ANNEX

≡-[*bis*MPA,G1]-(ketal)$_1$ (1)

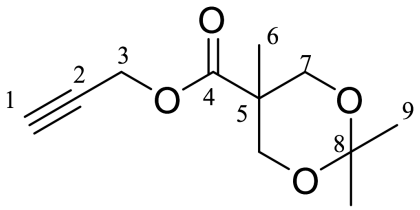

Figure A1: Structure of molecule 1.

Synthetic procedure: Steglich esterification

Propargyl alcohol (5 g, 89.19 mmol, 1 eq.) was dissolved into dry dichloromethane (120 mL), together with *bis*-MPA (monomer) (17.1 g, 98.11 mmol, 1.1 eq.) and DPTS (10.5 g, 35.68 mmol, 0.4 eq.). This mixture was stirred under argon atmosphere and cooled down to 0ºC. A solution of DCC (20.24 g, 98.11 mmol, 1.1 eq.) in dry dichloromethane (30 mL) was added drop wise. The reaction mixture was then allowed to stir under argon atmosphere overnight at room temperature. The white precipitate which appeared, *N,N'*-dicyclohexylurea (DCU), was removed by filtration. The solvent of the resulting mixture was evaporated under vacuum. Hexane was added to provoke further precipitation of the DCU, which was filtered off. The solvent was evaporated under reduced pressure. The crude product was purified through silica gel column chromatography (hexane : ethyl acetate = 8 : 2). A pale yellow oil was obtained (17.59 g, 93%).

Characterization

Mw = 212.24 g/mol.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.72 (d, *J* = 2.5 Hz, 2H, H-3), 4.19 (d, *J* = 12 Hz, 2H, H7), 3.64 (d, *J* = 12 Hz, 2H, H-7'), 2.46 (t, *J* = 2.5 Hz, 1H, H-1), 1.41 (d, *J* = 0.5 Hz, 3H, H-9), 1.37 (d, *J* = 0.5 Hz, 3H, H-9'), 1.20 (s, 3H, H-6).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.57 (C-4), 98.26 (C-8), 75.09 (C-1), 65.98 (C-7), 52.47 (C-3), 42.01 (C-5), 24.69 (C-9), 22.74 (C-9'), 18.55 (C-6).

MS (ESI$^+$) *m/z (%)*: found *234.9 (100)*, calculated for $[C_{11}H_{16}O_4,Na]^+$ *235.23*.

FTIR (ν_{max}/cm^{-1}, ATR): 3274 (≡C-H st), 2993 (C-H st), 1735 (C=O st), 1456 (CH_2, CH_3 δ).

EA (%): *Found:* C, 61.6; H, 7.7. *Calc. for* $C_{11}H_{16}O_4$: C, 62.3; H, 7.6.

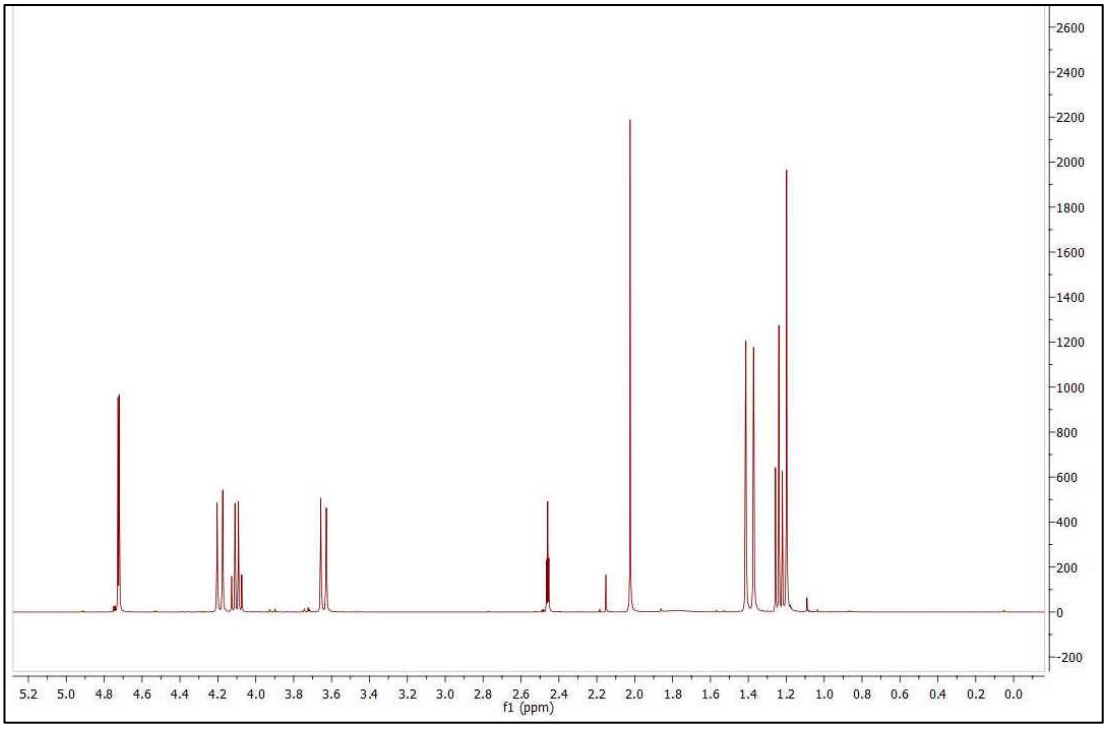

Figure A2: ^{1}H NMR spectrum of molecule 1.

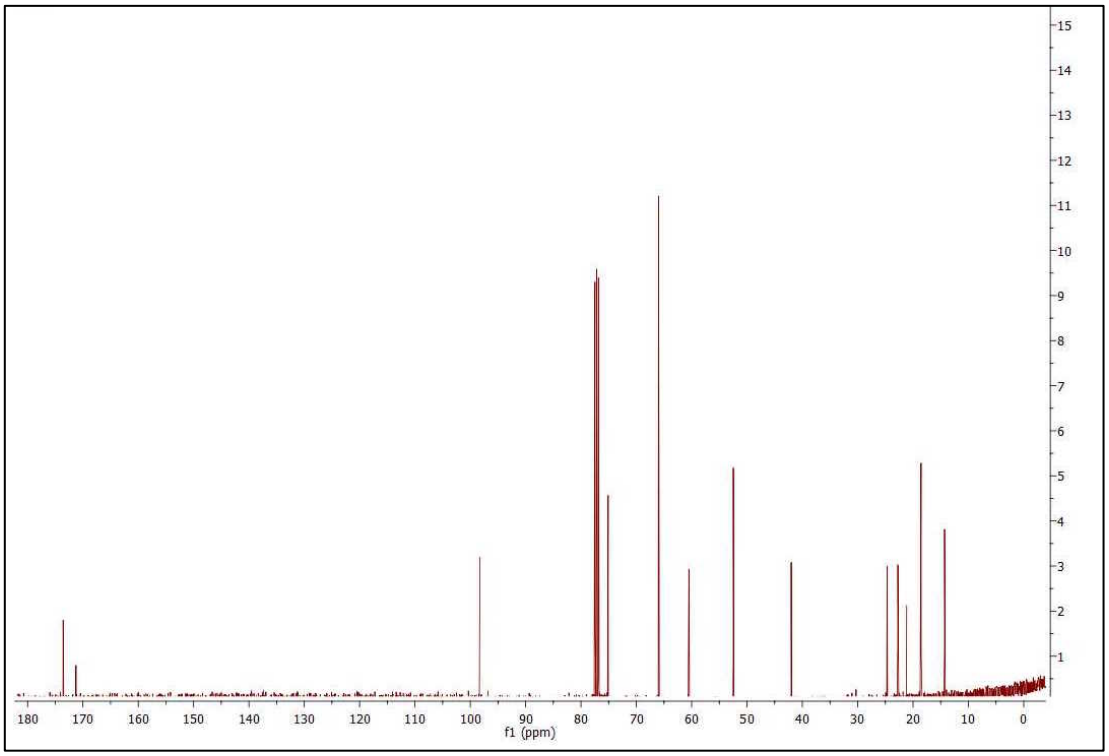

Figure A3: ^{13}C NMR spectrum of molecule 1.

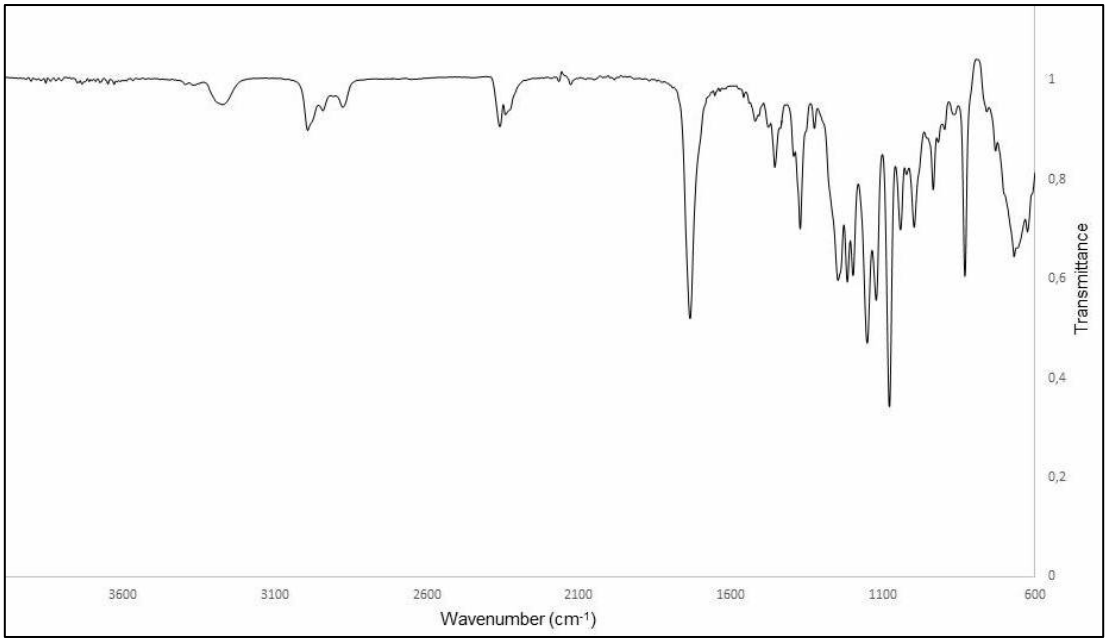

Figure A4: IR spectrum of molecule 1.

