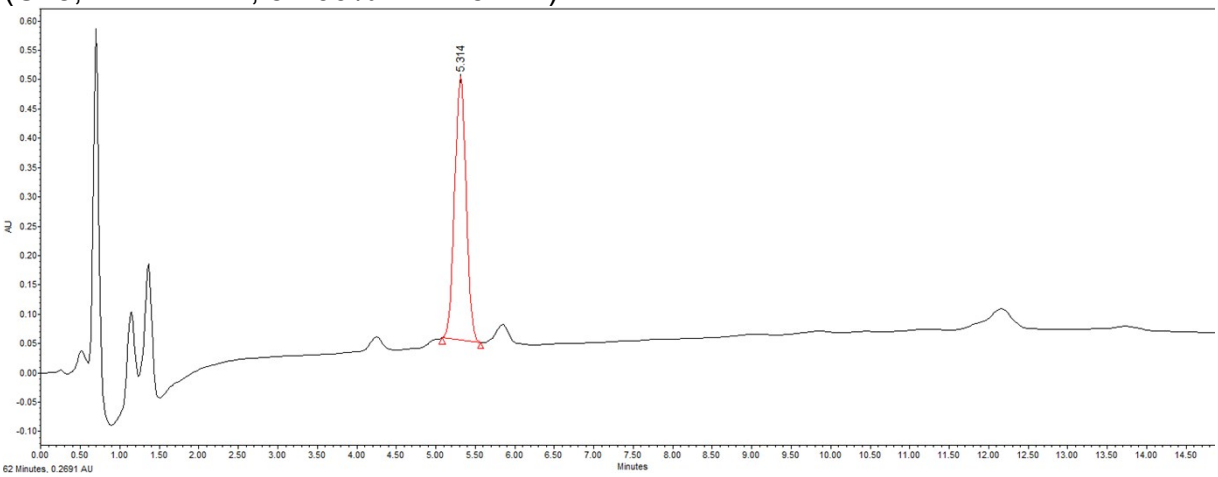

Figure S 36. RP-HPLC Spectrum of compound **16DD**

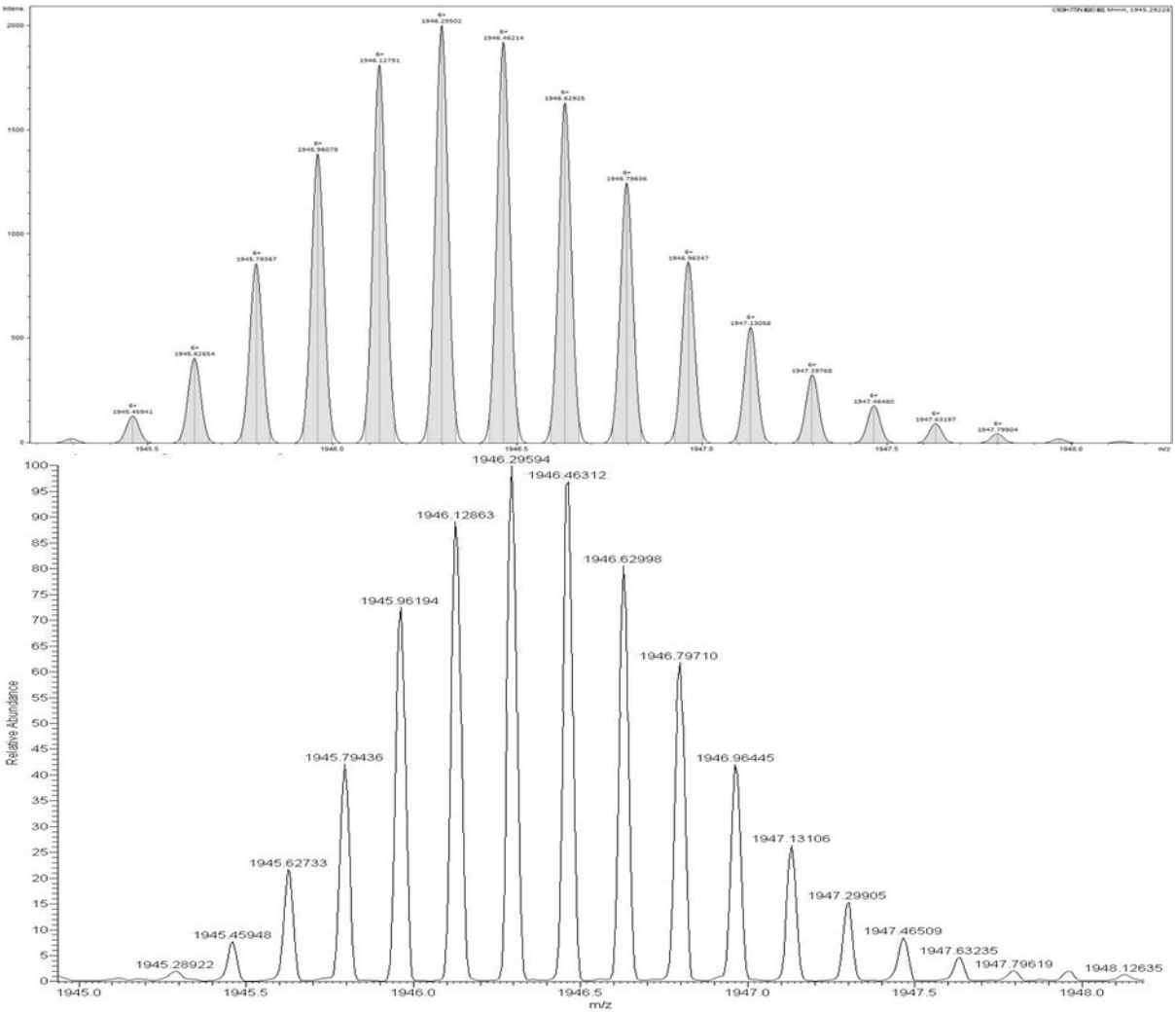

Figure S 37. HRMS spectrum of compound **16DD**

Compound **4C-P**

Prepared according general procedure **E** from **7** (4.1 mg, 1.9 µmol) and **10** (7.7 mg, 1.9 µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (10 mg, 1.6 µmol, 87%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{269}H_{407}N_{85}O_{76}$ $[M+4H]^{4+}$: 1511.0143, found 1511.0284; RP-HPLC: R_t = 5.49 min (C18, λ = 214 nm, 5-100% B in 15 min).

