SUPPORTING INFORMATION

Selective High-resolution DNP-enhanced NMR of Biomolecular Binding Sites

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1) Aromatic region of Sel-DNP spectra for k = 1, 0.8, and 0.7

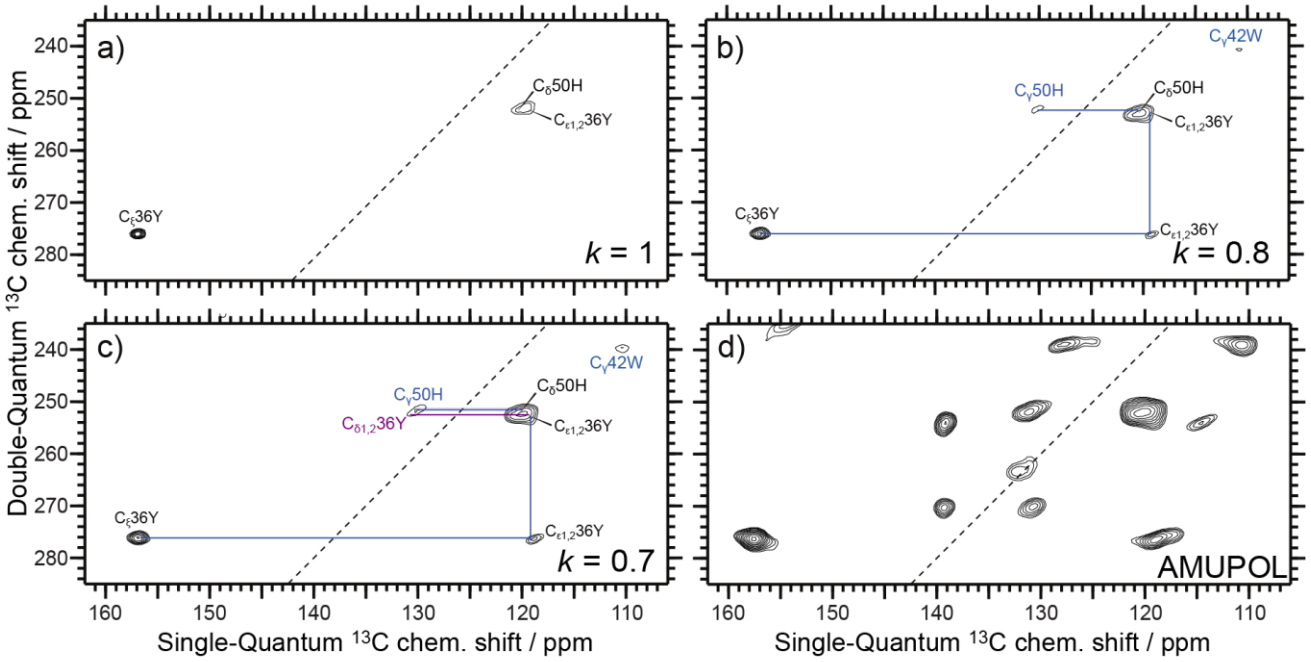

Figure S1. Aromatic region of Sel-DNP ^{13}C-^{13}C DQ/SQ one-bond correlation spectra obtained with k = 1 (a), k = 0.8 (b), and k = 0.7 (c), compared in (d) to the reference spectrum S_0 obtained with AMUPol as PA. Contours are set to the same levels as for **Fig. 3**. Further Sel-DNP spectra for k = 0.6 and 0.4 are given in **Fig. S2**.