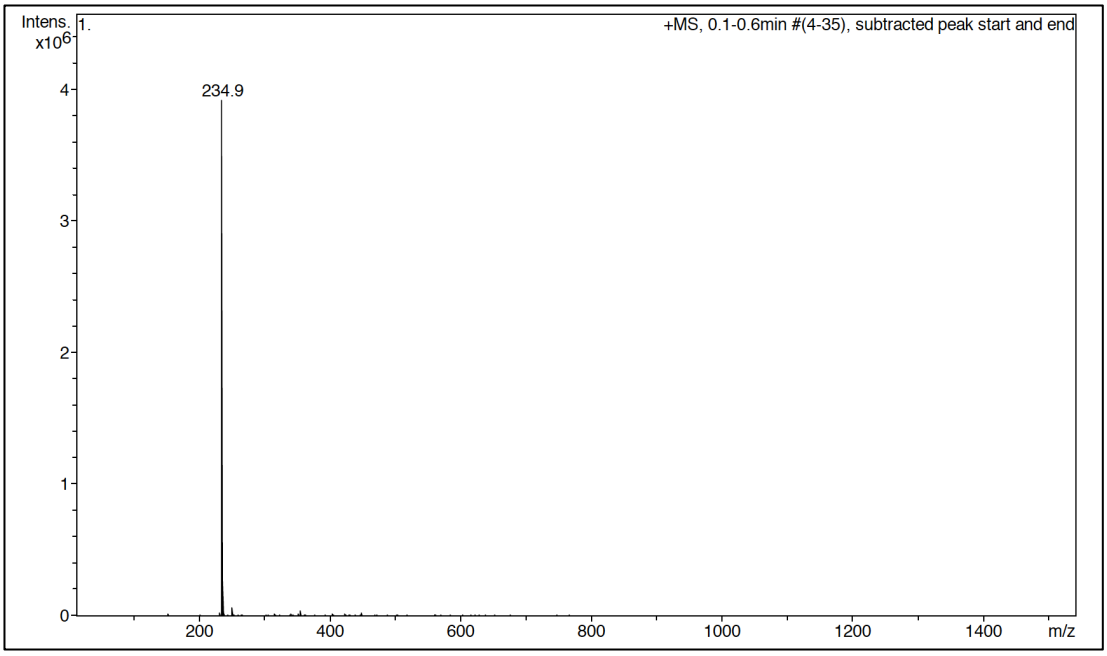

Figure A5: MS spectrum of molecule 1.

≡-[*bis*MPA,G1]-$(OH)_2$ (2)

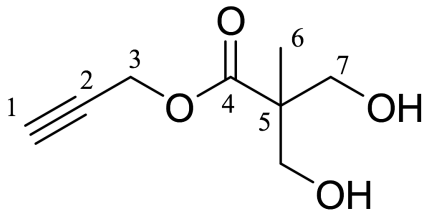

Figure A6: Structure of molecule 2.

Synthetic procedure: hydroxyl group deprotection

≡-[*bis*MPA,G1]-$(ketal)_1$ (17.5 g, 82.45 mmol, 1 eq.) was dissolved into MeOH (150 mL). Dowex® 50 WX2 hydrogen form 50.100 (mesh) (6.25 g, 50 wt%) was added. The Dowex resin was washed with methanol prior to use. The reaction mixture was stirred overnight at room temperature, after which the resin was removed by filtration. The solvent was evaporated under vacuum, obtaining a colourless, viscous oil (9.29 g, 65%).

Characterization

Mw = 172.18 g/mol.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.72 (d, *J* = 2.5 Hz, 2H, H-3), 3.87 (d, *J* = 11.2 Hz, 2H, H-7), 3.69 (d, *J* = 11.2 Hz, 2H, H-7'), 2.96 (s, 2H, -OH), 2.49 (t, *J* = 2.5 Hz, 1H, H-1), 1.09 (s, 3H, H-6).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 175.11 (C-4), 77.48 (C-2), 75.35 (C-1), 67.29 (C-7), 52.54 (C-3), 49.51 (C-5), 17.11 (C-6).

MS (ESI^+) *m/z (%)*: found *194.9 (100)*, calculated for $[C_8H_{12}O_4,Na]^+$ *195.17*.

FTIR (ν_{max}/cm^{-1}, ATR): 3387 (O-H st), 3292 (≡C-H st), 2947 and 2885 (C-H st), 2129 (C≡C st), 1724 (C=O st), 1460 (CH_2, CH_3 δ).

EA (%): *Found*: C, 55.4; H, 7.3; *Calc. for* $C_8H_{12}O_4$: C, 55.8, H, 7.0.

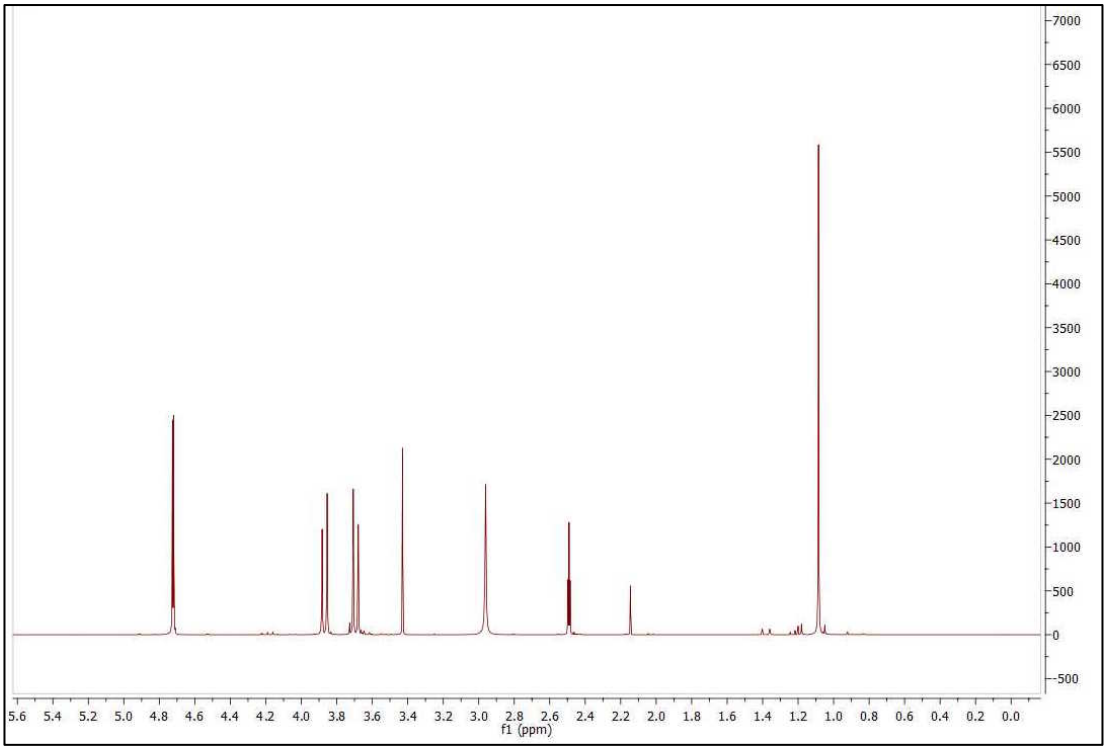

Figure A7: ^{1}H NMR spectrum of molecule 2.

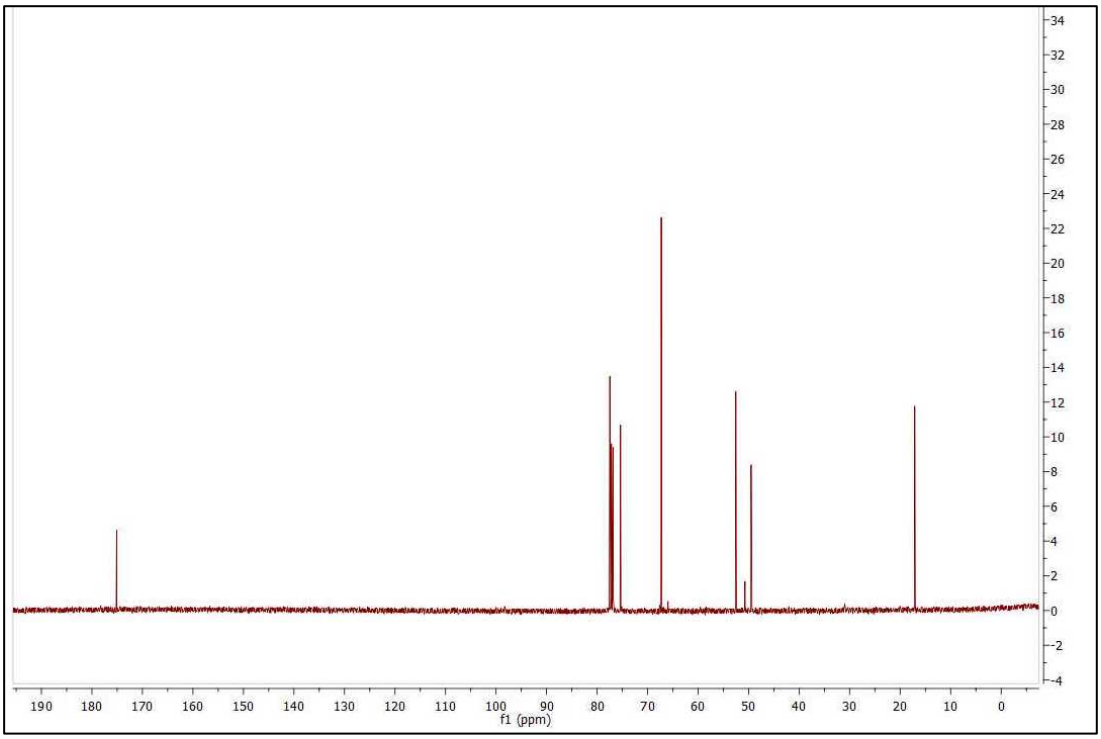

Figure A8: ^{13}C NMR spectrum of molecule 2.

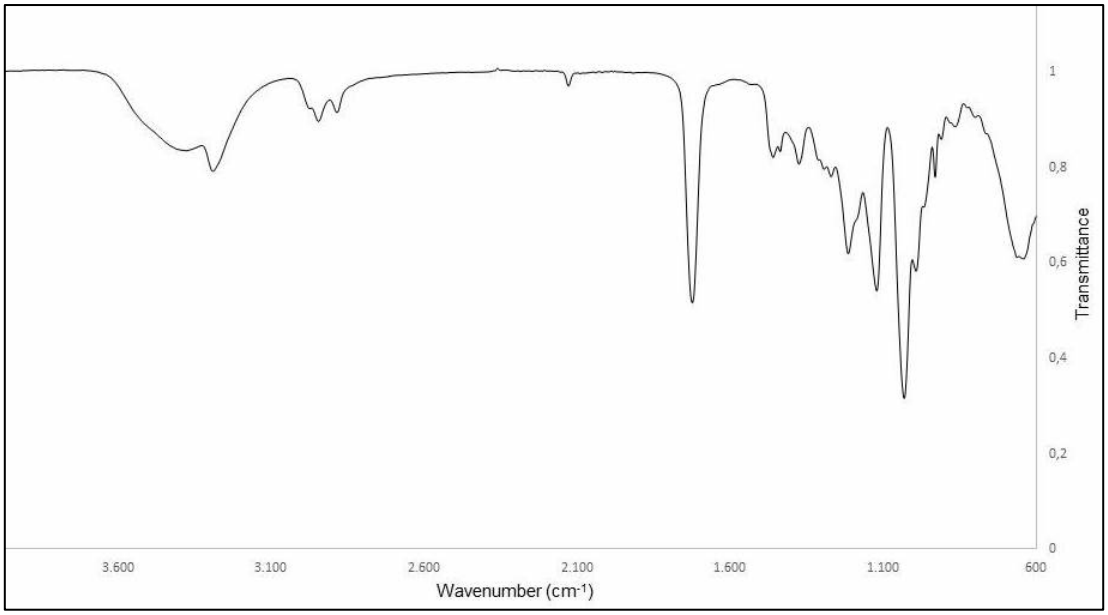

Figure A9: IR spectrum of molecule 2.

