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Electronic Supporting Information

Synthesis, evaluation and modification of heterofunctional polyester dendrimers with internally queued bromide groups

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General Information

Abbreviations

Ac	Acetonide
ATCC	American Tissue Culture Collection
BHP-Diol	2-(bromomethyl)-2-(hydroxymethyl)propane-1,3-diol
Boc	Di-tert-butyl dicarbonate
Boc-PA	Boc protected propargyl amine
br	Broad signal
CsF	Cesium Fluoride
DCC	N,N'-Dicyclohexylcarbodiimide
Dicl	Diclofenac
DCM	Dichloromethane
DCU	1,3-Dicyclohexyl urea
DTCB	Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile
DHB	2,5-dihydroxybenzoic acid
DMAP	2,2-Dimethoxy propane
DMEM	Dulbecco's modified Eagle medium
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtOAc	Ethyl acetate
EtOH	Ethanol
FBS	Fetal bovine serum
HaCaT	Human keratinocyte
HOBt	Hydroxybenzotriazole
MALDI-TOF	Matrix-assisted laser desorption ionization time-of-flight
mPEG	Methoxy poly(ethylene glycol)-propionic acid
NMR	Nuclear magnetic resonance
p-TSA	p-Toluenesulfonic acid
RAW 264.7	Mouse monocyte
SEC	Size Exclusion Chromatography

THF	Tetrahydrofuran

Materials

All chemical reagents and materials were procured from Sigma Aldrich and used without modifications unless stated otherwise. 2-(bromomethyl)-2-(hydroxymethyl)propane-1,3-diol (BHP-Diol) and Dowex™ 50WX2 50-100 (H) were purchased from Thermofisher Scientific and Acros Organics, respectively. Methoxy poly(ethylene glycol)-propionic acid (mPEG) was acquired from Polypure AS. Silica gel for column chromatography was purchased from ICN SiliTech (ICN Biomedicals GmbH, Eschwege, Germany). Bio-beads S-X8 beads and Sephadex LH-20 were bought from Bio-Rad Laboratories, Inc. and Cytiva, respectively.

Human keratinocytes (HaCaT) and mouse monocytes (RAW 264.7) cell lines were obtained from the ATCC (American Tissue Culture Collection). Dulbecco's modified Eagle medium (DMEM) with cell culture supplements including fetal bovine serum (FBS), and penicillin/streptomycin antibiotic mixture were purchased from Thermo Fisher Scientific for the purpose of cell viability studies.

Characterization methods

Nuclear Magnetic Resonance

^{1}H-NMR and ^{13}C-NMR analysis were performed using Bruker AM nuclear magnetic resonance (NMR) and the spectra were recorded at 400 and 101 MHz, respectively. ^{1}H-NMR spectra were obtained after 16 scans with a spectral window of 20 ppm and relaxation delay of 1 second, employing automatic lock and shimming. ^{13}C-NMR spectra were obtained between 256 to 1024 scans with a spectral window of 240 ppm and relaxation delay of 2 seconds. The acquired spectra were analyzed using MestReNova version 14.2.0-26256 (Mestrelab Research S.L 2020).

Matrix-Assisted Laser Desorption/Ionization

Matrix-assisted laser desorption ionization time-of-flight (MALDI TOF) spectra analysis was performed using a Bruker UltrafleXtreme MALDI TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) incorporated with a SmartbeamII laser (355 nm, UV) functioning in the positive mode. SpheriCal™ calibrants (Polymer Factory Sweden AB) were used to perform the calibration. FlexControl and FlexAnalysis Version 3.4 (Bruker Daltonics) were used to record and analyze the mass spectra. Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DTCB) and 2,5-dihydroxybenzoic acid (DHB) were used as the matrices and dissolved in tetrahydrofuran at a concentration of 20 mg ml^{-1}. MALDI sample spot was prepared by the sequential deposition of 1 µl of 1 mg ml^{-1} analyte solution and 2 µl of the matrix solution on an MPT 284 Target ground steel TF Target purchased from Bruker Daltonics. The spectra were recorded in reflector mode with an acceleration voltage of 25 kV and reflector voltage of 26.3 kV. The laser intensity was adjusted between 50-100% to obtain high resolution spectra.

Size Exclusion Chromatography (SEC)

SEC spectra measurements were conducted in dimethylformamide (DMF) as the mobile phase with 0.01 M LiBr at 35 °C. A TOSOH EcoSECHLC-8320GPC instrument constituting an EcoSEC RI detector and three columns (PSS PFG 5 µm; Microguard, 100, and 300 Å) (MW resolving range: 300–100,000 Da) from PSS GmbH was used to run the sample analysis. SEC samples were prepared for precursors and post-functionalized dendrimers within a concentration range of 3-4 mg ml^{-1}. A calibration method was prepared from Narrow linear poly(methyl methacrylate) standards purchased from PSS. The flow rate fluctuations were corrected using toluene as an internal standard. The data and graphs were processed by WinGPC Unity software version 7.2, which were normalized and plotted in Origin 9.1.0 Sr1.

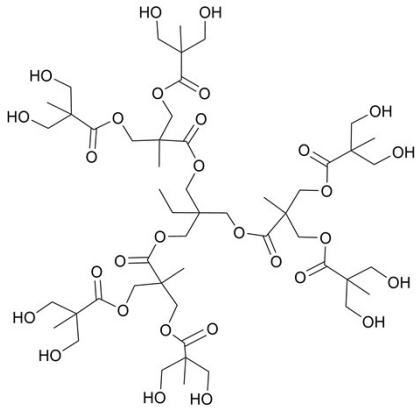
Degradation evaluation

0.5 mM solutions of TMP-G2-$(OH)_{12,}$ TMP-G2-$(Br)_9$-$(OH)_{12}$ and TMP-G2-$(N_3)_9$-$(OH)_{12}$ were prepared in phosphate-citrate buffers (pH 4.4, 5.4, 6.4 and 7.4) containing 10% Dimethyl sulfoxide (DMSO) with 0.1 M KCl and kept at 37°C. The pH of the prepared solutions was adjusted and re-confirmed after addition of dendrimers. Sample aliquots were prepared for the dendrimer solutions and analyzed by MALDI at different time intervals (0h, 1h, 3h, 5h, 9h, 24h, 48h, 5 days, 9 days, 15 days, 22 days and 50 days).

Cytotoxicity assessment

The cytotoxicity of TMP-G2-$(mPEG)_{12}$, TMP-G2-$(Br)_9$-$(mPEG)_{12}$ and TMP-G2-$(N_3)_9$-$(mPEG)_{12}$ was evaluated by the Alamar Blue assay. HaCaT and RAW 264.7 cells were maintained in DMEM containing 10% FBS and 100 units ml^{-1} each of penicillin and streptomycin under 5% carbon dioxide and 37°C. HaCaT cells were detached using trypsin and RAW 264.7 with a scraper. Both the cell lines were transferred and cultured into 96 well plates overnight with 5 x 10^5 cells per 100 µL DMEM. Next day, the old medium was replaced with fresh medium, followed by treatment with reference and test compounds dissolved in DMEM at different concentrations (0.1 µM, 1 µM, 10 µM and 100 µM), added per six wells each. After 24 hours, the wells containing different treatments were replaced with 100 µL of 90:10 fresh medium:Alamar Blue reagent and incubated for 4h, after which the fluorescent intensities were recorded using Tecan Infinite M200 Pro plate reader at a wavelength of 560/590 (excitation/emission) nm. The cytotoxicity measurements were performed thrice (n=3) with fresh stock of dendrimer solutions prepared each time.

Synthesis protocols



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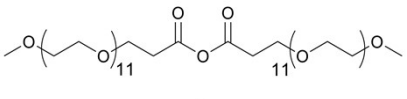

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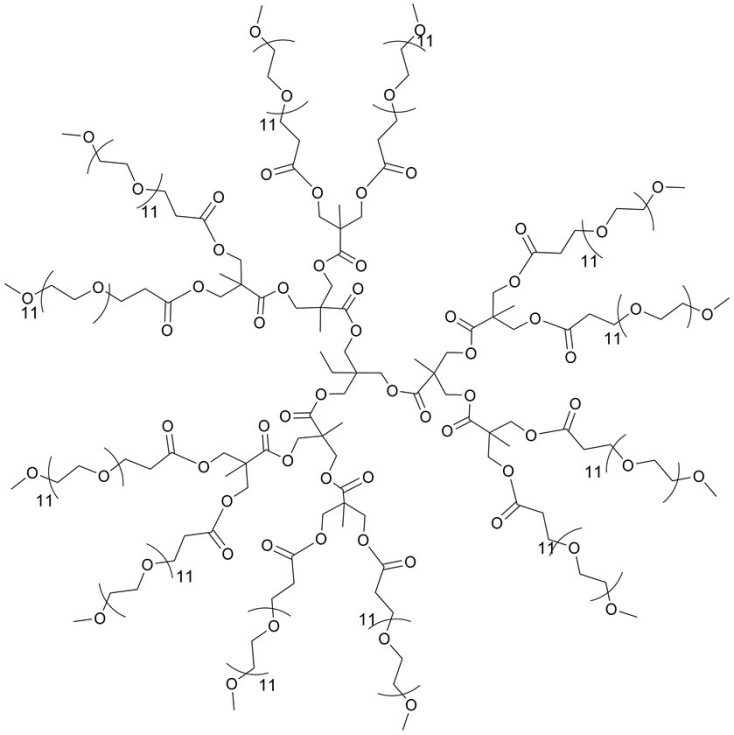
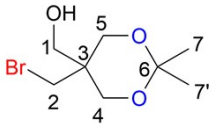


C

The following compounds were synthesized as previously published. A) TMP-G2-$(OH)_{12}$[1] B) mPEG anhydride[2] C) TMP-G2-$(mPEG)_{12}$[2]

Synthesis of AB_2C monomer based on BHP-diol

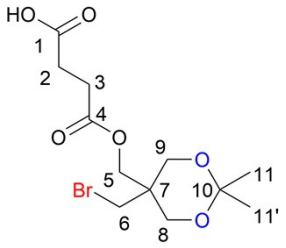
Acetonide protected BHP-diol



Acetonide protected BHP-diol was prepared as previously published[3] with slight modifications in synthetic protocol. Briefly, BHP-Diol (60.43 g, 0.30 mol) was dissolved in 400 mL acetone with consequent addition of 2,2-Dimethoxy propane (DMP) (55.89 mL, 0.455 mol) and catalytic amounts of p-Toluenesulfonic acid (p-TSA) (2.875 g, 0.017 mol) and the reaction was stirred overnight. Next day, the reaction was neutralized with a 50:50 mixture of 30% ammonia solution:EtOH, followed by

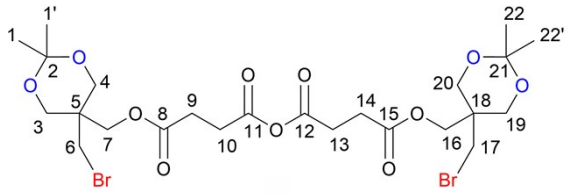
evaporation of acetone. The concentrated compound was dissolved in DCM and washed with H_2O four times, followed by drying with $MgSO_4$. The crude product was eluted in a mixture of 50:50 EtOAc:heptane and obtained as a colorless oil (60.18 g, 83%). $C_8H_{15}BrO_3$ (239.11 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 3.76 (4 H, s, H4, H5), 3.69 (2 H, d, J 5.3, H1), 3.54 (2 H, s, H2), 2.01 (1 H, br, OH), 1.41 (6 H, d, H7, H7').^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 98.77 (C6), 63.77 (C4, C5), 62.69 (C1), 38.85 (C3), 35.64 (C2), 23.77 (C7), 23.66 (C7').

Acid functionalized acetonide protected BHP-diol



Acetonide protected BHP-diol (110.05 g, 0.46 mol) was dissolved in 250 mL dichloromethane (DCM) followed by consequent addition of succinic anhydride (55.76 g, 0.56 mol) and DMAP (11.23 g, 0.09 mol). The reaction was left to stir overnight at room temperature. Next day, the anhydride was quenched with 100 mL tetrahydrofuran (THF) and 45 mL H_2O for an hour. The crude reaction mixture was then washed thrice with $NaHSO_4$ (10 wt%) in water and dried with $MgSO_4$. Acid functionalized acetonide protected BHP-diol was obtained as a white powder after removal of solvent (138.48 g, 89%). $C_{12}H_{19}BrO_6$ (339.18 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.19 (2 H, s, H5), 3.79 – 3.73 (4 H, m, H8, H9), 3.52 (2 H, s, H6), 2.72 – 2.63 (4 H, m, H2, H3), 1.41 (6 H, d, H11, H11').^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 177.22 (C1), 171.80 (C4), 98.95 (C10), 64.41 (C5), 63.69 (C8, C9), 37.63 (C7), 34.74 (C6), 28.94 (C2), 28.91 (C3), 23.88 (C11), 23.41 (C11').

Acetonide protected BHP-diol anhydride



Acid functionalized acetonide protected BHP-diol (81.35 g, 0.24 mol) was dissolved in a DCM (300 mL) containing flask which was placed on an ice bath. Next, a solution of DCC (24.87 g, 0.12 mol) in 300 mL DCM was added to the cold reaction mixture dropwise and the reaction was left running overnight. The following day, the reaction mixture was filtered through celite to remove DCU formed in the reaction. Acetonide protected BHP-diol anhydride was obtained as a colorless oil after removal of DCM (62 g, 78%). $C_{24}H_{36}Br_2O_{11}$ (660.35 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.23 (4 H, s, H7, H16), 3.79 (8 H, s, H3, H4, H19, H20), 3.54 (4 H, s, H6, H17), 2.83 (4 H, dd, J 7.1, 5.3, H9, H10), 2.77 – 2.67 (4 H, m, H13, H14), 1.44 (12 H, d, H1, H1', H22, H22').^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.33 (C8, C15), 167.93 (C11, C12), 98.91 (C2, C21), 64.56 (C7, C16), 63.69 (C3, C4, C19, C20), 37.59 (C5, C18), 34.73 (C6, C17), 30.29 (C9, C10), 28.48 (C13, C14), 23.67 (C1, C1'), 23.62 (C22, C22').

Synthesis of heterofunctional polyester dendrimers with bromide and azide groups

General esterification procedure for the synthesis of acetonide protected bromide dendrimers

The hydroxyl-functional compound is dissolved in DCM, to which bases including DMAP (0.2 eq/OH) and pyridine (5 eq/OH) are added. Consequently, acetonide protected BHP-diol anhydride (1.5 eq/OH) dissolved in DCM is slowly added to the reaction mixture and the reaction proceeds at room temperature overnight. The reaction progress is monitored through NMR and MALDI-TOF. Upon completion of the reaction, the excess of anhydride is quenched with a mixture of THF and water for 1 h. The reaction mixture is then diluted with DCM and washed 3-4 times with aqueous solutions of 10% $NaHSO_4$, 10% $NaHCO_3$ and once with brine before drying with $MgSO_4$. After washes, the crude product is eluted in a suitable eluent mixture to yield the pure compound.

General azidation procedure for the synthesis of acetonide protected azide dendrimers

The acetonide protected bromide dendrimer is dissolved in DMSO at 85°C, to which an excess of NaN_3 (5 eq/Br) is added. The reaction proceeds with constant heating and stirring at 85°C overnight. The complete azidation of bromide derivative is monitored with NMR and MALDI-TOF. Upon completion of the reaction, the reaction mixture is allowed to cool down to room temperature and H_2O is added to it. The acetonide-protected azide dendrimer is extracted from the DMSO:H_2O mixture to the ether phase repeatedly. The ether containing azide derivative is washed twice with water to remove any residual DMSO and concentrated.

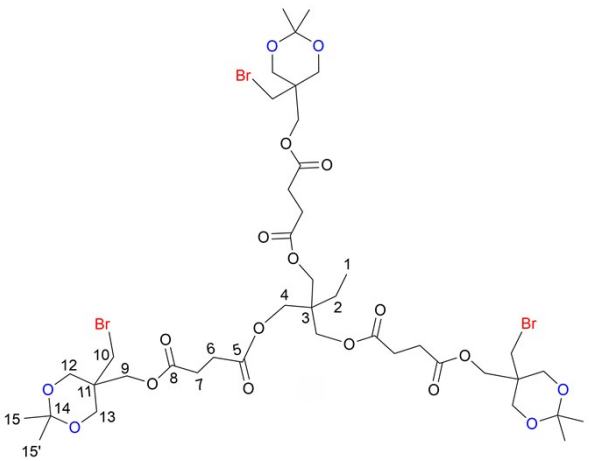
Hazards associated with NaN_3 during azidation

It is necessary to be cautious when handling NaN_3 during the azidation procedure as its highly explosive. NaN_3 can also be absorbed by the body through skin contact, inhalation and ingestion which can cause acutely toxicity to the central nervous system and cardiovascular system.

General deprotection procedure for the synthesis of hydroxyl-functional dendrimers

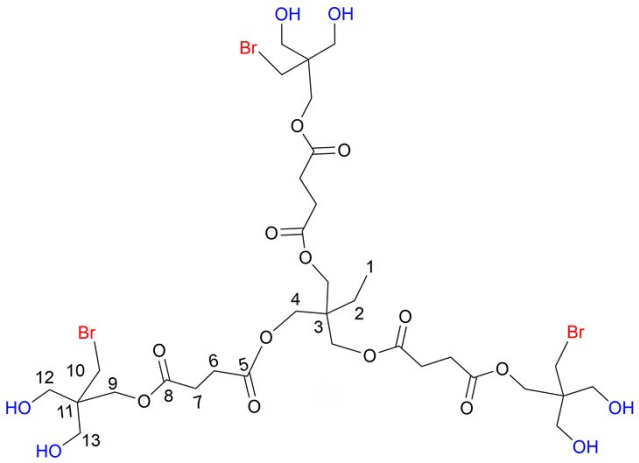
The acetonide-protected dendrimer is dissolved in MeOH, in the presence of an acidic resin Dowex™ 50WX2 50-100 (H). The deprotection reaction proceeds overnight with constant stirring and heating at 45°C overnight. The reaction is checked for completion using NMR and MALDI-TOF. Upon reaction completion, the DOWEX is filtered off and MeOH is evaporated to obtain the deprotected derivative.

TMP-G1-$(Br)_3$-$(Ac)_3$



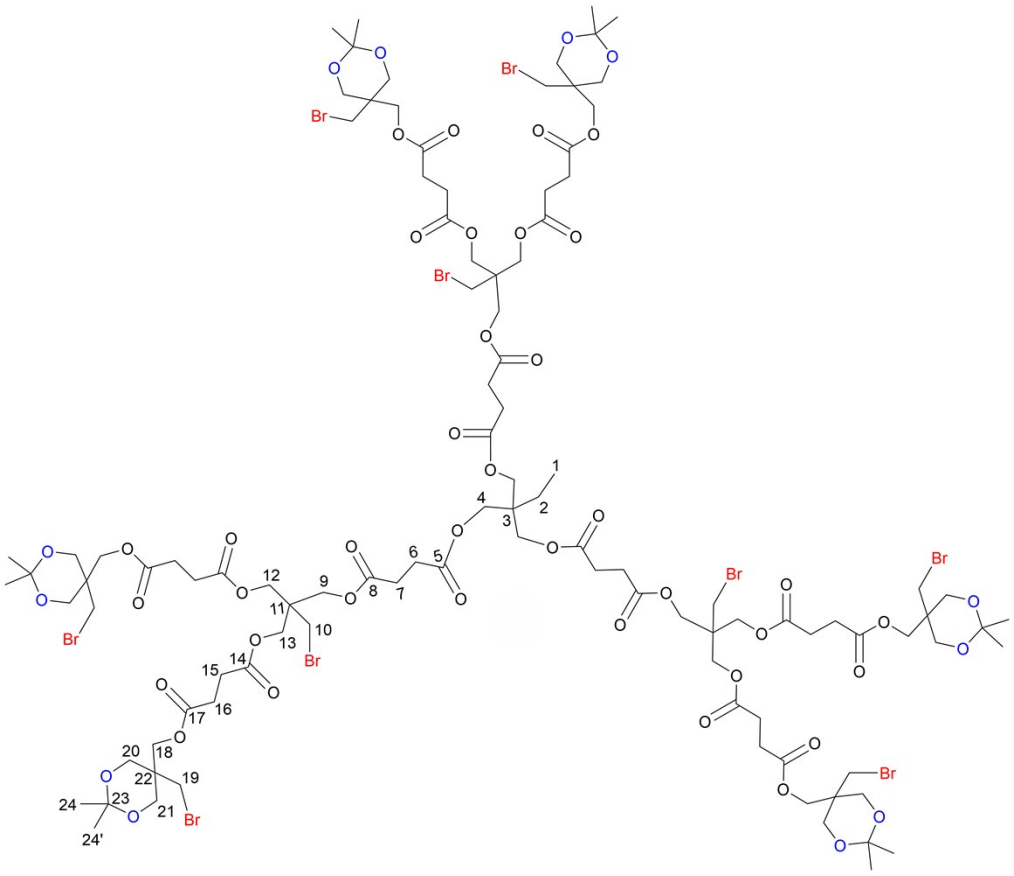
TMP-G1-$(Br)_3$-$(Ac)_3$ was synthesized according to the general esterification procedure, using the following reagents with their stated amounts: TMP (2.03 g, 15.14 mmol), acetonide protected BHP-diol anhydride (45 g, 68.15 mmol), DMAP (1.11 g, 9.08 mmol) and Pyridine (18.29 mL, 227.15 mmol). After the washing steps, TMP-G1-$(Br)_3$-$(Ac)_3$ was eluted in a mixture of 50:50 EtOAc:heptane and obtained as a colorless oil (16.0 g, 96%). $C_{42}H_{65}Br_3O_{18}$ (1097.68 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.18 (6 H, s, H9), 4.04 (6 H, s, H4), 3.77 (12 H, s, H12, H13), 3.53 (6 H, s, H10), 2.65 (12 H, s, H6, H7), 1.48 (2 H, d, J 7.6, H2), 1.42 (18 H, s, H15, H15'), 0.88 (3 H, t, J 7.5, H1).^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.96 (C5), 171.87 (C8), 98.90 (C14), 64.38 (C9), 64.19 (C4), 63.71 (C13), 40.90 (C3), 37.62 (C11), 34.88 (C10), 29.03 (C6), 28.97 (C7), 23.94 (C15), 23.42 (C15'), 23.08 (C2), 7.50 (C1). MALDI: Calc. [M+Na^+] = 1120.67 Da, Found [M+Na^+] = 1120.23 Da. SEC (DMF) M_n = 1041.7 g mol^{-1}, M_w= 1057.5 g mol^{-1}, Đ = 1.01.

TMP-G1-$(Br)_3$-$(OH)_6$



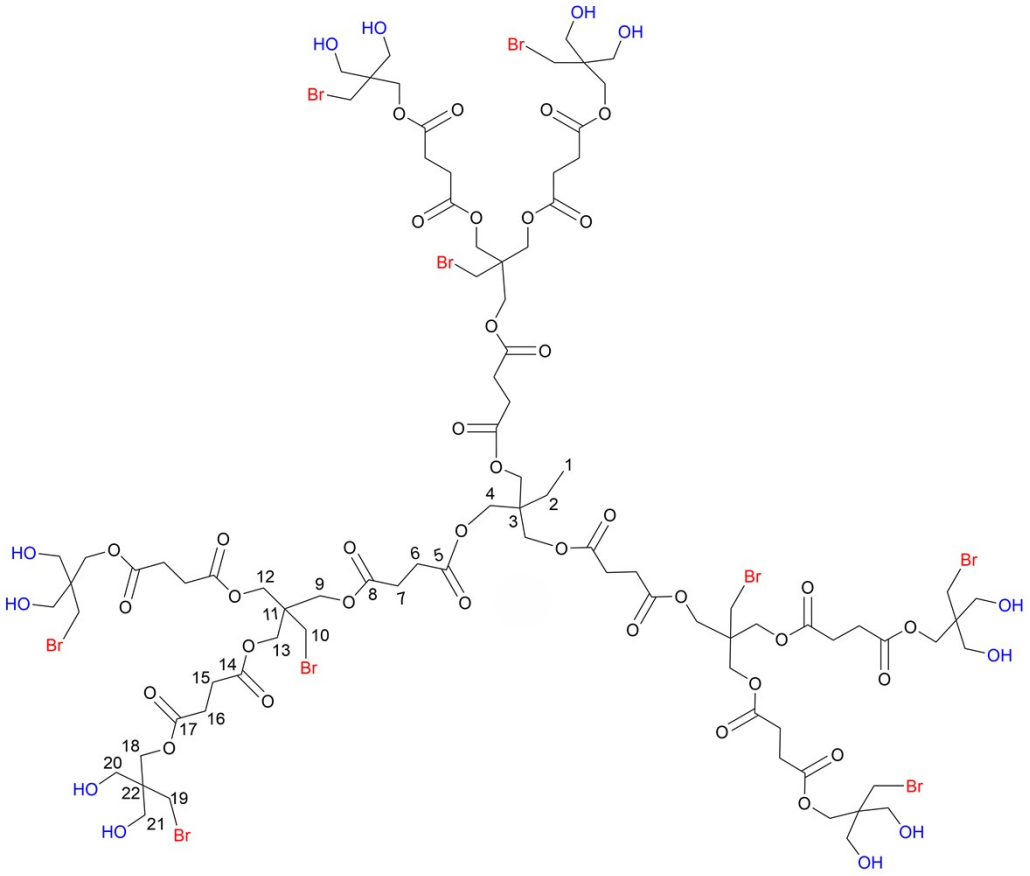
TMP-G1-$(Br)_3$-$(OH)_6$ was synthesized according to the general deprotection procedure, using the following reagents with their stated amounts: TMP-G1-$(Br)_3$-$(Ac)_3$ (8.00 g, 7.3 mmol) and DOWEX (8.00 g). TMP-G1-$(Br)_3$-$(OH)_6$ was obtained as a viscous oil after concentration (6 g, 84%). $C_{33}H_{53}Br_3O_{18}$ (977.48 g/mol). ^{1}H-NMR (400 MHz, CD_3OD) δ/ppm: 4.09 (12 H, d, J 9.1, H4, H9), 3.59 (12 H, s, H12, H13), 3.52 (6 H, s, H10), 2.68 (12 H, d, J 1.5, H6, H7), 1.51 (2 H, q, J 7.6, H2), 0.92 (3 H, t, J 7.6, H1).^{13}C-NMR (101 MHz, CD_3OD) δ/ppm: 173.87 (C5), 173.81 (C8), 65.14 (C4), 64.79 (C9), 61.91 (C12, C13), 45.70 (C11), 42.12 (C3), 35.09 (C10), 29.92 (C6), 29.87 (C7), 23.98 (C2), 7.71 (C1). MALDI: Calc. [M+Na^+] = 1000.47 Da, Found [M+Na^+] = 1000.10 Da. SEC (DMF) M_n = 1131 g mol^{-1}, M_w= 1158 g mol^{-1}, Đ = 1.02.

