Synthesis and Characterization of Water-Soluble and Photostable L-DOPA Dendrimers

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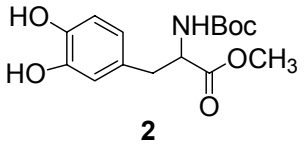
1. Detailed Experimental Procedures :

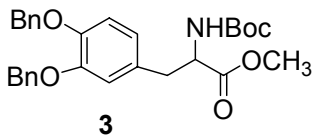
General procedures: All reactions were carried out under a nitrogen atmosphere with anhydrous and freshly distilled analytical grade solvents unless otherwise noted or unless aqueous solvents were used. Anhydrous dichloromethane (DCM) and pyridine were prepared by distillation from calcium hydride under a nitrogen atmosphere. Acetone was prepared by distillation under a nitrogen atmosphere. Dry tetrahydrofuran (THF) and methanol (MeOH) were purchased from Aldrich. All chemicals were purchased from Aldrich and Fisher as highest purity grade and used without further purification. 3,4-Dihydroxy-L-Phenylalanine (L-DOPA) was obtained at the highest commercial quality from Aldrich. 4-(dimethylamino)-pyridinium p-toluenesulfonate (DPTS) was synthesized using reported procedure. Reactions were monitored by thin-layer chromatography (TLC) performed on silica gel UV_{254} flexible plates. Column chromatography was carried out using silica gel (60-100 mesh). NMR spectra were recorded on Varian Mercury 300 MHz NMR spectrometer at a constant temperature of 25˚C. ^{1}H Chemical shifts (δ) were calibrated using $CDCl_3$ (δ =7.26 ppm) and CD_3OD (central peak, δ = 3.31 ppm) as an internal reference; ^{13}C Chemical shifts value (δ) were referenced to $CDCl_3$ (central peak, δ = 77.00 ppm) and CD_3OD (central peak, δ = 49.15 ppm) as the internal standard. Standard abbreviations indicating multiplicity were used as follows: s = singlet, d = doublet, t = triplet, m = multiplet, b = broad. Mass spectra were measured on a Bruker MALDI-TOF-MS spectrometer. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. DCC = dicyclohexylcarbodiimide, DCU = 1,3-dicyclohexylurea, Boc = tert-butyloxycarbonyl, Bn = benzyl.

Experimental details and spectral characterization data for compounds 2 - 17:

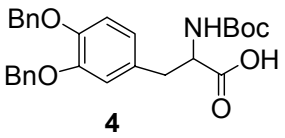


2

HO-DOPA-NH-Boc-COOMe 2: 80 mL methanol was cooled to 0˚C and thiony chloride (3.56 mL, 48.8 mmol) was added dropwise. The solution was allowed to warm to room temperature, and L- DOPA **1** (8.000 g, 40.4 mmol) was added wisely. The solution was stirred for 18 h at room temperature and concentrated to give a yellow powder. The resulting mixture was dissolved in THF (60 mL) and saturated aqueous $NaHCO_3$ (60 mL) was added. The resulting solution was cooled at 0˚C and di-tertbutyl-dicarbonate (1 M in THF) (44.4 mL, 44.4 mmol) was added dropwise. The solution was allowed to warm to room temperature and stirred for 1 h. The organic solvent was moved and the aqueous layer was extracted with ethyl acetate (2×60 mL). The combined organic extracts were washed subsequently with water (2×60 mL), 5%HCl (60 mL), water (60 mL), and brine (60 mL). The resulting solution was dried over $MgSO_4$, filtered and concentrated. Flash column chromatography (1:1 EtOAc/hexanes) afforded product **2** as a white solid (11.320 g, 90%). ^{1}H NMR ($CDCl_3$) δ^1H (300 MHz): 1.416 (9H, s, Boc-CH_3), 2.962 (2H, m, DOPA-CH_2), 3.690 (3H, s, OCH_3), 4.513 (1H, m, DOPA-CH), 5.175 (1H, d, J = 5.1 Hz, DOPA-NH), 6.526 (1H, d, J = 4.5 Hz, 6-ArH), 6.651 (1H, s, 2-ArH), 6.752 (1H, d, J = 5.1 Hz, 5-ArH); ^{13}C NMR ($CDCl_3$) δ^{13}C (75 MHz): 27.396 (Boc-CH_3), 37.750 (DOPA-CH_2), 52.316 (OCH_3), 54.552 (DOPA-CH), 80.289 (Boc-C), 115.289 (2-ArC) , 116.128 (6-ArC), 121.538 (5-ArC), 128.413 (1-ArC), 143.788 (3-ArC), 146.733 (4-ArC), 155.386 (Boc-CO), 172.623 (DOPA-CO); MALDI-TOF-MS found $[M+Na]^+$ 334.0; $C_{15}H_{21}NO_6$ requires 311.3.

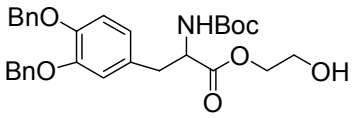


BnO-DOPA-NHBoc-COOMe 3: To HO-DOPA-NHBoc-COOMe **2** (9.432 g, 30.3 mmol) in acetone (90 mL) were added K_2CO_3 (12.442 g, 84.1 mmol,) and NaI (540 mg, 3.9 mmol) and stirred for 1 h. Benzyl bromide (10.1 mL, 81 mmol) was added. The solution was heated to reflux for 18 h and then cooled and concentrated. This sediment was dissolved in CH_2Cl_2 (100 mL). The solution was washed with water (2×100 mL), 5% HCl (100 mL), water (100 mL), brine (100 mL), dried over $MgSO_4$, filtered, and concentrated. Flash column chromatography (CH_2Cl_2 and then 3% CH_3OH in CH_2Cl_2) afforded product **3**, a white solid (11.767 g, 79%). ^{1}H NMR ($CDCl_3$) $\delta_{^1H}$ (300 MHz): 1.424 (9H, s, Boc-CH_3), 2.976 (2H, m, DOPA-CH_2), 3.643 (3H, s, OCH_3), 4.523 (1H, m, DOPA-CH), 4.940 (1H, d, J = 7.8 Hz, DOPA-NH), 5.121 and 5.126 (4H, s, 2Benzyl-CH_2), 6.639 (1H, d-d, J = 8.2 and 1.9 Hz, 6-ArH), 6.731 (1H, d, J = 2.1 Hz, 2-ArH), 6.858 (1H, d, J = 8.1 Hz, 5-ArH), 7.296-7.460 (10H, m, Benzyl-ArH); ^{13}C NMR ($CDCl_3$) $\delta_{^{13}C}$ (75 MHz): 28.296 (Boc-CH_3), 37.765 (DOPA-CH_2), 52.148 (OCH_3), 54.376 (DOPA-CH), 71.270 and 71.300 (2Benzyl-CH_2), 79.915 (Boc-C), 115.060 (2-ArC) , 116.128 (6-ArC), 122.263 (5-ArC), 127.245 and 127.306 (2-C, 2′-C, Benzyl), 127.749 and 127.802 (3-C, 3′-C, Benzyl), 128.443 and128.474 (4-C, 4′-C , Benzyl), 129.191 (1-ArC), 137.150 and 137.272 (1-, 1′-C, Benzyl), 148.061 (3-ArC), 148.862 (4-ArC), 155.050 (Boc-CO), 172.325 (DOPA-CO). MALDI-TOF-MS found $[M+Na]^+$ 514.2 and $[M+K]^+$ 550.6; $C_{29}H_{33}NO_6$ requires 491.6.