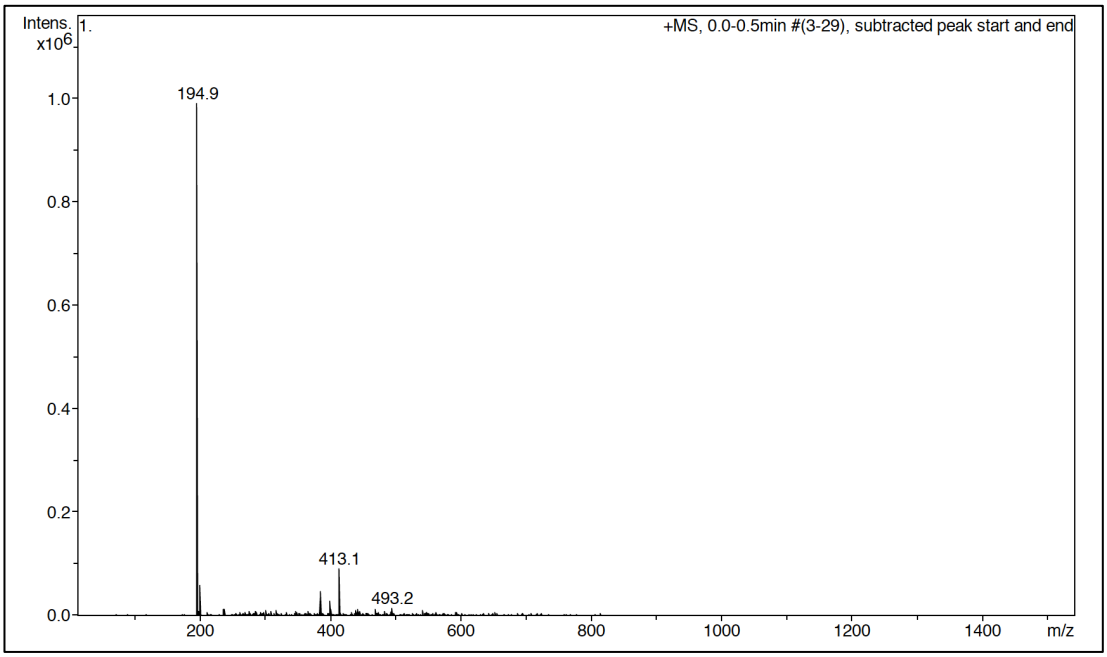

Figure A10: MS spectrum of molecule 2.

≡-[bisGMPA,G1]-(NHBoc)$_2$ (3)

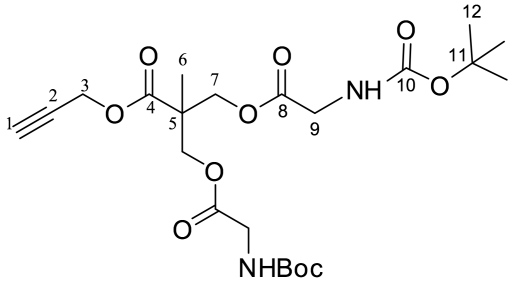

Figure A11: Structure of molecule 3.

Synthetic procedure: Steglich esterification

≡-[*bis*MPA,G1]-(OH)$_2$ (8 g, 46.46 mmol, 1 eq.) was dissolved into dry dichloromethane (220 mL), together with Boc-Gly-OH (20.35 g, 116.15 mmol, 2.5 eq.), DPTS (6.7 g, 22.76 mmol, 0.5 eq.) and DMAP (3.7 g, 30.29 mmol, 0.7 eq.). This mixture was stirred under argon atmosphere and cooled down to 0ºC. A solution of DCC (23.97 g, 116.15 mmol, 2.5 eq.) in dry dichloromethane (30 mL) was added drop wise. The reaction mixture was then allowed to stir under argon atmosphere overnight at room temperature. The white precipitate which appeared, N,N'-dicyclohexylurea (DCU), was removed by filtration. The solvent of the resulting mixture was evaporated under vacuum. A mixture of hexane and ethyl acetate (4:1) was added to provoke further precipitation of the DCU, which was filtered off. The solvent was evaporated under reduced pressure. The crude product was purified through silica gel column chromatography (hexane : ethyl acetate = ramp from 9:1 to 7:3). A yellow paste was obtained (19.1 g, 84.5%).

Characterization

Mw = 486.51 g/mol.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.19 – 5.11 (bs, 2H, -NH), 4.69 (d, *J* = 2.5 Hz, 2H, H-3), 4.28 (dd, *J* = 26.4, 11.1 Hz, 4H, H-7), 3.86 (d, *J* = 5.9 Hz, 4H, H-9), 2.50 (t, *J* = 2.4 Hz, 1H, H-1), 1.40 (s, 18H, H-12), 1.25 (s, 3H, H-6).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 171.73 (C-4), 170.06 (C-8), 155.84 (C-10), 80.11 (C-11), 77.48 (C-2), 75.52 (C-1), 65.68 (C-7), 52.80 (C-3), 46.40 (C-5), 42.31 (C-9), 28.36 (C-12), 17.85 (C-6).

FTIR (ν_{max}/cm^{-1}, ATR): 3383 (N-H st), 3283 (≡C-H st), 2980 and 2939 (C-H st), 2127 (C≡C st), 1744 (C=O st ester), 1699 (C=O st carbamate), 1514 (N-H δ), 1367 (C-N st), 1248 (CO-O st), 1155 (O-C-C st).

MS (ESI$^+$) *m/z (%)*: found *509.0 (100)*, calculated for $[C_{22}H_{34}N_2O_{10},Na]^+$ *509.5*.

EA (%): *Found*: C, 54.28; H, 7.55; N, 5.13; *Calc. for* $C_{22}H_{34}N_2O_{10}$: C, 54.31; H, 7.04; N, 5.76.

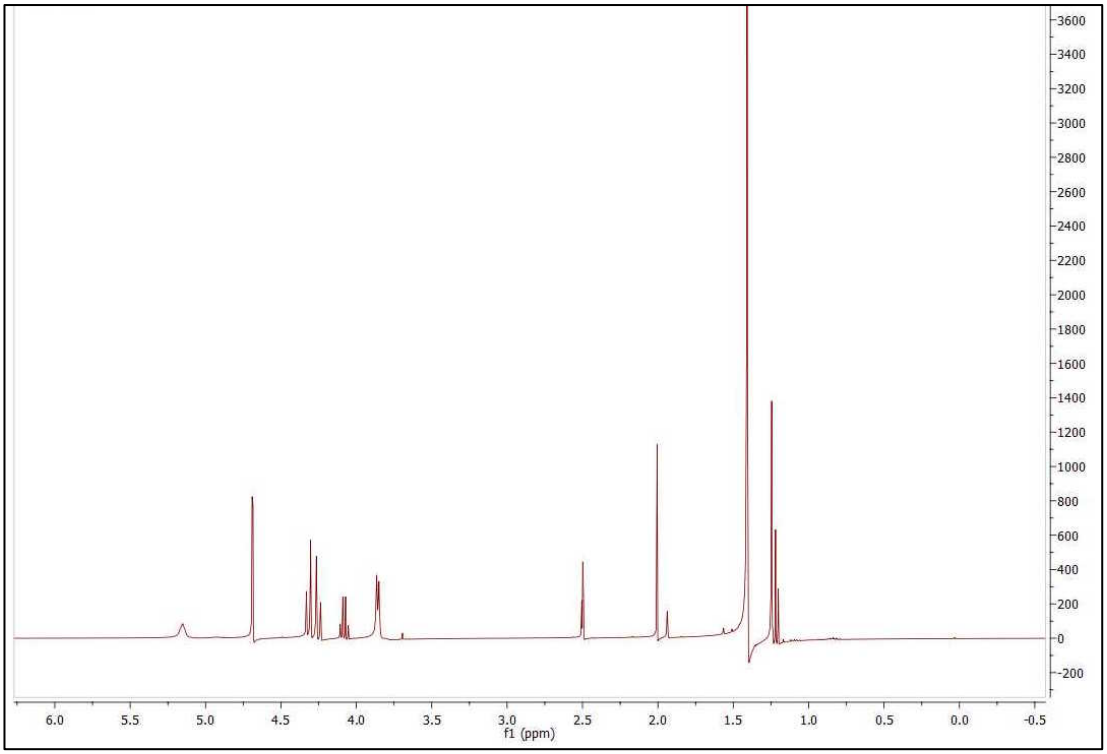

Figure A12: ^{1}H NMR spectrum of molecule 3.

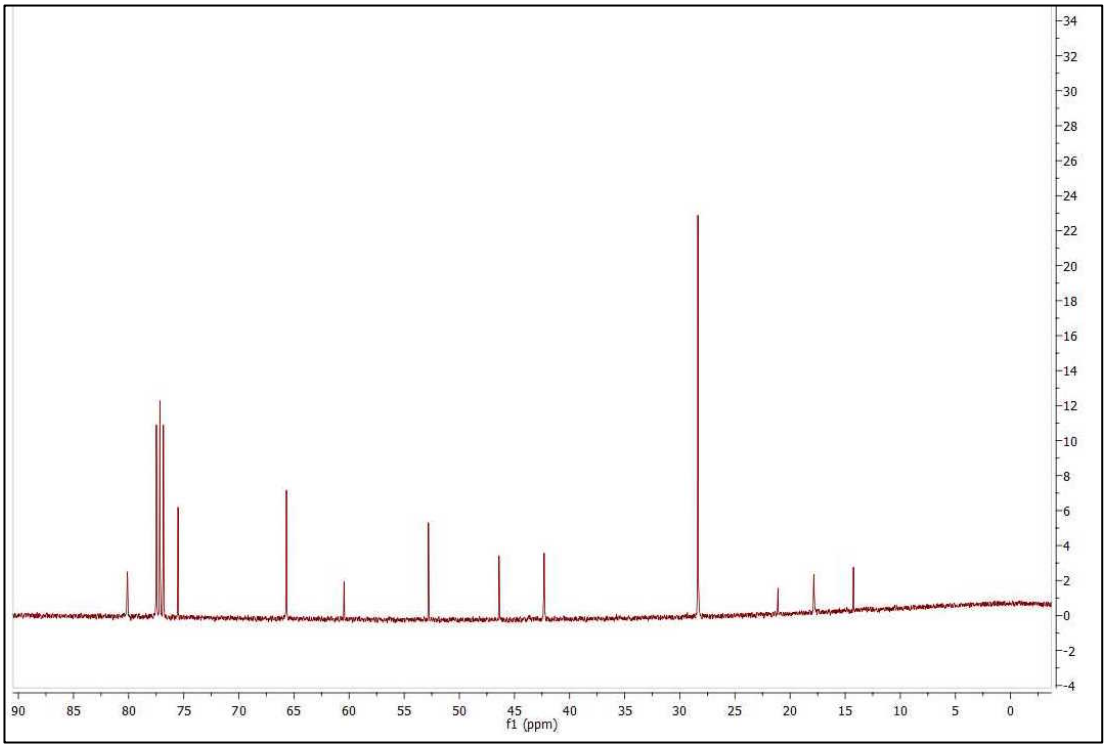

Figure A13: ^{13}C NMR spectrum of molecule 3.