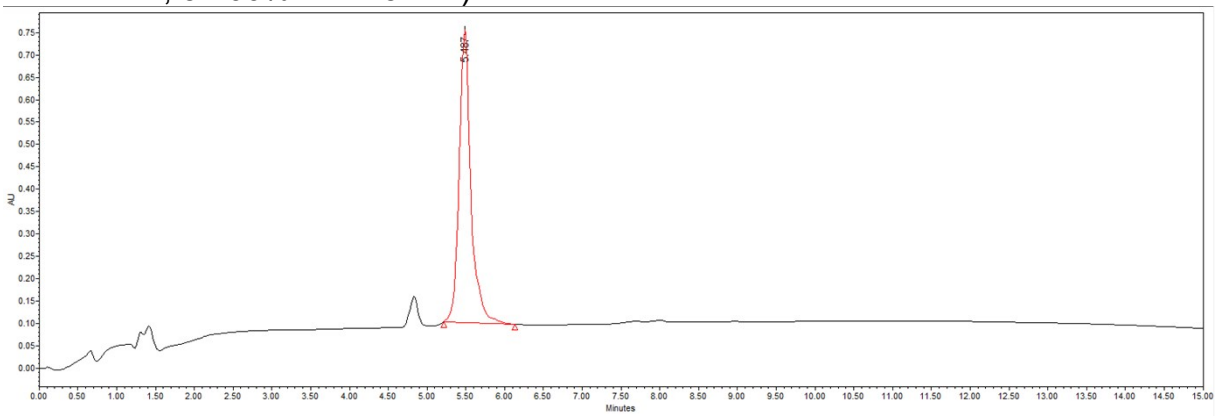

Figure S 38. RP-HPLC Spectrum of compound **4C-P**

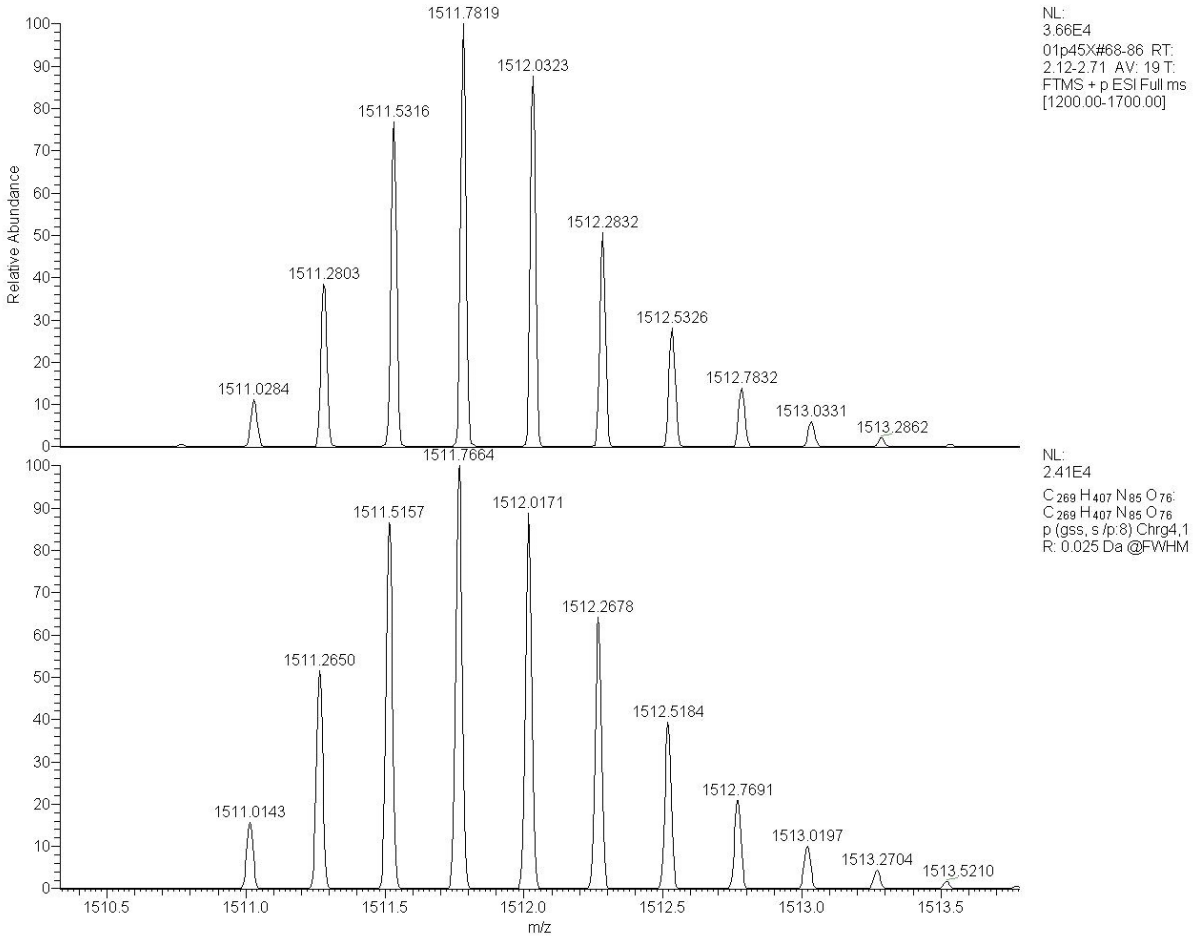

Figure S 39. HRMS spectrum of compound **4C-P**

Compound **16CC-P**

Prepared according general procedure **E** from **18** (5.2 mg, 0.5 µmol) and **10** (2.2 mg, 0.5µmol). The crude mixture was purified to afford the title compound as a white fluffy solid after lyophilization (5.1 mg, 0.38 µmol, 77%). HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{585}H_{895}N_{177}O_{180}$ $[M+7H]^{7+}$: 1898.9571, found 1898.9592; RP-HPLC: R_t = 5.30 min (C18, λ = 214 nm, 5-100% B in 15 min).

