

Supporting information

Preparation and self-assembly of amphiphilic polylysine dendrons

Sahar Mirsharghi,^a Kenneth D. Knudsen,^b Shahla Bagherifam,^{c,d} Bo Nyström^c and
Ulrik Boas*^a

^a *Section of Immunology and Vaccinology, National Veterinary Institute, Technical University of Denmark (DTU), Bülowsvej 27, DK-1870 Frederiksberg C, Denmark. E-mail: uboa@vet.dtu.dk*

^b *Department of Physics, Institute for Energy Technology, PO Box 40, N-2027 Kjeller, Norway*

^c *Department of Chemistry, University of Oslo, PO Box 1033, Blindern N-0315 Oslo, Norway*

^d *Department of Biology, University of Oslo, Blindernveien 31, N-0316 Oslo, Norway*

List of contents

Table of compounds with page numbers of spectra and chromatograms	3
Zeta potential data	4
Selected IR data	5
NMR spectra and UPLC-MS data on synthesized compounds	7
Background signals observed in UPLC-MS	7
G1-C1	8
G1-C8	11
G1- C12	14
G1-C14	17
G1-C16	20
G1-C18	23
G1(acetyl)-C4	26
G1(acetyl)-C6	29
G1(acetyl)-C10	32
G1(acetyl)-C16	35
G2-C1	38
G2-C12	42
G2-C16	46
Notes	50

Table of compounds with page numbers of spectra and chromatograms

#	Compound	UPLC-MS	¹ H-NMR	¹³ C-NMR	DEPT-135	HSQC	COSY
1	G1-C1	8	8	9	9	10	10
2	G1-C8	11	11	12	12	13	13
3	G1-C12	14	14	15	15	16	16
4	G1-C14	17	17	18	18	19	19
5	G1-C16	20	20	21	21	22	22
6	G1-C18	23	23	24	24	25	25
7	G1(acetyl)-C4	26	26	27	27	28	28
8	G1(acetyl)-C6	29	29	30	30	31	31
9	G1(acetyl)-C10	32	32	33	33	34	34
10	G1(acetyl)-C16	35	35	36	36	37	37
11	G2-C1	38	39	39	40	40	41
12	G2-C12	42	43	43	44	44	45
13	G2-C16	46	47	47	48	48	49

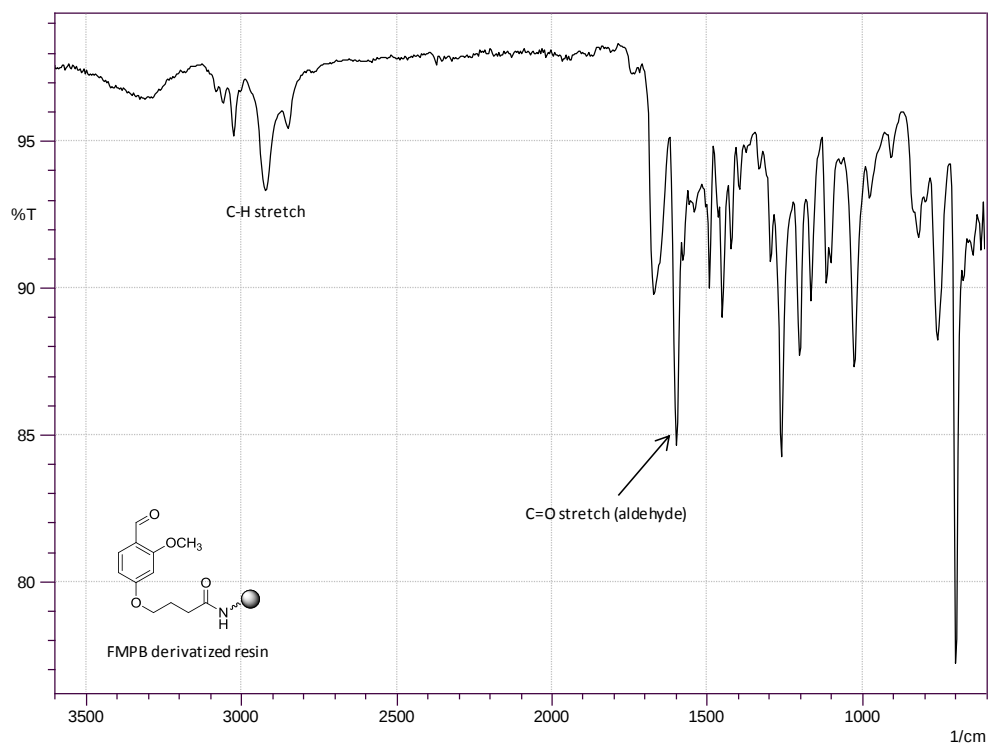
Zeta potential data

Table S1 G1 and G2 dendrons together with zeta potentials and DLS measurements.

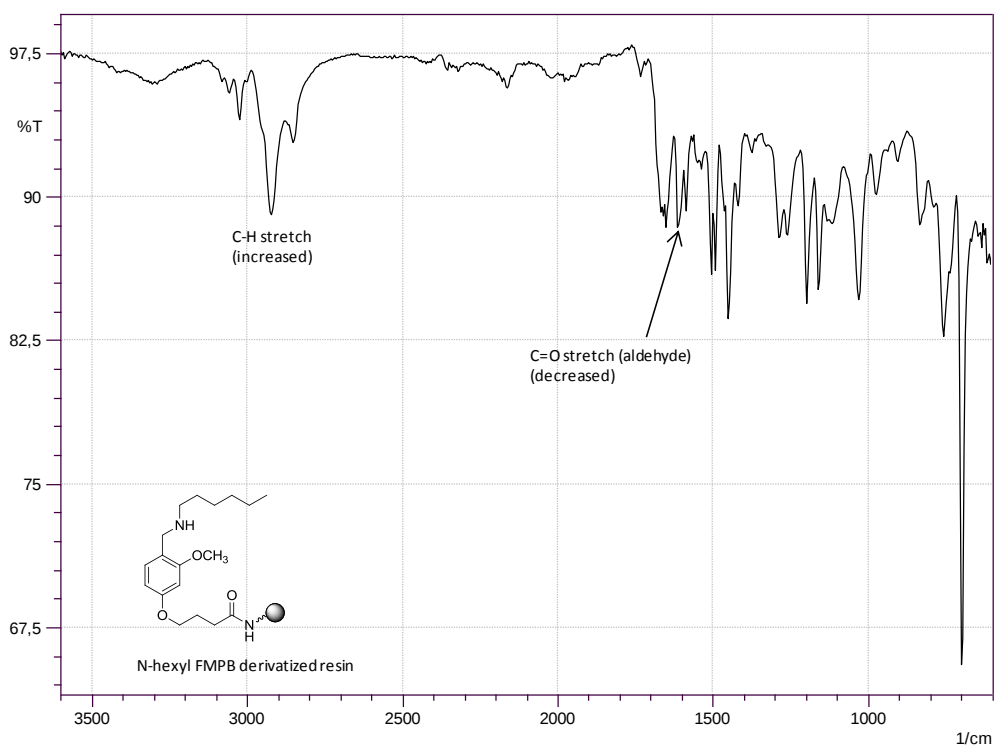
Compound	Zeta potential (mV) ^a	DLS
G1-C1	12	÷
G1-C8	12	+
G1-C12	20	÷
G1-C14	ND	+
G1-C16	55	+
G1-C18	52	+
G1(acetyl)-C4	11	÷
G1(acetyl)-C6	18	÷
G1(acetyl)-C10	42	+
G1(acetyl)-C16	54	+
G2-C1	25	÷
G2-C12	34	+
G2-C16	45	+

Selected IR data

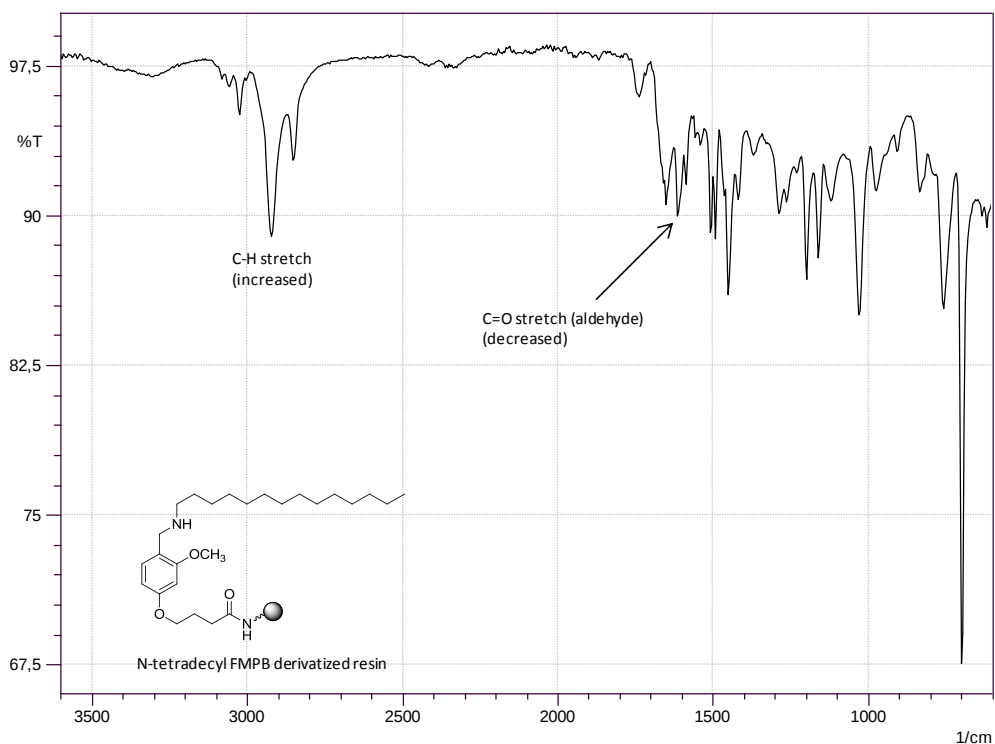
FMPB derivatized resin:



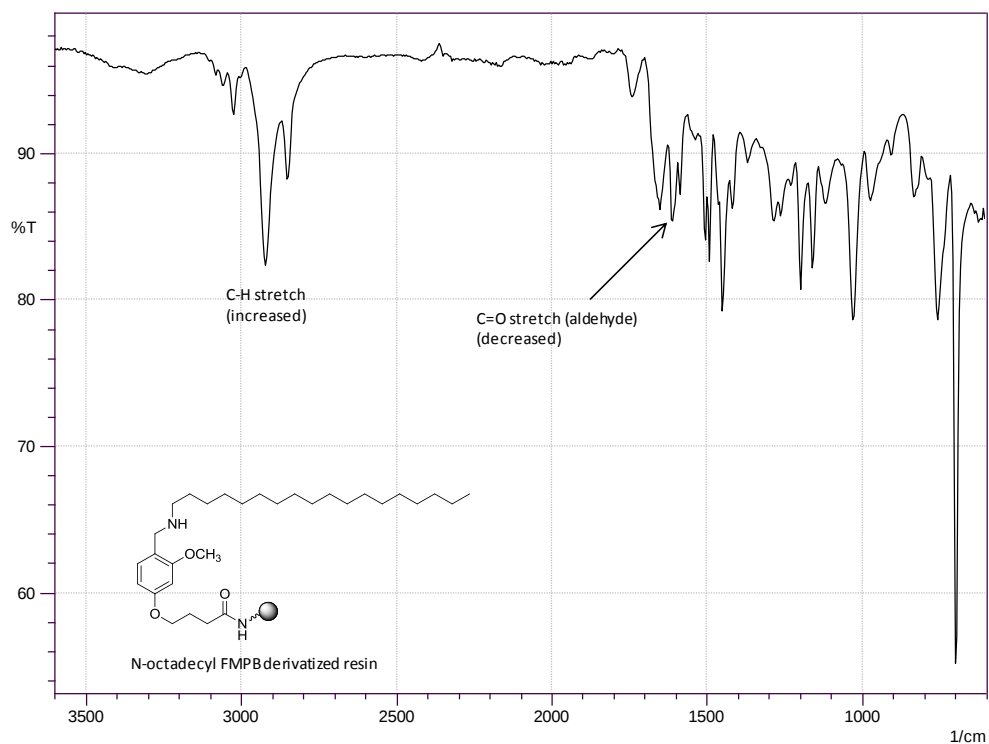
N-hexyl FMPB derivatized resin:



N-tetradecyl FMPB derivatized resin:



N-octadecyl FMPB derivatized resin:

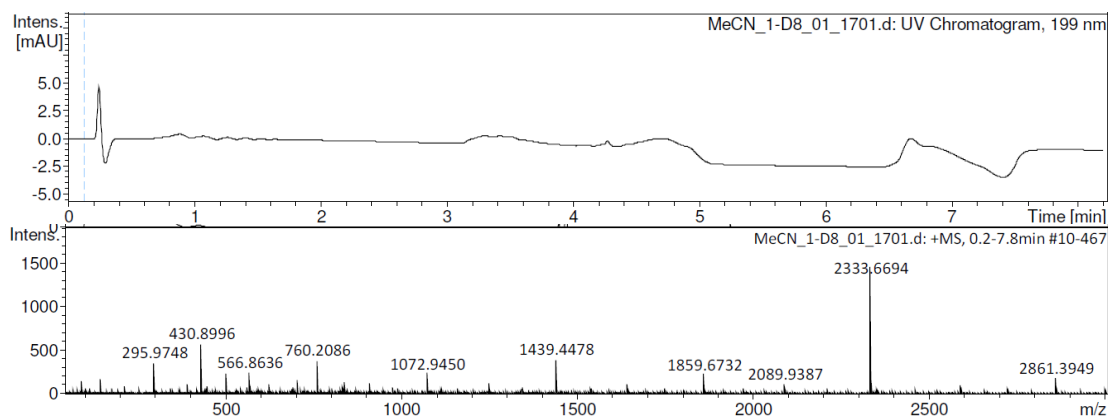


NMR spectra and UPLC-MS data on synthesized compounds

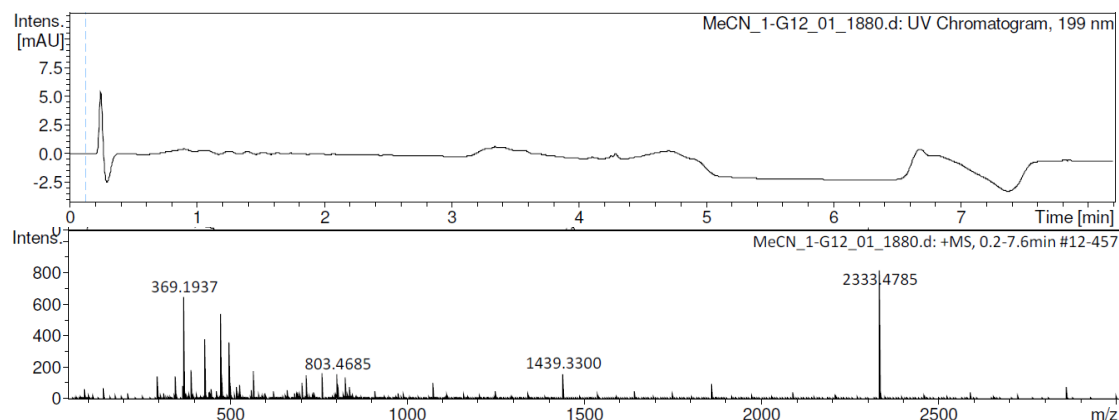
Background signals observed in UPLC-MS

Peaks 295; 369; 430; 760; 1072; 1859 and 2333 on some spectras are due to some residual impurity in the system (capillary (nebulizer), column, hexapole).

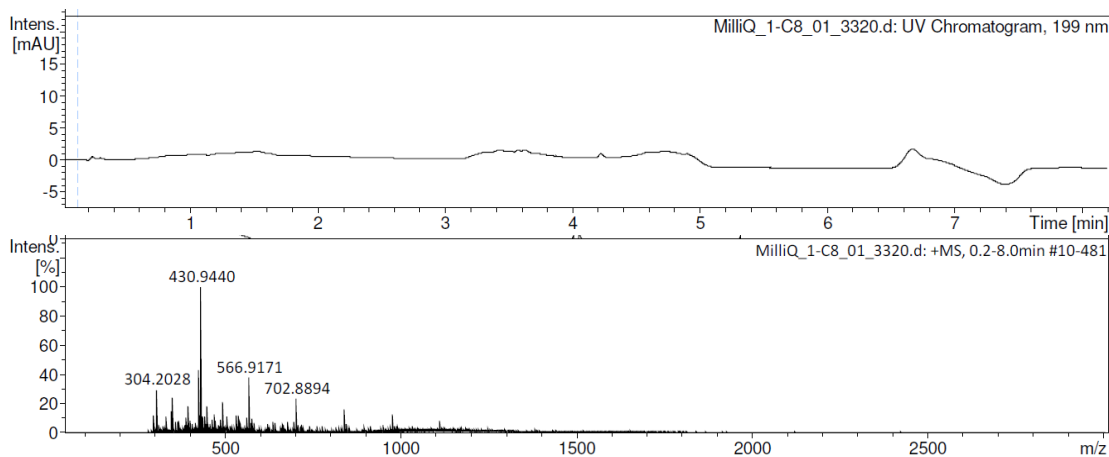
UPLC-MS (Acetonitril)



UPLC-MS (Acetonitril)

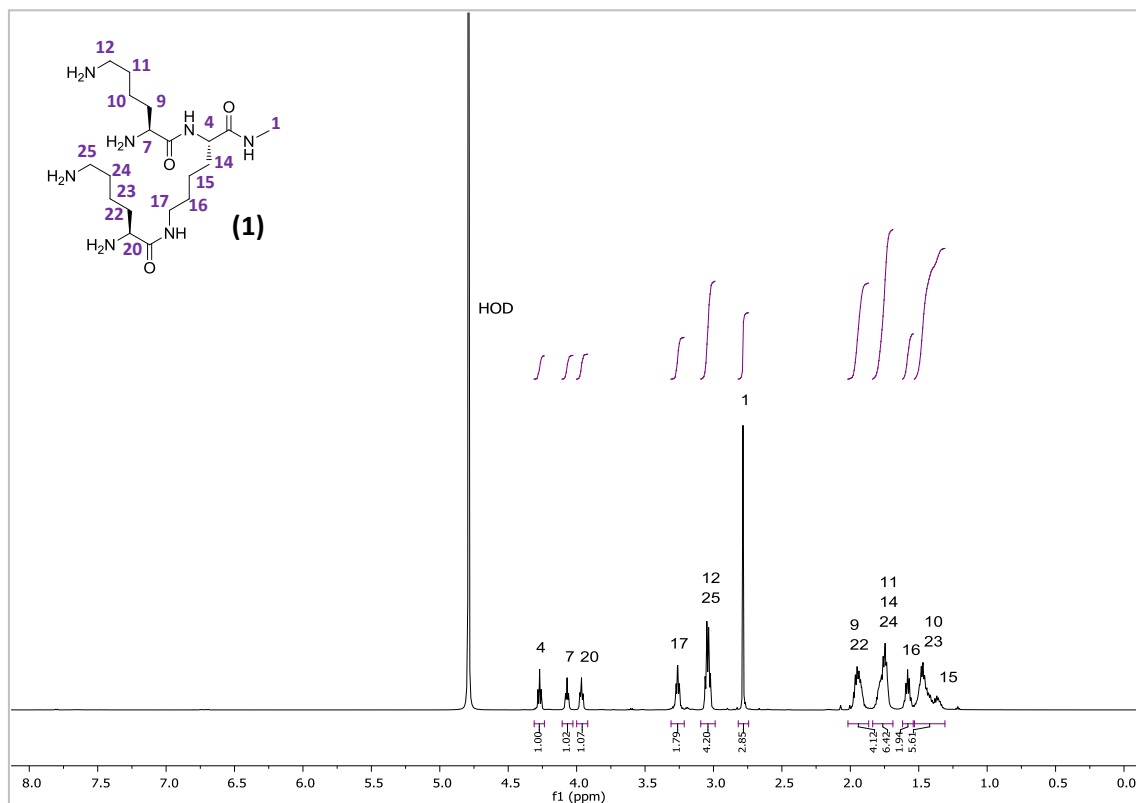
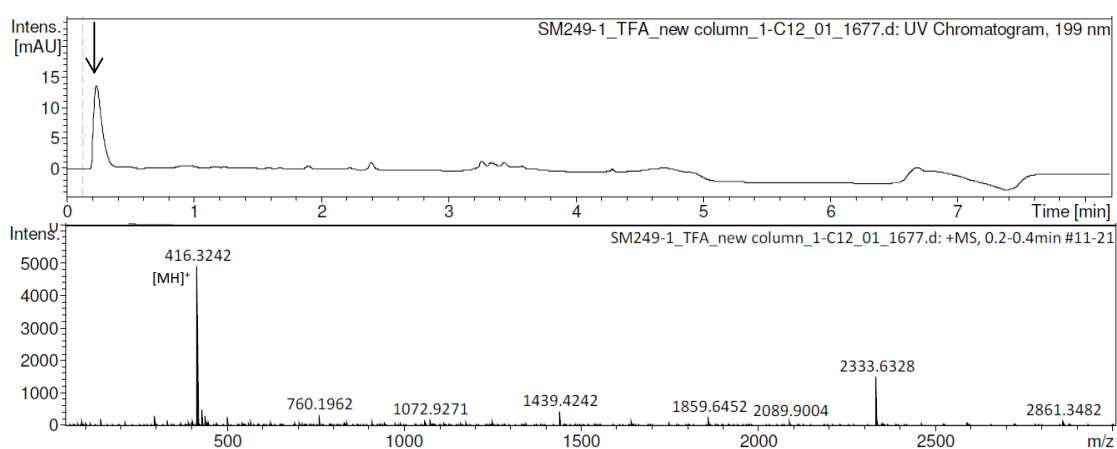


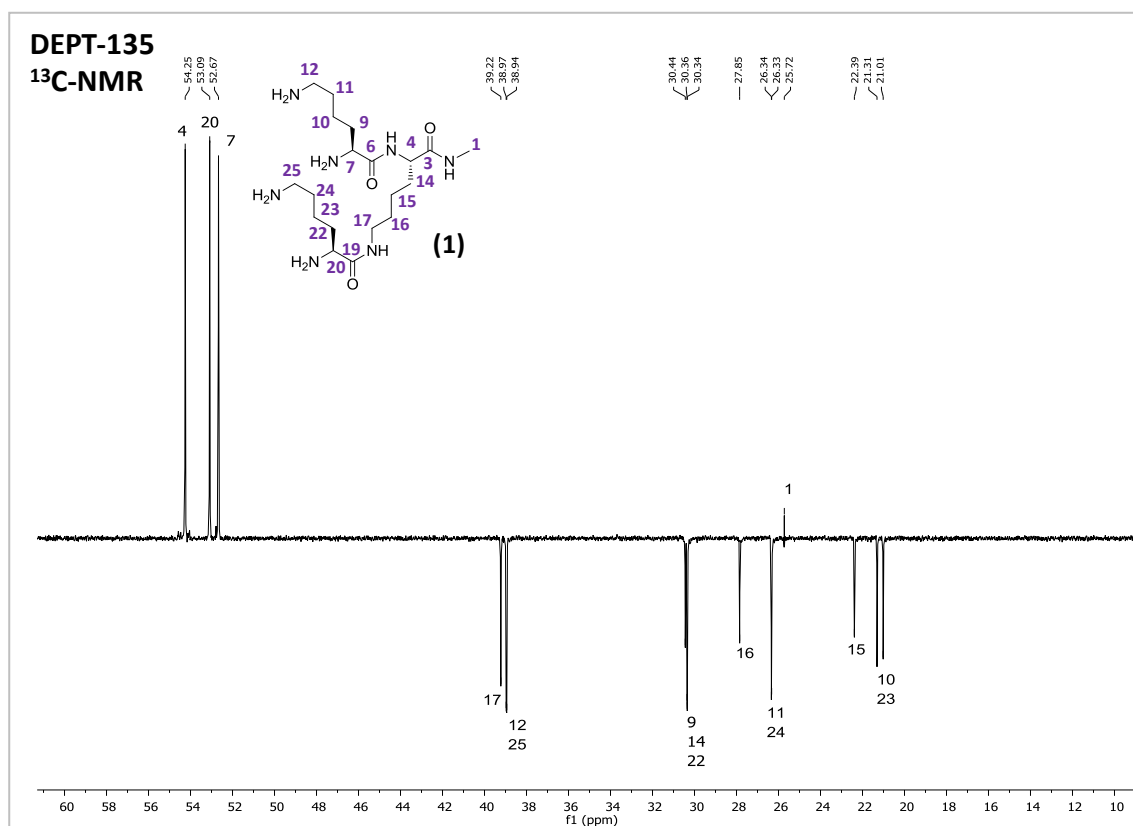
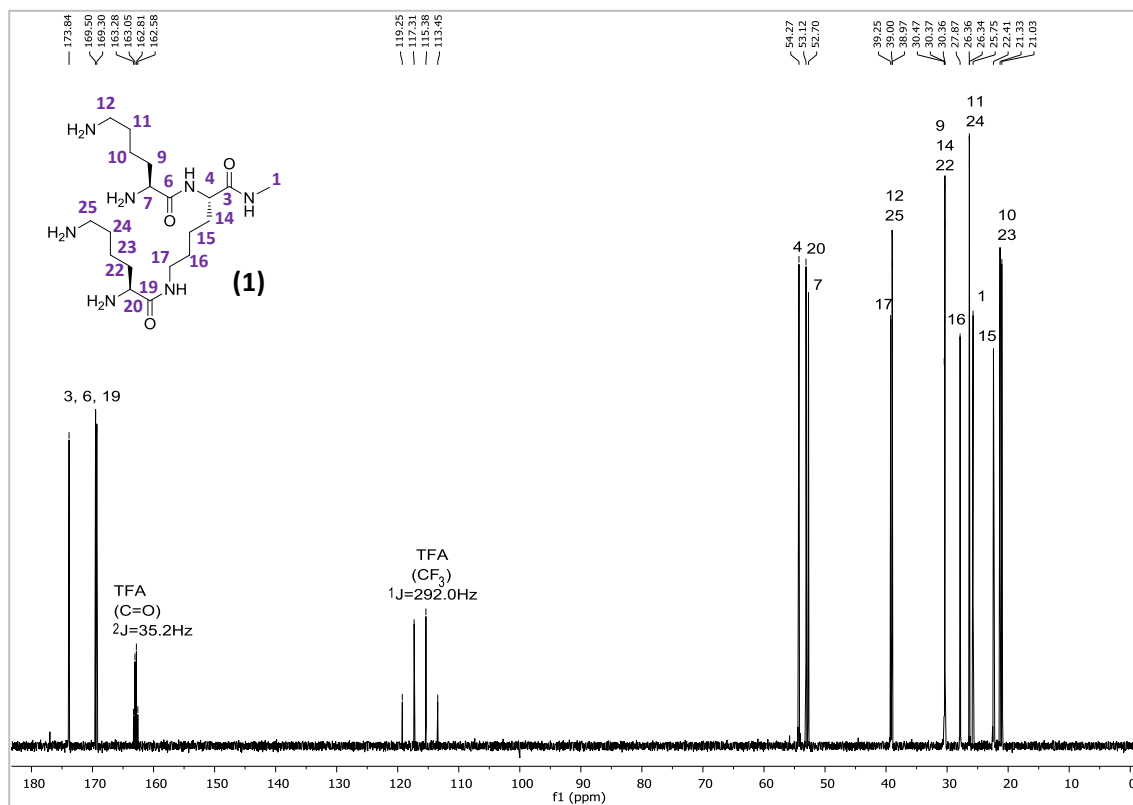
UPLC-MS (MilliQ water)



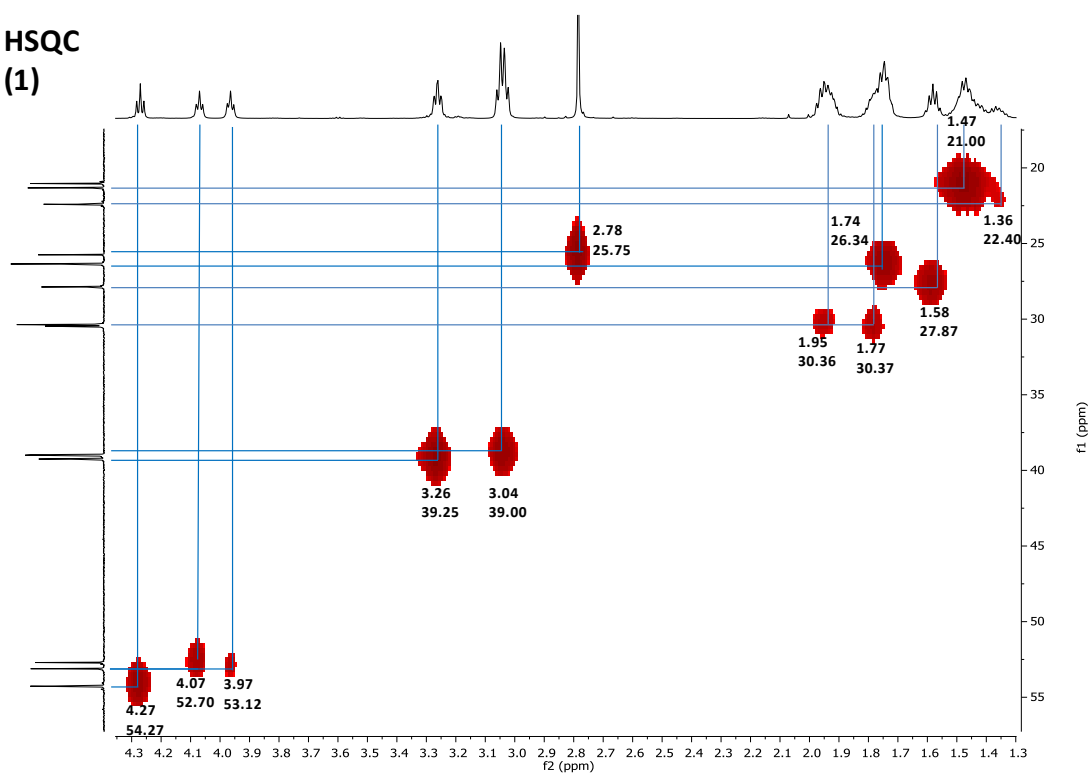
G1-C1 (1)

Yield (TFA salt; loading: 0.25 mmol/g): 56.6 mg (70 %). LC-MS data R_t : 0.2-0.4 min. MS(ESI^+), calcd $\text{C}_{19}\text{H}_{41}\text{N}_7\text{O}_3$: 415.33 g/mol, found: m/z 416.32 $[\text{MH}]^+$. ^1H NMR (600 MHz, D_2O): δ 4.27 (t, $J = 7.38$ Hz, 1H), 4.07 (t, $J = 6.56$ Hz, 1H), 3.96 (t, $J = 6.71$ Hz, 1H), 3.26 (td, $J = 7.13, 1.92$ Hz, 2H), 3.04 (q, $J = 7.80$ Hz, 4H), 2.78 (s, 3H), 2.02 – 1.87 (m, 4H), 1.84 – 1.69 (m, 6H), 1.58 (p, $J = 7.48$ Hz, 2H), 1.53 – 1.31 (m, 6H). ^{13}C NMR (151 MHz, D_2O): δ 173.84, 169.50, 169.30, 54.27, 53.12, 52.70, 39.25, 39.00, 38.97, 30.47, 30.37, 30.36, 27.87, 26.36, 26.34, 25.75, 22.41, 21.33, 21.03.