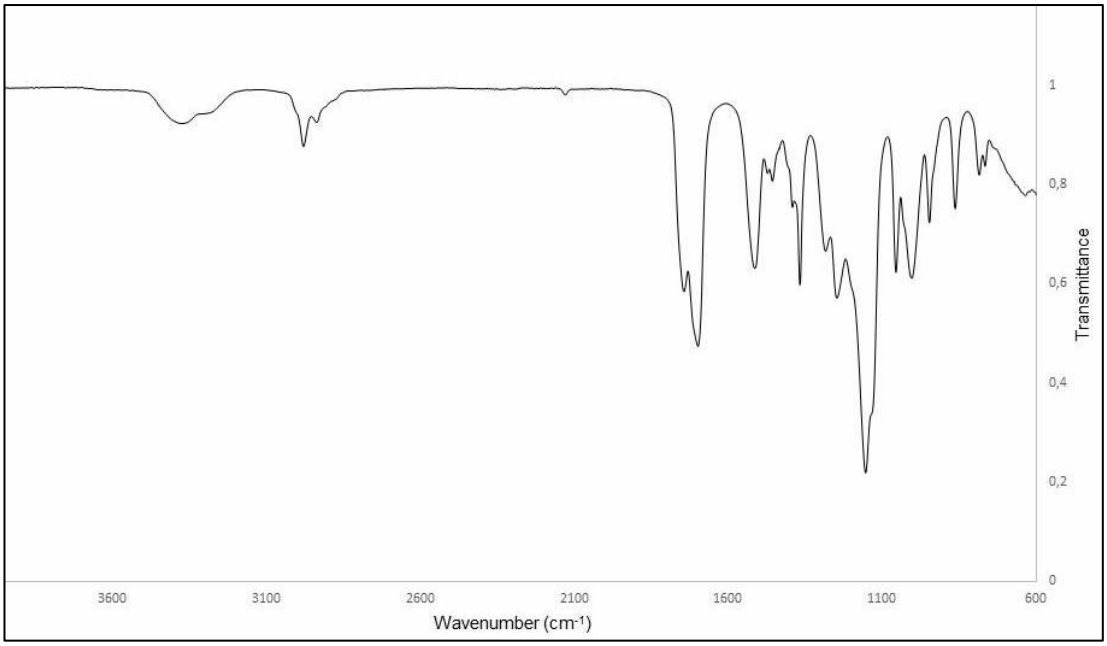

Figure A14: IR spectrum of molecule 3.

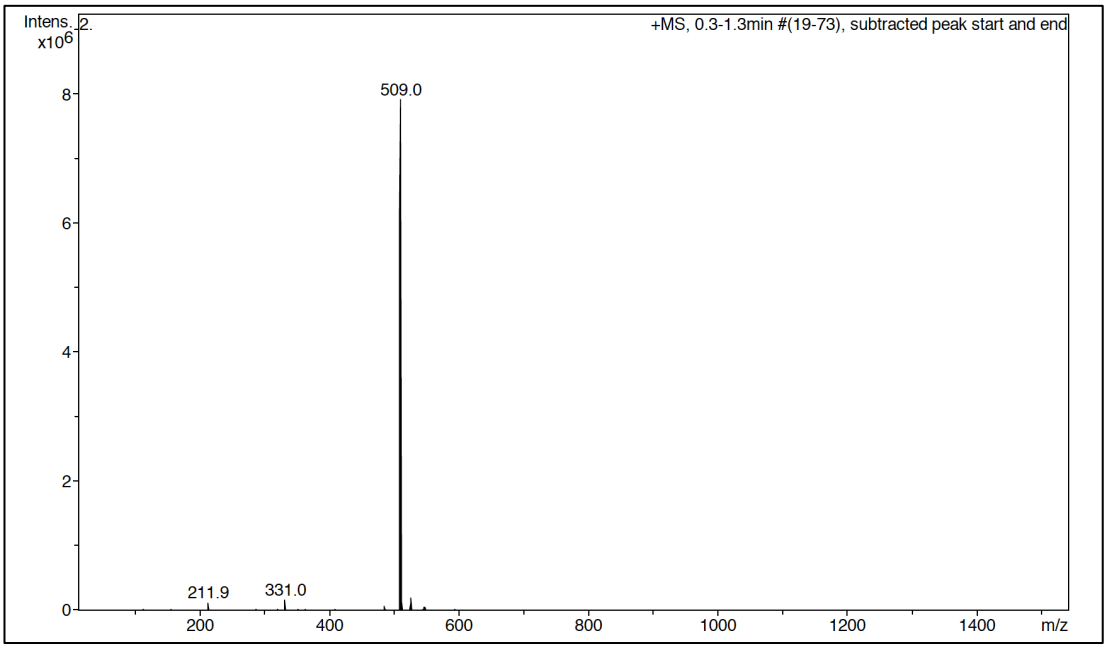

Figure A15: MS spectrum of molecule 3.

≡-[bisGMPA,G1]-($NH_3^+Cl^-$)$_2$ (4)

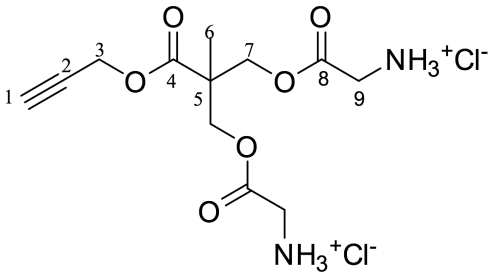

Figure A16: Structure of molecule 4.

Synthetic procedure: deprotection of the amino terminal groups

≡-[bisGMPA,G1]-(NHBoc)$_2$ (9.98 g, 20.51 mmol) was dissolved into a minimal volume of ethyl acetate. Hydrochloric acid into ethyl acetate (3M, 20 mL) was added to it. The reaction mixture was stirred at room temperature during 50 minutes. It was then diluted into ethyl acetate (15 mL) and stirred during another 15 minutes. The hydrochloric acid was removed under vacuum. The reaction mixture was centrifuged and the precipitate recovered. The supernatant was dried separately. It was then used to repeat the reaction, allowing it to stir at room temperature with hydrochloric acid into ethyl acetate (3M, 15 mL) for two and a half hours. Ethyl acetate (15 mL) was added and the mixture was stirred for a half hour. The acid was likewise removed under vacuum, and the reaction mixture centrifuged. The precipitates from both centrifugations were washed twice with pure ethyl acetate, finally yielding a white powder (5.74 g, 78%).

Characterization

Mw = 359.20 g/mol.

^{1}H NMR (400 MHz, DMSO) δ (ppm): 8.56 (bs, -NH), 4.73 (s, 2H, H-3), 4.32 (s, 4H, H-7), 3.77 (s, 4H, H-9), 3.43 (s, 1H, H-1), 1.22 (s, 3H, H-6).

^{13}C NMR (100 MHz, DMSO) δ (ppm): 171.23 (C-4), 167.09 (C-8), 78.18 (C-2), 78.00 (C-1), 65.77 (C-7), 52.83 (C-3), 45.88 (C-5), 39.52 (C-9), 17.19 (C-6).

FTIR (ν_{max}/cm^{-1}, ATR): 3300-2560 (bs N-H$^+$ st), 1761 and 1726 (C=O st ester), 1555 (N-H$^+$ δ), 1502 (CH_2, CH_3 δ), 1406 (C-N st), 1170 (O-C-C st).

MS (ESI$^+$) *m/z (%)*: found *286.9 (100)*, calculated for $[C_{12}H_{18}N_2O_{6,}H]^+$ *287,1*.

EA (%): *Found*: C, 39.72; H, 5.96; N, 7.76; *Calc. for* $C_{12}H_{20}N_2O_6Cl_2$: C, 40.12; H, 5.61; N, 7.8.

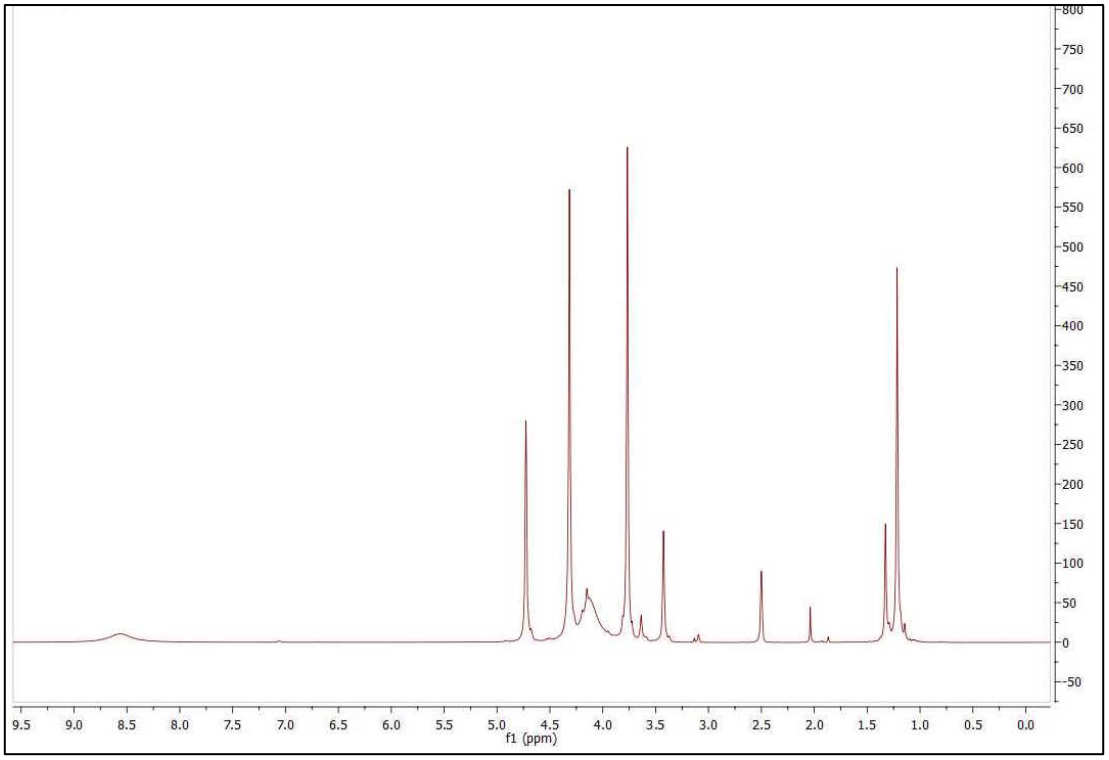

Figure A17: ^{1}H NMR spectrum of molecule 4.