Figure S 40. RP-HPLC Spectrum of compound **16CC-P**

Figure S 41. HRMS spectrum of compound **16CC-P**

NMR spectra of final compounds

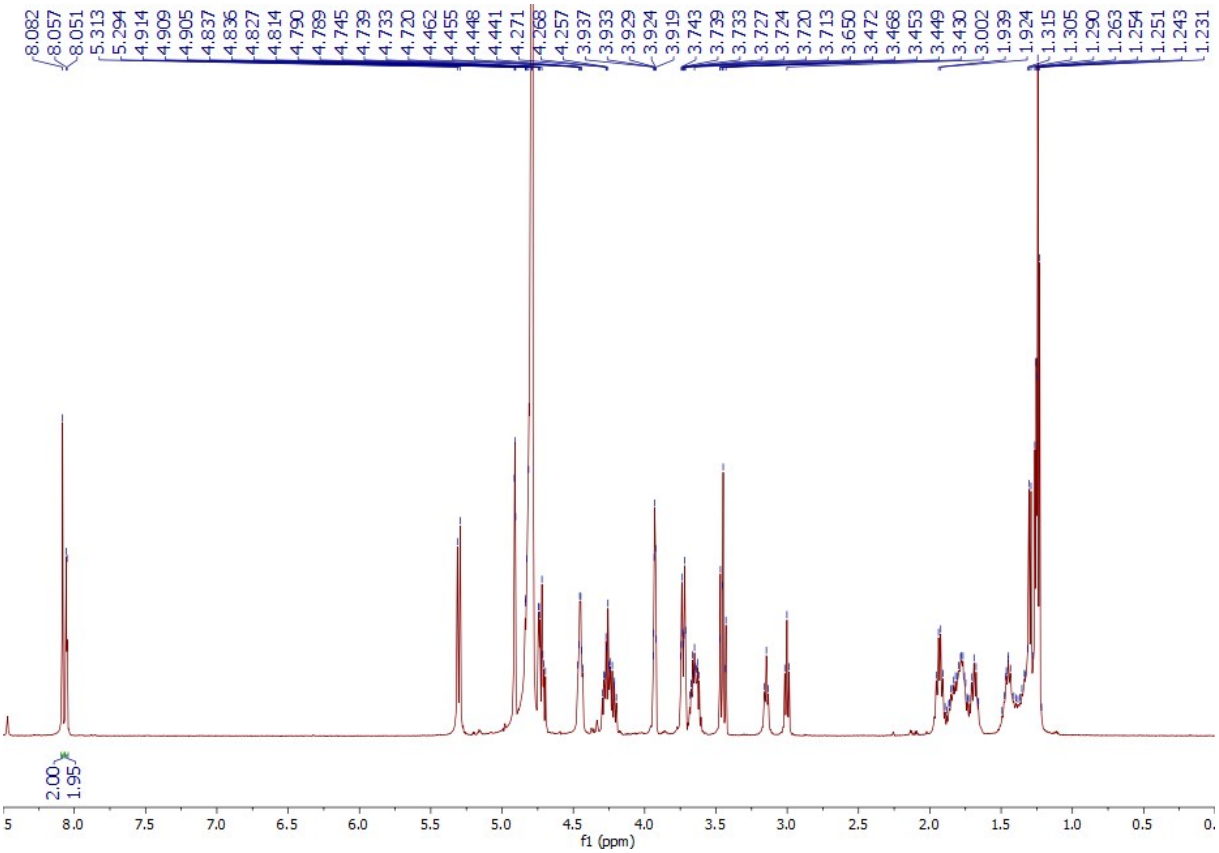

Figure S 42. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **5**

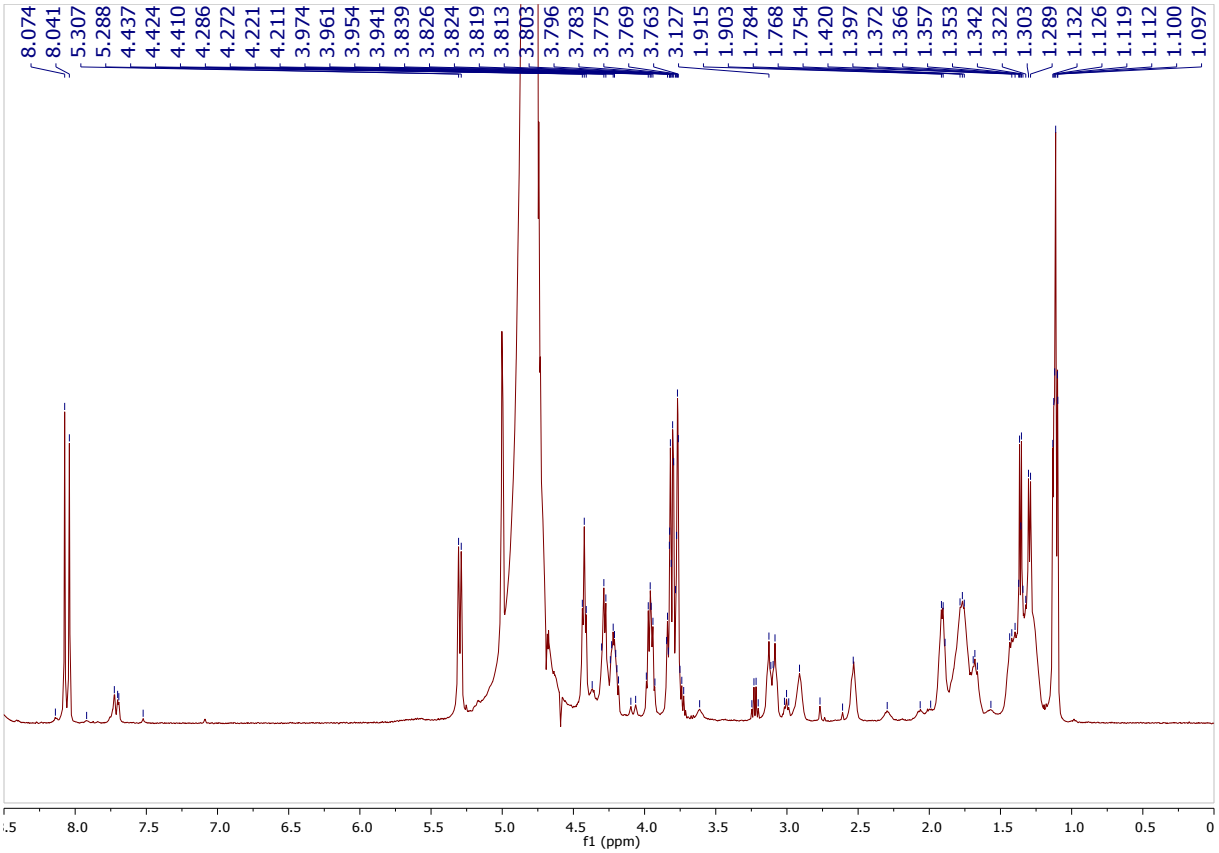

Figure S 43. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **12**

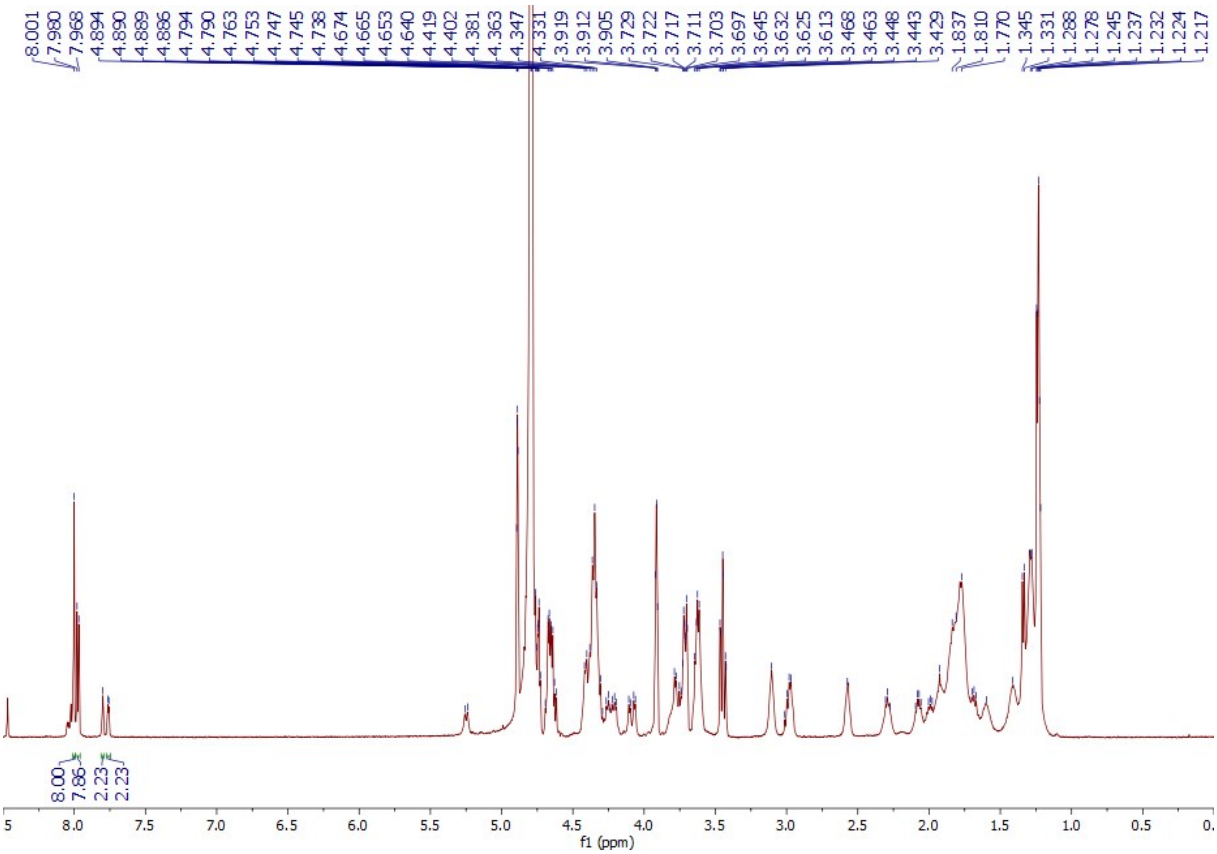