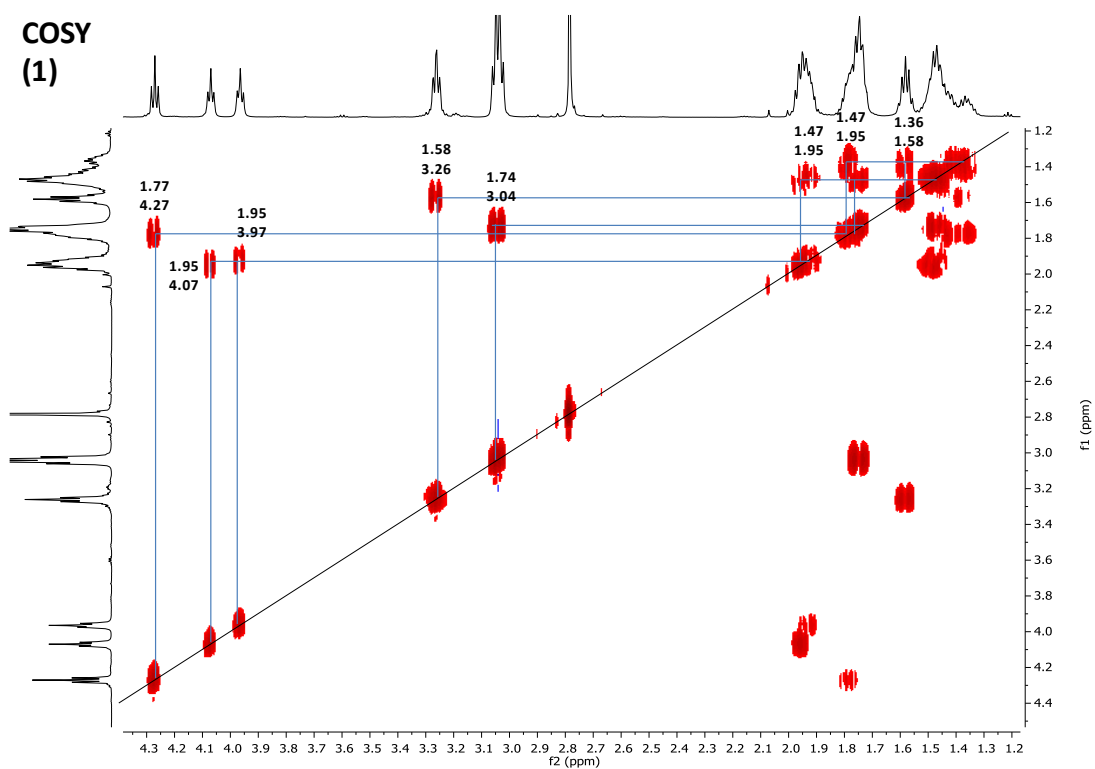
UPLC-MS(1)



HSQC
(1)

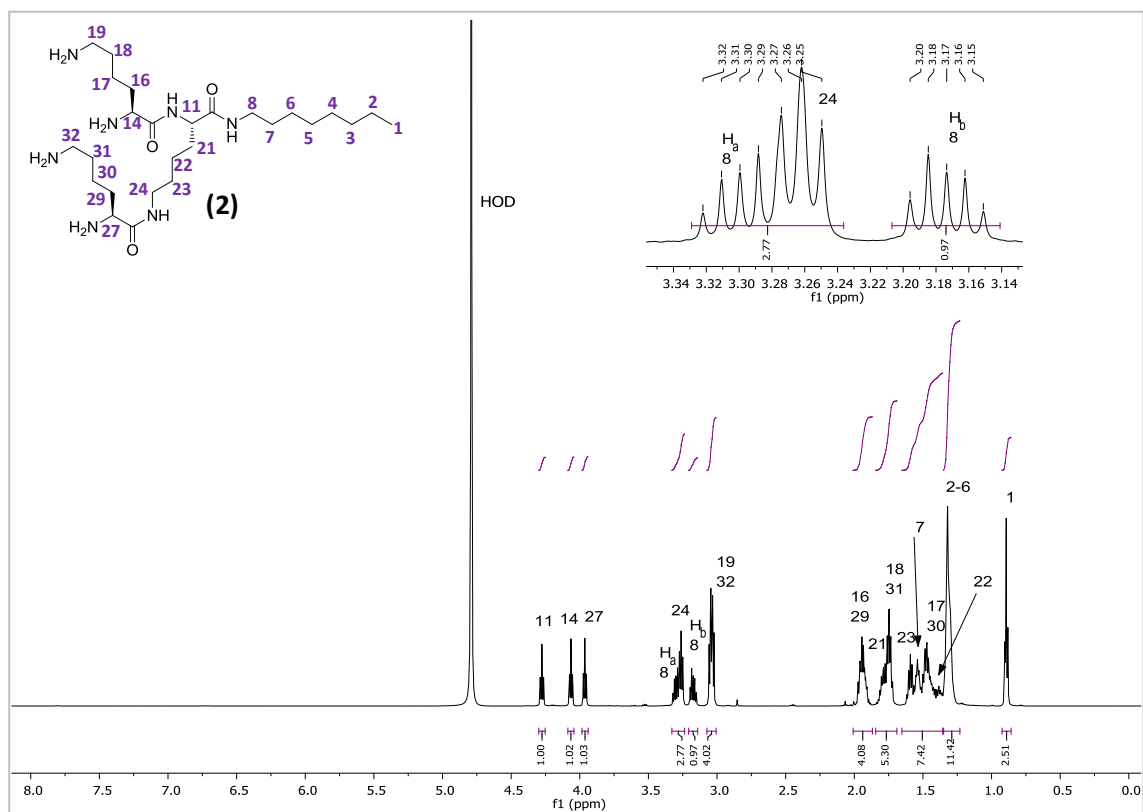
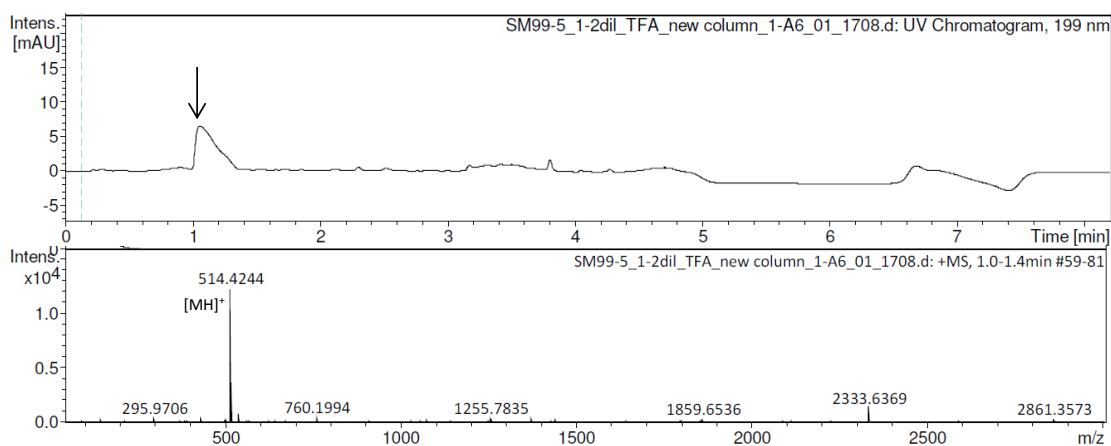


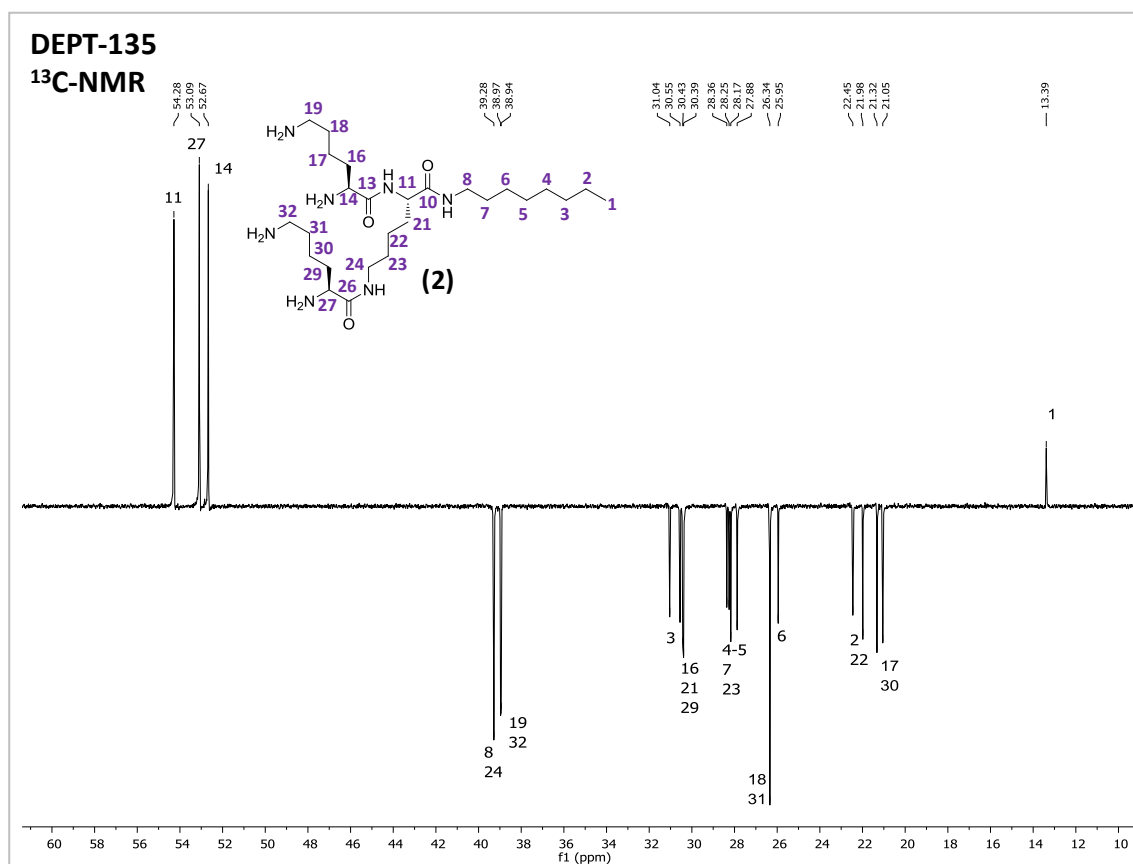
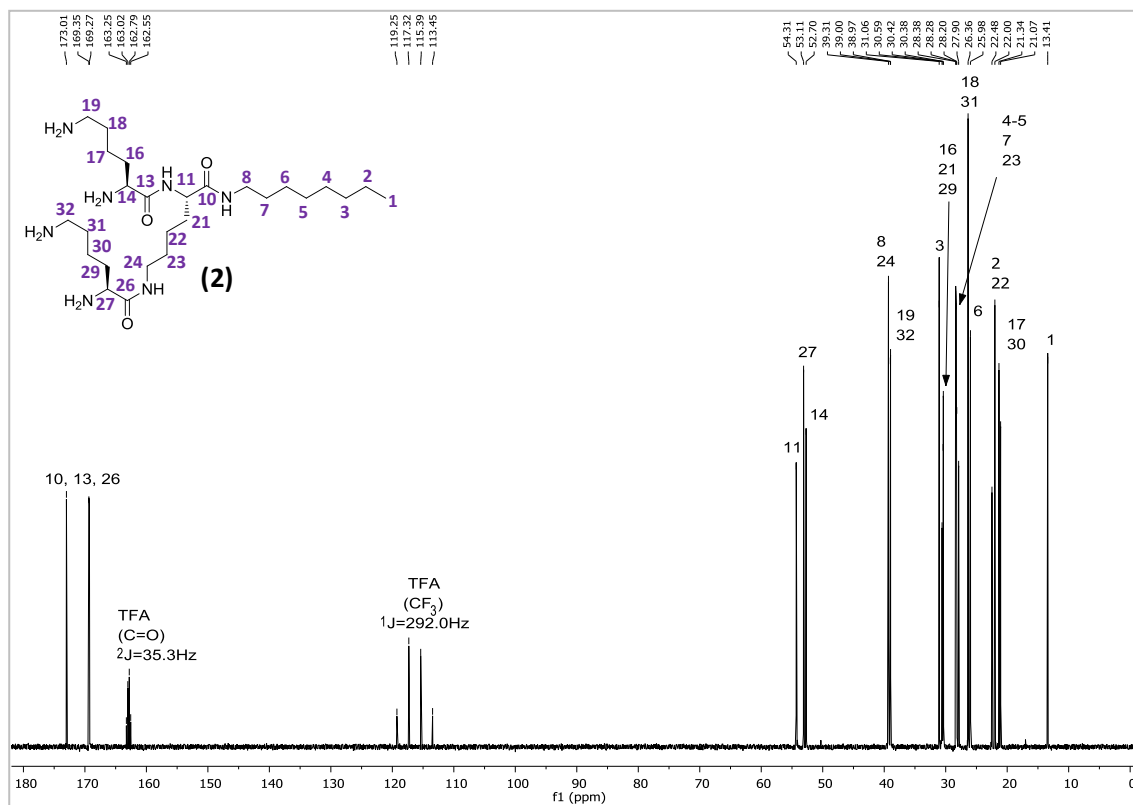
COSY
(1)



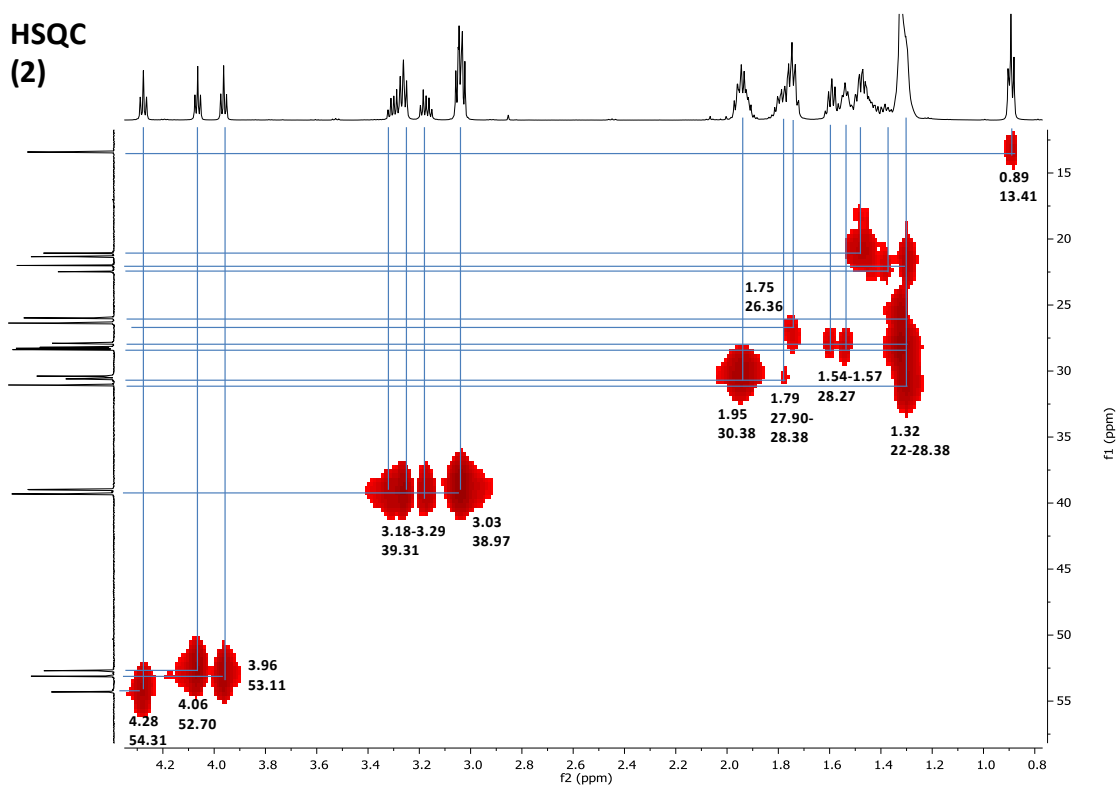
G1-C8 (2)

Yield (TFA salt; loading: 0.33 mmol/g): 102.8 mg (64 %). LC-MS data R_t : 1.0-1.4 min. MS(ESI⁺), calcd C₂₆H₅₅N₇O₃: 513.44 g/mol, found: m/z 514.42 [MH]⁺. ¹H NMR (600 MHz, D₂O): δ 4.28 (t, J = 7.39 Hz, 1H), 4.06 (t, J = 6.55 Hz, 1H), 3.96 (t, J = 6.73 Hz, 1H), 3.33 – 3.24 (m, 3H), 3.17 (dt, J = 13.42, 6.68 Hz, 1H), 3.07 – 3.01 (m, 4H), 2.01 – 1.87 (m, 4H), 1.84 – 1.69 (m, 5H)¹, 1.65 – 1.36 (m, 7H), 1.35 – 1.23 (m, 11H), 0.92 – 0.86 (m, 3H). ¹³C NMR (151 MHz, D₂O): δ 173.01, 169.35, 169.27, 54.31, 53.11, 52.70, 39.31, 39.00, 38.97, 31.06, 30.59, 30.42, 30.38, 28.38, 28.28, 28.20, 27.90, 26.36, 25.98, 22.48, 22.00, 21.34, 21.07, 13.41.

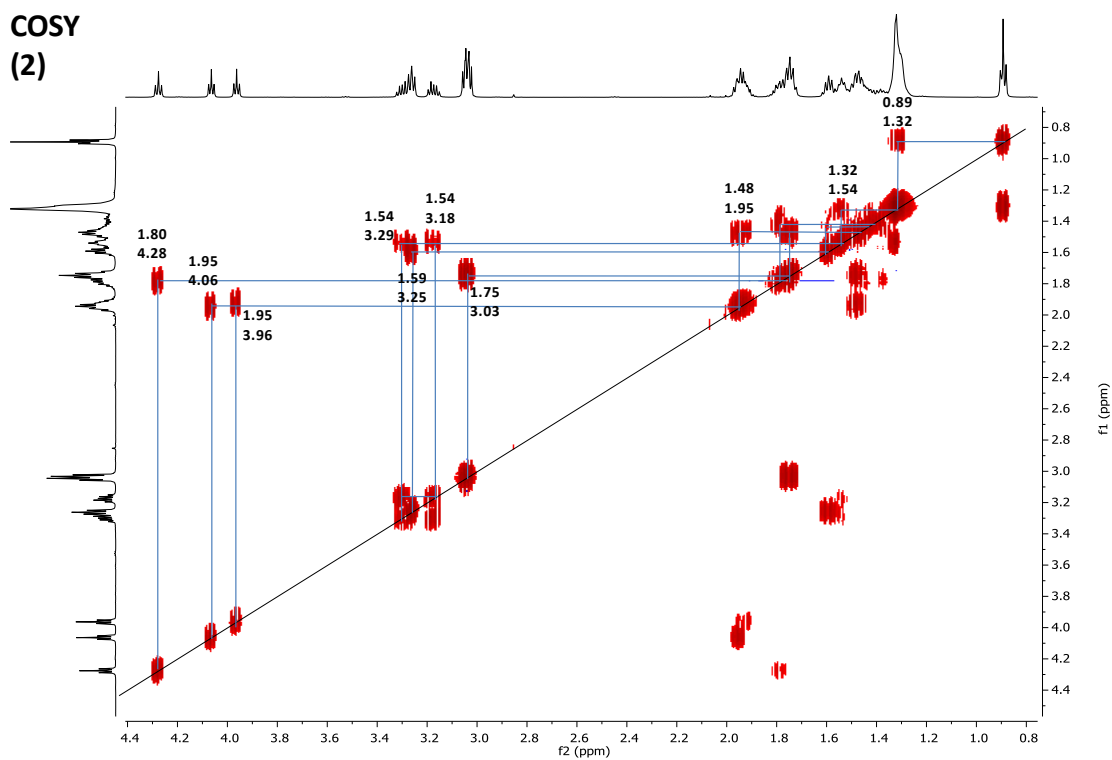
UPLC-MS (2)



**HSQC
(2)**

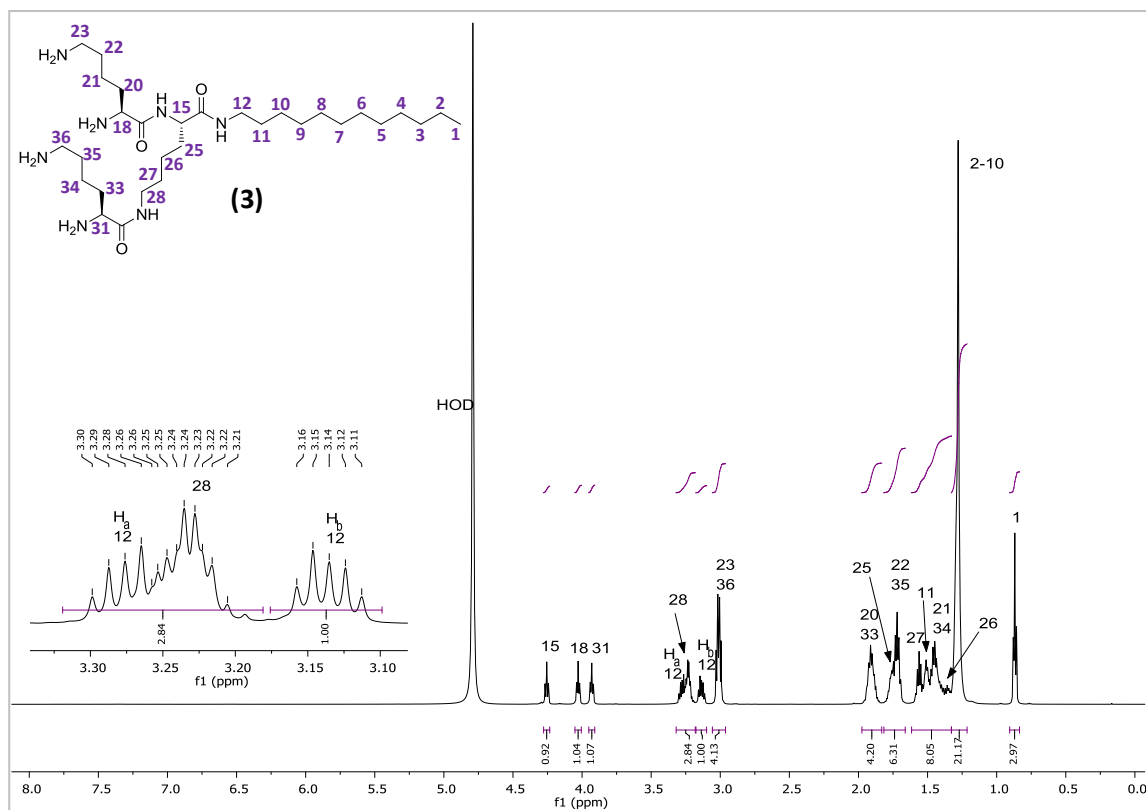
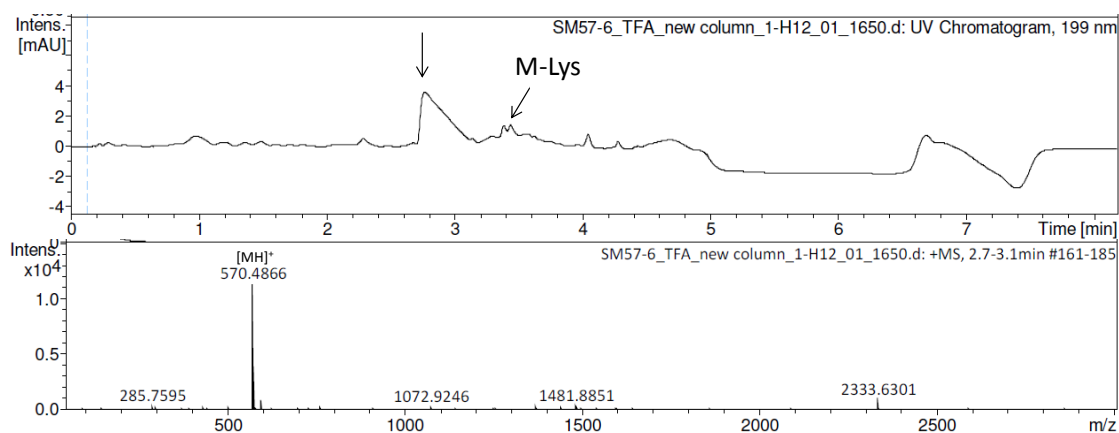


**COSY
(2)**



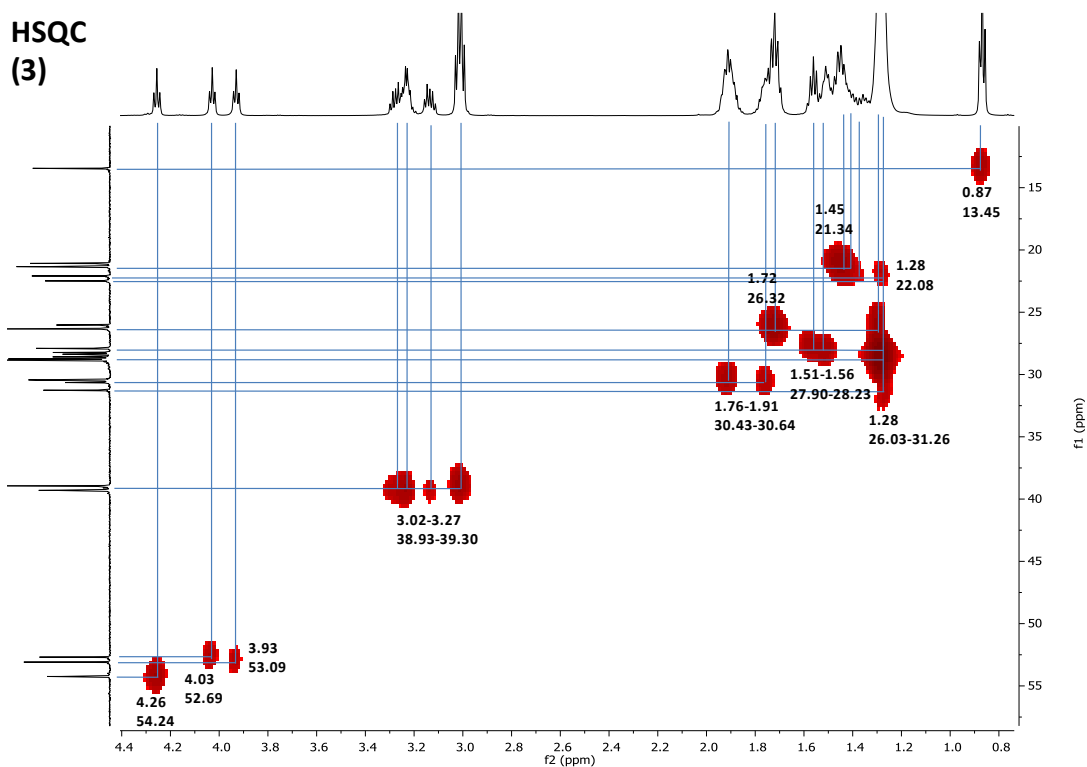
G1-C12 (3)

Yield (TFA salt; loading: 0.34 mmol/g): 93.3 mg (54 %). LC-MS data R_t : 2.7-3.1 min. MS(ESI⁺), calcd C₃₀H₆₃N₇O₃: 569.50 g/mol, found: m/z 570.49 [MH]⁺. ¹H NMR (600 MHz, D₂O): δ 4.26 (t, J = 7.36 Hz, 1H), 4.03 (t, J = 6.59 Hz, 1H), 3.93 (t, J = 6.74 Hz, 1H), 3.32 – 3.18 (m, 3H), 3.14 (dt, J = 13.45, 6.71 Hz, 1H), 3.06 – 2.96 (m, 4H), 1.98 – 1.83 (m, 4H), 1.82 – 1.66 (m, 6H), 1.62 – 1.33 (m, 8H)², 1.33 – 1.21 (m, 21H), 0.87 (t, J = 6.94 Hz, 3H). ¹³C NMR (151 MHz, D₂O): δ 172.94, 169.48, 169.34, 54.24, 53.09, 52.69, 39.30, 39.27, 38.97, 38.93, 31.26, 30.64, 30.46, 30.43, (28.85, 28.84, 28.75, 28.73, 28.59, 28.37, 28.23, 27.90, 26.34, 26.32, 26.03, 22.48, 22.09, 21.34, 21.08, 13.45.