TMP-G2-$(Br)_9$-$(Ac)_6$



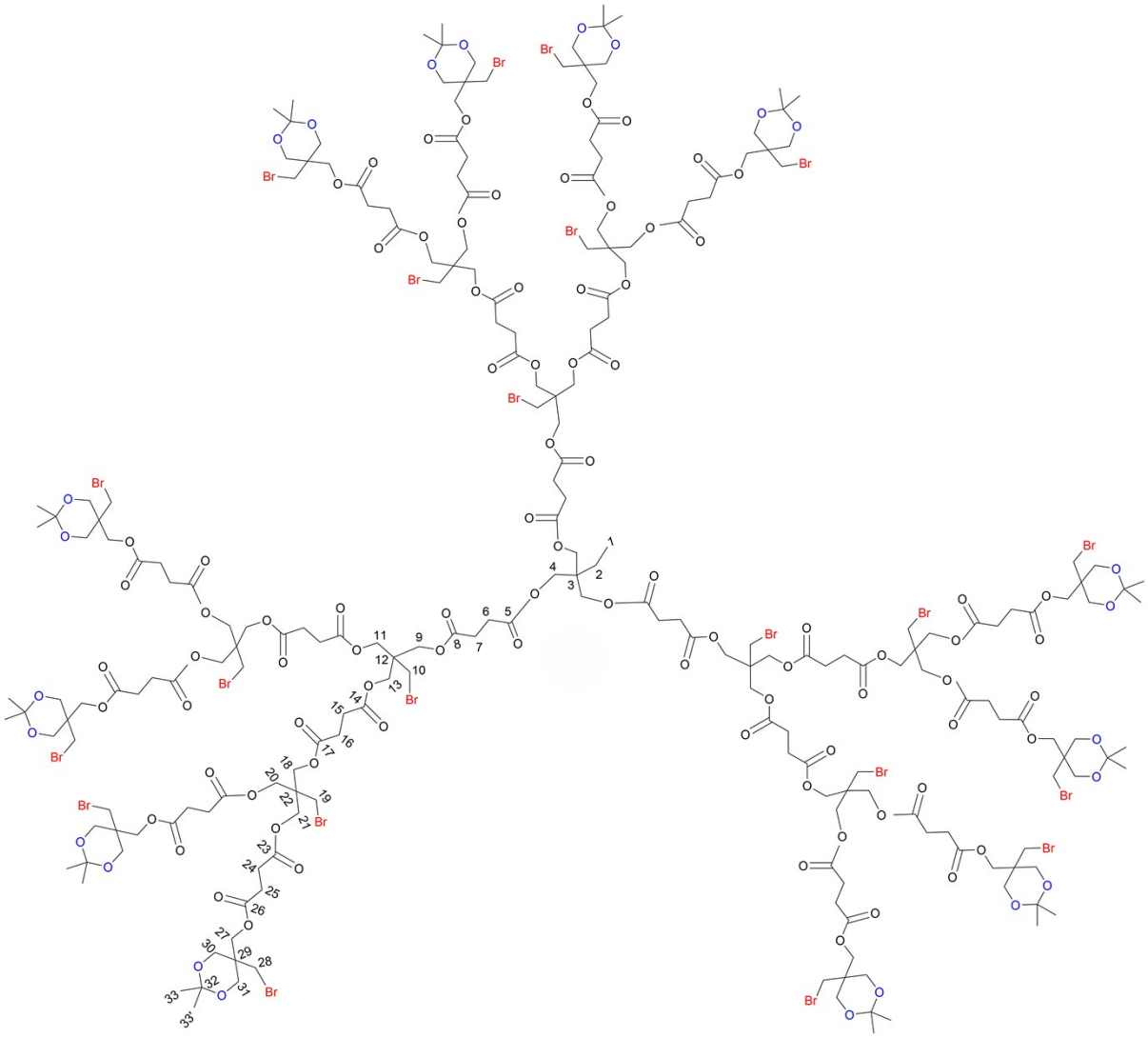
TMP-G2-$(Br)_9$-$(Ac)_6$ was synthesized according to the general esterification procedure, using the following reagents with their stated amounts: TMP-G1-$(Br)_3$-$(OH)_6$ (5.56 g, 5.69 mmol), acetonide protected BHP-diol anhydride (33.8 g, 51.19 mmol), DMAP (0.834 g, 6.82 mmol) and Pyridine (13.75 mL, 170.64 mmol). After the washing steps, TMP-G2-$(Br)_9$-$(Ac)_6$ was first eluted in a mixture of 40:40:20 EtOAc:heptane:DCM and later in 95:5 EtOAc:MeOH. TMP-G2-$(Br)_9$-$(Ac)_6$ was obtained as a colorless oil after concentration (11.5 g, 70%). $C_{105}H_{155}Br_9O_{48}$ (2904.48 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.21 – 4.13 (30 H, m, H9, H12, H13, H18), 4.04 (6 H, s, H4), 3.77 (24 H, s, H20, H21), 3.55 – 3.45 (18 H, m, H19, H10), 2.65 (36 H, d, J 7.6, H15, H16, H6, H7), 1.41 (38 H, s, H2, H24, H24'), 0.88 (3 H, t, J 7.6, H1). ^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.94 (C5), 171.82 (C8), 171.70 (C14), 171.66 (C17), 98.89 (C23), 64.41 (C9, C18), 64.19 (C4), 63.70 (C20, C21), 63.19 (C12, C13), 42.40 (C11), 40.86 (C3), 37.59 (C22), 34.89 (C19), 32.59 (C10), 28.96 (C6, C7), 28.92 (C15), 28.90 (C16), 23.91 (C24), 23.45 (C24'), 23.04 (C2), 7.54 (C1). MALDI: [M+Na^+] = 2927.47 Da, Found [M+Na^+] = 2929.2 Da. SEC (DMF) M_n = 2734.5 g mol^{-1}, M_w= 2839.3 g mol^{-1}, Đ = 1.03.

TMP-G2-$(Br)_9$-$(OH)_{12}$



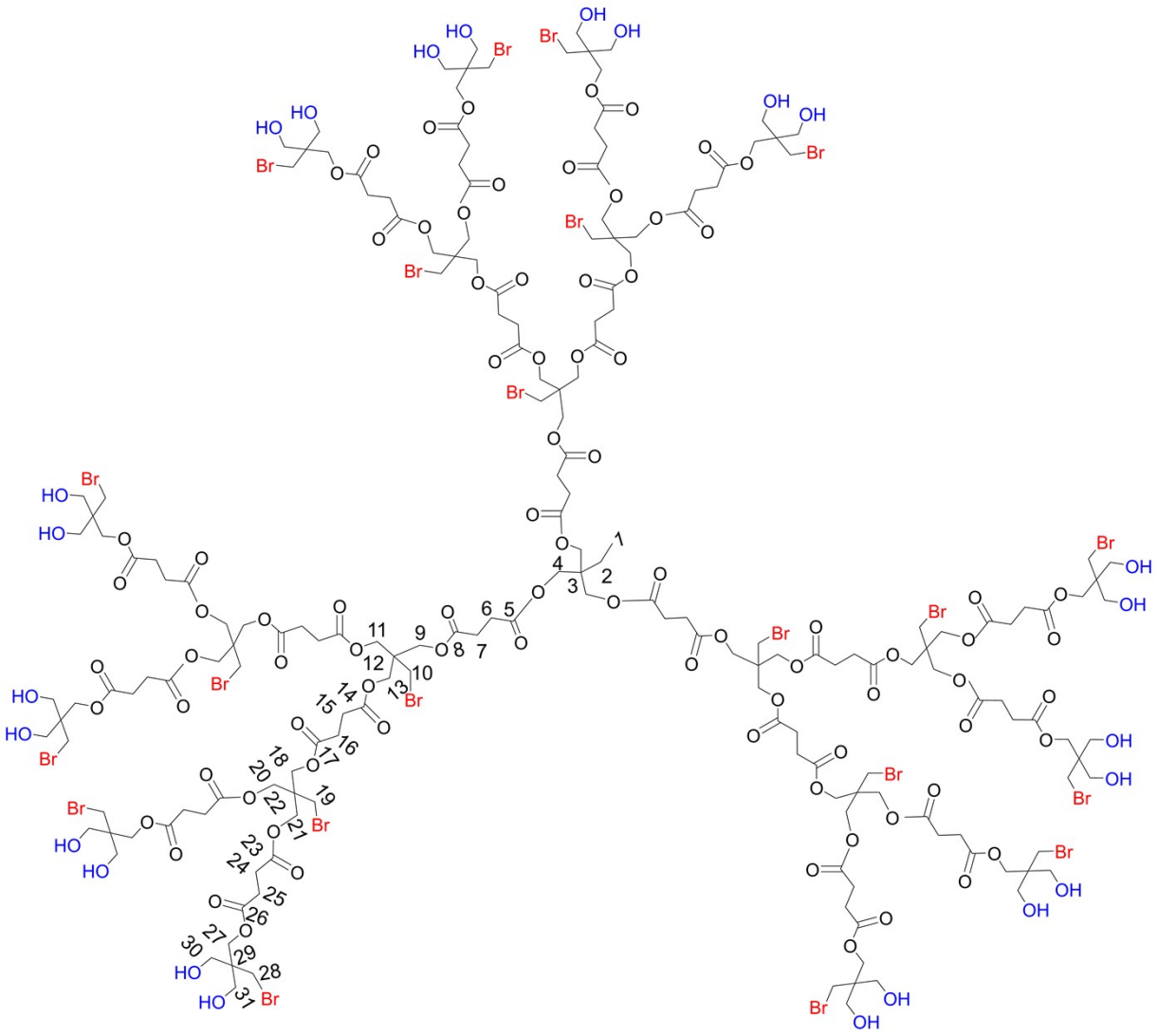
TMP-G2-$(Br)_9$-$(OH)_{12}$ was synthesized according to the general deprotection procedure, using the following reagents with their stated amounts: TMP-G2-$(Br)_9$-$(Ac)_6$ (6.00 g, 2.06 mmol) and DOWEX (6.00 g). TMP-G2-$(Br)_9$-$(OH)_{12}$ was obtained as a viscous oil after concentration (4.5 g, 82%). $C_{87}H_{131}Br_9O_{48}$ (2664.09 g/mol). ^{1}H-NMR (400 MHz, CD_3OD) δ/ppm: 4.21 (18 H, s, H9, H12, H13), 4.10 (18 H, d, J 13.2, H4, H18), 3.60 (30 H, d, J 5.1, H10, H20, H21), 3.53 (12 H, s, H19), 2.69 (36 H, d, J 3.3, H6, H7, H15, H16), 1.53 (2 H, q, J 7.5, H2), 0.93 (4 H, t, J 7.6, H1).^{13}C-NMR (101 MHz, CD_3OD) δ/ppm: 173.77 (C5), 173.65 (C8), 173.43 (C14), 173.39 (C17), 65.37 (C4), 64.86 (C12, C13), 64.37 (C18), 62.78 (C9), 62.05 (C20, C21), 45.72 (C22), 43.57 (C11), 42.12 (C3), 35.28 (C19), 33.92 (C10), 29.99 (C6, C7), 29.93 (C15, C16), 24.20 (C2), 7.94 (C1). MALDI: $[M+Na^+]$ = 2687.07 Da, Found $[M+Na^+]$ = 2687.06 Da. SEC (DMF) M_n = 3624 g mol^{-1}, M_w= 3725 g mol^{-1}, Đ = 1.02.

TMP-G3-$(Br)_{21}$-$(Ac)_{12}$



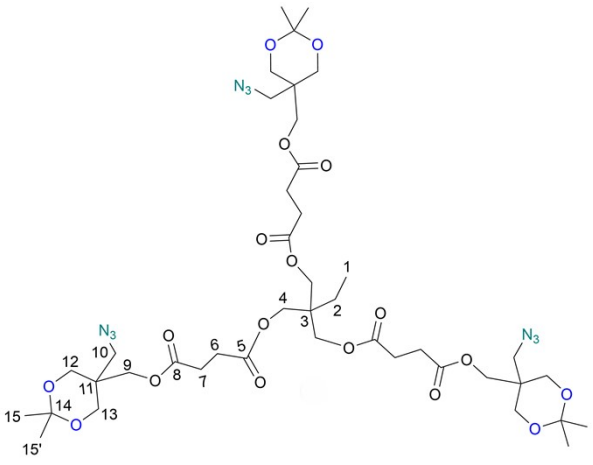
TMP-G3-$(Br)_{21}$-$(Ac)_{12}$ was synthesized according to the general esterification procedure, using the following reagents with their stated amounts: TMP-G2-$(Br)_9$-$(OH)_{12}$ (4.08 g, 1.53 mmol), acetonide protected BHP-diol anhydride (18.2 g, 27.56 mmol), DMAP (0.45 g, 3.7 mmol) and Pyridine (7.4 mL, 91.86 mmol). After the washing steps, TMP-G3-$(Br)_{21}$-$(Ac)_{12}$ was slowly eluted in a mixture of 50:50 EtOAc:heptane with gradual increase in polarity to 65:35 EtOAc:heptane and ultimately to 70:30 EtOAc:heptane. TMP-G3-$(Br)_{21}$-$(Ac)_{12}$ was obtained as a colorless oil after concentration (3.98 g, 40%). $C_{231}H_{335}Br_{21}O_{108}$ (6503.06 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.21 – 4.14 (78 H, m, H9, H11, H13, H18, H20, H21, H27), 4.04 (6 H, s, H4), 3.77 (48 H, s, H30, H31), 3.56 – 3.45 (42 H, m, H10, H19, H28), 2.66 (84 H, d, J 2.9, H6, H7, H15, H16, H24, H25), 1.41 (74 H, s, H2, H33, H33'), 0.88 (3 H, t, J 7.5, H1).^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.96 (C5, C8), 171.83 (C14), 171.72 (C17), 171.66 (C23), 171.64 (C26), 98.89 (C32), 64.41 (C11, C13, C20, C21), 64.19 (C4), 63.69 (C30, C31), 63.19 (C9, C18, C27), 42.40 (C12), 42.37 (C22), 40.85 (C3), 37.59 (C29), 34.92 (C28), 32.62 (C10, C19), 28.97 (C6, C7), 28.93 (C15, C16), 28.87 (C24, C25), 23.91 (C33), 23.47 (C33'), 7.57 (C1). MALDI: $[M+K^+]$ = 6557.19 Da, Found $[M+K^+]$ = 6552.05 Da. SEC (DMF) M_n = 5545 g mol^{-1}, M_w= 5598 g mol^{-1}, Đ = 1.0.

TMP-G3-$(Br)_{21}$-$(OH)_{24}$



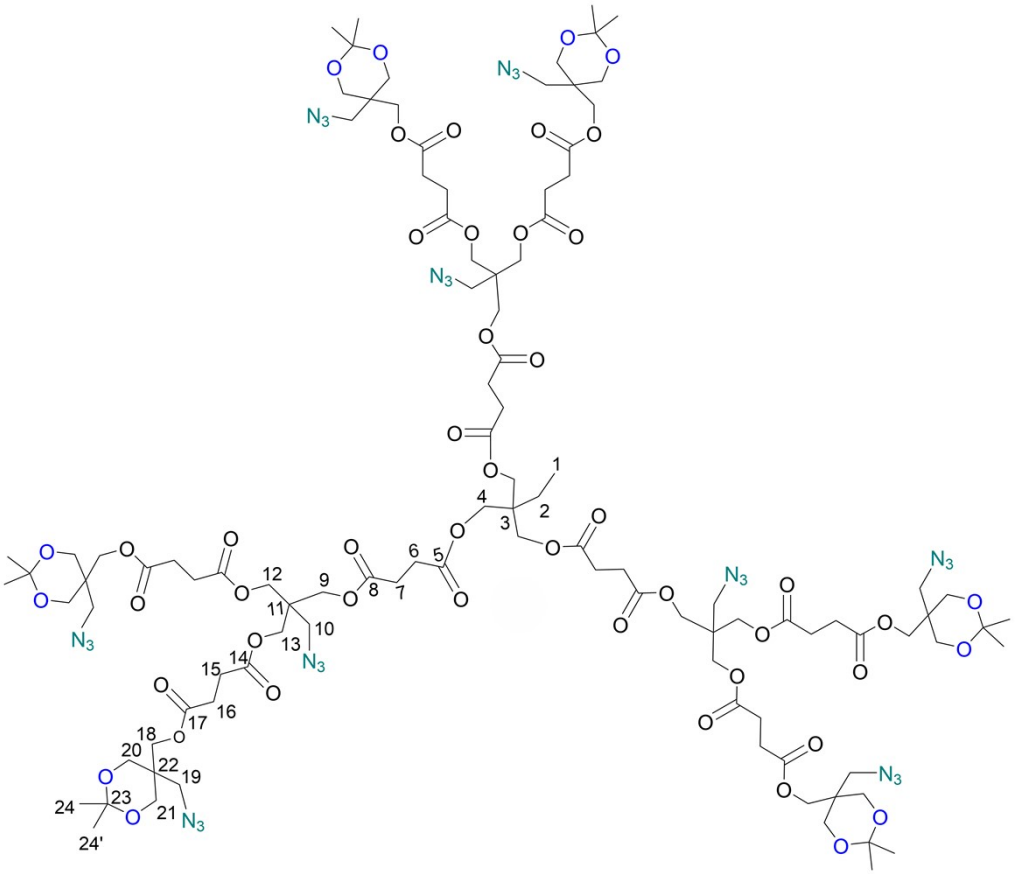
TMP-G3-$(Br)_{21}$-$(OH)_{24}$ was synthesized according to the general deprotection procedure, using the following reagents with their stated amounts: TMP-G3-$(Br)_{21}$-$(Ac)_{12}$ (0.05 g, 0.008 mmol) and DOWEX (0.05 g). TMP-G3-$(Br)_{21}$-$(OH)_{24}$ was obtained as a viscous oil after concentration (0.04 g, 83%). $C_{198}H_{291}Br_{21}O_{108}$ (6077.38 g/mol). ^{1}H-NMR (400 MHz, CD_3OD) δ/ppm: 4.22 (54 H, s, H9, H11, H13, H18, H20, H21), 4.12 (30 H, s, H27, H4), 3.64 – 3.57 (72 H, m, H30, H31, H28), 3.54 (18 H, s, H10, H28), 2.71 (84 H, d, J 4.9, H6, H7, H15, H16, H24, H25), 1.54 (2 H, d, J 7.5, H2), 0.94 (3 H, t, J 7.6, H1).^{13}C-NMR (101 MHz, CD_3OD) δ/ppm: 173.83 (C5, C8), 173.68 (C14, C17), 173.49 (C23), 173.42 (C26), 65.50 (C4), 64.92 (C20, C21), 64.47 (C11, C13), 62.79 (C9, C18, C27), 62.11 (C30, C31), 46.46 (C12), 45.77 (C29), 43.63 (C22), 42.18 (C3), 35.90 (C10), 35.37 (C28), 34.04 (C19), 30.01 (C6, C7, C15, C16, C24, C25). SEC (DMF) M_n = 5425 g mol^{-1}, M_w= 5640 g mol^{-1}, Đ = 1.03.

TMP-G1-$(N_3)_3$-$(Ac)_3$



TMP-G1-$(N_3)_3$-$(Ac)_3$ was synthesized according to the general azidation procedure, using the following reagents with their stated amounts: TMP-G1-$(Br)_3$-$(Ac)_3$ (1.5 g, 1.37 mmol) and NaN_3 (1.33 g, 20.5 mmol). TMP-G1-$(N_3)_3$-$(Ac)_3$ was obtained as a pale-yellow oil after concentration (0.81 g, 61%). $C_{42}H_{65}N_9O_{18}$ (984.3 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.08 (6 H, s, H9), 4.03 (6 H, s, H4), 3.74 – 3.64 (12 H, m, H12, H13), 3.51 (6 H, s, H10), 2.64 (12 H, s, H6, H7), 1.47 (2 H, q, J 7.5, H2), 1.40 (18 H, d, H15, H15'), 0.87 (3 H, t, J 7.6, H1).^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.99 (C5, C8), 98.74 (C14), 64.16 (C4), 63.95 (C9), 62.87 (C12, C13), 52.01 (C10), 40.85 (C3), 37.90 (C11), 28.97 (C6), 28.93 (C7), 24.49 (C15), 23.02 (C2), 22.94 (C15'), 7.46 (C1). MALDI: [M+Na^+] = 1007.29 Da, Found [M+Na^+] = 1007.96 Da. SEC (DMF) M_n = 1102.4 g mol^{-1}, M_w= 1119.1 g mol^{-1}, Đ = 1.01.

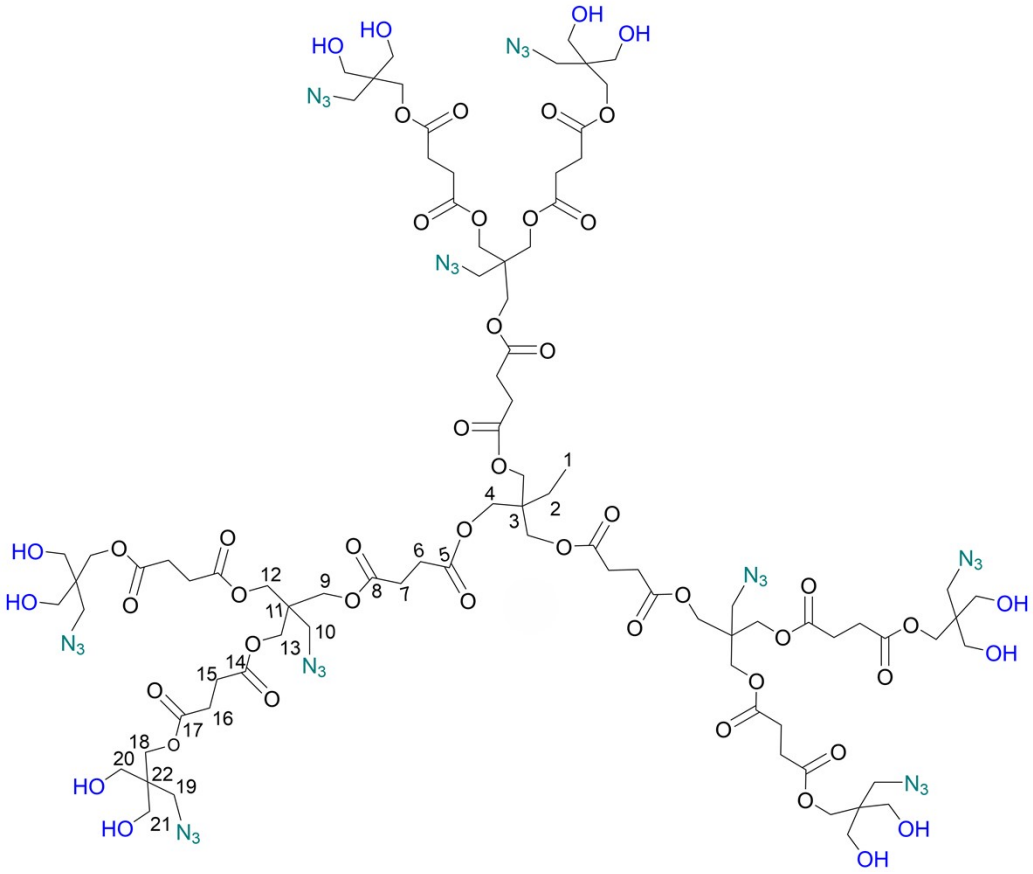
TMP-G2-$(N_3)_9$-$(Ac)_6$



TMP-G2-$(N_3)_9$-$(Ac)_6$ was synthesized according to the general azidation procedure, using the following reagents with their stated amounts: TMP-G2-$(Br)_9$-$(Ac)_6$ (4.57 g, 1.57 mmol) and NaN_3 (4.6 g, 70.8 mmol). TMP-G2-$(N_3)_9$-$(Ac)_6$ was obtained as a pale-yellow oil after concentration (3 g, 75%). $C_{105}H_{155}N_{27}O_{48}$ (2563.54 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.09 (30 H, d, J 5.2, H9, H12, H13,