BnO-DOPA-NHBoc-COOH 4: To a solution of BnO-DOPA-NHBoc-COOMe **3** (10.560 g, 21.48 mmol) in 1:1 THF/Methanol (60 mL) was added aqueous NaOH (3 M, 30

mL) dropwise. The solution was stirred at room temperature for 6 h. The solution was then acidified to pH = 3 with aqueous HCl (5 %) and extracted with CH_2Cl_2 (3×60 mL). The combined organic extracts were washed with water (180 mL), brine (180 mL), dried over $MgSO_4$, filtered, and concentrated. Flash column chromatography (2:1 EtOAc /hexanes) afforded product **4** as a white solid (9.539 g, 93%). ^{1}H NMR ($CDCl_3$) $\delta_{^1H}$ (300 MHz): 1.419 (9H, s, Boc-CH_3), 3.025 (2H, m, DOPA-CH_2), 4.505 (1H, m, DOPA-CH), 4.874 (1H, d, *J* = 6.9 Hz, DOPA-NH), 5.121 (4H, s, 2Benzyl-CH_2), 6.678 (1H, d-d, *J* = 8.1 and 1.8 Hz, 6-ArH), 6.756 (1H, d, *J* = 2.1 Hz, 2-ArH), 6.854 (1H, d, *J* = 8.4 Hz, 5-ArH), 7.294-7.450 (10H, m, BnO-ArH); ^{13}C NMR ($CDCl_3$) $\delta_{^{13}C}$ (75 MHz): 28.273 (Boc-CH_3), 37.101 (DOPA-CH_2), 54.254 (DOPA-CH), 71.239 (Benzyl-CH_2), 80.441 (Boc-C), 115.014 (2-ArC) , 116.227 (6-ArC), 122.309 (5-ArC), 127.126 and 127.368 (2-C, 2′-C, Benzyl), 127.772 (3-C, 3′-C, Benzyl), 128.466 and128.504 (4-C, 4′-C , Benzyl), 128.871 (1-ArC), 137.241 (1-C, 1′-C, Benzyl), 148.152 (3-ArC), 148.770 (4-ArC), 155.508 (Boc-CO), 174.843 (DOPA-CO). MALDI-TOF-MS found $[M+Na]^+$ 500.2 and $[M+K]^+$ 522.2; $C_{28}H_{31}NO_6$ requires 477.5.



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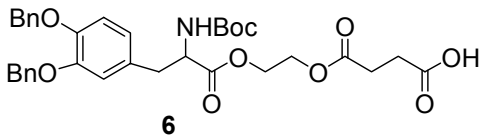
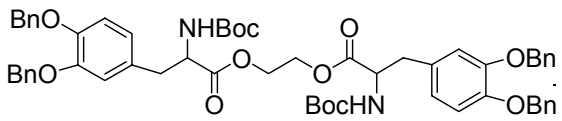
BnO-DOPA-NHBoc-COOCH$_2$CH$_2$-OH 5: To BnO-DOPA-NHBoc-COOH **4** (7.042 g, 14.75 mol) in CH_2Cl_2 (60 mL) were added ethylene glycol (4.115g, 66.38 mmol) and DPTS (860 mg, 4.4 mmol). The mixture was cooled over ice and DCC (1 M in CH_2Cl_2) (16.22 mL, 16.22 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 6 h. The reaction was quenched with water (60 mL).The solid (DCU) was filtered off and washed with cool CH_2Cl_2 (5 mL). The organic layer was separated and washed with water (60 mL), brine (60 mL), dried over

$MgSO_4$, filtered and then concentrated. The sediment was dissolved in 1:1 EtOAc/hexanes (100 mL) to precipitate extra DCU. The solution was filtered and concentrated. A flash chromatography (1:2 to 1:1 EtOAc /hexane) afforded product **5** as a colorless solid (6.616 g, 86%). ^{1}H NMR ($CDCl_3$) $\delta_{^1H}$ (300 MHz): 1.425 (9H, s, Boc-CH_3), 2.990 (2H, d, J = 5.7 Hz, DOPA-CH_2), 3.869-4.073 (4H, m, OCH_2CH_2O), 4.444 (1H, m, DOPA-CH), 4.937 (1H, d, J = 7.2 Hz, DOPA-NH), 5.131 (4H, s, 2Benzyl-CH_2), 6.674 (1H, d-d, J = 7.2 and 0.9 Hz, 6-ArH), 6.779 (1H, d, J = 1.8 Hz, 2-ArH), 6.870 (1H, d, J = 8.1 Hz, 5-ArH), 7.299-7.462 (10H, m, Benzyl-ArH); ^{13}C NMR ($CDCl_3$) $\delta_{^{13}C}$ (75 MHz): 28.281 (Boc-CH_3), 37.521 (DOPA-CH_2), 54.758 (DOPA-CH), 61.277 and 67.282 (OCH_2CH_2O), 71.239 and 71.346 (2Benzyl -CH_2), 80.281 (Boc-C), 115.113 (2-ArC) , 116.082 (6-ArC), 122.171 (5-ArC), 127.245 and 127.337 (2-C, 2′-C, Benzyl), 127.772 and 127.833 (3-C, 3′-C, Benzyl), 128.459 and 128.482 (4-C, 4′-C , Benzyl), 129.077 (1-ArC), 137.111 and 137.226 (1-C, 1′-C, Benzyl), 148.213 (3-ArC), 148.999 (4-ArC), 155.401 (Boc-CO), 171.967 (DOPA-CO). MALDI-TOF-MS found $[M+Na]^+$ 544.2; $C_{30}H_{35}NO_7$ requires 521.6.