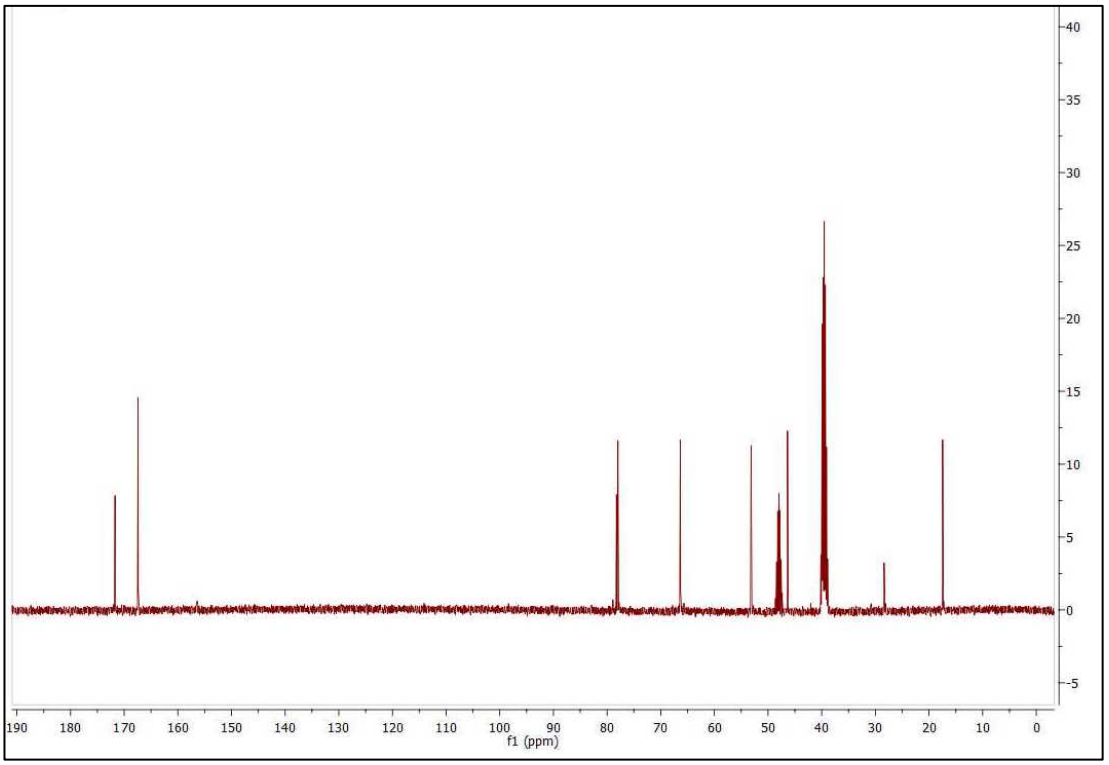

Figure A18: ^{13}C NMR spectrum of molecule 4.

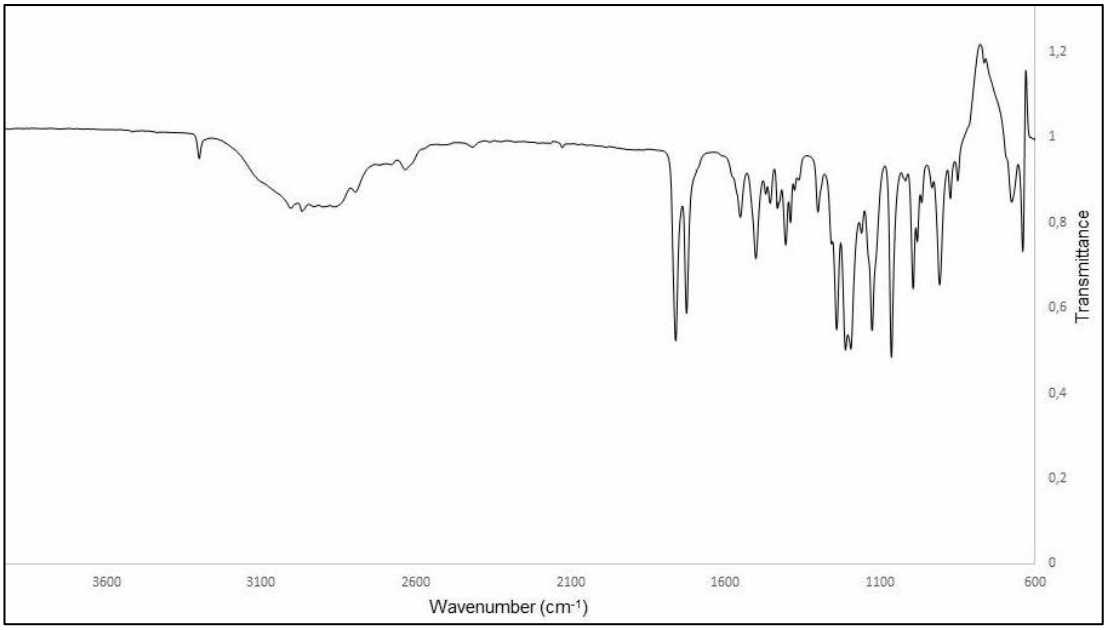

Figure A19: IR spectrum of molecule 4.

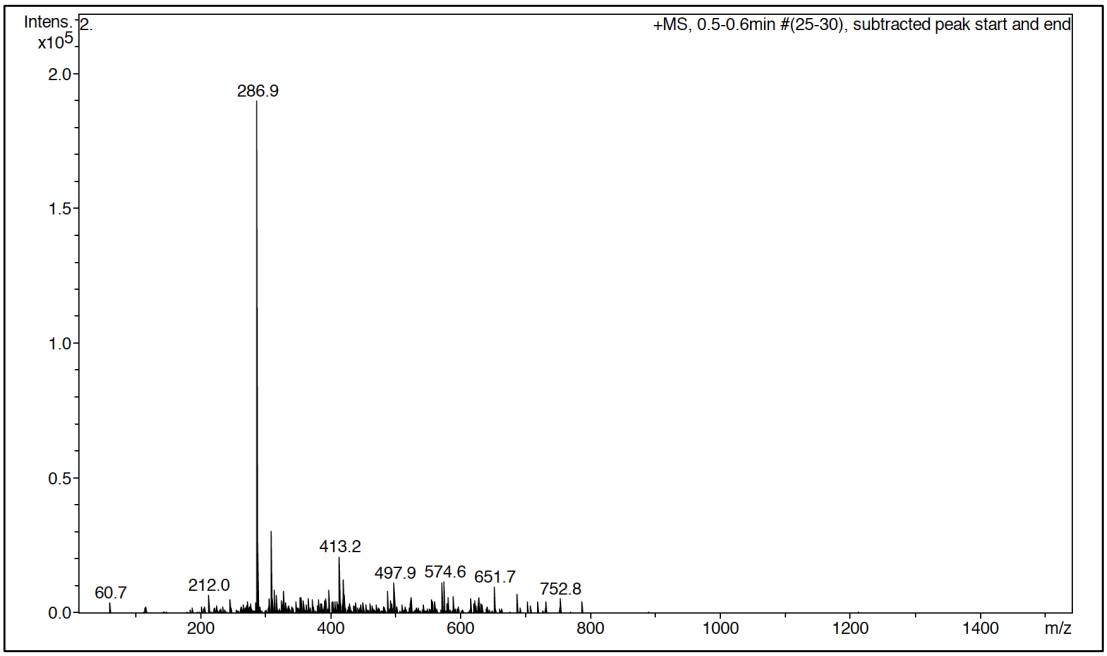

Figure A20: MS spectrum of molecule 4.

≡-[bisGMPA,G0]-(C17)$_2$ (5)

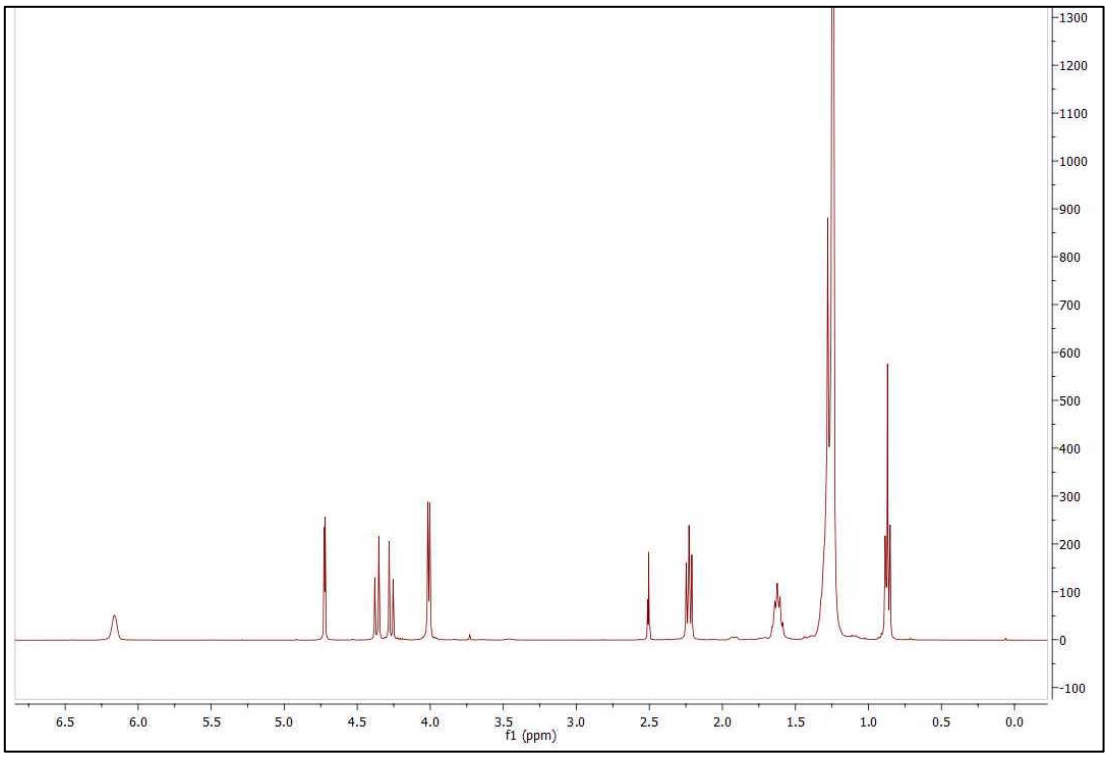

Figure A21: ^{1}H NMR spectrum of molecule 5.