2) Sel-DNP spectra for *k* = 0.6 and *k* = 0.4

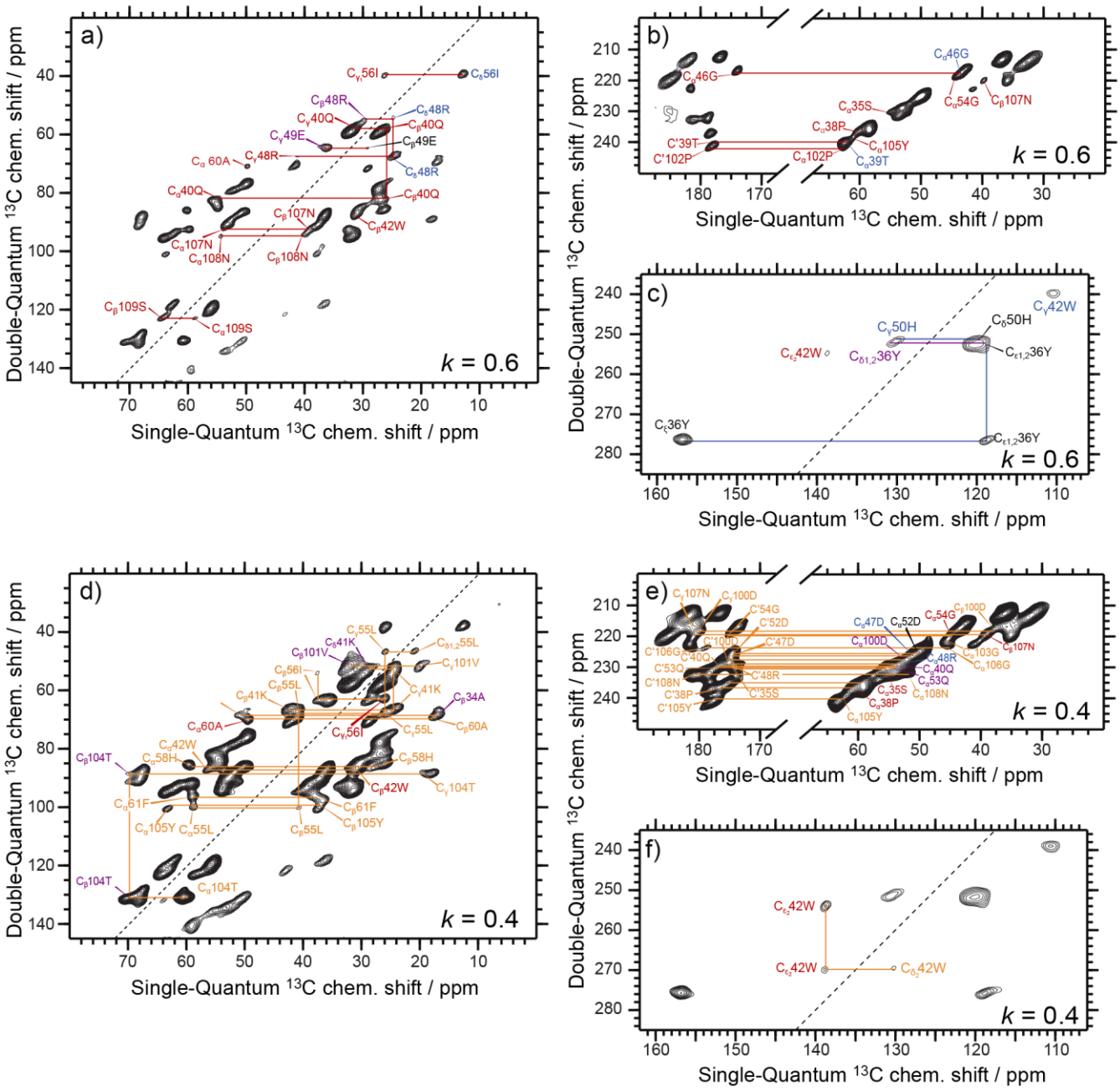

Figure S2. Sel-DNP ^{13}C-^{13}C DQ/SQ one-bond correlation spectra obtained with *k* = 0.6 and 0.4. (a) and (d) aliphatic region, (b) and (e) CO-aliphatic region, (c) and (f) aromatic region. New residues appearing in the *k* = 0.6, respectively *k* = 0.4, Sel-DNP spectra are labelled in burgundy, respectively in orange. Resonances belonging to residues, which already partially appear in Sel-DNP spectra of higher *k* are labelled in black (*k* = 1), blue (*k* = 0.8) or purple (*k* = 0.7). Contours are set at the same levels as for **Fig. 3.**

3) Conformations of the PA-ligand in the LecA binding site

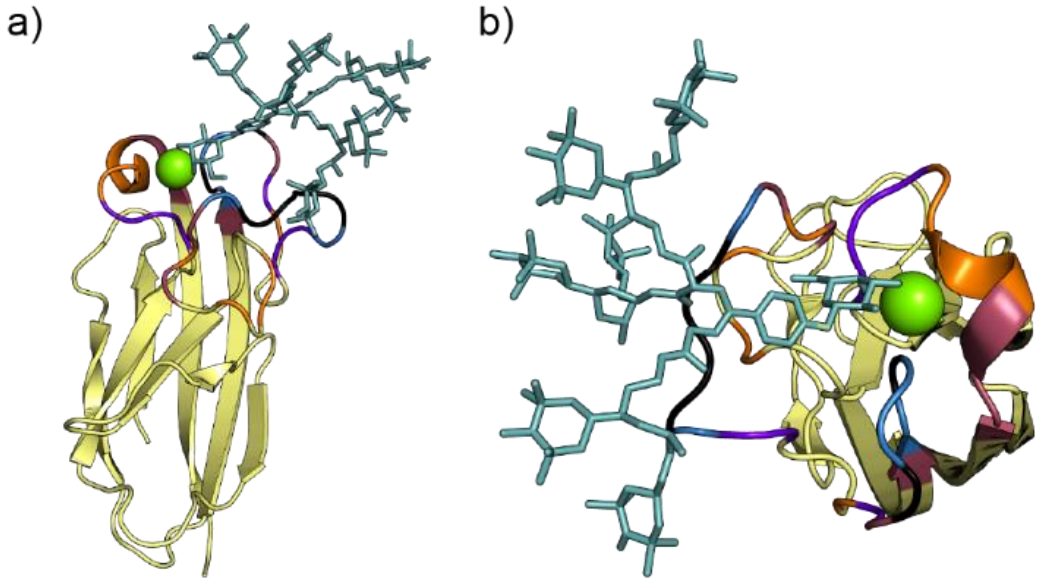

Figure S3. Representation of three possible low-energy conformations of the PA-ligand in the LecA binding site with the phenyl-galactoside moiety in the position observed in the crystal state. a) Lateral view and b) top view.

4) DQ/SQ 2D dipolar recoupling pulse sequence using $R16_2^7$

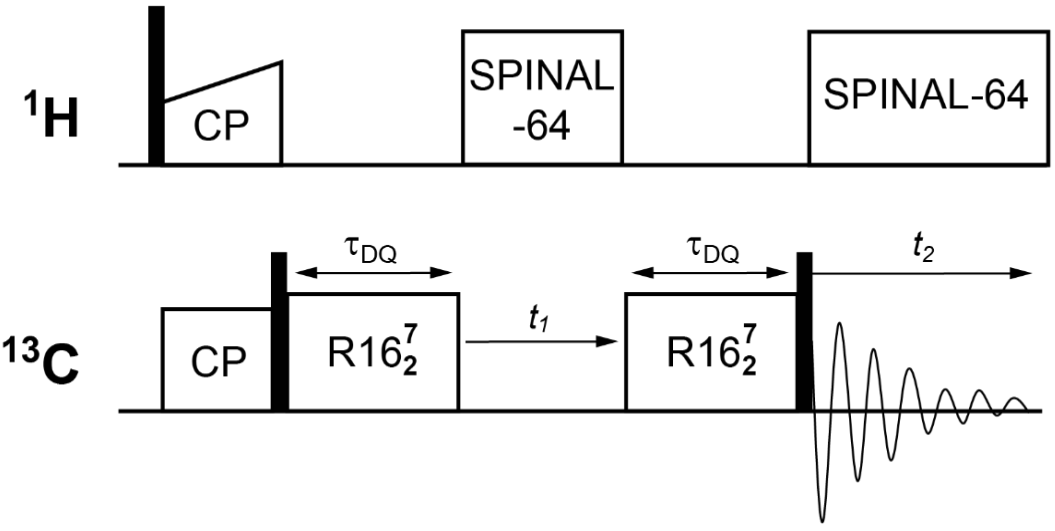

Figure S4. Pulse sequence used to record the ^{13}C-^{13}C DQ-SQ dipolar correlation experiments of **Fig. 1c and d**. For DNP-enhanced spectra, microwave irradiation is on during the entire experiment.

5) Table of residues involved in the binding of D-galactose

Table S1. Amino acid residues of LecA involved in the binding of galactose according to the X-ray structure PDB ID 10K0 (Ref: Cioci, G.; Mitchell, E. P.; Gautier, C.; Wimmerová, M.; Sudakevitz, D.; Pérez, S.; Gilboa-Garber, N.; Imberty, A. FEBS Lett. 2003, 555, 297.)