BnO-DOPA-NHBoc-$COOCH_2CH_2OOCCH_2CH_2COOH$ (the building block) 6: To BnO-DOPA-NHBoc-$COOCH_2CH_2$-OH **5** (5.865 g, 11.24 mmol) in pyridine (30 mL) was added a solution of succinic anhydride (1.69 g, 16.9 mmol) in pyridine (30 mL). The resulting reaction solution was stirred at room temperature for 18 h and concentrated. This material was dissolved in CH_2Cl_2 (60 mL). The solution was washed with saturated aqueous $KHSO_4$ (60 mL), water (60 mL), brine (60 mL), dried over $MgSO_4$, filtered and then concentrated.

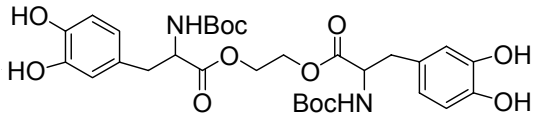
A flash chromatography (1:1 EtOAc /hexane) afforded product **6**, a white solid (4.753 g, 68%). ^{1}H NMR ($CDCl_3$) $\delta_{^1H}$ (300 MHz): 1.417 (9H, s, Boc-CH_3), 2.628 (4H, m, succ.-CH_2), 2.949-3.020 (2H, m, DOPA-CH_2), 4.246-4.365 (4H, m, OCH_2CH_2O), 4.597 (1H, m, DOPA-CH), 5.035 (1H, d, J = 4.8 Hz, DOPA-NH), 5.123 and 5.130 (4H, s, 2 Benzyl-CH_2), 6.664 (1H, d, J = 4.8 Hz, 6-ArH), 6.772 (1H, J = 1.8, 2-ArH), 6.865 (1H, d, J = 4.5 Hz, 5-ArH), 7.294-7.453 (10H, m, 2Benzyl-ArH); ^{13}C NMR ($CDCl_3$) $\delta_{^{13}C}$ (75 MHz): 28.235 (Boc-CH_3), 28.799 and 29.074 (succ.-CH_2), 37.574 (DOPA-CH_2), 54.124 (DOPA-CH), 61.831 and 62.724 (OCH_2CH_2O), 71.193 and 71.206 (2Benzyl -CH_2), 80.563 (Boc-C), 114.999 (2-ArC) , 116.090 (6-ArC), 122.209 (5-ArC), 127.223 and 127.314 (2-C, 2′-C, Benzyl), 127.726 and 127.764 (3-C, 3′-C, Benzyl), 128.413 (4-C, 4′-C , Benzyl), 128.916 (1-ArC), 137.134 and 137.203 (1-C, 1′-C, Benzyl), 148.061 (3-ArC), 148.862 (4-ArC), 155.607 (Boc-CO), 171.699 (DOPA-CO), 175.492 (succ.-CO); MALDI-TOF-MS found $[M+Na]^+$ 644.3, $[M+K]^+$ 660.3 ; $C_{34}H_{39}NO_{10}$ requires 621.6.



7

BnO-G0-NHBoc 7: To BnO-DOPA-NHBoc-COOH **4** (1.163 g, 2.43 mmol) in CH_2Cl_2 (10 mL) were added ethylene glycol (68 mg, 1.10 mmol) and DPTS (142 mg, 0.73 mmol). The mixture was cooled at 0˚C and DCC (1 M in CH_2Cl_2) (2.67 mL, 2.67 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred 16 h. The reaction was quenched with water (10 mL).The solid (DCU) was filtered off and washed with cool CH_2Cl_2 (1 mL). The organic layer was separated and washed with water (10 mL), brine (60 mL), dried over $MgSO_4$, filtered and then concentrated. A flash chromatography (2% methanol in CH_2Cl_2) afforded product **7** as a colorless waxy solid (841 mg, 78%). ^{1}H

NMR ($CDCl_3$) $\delta^{1}H$ (300 MHz): 1.401(18H, s, Boc-CH_3), 2.962 (4H, m, DOPA-CH_2), 4.166 (4H, t, J = 3.3 Hz, core-OCH_2), 4.520(2H, m, DOPA-CH), 4.950 (2H, d, J = 8.1 Hz, DOPA-NH), 5.089 and 5.099 (8H, s, 2BnO-CH_2), 6.634 (2H, d-d, J = 8.2 and 2.0 Hz, 6-ArH), 6.778 (2H, d, J = 2.1 Hz, 2-ArH), 6.842 (2H, d, J = 8.4 Hz, 5-ArH), 7.285-7.435 (20H,m, BnO-ArH); ^{13}C NMR ($CDCl_3$) $\delta^{13}C$ (75 MHz): 28.285 (Boc-CH_3), 37.567 (DOPA-CH_2), 54.361 (DOPA-CH), 62.563 (core-OCH_2), 71.178 and 71.206 (2 Benzyl-CH_2), 79.991 (Boc-C), 115.655 (2-ArC) , 114.961 (6-ArC), 116.136 (5-ArC), 122.240 (1-ArC), 127.253 and 127.322 (2-C, 2′-C, Benzyl), 127.749 and 127.787 (3-C, 3′-C, Benzyl), 128.443 and 128.459 (4-C, 4′-C , Benzyl), 128.985 (1-ArC), 137.134 and 137.233 (1-, 1′-C, Benzyl), 148.122 (3-ArC), 148.839 (4-ArC), 155.065 (Boc-CO), 171.684 (DOPA-CO); MALDI-TOF-MS found $[M+Na]^+$ 1003.7 and $[M+K]^+$ 1019.7; $C_{58}H_{64}N_2O_{12}$ requires 981.1.