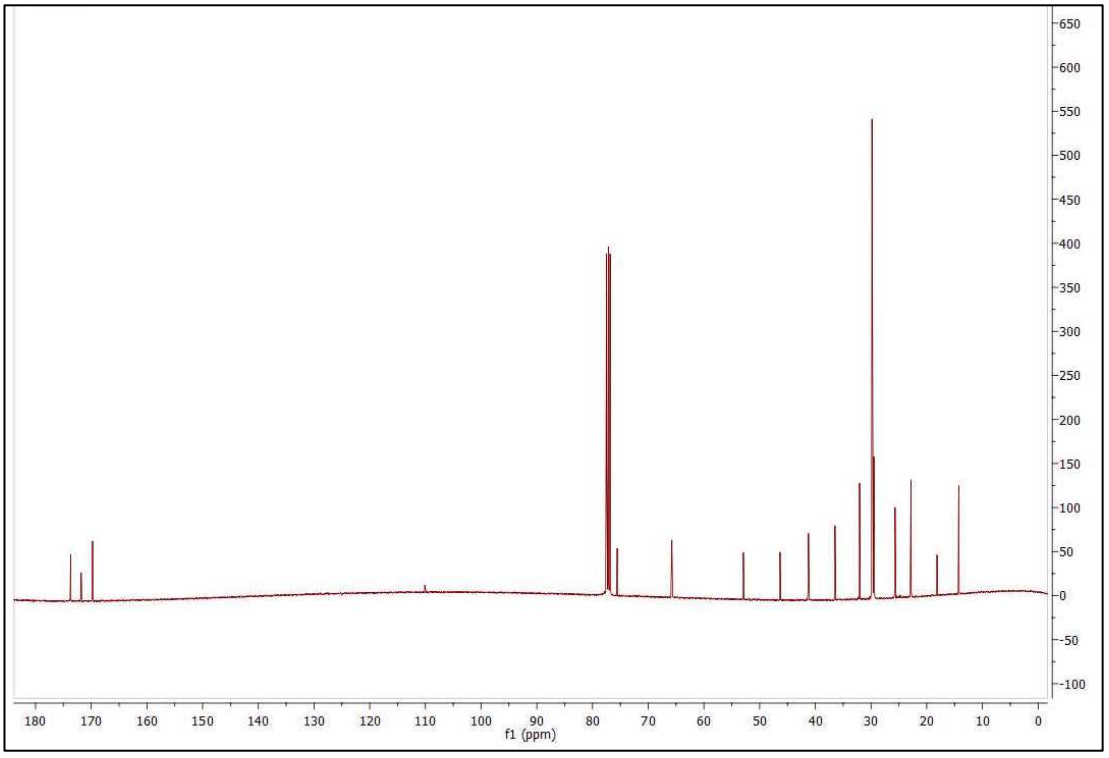

Figure A22: ^{13}C NMR spectrum of molecule 5.

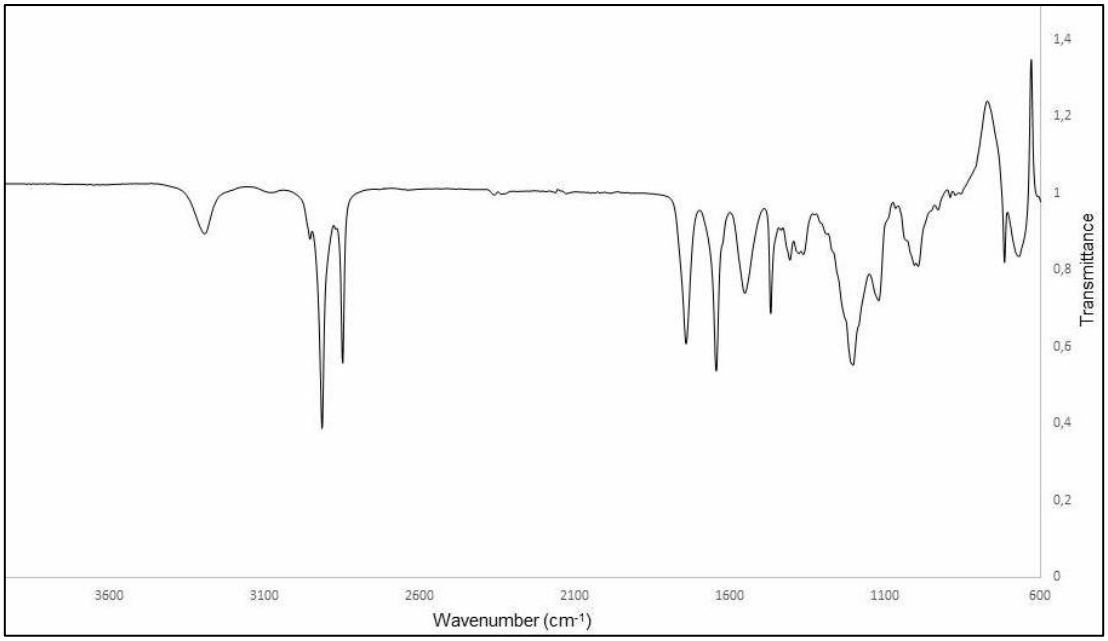

Figure A23: IR spectrum of molecule 5.

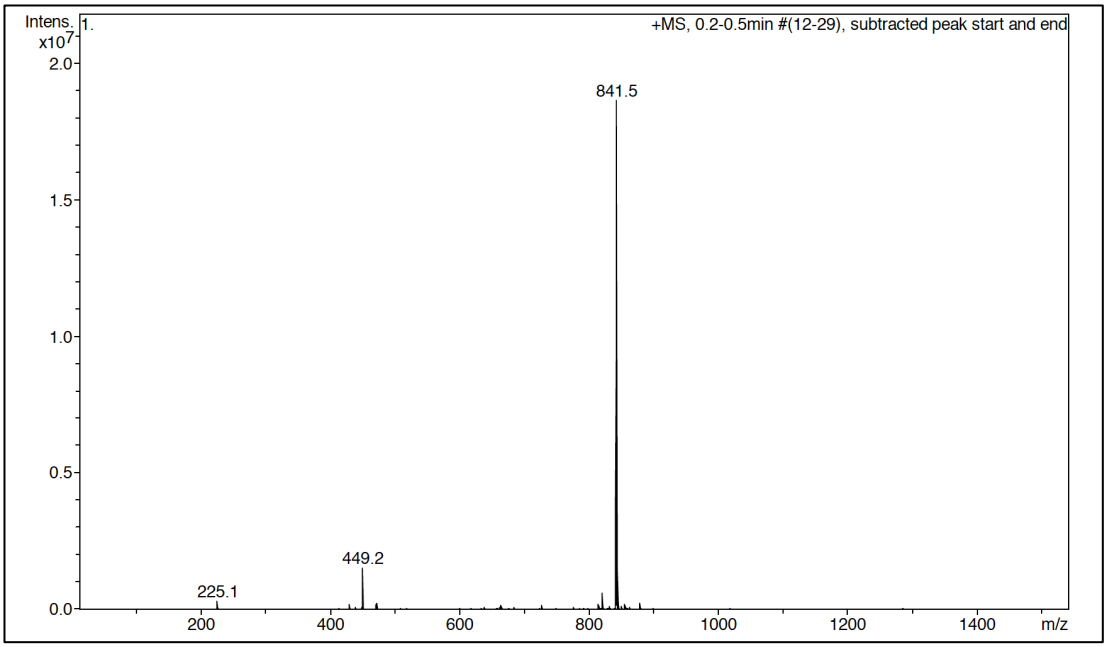

Figure A24: MS spectrum of molecule 5.

(NHBoc)$_8$[*bis*MPA,G3]-[*bis*GMPA,G0](C17)$_2$ (7)

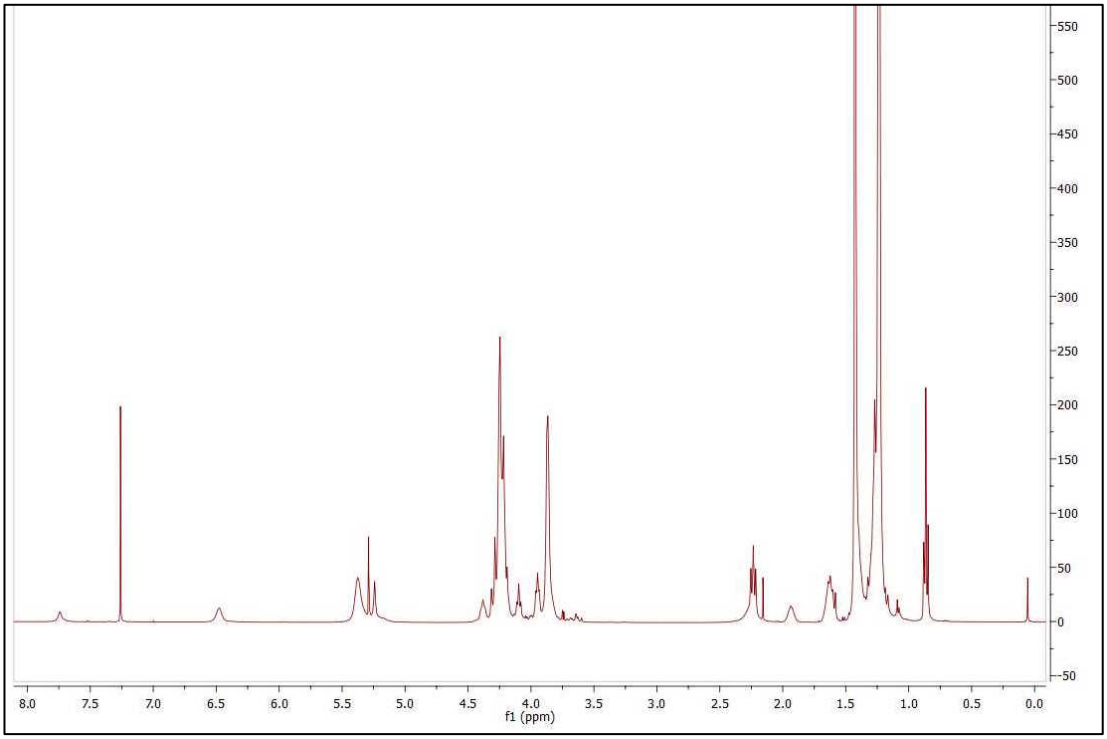

Figure A25: ^{1}H NMR spectrum of molecule 7.