Figure S 44. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **13**

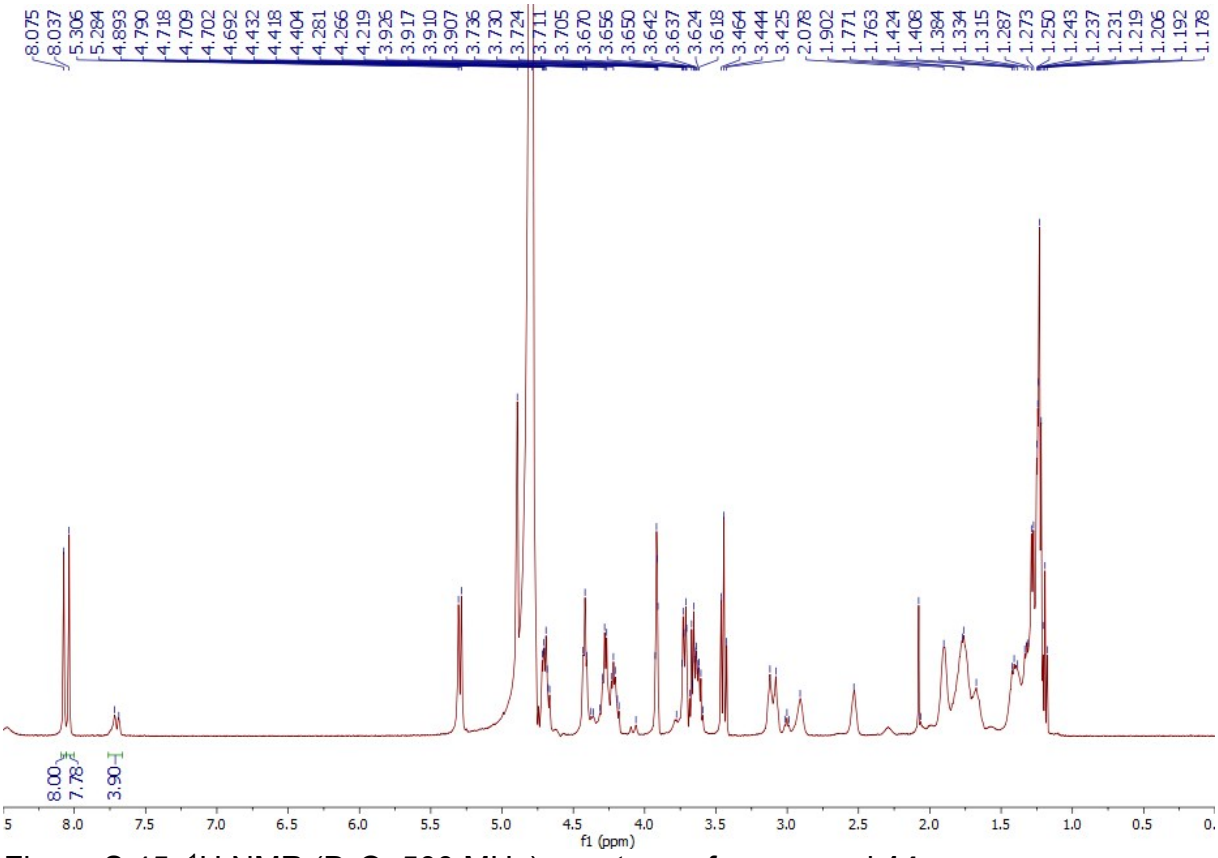

Figure S 45. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **14**

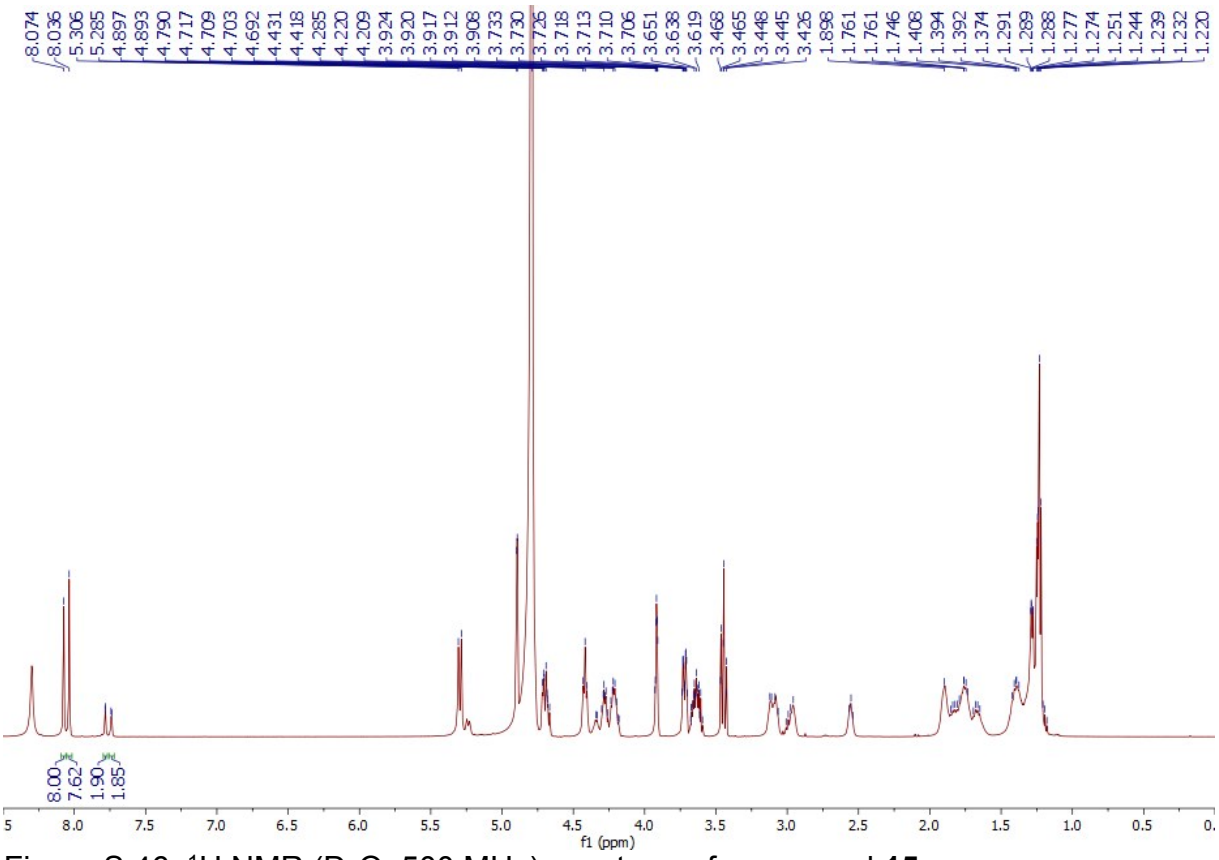

Figure S 46. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **15**

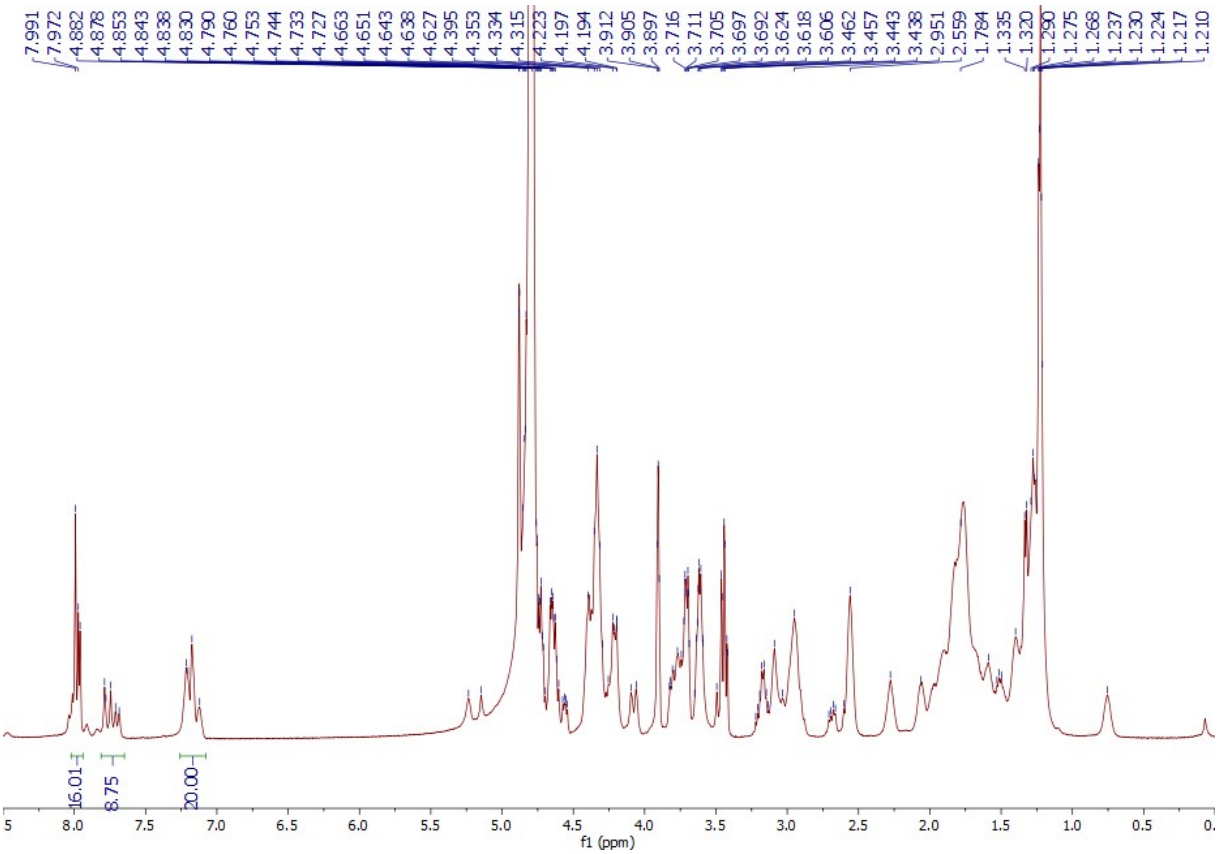

Figure S 47. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **16DC**

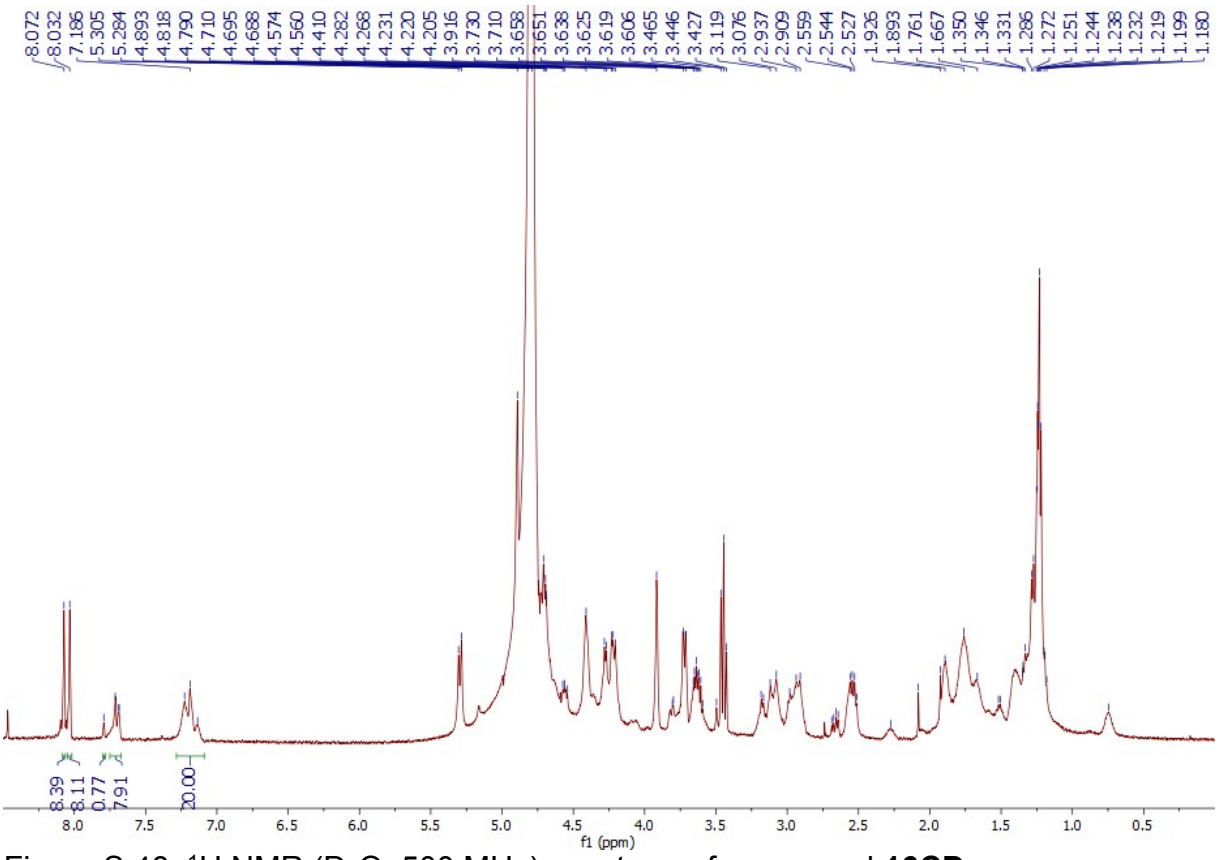