AA Type	Ca^{2+}	Ca^{2+} / D-Gal	D-Gal
Thr	-	T104	-
Tyr	Y105	Y36	-
Gly	G106	-	-
Asn	N108	N107	-
Asp	D100	-	-
His	-	-	H50
Gln	-	-	Q53
Pro	-	-	P51
Val	-	-	V101

6) Assignment table of LecA based on Sel-DNP

Table S2. Chemical shift values for the assigned residues of LecA (same color code as for **Fig. 3, S1, and S2**).

δ(ppm) / Residues	C_α	C_β	C_γ	$C_{\delta1}$	$C_{\delta2}$	$C_{\varepsilon1}$	$C_{\varepsilon2}$	$C_{\varepsilon3}$	$C_{\eta1}$	$C_{\eta2}$	C_ζ	C´
^{34}A	49.2	21.0	-	-	-	-	-	-	-	-	-	-
^{35}S	55.7	61.6	-	-	-	-	-	-	-	-	-	175.6
^{36}Y	63.2	37.5	132.3	131.6	131.6	119.0	119.0	-	-	-	156.9	178.1
^{37}G	42.6	-	-	-	-	-	-	-	-	-	-	173.3
^{38}P	61.5	31.6	26.6	49.7	-	-	-	-	-	-	-	176.3
^{39}T	61.4	67.2	19.4	-	-	-	-	-	-	-	-	178.0
^{40}Q	55.7	26.0	30.7	181.8	-	-	-	-	-	-	-	177.1
^{41}K	-	28.9	24.2	29.6	-	41.2	-	-	-	-	-	-
^{42}W	56.4	30.9	110.7	-	130.0	-	138.9	-	-	-	-	-
^{46}G	43.6	-	-	-	-	-	-	-	-	-	-	173.9
^{47}D	51.0	36.4	176.9	-	-	-	-	-	-	-	-	174.8
^{48}R	54.4	29.3	42.7	23.7	-	-	-	-	-	-	-	174.7
^{49}E	54.8	28.0	35.7	185.3	-	-	-	-	-	-	-	175.2
^{50}H	59.7	25.7	129.1	121.3	-	-	-	-	-	-	-	178.4
^{51}P	63.7	31.5	26.8	51.6	-	-	-	-	-	-	-	178.1
^{52}D	50.5	35.8	176.3	-	-	-	-	-	-	-	-	174.6
^{53}Q	54.6	26.9	31.0	182.5	-	-	-	-	-	-	-	176.1
^{54}G	44.3	-	-	-	-	-	-	-	-	-	-	174.5
^{55}L	58.8	41.0	26.7	21.0	-	-	-	-	-	-	-	178.3
^{56}I	59.3	37.4	26.0	12.3	-	-	-	-	-	-	-	175.1
^{58}H	59.4	27.5	-	-	-	-	-	-	-	-	-	178.8
^{60}A	50.1	17.1	-	-	-	-	-	-	-	-	-	-
^{61}F	56.8	38.8	-	-	-	-	-	-	-	-	-	177.5
^{100}D	53.0	37.5	181.0	-	-	-	-	-	-	-	-	175.2
^{101}V	59.3	32.3	20.1	-	-	-	-	-	-	-	-	175.8
^{102}P	62.9	32.1	24.5	49.8	-	-	-	-	-	-	-	178.9
^{103}G	45.5	-	-	-	-	-	-	-	-	-	-	-
^{104}T	60.9	70.4	18.0	-	-	-	-	-	-	-	-	177.4
^{105}Y	62.5	36.4	-	-	-	-	-		-	-	-	177.3
^{106}G	45.6	-	-	-	-	-	-	-	-	-	-	179.0
^{107}N	54.1	39.9	181.7	-	-	-	-	-	-	-	-	178.7
^{108}N	52.5	44.0	180.8	-	-	-	-	-	-	-	-	181.2
^{109}S	58.8	64.4	-	-	-	-	-	-	-	-	-	173.4

7) Chemical analysis of compounds 1, 2, 4, and 5

Data for compound 1.

Obtained as a white solid after lyophilization (489 mg, 0.86 mmol, 83%); RP-HPLC: R_t = 6.61 min (C_{18}, λ = 214 nm, 0-40% B in 15 min); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.90 (d, 2H, *J* = 8.6 Hz, 2×H-Ar), 7.11 (d, 2H, *J* = 8.5 Hz, 2×H-Ar), 5.47 (d, 1H, *J* = 2.7 Hz, H-4), 5.35-5.45 (m, 2H, H-1, H-2), 5.27 (dd, 1H, *J* = 9.8, 3.0 Hz, H-3), 4.35 (t, 1H, *J* = 6.4 Hz, H-5), 4.18 (d, 2H, *J* = 6.6 Hz, 2×H-6), 4.01 (s, 2H, CH_2-Gly), 3.51 (t, 2H, *J* = 5.5 Hz, $CH_2CH_2NH_2$), 3.08 (t, 2H, *J* = 5.6 Hz, $CH_2CH_2NH_2$), 2.18, 2.05, 2.04, 1.98 (4s, 12H, 4×CH_3-acetyl); ^{13}C NMR (CD_3OD d_6, 100 MHz) δ 173.2, 172.0, 171.9, 171.4, 171.2, 170.0 (7C, C=O), 161.1 (C-Ar), 130.5 (2×CH-Ar), 129.2 (C-Ar), 117.3 (2×CH-Ar), 99.4 (C-1), 72.3 (C-5), 72.2 (C-3), 70.1 (C-2), 68.7 (C-4), 62.6 (C-6), 44.3 (CH_2-Gly), 41.0 ($CH_2CH_2NH_2$), 38.2 ($CH_2CH_2NH_2$), 20.6, 20.6, 20.5, 20.5 (4×CH_3-acetyl); HMRS [ESI +] $(M+H)^+$ *m/z* calcd. for $C_{25}H_{34}N_3O_{12}$, 568.2142 found 568.2140.