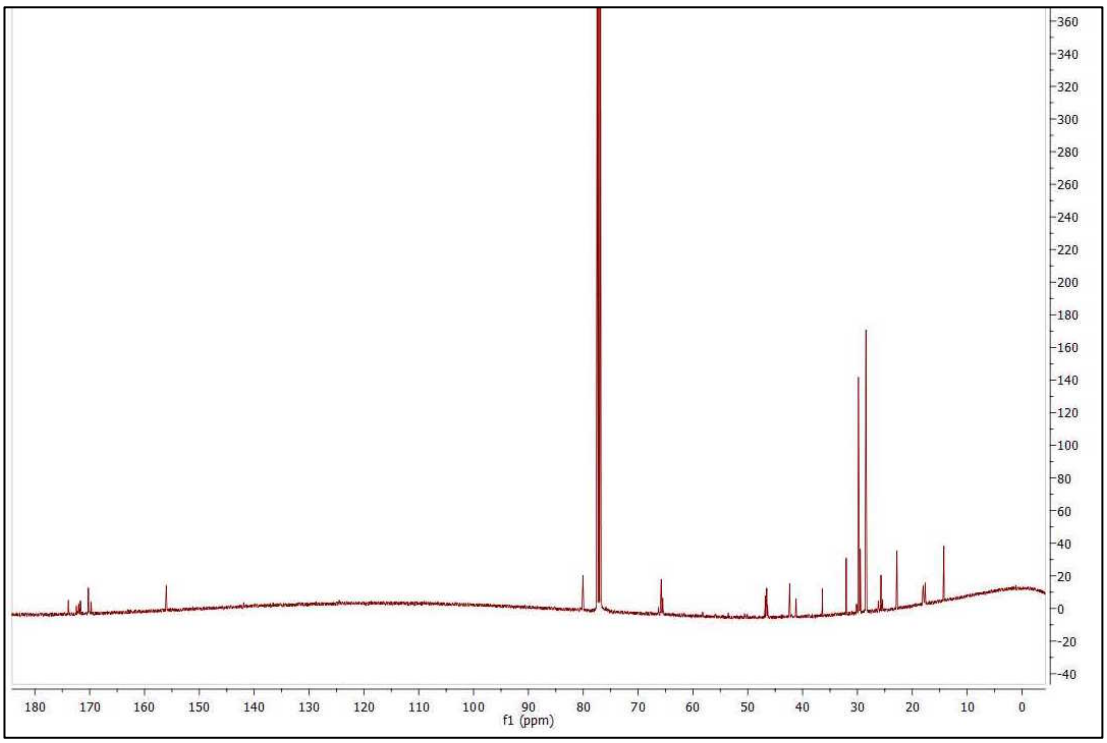

Figure A26: ^{13}C NMR spectrum of molecule 7.

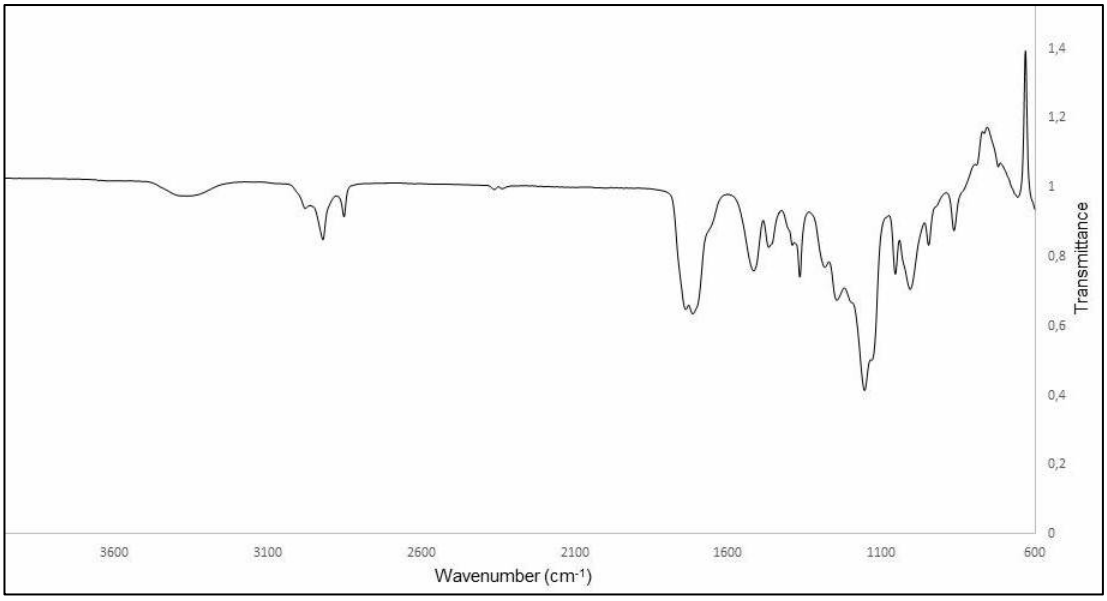

Figure A27: IR spectrum of molecule 7.

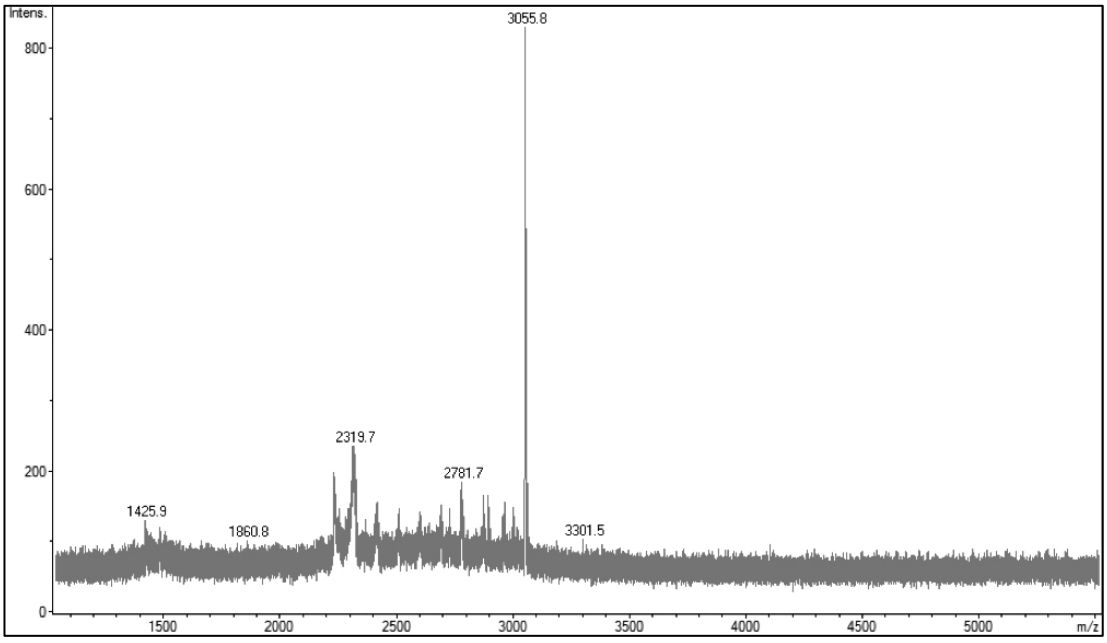

Figure A28: MS spectrum of molecule 7.

$(NH_3^+Cl^-)_8$[*bis*MPA,G3]-[*bis*GMPA,G0]$(C17)_2$ (8)

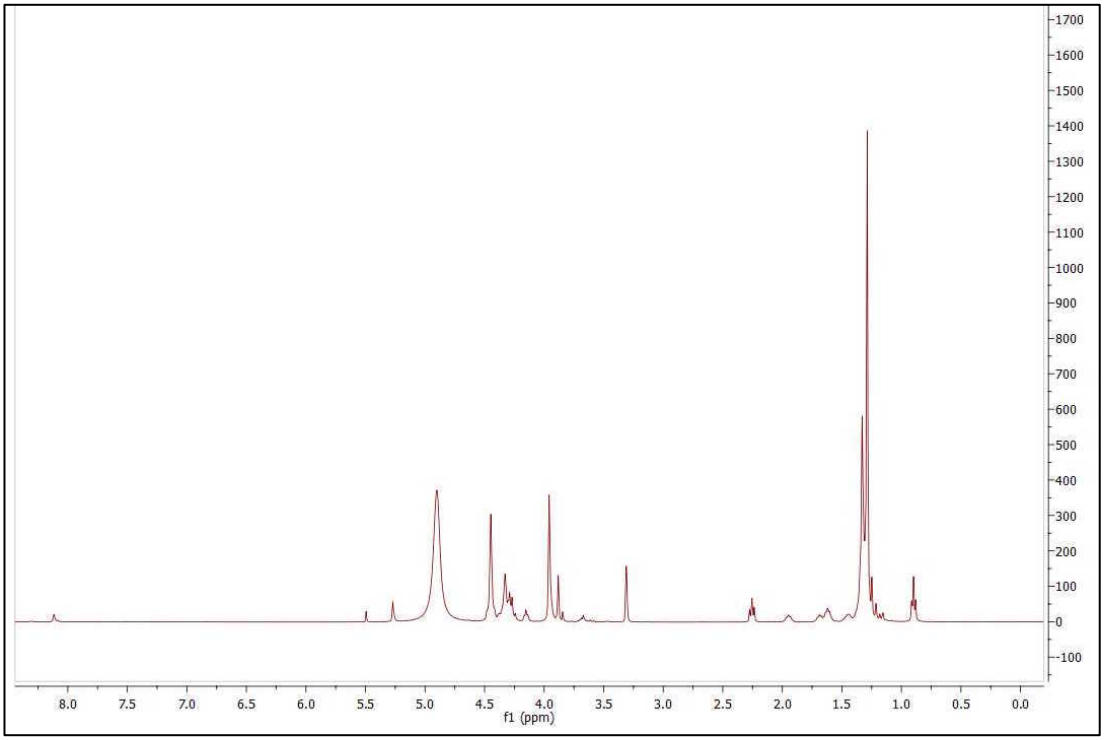

Figure A29: ^{1}H NMR spectrum of molecule 8.