Figure S 48. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **16CD**

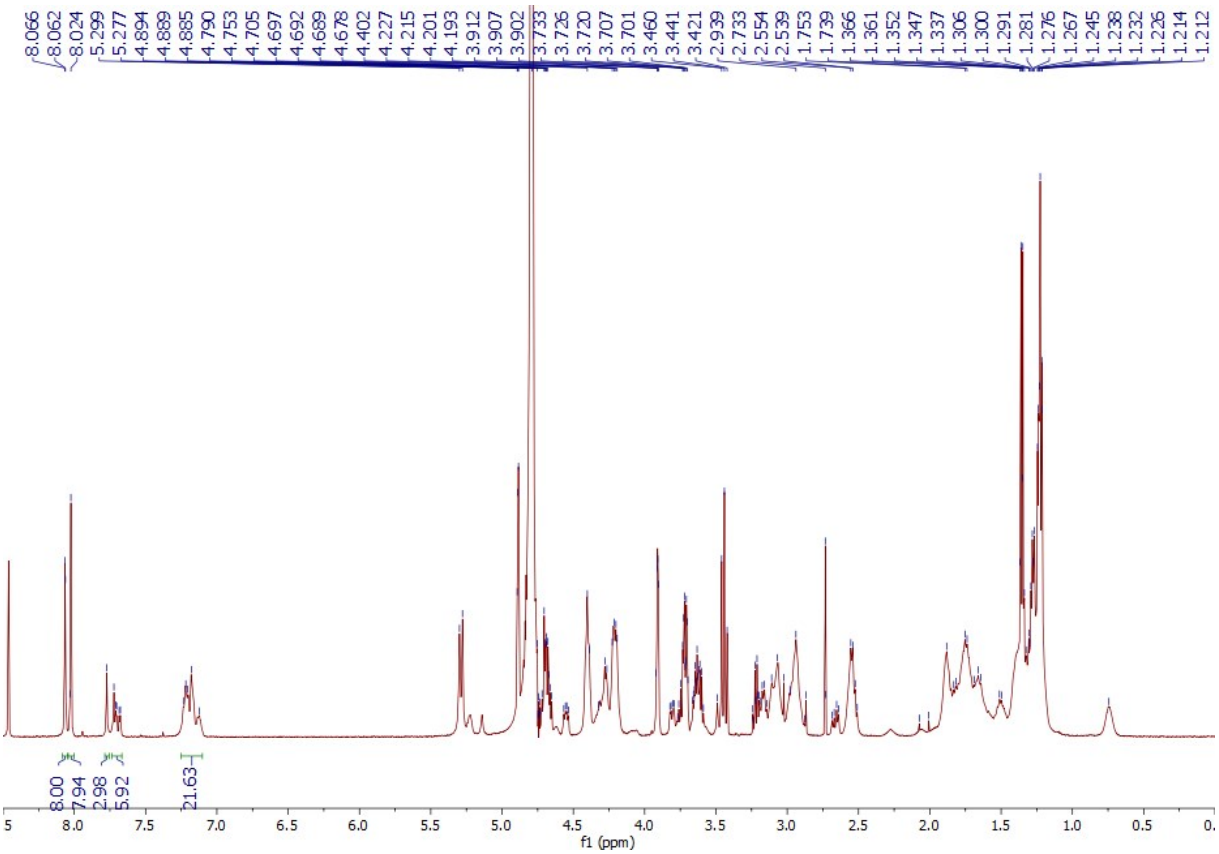

Figure S 49. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **16DD**

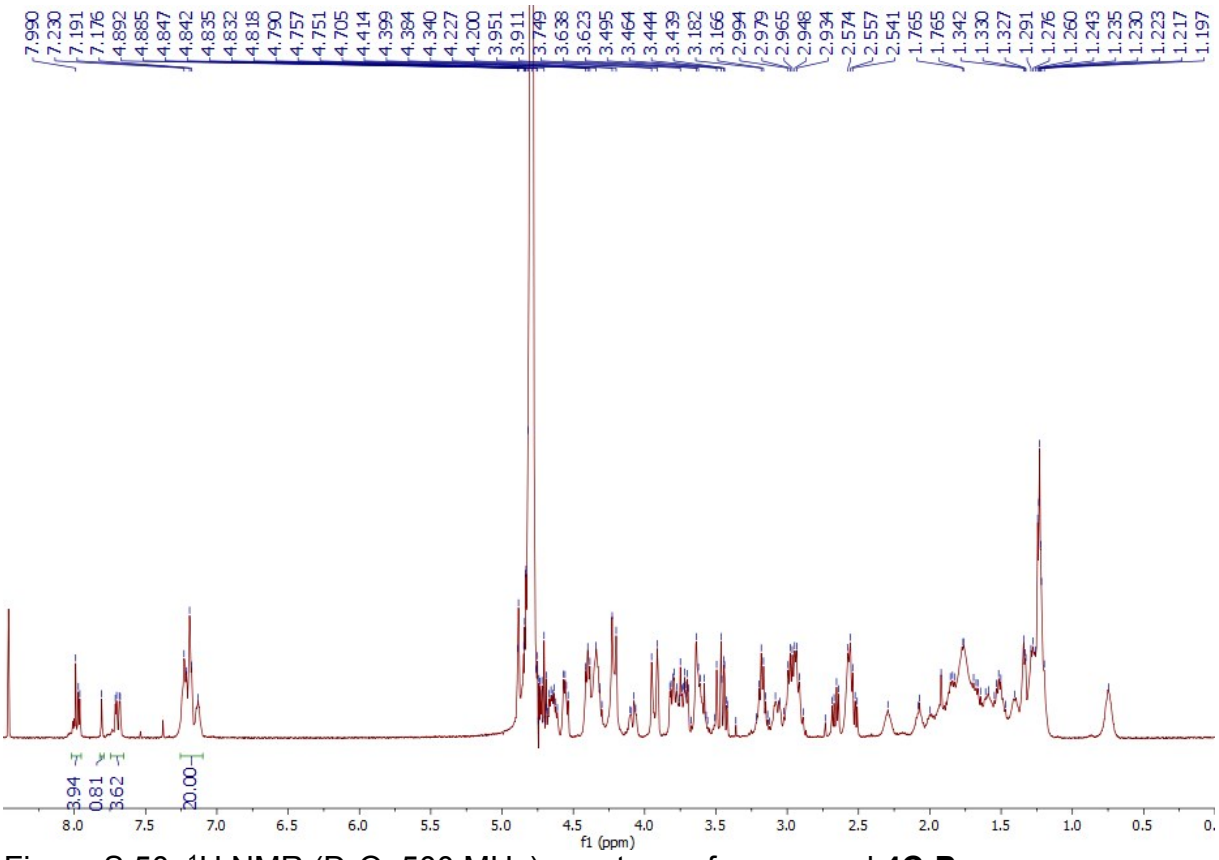

Figure S 50. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **4C-P**

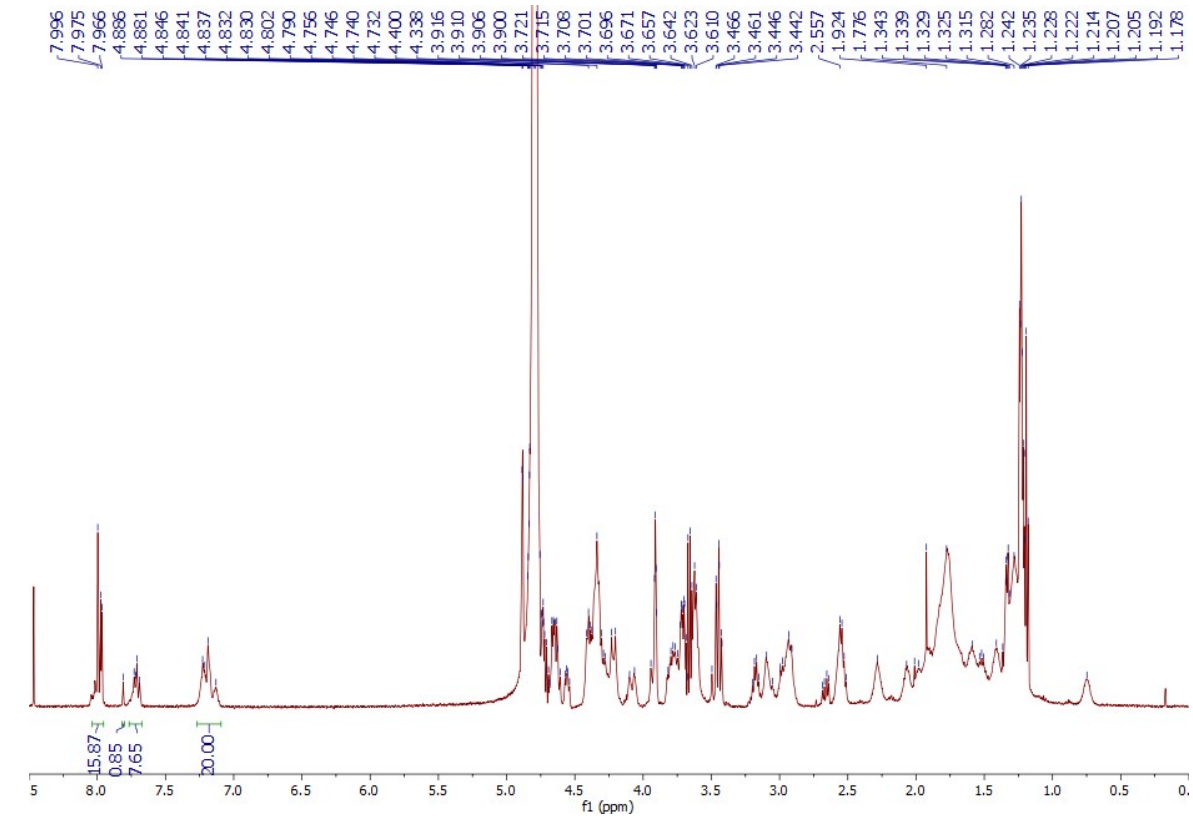

Figure S 51. ^{1}H NMR (D_2O, 500 MHz) spectrum of compound **16CC-P**