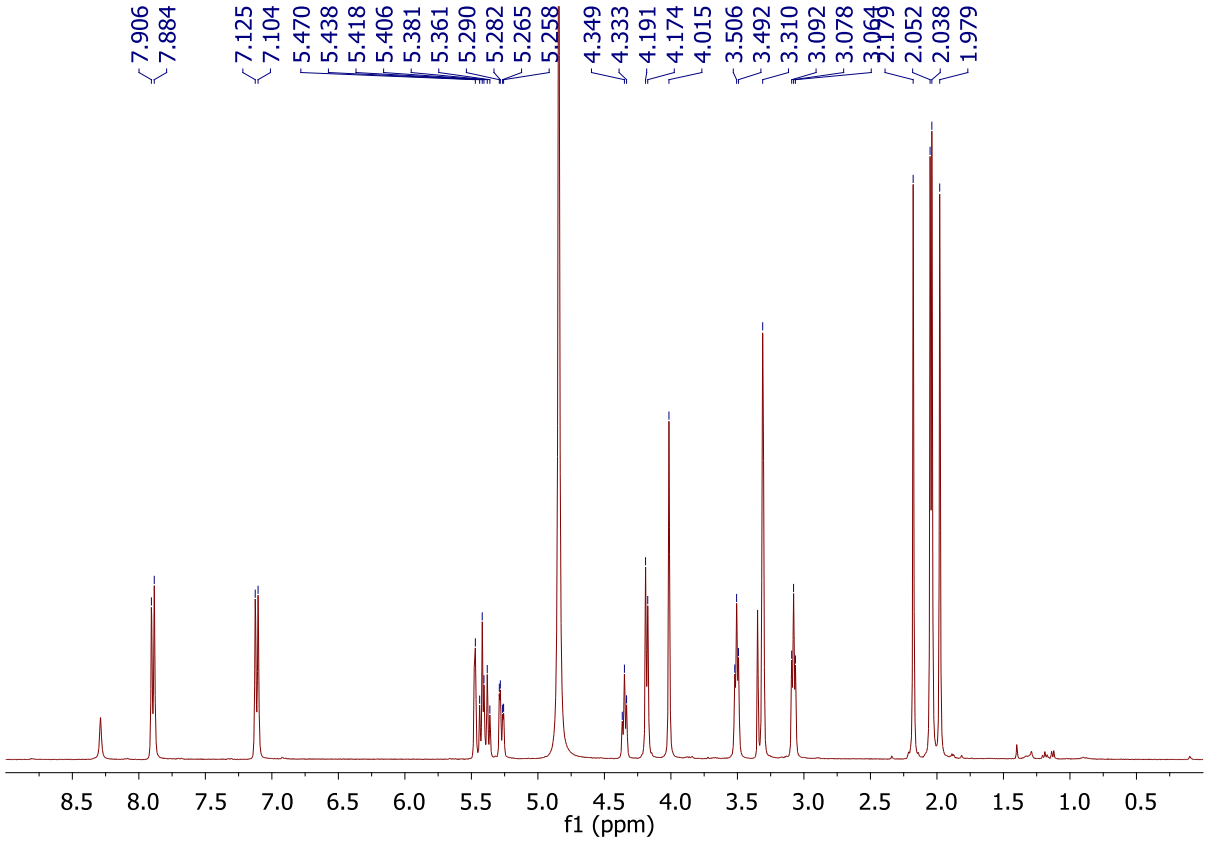

Figure S5. ^{1}H NMR spectrum of compound **1**.

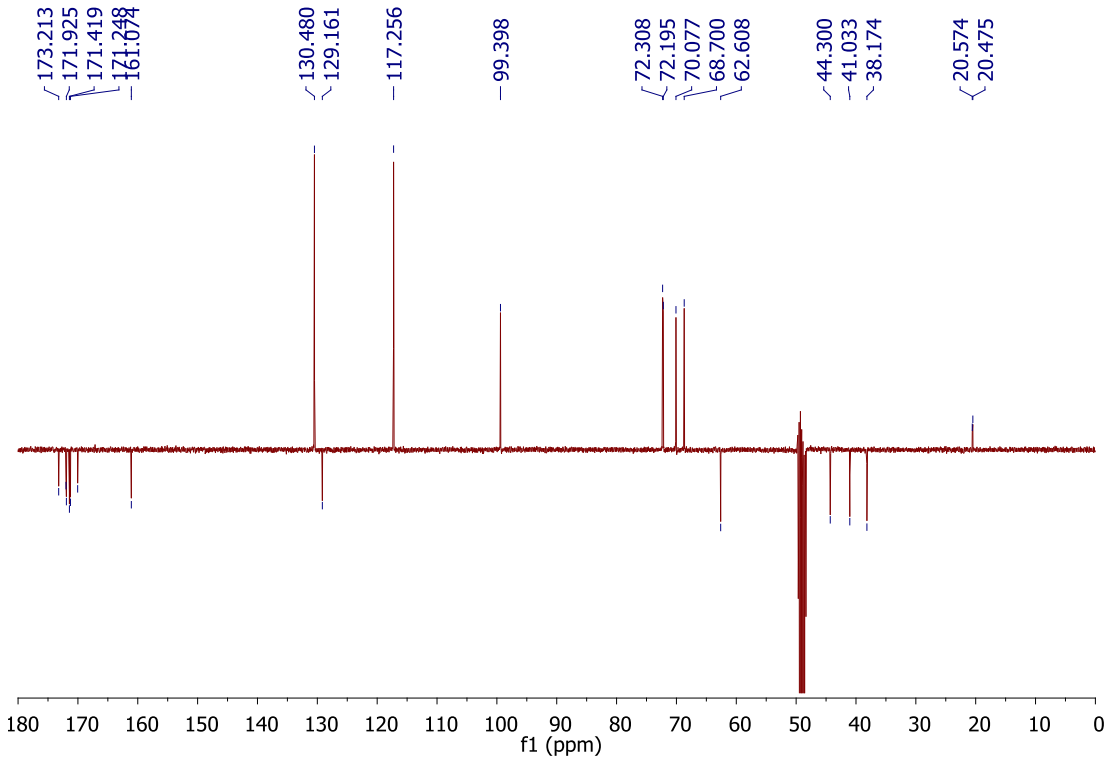

Figure S6. ^{13}C NMR spectrum of compound **1**.

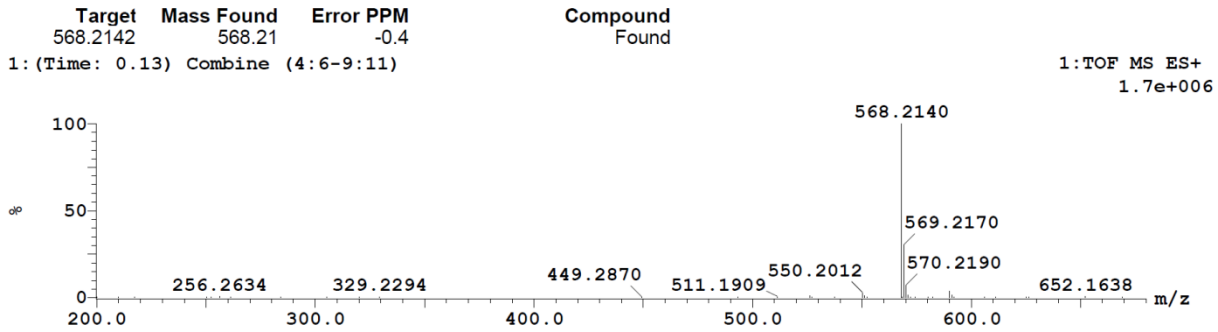

Figure S7. HRMS spectrum of compound **1**.