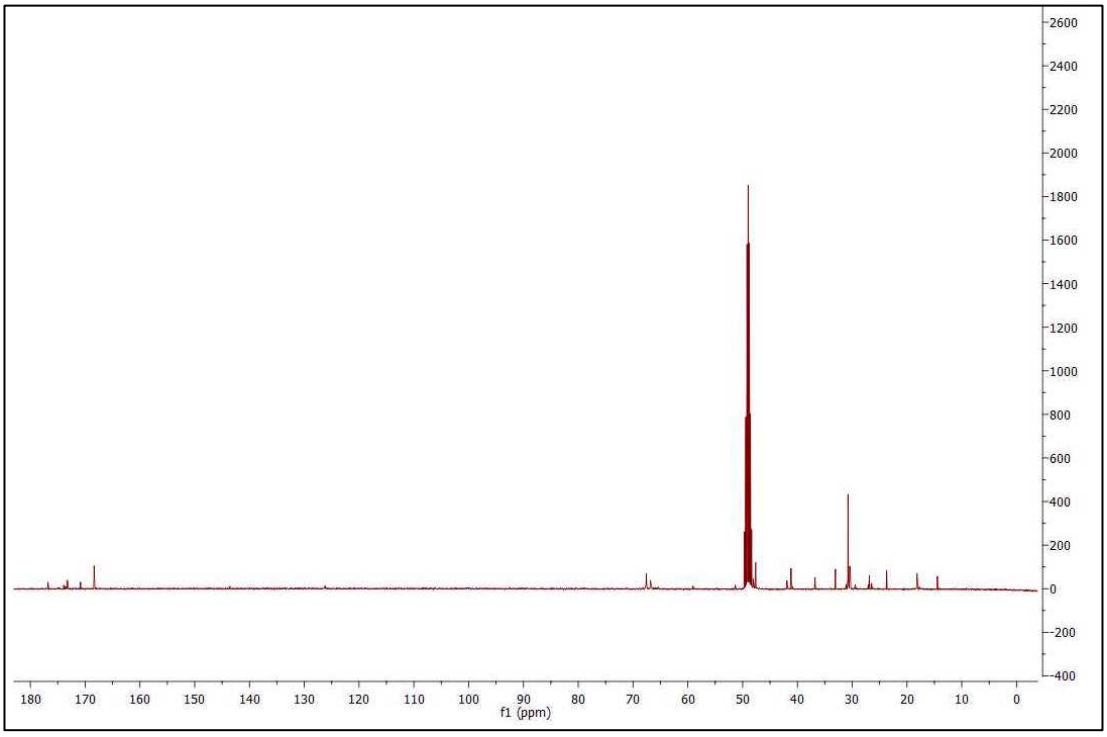

Figure A30: ^{13}C NMR spectrum of molecule 8.

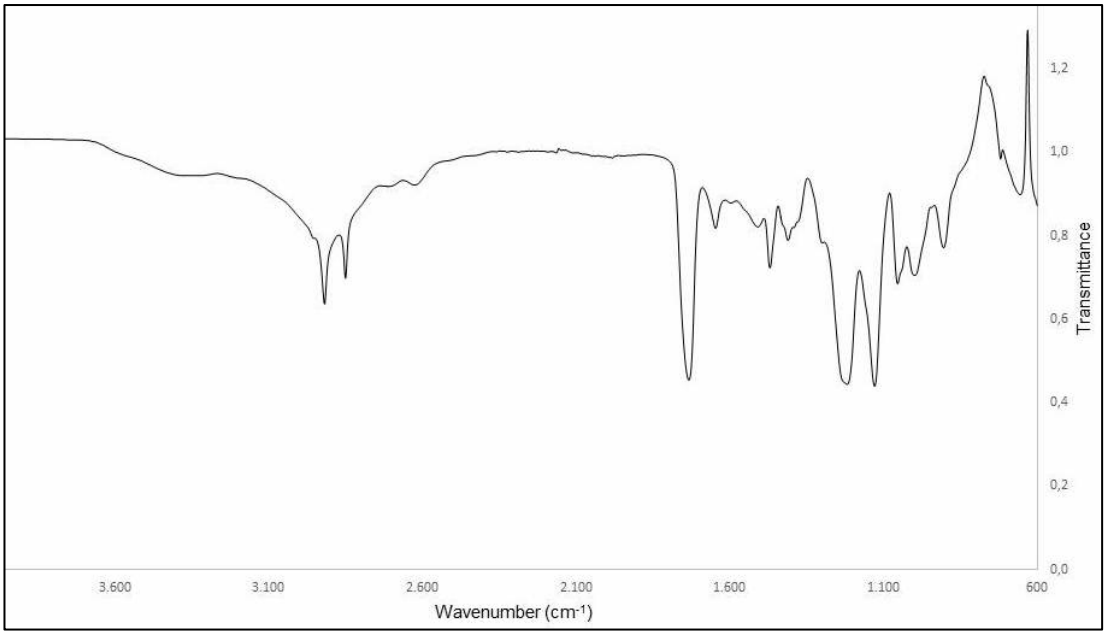

Figure A31: IR spectrum of molecule 8.

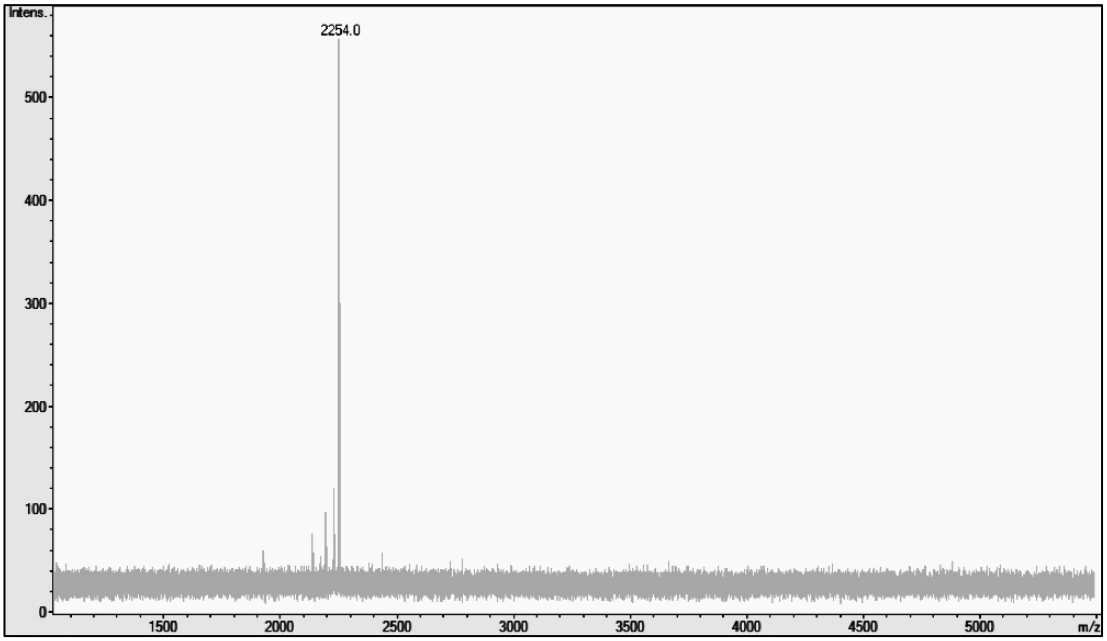

Figure A32: MS spectrum of molecule 8.

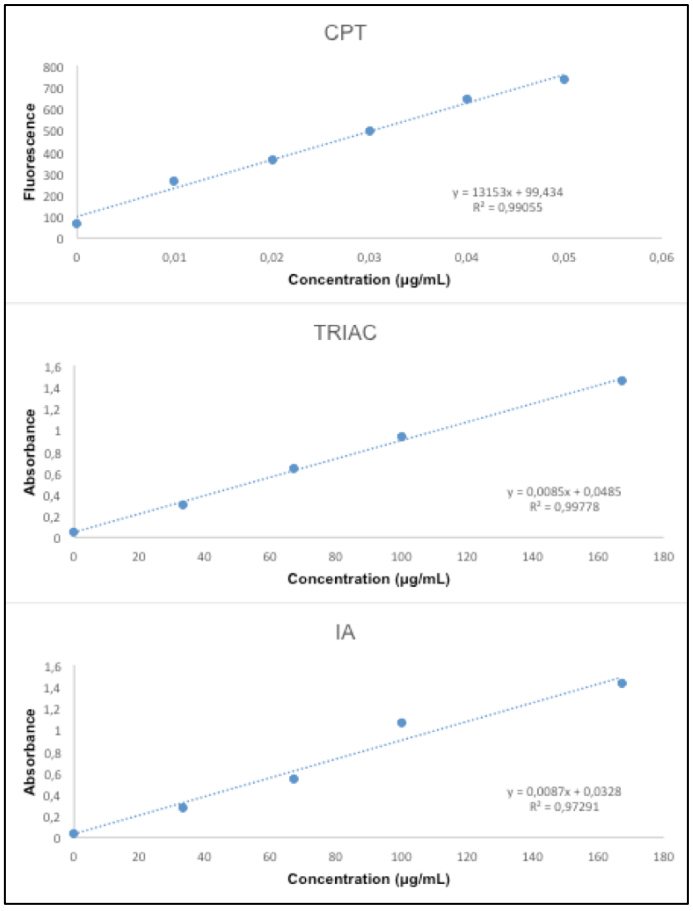

Figure A33: Fluorescence/absorbance calibration curves for CPT, TRIAC and IA (section 4.3).

	Free drug (drug concentration, µM)			Drug/Dendrimer conjugate (drug concentration, µM)			Dendrimer aggregate (dendrimer concentration, µM)					
CPT	2	2	2	2	2	2	13,57	13,57	13,57	-	-	-
	1	1	1	1	1	1	6,78	6,78	6,78	-	-	-
	0,50	0,50	0,50	0,50	0,50	0,50	3,39	3,39	3,39	-	-	-
	0,25	0,25	0,25	0,25	0,25	0,25	1,70	1,70	1,70	-	-	-
	0,13	0,13	0,13	0,13	0,13	0,13	0,85	0,85	0,85	-	-	-
	0,06	0,06	0,06	0,06	0,06	0,06	0,42	0,42	0,42	-	-	-
	0,03	0,03	0,03	0,03	0,03	0,03	0,21	0,21	0,21	-	-	-
	0	0	0	0	0	0	0	0	0	-	-	-
Tiratricol	120	120	120	20	20	20	13,57	13,57	13,57	-	-	-
	60	60	60	10	10	10	6,78	6,78	6,78	-	-	-
	30	30	30	5	5	5	3,39	3,39	3,39	-	-	-
	15,00	15,00	15,00	2,50	2,50	2,50	1,70	1,70	1,70	-	-	-
	7,50	7,50	7,50	1,25	1,25	1,25	0,85	0,85	0,85	-	-	-
	3,75	3,75	3,75	0,63	0,63	0,63	0,42	0,42	0,42	-	-	-
	1,88	1,88	1,88	0,31	0,31	0,31	0,21	0,21	0,21	-	-	-
	0	0	0	0	0	0	0	0	0	-	-	-
Iopanoic acid	240	240	240	20	20	20	13,57	13,57	13,57	-	-	-
	120	120	120	10	10	10	6,78	6,78	6,78	-	-	-
	60	60	60	5	5	5	3,39	3,39	3,39	-	-	-
	30	30	30	2,50	2,50	2,50	1,70	1,70	1,70	-	-	-
	15,00	15,00	15,00	1,25	1,25	1,25	0,85	0,85	0,85	-	-	-
	7,50	7,50	7,50	0,63	0,63	0,63	0,42	0,42	0,42	-	-	-
	3,75	3,75	3,75	0,31	0,31	0,31	0,21	0,21	0,21	-	-	-
	0	0	0	0	0	0	0	0	0	-	-	-

Figure A34: Setting of the plates for the cell viability and drug inhibition assays (section 4.8).