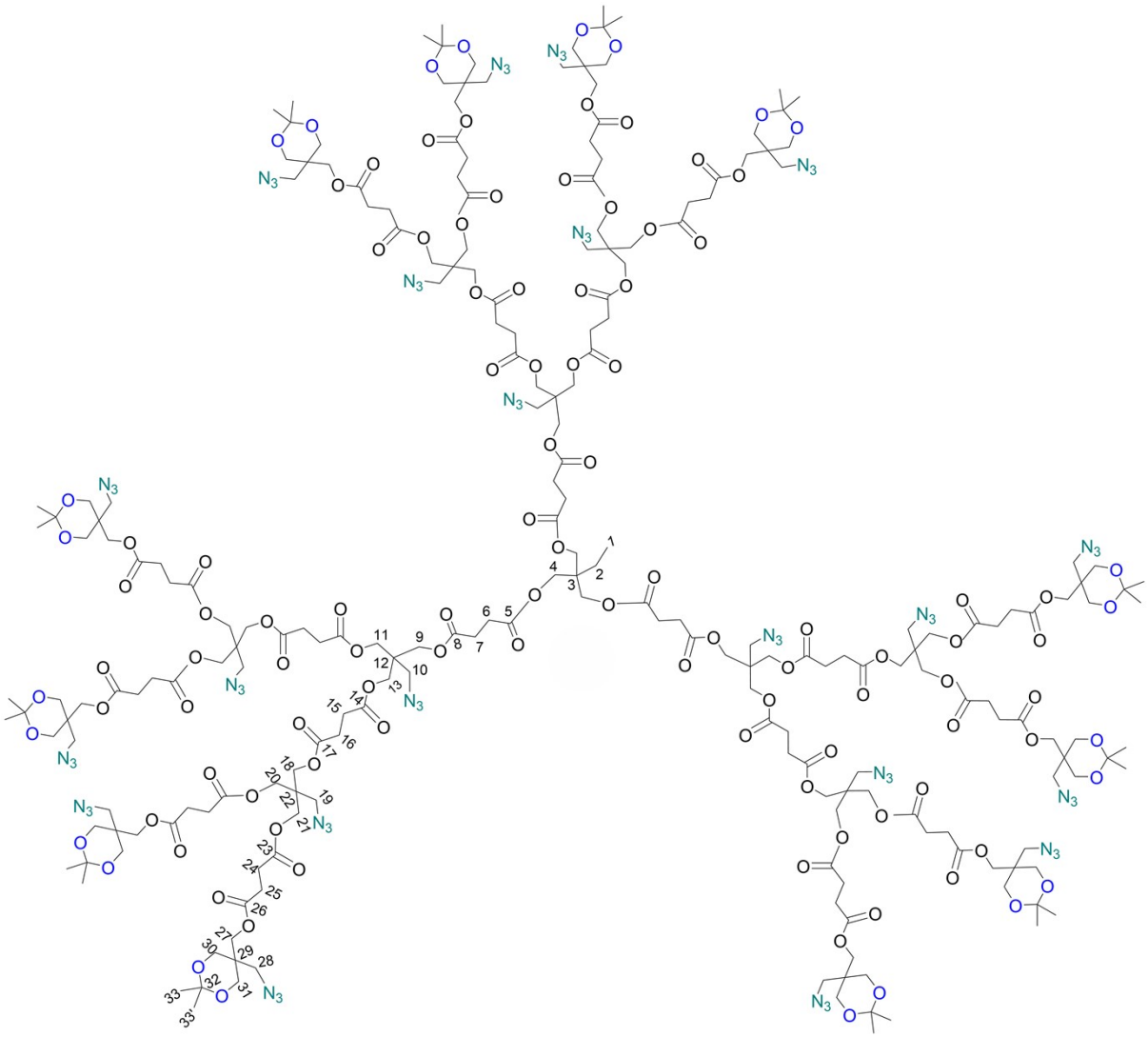
H18), 4.04 (6 H, s, H4), 3.74 – 3.65 (24 H, m, H20, H21), 3.49 (18 H, d, J 16.2, H19, H10), 2.65 (36 H, d, J 7.5, H15, H16, H6, H7), 1.41 (38 H, d, H2, H24, H24'), 0.88 (3 H, t, J 7.5, H1).^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.95 (C5, C8), 171.81 (C14), 171.79 (C17), 98.77 (C23), 64.21 (C4), 64.03 (C9, C18), 62.88 (C20, C21), 62.84 (C12, C13), 52.06 (C19), 51.23 (C10), 42.73 (C11), 40.86 (C3), 37.93 (C22), 28.95 (C15, C16), 28.92 (C6, C7), 24.47 (C24), 22.99 (C24'), 7.50 (C1). MALDI: [M+Na$^+$] = 2586.53 Da, Found [M+Na$^+$] = 2587.23 Da, [M+K$^+$] = 2602.63 Da, Found [M+K$^+$] = 2603.21 Da. SEC (DMF) M_n = 2873.6 g mol^{-1}, M_w= 2933.4 g mol^{-1}, Đ = 1.02.

TMP-G2-$(N_3)_9$-$(OH)_{12}$



TMP-G2-$(N_3)_9$-$(OH)_{12}$ was synthesized according to the general deprotection procedure, using the following reagents with their stated amounts: TMP-G2-$(N_3)_9$-$(Ac)_6$ (0.09 g, 0.037 mmol) and DOWEX (0.09 g). TMP-G2-$(N_3)_9$-$(OH)_{12}$ was obtained as a viscous oil after concentration (0.06 g, 71%). $C_{87}H_{131}N_{27}O_{48}$ (2323.15 g/mol). ^{1}H-NMR (400 MHz, CD_3OD) δ/ppm: 4.17 – 4.05 (36 H, m, H9, H12, H13, H18, H4), 3.54 (30 H, d, J 11.3, H10, H20, H21), 3.44 (12 H, s, H19), 2.69 (36 H, d, J 2.4, H15, H16, H6, H7), 1.53 (2 H, q, J 7.5, H2), 0.93 (3 H, t, J 7.6, H1).^{13}C-NMR (101 MHz, CD_3OD) δ/ppm: 173.89 (C5), 173.66 (C8), 173.54 (C14), 173.50 (C17), 65.33 (C4), 64.62 (C12, C13), 64.02 (C18), 62.64 (C9), 61.85 (C20, C21), 52.41 (C19), 52.34 (C19), 46.82 (C10), 46.10 (C22), 43.90 (C11), 42.11 (C3), 29.94 (C15), 29.91 (C16), 29.71 (C6, C7), 24.15 (C2), 7.84 (C1). MALDI: [M+Na$^+$] = 2346.14 Da, Found [M+Na$^+$] = 2346 Da, [M+K$^+$] = 2362.25 Da, Found [M+K$^+$] = 2362 Da. SEC (DMF) M_n = 2873.6 g mol^{-1}, M_w= 2933.4 g mol^{-1}, Đ = 1.02.

TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$



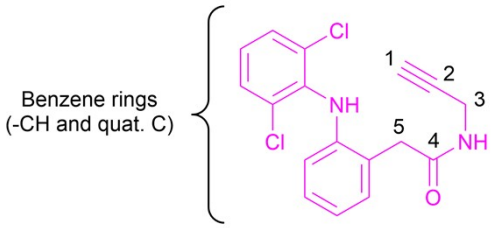
TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$ was synthesized according to the general azidation procedure, using the following reagents with their stated amounts: TMP-G3-$(Br)_{21}$-$(Ac)_{12}$ (0.05 g, 0.008 mmol) and NaN_3 (0.052 g, 0.8 mmol). The purification method for TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$ was slightly different from the previously mentioned general azidation procedure. Upon completion of the reaction, TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$ was extracted thrice in EtOAc from the DMSO:H_2O mixture. EtOAc containing the TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$ was then washed twice with water to remove the residual DMSO and dried with $MgSO_4$. Consequently, a concentrated volume of the crude TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$ in EtOAc was precipitated thrice with cold ether. TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$ was obtained as a colorless oil after removal of solvent (0.032 g, 70%). $C_{231}H_{335}N_{63}O_{108}$ (5722.5 g/mol).^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.09 (84 H, d, J 5.0, H9, H11, H13, H18, H20, H21, H27), 3.75 – 3.64 (48 H, m, H30, H31), 3.49 (42 H, d, J 16.5, H28, H10, H19), 2.65 (84 H, d, J 2.7, H6, H7, H15, H16, H24, H25), 1.41 (74 H, s, H2, H33, H33'), 0.91 – 0.86 (3 H, m, H1).^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.92 (C5), 171.89 (C8), 171.78 (C14), 171.73 (C17), 171.70 (C23, C26), 98.71 (C32), 64.15 (C4), 63.97 (C11, C13, C20, C21), 62.82 (C30, C31), 62.78 (C9, C18, C27), 52.01 (C28), 51.19 (C10, C19), 42.68 (C12), 42.64 (C22), 40.80 (C3), 37.88 (C29), 28.90 (C6, C7), 28.88 (C15, C16), 28.82 (C24, C25), 24.42 (C33), 22.95 (C33'), 7.46 (C1). MALDI: $[M+Na^+]$ = 5745.49 Da, Found $[M+Na^+]$ = 5742.00 Da. SEC (DMF) M_n = 5619.7 g mol^{-1}, M_w= 5686.6 g mol^{-1}, Đ = 1.01.

Post-functionalization of heterofunctional dendrimers

Click probes with alkyne functionality including Dicl-alkyne and Boc protected propargyl amine (Boc-PA), were employed to evaluate the feasibility of modifying azide groups in the synthesized dendrimers with copper click chemistry. 1-Propanethiol with a thiol functionality was selected to post-functionalize the bromide groups in a bromide-based dendrimer with thiol-bromo click chemistry. Monodisperse mPEG with carboxylic acid functionality was used to post-functionalize the peripheral hydroxyl groups of azide and bromide-based dendrimers. mPEG anhydride was prepared according to previously published protocol.[2]

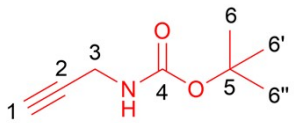
Synthesis of click probes

Dicl-alkyne



Dicl (1.5 g, 4.7 mmol) was dissolved in an ice-cooled flask containing THF, followed with subsequent addition of Hydroxybenzotriazole (HOBt) (756 mg, 5.6 mmol) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (1.084 g, 5.6 mmol). Next, a solution of propargyl amine (644 µ L, 9.4 mmol) in THF was added to this reaction mixture. The reaction was left stirring for 1 h in the ice-cold bath. Upon the reaction completion, THF was evaporated and the crude product dissolved in DCM was washed once with $NaHSO_4$, $NaHCO_3$ and brine and dried with $MgSO_4$. The crude product was first eluted in 100% heptane and later in 10:90 EtOAc:heptane mixture. Dicl-alkyne was obtained as a white powder after concentration (0.76 g, 48%). $C_{17}H_{14}Cl_2N_2O$ (333.21 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.35 (2 H, dd, J 8.1, 1.6, C*H*), 7.23 – 6.89 (6 H, m, C*H*), 6.51 (1 H, d, J 8.0, C*H*), 6.03 (1 H, s, br: NH), 4.06 (2 H, dd, J 5.2, 2.6, H3), 3.71 (2 H, s, H5), 2.21 (1 H, t, J 2.5, H1). ^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.10 (C4), 142.89, 137.46, 130.93, 130.24, 129.00, 128.35, 124.60, 124.05, 121.81, 117.62 (Carbon signals from benzene rings: C*H* and quat. C), 79.30 (C2), 72.08 (C1), 40.82 (C5), 29.67 (C3).

Boc-PA



Propargyl amine (4.07 mL, 63.5 mmol) was dissolved in an ice-cooled mixture of 1:1 H_2O:acetone, to which 1M sodium hydroxide (NaOH) was added until the pH of the solution was >10. Consequently, Di-tert-butyl dicarbonate (21.9 mL, 95.3 mmol) was added to the reaction and stirred overnight. The crude product was extracted five times in DCM. The organic phase containing the crude product was dried with $MgSO_4$ and concentrated. The crude compound was eluted in a mixture of 20:80 EtOAc:heptane and Boc-PA was obtained after concentration (8 g, 81%). $C_8H_{13}NO_2$ (155.2 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.73 (1 H, br, s, NH), 3.91 (2 H, dd, J 5.4, 2.6, H3), 2.21 (1 H, t, J 2.5, H1), 1.44 (9 H, s, H6, H6', H6'').^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 155.38 (C4), 80.22 (C2), 71.36 (C1), 30.52 (C3), 28.47 (C6, C6', C6'').

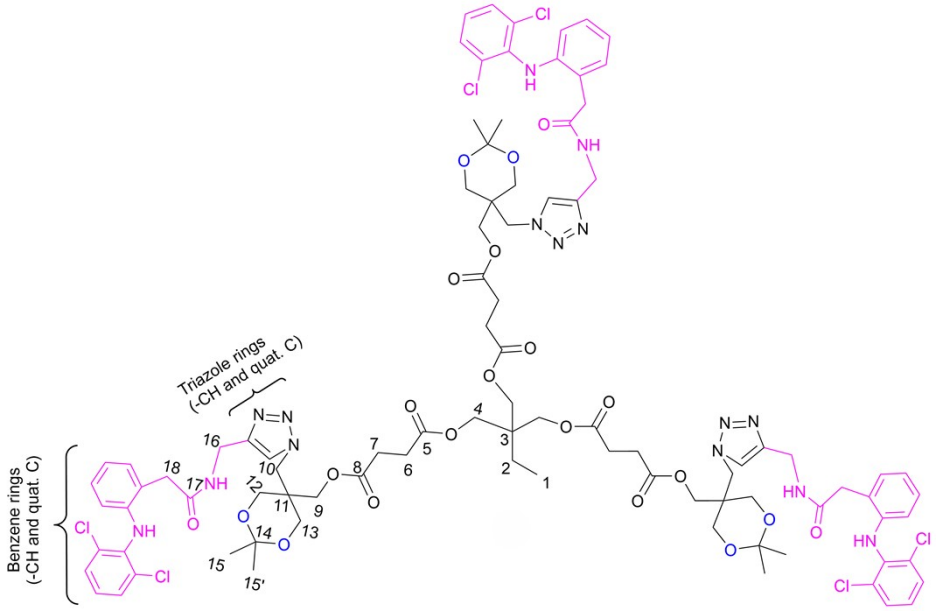
General procedure for CuAAC functionalization

The azide functional dendrimer and alkyne-functional probe (1.5 eq/N_3 group) are dissolved in THF. Respective solutions of copper sulfate ($CuSO4.5H_2O$) (0.2 eq/N_3 group) and Na ascorbate (0.4 eq/N_3 group) in water are sequentially added to finally have 1:1 THF:H_2O in the reaction mixture. The reaction is left to stir overnight at room temperature. Upon completion of the reaction, the solvent mixture is evaporated and the crude product dissolved in acetone is purified through size exclusion chromatography (Bio-beads S-X8) to remove $CuSO_4$ and sodium ascorbate.

General procedure for anhydride-based esterification

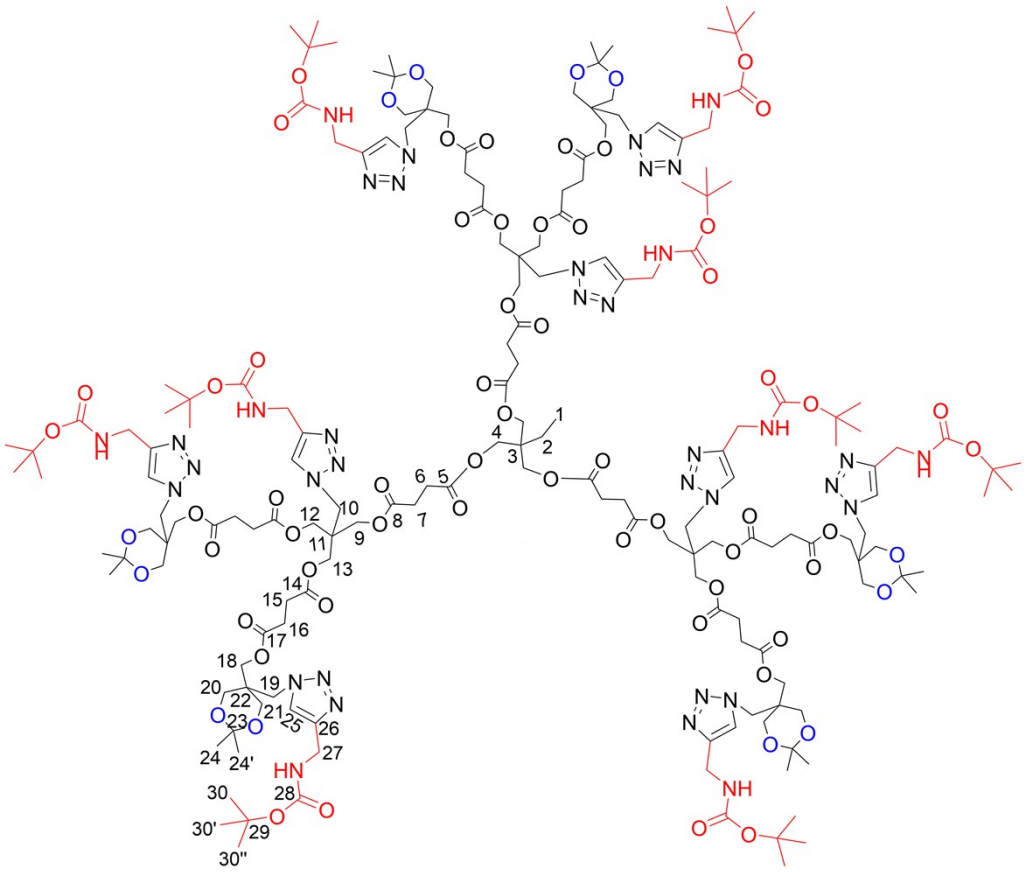
The post-functionalization of hydroxyl groups through anhydride-based esterification follows the same reaction procedure as seen for dendrimer growth. Upon completion of the reaction, the DCM is evaporated and the crude product dissolved in acetone is purified through size exclusion chromatography (Sephadex LH-20) to remove excess mPEG acid. The obtained product is then precipitated in ether (1x) after dissolution in EtOAc followed by removal of solvent.

TMP-G1-(Dicl)$_3$-(Ac)$_3$



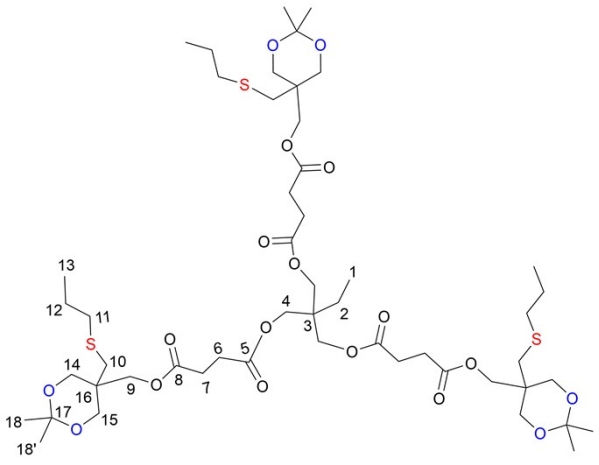
TMP-G1-(N_3)$_3$-(Ac)$_3$ (30 mg, 0.03 mmol) was post-functionalized with Dicl-alkyne (36 mg, 0.106 mmol) in 1.4 mL of 1:1 THF:H_2O through the general CuAAC procedure using the following reagents: $CuSO4.5H_2O$ (5 mg, 0.02 mmol) and Na ascorbate (7.2 mg, 0.037 mmol). $C_{92}H_{105}Cl_6N_{15}O_{21}$ (1969.64 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.62 (3 H, s, H19), 7.55 (2 H, s), 7.32 (6 H, d, J 8.1), 7.24 (4 H, d, J 5.7), 7.15 (3 H, dd, J 7.5, 1.6), 7.06 (3 H, td, J 7.7, 1.6), 6.97 (4 H, t, J 8.1), 6.86 (3 H, td, J 7.4, 1.2), 6.52 – 6.43 (3 H, m) (Proton signals from Dicl: Benzene and triazole rings), 4.54 – 4.44 (12 H, m, H4, H9), 4.04 (6 H, s, CH_2 Dicl), 3.87 (6 H, s, CH_2 Dicl), 3.75 – 3.65 (12 H, m, CH_2 Dicl, H12, H13), 3.56 (6 H, d, J 12.2, H10), 2.60 (12 H, s, H6, H7), 1.41 (20 H, d, H2, H15, H15'), 0.84 (3 H, t, J 7.5, H1).^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 172.22 (-CONH Dicl), 172.00 (C5), 171.90 (C8), 144.49, 143.11, 137.80, 130.84, 130.14, 128.96, 127.95, 124.77, 124.61, 124.25, 121.52, 117.48 (Carbon signals from benzene and triazole rings: C*H* and quat. C), 99.00 (C14), 64.39 (C4), 63.56 (C9), 62.97 (C12, C13), 50.05 (C10), 40.89 (CH_2 Dicl), 40.74 (C3), 38.23 (C11), 35.08 (CH_2 Dicl), 28.93 (C6), 28.90 (C7), 25.55 (C15), 23.17 (C2), 21.97 (C15'), 7.49 (C1). MALDI: [M+H$^+$] = 1970.65 Da, Found [M+H$^+$] = 1970.44 Da, [M+K$^+$] = 2008.7 Da, Found [M+K$^+$] = 2008.49 Da. SEC (DMF) M_n = 2350.7 g mol^{-1}, M_w= 2429.6 g mol^{-1}, Đ = 1.03.

TMP-G2-(Boc-PA)$_9$-(Ac)$_6$



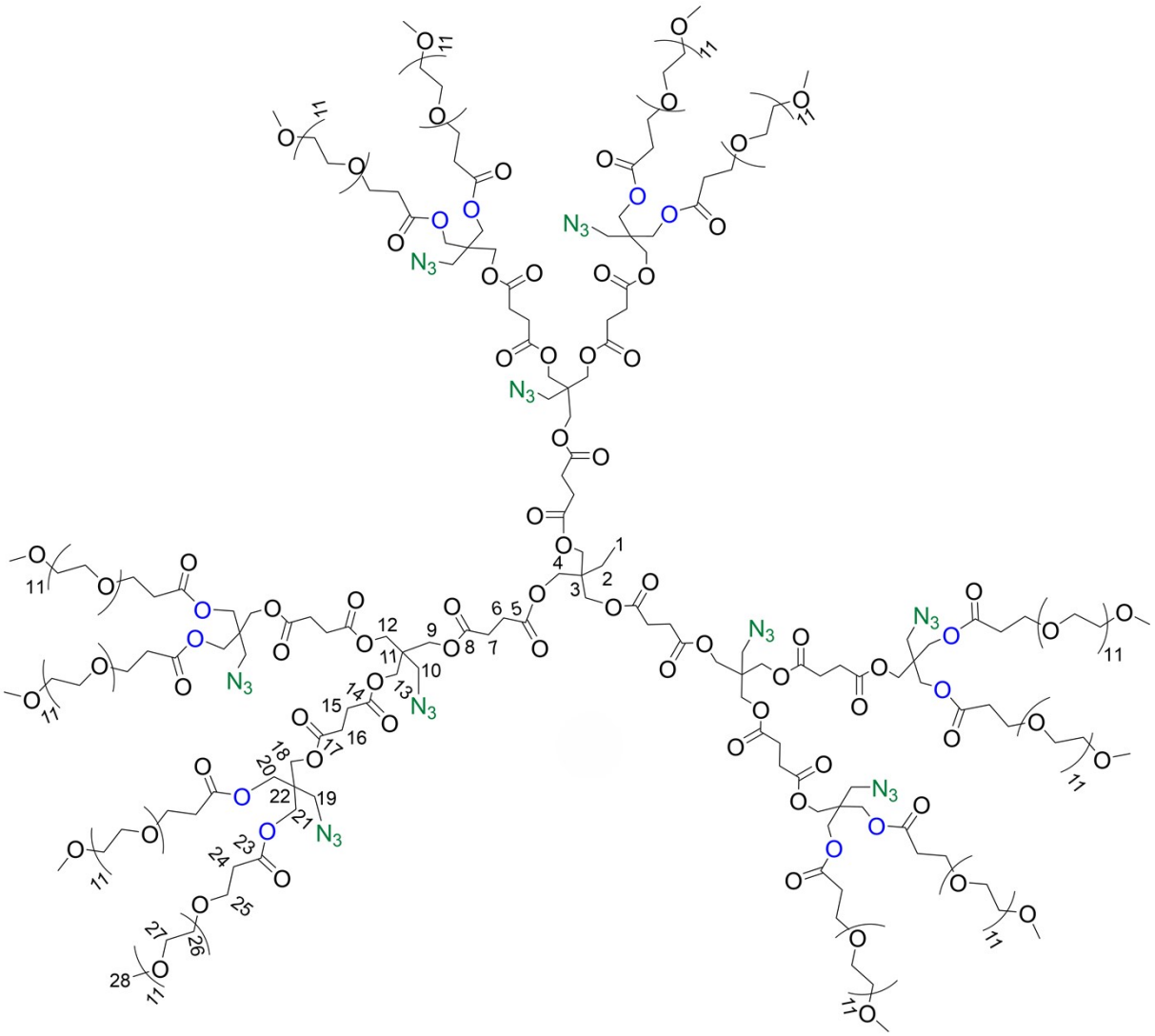
TMP-G2-(N_3)$_9$-(Ac)$_6$ (20 mg, 0.008 mmol) was post-functionalized with Boc-PA (13.6 mg, 0.09 mmol) in 1 mL of 1:1 THF:H_2O through the general CuAAC procedure using the following reagents: CuSO4.5H_2O (4 mg, 0.014 mmol) and Na ascorbate (5.6 mg, 0.028 mmol). $C_{177}H_{272}N_{36}O_{66}$ (3960.31 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.74 – 7.55 (9 H, m, H25), 5.60 – 5.29 (9 H, m, br: NH), 4.54 (18 H, d, J 11.8, H19), 4.36 (18 H, d, J 5.6, H27), 4.10 (18 H, s, H9, H12), 4.06 – 3.93 (18 H, m, H4, H13, H18), 3.77 (12 H, d, J 12.3, H20), 3.57 (12 H, d, J 12.2, H21), 2.63 (36 H, d, J 7.7, H6, H7, H15, H16), 1.41 (119 H, d, H2, H24, 24', H30, H30', H30''), 0.86 (2 H, t, J 7.5).^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 172.10 (C5, C8), 171.99 (C14), 171.77 (C17), 155.98 (C28), 124.11 (C25), 99.01 (C23), 79.69 (C29), 64.24 (C4), 63.86 (C9, C12), 62.93 (C20, C21, C13, C18), 49.90 (C19, C10), 42.97 (C11), 38.25 (C22), 36.14 (C27), 28.95 (C6, C7), 28.89 (C15, C16), 28.51 (C30, C30', C30''), 25.93 (C24), 21.71 (C24'), 7.50 (C1). MALDI: [M+K^+] = 3999.40 Da, Found [M+K^+] = 4000.90 Da. SEC (DMF) M_n = 4107.0 g mol^{-1}, M_w= 4156.0 g mol^{-1}, Đ = 1.01.