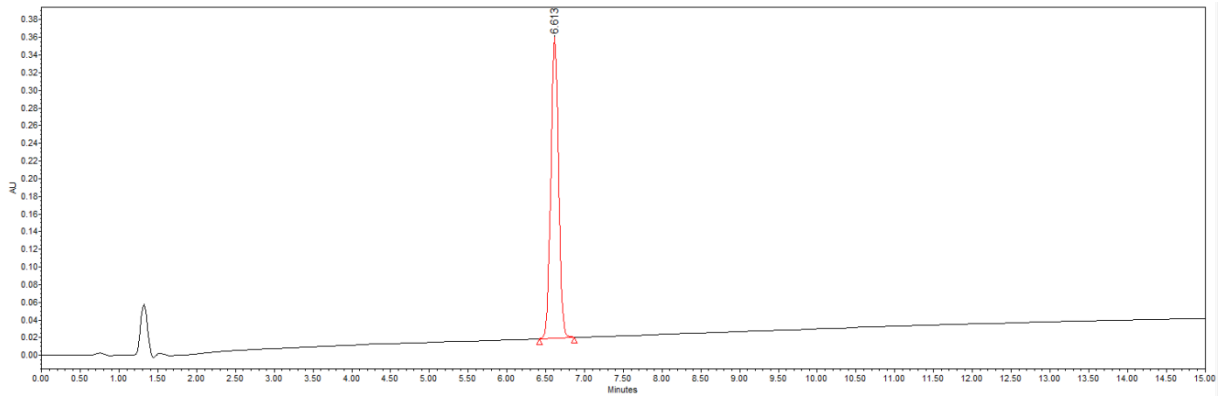

Figure S8. Analytical RP-HPLC spectrum of compound **1**.

Data for compound 2.

Obtained as a slightly orange solid after lyophilization (151 mg, 0.21 mmol, 72%); RP-HPLC: R_t = 8.53 min (C_{18}, λ = 214 nm, 5-40% B in 15 min); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.90 (d, 2H, *J* = 8.5 Hz, 2×H-Ar), 7.12 (d, 2H, *J* = 8.6 Hz, 2×H-Ar), 5.47 (d, 1H, *J* = 3.0 Hz, H-4), 5.36-5.44 (m, 2H, H-1, H-2), 5.27 (dd, 1H, *J* = 9.9, 3.1 Hz, H-3), 4.35 (t, 1H, *J* = 6.5 Hz, H-5), 4.18 (d, 2H, *J* = 6.6 Hz, 2×H-6), 3.99 (s, 2H, CH_2-Gly), 3.55 (t, 2H, *J* = 5.4 Hz, $CH_2CH_2NH_2$), 3.48-3.51 (m, 1H, CH-TEMPO), 3.19 (t, 2H, *J* = 5.3 Hz, $CH_2CH_2NH_2$), 2.18, 2.05, 2.04, 2.01, 1.98 (4s, 12H, 4×CH_3-acetyl), 1.99-2.01 (m, 2H, 2×CH-TEMPO), 1.57 (t, 2H, *J* = 12.2 Hz, 2×CH-TEMPO), 1.21, 1.17 (2s, 12H, 2×CH_3-TEMPO); ^{13}C NMR (CD_3OD *d*$_6$, 100 MHz) δ 173.6, 172.0, 171.9, 171.4, 171.3, 170.1 (6C, C=O), 161.1 (C-Ar), 130.5 (2×CH-Ar), 129.0 (C-Ar), 117.3 (2×CH-Ar), 99.4 (C-1), 72.3 (C-5), 72.2 (C-3), 70.1 (C-2), 68.7 (C-4), 62.6 (C-6), 59.6 (2×C-TEMPO), 51.4 (CH-TEMPO), 46.0 (CH_2CH_2NH), 44.6 (CH_2-Gly), 42.3 (2×CH_2-TEMPO), 37.1 (CH_2CH_2NH), 32.8 (2×CH_3-TEMPO), 20.6, 20.6, 20.5, 20.5 (4×CH_3-acetyl), 20.0 (2×CH_3-TEMPO); HMRS [ESI +] $(M+H)^+$ *m/z* calcd. for $C_{34}H_{50}N_4O_{13}$, 722.3374 found 722.3360.

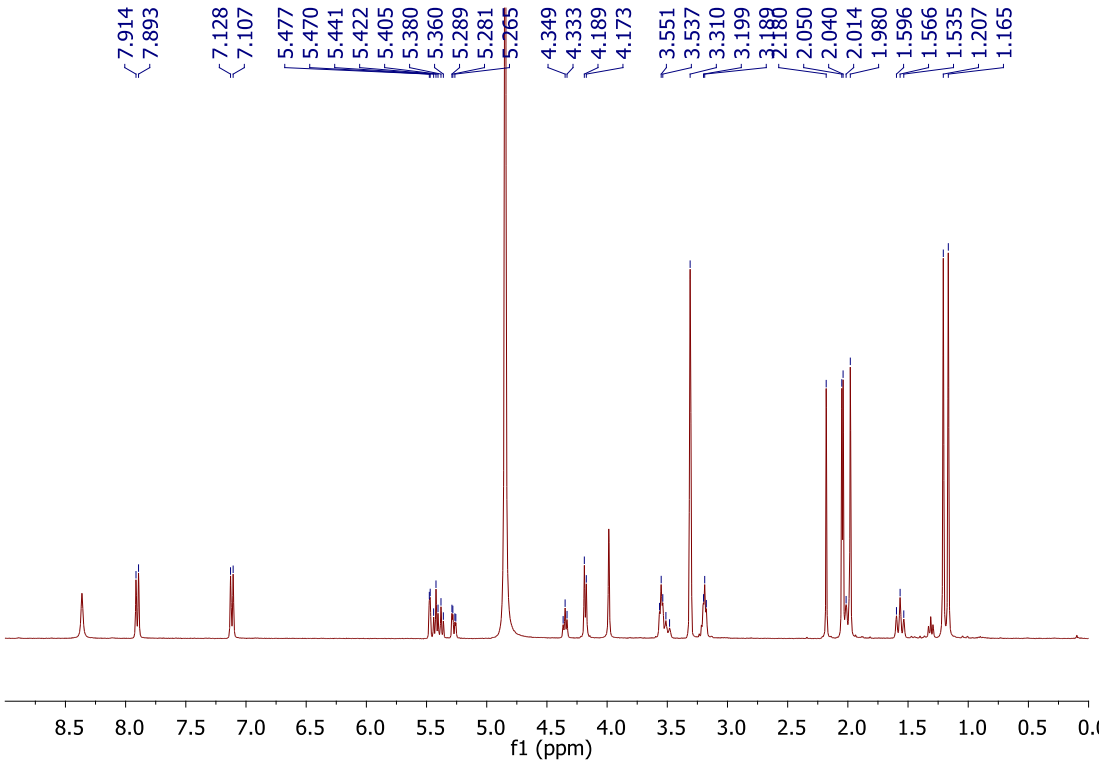

Figure S9. ^{1}H NMR spectrum of compound **2**.