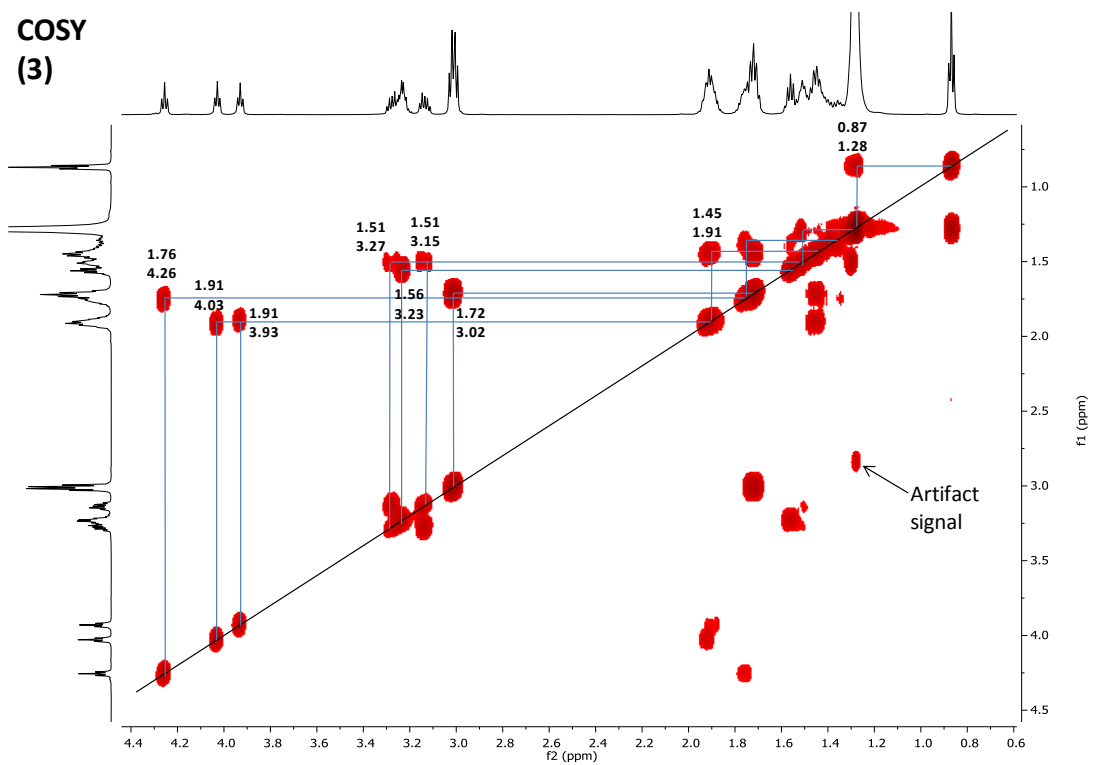
UPLC-MS (3)



HSQC (3)

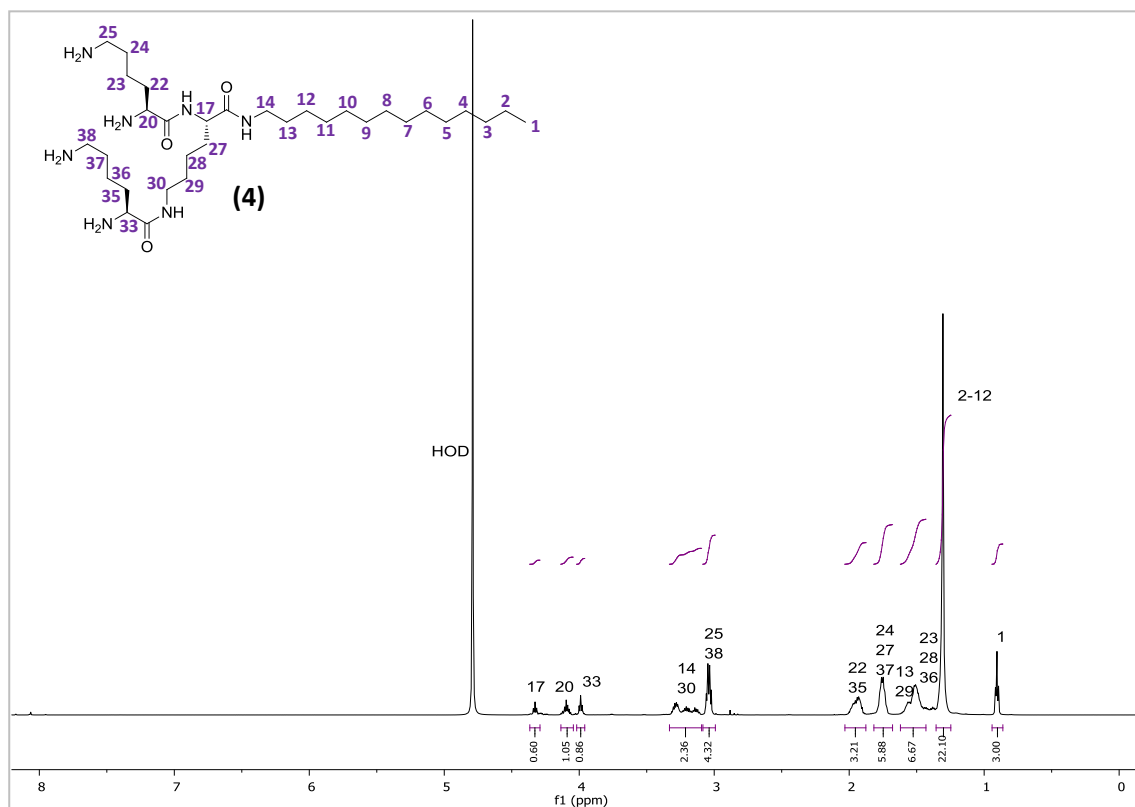
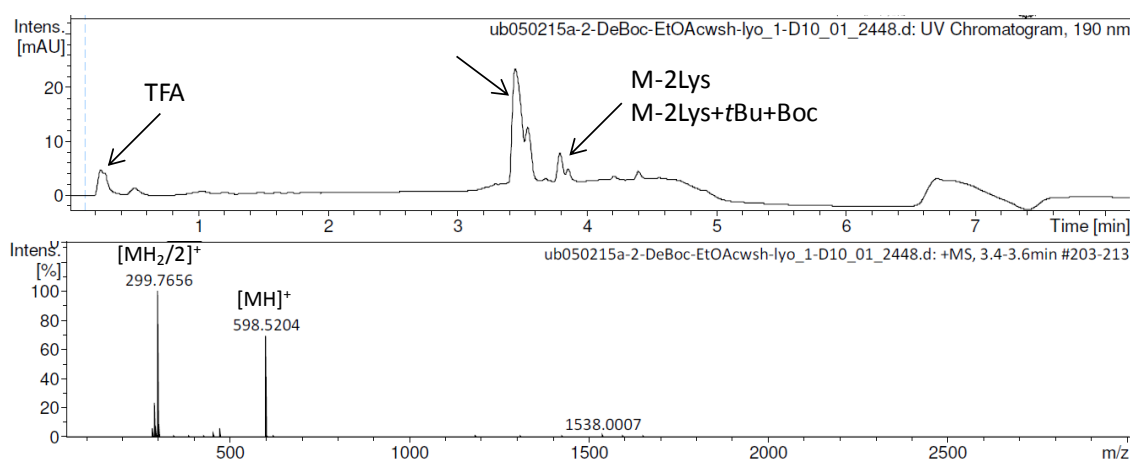


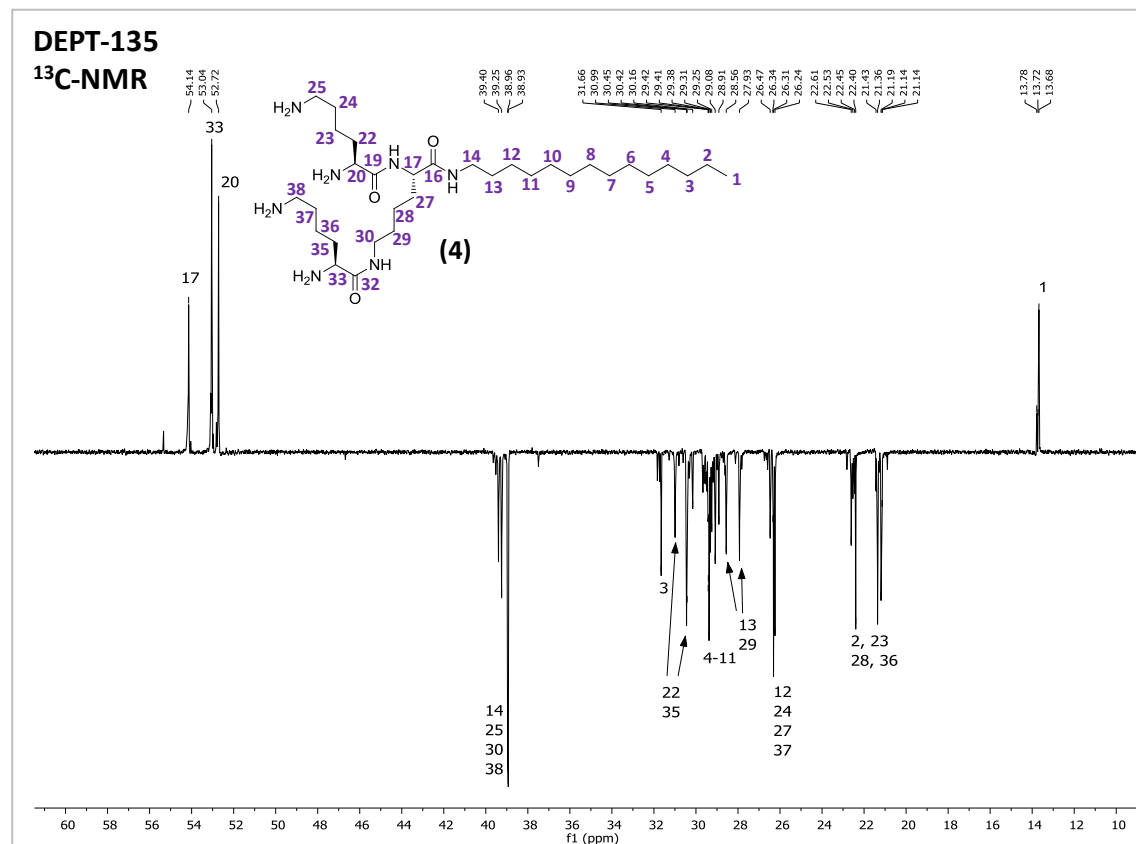
COSY (3)



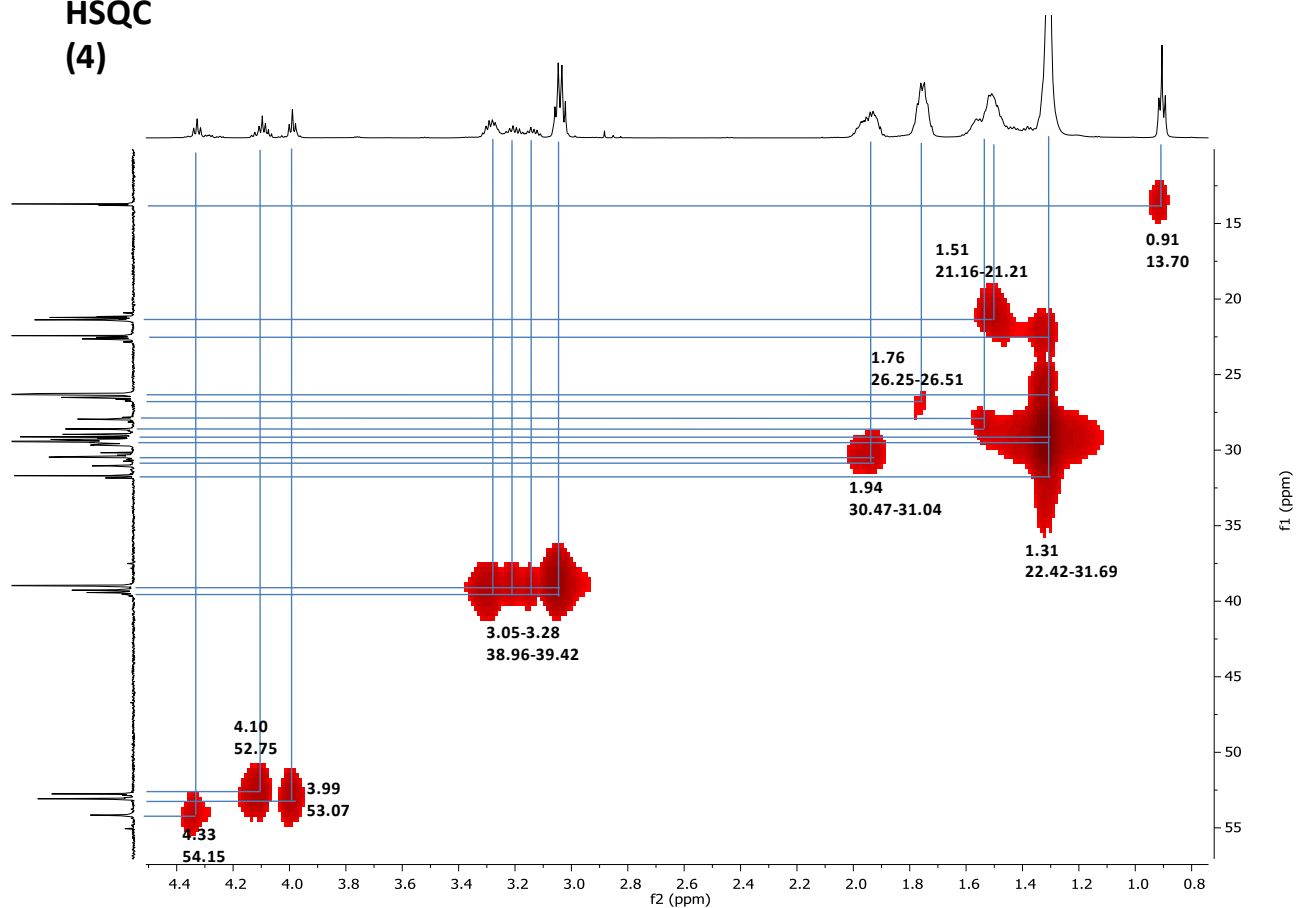
G1-C14 (4)

Yield (TFA salt; loading: 0.22 mmol/g): 147.1 mg (quant.). LC-MS data R_t : 3.4-3.6 min. MS(ESI⁺), calcd C₃₂H₆₇N₇O₃: 597.53 g/mol, found: m/z 598.52 [MH]⁺; 299.77 [MH₂/2]⁺. ¹H NMR (600 MHz, D₂O): δ 4.33 (t, J = 7.29 Hz, 1H), 4.14 – 4.04 (m, 1H), 3.99 (t, J = 6.71 Hz, 1H), 3.33 – 3.09 (m, 2H)², 3.04 (q, J = 7.23 Hz, 4H), 2.03 – 1.88 (m, 3H), 1.75 (m, 6H), 1.62 – 1.43 (m, 7H), 1.31 (s, 22H), 0.93 – 0.88 (m, 3H). ¹³C NMR (151 MHz, D₂O): δ 172.75, 169.37, 169.21, 54.15, 53.07, 52.75, 39.42, 39.27, 38.99, 38.96, 31.69, 31.04, 30.47, 30.43, 29.67, 29.55, 29.45, 29.42, 29.36, 29.29, 29.12, 28.95, 28.60, 26.51, 26.32, 26.25, 22.42, 21.37, 21.22, 21.16, 13.70.

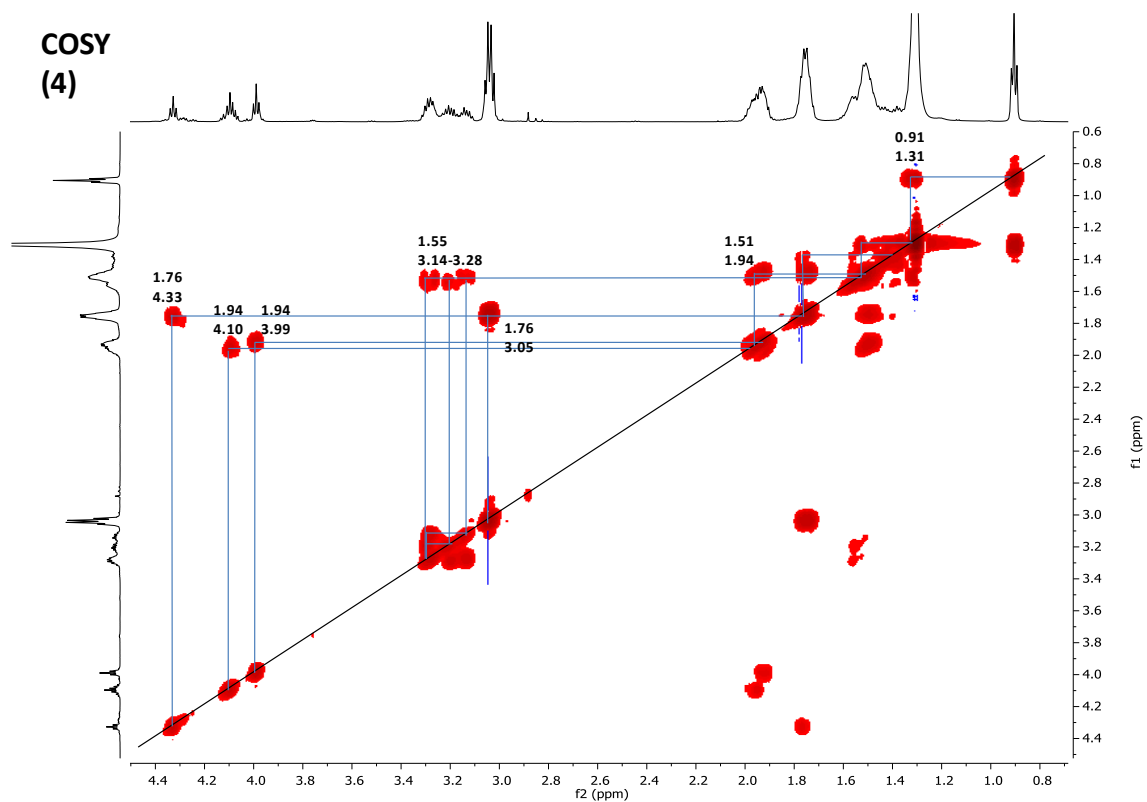
UPLC-MS (4)



HSQC (4)

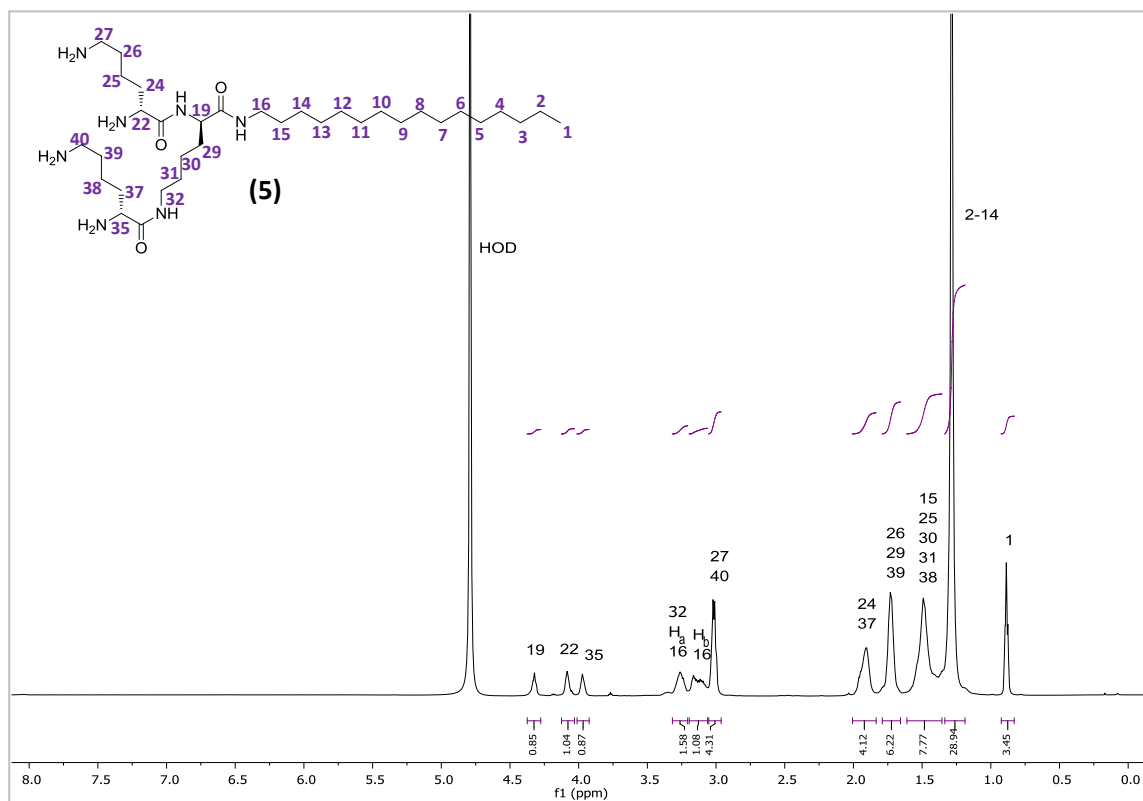
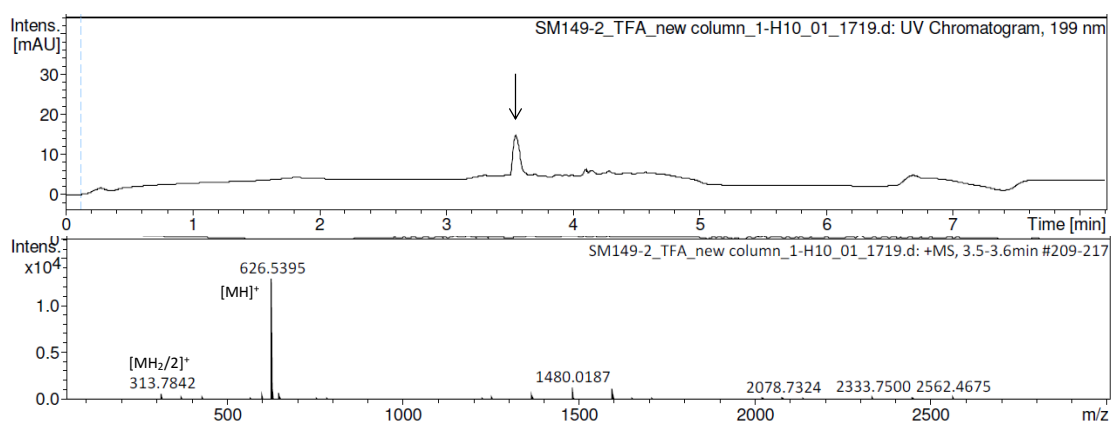


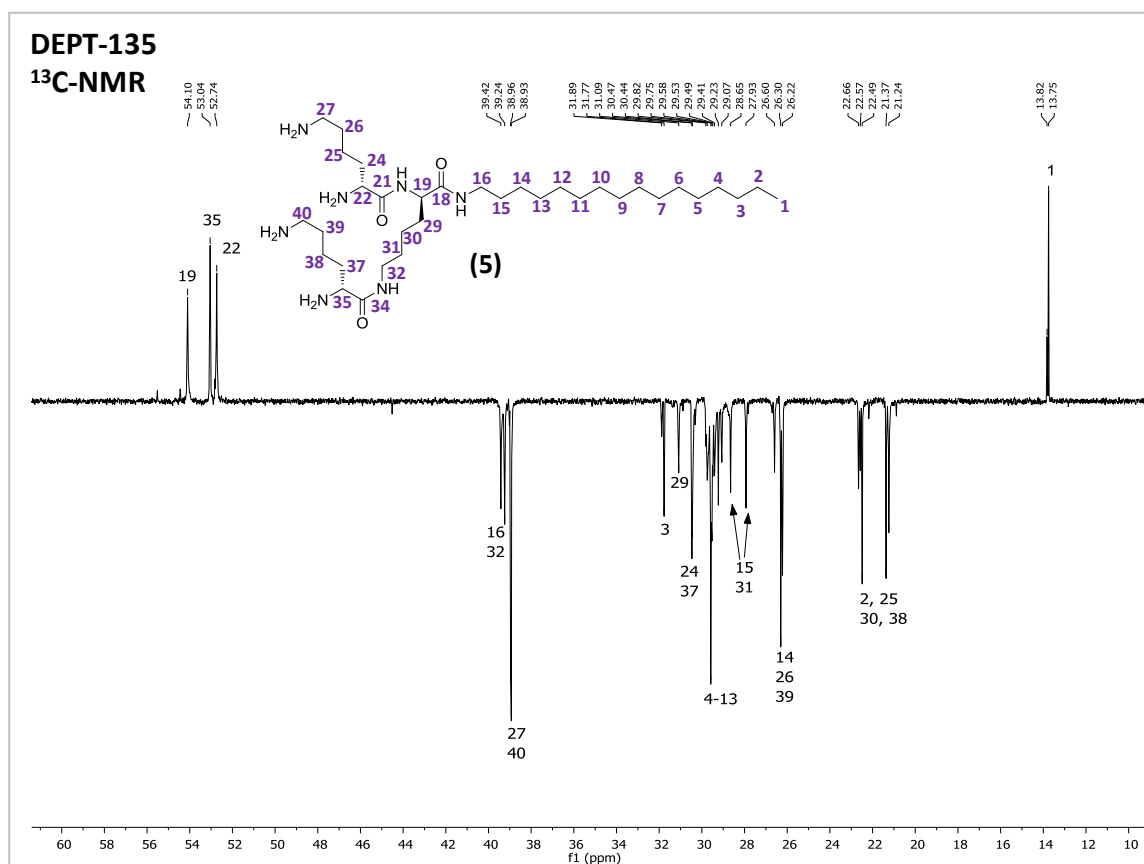
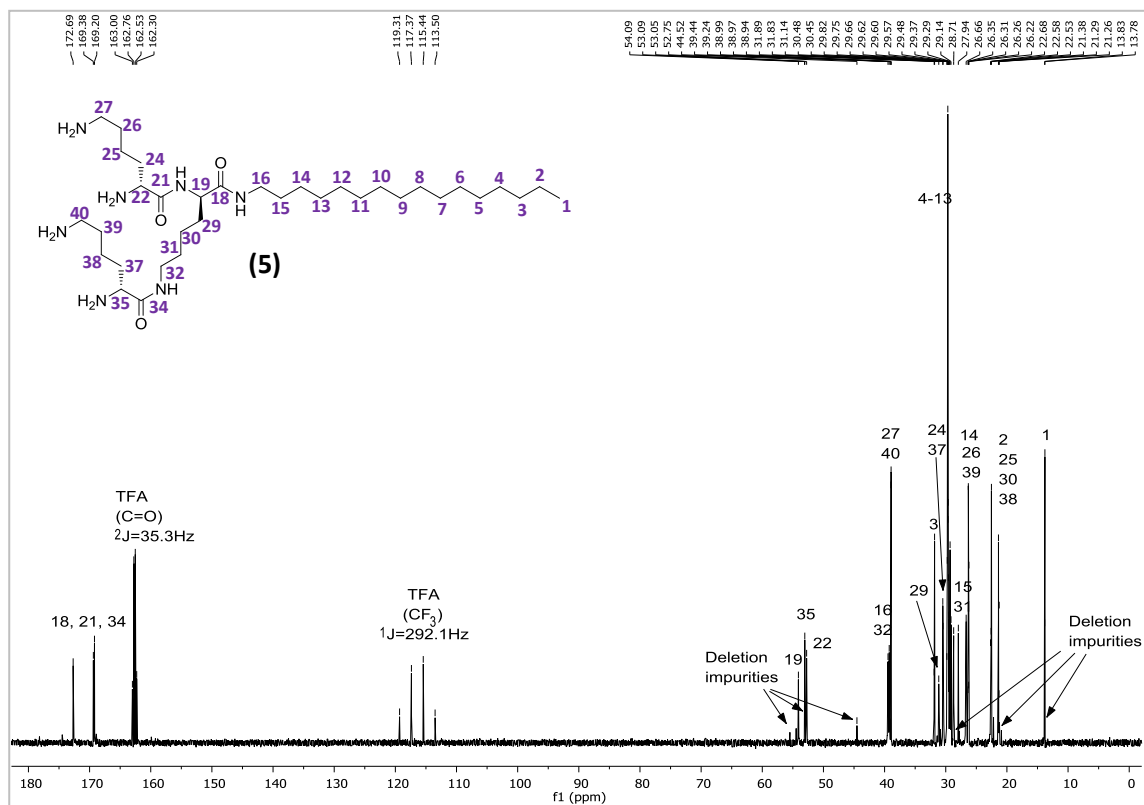
COSY (4)