TMP-G1-(S-(CH_2)$_2$-CH_3)$_3$-(Ac)$_3$



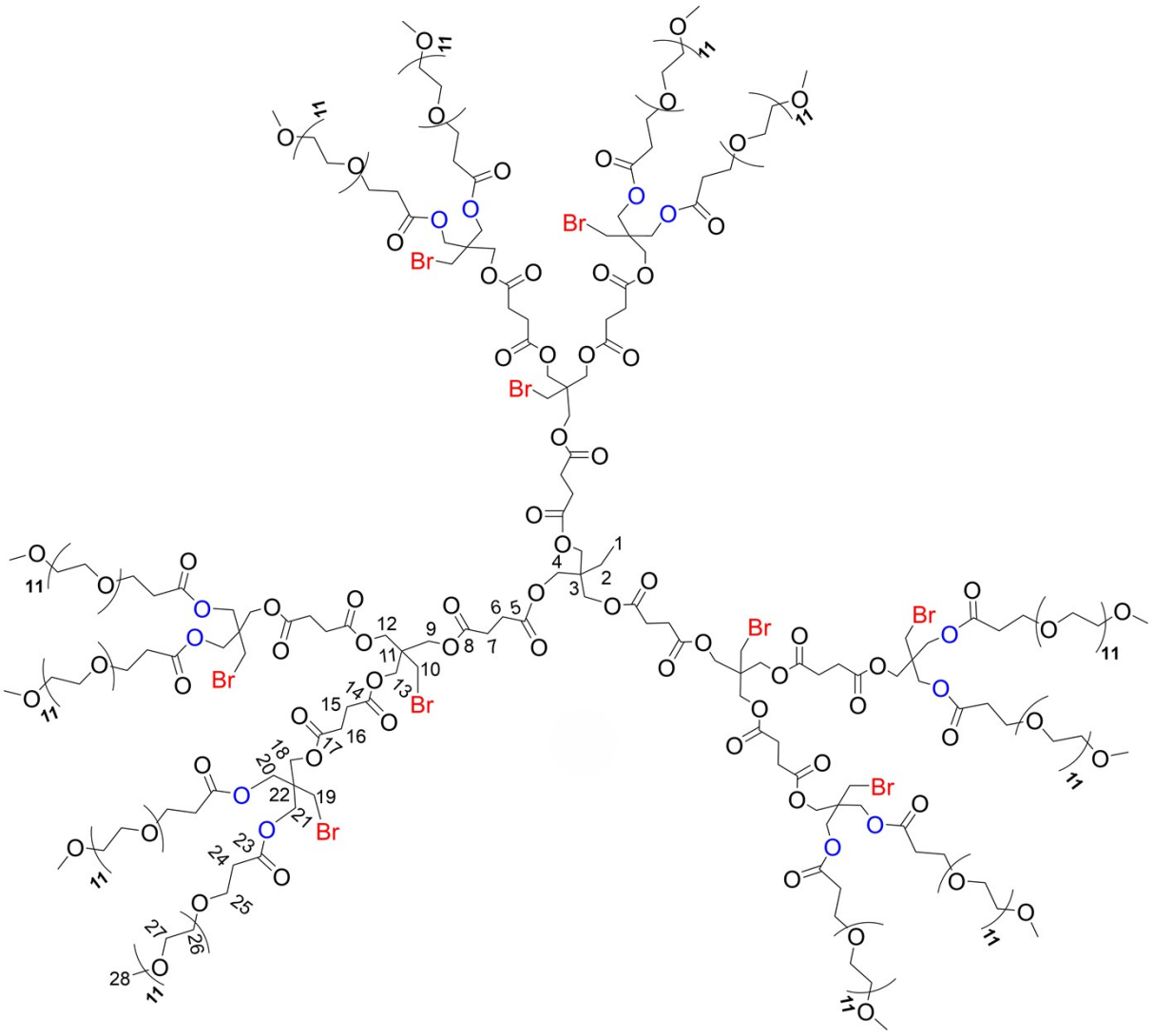
TMP-G1-$(Br)_3$-$(Ac)_3$ (0.0505 g, 0.046 mmol) was dissolved in a flask containing acetonitrile (4 mL) to which cesium fluoride (CsF) (0.031 g, 0.21 mmol) was added and magnetically stirred. Next, 1-Propanethiol (63 µL, 0.69 mmol) was added to the reaction mixture and refluxed for 96 h at 82°C. The reaction was monitored with NMR spectroscopy and MALDI until it reached full conversion. Upon completion of the reaction, the reaction mixture was filtered and evaporated to obtain pure TMP-G1-(S-$(CH_2)_2$-$CH_3)_3$-$(Ac)_3$ as a yellow coloured oil. $C_{51}H_{86}O_{18}S_3$ (1083.41 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.17 (6H, s, H9), 4.03 (6H, s, H4), 3.74 (12H, q, J = 11.8 Hz, H14, H15), 2.63 (18H, d, J = 2.5 Hz, H6, H7, H10), 2.48 (6H, t, J = 7.3 Hz, H11), 1.65 – 1.54 (6H, m, H12), 1.50 – 1.36 (20H, m, H2, H18, H18'), 0.97 (9H, t, J = 7.4 Hz, H13), 0.87 (3H, t, J = 7.5 Hz, H1). ^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.95 (C5, C8), 98.50 (C17), 65.02 (C9), 64.32 (C14, C15), 64.13 (C4), 40.87 (C3), 37.70 (C16), 36.44 (C11), 34.48 (C10), 29.00 (C6, C7), 24.58 (C18), 23.13 (C18'), 23.06 (C12), 13.50 (C13), 7.46 (C1). MALDI: $[M+Na^+]$ = 1106.40 Da, Found $[M+Na^+]$ = 1105.50 Da, $[M+K^+]$ = 1122.50 Da , Found $[M+K^+]$ = 1121.50. SEC (DMF) M_n = 1152.3 g mol^{-1}, M_w= 1184.7 g mol^{-1}, Đ = 1.02.

TMP-G2-$(N_3)_9$-$(mPEG)_{12}$



TMP-G2-$(N_3)_9$-$(OH)_{12}$ (42 mg, 0.017 mmol) was post-functionalized with mPEG anhydride (303 mg, 0.25 mmol) using the general esterification procedure with the following reagents: DCM (1 mL), DMAP (13 mg, 0.104 mmol) and Pyridine (281 µL, 3.33 mmol). TMP-G2-$(N_3)_9$-$(mPEG)_{12}$ was was obtained as a colorless oil after purification. $C_{399}H_{731}N_{27}O_{204}$ (9171.22 g/mol). ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.09 (60 H, d, J 4.0, H9, H12, H13, H18, H20, H21), 3.71 – 3.57 (507 H, m, H25, H26, H27), 3.47 (21 H, d, J 6.1, H10, H19), 3.37 (35 H, s, H28), 2.68 – 2.57 (60 H, m, H6, H7, H15, H16, H24), 1.48 (2 H, d, J 7.7, H2), 0.88 (3 H, t, J 7.5, H1).^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.80 (C5), 171.65 (C8), 171.56 (C14), 171.54 (C17), 170.88 (C23), 71.93 (C26), 70.60 (C27), 70.56 (C27), 70.55 (C27), 70.50 (C27), 70.46 (C27), 70.38 (C27), 66.38 (C25), 64.03 (C4), 62.77 (C12, C13, C18), 62.72 (C9), 62.33 (C20, C21), 59.01 (C28), 51.15 (C10), 51.11 (C19), 42.64 (C11), 42.55 (C22), 40.70 (C3), 34.89 (C24), 28.70 (C6, C7, C15, C16), 7.35 (C1). SEC (DMF) M_n = 10,206 g mol^{-1}, M_w= 10,697 g mol^{-1}, Đ = 1.04.

TMP-G2-$(Br)_9$-$(mPEG)_{12}$



TMP-G2-$(Br)_9$-$(OH)_{12}$ (60 mg, 0.022 mmol) was post-functionalized with mPEG anhydride (467.6 mg, 0.4 mmol) using the general esterification procedure with the following reagents: DCM (1 mL), DMAP (16 mg, 0.134 mmol) and Pyridine (347 µL, 4.3 mmol). TMP-G2-$(Br)_9$-$(mPEG)_{12}$ was obtained as a colorless oil after purification. $C_{399}H_{731}Br_9O_{204}$ (9512.17 g/mol). Structural characterization through various techniques including DOSY NMR, ^{1}H NMR, ^{13}C NMR and SEC verified the complete post-functionalization of the dendrimer. ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.15 (54 H, d, J 4.9, H9, H12, H13, H18, H20, H21), 4.03 (6 H, s, H4), 3.67 – 3.56 (527 H, m, H25, H26, H27), 3.46 (20 H, s, H10, H19), 3.36 (40 H, s, H28), 2.66 – 2.57 (61 H, m, H6, H7, H15, H16, H24), 1.47 (2 H, d, J 7.9, H2), 0.87 (4 H, t, J 7.5, H1).^{13}C-NMR (101 MHz, $CDCl_3$) δ/ppm: 171.91 (C5), 171.67 (C8), 171.57 (C14), 171.55 (C17), 170.94 (C23), 72.06 (C26), 70.73 (C27), 70.69 (C27), 70.63 (C27), 70.59 (C27), 70.52 (C27), 66.52 (C25), 66.48 (C25), 64.17 (C4), 63.29 (C12, C13), 63.19 (C18), 62.80 (C9, C20, C21), 59.14 (C28), 42.45 (C11), 42.35 (C22), 40.84 (C3), 35.03 (C24), 32.65 (C10), 32.57 (C19), 28.88 (C6, C7), 28.82 (C15, C16), 23.00 (C2), 7.52 (C1). SEC (DMF) M_n = 10,017 g mol^{-1}, M_w= 10,274 g mol^{-1}, Đ = 1.02.

Figures

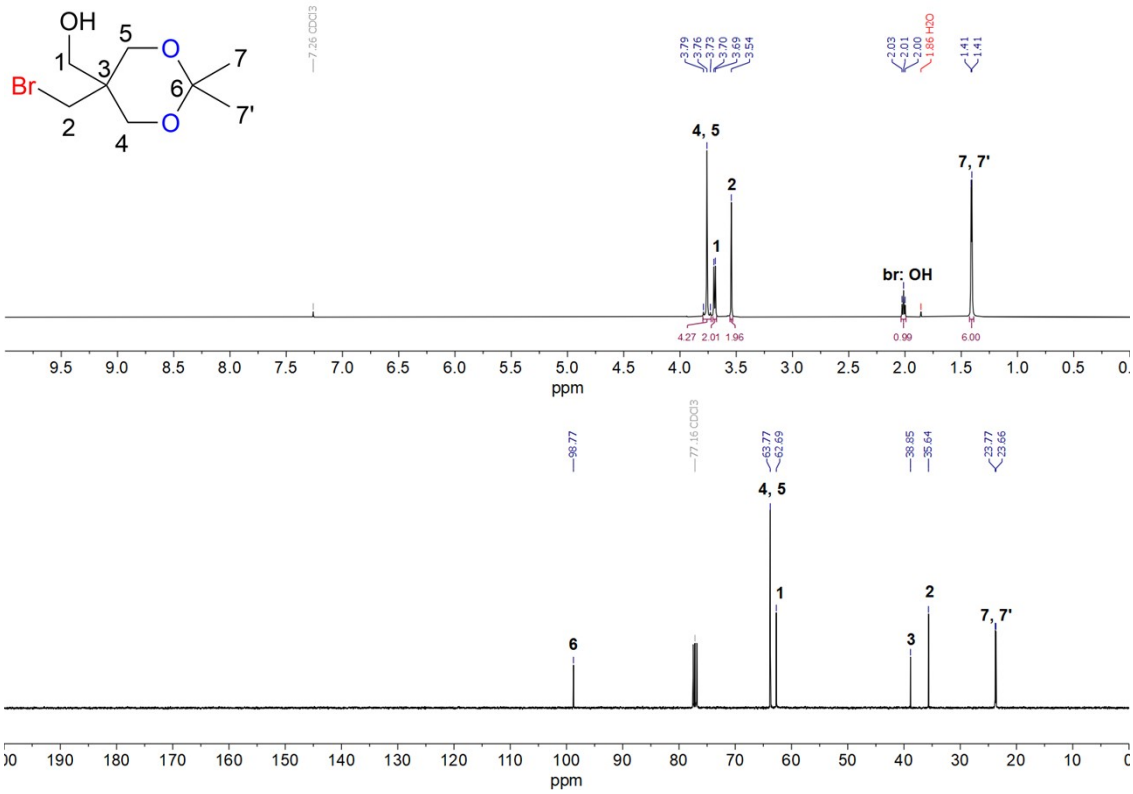


Figure S1. ^{1}H and ^{13}C NMR spectra of acetonide protected BHP-diol in $CDCl_3$.

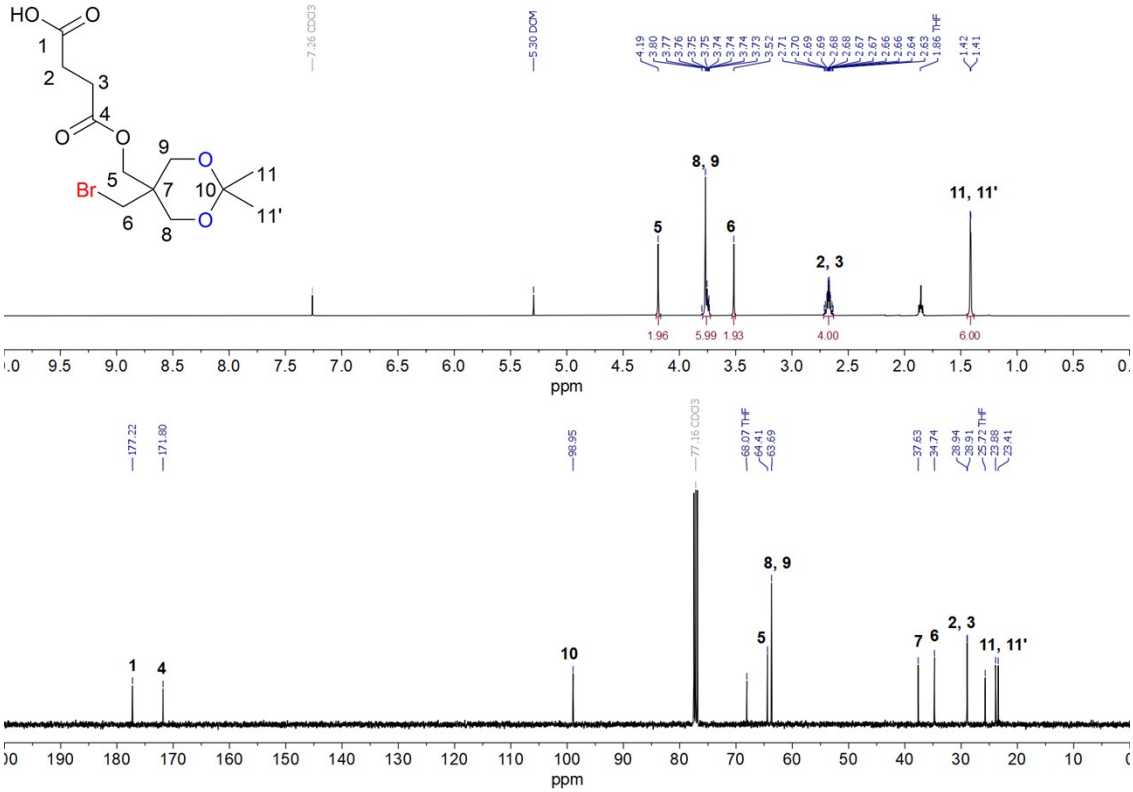


Figure S2. ^{1}H and ^{13}C NMR spectra of acid functionalized acetonide protected BHP-diol in $CDCl_3$. DCM and THF are indicated as residual solvents.

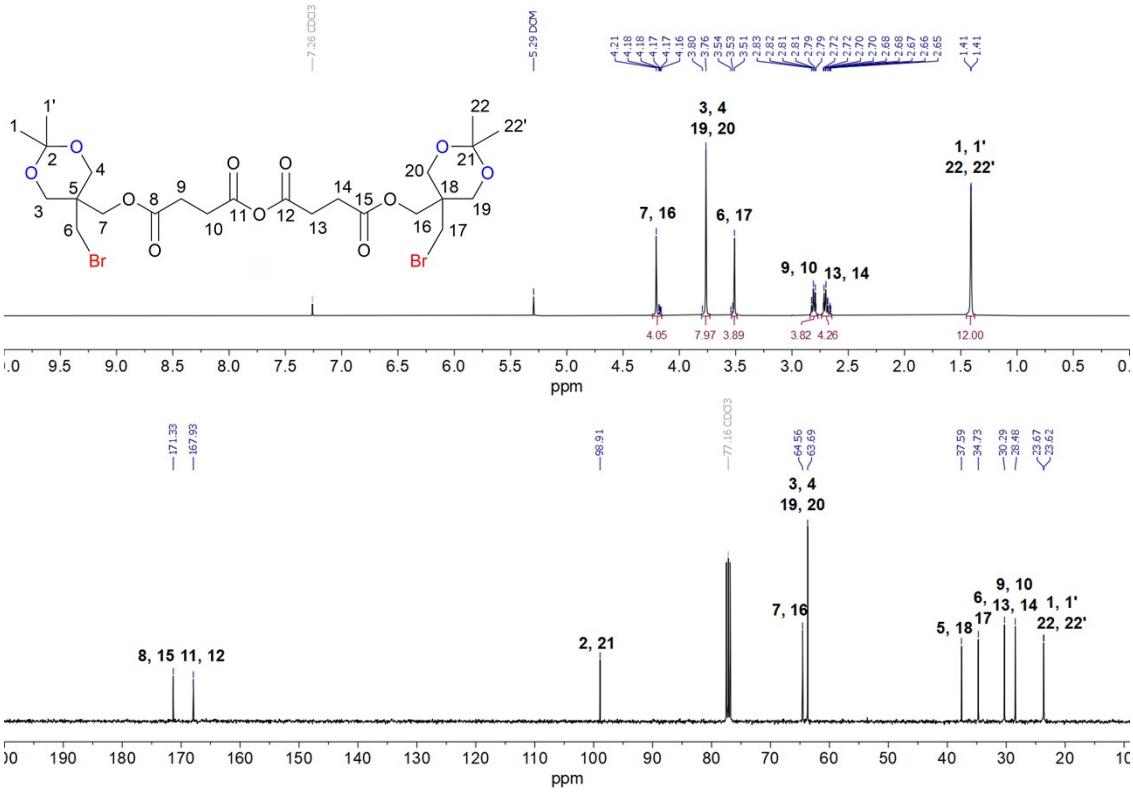

7.26 CDCl3
5.29 DCM
1'
1
2
22
22'
3, 4
19, 20
1, 1'
22, 22'
7, 16
6, 17
9, 10
13, 14
4.05
7.97
3.89
3.82
4.26
12.00
ppm
171.33
167.93
98.91
77.16 CDCl3
64.56
63.69
37.59
34.73
30.29
28.48
23.67
23.62
8, 15
11, 12
2, 21
5, 18
6, 17
ppm

Figure S3. ^{1}H and ^{13}C NMR spectra of acetonide protected BHP-diol anhydride in $CDCl_3$. DCM is indicated as residual solvent.

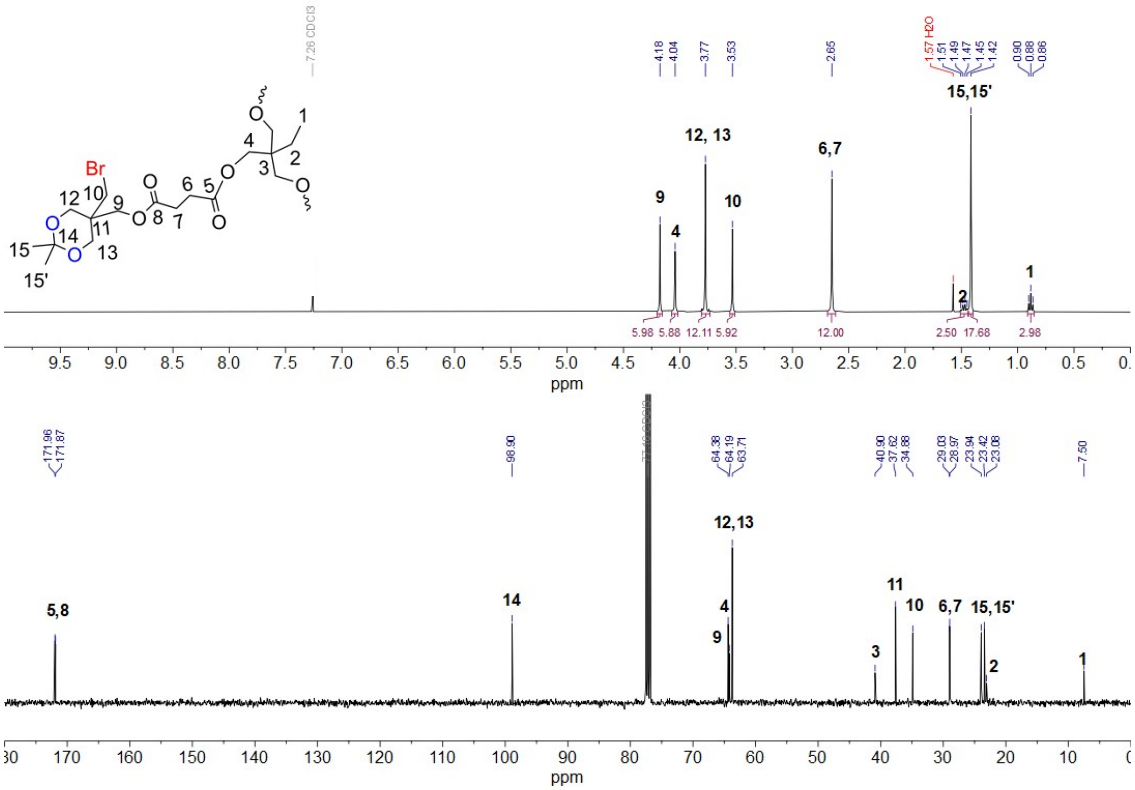


Figure S4. ^{1}H and ^{13}C NMR spectra of TMP-G1-$(Br)_3$-$(Ac)_3$ in $CDCl_3$.

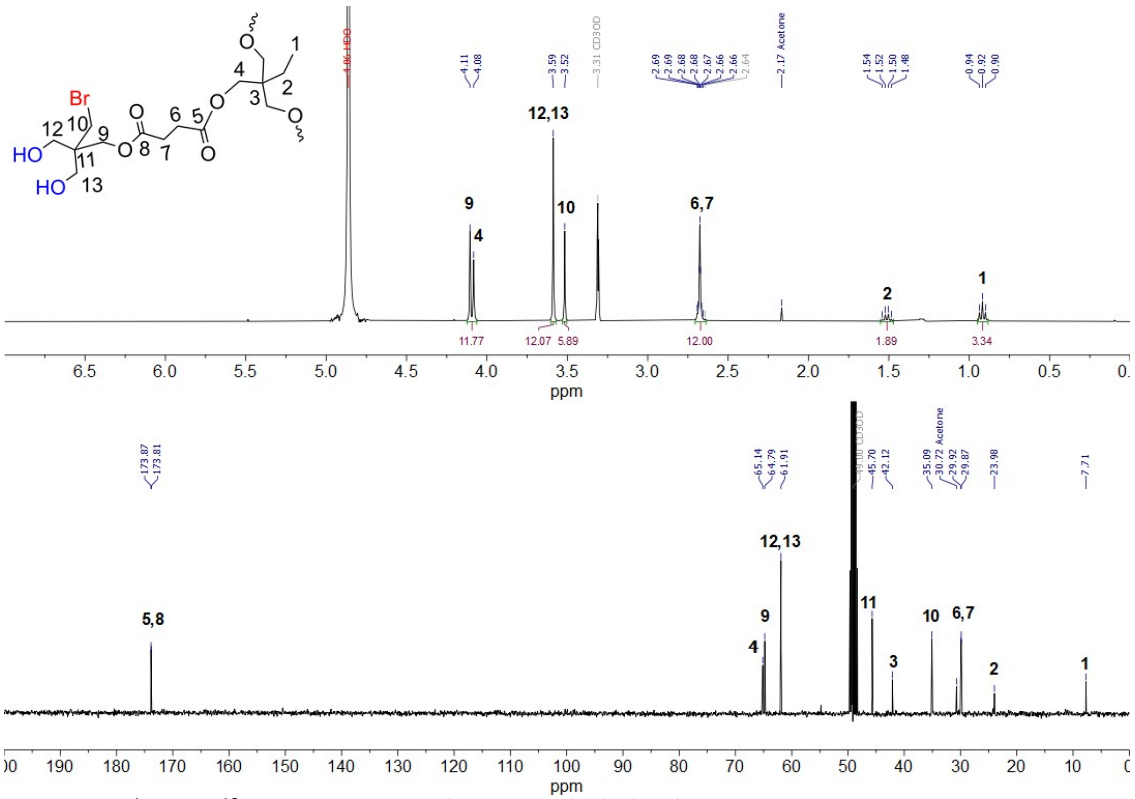


Figure S5. ^{1}H and ^{13}C NMR spectra of TMP-G1-$(Br)_3$-$(OH)_6$ in CD_3OD. Acetone is indicated as residual solvent.