^{1}H NMR (D_2O, 500 MHz) spectrum of compounds **11, 15, 31, 32** were in agreement with the literature.[2]

Flow cytometry and cell viability assays

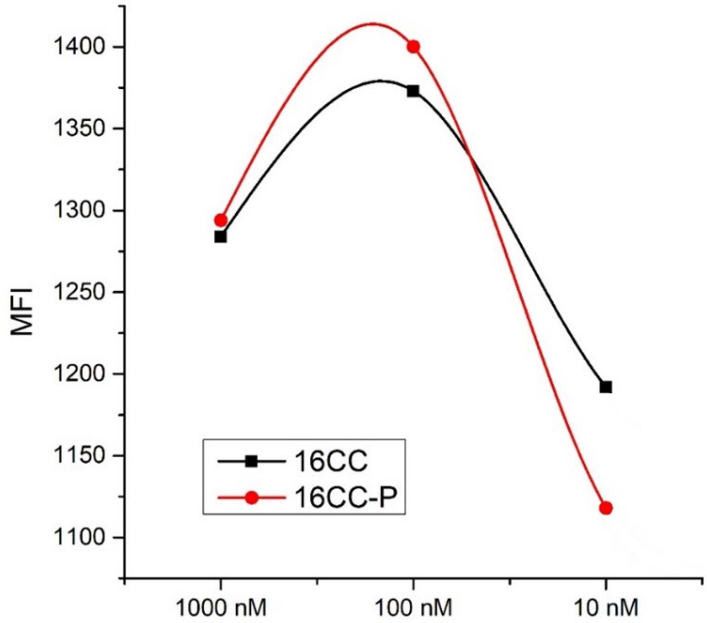

Figure S 52. Dose-dependent recruitment of IgM anti-Rha antibodies present in human serum to the M21 cell surface following cell treatment with various concentrations of **16CC** (10nM to 1µM). The anti-Rha antibodies recruitment was revealed with Alexa-Fluor488-conjugated anti-human IgM secondary antibody.