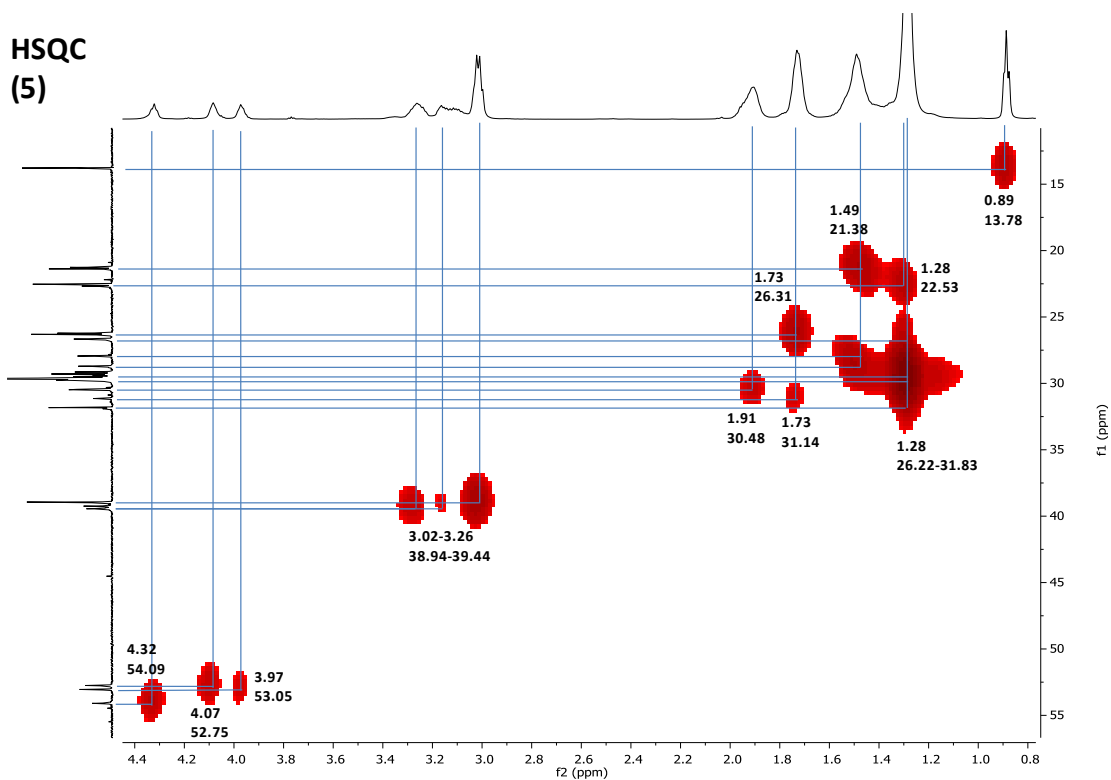
G1-C16 (5)

Yield (TFA salt; loading: 0.21 mmol/g): 71.4 mg (79 %). LC-MS data R_t : 3.5-3.6 min. MS(ESI⁺), calcd C₃₄H₇₁N₇O₃: 625.56 g/mol, found: m/z 626.54 [MH]⁺; 313.78 [MH₂/2]⁺. ¹H NMR (600 MHz, D₂O): δ 4.32 (t, J = 7.39 Hz, 1H), 4.13 – 4.03 (m, 1H), 3.97 (t, J = 6.80 Hz, 1H), 3.32 – 3.21 (m, 2H), 3.20 – 3.06 (m, 1H), 3.02 (q, J = 7.44 Hz, 4H), 2.01 – 1.83 (m, 4H), 1.79 – 1.66 (m, 6H), 1.61 – 1.36 (m, 8H)², 1.28 (s, 29H), 0.89 (t, J = 6.68 Hz, 3H). ¹³C NMR (151 MHz, D₂O): δ 172.69, 169.38, 169.20, 54.09, 53.05, 52.75, 39.44, 39.24, 38.97, 38.94, 31.83, 31.14, 30.48, 30.45, 29.82, 29.75, 29.66, 29.62, 29.60, 29.57, 29.48, 29.38, 29.29, 29.14, 28.71, 27.94, 26.66, 26.31, 26.22, 22.53, 21.38, 21.26, 13.78.

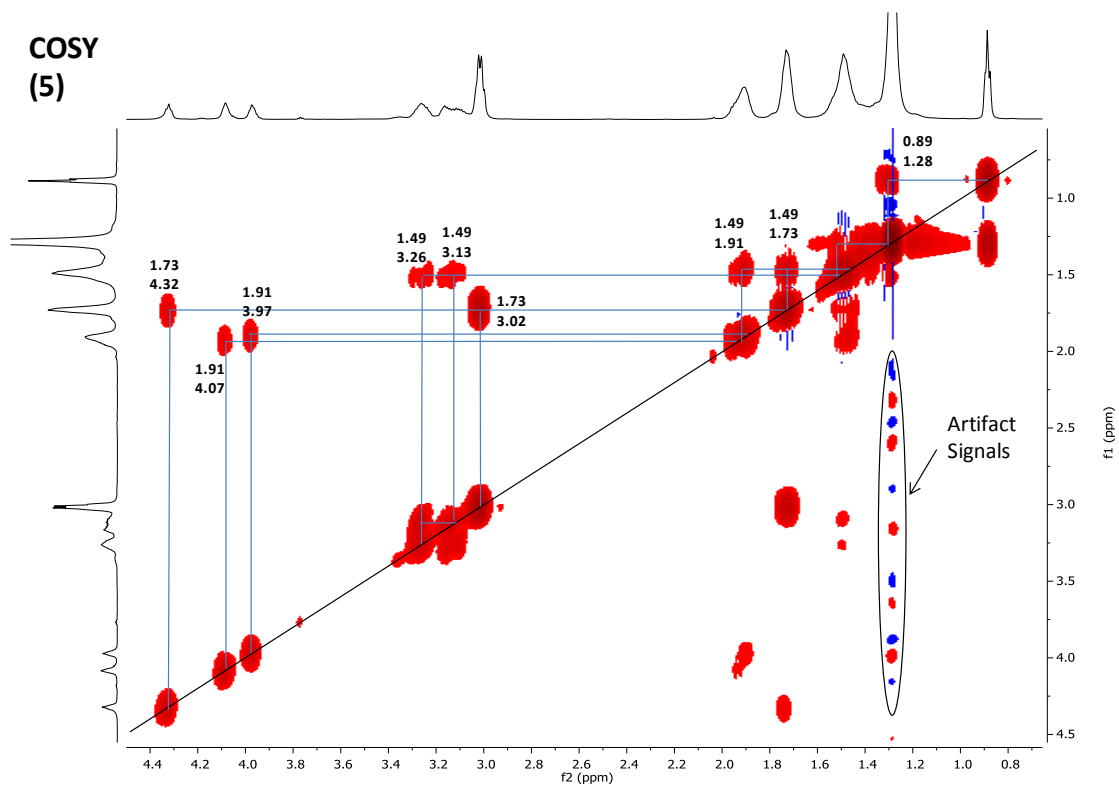
UPLC-MS (5)



HSQC (5)

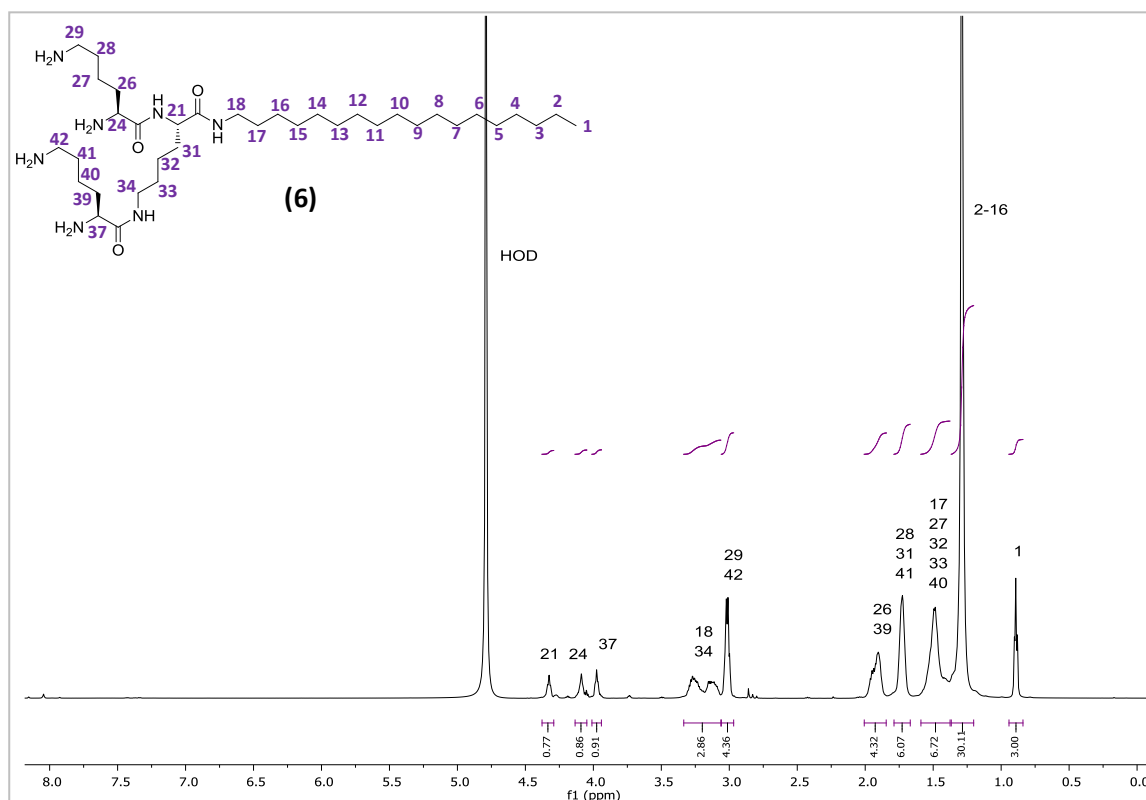
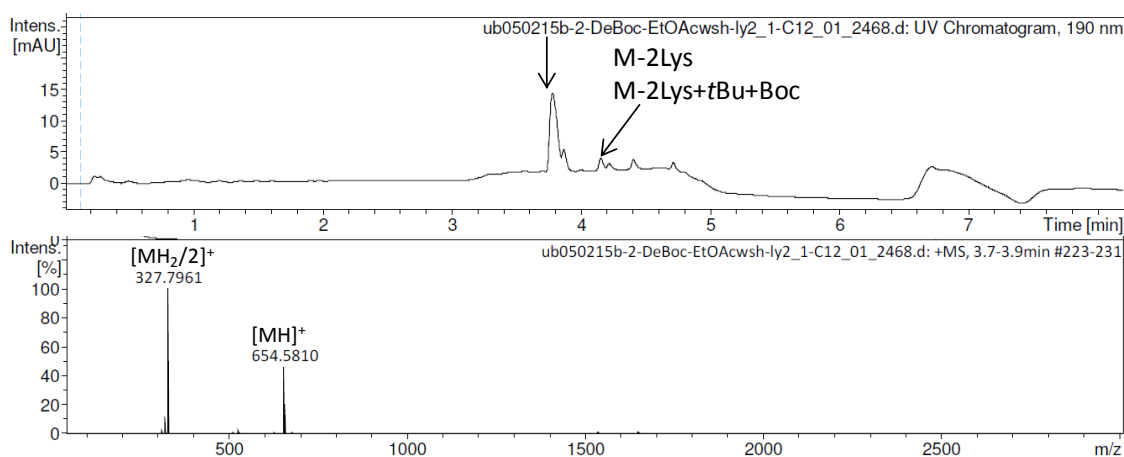


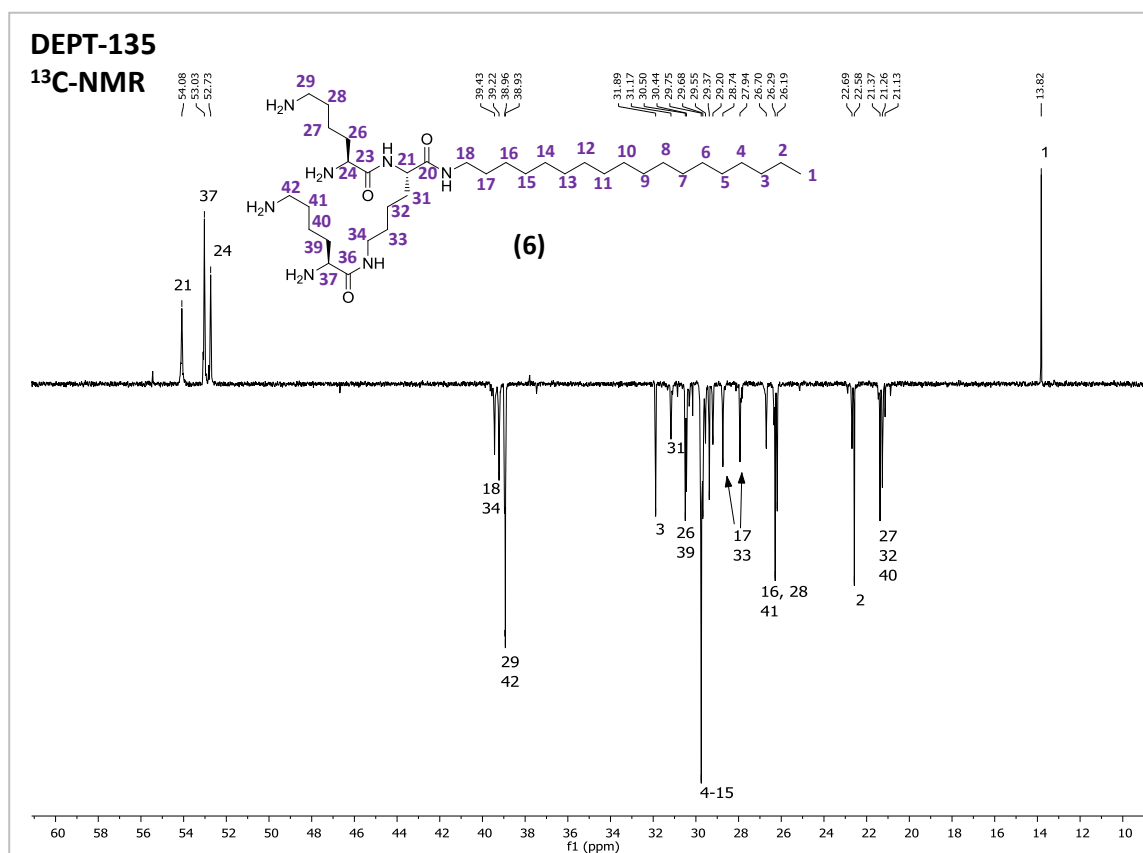
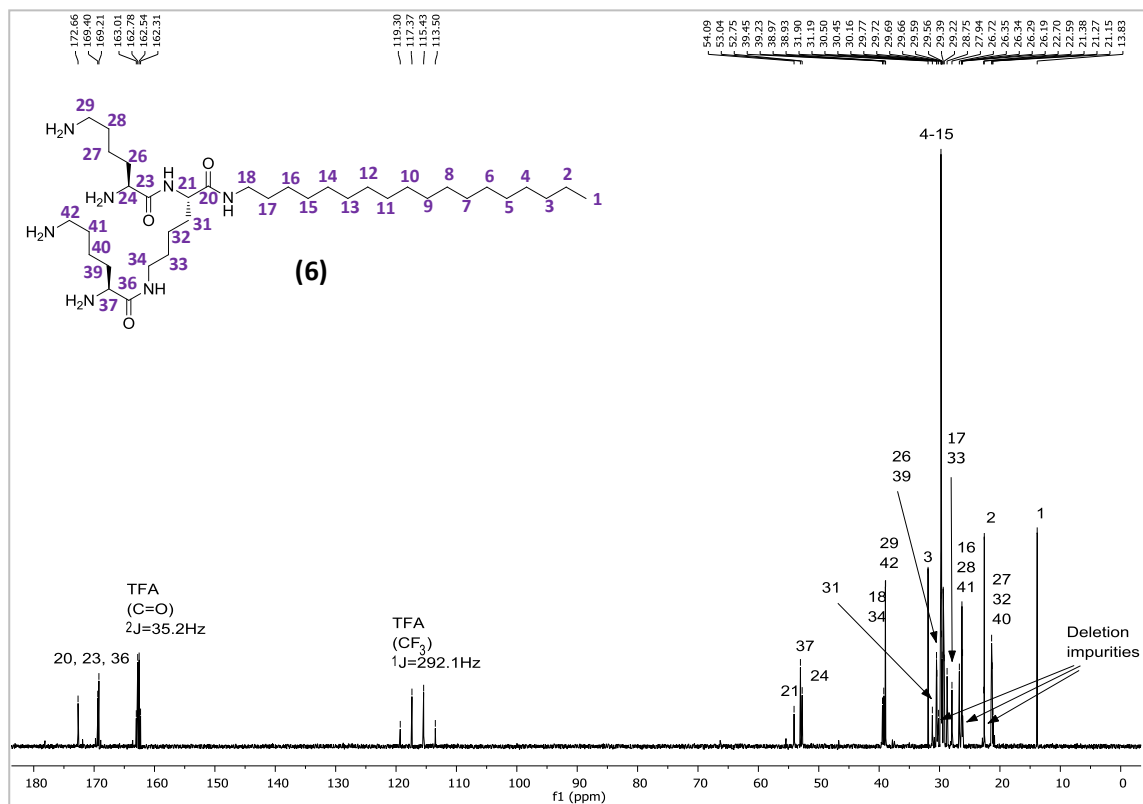
COSY (5)

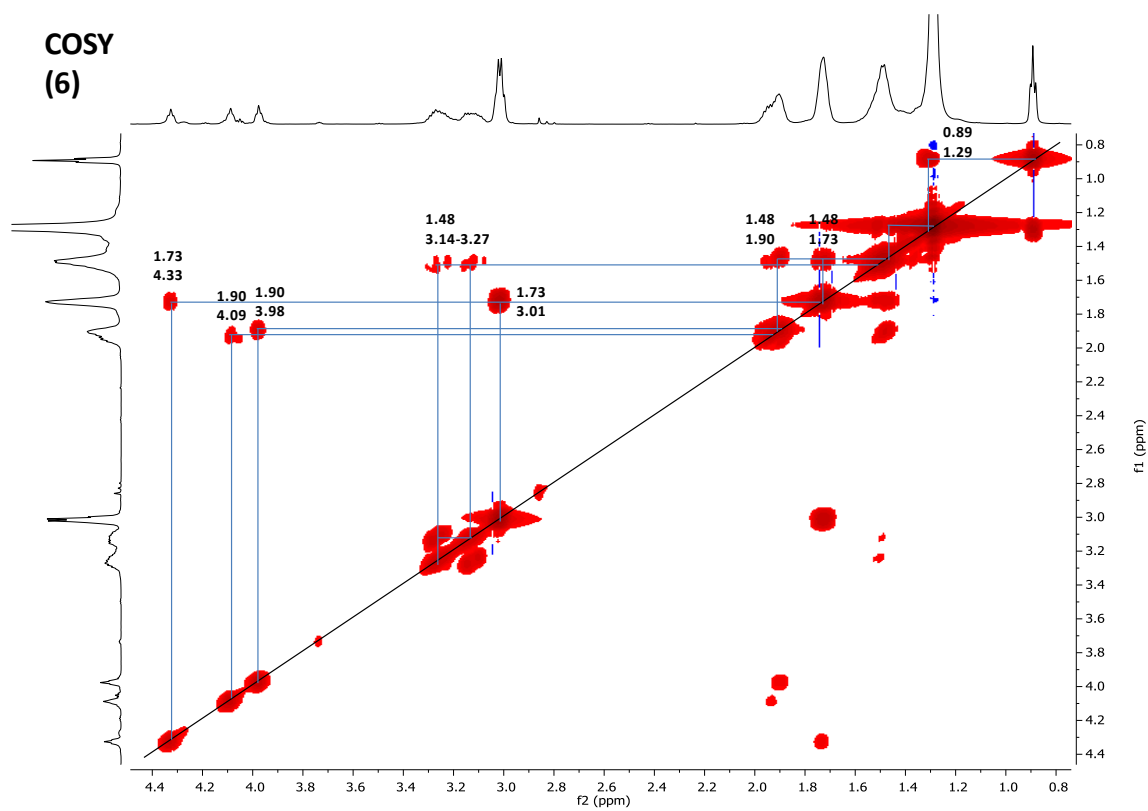
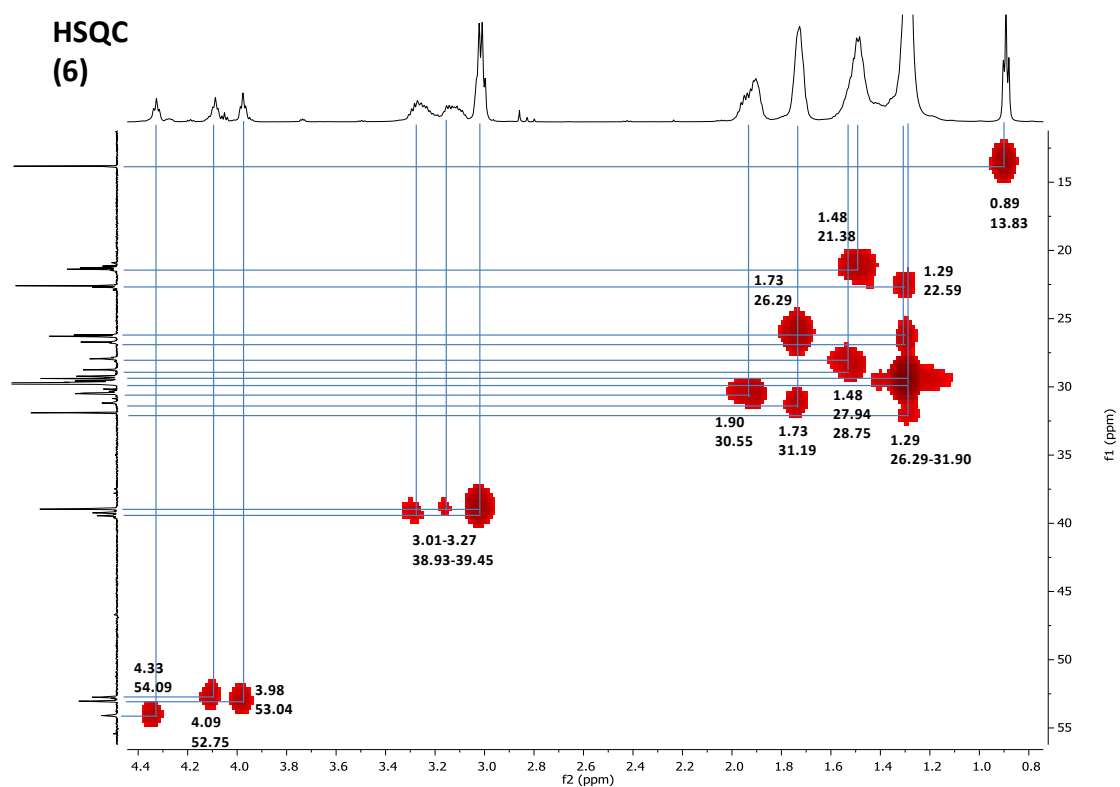


G1-C18 (6)

Yield (TFA salt; loading: 0.14 mmol/g): 101.2 mg (quant.). LC-MS data R_t : 3.7-3.9 min. MS(ESI⁺), calcd C₃₆H₇₅N₇O₃: 653.59 g/mol, found: m/z 654.58 [MH]⁺; 327.80 [MH₂/2]⁺. ¹H NMR (600 MHz, D₂O): δ 4.33 (t, J = 7.20 Hz, 1H), 4.09 (t, J = 7.07 Hz, 1H), 3.97 (t, J = 7.29 Hz, 1H), 3.33 – 3.06 (m, 3H)², 3.02 (q, J = 7.34 Hz, 4H), 2.01 – 1.85 (m, 4H), 1.79 – 1.67 (m, 6H), 1.59 – 1.38 (m, 7H), 1.29 (s, 30H), 0.89 (t, J = 6.76 Hz, 3H). ¹³C NMR (151 MHz, D₂O): δ 172.66, 169.40, 169.21, 54.09, 53.04, 52.75, 39.45, 39.23, 38.97, 38.93, 31.90, 31.19, 30.50, 30.45, 29.77, 29.72, 29.69, 29.66, 29.59, 29.56, 29.39, 29.22, 28.75, 27.94, 26.72, 26.29, 26.19, 22.59, 21.38, 21.27, 21.15, 13.83.

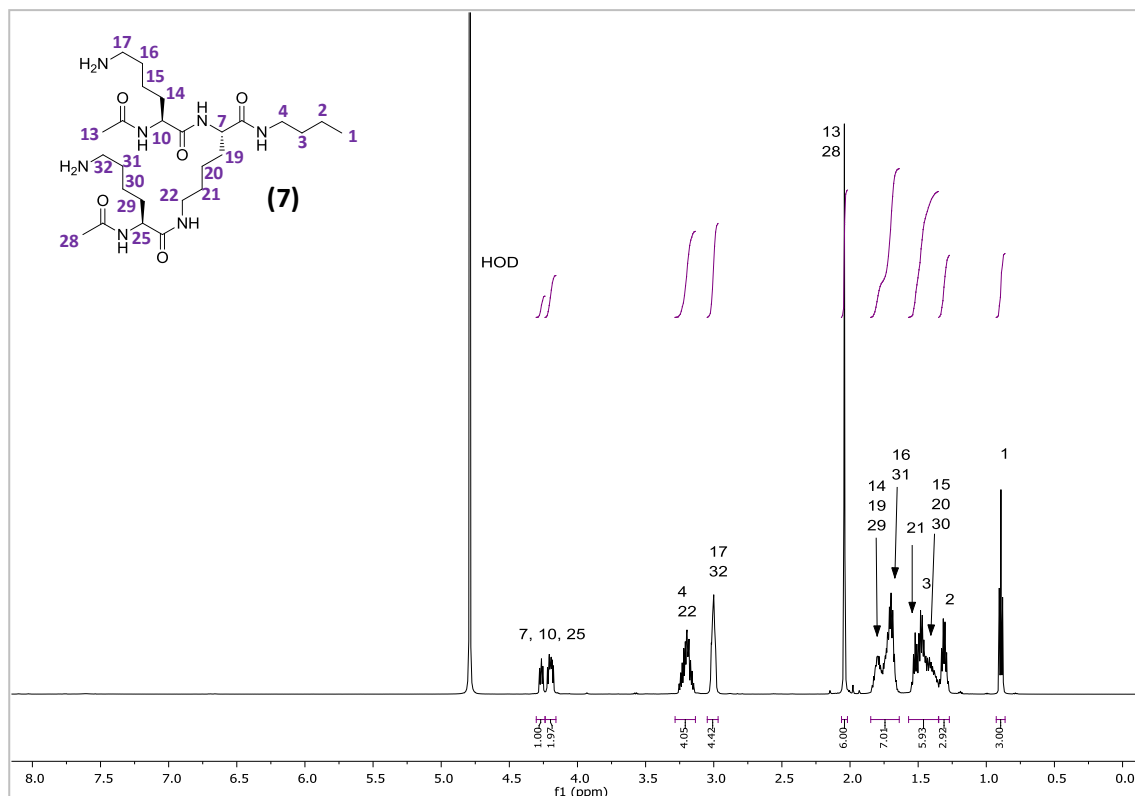
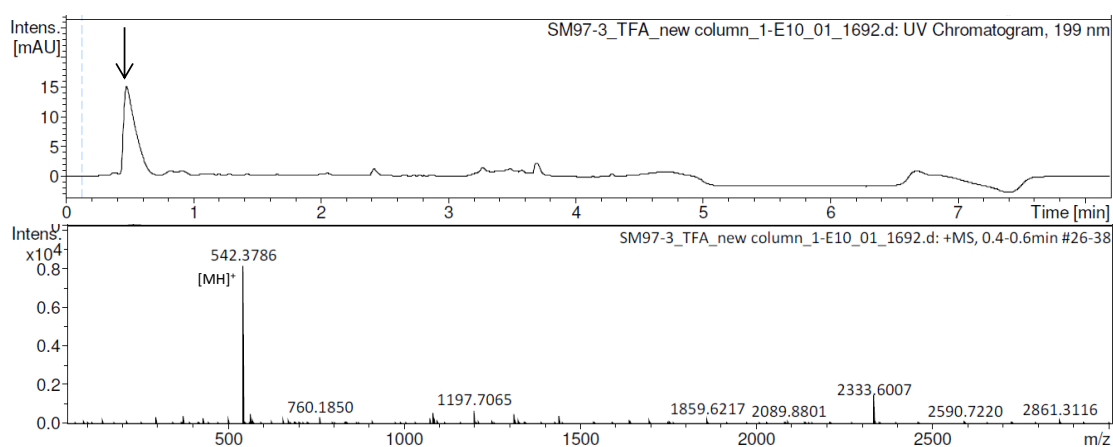
UPLC-MS (6)