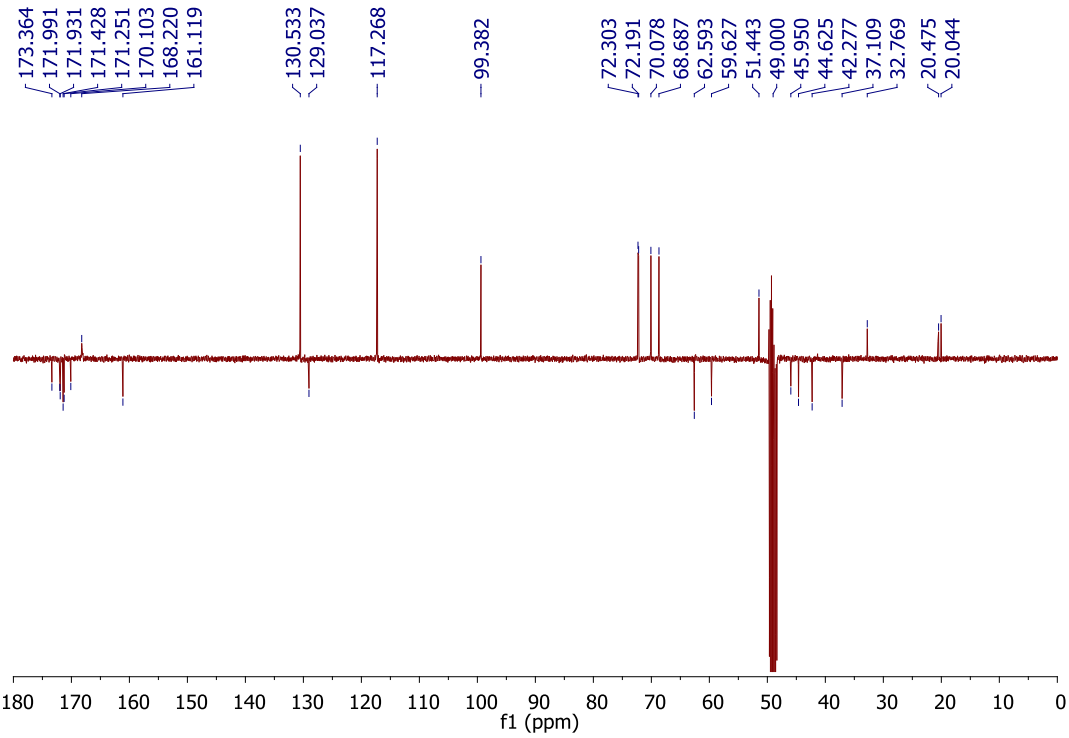

Figure S10. ^{13}C NMR spectrum of compound **2**.

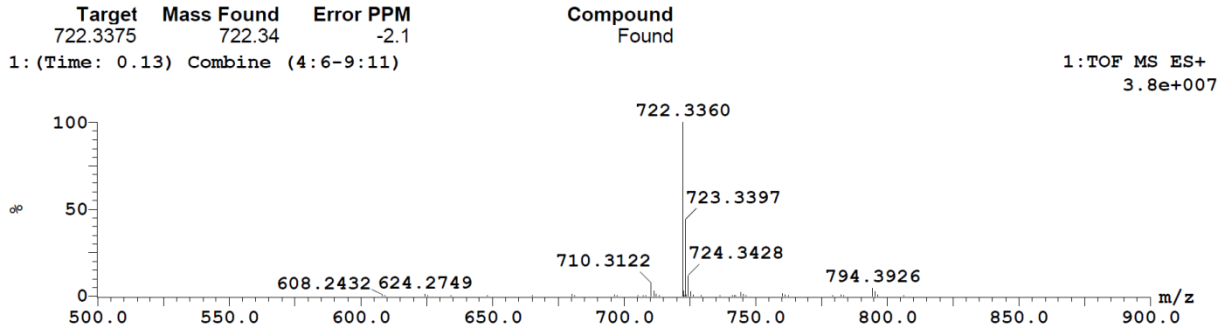

Figure S11. HRMS spectrum of compound **2**.

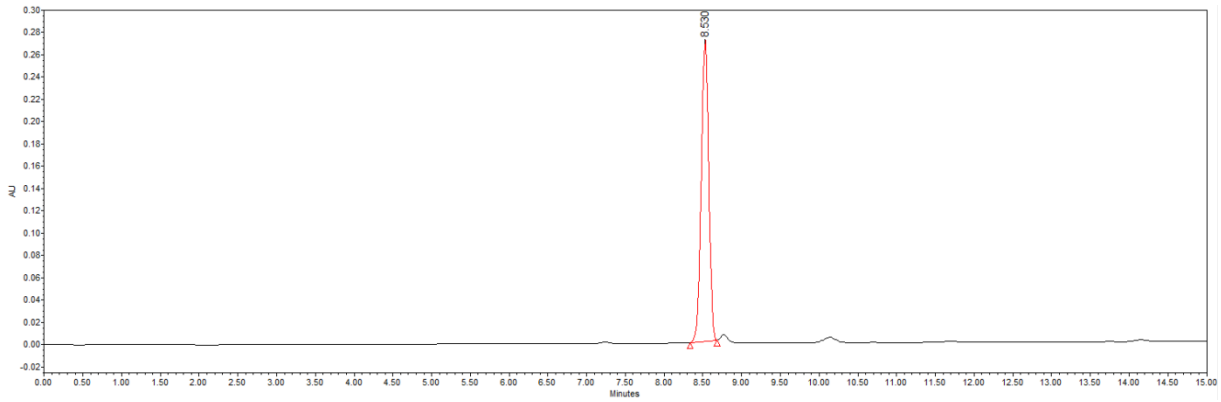

Figure S12. Analytical RP-HPLC of compound **2**.