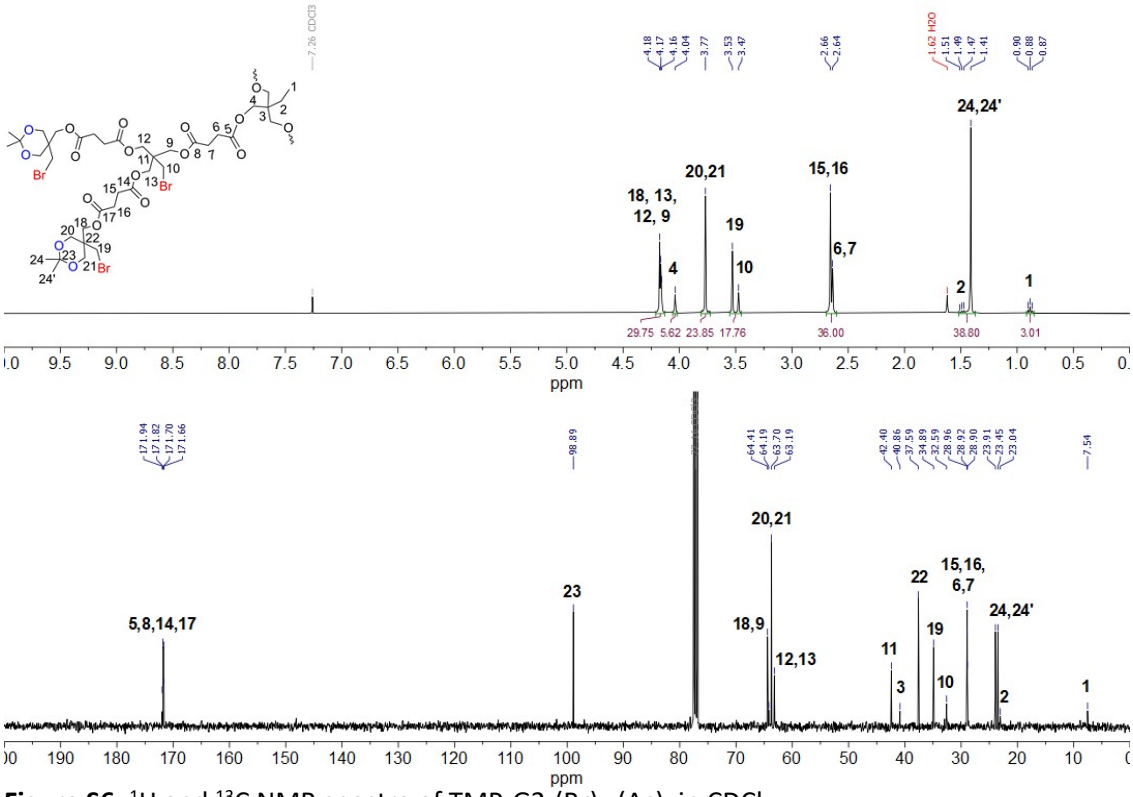


Figure S6. ^{1}H and ^{13}C NMR spectra of TMP-G2-$(Br)_9$-$(Ac)_6$ in $CDCl_3$.

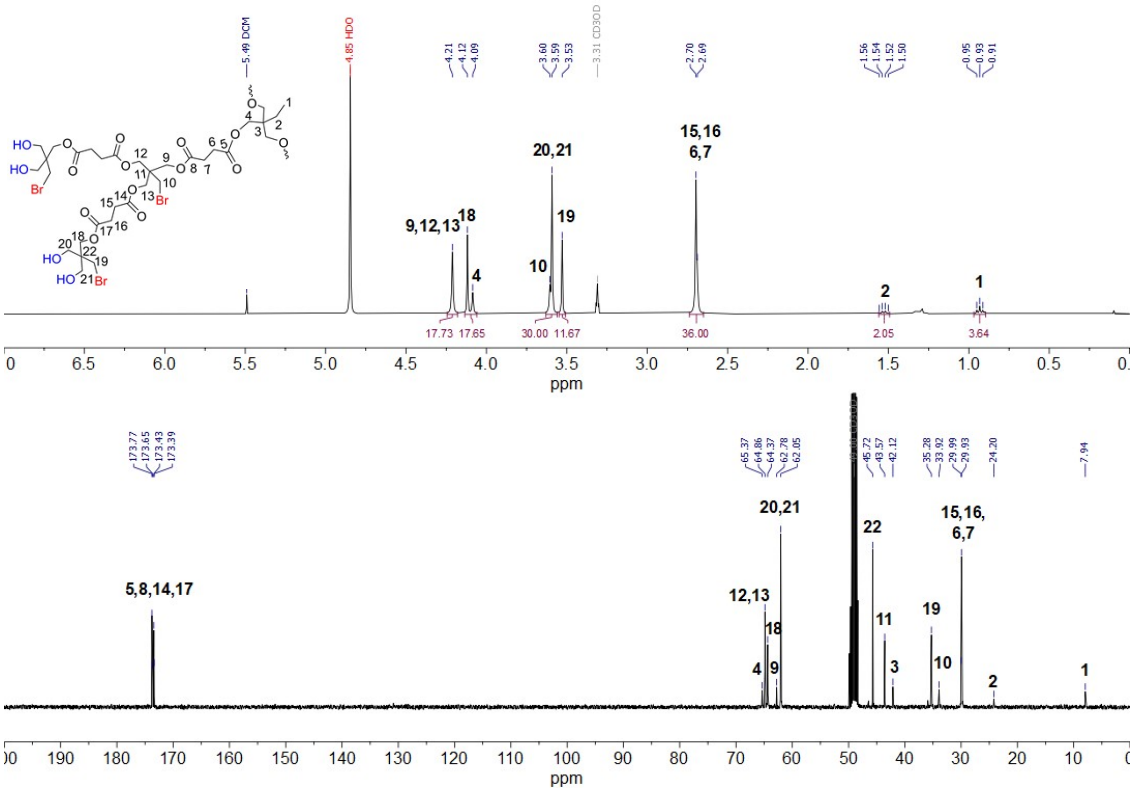

5.49 DCM
4.85 HDO
4.21
4.12
4.09
3.60
3.59
3.53
3.31 CD3OD
2.70
2.69
1.56
1.54
1.52
1.50
0.95
0.93
0.91
9,12,13
18
4
20,21
10
19
15,16
6,7
2
1
17.73
17.65
30.00
11.67
36.00
2.05
3.64
ppm
173.77
173.65
173.43
173.39
65.37
64.86
64.37
62.78
62.05
45.72
43.57
42.12
35.28
33.92
29.99
29.93
24.20
7.94
5,8,14,17
20,21
22
15,16,
6,7
12,13
18
11
19
4
9
3
10
2
1
ppm

Figure S7. ^{1}H and ^{13}C NMR spectra of TMP-G2-$(Br)_9$-$(OH)_{12}$ in CD_3OD. DCM is indicated as residual solvent.

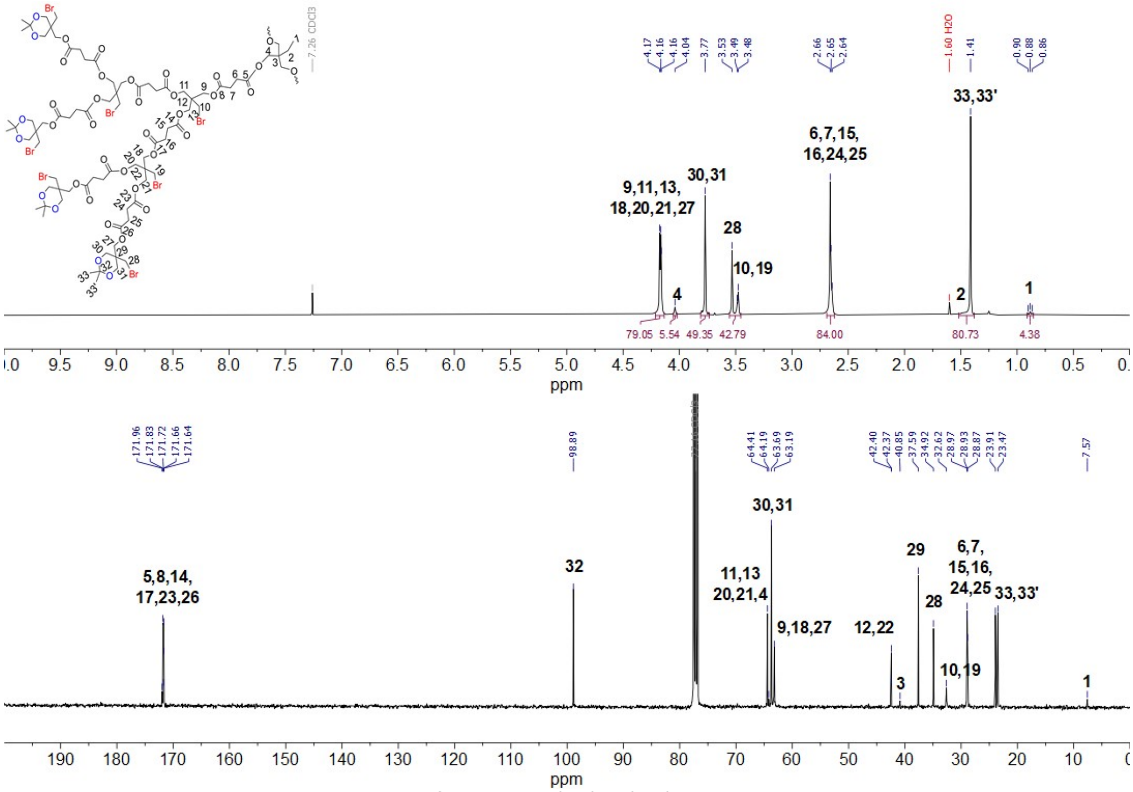


Figure S8. ^{1}H and ^{13}C NMR spectra of TMP-G3-$(Br)_{21}$-$(Ac)_{12}$ in $CDCl_3$.

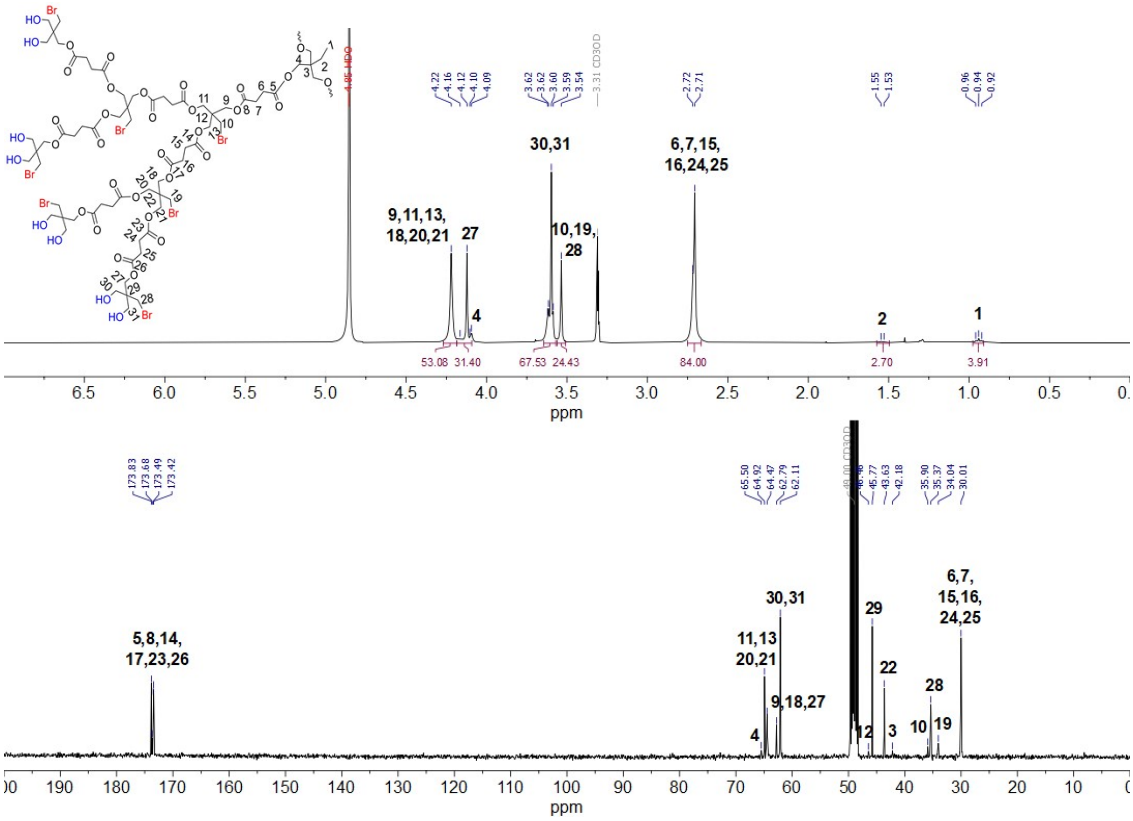

9,11,13,
18,20,21
27
4
30,31
10,19,
28
6,7,15,
16,24,25
2
1
53.08
31.40
67.53
24.43
84.00
2.70
3.91
ppm
5,8,14,
17,23,26
30,31
11,13
20,21
9,18,27
4
12
3
29
22
28
10
19
6,7,
15,16,
24,25
ppm

Figure S9. ^{1}H and ^{13}C NMR spectra of TMP-G3-$(Br)_{21}$-$(OH)_{24}$ in CD_3OD.

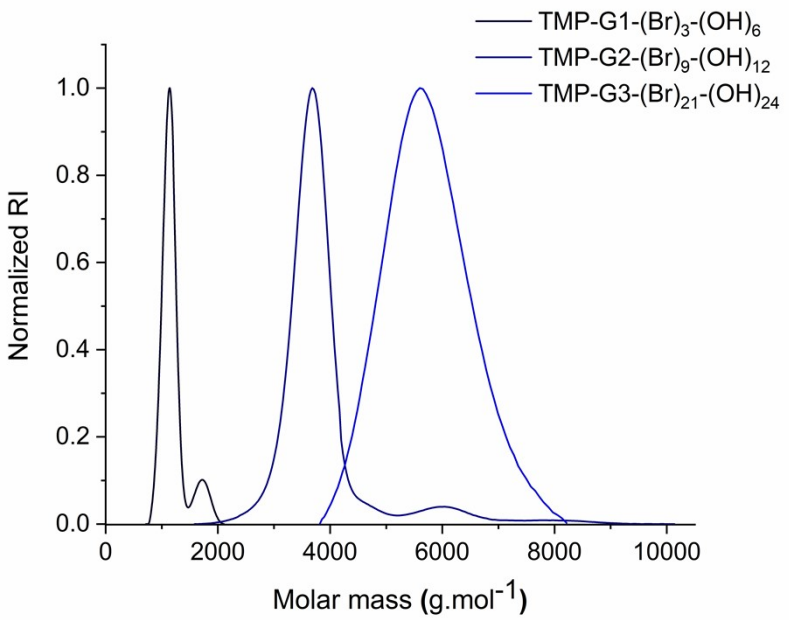


Figure S10. SEC overlay of TMP-G1-$(Br)_3$-$(OH)_6$, TMP-G2-$(Br)_9$-$(OH)_{12}$ and TMP-G3-$(Br)_{21}$-$(OH)_{24}$. Aggregation phenomenon was observed probably due to physical interaction of the dendrimers.

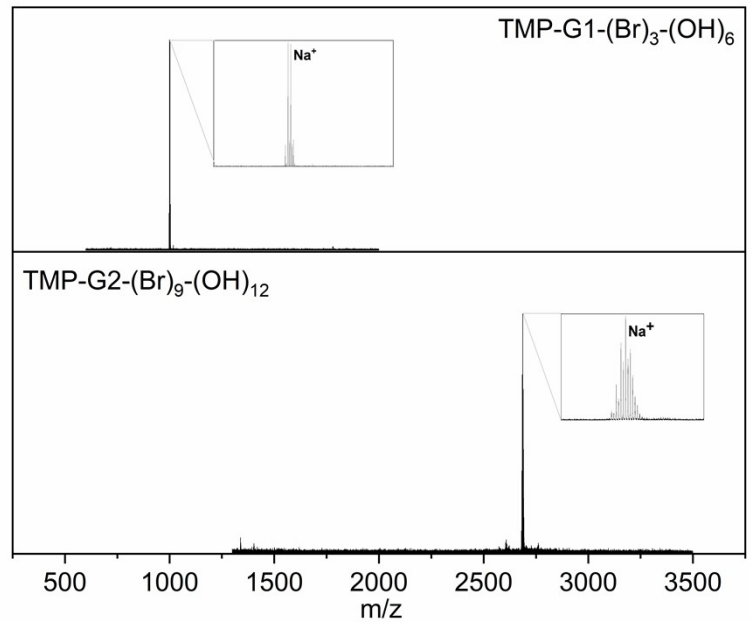


Figure S11. Stacked MALDI-TOF spectra of TMP-G1-$(Br)_3$-$(OH)_6$ in DHB and TMP-G2-$(Br)_9$-$(OH)_{12}$ in DCTB.

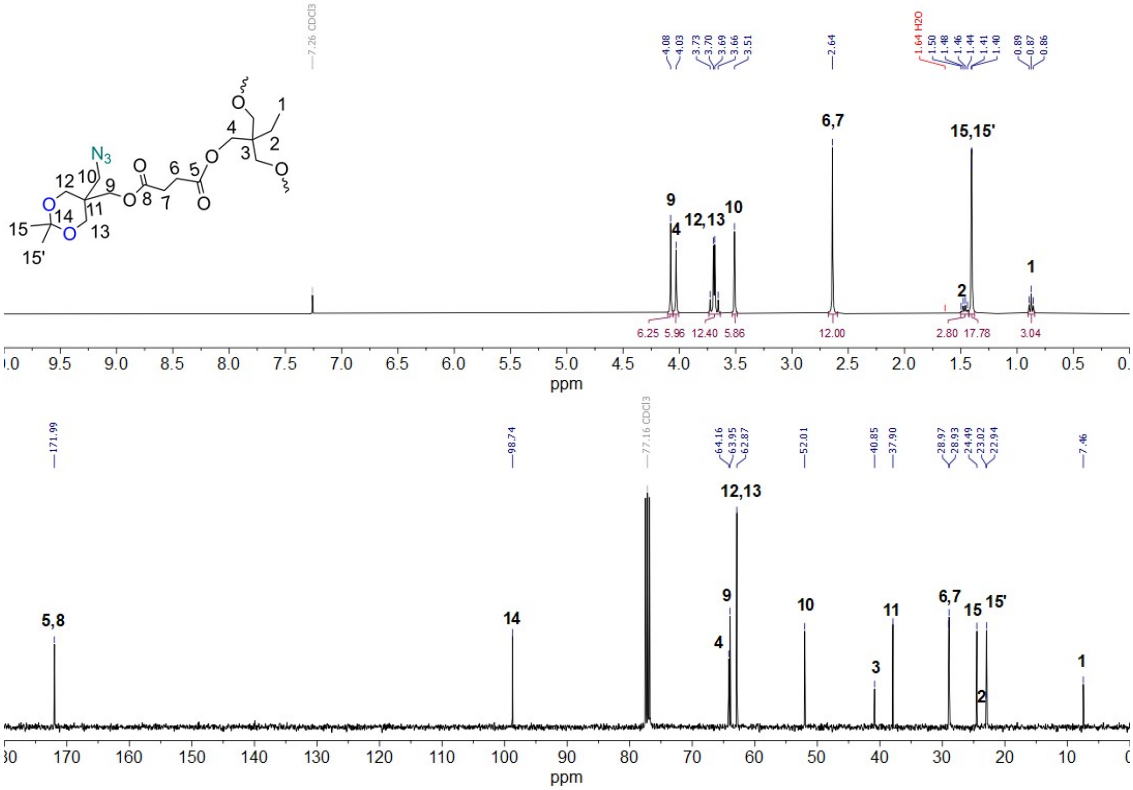
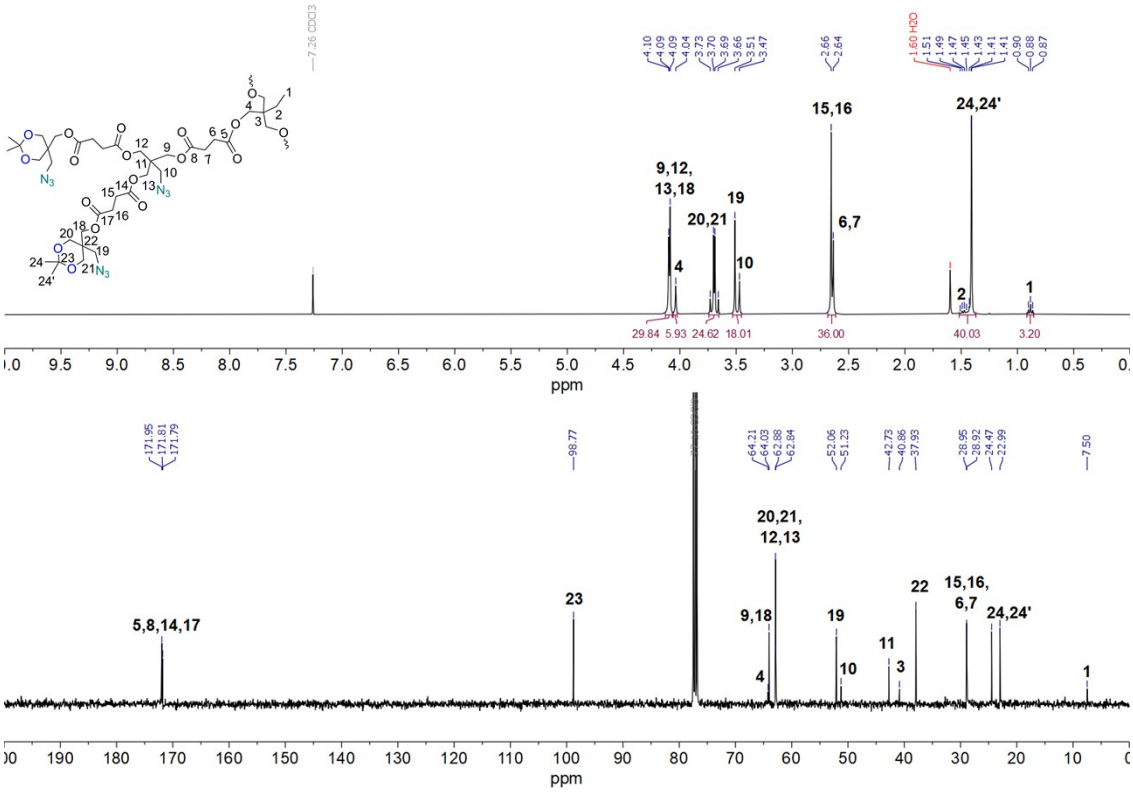

7.26 CDCl3
4.08
4.03
3.73
3.70
3.69
3.66
3.51
2.64
1.64 H2O
1.50
1.48
1.46
1.44
1.41
1.40
0.89
0.87
0.86
N3
O
O
O
O
O
O
O
O
O
O
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
15'
6,7
15,15'
9
4
12,13
10
1
2
6.25
5.96
12.40
5.86
12.00
2.80
17.78
3.04
ppm
171.99
98.74
77.16 CDCl3
64.16
63.95
62.87
52.01
40.85
37.90
28.97
28.93
24.49
23.02
22.94
7.46
12,13
5,8
14
9
4
10
11
6,7
15
15'
3
1
2
ppm

Figure S12. ^{1}H and ^{13}C NMR spectra of TMP-G1-$(N_3)_3$-$(Ac)_3$ in $CDCl_3$.