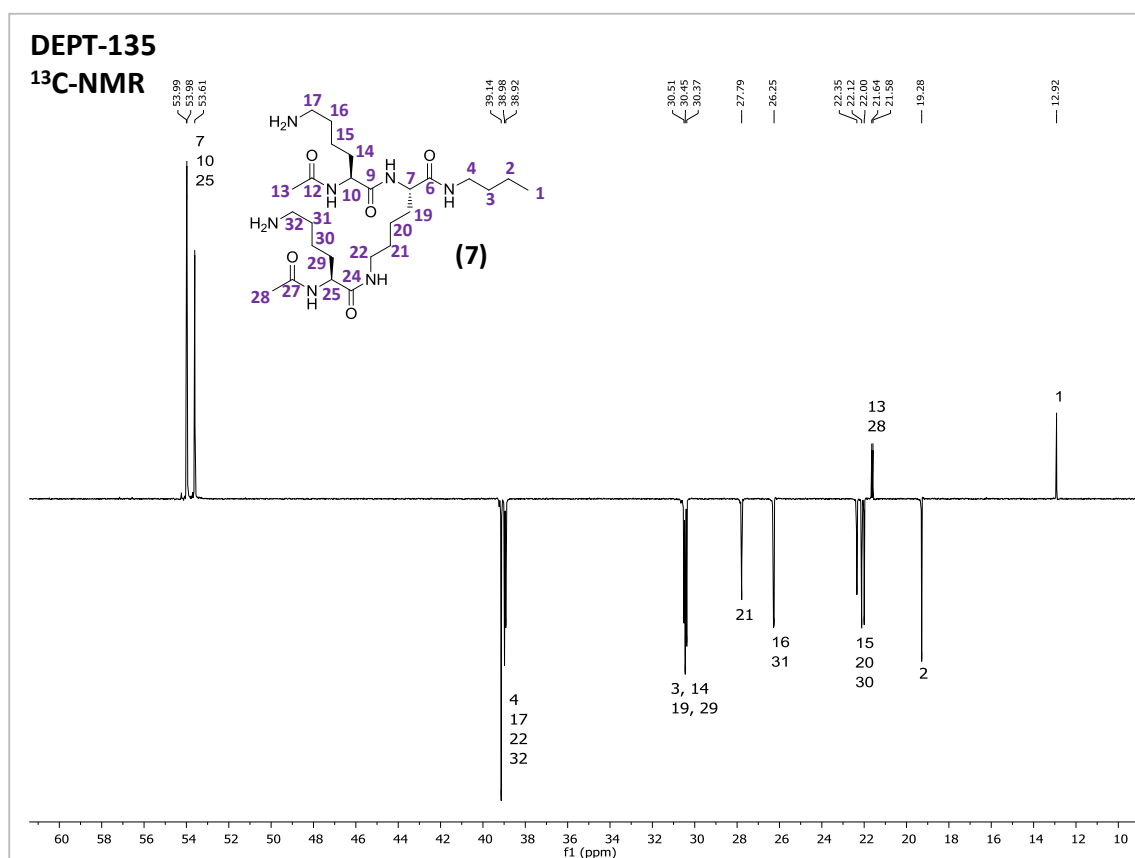
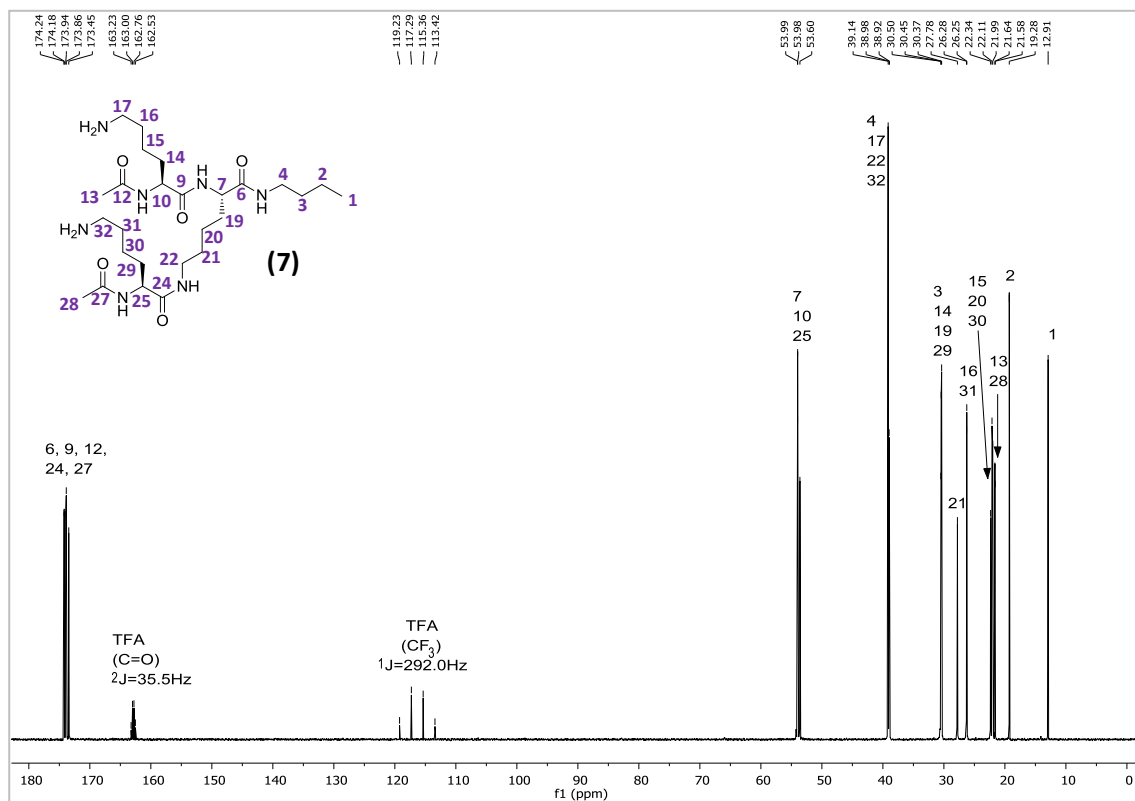


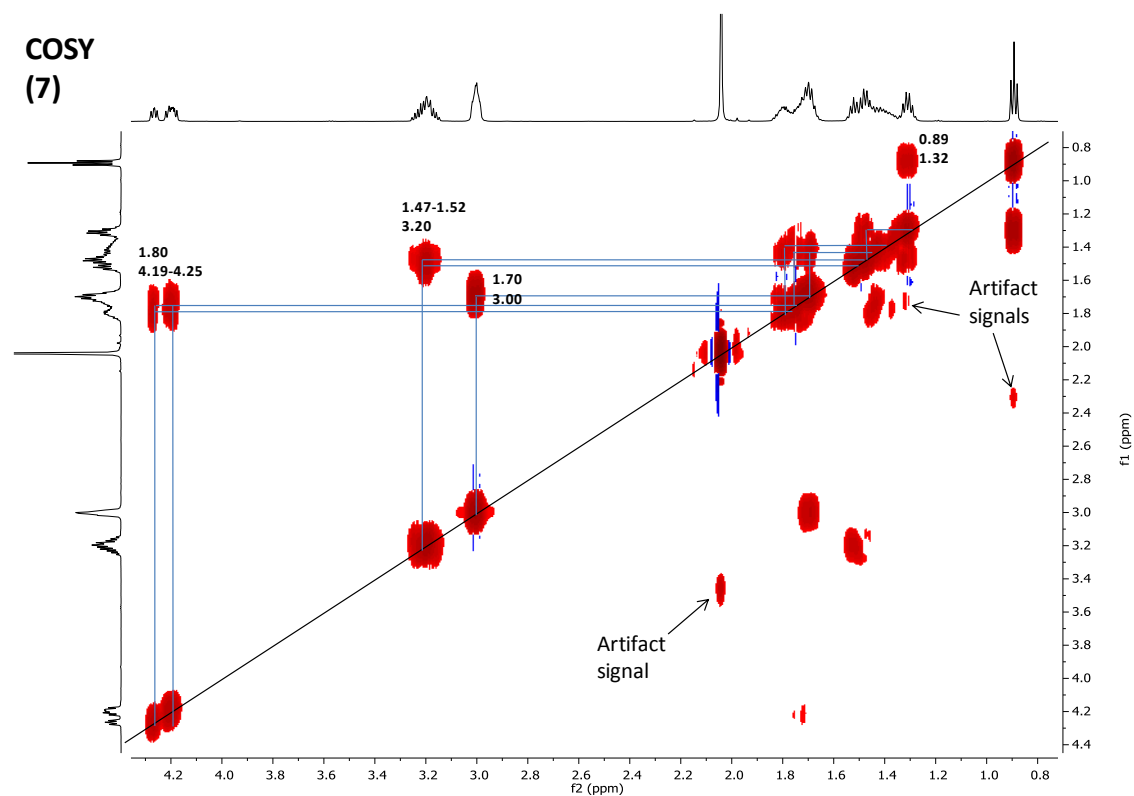
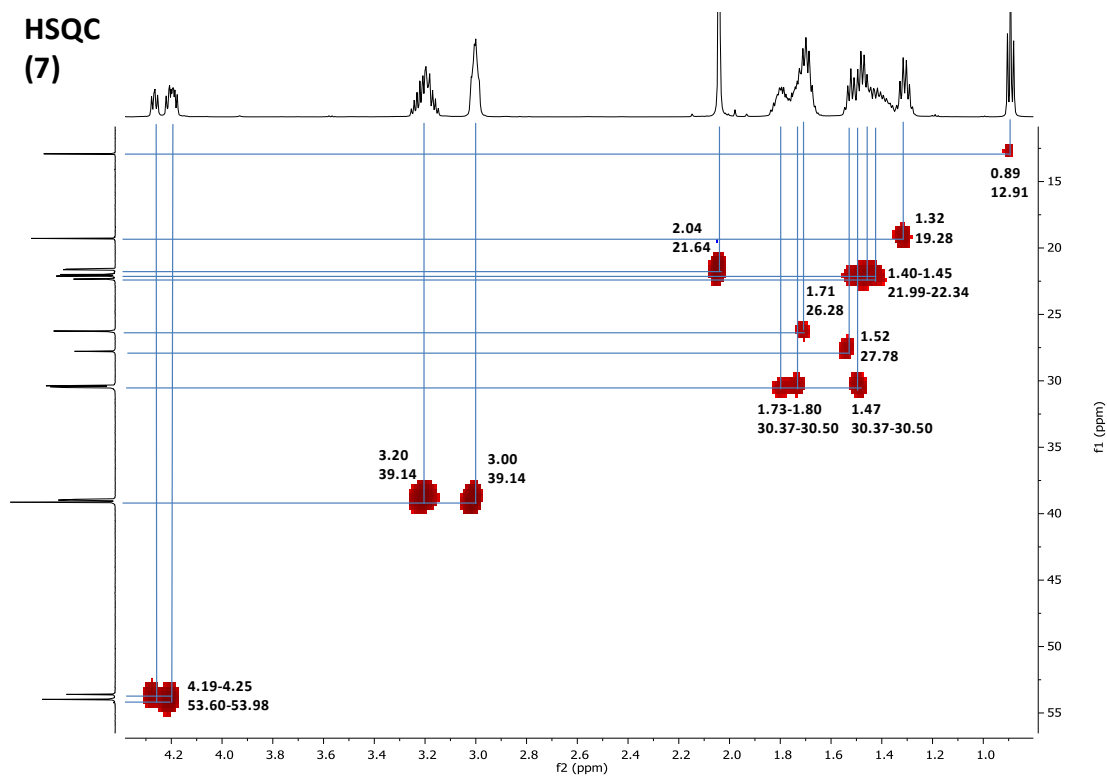


G1(acetyl)-C4 (7)

Yield (TFA salt; loading: 0.32 mmol/g): 99.8 mg (81 %). LC-MS data R_t : 0.4-0.6 min. MS(ESI⁺), calcd C₂₆H₅₁N₇O₅: 541.40 g/mol, found: m/z 542.38 [MH]⁺. ¹H NMR (600 MHz, D₂O): δ 4.27 (dd, J = 8.39, 5.96 Hz, 1H), 4.24 – 4.16 (m, 2H), 3.28 – 3.13 (m, 4H), 3.00 (td, J = 7.76, 3.80 Hz, 4H), 2.04 (s, 6H), 1.85 – 1.64 (m, 7H)¹, 1.57 – 1.35 (m, 6H), 1.31 (h, J = 7.36 Hz, 3H), 0.89 (t, J = 7.38 Hz, 3H). ¹³C NMR (151 MHz, D₂O): δ 174.24, 174.18, 173.94, 173.86, 173.45, 53.99, 53.98, 53.60, 39.14, 38.98, 38.92, 30.50, 30.45, 30.37, 27.78, 26.28, 26.25, 22.34, 22.11, 21.99, 21.64, 21.58, 19.28, 12.91.

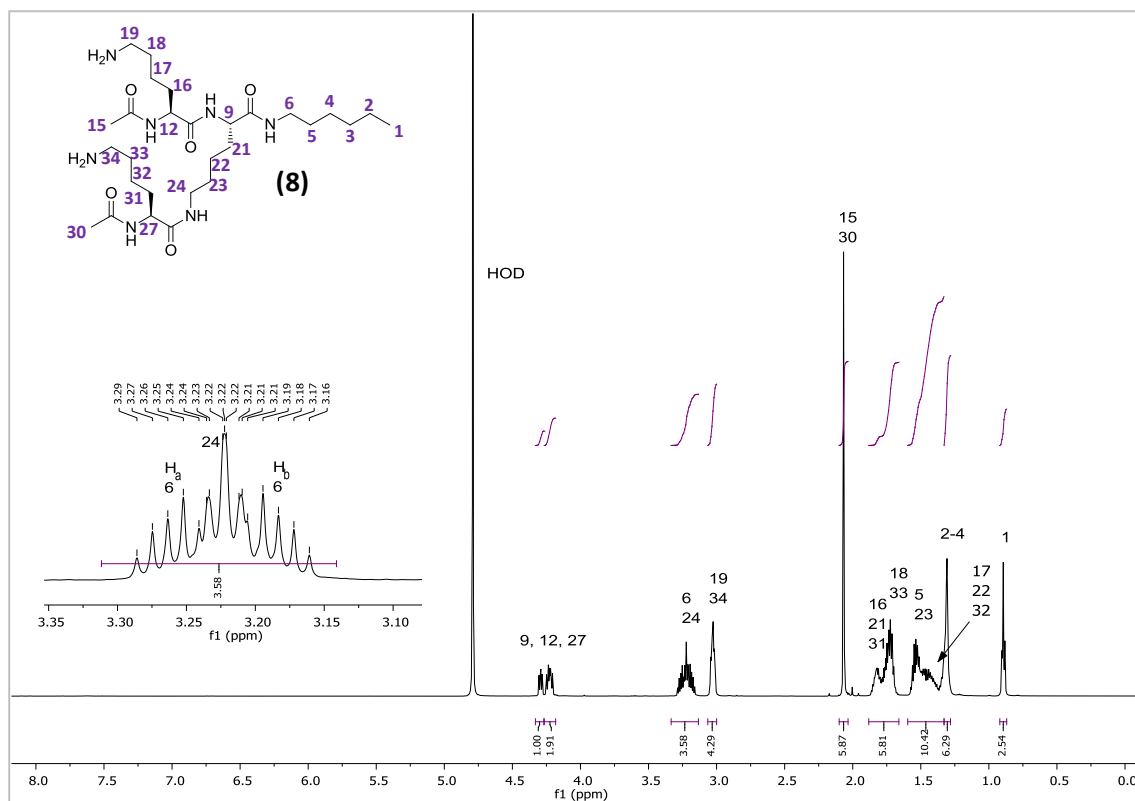
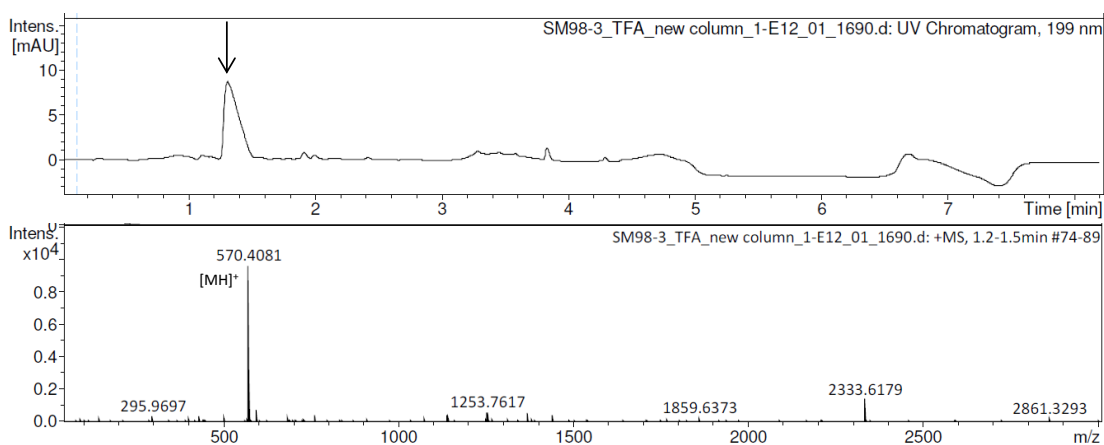
UPLC-MS (7)

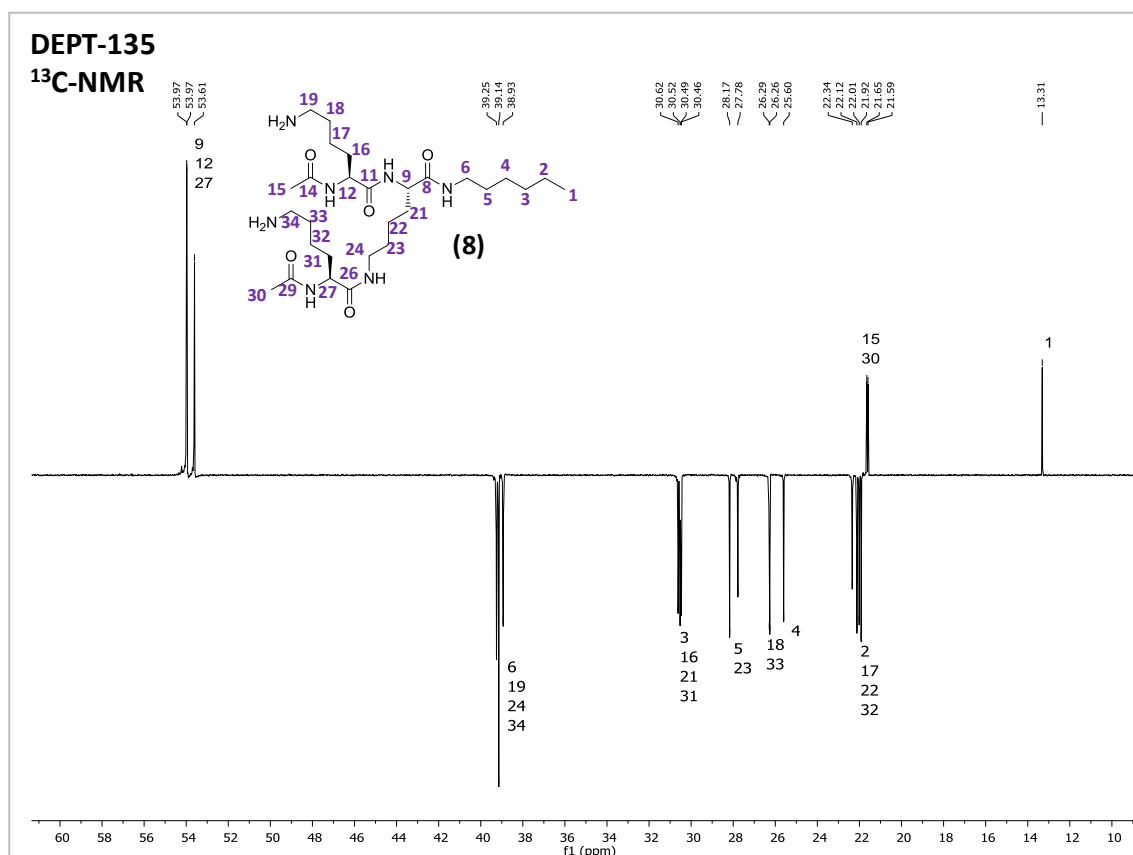
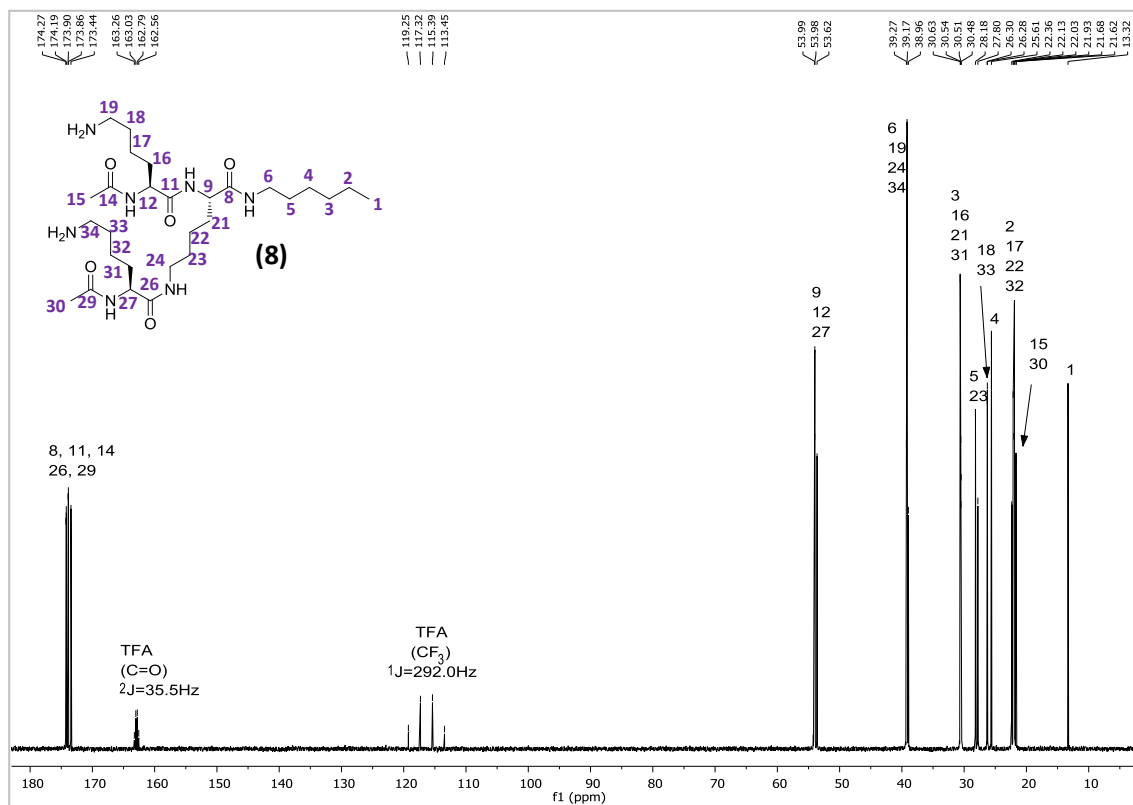


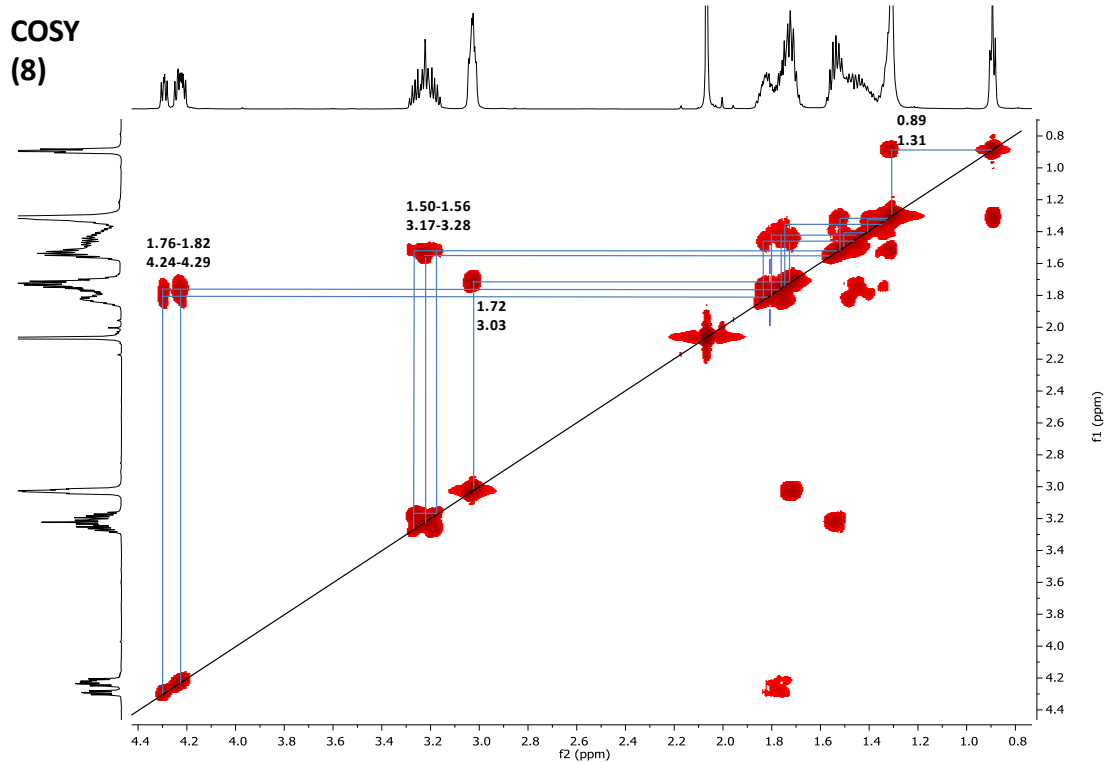
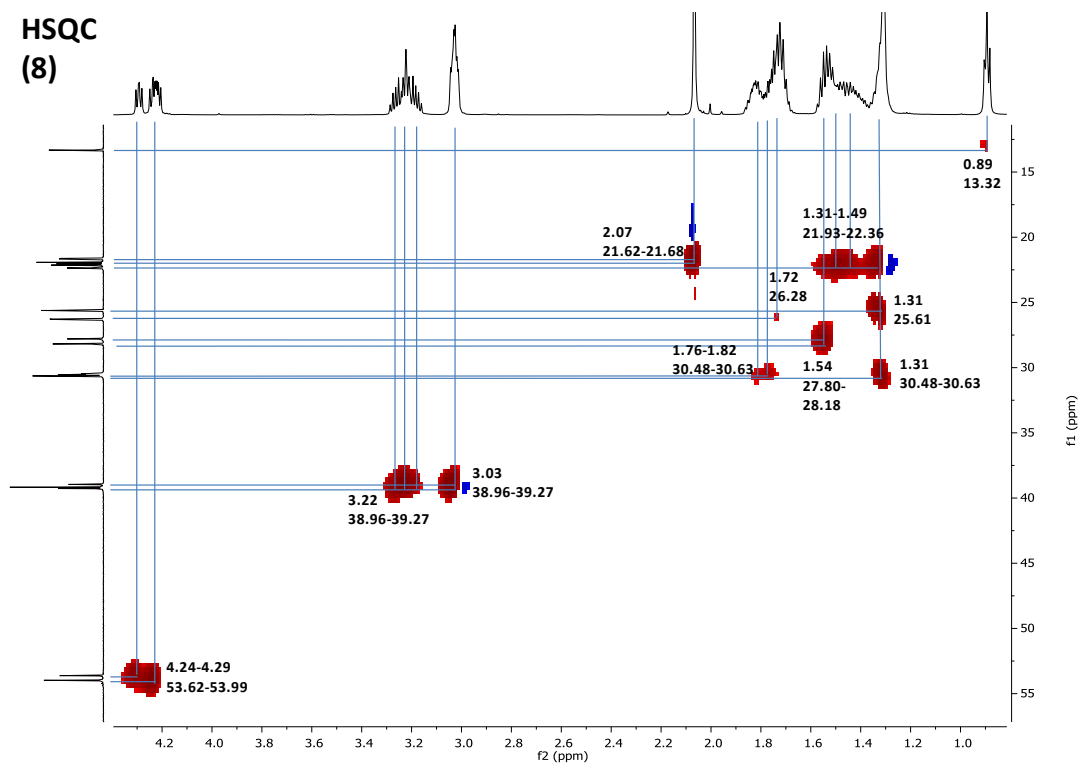


G1(acetyl)-C6 (8)

Yield (TFA salt; loading: 0.34 mmol/g): 98.8 mg (73 %). LC-MS data R_t : 1.2-1.5 min. MS(ESI^+), calcd $\text{C}_{28}\text{H}_{55}\text{N}_7\text{O}_5$: 569.43 g/mol, found: m/z 570.41 $[\text{MH}]^+$. ^1H NMR (600 MHz, D_2O): δ 4.29 (dd, $J = 8.44, 5.91$ Hz, 1H), 4.27 – 4.18 (m, 2H), 3.33 – 3.13 (m, 4H), 3.03 (td, $J = 7.49, 3.43$ Hz, 4H), 2.10 – 2.03 (m, 6H), 1.88 – 1.66 (m, 6H)¹, 1.60 – 1.33 (m, 10H), 1.33 – 1.28 (m, 6H), 0.92 – 0.87 (m, 3H). ^{13}C NMR (151 MHz, D_2O): δ 174.27, 174.19, 173.90, 173.86, 173.44, 53.99, 53.98, 53.62, 39.27, 39.17, 38.96, 30.63, 30.54, 30.51, 30.48, 28.18, 27.80, 26.30, 26.28, 25.61, 22.36, 22.13, 22.03, 21.93, 21.68, 21.62, 13.32.