Data for compound 4.

Obtained as a slightly orange solid after lyophilization (39 mg, 0.041 mmol, 41%); RP-HPLC: R_t = 10.59 min (C_{18}, λ = 214 nm, 5-40% B in 15 min); HMRS [ESI +] $(M+H)^+$ *m/z* calcd. for $C_{46}H_{72}N_5O_{16}$, 950.4974 found 950.4941.

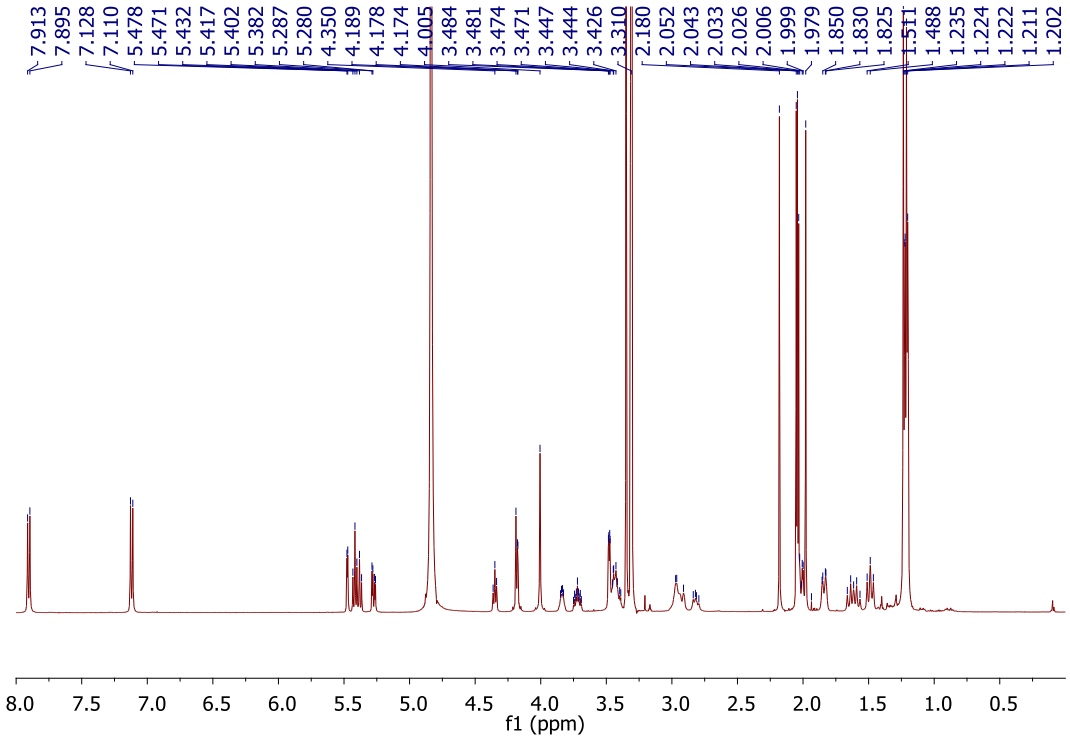

Figure S13. ^{1}H NMR of compound **4**.

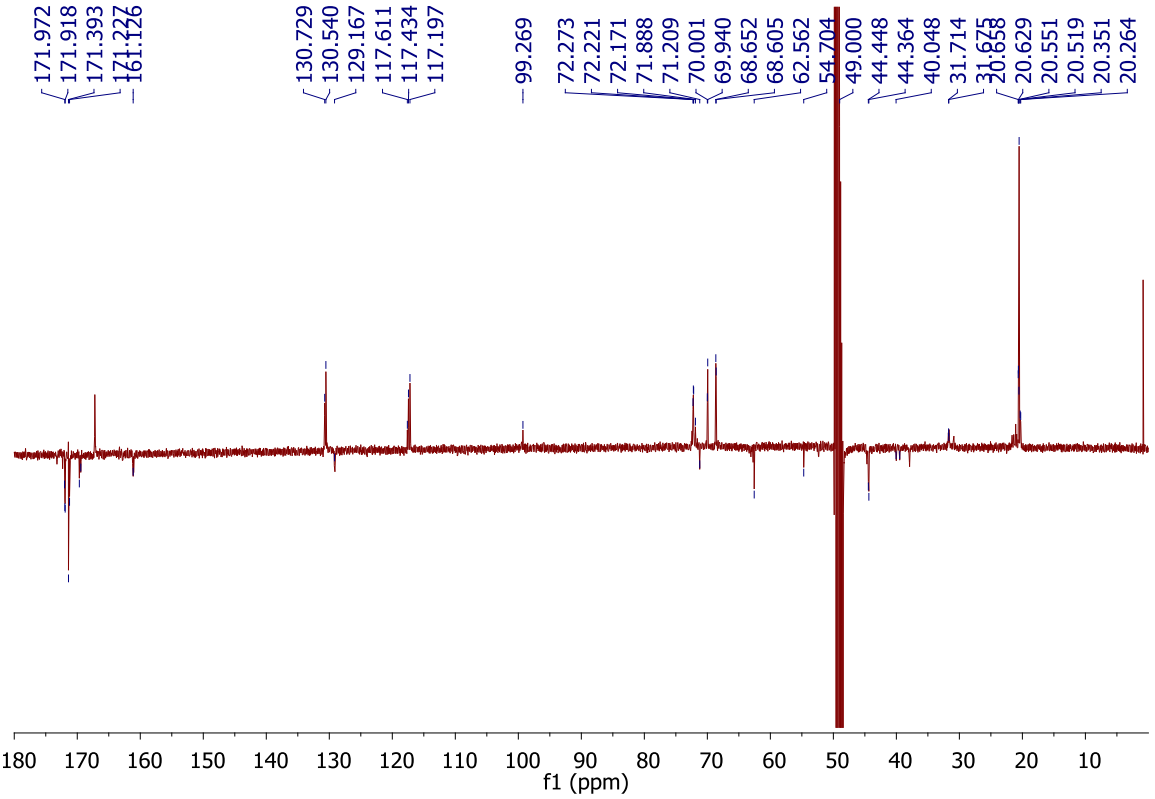

Figure S14. ^{13}C NMR of compound **4**.