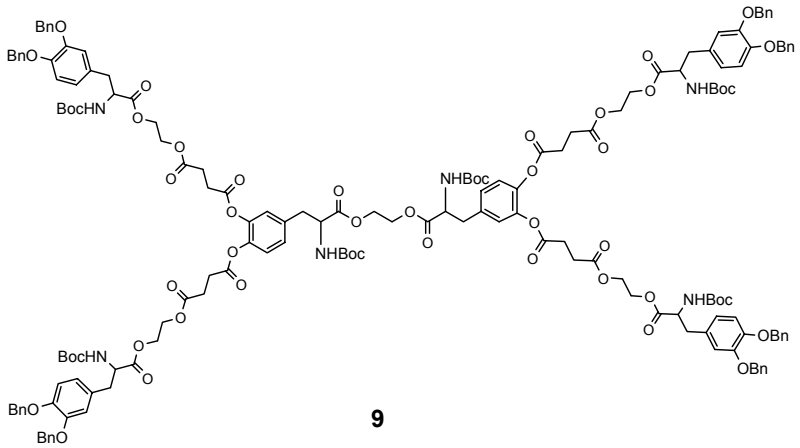
8

HO-G0-NHBoc 8: To a solution of BnO-G0-NHBoc **7** (822 mg, 0.84 mmol) in THF (10 mL) was added 5% palladium on charcoal (150 mg). The suspension was then placed in a Parr tube on a hydrogenator, evacuated, flushed, and shaken under 50 psi of H_2 for 4h. The solution was filtered and concentrated. A flash chromatography (1:1 ethyl acetate/hexane) afforded HO-G0-NHBoc **8** as a white solid (490 mg, 94%). ^{1}H NMR ($CDCl_3$) $\delta^{1}H$ (300 MHz): 1.429(18H, s, Boc-CH_3), 2.876 (4H, m, DOPA-CH_2), 4.131 (4H, t, J = 3.3 Hz, core-OCH_2), 4.423 (2H, m, DOPA-CH), 5.257 (2H, d, J = 8.4 Hz, DOPA-NH), 6.489 (2H, d-d, J = 8.1 and 1.5 Hz, 6-ArH), 6.654 (2H, s, 2-ArH), 6.770 (2H, d, J = 8.1 Hz, 5-ArH), 6.900 (4H, b, ArOH); ^{13}C NMR ($CDCl_3$) $\delta^{13}C$ (75 MHz): 28.265 (Boc-CH_3), 38.101 (DOPA-CH_2),

54.697 (DOPA-CH), 62.602 (core-OCH_2), 115.655 (2-ArC) , 116.052 (6-ArC), 121.393 (5-ArC), 127.772 (1-ArC), 143.216 (3-ArC), 144.246 (4-ArC), 155.867 (Boc-CO), 172.112 (DOPA-CO); MALDI-TOF-MS found $[M+Na]^+$ 643.4 and $[M+K]^+$ 659.4; $C_{30}H_{40}N_2O_{12}$ requires 620.6.

BnO-G1-NHBoc 9: To HO-G0-NHBoc **8** (246 mg, 0.40 mmol) in CH_2Cl_2 (15 mL) were added building block **6** (1.184 g, 1.9 mmol) and DPTS (111 mg, 0.57 mmol). The mixture was cooled at 0˚C and DCC (1 M in CH_2Cl_2) (2.0 mL, 2.0 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for18 h. The reaction was quenched with water (10 mL).The solid (DCU) was filtered off and washed with cool CH_2Cl_2 (1 mL). The organic layer was separated and washed with water (15 mL), brine (15 mL), dried over $MgSO_4$, filtered and then concentrated. The sediment was dissolved in 1:1 EtOAc/hexanes (20 mL) to precipitate extra DCU. The solution was filtered and concentrated. A flash chromatography (1:2 and then 1:1 EtOAc /hexane) afforded product **9** as a white waxy solid (922 mg, 76%). ^{1}H NMR ($CDCl_3$) $\delta_{^1H}$ (300 MHz): 1.386, and 1.405 (54H, b, Boc-CH_3), 2.668- 2.712 (8H, m, succ.-CH_2), 2.826- 2.868 (8H, m, succ.-CH_2), 2.935-3.037 (12H, m, DOPA-CH_2), 4.223(20H, b, OCH_2CH_2O), 4.507-4.528 (6H, m, DOPA-CH), 4.962 (6H, b, DOPA-NH), 5.098(16H, b, 8Benzyl-CH_2), 6.638(4H,d-d, J = 8.4 and J = 2.1 Hz, exterior 6-ArH), 6.743 (4H, d, J = 1.2 Hz, exterior 2-ArH), 6.844 (4H, d, J = 8.4 Hz, exterior 5-ArH), 6.955-7.082 (6H, m, core-ArH), 7.281-7.434 (40H, m, 8Benzyl-ArH); ^{13}C NMR ($CDCl_3$) $\delta_{^{13}C}$ (75 MHz) : 28.258 and 28.311 (core and exterior Boc-CH_3), 28.624 (succ.-CH_2), 37.620 (DOPA-CH_2), 54.353 (DOPA -CH), 62.258, and 62.792 (OCH_2CH_2O), 71.277 and 71.399 (Benzyl-CH_2), 80.044 (Boc-C), 115.106, 116.349, 122.339, 123.369, 124.170, and

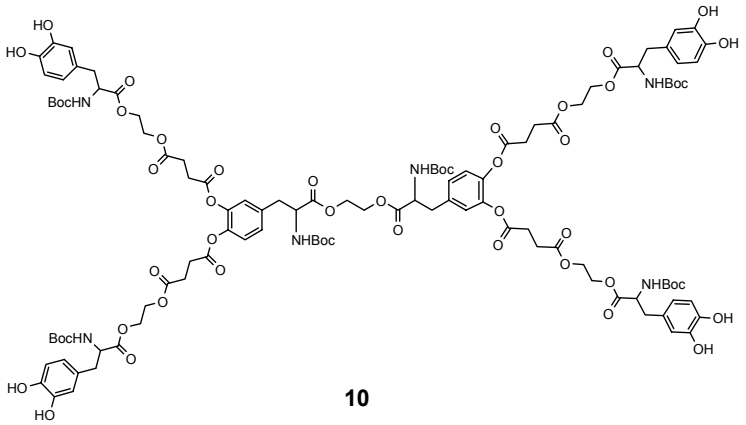
129.138(Ar-CH), 127.276 and 127.352 (2-C, 2′-C, Benzyl), 127.764 and 127.818 (3-C, 3′-C, Benzyl), 128.451 and128.474 (4-C, 4′-C , Benzyl), 137.195 and 137.287 (1-, 1′-C, Benzyl), 141.003, 141.873, 148.1191, 148.915 (Ar-C), 155.065 (Boc-CO), 169.784, 169.853 (DOPA-CO), 169.784, 169.853, 171.364,171.860, 173.027 (succ.-CO); MALDI-TOF-MS found $[M+Na]^+$ 3059.8 and $[M+K]^+$ 3075.8 ; $C_{166}H_{188}N_6O_{48}$ requires 3035.3.