7.26 CDCl3
4.10
4.09
4.09
4.04
3.73
3.70
3.69
3.66
3.51
3.47
2.66
2.64
1.60 H2O
1.51
1.49
1.47
1.45
1.43
1.41
1.41
0.90
0.88
0.87
9,12,
13,18
19
20,21
15,16
6,7
24,24'
4
10
2
1
29.84
5.93
24.62
18.01
36.00
40.03
3.20
ppm
171.95
171.81
171.79
98.77
64.21
64.03
63.88
62.84
52.06
51.23
42.73
40.86
37.93
28.95
28.92
24.47
22.99
7.50
20,21,
12,13
22
15,16,
6,7
24,24'
5,8,14,17
23
9,18
19
11
3
4
10
1
ppm

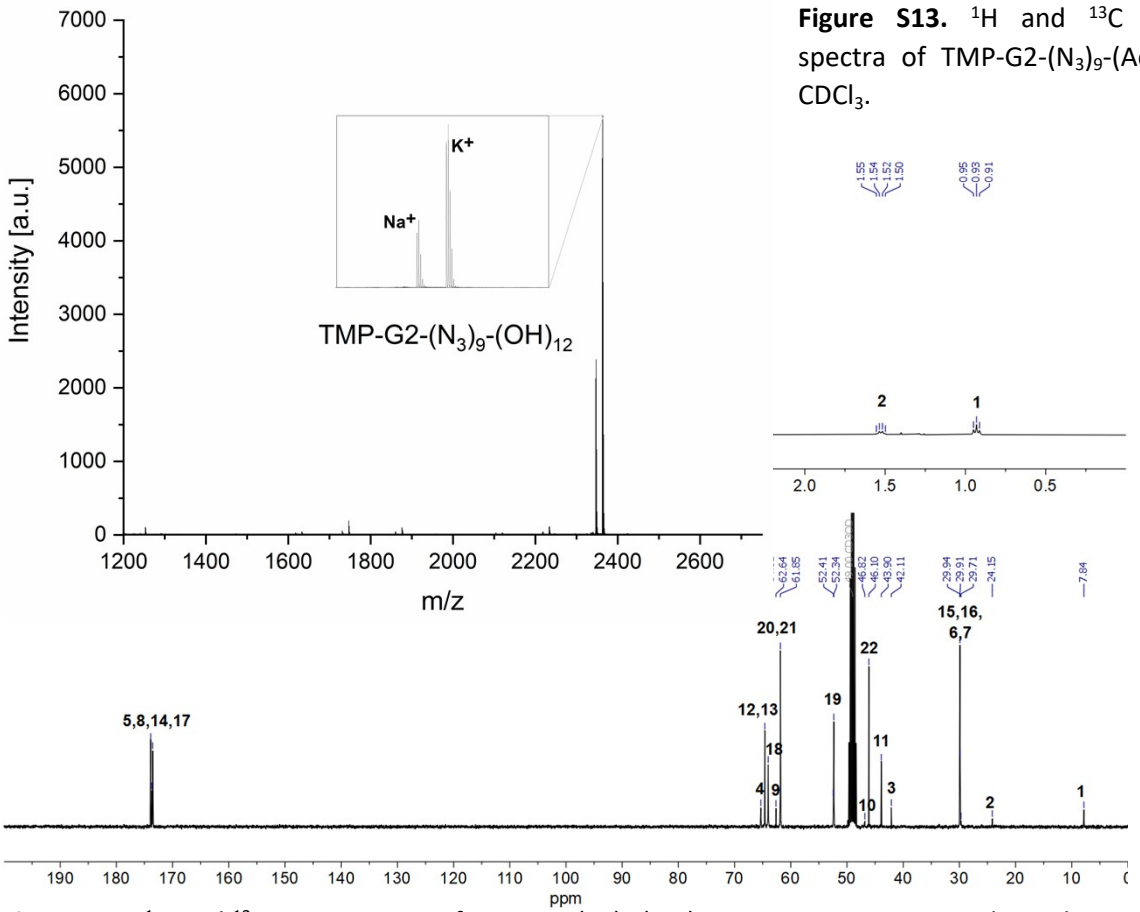


Figure S13. ^{1}H and ^{13}C NMR spectra of TMP-G2-$(N_3)_9$-$(Ac)_6$ in $CDCl_3$.

Figure S14. ^{1}H and ^{13}C NMR spectra of TMP-G2-$(N_3)_9$-$(OH)_{12}$ in CD_3OD. MeOH is indicated as residual solvent.

Figure S15. MALDI-TOF spectra of TMP-G2-$(N_3)_3$-$(OH)_{12}$ in DCTB.

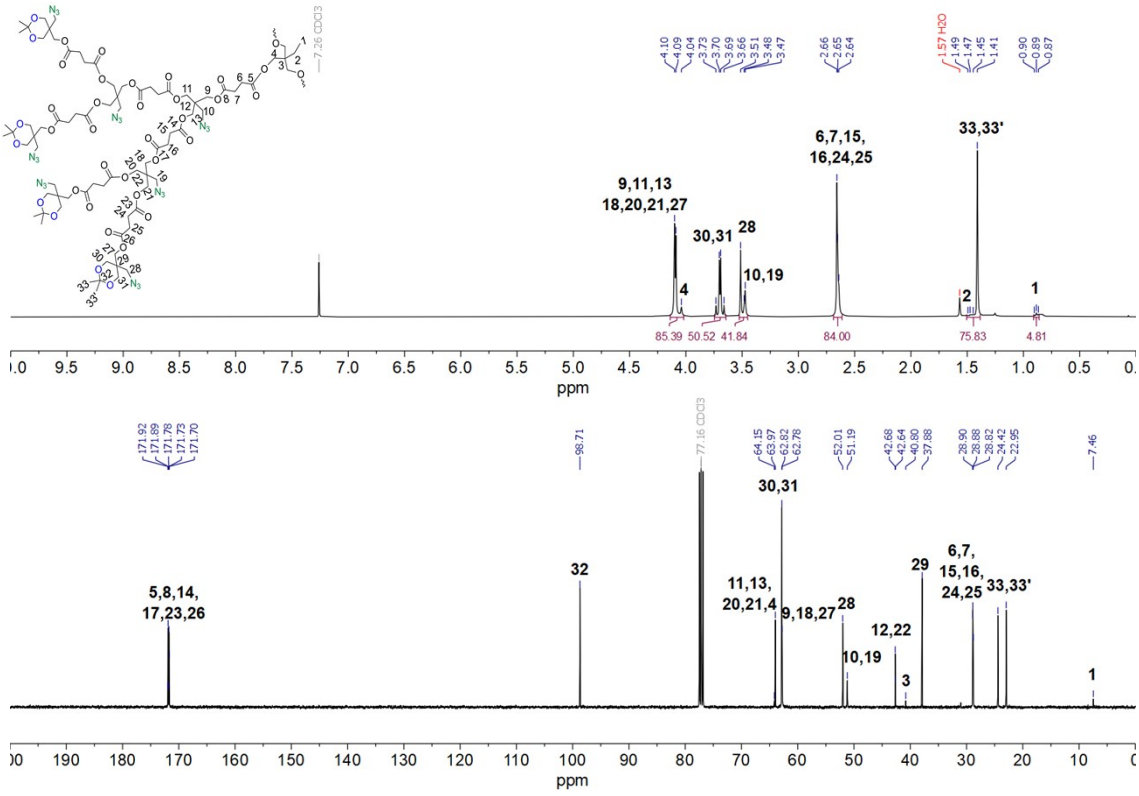

7.26 CDCl3
4.10
4.09
4.04
3.73
3.70
3.69
3.66
3.51
3.48
3.47
2.66
2.65
2.64
1.57 H2O
1.49
1.47
1.45
1.41
0.90
0.89
0.87
9,11,13
18,20,21,27
30,31
28
10,19
4
6,7,15,
16,24,25
33,33'
2
1
85.39
50.52
41.84
84.00
75.83
4.81
ppm
171.92
171.89
171.78
171.73
171.70
98.71
77.16 CDCl3
64.15
63.97
63.82
62.78
52.01
51.19
42.68
42.64
40.80
37.88
28.90
28.88
28.82
24.42
22.95
7.46
30,31
32
5,8,14,
17,23,26
11,13,
20,21,4
9,18,27
28
29
6,7,
15,16,
24,25
33,33'
12,22
10,19
3
1
ppm

Figure S16. ^{1}H and ^{13}C NMR spectra of TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$ in $CDCl_3$.

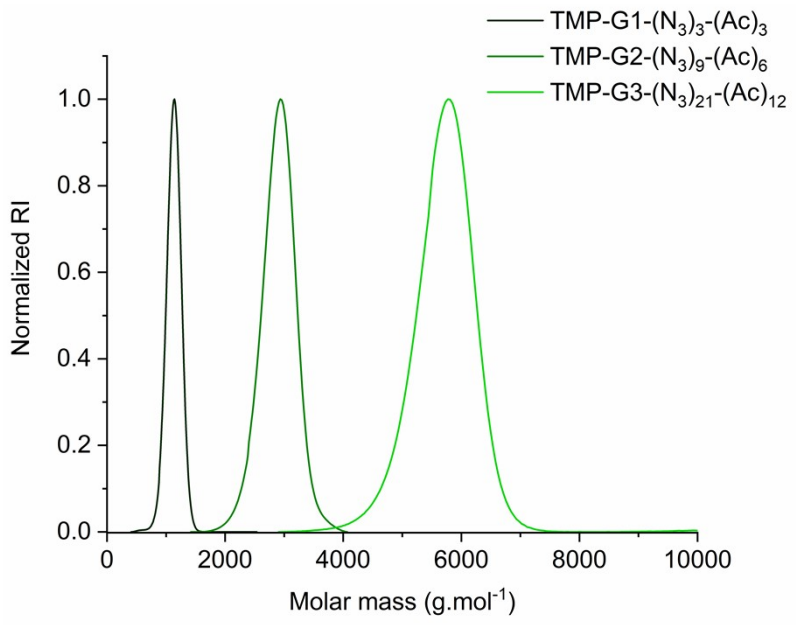


Figure S17. SEC overlay of TMP-G1-$(N_3)_3$-$(Ac)_3$, TMP-G2-$(N_3)_9$-$(Ac)_6$ and TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$.

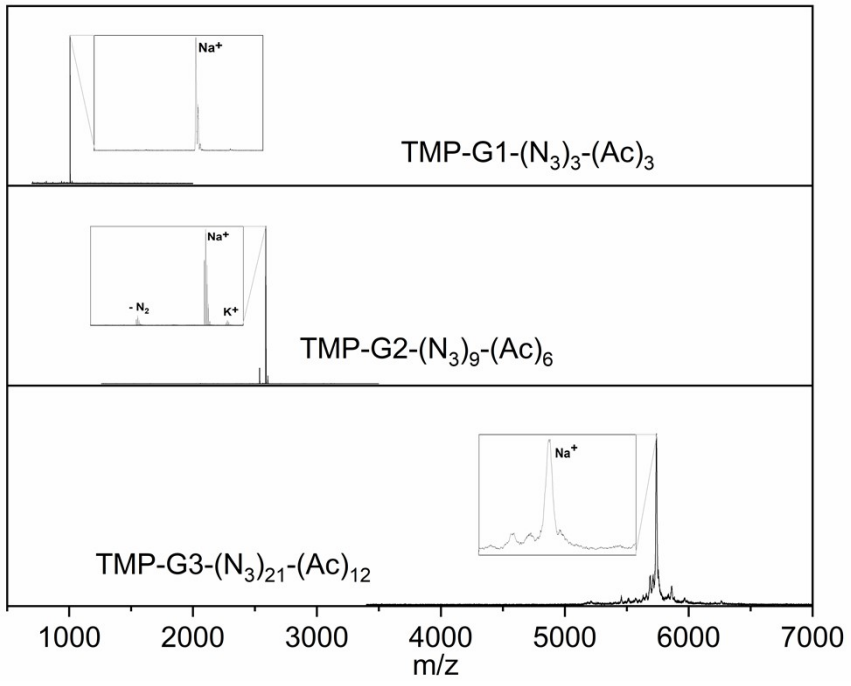


Figure S18. Stacked MALDI-TOF spectra of TMP-G1-$(N_3)_3$-$(Ac)_3$, TMP-G2-$(N_3)_9$-$(Ac)_6$ and TMP-G3-$(N_3)_{21}$-$(Ac)_{12}$.

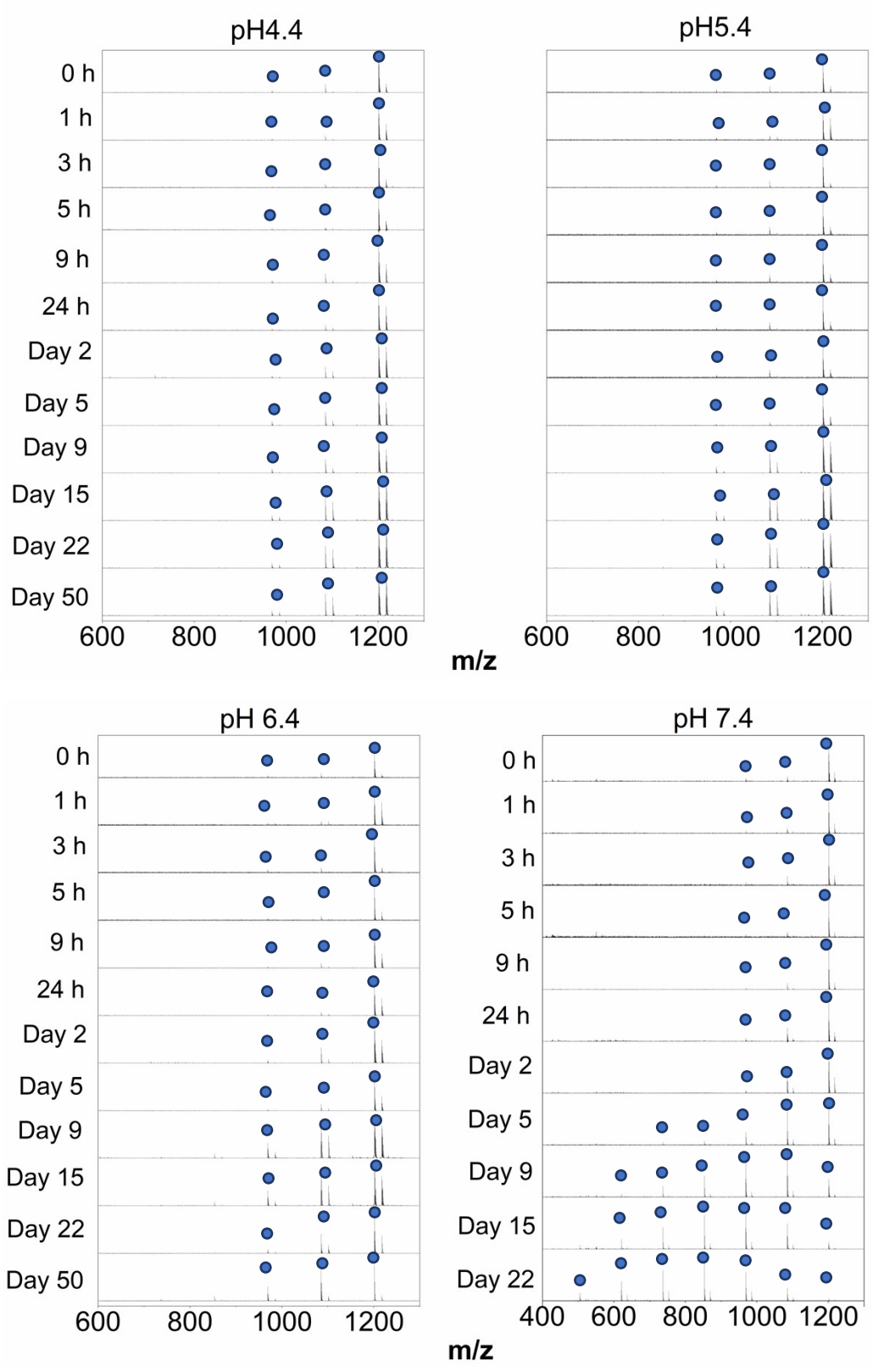


Figure S19. Degradation evaluation of TMP-G2-$(OH)_{12}$ at varying pH (4.4-7.4) and time intervals (up to day 50) by MALDI-TOF in DCTB.

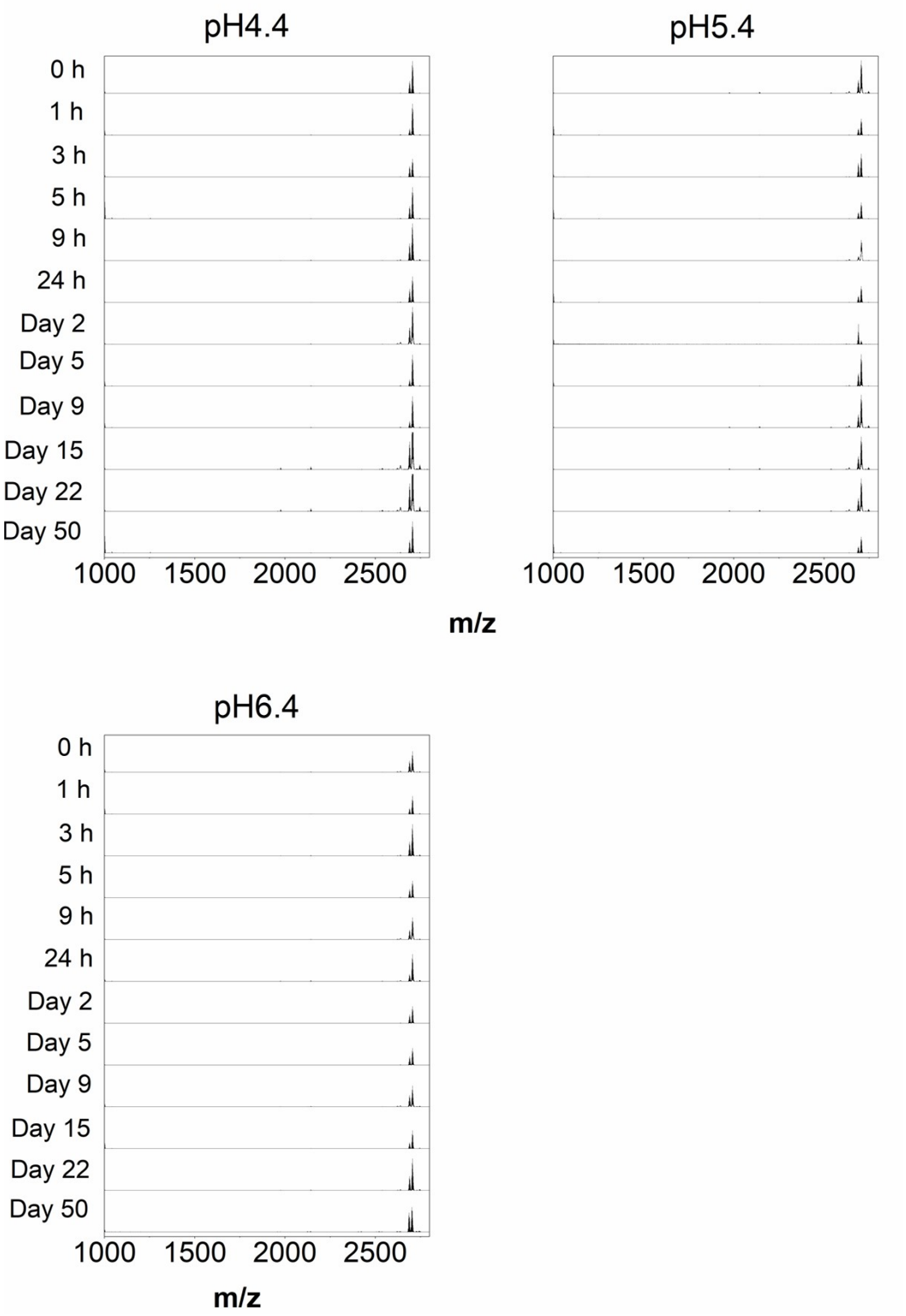


Figure S20. Degradation evaluation of TMP-G2-$(Br)_9$-$(OH)_{12}$ at varying pH (4.4-6.4) and time intervals (up to day 50) by MALDI-TOF in DCTB.

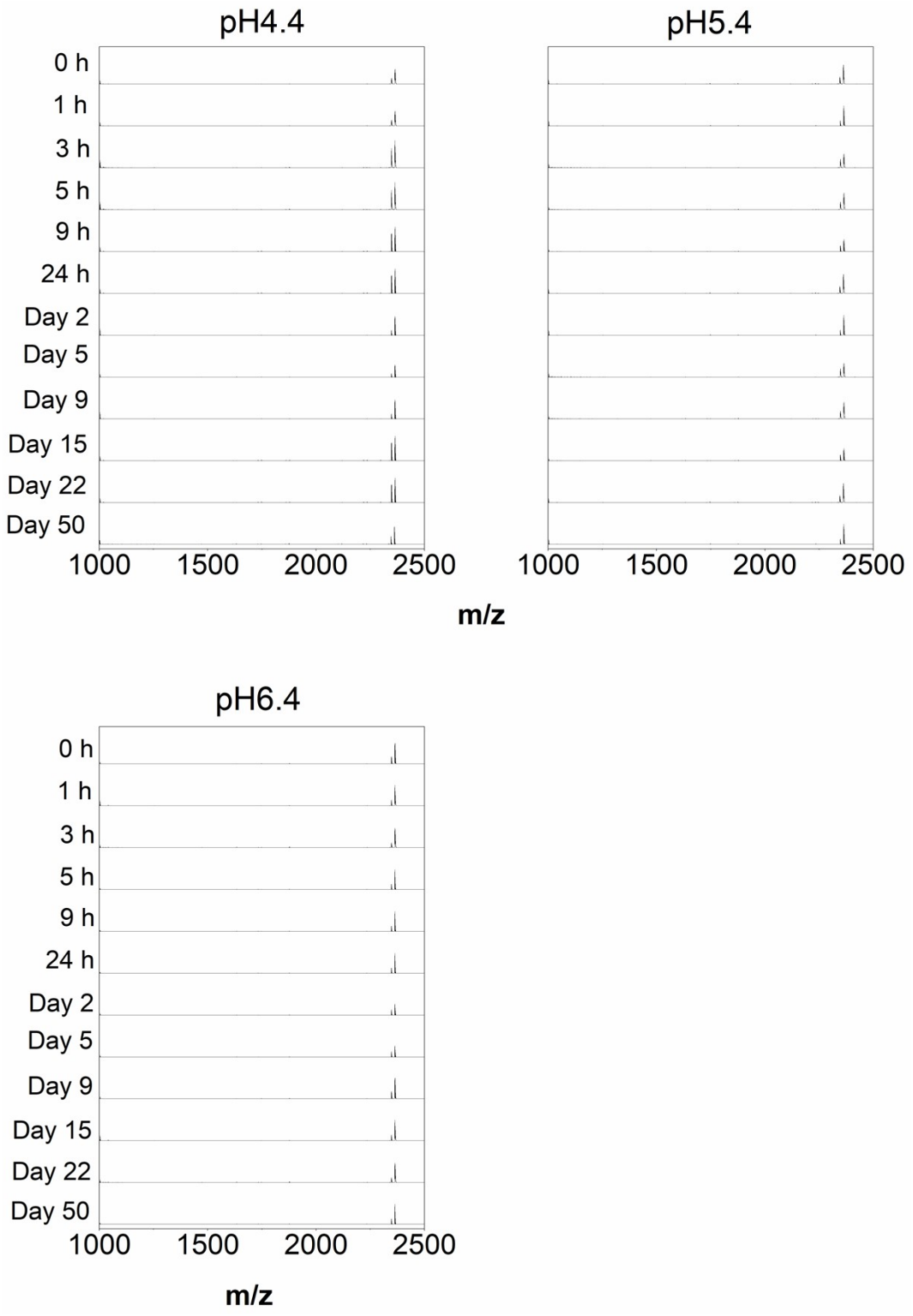


Figure S21. Degradation evaluation of TMP-G2-$(N_3)_9$-$(OH)_{12}$ at varying pH (4.4-6.4) and time intervals (up to day 50) by MALDI-TOF in DCTB.

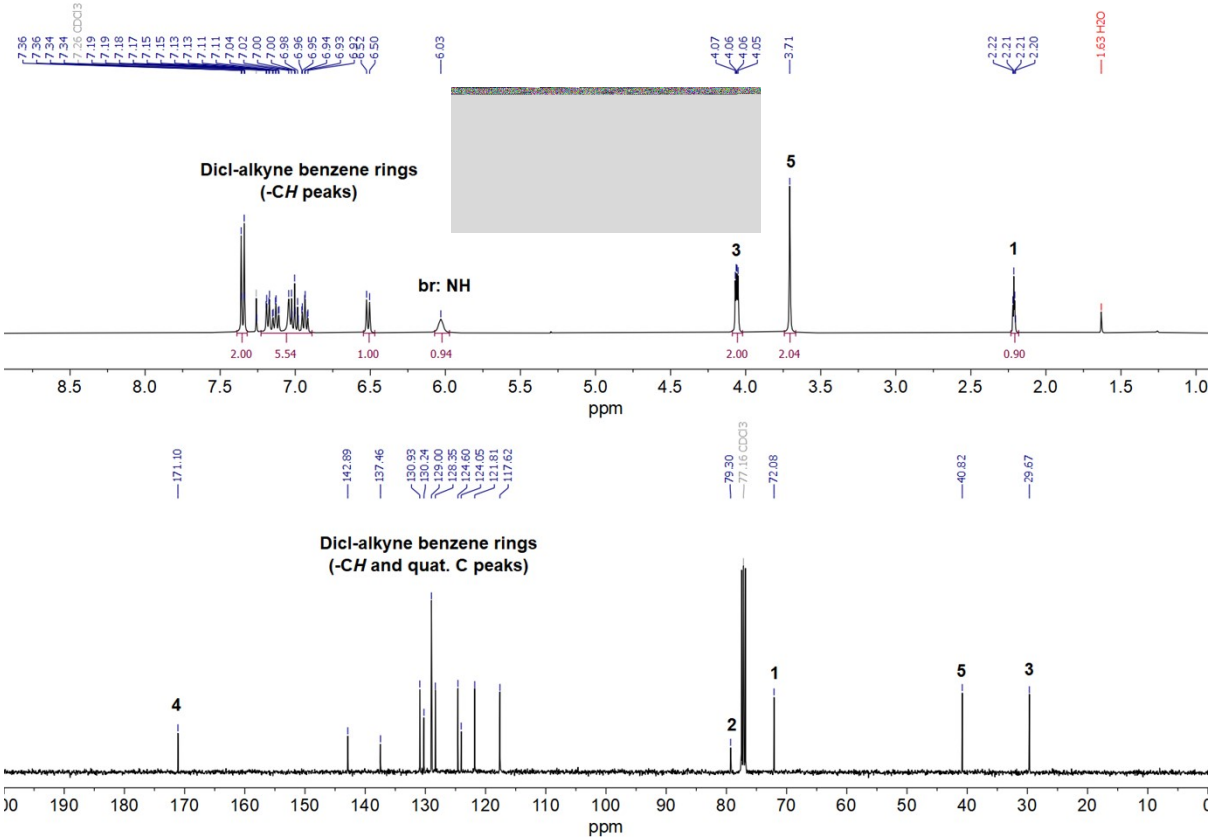


Figure S22. ^{1}H and ^{13}C NMR spectra of Dicl-alkyne in $CDCl_3$.