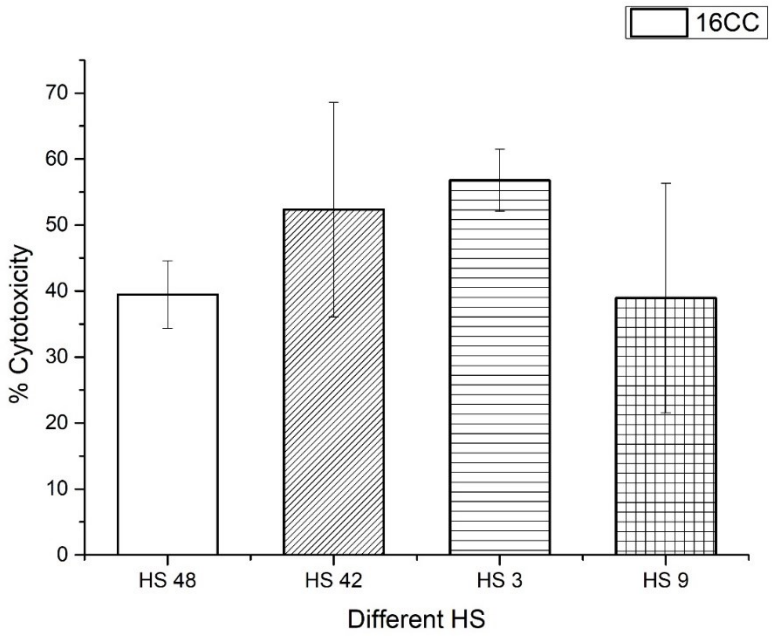

Figure S 53. Comparison of the cytotoxicity induced by **16CC** and 4 four different human serums against M21 cells (HS 48 and HS 42 were commercially purchased from Sigma. HS 3 and HS 9 were obtained from EFS). M21 cells were treated with 100nM of **16CC** and human serum 50% (used as source of anti-rhamnose and complement).