9

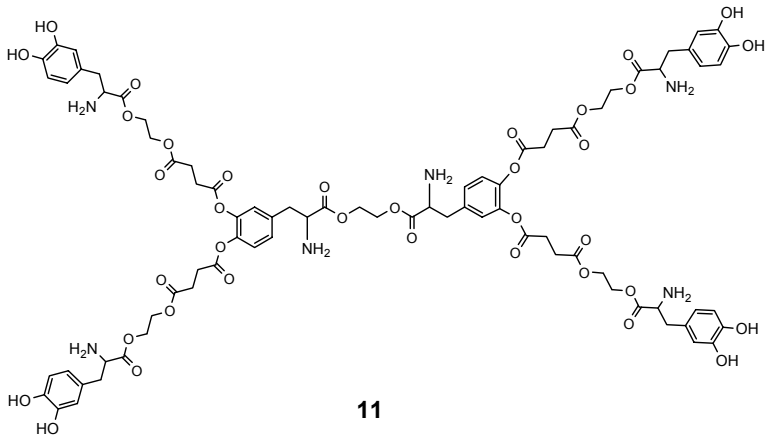
HO-G1-NHBoc 10: To a solution of BnO-G1-NHBoc **9** (505 mg, 0.17 mmol) in THF (8 mL) in a Parr tube was added 5% palladium on charcoal (100 mg). The suspension was charged with hydrogen, evacuated, and then agitated under 50 psi of H_2 for 6h. The solid (Pd/C) was filtered off and washed with THF (2 mL). The filtrate was concentrated. A flash chromatography (2:1 ethyl acetate/hexane) afforded HO-G1-NHBoc **10** as a white solid (355 mg, 92%). ^{1}H NMR ($CDCl_3$) δ^{1H} (300 MHz): 1.381and 1.420 (54H, b, Boc-CH_3), 2.704-2.715 (8H, m, succ.-CH_2), 2.879-2.890 (8H, m, succ.-CH_2), 2.944-3.011 (12H, m, DOPA-CH_2), 4.217(16H, m, exterior OCH_2CH_2O), 4.341 (4H, m, core OCH_2CH_2O), 4.490 (4H, m, exterior DOPA-CH), 4.558 (2H, m, core DOPA-CH), 5.078-5.128 (4H, m, exterior DOPA-NH), 5.297-5.332 (2H, m, core DOPA-NH), 6.484-6.498 (4H,b, exterior 6-ArH), 6.580-6.606 (4H, b, exterior 2-ArH), 6.698-6.725 (4H, b, exterior 5-ArH), 6.952-7.065 (6H, b, core-ArH); ^{13}C NMR ($CDCl_3$) δ^{13C} (75 MHz) :

28.189 and 28.281 (core and exterior Boc-CH_3), 29.593 (succ.-CH_2), 37.521 (DOPA-CH_2), 54.491 (DOPA -CH), 62.419 and 62.831 (OCH_2CH_2O), 80.304 (Boc-C), 115.312, 116.113, 121.340, 123.377, 124.178, 127.726, 135.196 (Ar-CH), 140.888, 141.720, 143.345, 144.063 (Ar-C), 155.439(Boc-CO), 170.730, 171.219(DOPA-CO), 172.020, 172.249 (succ.-CO); MALDI-TOF-MS found $[M+Na]^+$] 2337.2 and $[M+K]^+$ 2353.2 ; $C_{110}H_{140}N_6O_{48}$ requires 2314.3.



HO-G1-NH_2 11: To HO-G1-NHBoc **10** (132 mg, 0.057 mmol) a solution of 4 M HCl in dioxane (2 mL) was added. The reaction mixture was stirred at room temperature for 5 minutes. The chlorate of **11** was precipitated. The solvent and the remaining HCl were removed and the sediment was washed with DCM (2×2 mL). Drying gave HO-G1-NH_2 **11** as a colorless powder (96 mg, 87%). ^{1}H NMR (CD_3OD) δ^{1H} (300 MHz): 2.646 (8H, m, succ.-CH_2), 2.830 (8H, m, succ.-CH_2), 2.970 (8H, m, exterior DOPA-CH_2), 3.142-3.170 (4H, m, core DOPA-CH_2), 4.138 (4H, m, core OCH_2CH_2O), 4.240 (8H, m, exterior OCH_2CH_2O), 4.310 (8H, m, exterior OCH_2CH_2O), 4.359 (6H, m, DOPA-CH), 6.473 (4H,d-d, J = 4.6 and 1.1 Hz, exterior 6-ArH), 6.609 (4H, t, J= 1.2 Hz, exterior 2-ArH), 6.660 (4H, d, J = 4.8 Hz, exterior 5-ArH), 7.085-7.130 (6H, m, core-ArH); ^{13}C NMR (CD_3OD) δ^{13C} (75 MHz) : 29.583, 29.663, and 29.709 (succ.-CH_2), 36.440 and 36.707