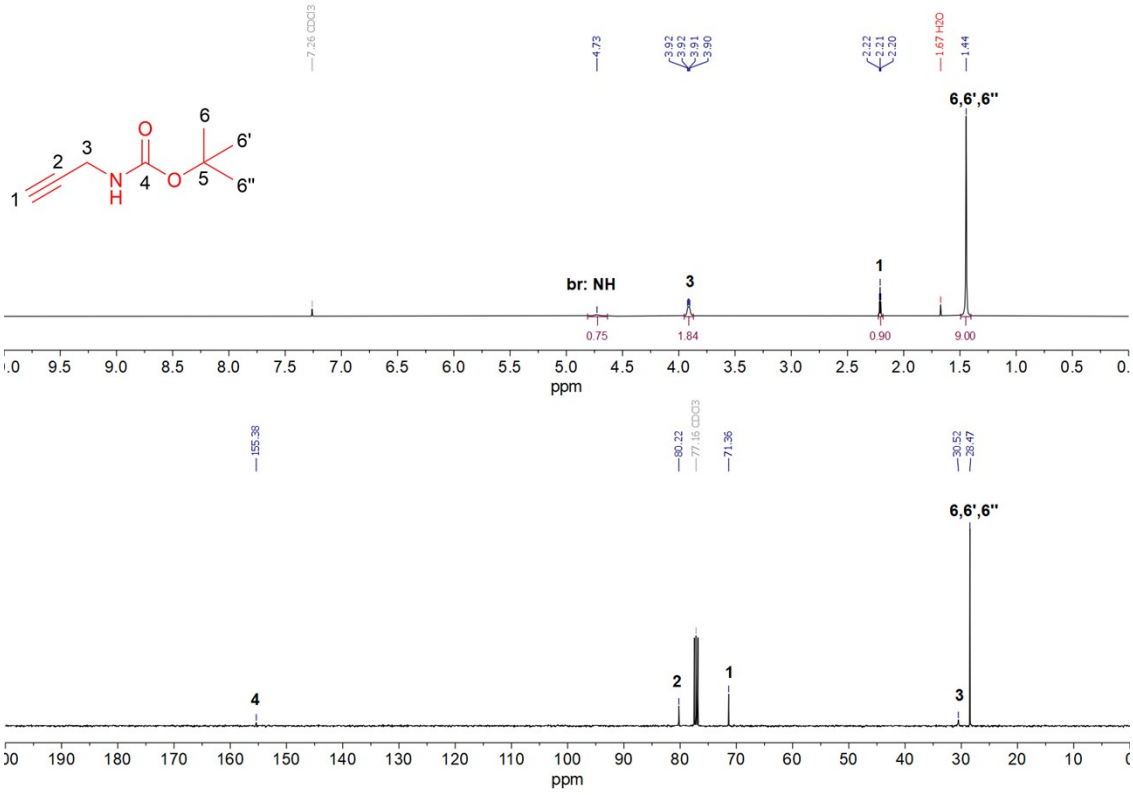

7.26 CDCl3
4.73
3.92
3.92
3.91
3.90
2.22
2.21
2.20
1.67 H2O
1.44
6,6',6''
br: NH
3
1
0.75
1.84
0.90
9.00
ppm
155.38
80.22
77.16 CDCl3
71.36
30.52
28.47
6,6',6''
4
2
1
3
ppm

Figure S23. ^{1}H and ^{13}C NMR spectra of Boc-PA in $CDCl_3$.

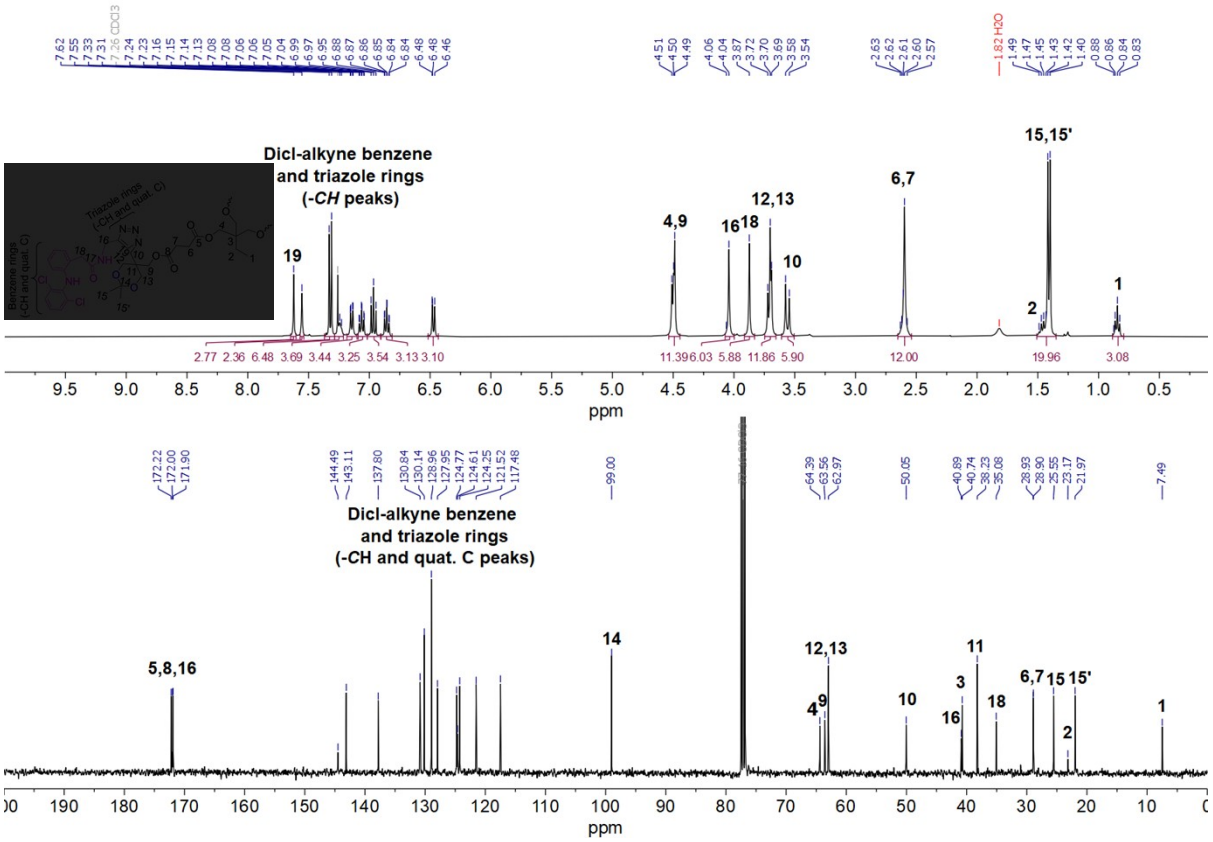


Figure S24. ^{1}H and ^{13}C NMR spectra of TMP-G1-$(DicI)_3$-$(Ac)_3$ in $CDCl_3$.

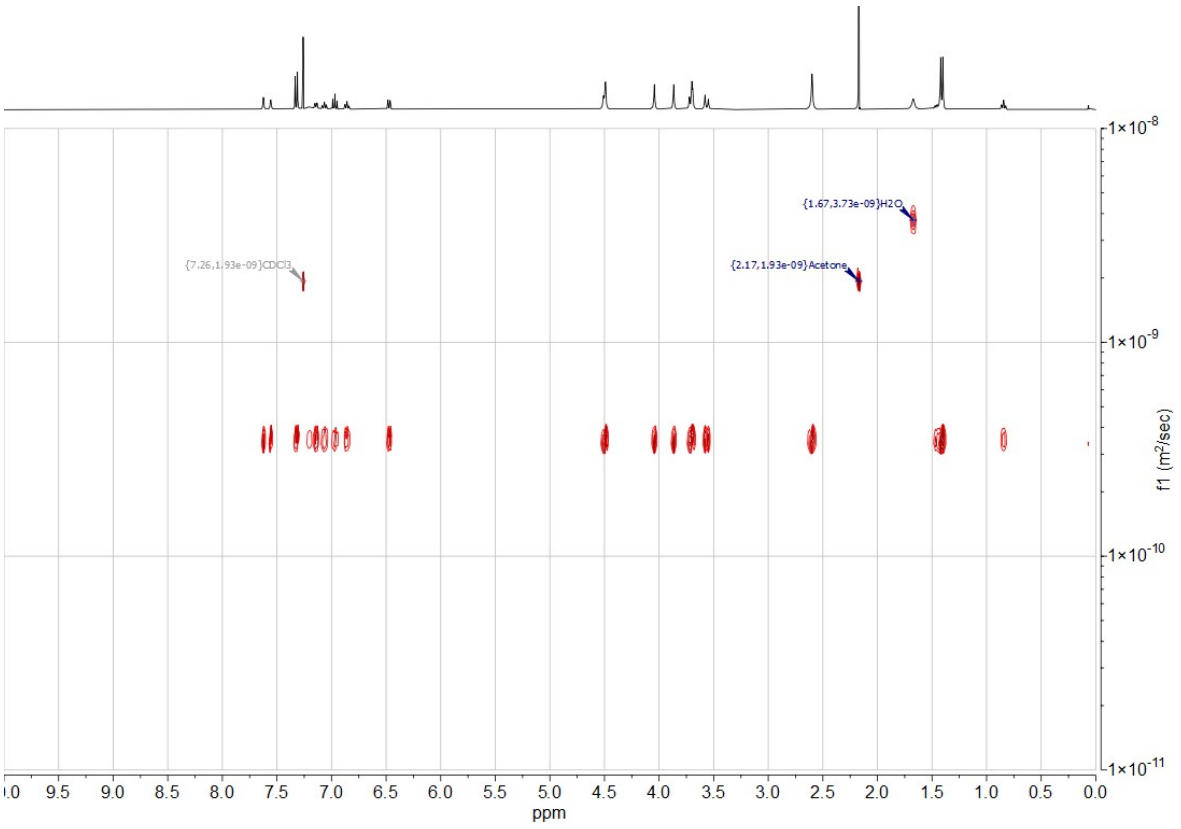


Figure S25. DOSY spectra of TMP-G1-$(Dicl)_3$-$(Ac)_3$ in $CDCl_3$.

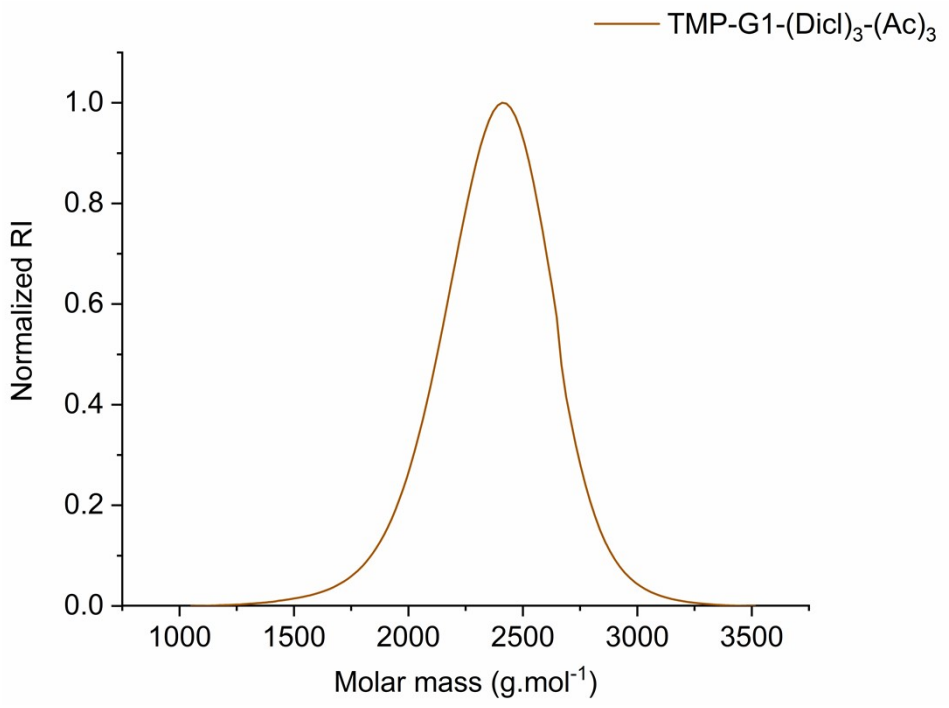


Figure S26. SEC of TMP-G1-$(Dicl)_3$-$(Ac)_3$.

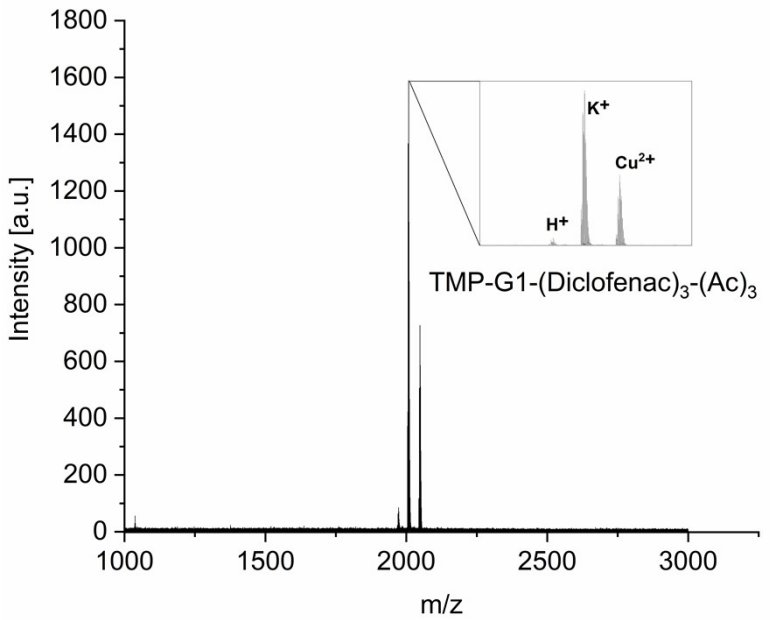
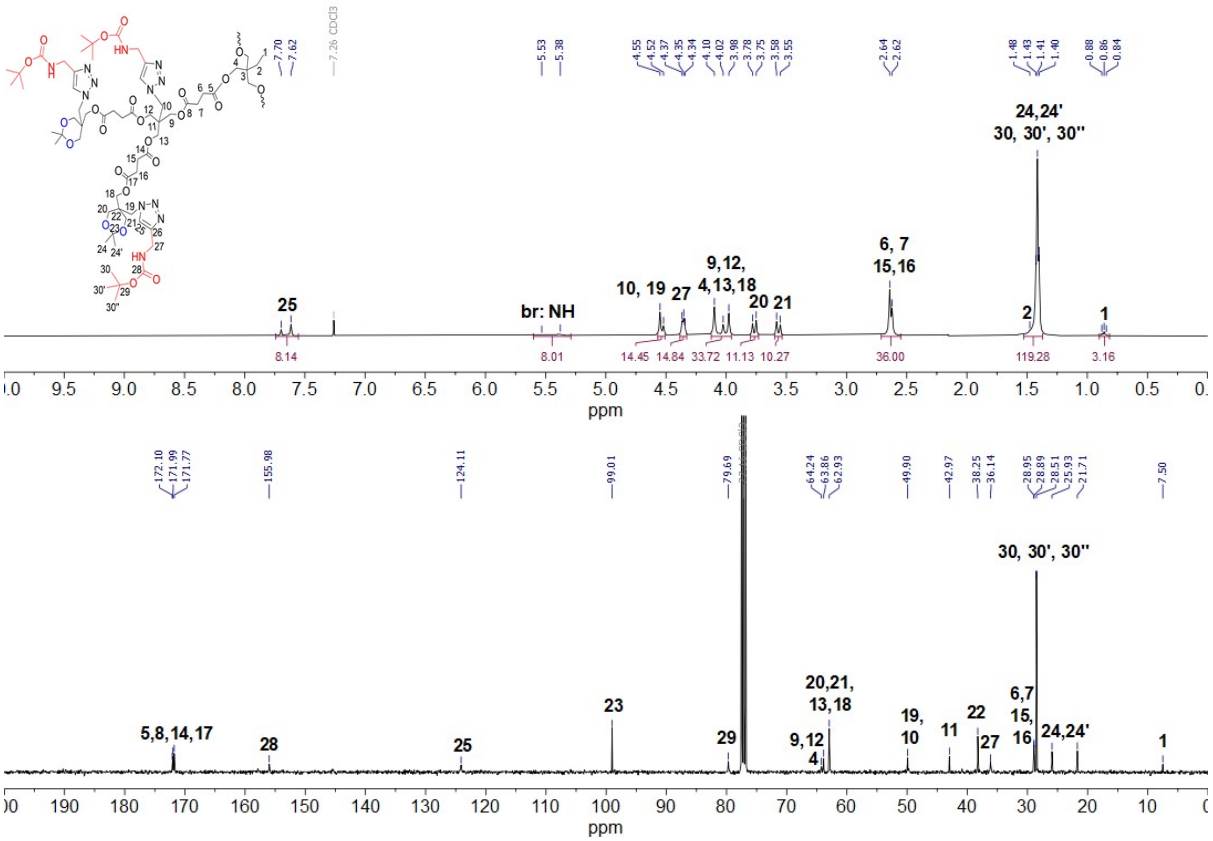


Figure S27. MALDI-TOF spectra of TMP-G1-$(Dicl)_3$-$(Ac)_3$ in DHB.


7.70
7.62
7.26 CDCl3
5.53
5.38
4.55
4.52
4.37
4.35
4.34
4.10
4.02
3.98
3.78
3.75
3.58
3.55
2.64
2.62
1.48
1.43
1.41
1.40
0.88
0.86
0.84
24,24'
30, 30', 30''
6, 7
15,16
9,12,
4,13,18
10, 19
27
20 21
25
br: NH
2
1
8.14
8.01
14.45
14.84
33.72
11.13
10.27
36.00
119.28
3.16
ppm
172.10
171.99
171.77
155.98
124.11
99.01
79.69
64.24
63.86
62.93
49.90
42.97
38.25
36.14
28.95
28.89
28.51
25.93
21.71
7.50
30, 30', 30''
20,21,
13,18
6,7
15,
16
24,24'
5,8,14,17
28
25
23
29
9,12
4
19,
10
11
22
27
1
ppm

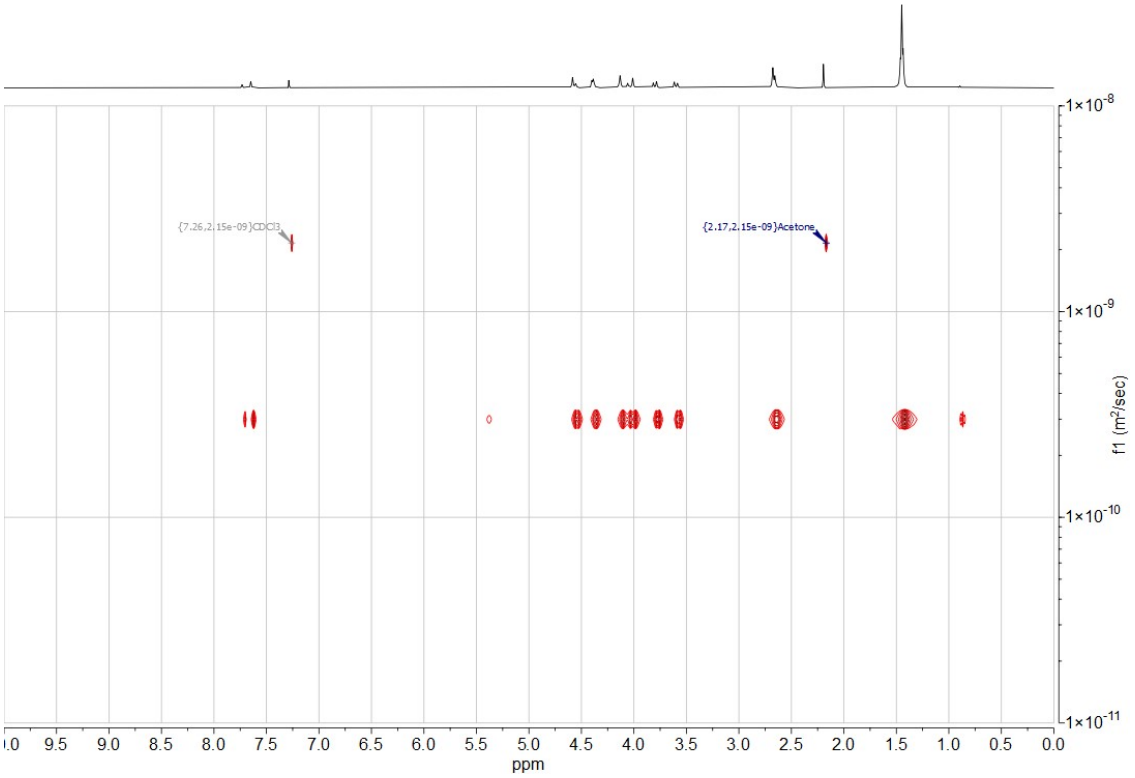


Figure S29. DOSY spectra of TMP-G2-(Boc-PA)$_9$-(Ac)$_6$ in $CDCl_3$.

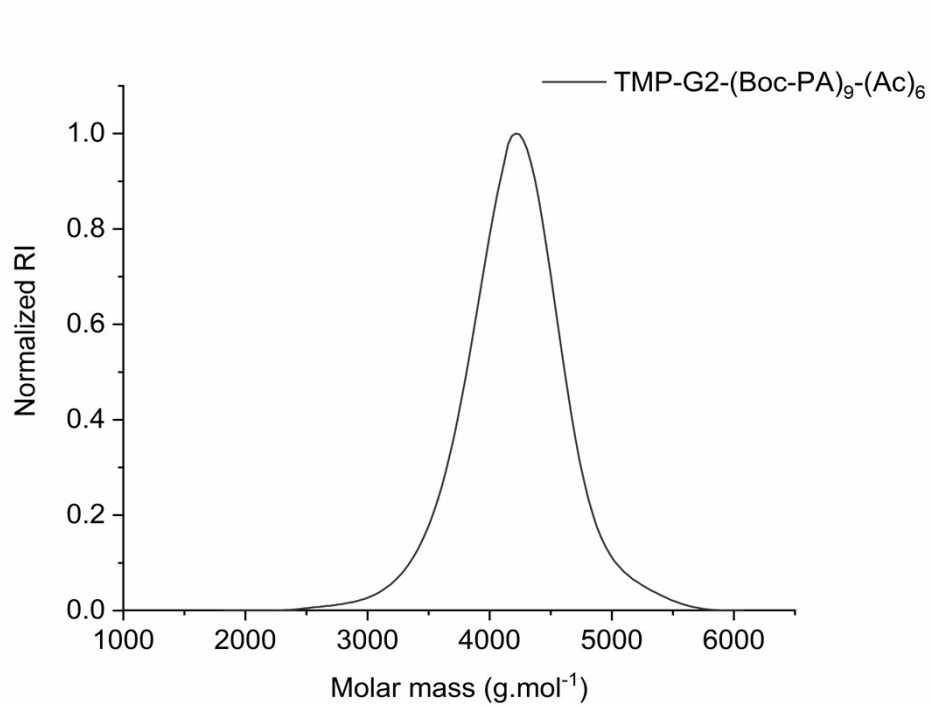


Figure S30. SEC of TMP-G2-(Boc-PA)$_9$-(Ac)$_6$.

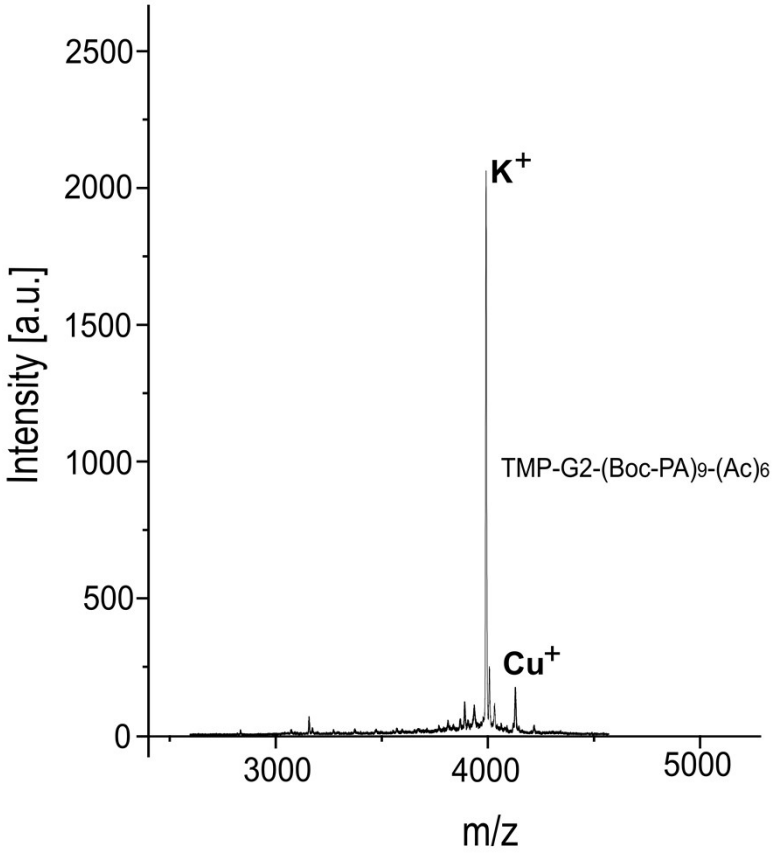


Figure S31. MALDI-TOF spectra of TMP-G2-(Boc-PA)$_9$-(Ac)$_6$ in DCTB.