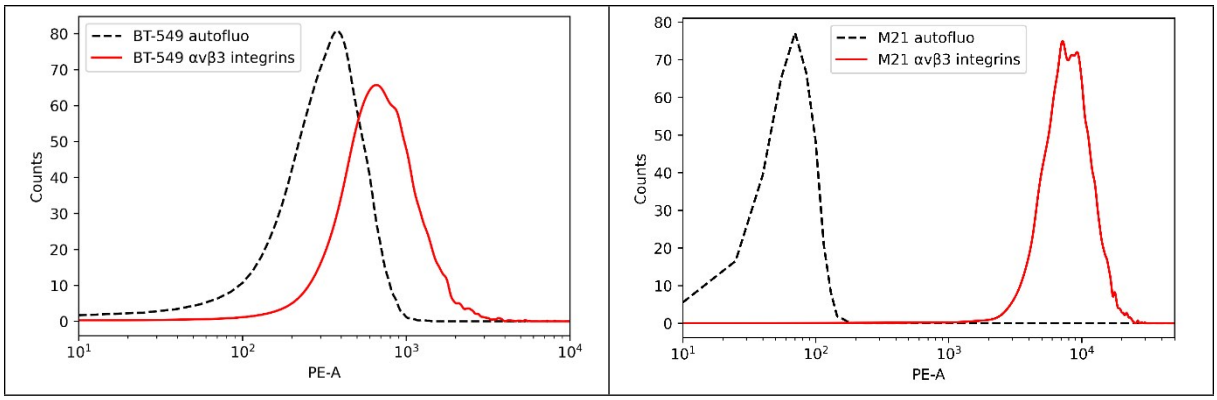

Figure S 54. Immunostaining of $\square_v\square_3$ integrins expressed on BT-549 and M21 cells by treatment with an anti-CD51/CD61 conjugated phycoerythrin (PE) antibody. The result is reported as phycoerythrin fluorescence (PE-A) histogram counts.

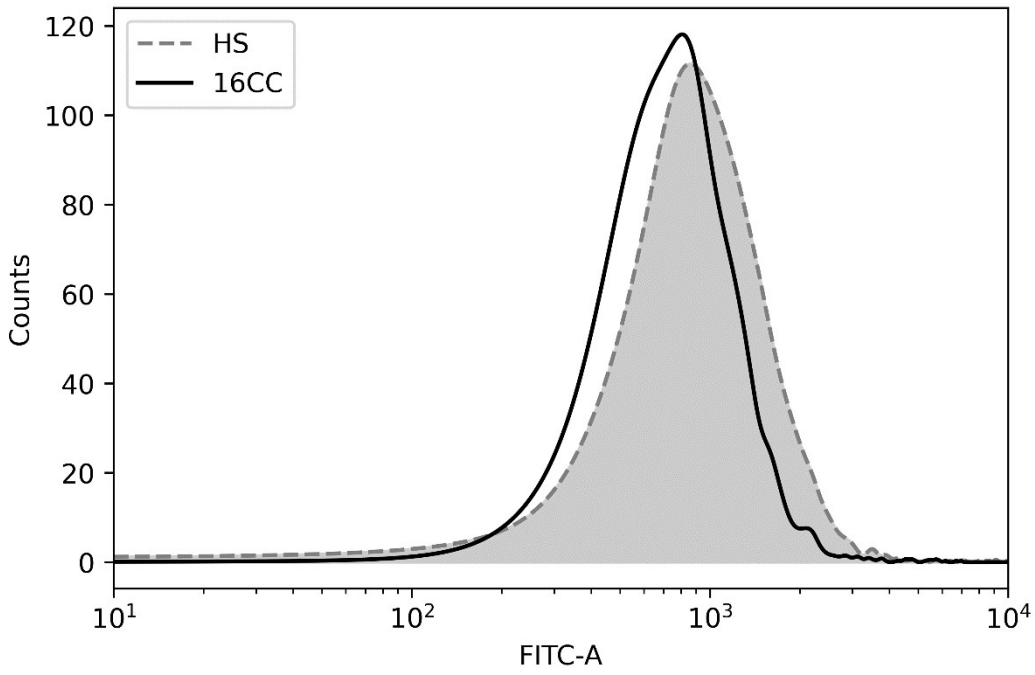

Figure S 55. Representative flow cytometry plots illustrating the absence of recruitment of IgM anti-Rha antibodies present in human serum (HS) to the BT-549 cell surface after cell treatment with 16CC. Cells were successively incubated with ARG, HS then Alexa-Fluor488-conjugated anti-human IgM secondary antibody to reveal human anti-Rha binding at the cell surface. Cells incubated with HS without ARG was used as control.

Cytotoxicity towards BT-549 cells

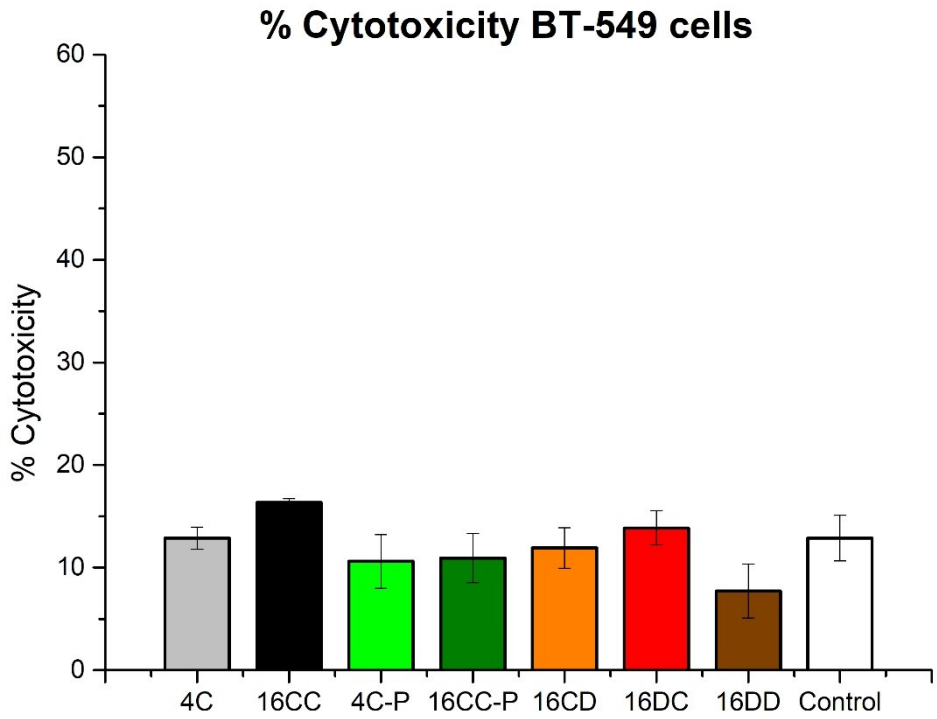

Figure S 56. Cytotoxicity induced by ARGs (100nM) and human serum (50%) towards BT-549 cells that have a low $\alpha_v\beta_3$ integrin expression (average value of 4 successive experiments). Cells incubated with unglycosylated compound **9** and human serum was used as control.

References

(1) I. Bossu, N. Berthet, P. Dumy and O. Renaudet, *J. Carbohydr. Chem.,* 2011, **30**, 458–468.

(2) B. Liet, E. Laigre, D. Goyard, B. Todaro, C. Tiertant, D. Boturyn, N. Berthet and O. Renaudet, *Chem. – A Eur. J.*, 2019, **25**, 15508–15515.