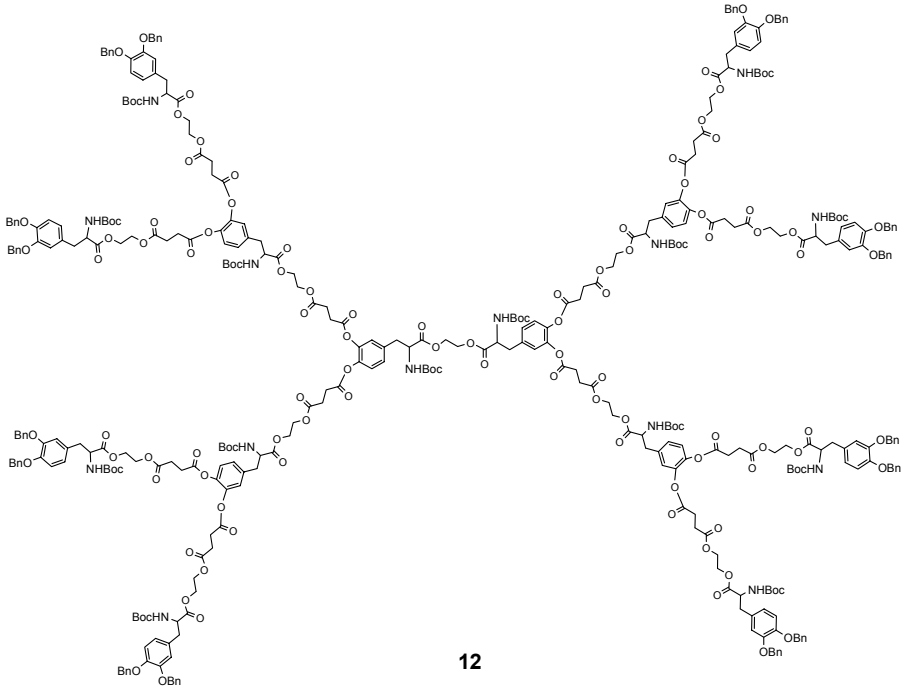
(DOPA-CH_2), 55.007 and 55.324 (DOPA -CH), 63.225, 63.356 and 65.248 (OCH_2CH_2O), 116.859, 117.454, 121.947, 125.258, 125.776, 126.268, 129.201, 134.739 (Ar-CH), 143.191, 143.737, 146.158, 146.780 (Ar-C), 169.718, 170.091, 172.124, 172.269 (DOPA-CO), 173.806, 173.825 (succ.-CO); MALDI-TOF-MS found $[M+Na]^+$ 1735.7 and $[M+K]^+$ 1751.7; $C_{80}H_{92}N_6O_{36}$ requires 1713.6.



11

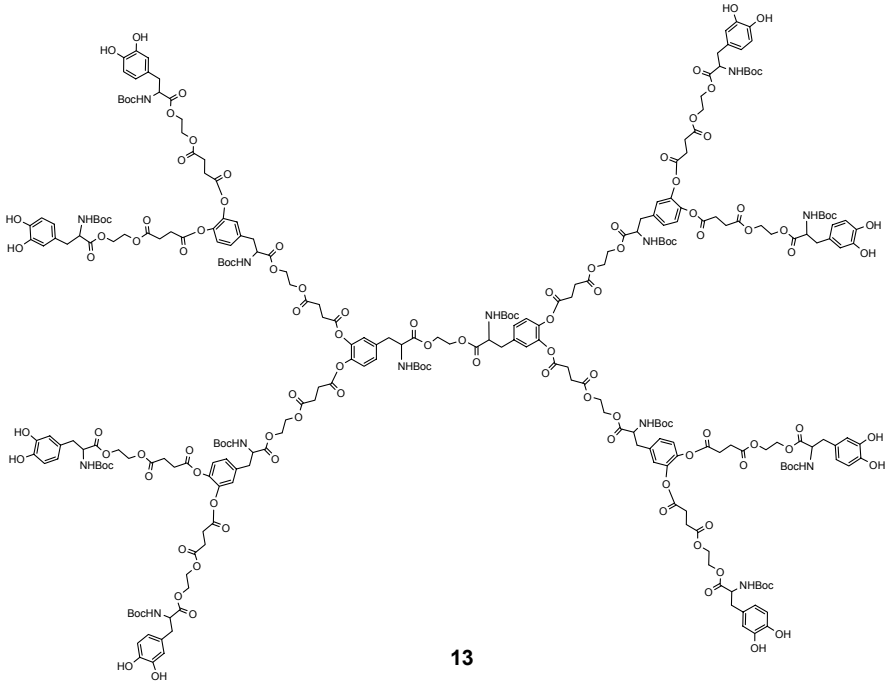
BnO-G2-NHBoc 12: To HO-G1-NHBoc **10** (175 mg, 0.075 mmol) in CH_2Cl_2 (10 mL) were added building block **6** (451 mg, 0.72 mmol) and DPTS (43 mg, 0.22 mmol). The mixture was cooled at 0˚C and DCC (1 M in CH_2Cl_2) (0.76 mL, 0.76 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred 36 h. The reaction was quenched with water (10 mL).The solid (DCU) was filtered off and washed with cool CH_2Cl_2 (2 mL). The organic layer was separated and washed with water (10 mL), brine (10 mL), dried over $MgSO_4$, filtered and then concentrated. The sediment was dissolved in 1:1 EtOAc/hexanes (10 mL) to precipitate extra DCU. The solution was filtered and concentrated. A flash chromatography (5:3 EtOAc /hexane) afforded BnO-G2-NHBoc **12** as a colorless solid (385 mg, 72%). ^1H NMR ($CDCl_3$) δ^{1H} (300 MHz): 1.414, 1.434, and 1.437 (126H, b, Boc-CH_3), 2.702 (24H, m, succ.-CH_2), 2.852 (24H, m, succ.-CH_2), 2.986 (28H, b, DOPA-CH_2), 4.227 (52H, b, OCH_2CH_2O),

4.515 (14H, b, DOPA-CH), 4.880 (14H, b, DOPA-NH), 5.130 (32H, b, Benzyl-CH_2), 6.444 (8H, d-d, *J*= 5.1 and 1.2 Hz, exterior 6-ArH), 6.747 (8H, s, exterior 2-ArH), 6.850 (8H, d, *J* = 5.1 Hz, exterior 5-ArH), 7.016 (12H, m, interior-ArH), 7.061(6H, m, core-ArH); ^{13}C NMR ($CDCl_3$) $\delta_{^{13}C}$ (75 MHz): 28.235 and 28.288 (Boc-CH_3), 28.601 (succ.-CH_2), 37.307 and 37.547 (DOPA-CH_2), 54.323 (DOPA-CH), 62.251, 62.762, and 63.021 (OCH_2CH_2O), 71.254 and 71.369 (40H, Benzyl-CH_2), 79.922 and 80.022 (Boc-C), 115.083, 116.326, 122.324, 123.323, 124.178, and 129.145(Ar-CH), 127.261 and 127.329 (2-C, 2′-C, Benzyl), 127.749 and 127.795 (3-C, 3′-C, Benzyl), 128.428 and128.459 (4-C, 4′-C , Benzyl), 137.172 and 137.264 (1-, 1′-C, Benzyl), 135.082, 140.980, 141.850, 148.160, 148.893 (Ar-C), 155.058 (Boc-CO), 169.769, 169.861 (DOPA-CO), 171.387, 171.715,171.860 (succ.-CO).