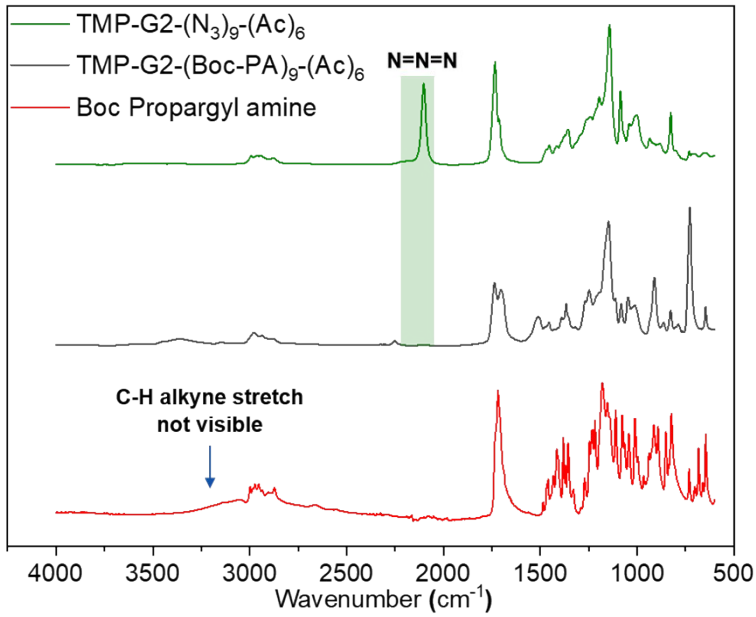


Figure S32. Stacked FTIR spectra of TMP-G2-(N$_3$)$_9$-(Ac)$_6$ (Top), TMP-G2-(Boc-PA)$_9$-(Ac)$_6$ (Middle) and free Boc-PA (Bottom).

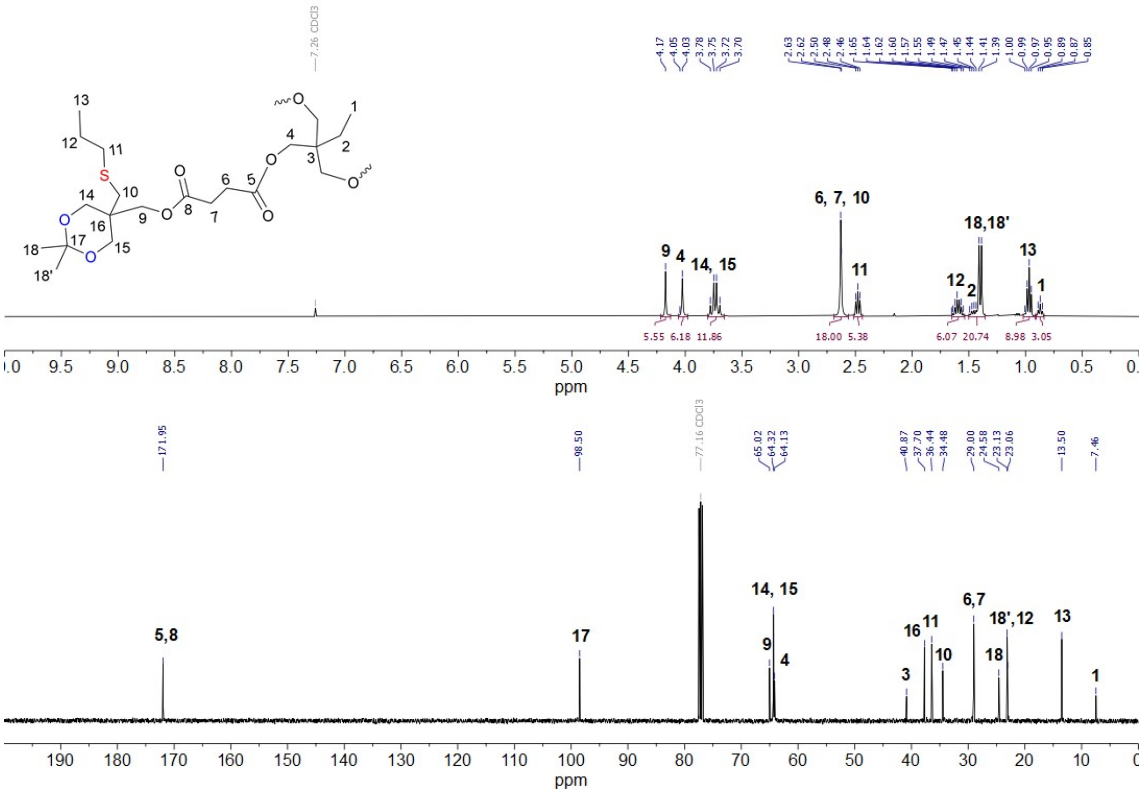

7.26 CDCl3
4.17
4.05
4.03
3.78
3.75
3.72
3.70
2.63
2.62
2.50
2.48
2.46
1.65
1.64
1.62
1.60
1.57
1.55
1.49
1.47
1.45
1.44
1.41
1.39
1.00
0.99
0.97
0.95
0.89
0.87
0.85
13
12
11
S
10
14
16
17
18
18'
15
O
9
8
7
6
5
4
3
2
1
6, 7, 10
18,18'
9
4
14, 15
11
13
12
2
1
5.55 6.18 11.86
18.00 5.38
6.07 20.74
8.98 3.05
ppm
171.95
98.50
77.16 CDCl3
65.02
64.32
64.13
40.87
37.70
36.44
34.48
29.00
24.58
23.13
23.06
13.50
7.46
5,8
17
14, 15
9
4
6,7
18',12
13
16
11
10
18
3
1
ppm

Figure S33. ^{1}H and ^{13}C NMR spectra of TMP-G1-(S-$(CH_2)_2$-$CH_3)_{21}$-$(Ac)_{12}$ in $CDCl_3$.

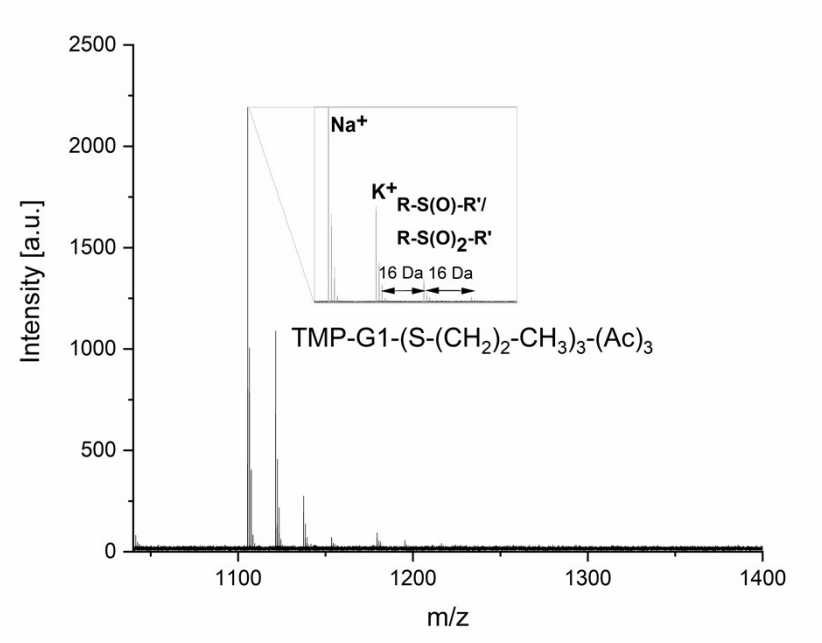


Figure S34. MALDI-TOF spectra of TMP-G1-(S-$(CH_2)_2$-$CH_3)_3$-$(Ac)_3$ in DCTB. The additional peaks with a difference of 16 Da represent the oxidation of sulfides to sulfoxides or sulfones in MALDI.

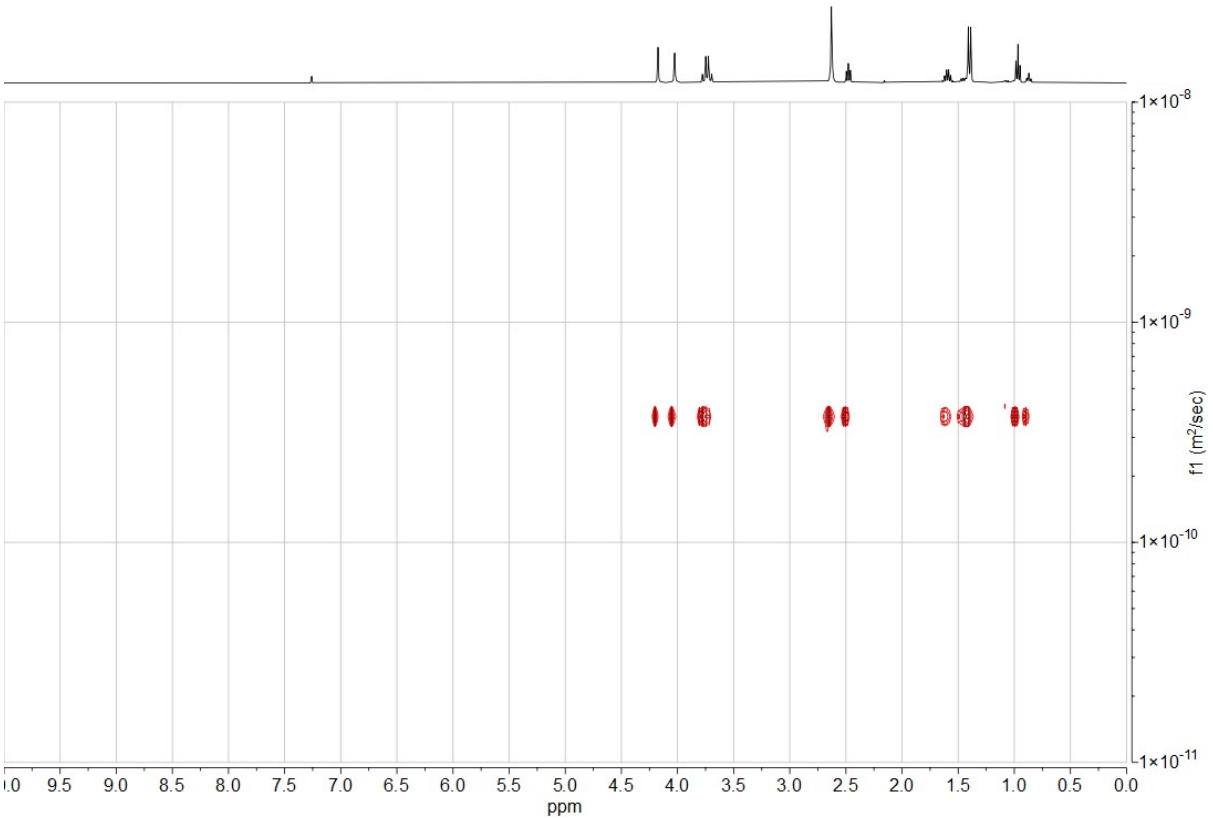


Figure S35. DOSY of TMP-G1-(S-$(CH_2)_2$-$CH_3)_3$-$(Ac)_3$.

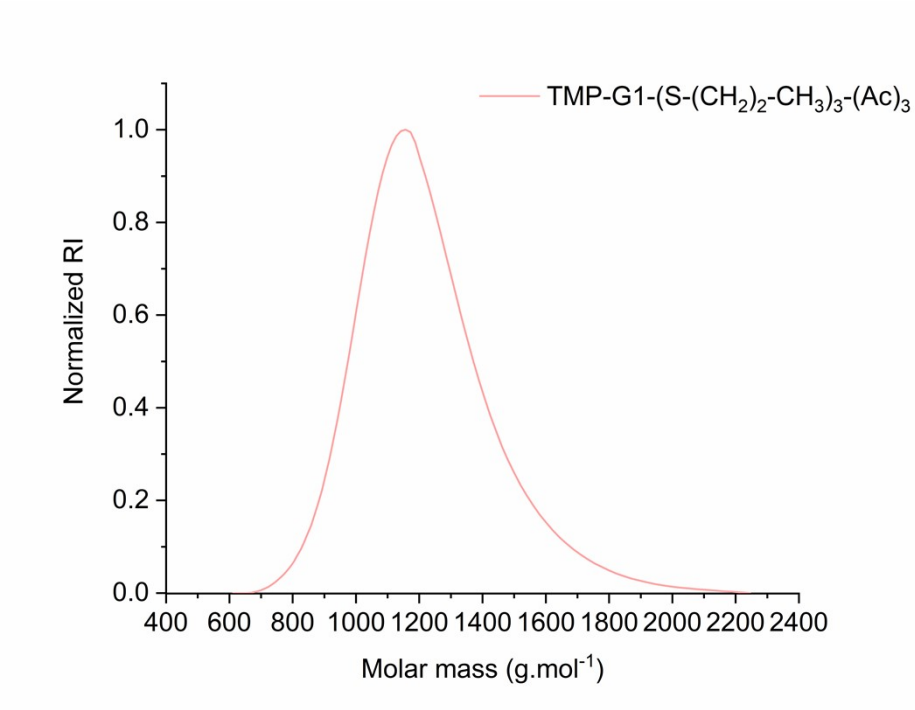


Figure S36. SEC of TMP-G1-(S-$(CH_2)_2$-$CH_3)_3$-$(Ac)_3$.

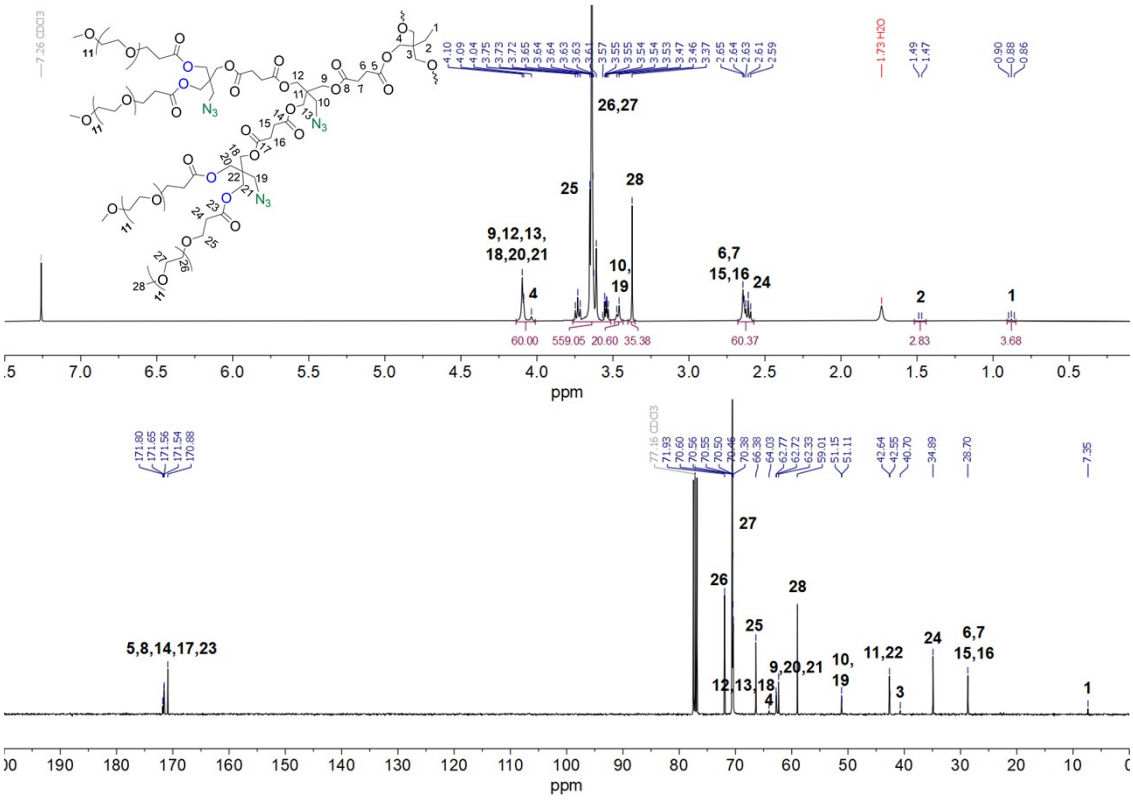


Figure S37. ^{1}H and ^{13}C NMR spectra of TMP-G2-$(N_3)_9$-$(mPEG)_{12}$ in $CDCl_3$.

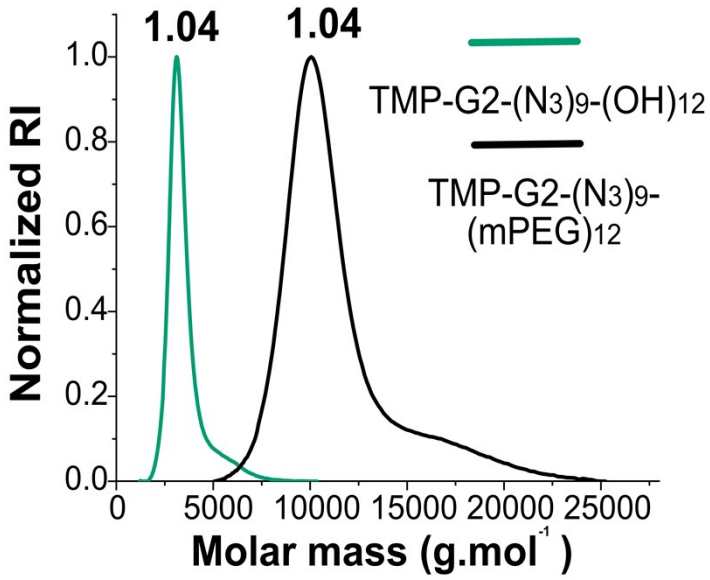


Figure S38. SEC overlay of TMP-G2-$(N_3)_9$-$(OH)_{12}$ and TMP-G2-$(N_3)_9$-$(mPEG)_{12}$.

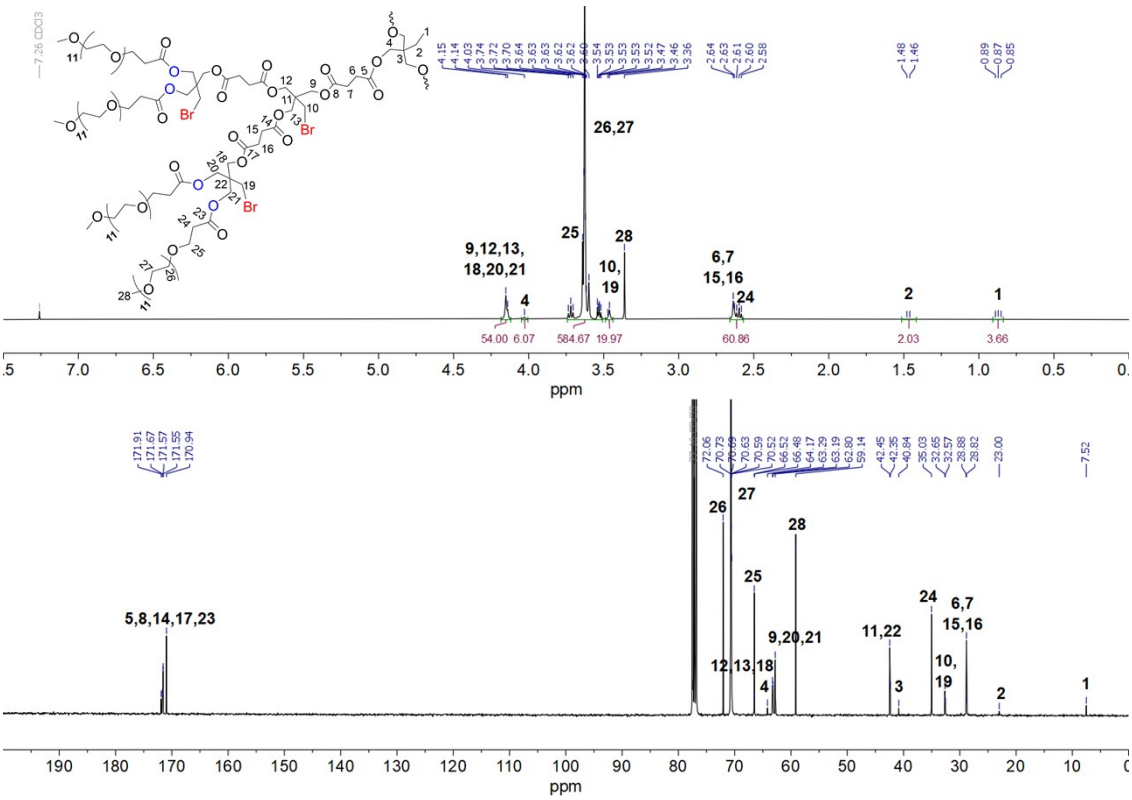


Figure S39. ^{1}H and ^{13}C NMR spectra of TMP-G2-$(Br)_9$-$(mPEG)_{12}$ in $CDCl_3$.

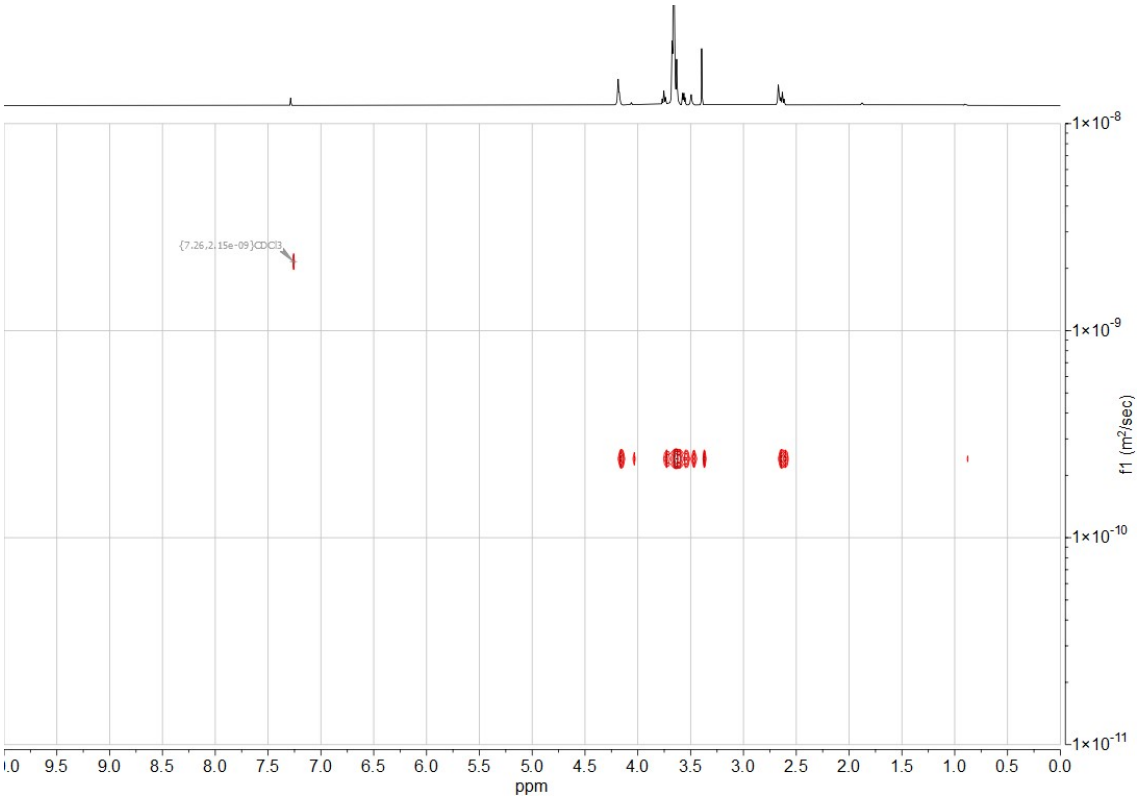


Figure S40. DOSY of TMP-G2-$(Br)_9$-$(mPEG)_{12}$.

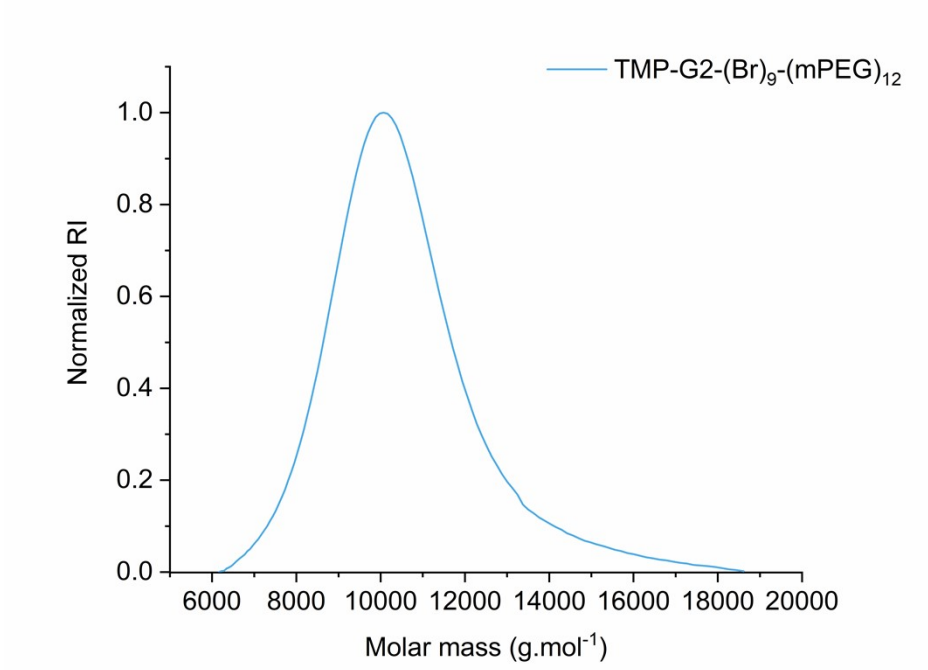


Figure S41. SEC of TMP-G2-$(Br)_9$-$(mPEG)_{12}$.

References

1. S. García-Gallego, D. Hult, J. V. Olsson and M. Malkoch, *Angew. Chem. Int. Ed.*, 2015, **54,** 2416-2419.
2. Y. Zhang, P. Mesa-Antunez, L. Fortuin, O. C. J. Andrén and M. Malkoch, *Biomacromolecules*, 2020, **21**, 4294-4301.
3. P. Antoni, Y. Hed, A. Nordberg, D. Nyström, H. Von Holst, A. Hult and M. Malkoch, *Angew. Chem. Int. Ed.*, 2009, **48**, 2126-2130.