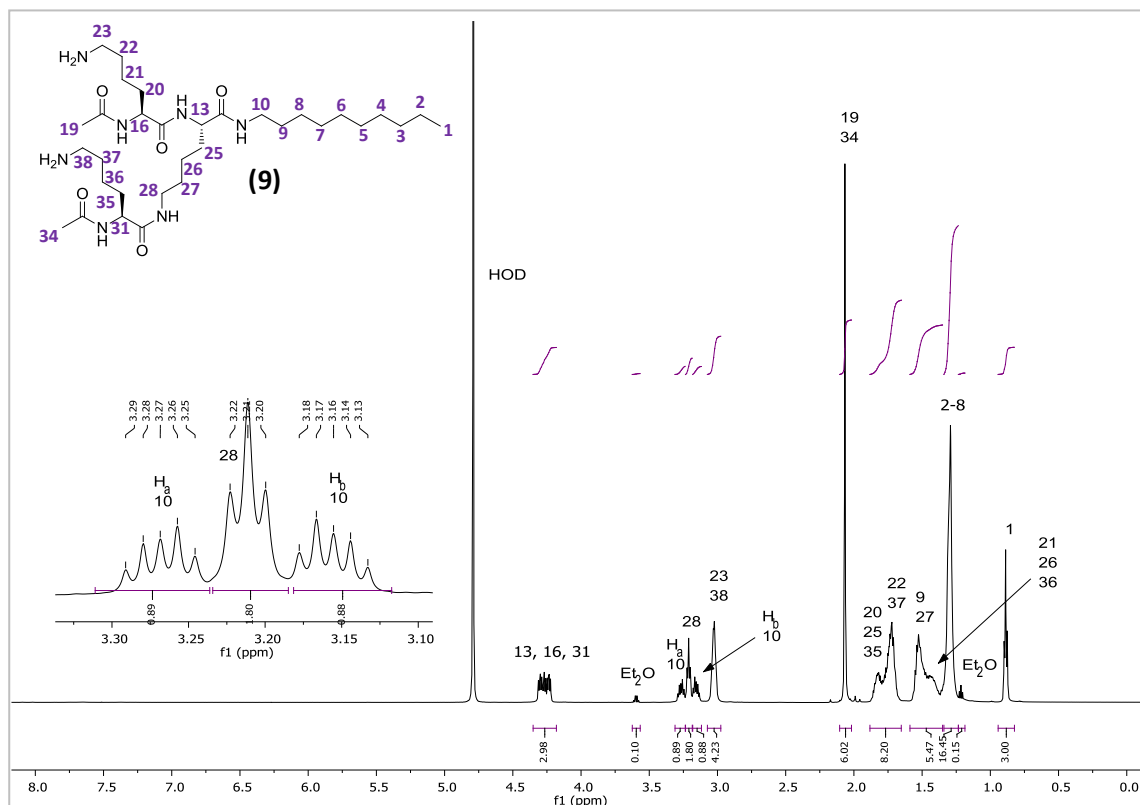
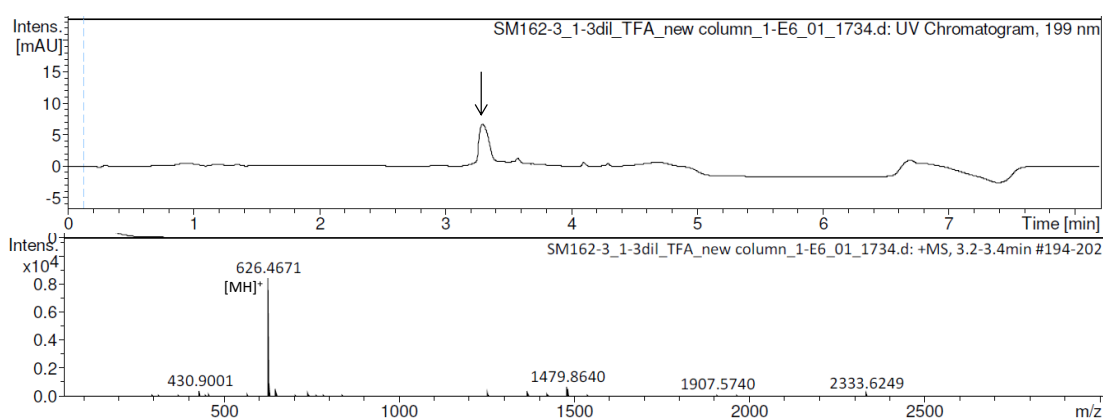
UPLC-MS (8)

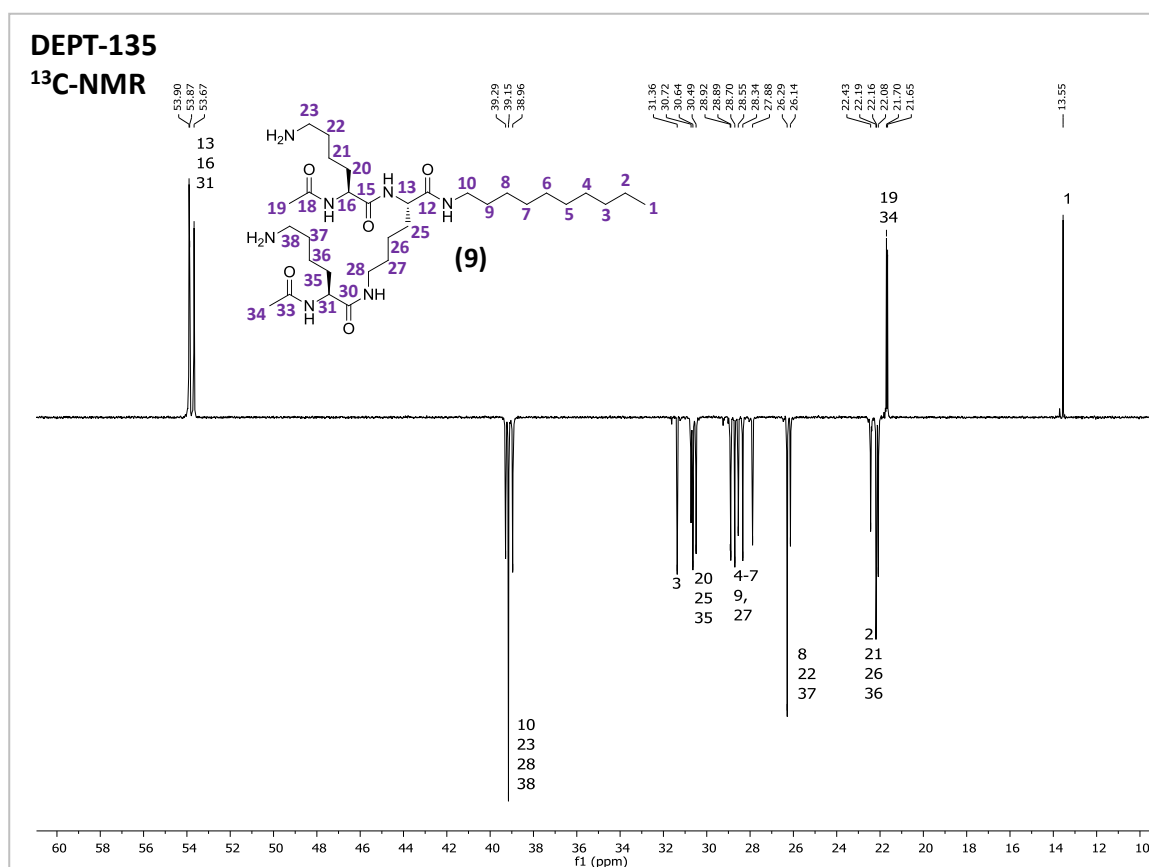
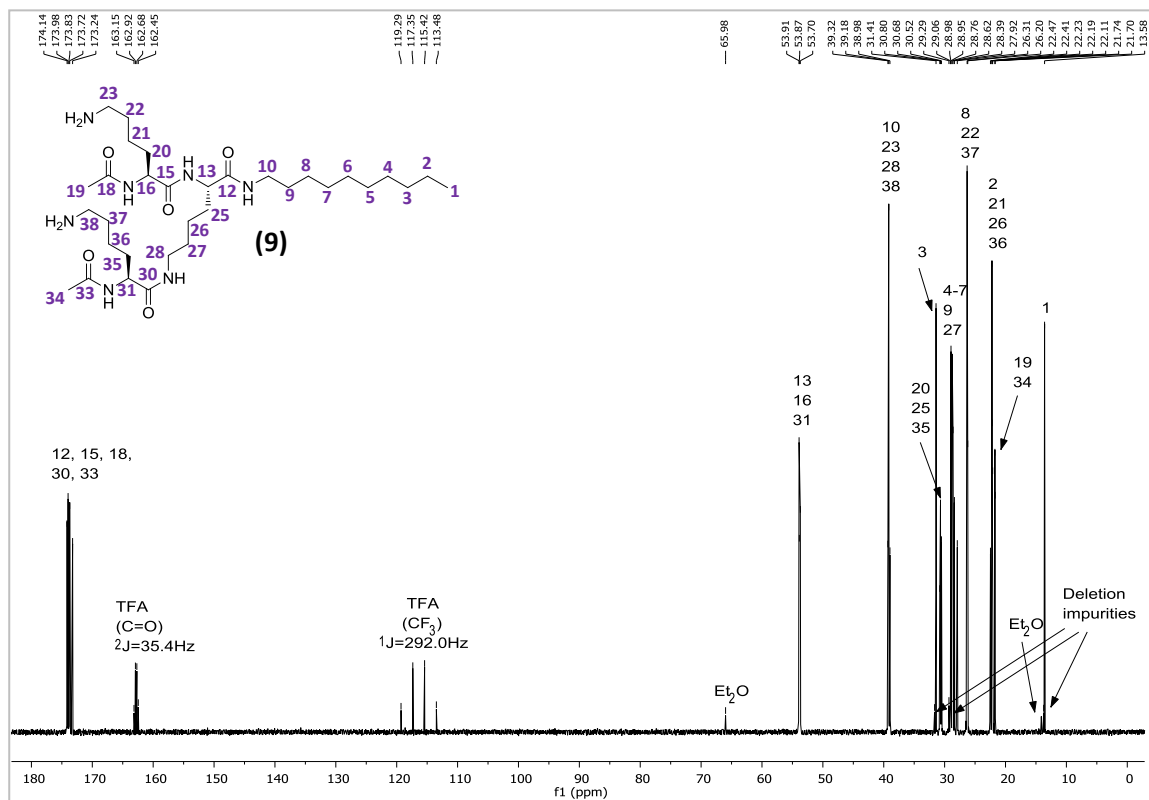


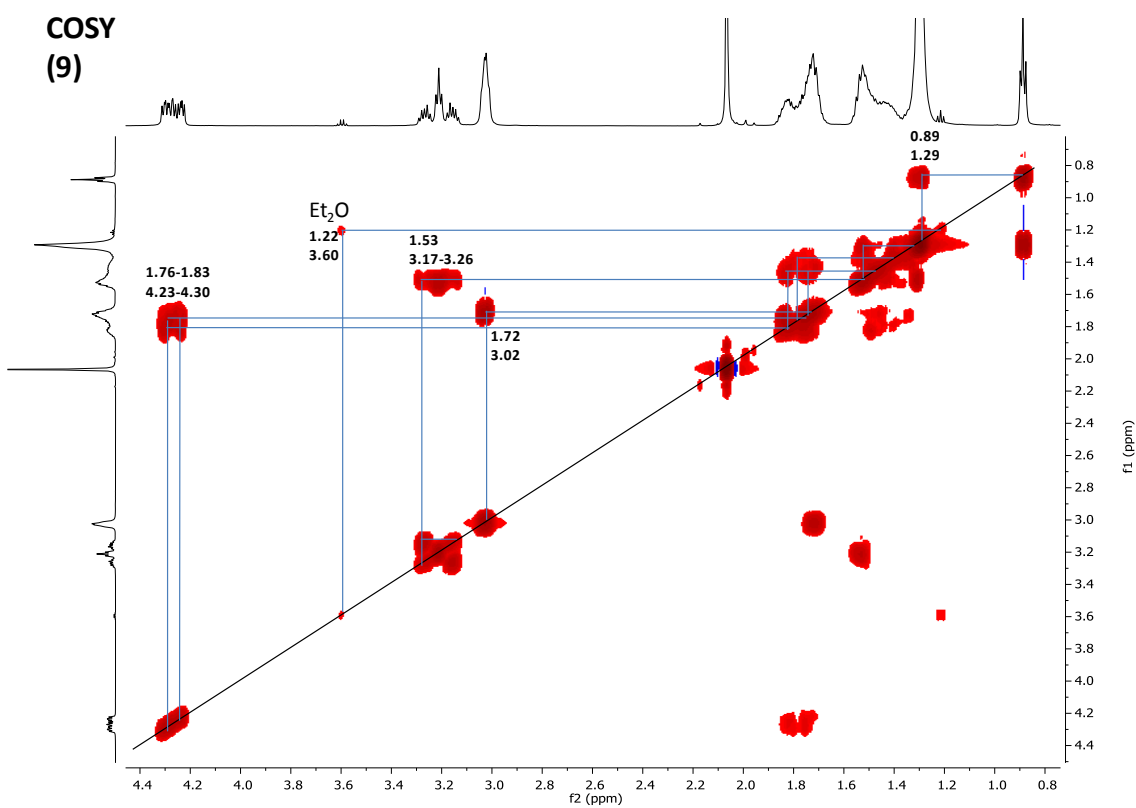
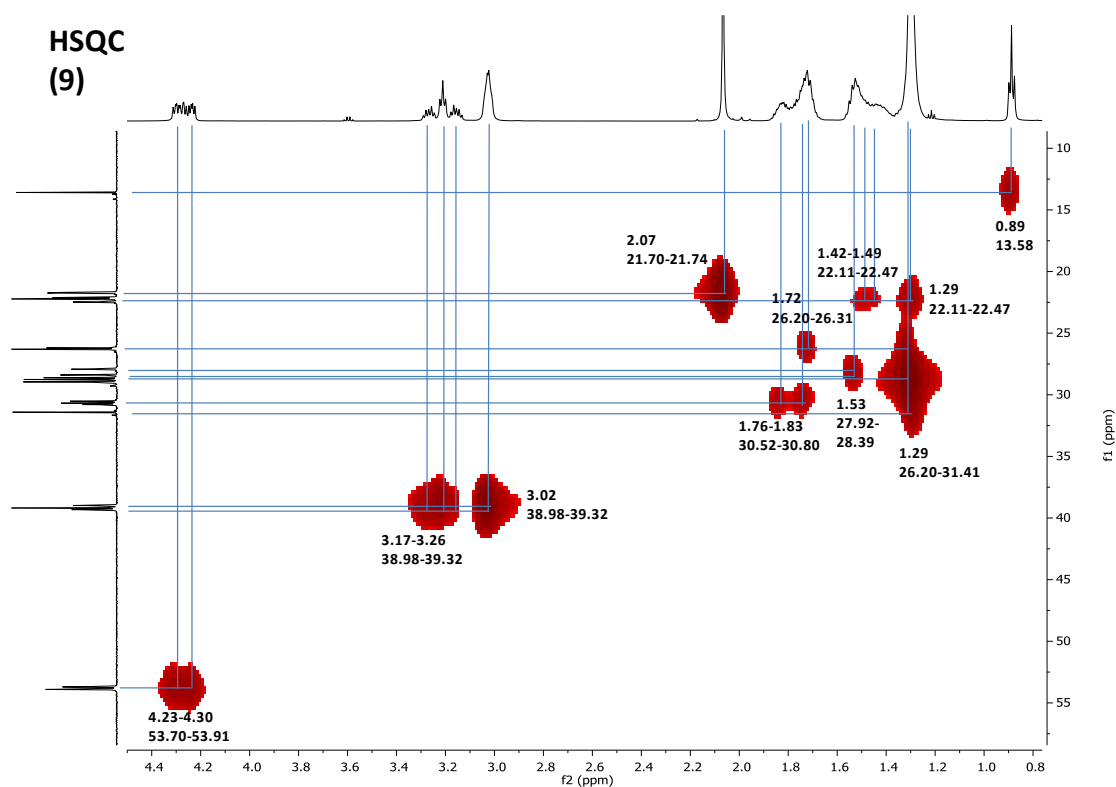


G1(acetyl)-C10 (9)

Yield (TFA salt; loading: 0.32 mmol/g): 139.8 mg (quant.). LC-MS data R_t : 3.2-3.4 min. MS(ESI⁺), calcd C₃₂H₆₃N₇O₅: 625.49 g/mol, found: m/z 626.47 [MH]⁺. ¹H NMR (600 MHz, D₂O): δ 4.35 – 4.18 (m, 3H), 3.27 (dt, J = 13.64, 6.90 Hz, 1H), 3.21 (t, J = 6.98 Hz, 2H), 3.16 (dt, J = 13.45, 6.75 Hz, 1H), 3.03 (td, J = 7.74, 4.08 Hz, 4H), 2.07 (s, 6H), 1.88 – 1.65 (m, 8H), 1.59 – 1.35 (m, 5H)², 1.34 – 1.23 (m, 16H), 0.89 (t, J = 6.91 Hz, 3H). ¹³C NMR (151 MHz, D₂O): δ 174.14, 173.98, 173.83, 173.72, 173.24, 53.91, 53.87, 53.70, 39.32, 39.18, 38.98, 31.41, 30.80, 30.68, 30.52, 28.98, 28.95, 28.76, 28.62, 28.39, 27.92, 26.31, 26.20, 22.47, 22.23, 22.19, 22.11, 21.74, 21.70, 13.58.

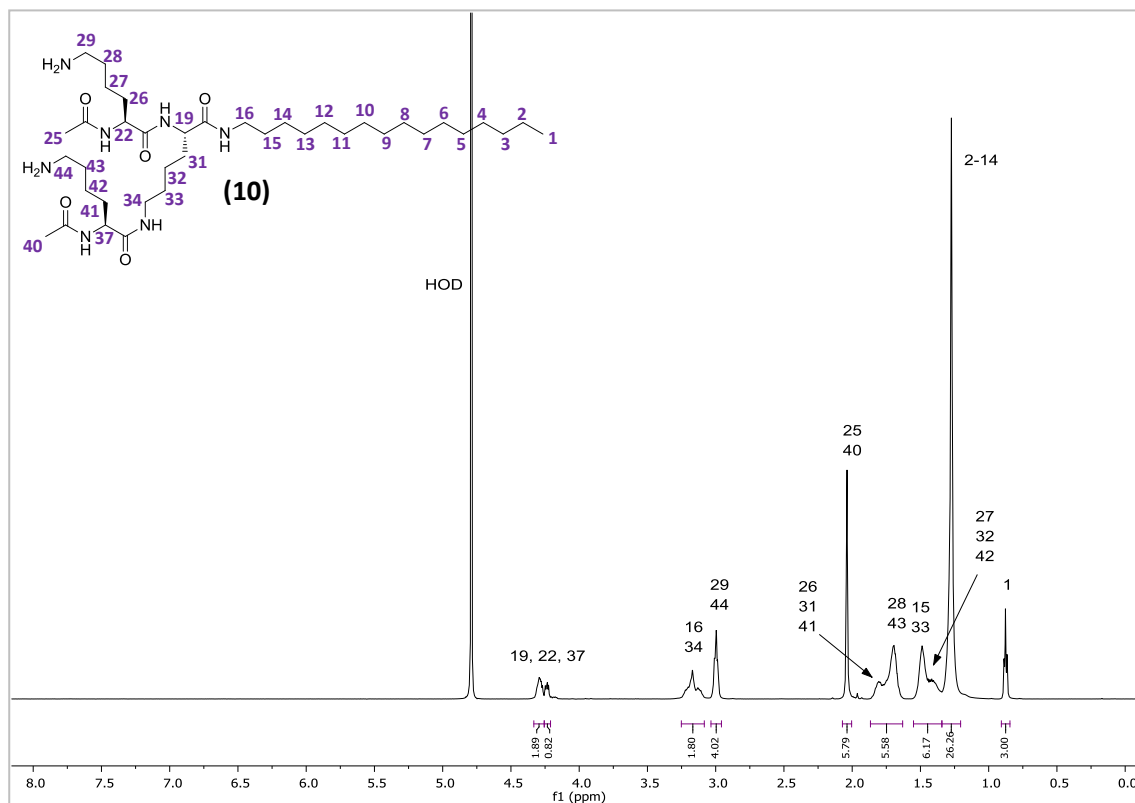
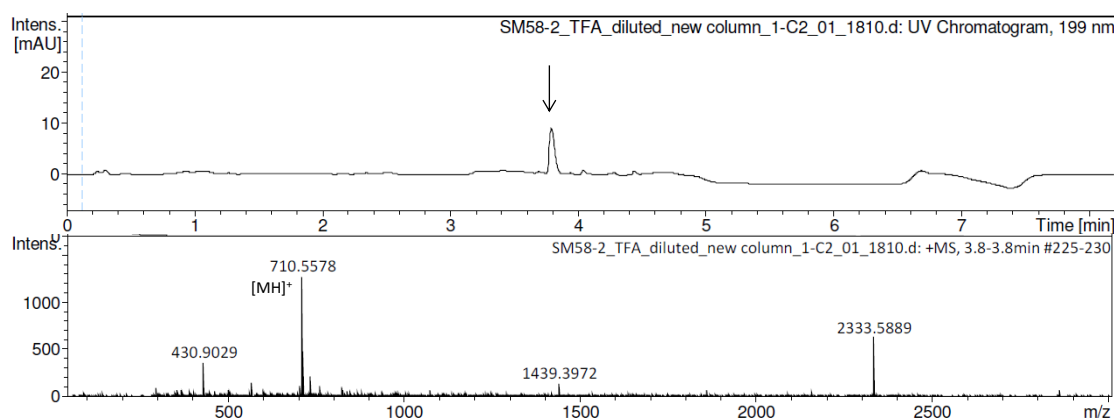
UPLC-MS (9)

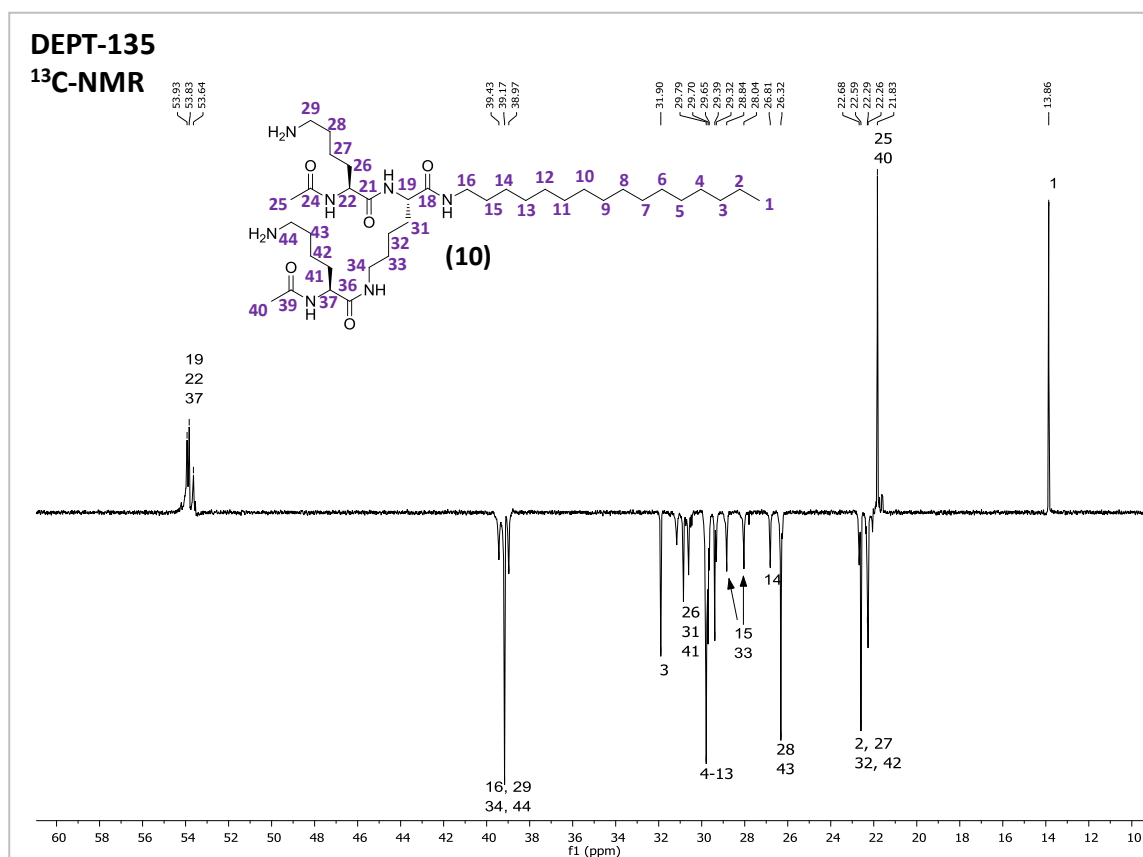
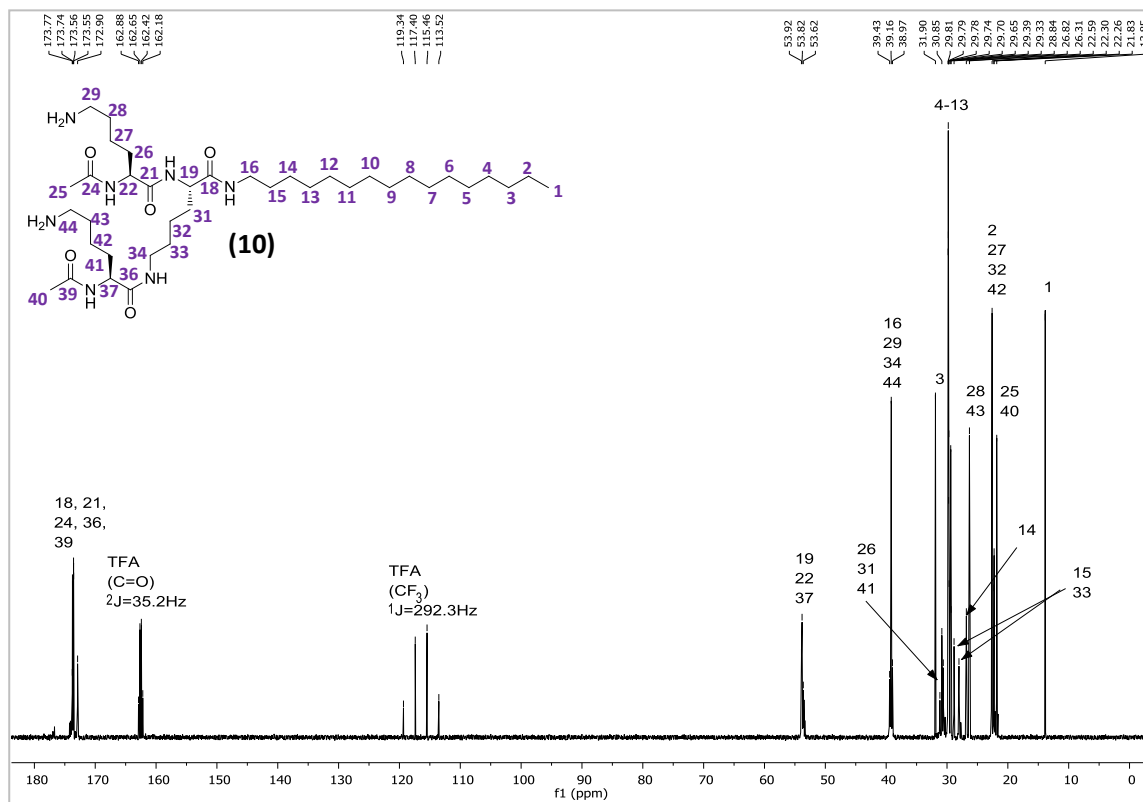