HO-G2-NHBoc 13: To a solution of BnO-G2-NHBoc **12** (312 mg, 0.043 mmol) in THF (6 mL) in a Parr tube was added 5% palladium on charcoal (100 mg). The Parr tube was evacuated, and filled with 50 psi of H_2. The solution was shaken for 6 h. The catalyst was

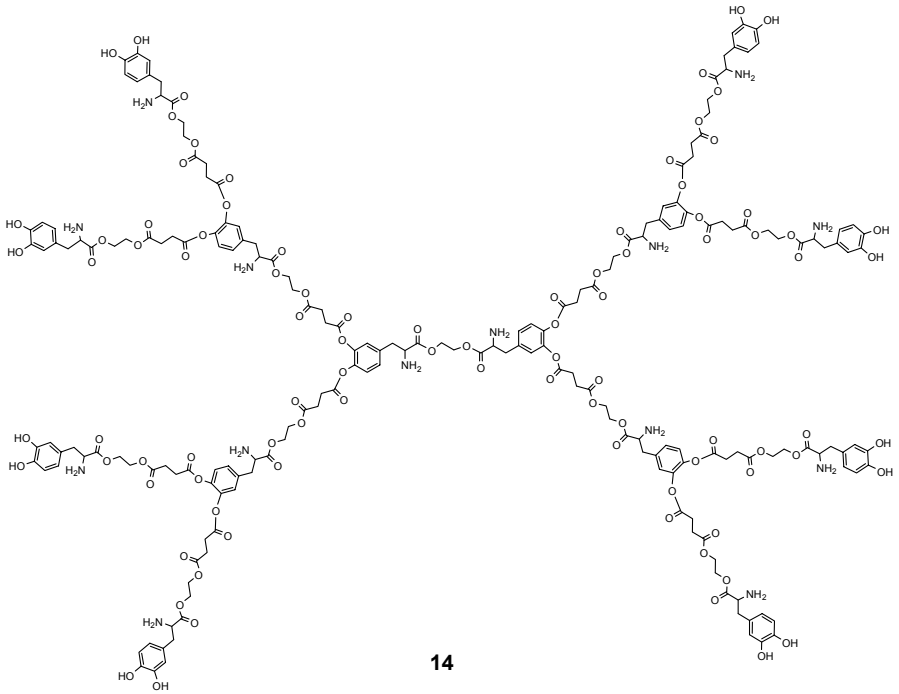
filtered off and washed with THF (2 mL). The filtrate was concentrated. A flash chromatography (5% methanol in ethyl acetate) afforded HO-G2-NHBoc **13** as a white solid (224 mg, 90%). ^{1}H NMR ($CDCl_3$) $\delta_{^1H}$ (300 MHz): 1.375, and 1.412 (126H, b, Boc-CH_3), 2.696 (24H, b, succ.-CH_2), 2.882 (24H, b, succ.-CH_2), 3.009 (28H, b, DOPA-CH_2), 4.221 and 4.303 (52H, b, OCH_2CH_2O), 4.497 (14H, b, DOPA-CH), 5.144 and 5.324 (14H, b, DOPA-NH), 6.485 (8H, d, *J*= 7.8 Hz, exterior 6-ArH), 6.587 (8H, d, *J*= 6.0, exterior 2-ArH), 6.705 (8H, d, *J* = 6.6 Hz, exterior 5-ArH), 6.948-6.986 (12H, b, interior-ArH), 7.023-7.049(6H, m, core-ArH); ^{13}C NMR ($CDCl_3$) $\delta_{^{13}C}$ (75 MHz): 28.189 and 28.281(Boc-CH_3), 28.578 (succ.-CH_2), 37.452 (DOPA-CH_2), 54.498 (DOPA-CH), 62.403, 62.846, and 63.182 (OCH_2CH_2O), 80.251 (Boc-C), 115.304, 116.067, 121,301, 123.362, 124.224, 127.741 (Ar-CH), 135.265, 140.835, 141.728, 143.330, 144.116 (Ar-C), 155.439 (Boc-CO), 170.616, 170. 686 (DOPA-CO), 171.989 172.249 (succ.-CO);



13

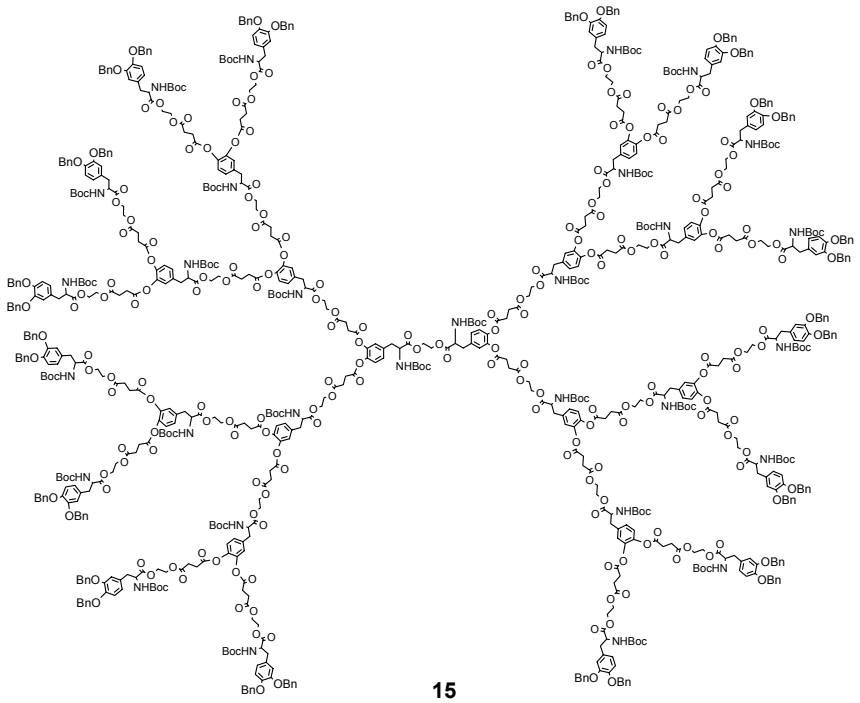
HO-G2-NH$_2$ 14: To HO-G2-NHBoc **13** (132 mg, 0.023 mmol) a solution of 4 M HCl in dioxane (2 mL) was added. The reaction mixture was stirred at room temperature for 5