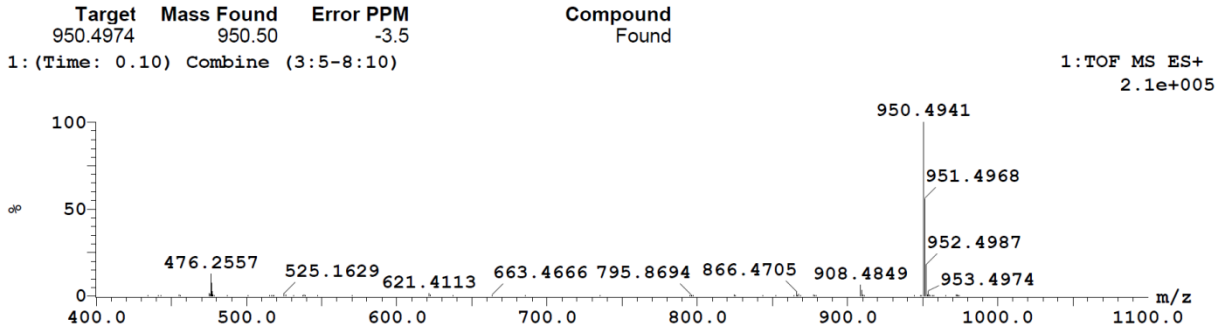

Figure S15. HRMS spectrum of compound **4**.

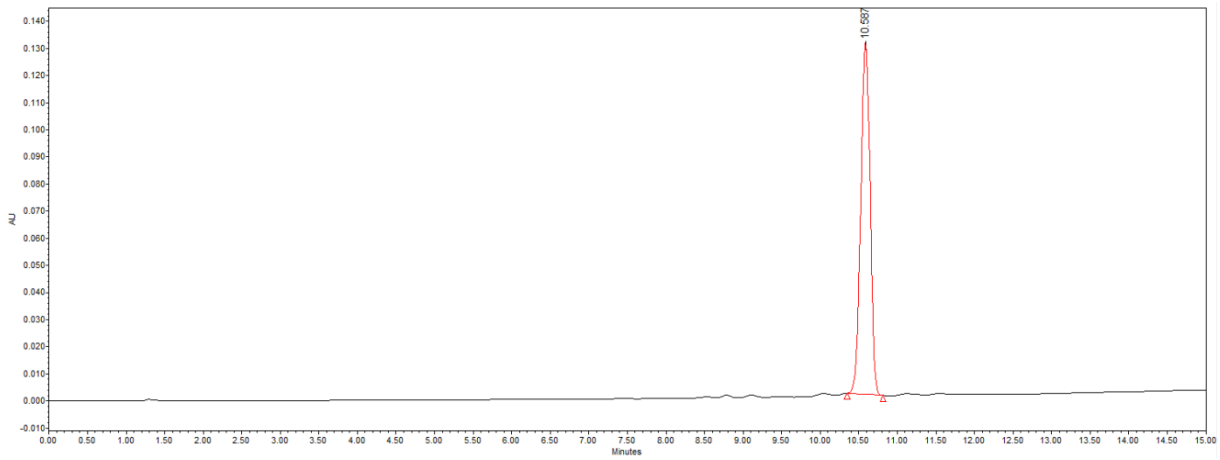

Figure S16. Analytical RP-HPLC of compound **4**.

Data for compound 5.

Obtained as a slightly orange solid after lyophilization (15 mg, 0.019 mmol, 92%); RP-HPLC: R_t = 7.21 min (C_{18}, λ = 214 nm, 5-40% B in 15 min); HMRS [ESI +] $(M+H)^+$ *m/z* calcd. for $C_{38}H_{64}N_5O_{12}$, 782.4551 found 782.4537.

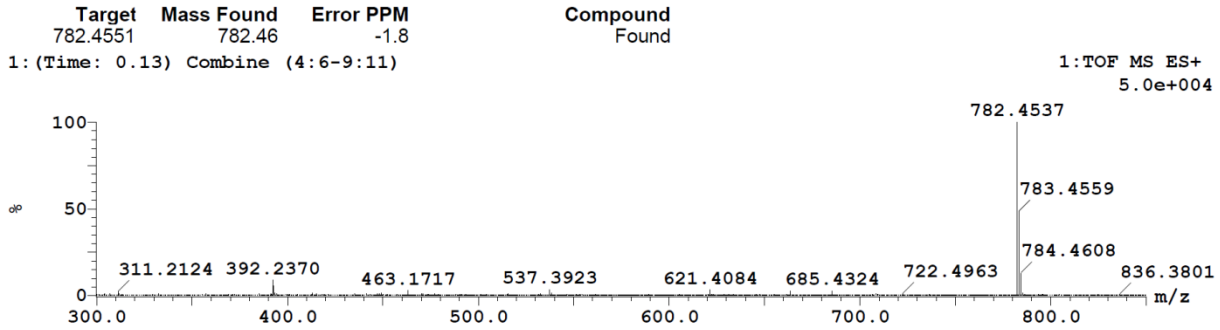

Figure S17. HRMS spectrum of compound **5**.

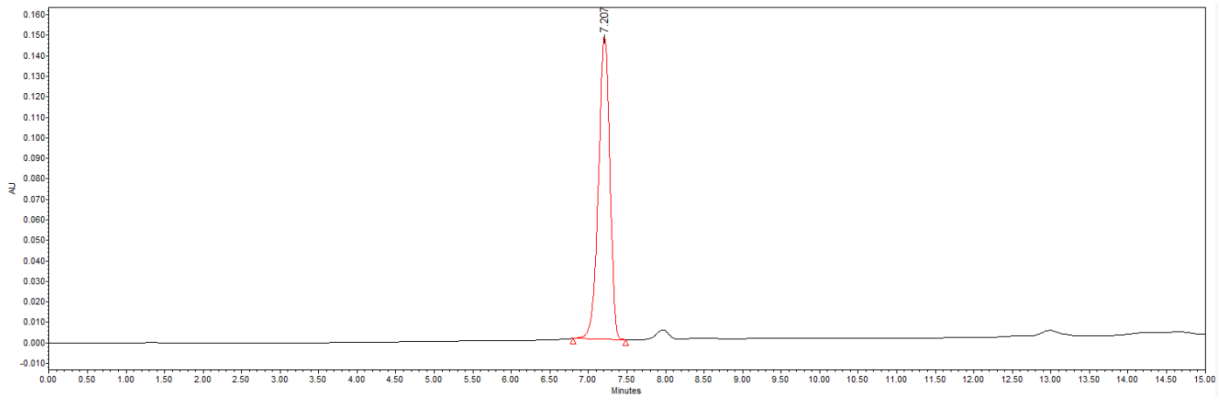

Figure S18. Analytical RP-HPLC of compound **5**.