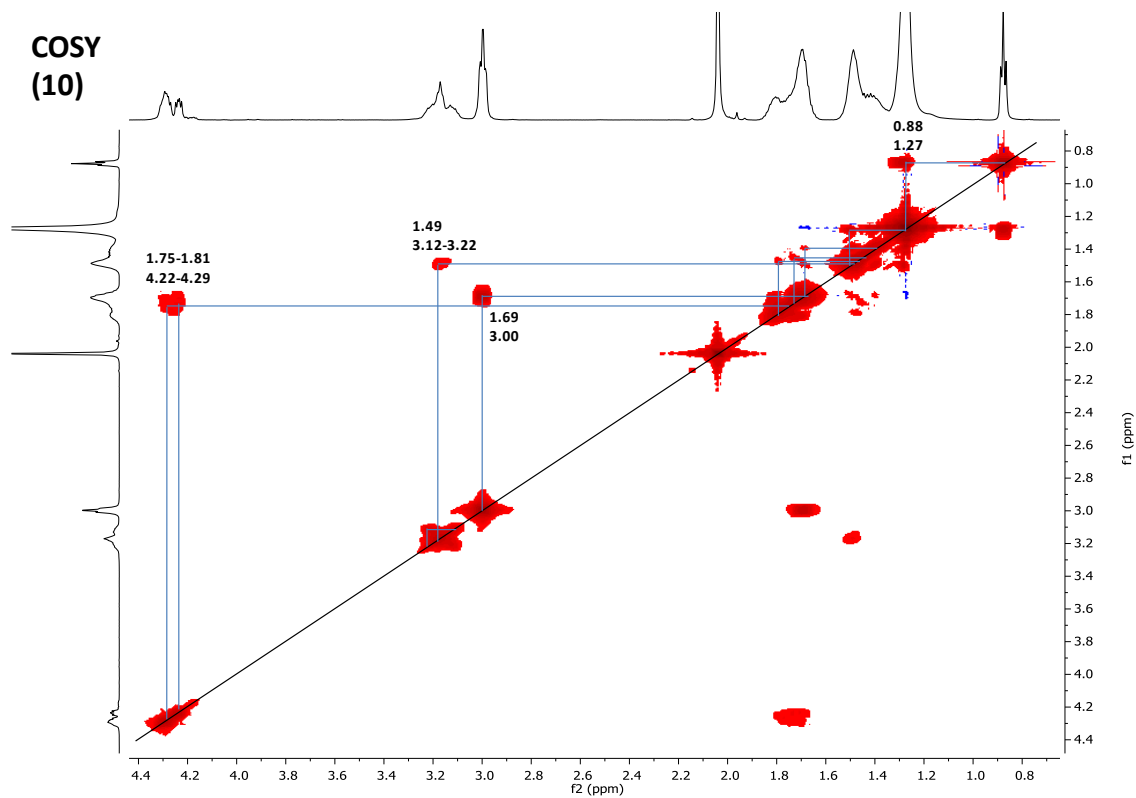
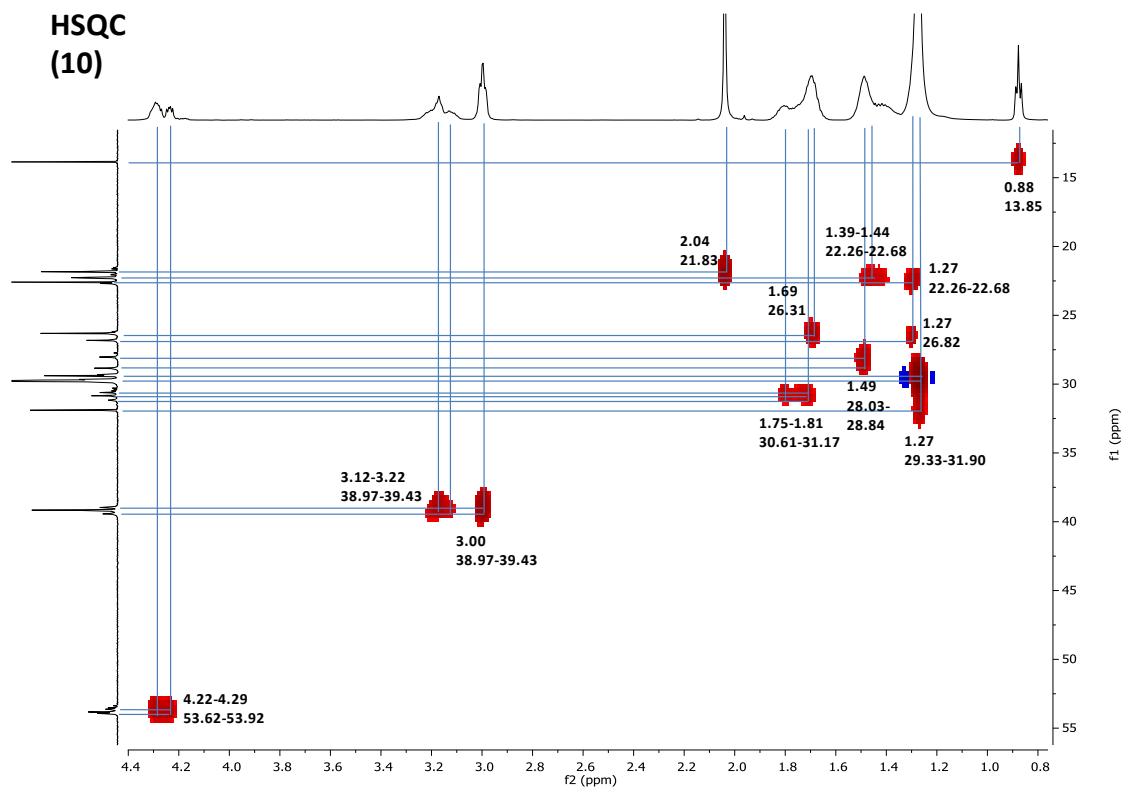


G1(acetyl)-C16 (10)

Yield (TFA salt; loading: 0.25 mmol/g): 84.9 mg (72 %). LC-MS data R_t : 3.8 min. MS(ESI⁺), calcd $C_{38}H_{75}N_7O_5$: 709.58 g/mol, found: m/z 710.55 [MH]⁺. ¹H NMR (600 MHz, D₂O): δ 4.33 – 4.26 (m, 2H), 4.24 (dd, J = 9.12, 5.26 Hz, 1H), 3.25 – 3.08 (m, 2H) ¹, 3.00 (td, J = 7.67, 2.68 Hz, 4H), 2.04 (m, 6H), 1.87 – 1.63 (m, 6H), 1.55 – 1.34 (m, 6H), 1.27 (s, 26H), 0.88 (t, J = 6.74 Hz, 3H). ¹³C NMR (151 MHz, D₂O): δ 173.77, 173.74, 173.56, 173.55, 172.90, 53.92, 53.82, 53.62, 39.43, 39.16, 38.97, 31.90, 31.16, 30.85, 30.61, 29.81, 29.79, 29.78, 29.74, 29.70, 29.65, 29.39, 29.33, 28.84, 28.03, 26.82, 26.31, 22.68, 22.59, 22.30, 22.26, 21.83, 13.85.

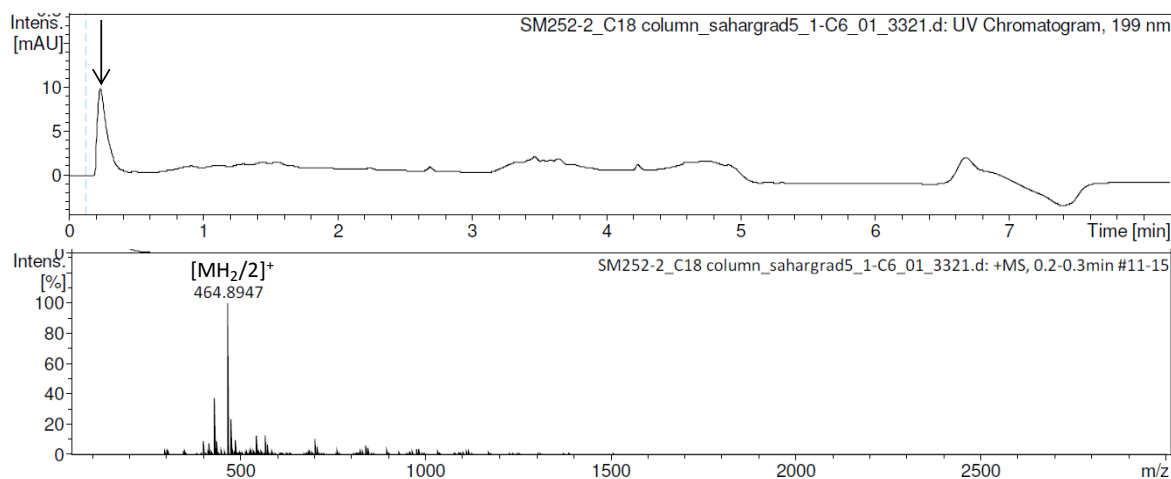
UPLC-MS (10)

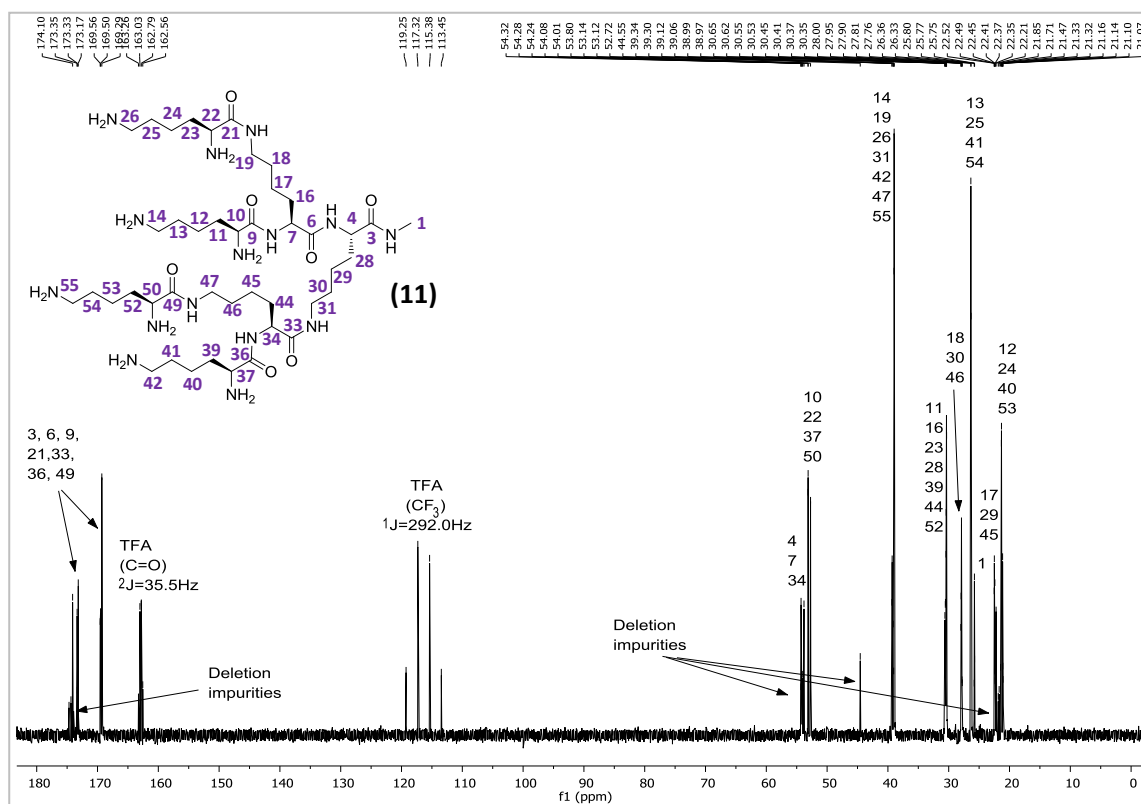
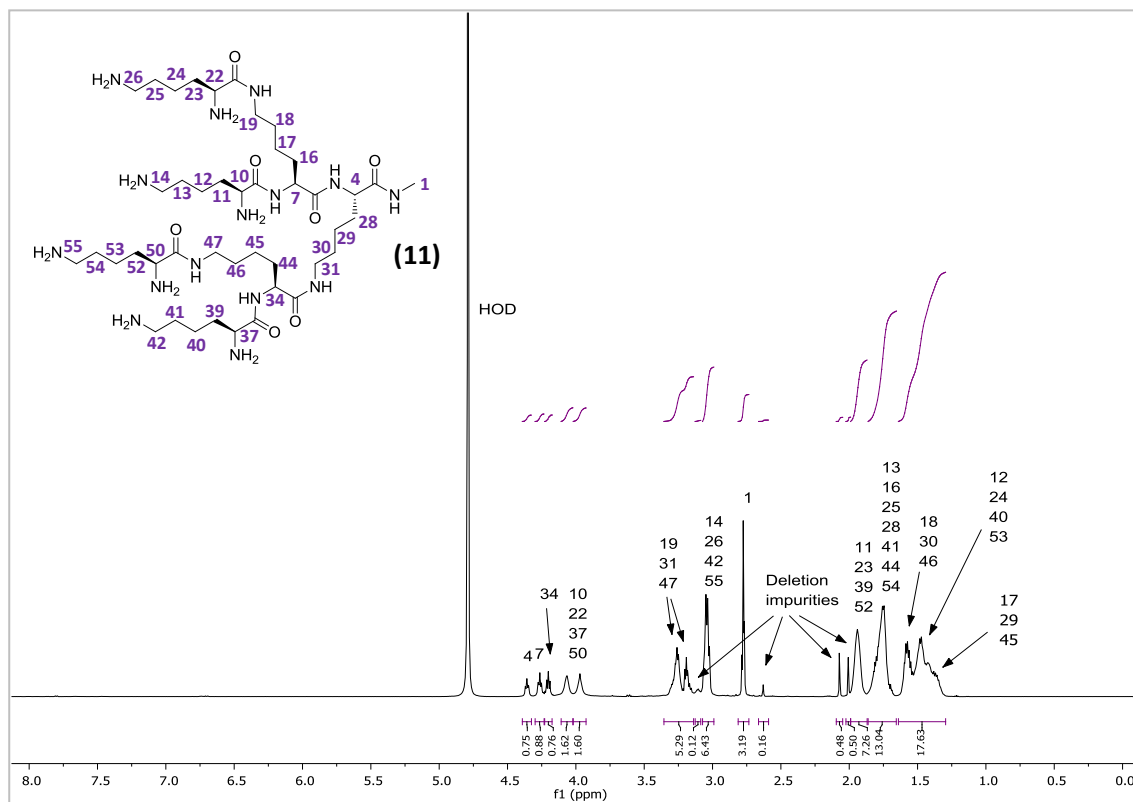


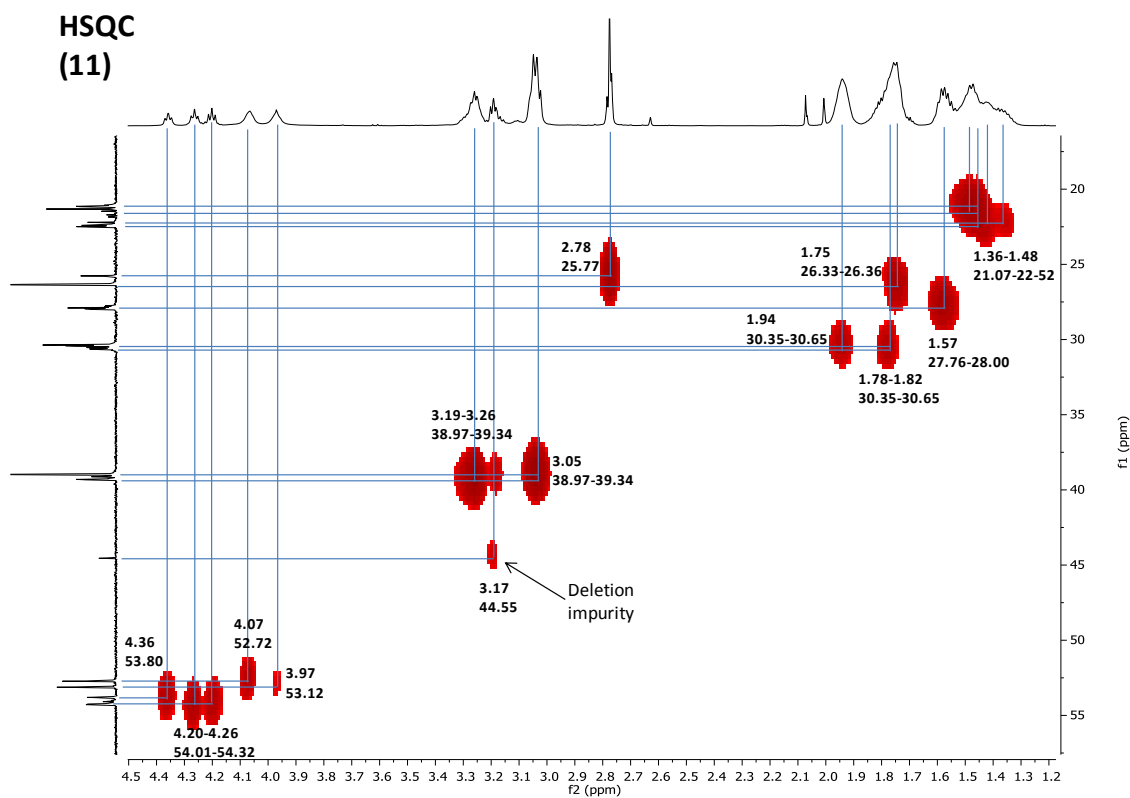
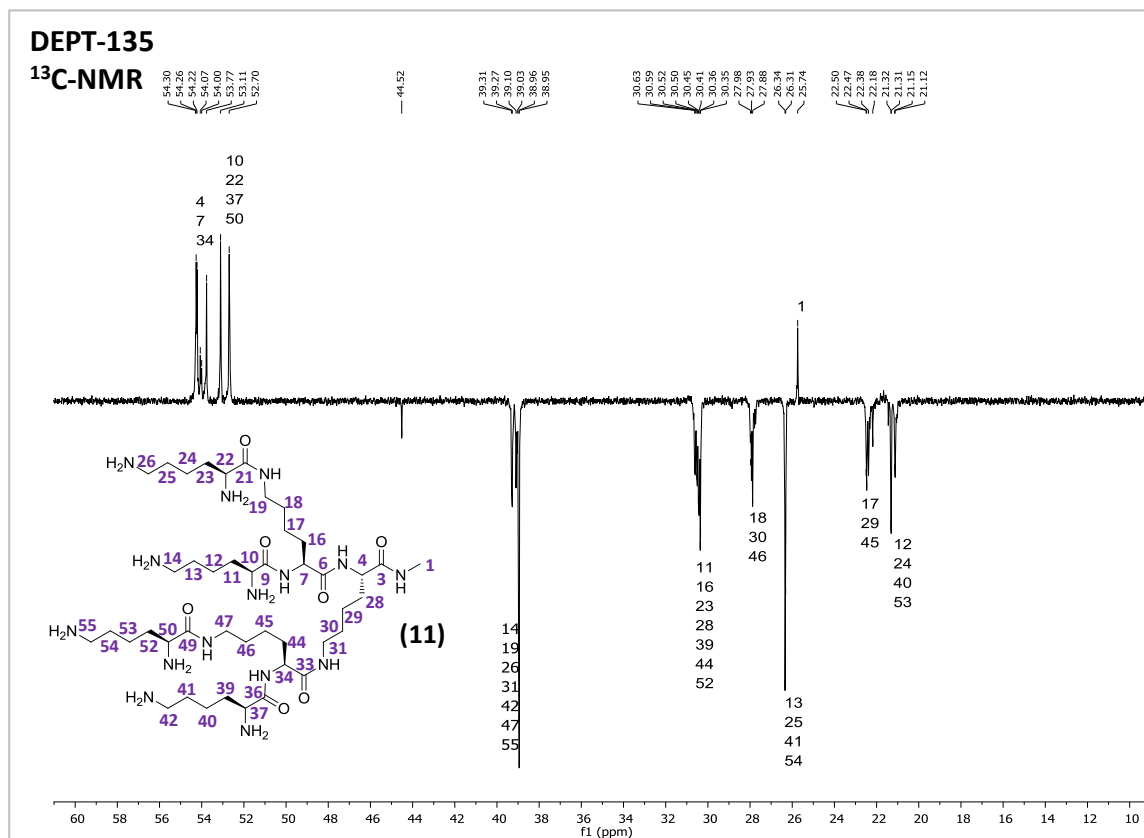


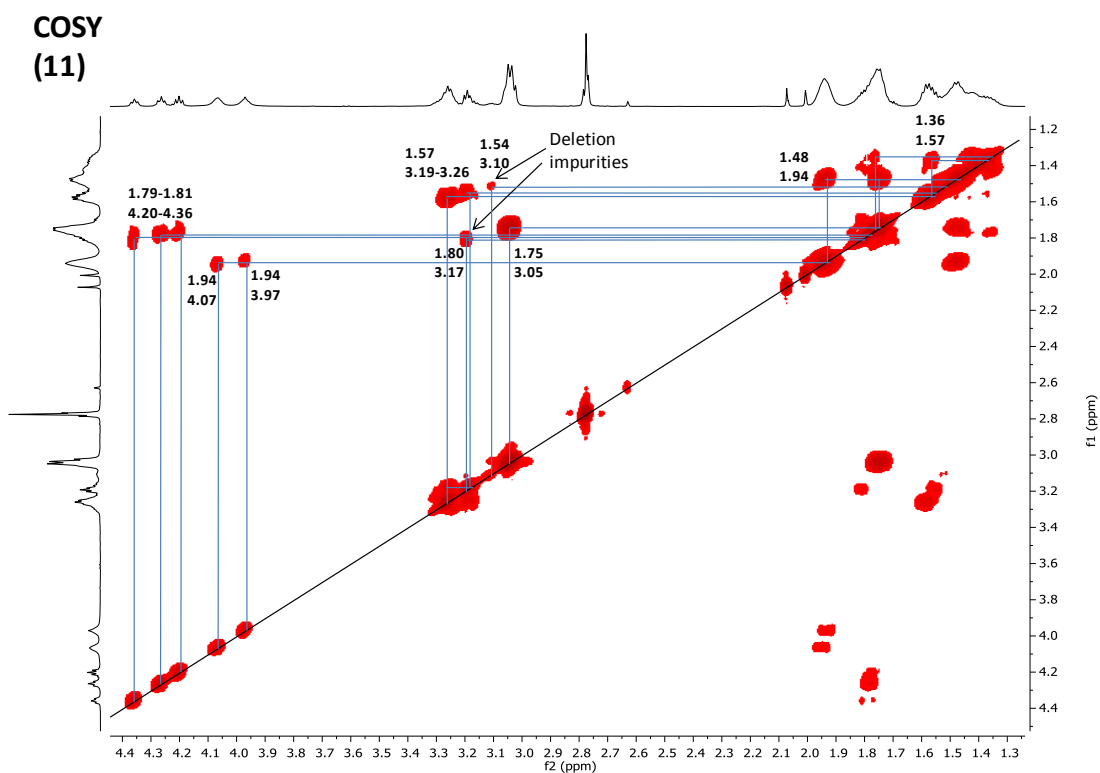
G2-C1 (11)

Yield (TFA salt; loading: 0.24 mmol/g): 77.3 mg (35 %). LC-MS data R_t : 0.2-0.3 min. MS(ESI⁺), calcd C₄₃H₈₉N₁₅O₇: 927.71 g/mol, found: m/z 464.89 [MH₂/2]⁺. ¹H NMR (600 MHz, D₂O): δ 4.39 – 4.33 (m, 1H), 4.26 (t, J = 7.34 Hz, 1H), 4.20 (t, J = 7.32 Hz, 1H), 4.07 (s, 2H), 3.97 (s, 2H), 3.33 – 3.13 (m, 5H)², 3.04 (q, J = 7.93 Hz, 6H), 2.81 – 2.73 (m, 3H), 1.99 – 1.87 (m, 7H), 1.86 – 1.66 (m, 13H), 1.64 – 1.29 (m, 18H). ¹³C NMR (151 MHz, D₂O): δ 174.10, 173.35, 173.17, 169.56, 169.50, 169.45, 169.29, 54.28, 54.24, 54.08, 53.80, 53.14, 53.12, 52.72, 39.34, 39.30, 39.12, 39.06, 38.99, 38.97, 30.62, 30.55, 30.53, 30.45, 30.41, 30.37, 30.35, 28.00, 27.95, 27.90, 26.36, 26.33, 25.77, 22.52, 22.49, 22.41, 21.85, 21.71, 21.47, 21.33, 21.32, 21.16, 21.14.

UPLC-MS (11)

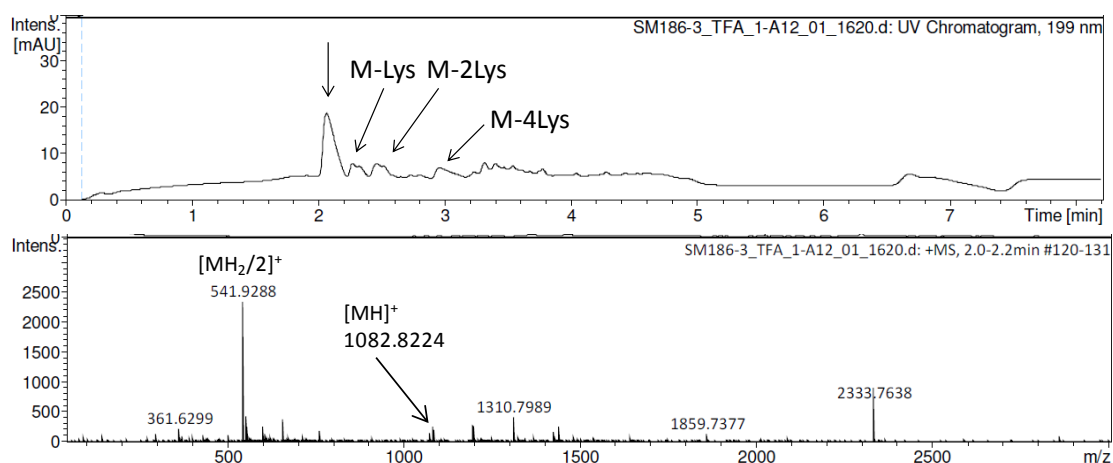


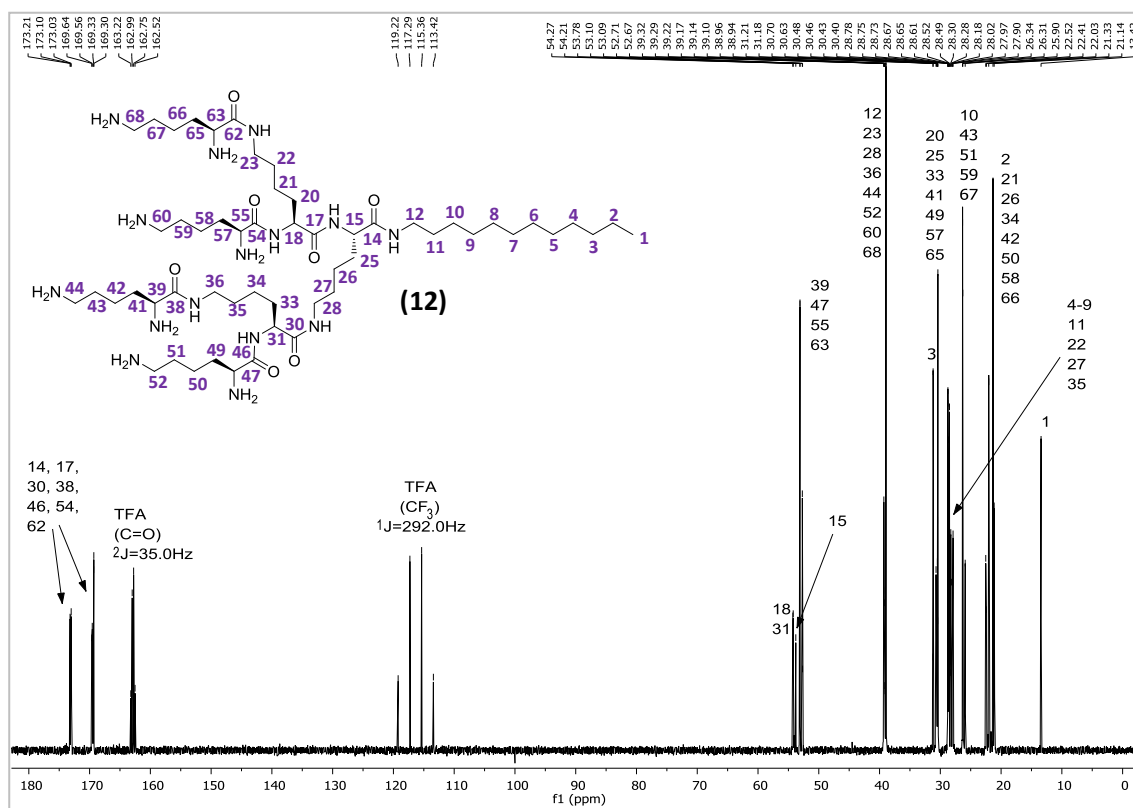
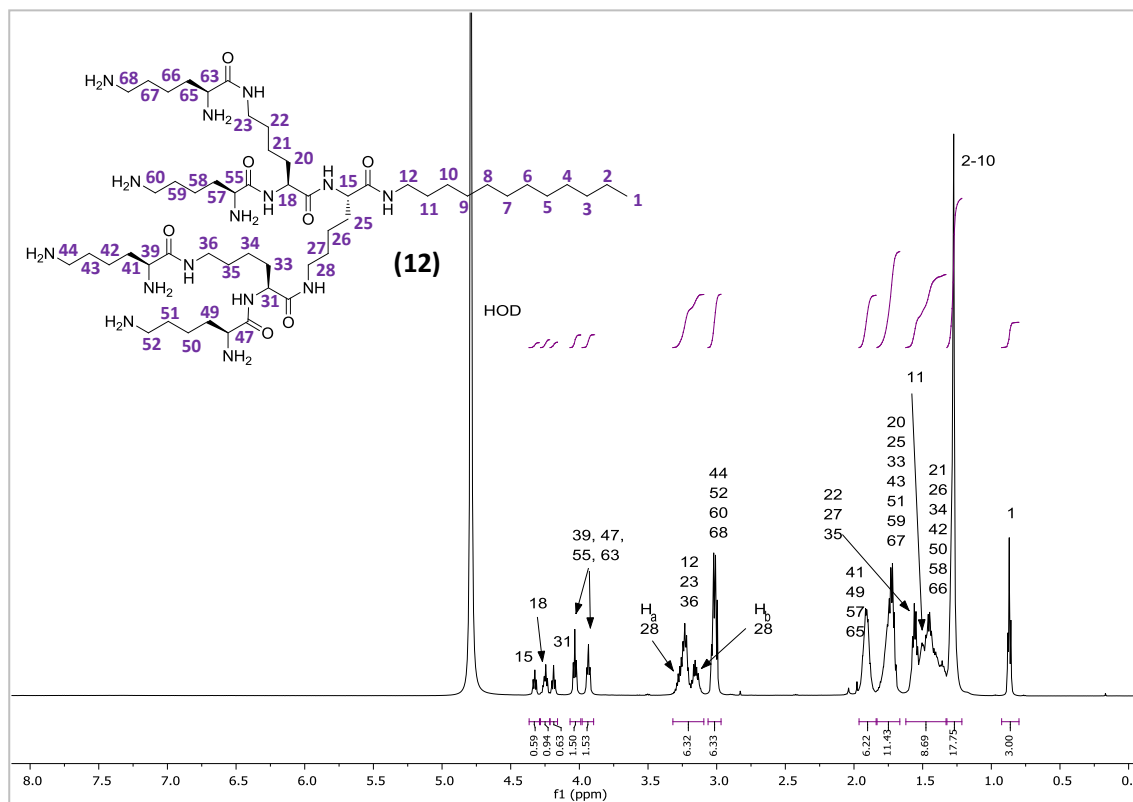


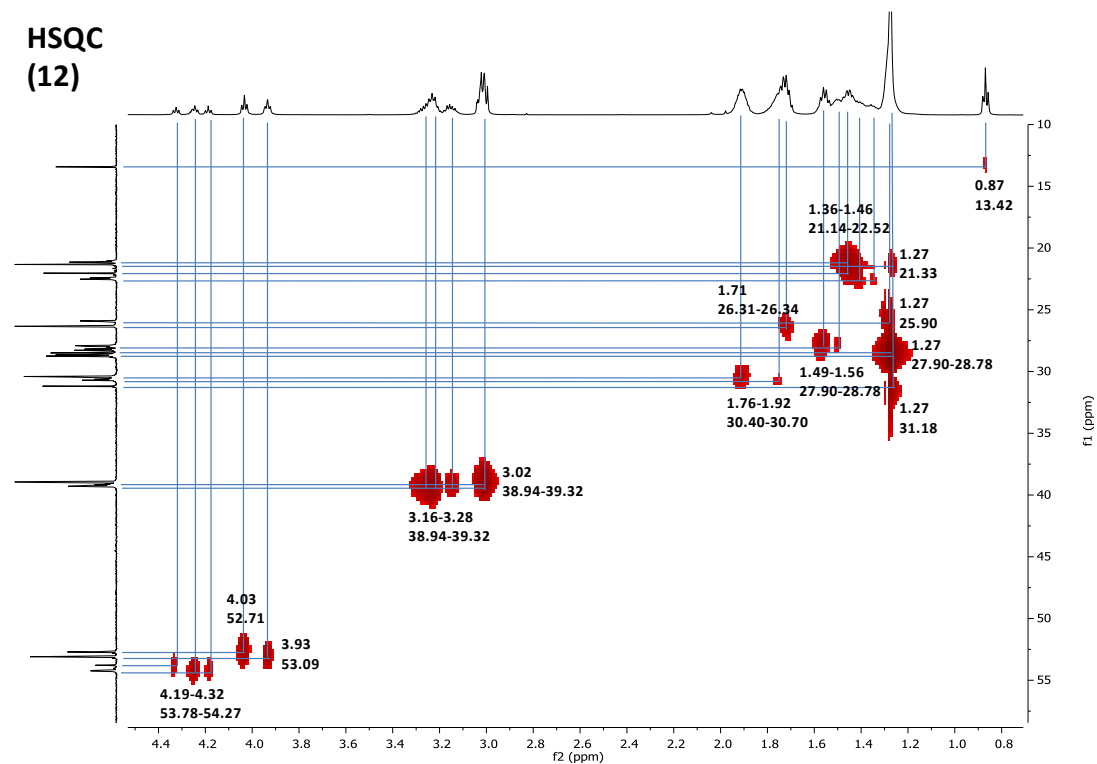
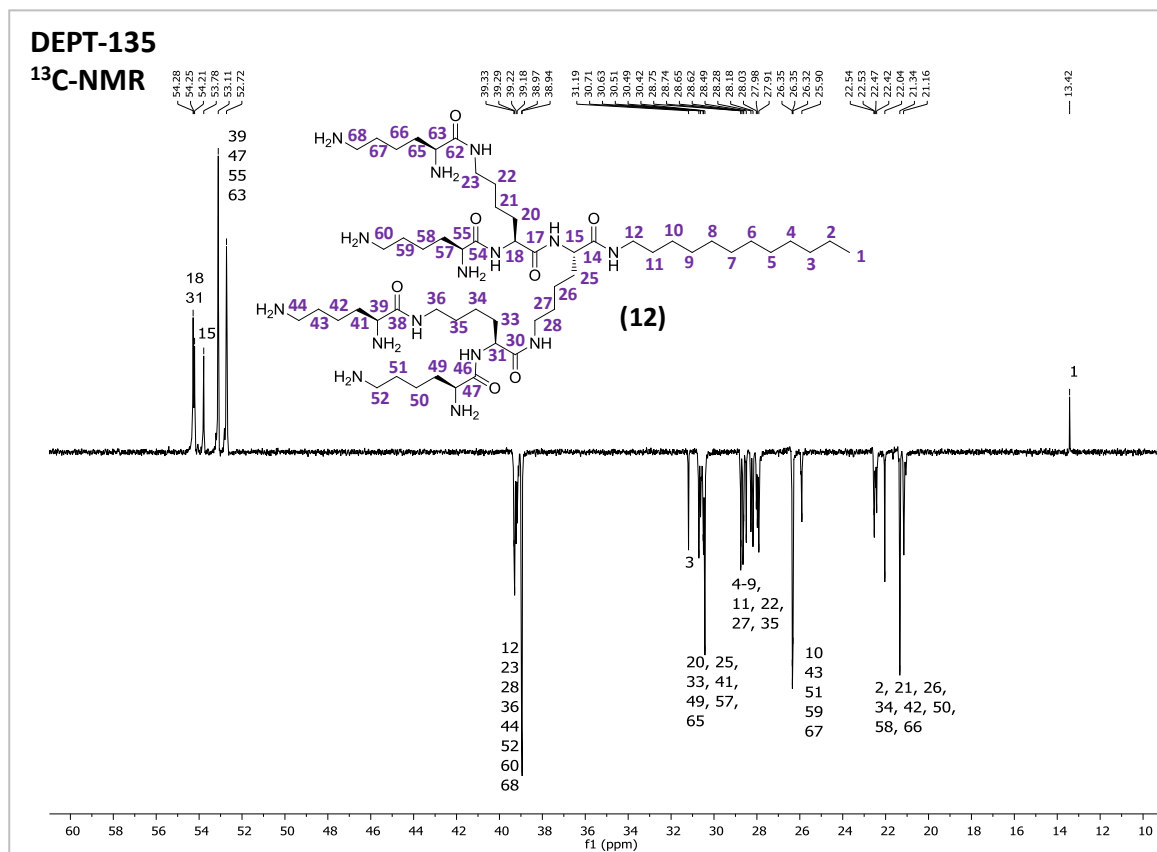


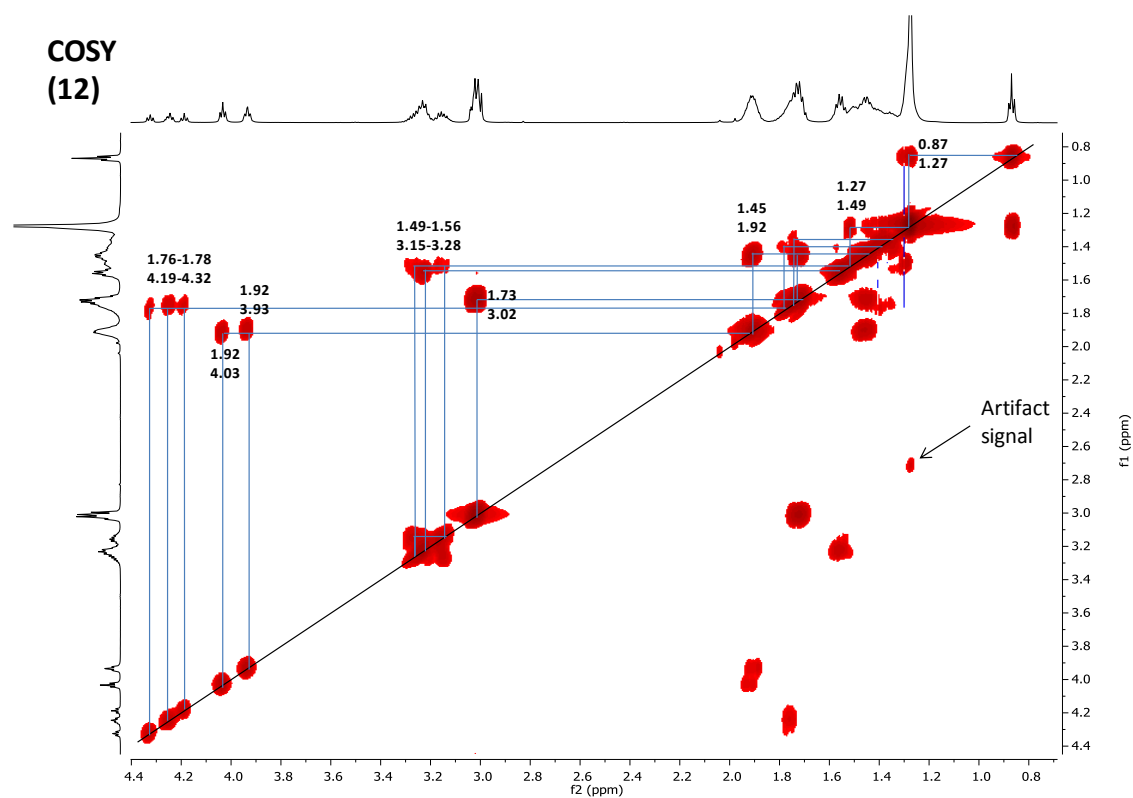
G2-C12 (12)

Yield (TFA salt; loading: 0.35 mmol/g): 151.4 mg (43 %). LC-MS data R_t : 2.0-2.2 min. MS(ESI^+), calcd $\text{C}_{54}\text{H}_{111}\text{N}_{15}\text{O}_7$: 1081.88 g/mol, found: m/z 1082.82 $[\text{MH}]^+$; 541.93 $[\text{MH}_2/2]^+$. ^1H NMR (600 MHz, D_2O): δ 4.32 (t, $J = 7.15$ Hz, 1H), 4.25 (td, $J = 7.16$ Hz, 4.53 Hz, 1H), 4.19 (t, $J = 7.40$ Hz, 1H), 4.03 (t, $J = 6.67$ Hz, 2H), 3.93 (td, $J = 6.77$ Hz, 2.0 Hz, 2H), 3.32 – 3.09 (m, 6H)², 3.06 – 2.97 (m, 6H), 1.96 – 1.84 (m, 6H), 1.83 – 1.67 (m, 11H), 1.62 – 1.33 (m, 9H), 1.32 – 1.21 (m, 18H), 0.87 (t, $J = 6.81$ Hz, 3H). ^{13}C NMR (151 MHz, D_2O): δ 173.21, 173.10, 173.03, 169.64, 169.56, 169.33, 169.30, 54.27, 54.21, 53.78, 53.10, 52.71, 52.67, 39.32, 39.29, 39.22, 39.17, 39.14, 39.10, 38.96, 38.94, 31.21, 31.18, 30.70, 30.63, 30.48, 30.46, 30.43, 30.40, 28.78, 28.75, 28.73, 28.67, 28.65, 28.61, 28.52, 28.49, 28.30, 28.28, 28.18, 28.02, 27.97, 27.90, 26.34, 26.31, 25.90, 22.52, 22.41, 22.03, 21.33, 21.14, 13.42.

UPLC-MS (12)

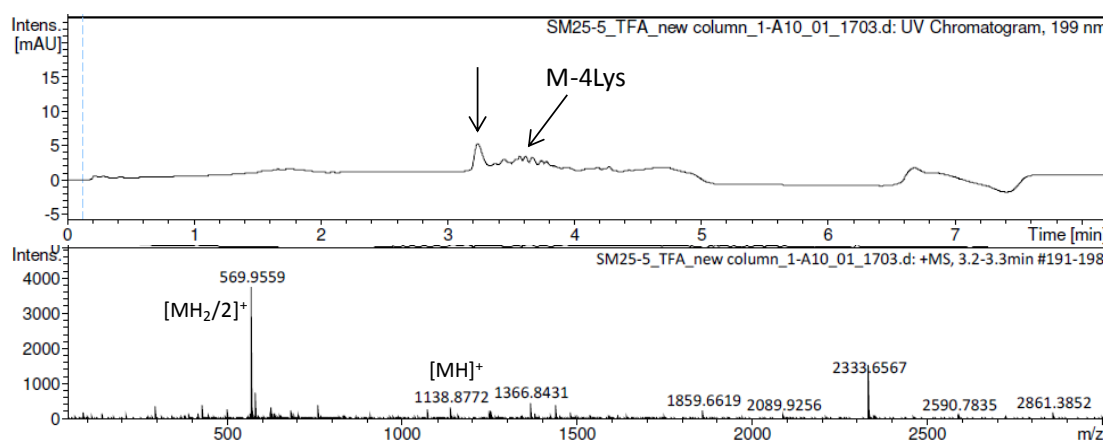


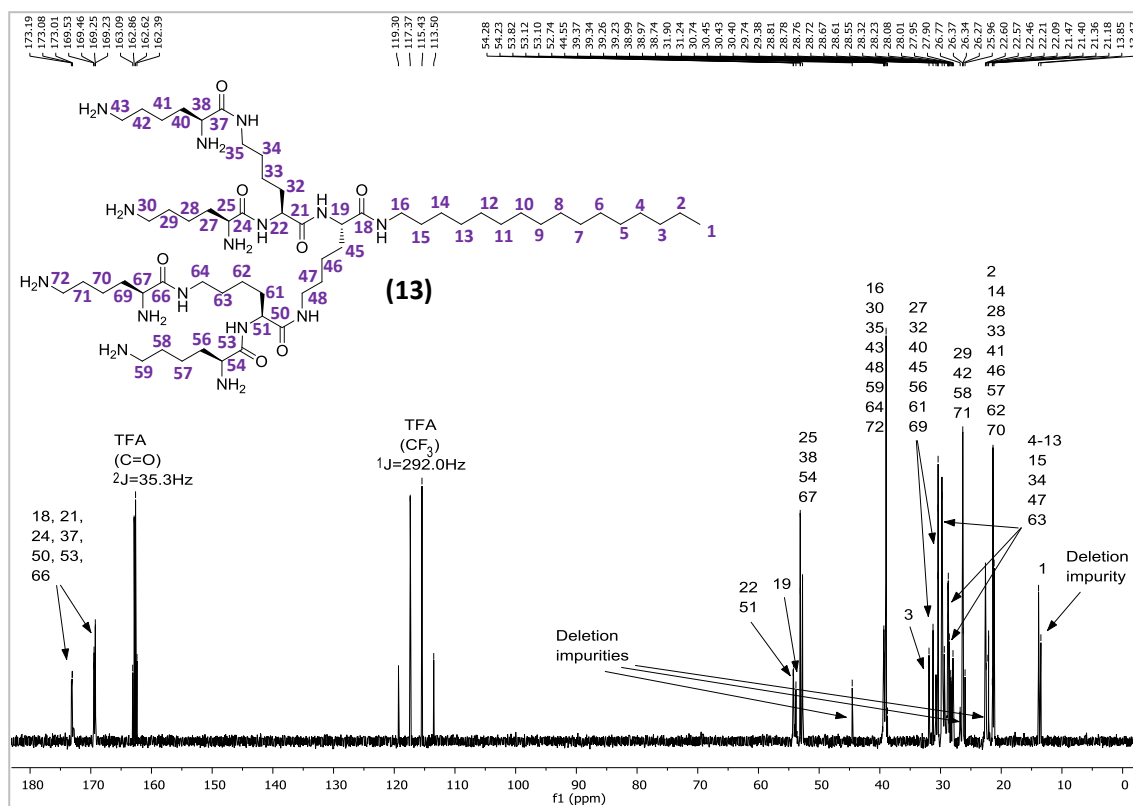
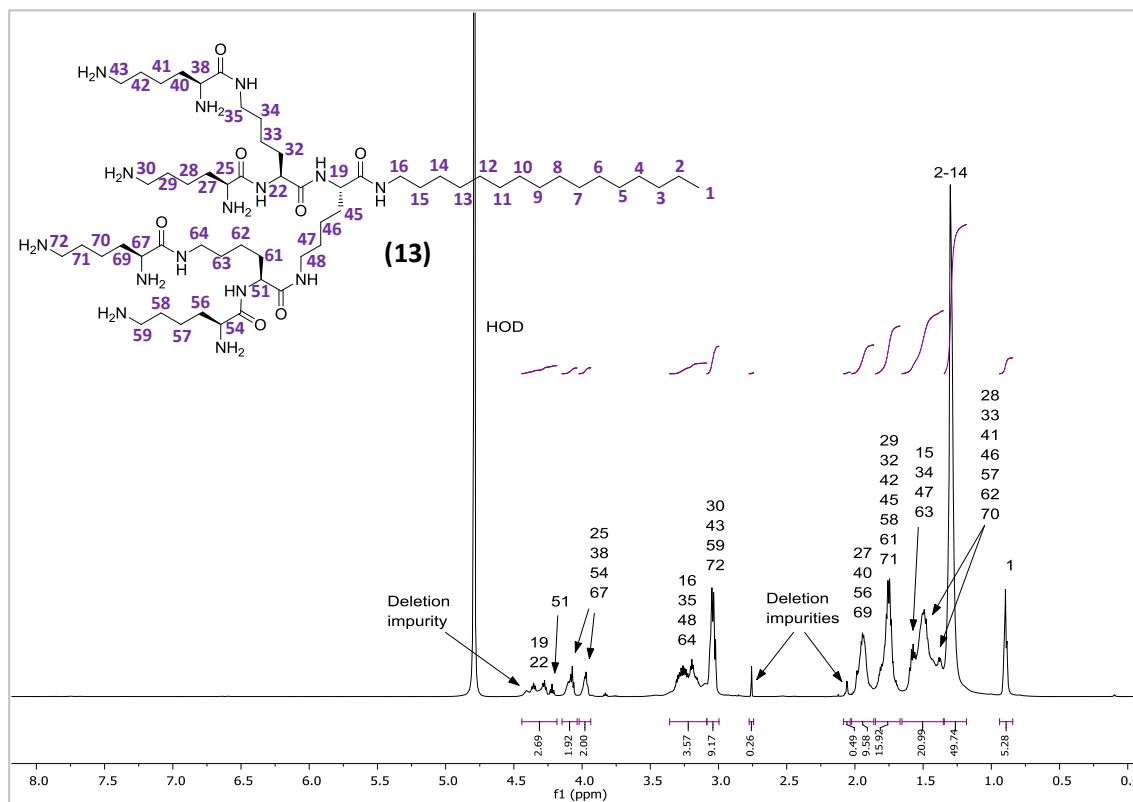


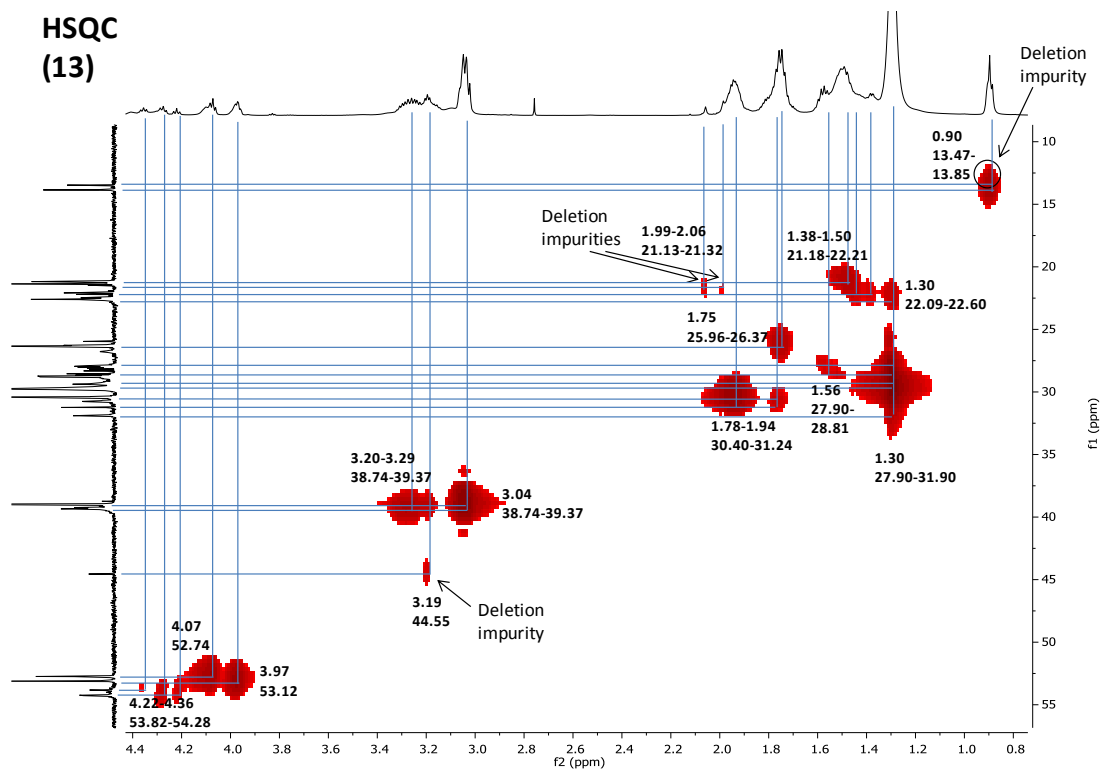
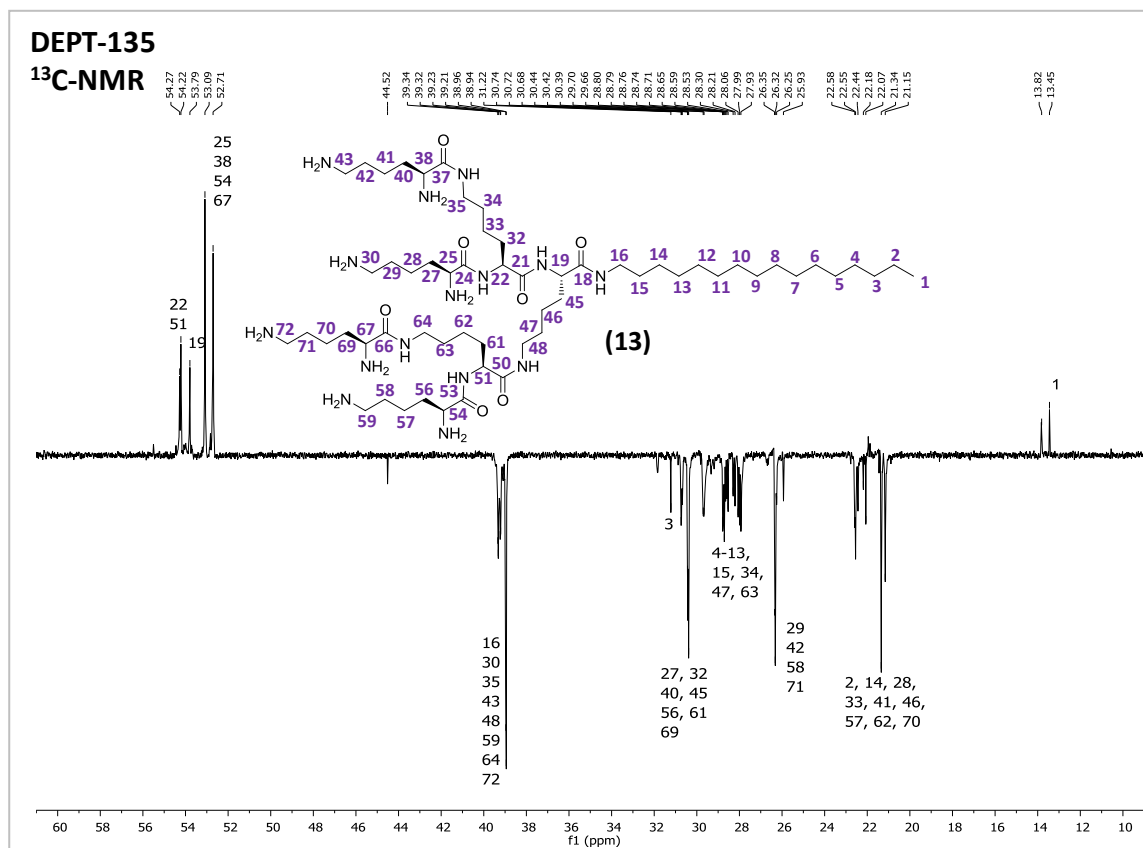


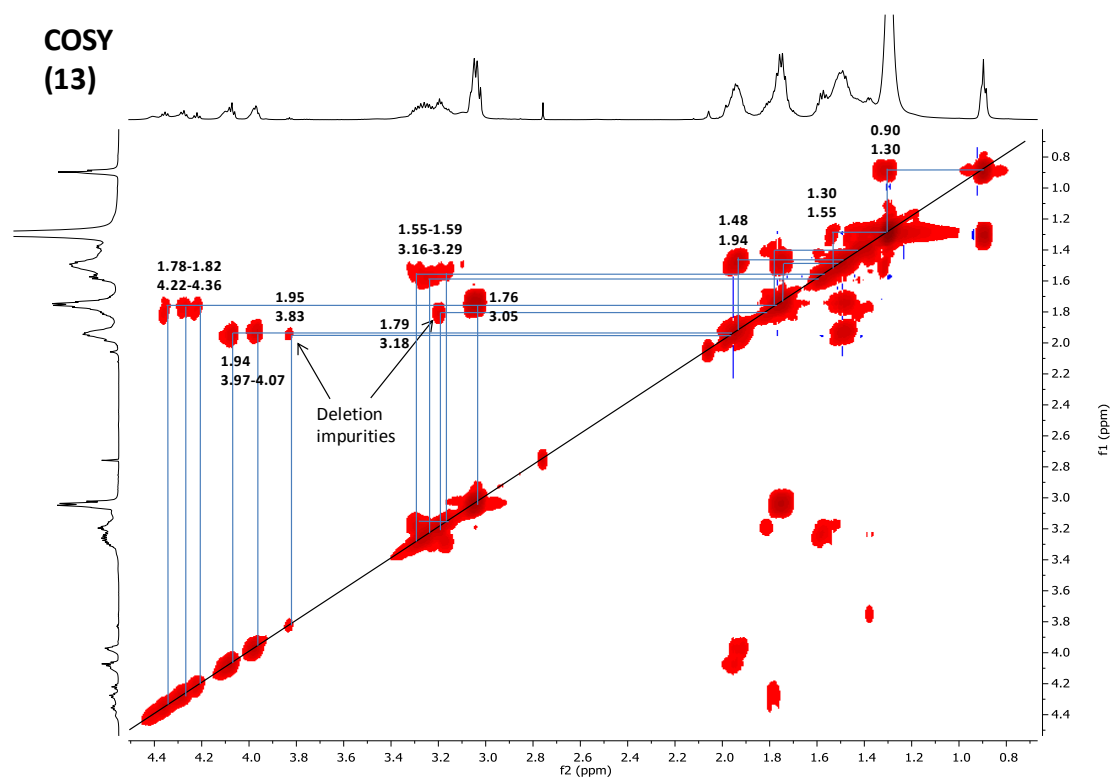
G2-C16 (13)

Yield (TFA salt; loading: 0.23 mmol/g): 152.6 mg (65 %). LC-MS data R_t : 3.2-3.3 min. MS(ESI⁺), calcd C₅₈H₁₁₉N₁₅O₇: 1137.84 g/mol, found: m/z 1138.88 [MH]⁺; 569.96 [MH₂/2]⁺. ¹H NMR (600 MHz, D₂O): δ 4.44 – 4.18 (m, 3H)¹, 4.15 – 4.04 (m, 2H), 4.02 – 3.94 (m, 2H), 3.36 – 3.09 (m, 4H), 3.09 – 3.00 (m, 9H), 2.02 – 1.86 (m, 10H), 1.85 – 1.67 (m, 16H), 1.65 – 1.35 (m, 21H), 1.34 – 1.18 (m, 50H), 0.90 (t, J = 5.94 Hz, 5H). ¹³C NMR (151 MHz, D₂O): δ 173.19, 173.08, 173.01, 169.53, 169.46, 169.25, 169.23, 54.28, 54.23, 53.82, 53.12, 53.10, 52.74, 39.37, 39.34, 39.26, 39.23, 38.99, 38.97, 31.90, 31.24, 30.74, 30.45, 30.43, 30.40, 29.74, 29.38, 28.81, 28.78, 28.76, 28.72, 28.67, 28.61, 28.55, 28.32, 28.23, 28.08, 28.01, 27.95, 27.90, 26.37, 26.34, 26.27, 25.96, 22.60, 22.57, 22.46, 22.21, 22.09, 21.47, 21.40, 21.36, 21.18, 13.85.

UPLC-MS (13)







Notes

- 1 In some of the compounds of high generation with long alkyl chains the ^1H integrals on some protons sometimes deviates from the proposed structure, presumably due to slow relaxation. However, UPLC-MS chromatograms and ^{13}C spectra correlate nicely with the proposed structure.
- 2 In some of the compounds deviations in the ^1H integrals are seen due to the presence of lysine deletion adducts with/without Boc/*t*Bu impurities.