minutes. The chlorate of **11** was precipitated. The solvent and the remaining HCl were removed and the sediment was washed with DCM (2×2 mL). Drying gave the product HO-G2-NH_2 **14** as a white powder (95 mg, 86%). ^{1}H NMR (CD_3OD) δ^1H (300 MHz): 2.622 (24H, b, succ.-CH_2), 2.817 (24H, b, succ.-CH_2), 2.937 (16H, b, exterior DOPA-CH_2), 3.176-3.198 (12H, b, interior and core DOPA-CH_2), 4.115 (4H, m, core OCH_2CH_2O), 4.220-4.232 (24H, m, exterior OCH_2CH_2O), 4.298 (24, m, 24H, exterior OCH_2CH_2O), 4.336 (14, m, DOPA-CH), 6.442 (8H, d-d, J = 7.8 and 1.8 Hz, exterior 6-ArH), 6.570 (8H, s, exterior 2-ArH), 6.630 (8H, d, J = 8.1 Hz, exterior 5-ArH), 7.047 (6H, b, core-ArH), 7.081(12H, b, interior-ArH); ^{13}C NMR (CD_3OD) δ^{13}C (75 MHz): 29.642 (succ.-CH_2), 36.753 (DOPA-CH_2), 55.295 (DOPA-CH), 63.353, 65.260, and 68.129 (OCH_2CH_2O), 116.833, 117.413, 121.922, 125.333, 125.844, 126.249, 129.232 and 134.634 (Ar-CH), 143.280, 143.791, 146.210, 146.843 (Ar-C), 169.818, 170.176, 172.160, 172.336 (DOPA-CO), 173.839 (succ.-CO); MALDI-TOF-MS found $[M+Na]^+$ 4324.2; $C_{200}H_{228}N_{14}O_{92}$ requires 4300.2



14

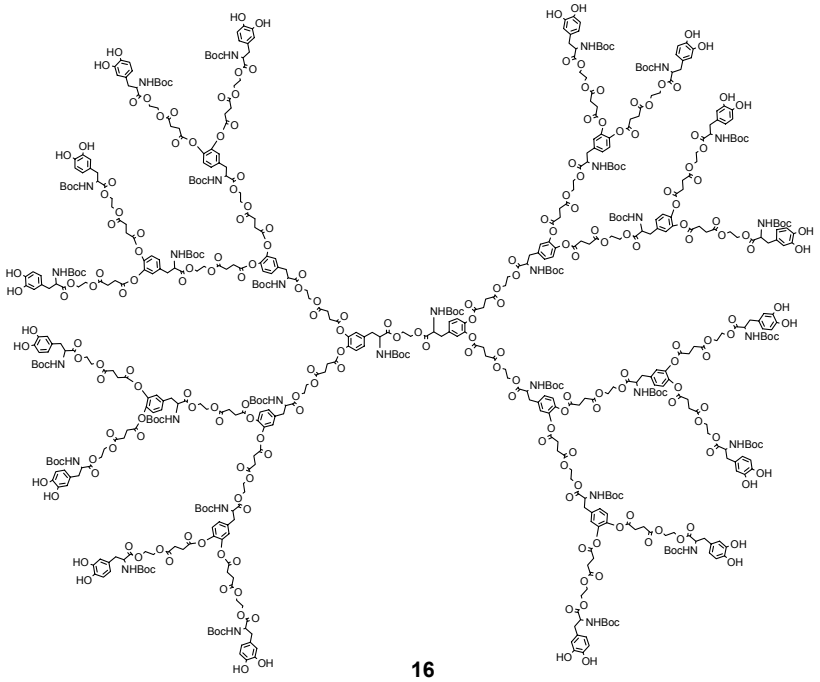
BnO-G3-NHBoc 15: To HO-G2-NHBoc **13** (81 mg, 0.014 mmol) in CH_2Cl_2 (10 mL) were added building block **6** (169 mg, 0.27 mmol) and DPTS (20 mg, 0.08 mmol). The mixture was cooled at 0˚C and DCC (1 M in CH_2Cl_2) (0.3 mL, 0.3 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred 72 h. The DCU was filtered off and washed with cool CH_2Cl_2 (2 mL). The filtrate was concentrated to dryness and the residue was resuspended in 1:1 EtOAc/hexanes (10 mL). The solution was filtered and concentrated. A flash chromatography (1:1 to 2:1 EtOAc /hexane) afforded BnO-G3-NHBoc **15** as a colorless solid (146 mg, 68%). ^{1}H NMR ($CDCl_3$) δ^{1H} (300 MHz): 1.388 (270H, b, Boc-CH_3), 2.689 (56H, b, succ.-CH_2), 2.847 (56H, b, succ.-CH_2), 2.966 (60H, b, DOPA-CH_2), 4.218 and 4.268 (116H, b, OCH_2CH_2O), 4.518 (30H, b, DOPA-CH), 4.979(30H, b, DOPA-NH), 5.093 (64H, b, Benzyl -CH_2), 6.636 (16H, d, *J*= 7.5 Hz, exterior 6-ArH), 6.741 (16H, s, exterior 2-ArH), 6.840 (16H, d, *J* = 8.4 Hz, exterior 5-ArH), 6.967-7.071 (42H, b, interior-ArH),7.308-7.405 (160H, b, Benzyl-ArH); ^{13}C NMR ($CDCl_3$) δ^{13C} (75 MHz): 28.227 and 28.281 (Boc-CH_3), 28.593 (succ.-CH_2), 37.261 and 37.559 (DOPA-CH_2), 54.315 (DOPA-CH), 62.243, 62.754, and 63.006 (OCH_2CH_2O), 71.239 and 71.361 (Benzyl-CH_2), 79.900 and 79.999 (Boc-C), 115.068, 116.311, 122.316, 123.323, 124.178, and 129.145(Ar-CH), 127.245 and 127.322 (2-C, 2′-C, Benzyl), 127.741 and 127.787 (3-C, 3′-C, Benzyl), 128.421 and128.443 (4-C, 4′-C , Benzyl), 137.165 and 137.256 (1-, 1′-C, Benzyl), 135.097, 140.972, 141.842, 148.152, 148.885 (Ar-C), 155.050 (Boc-CO), 169.754, 169.845 (DOPA-CO), 171.402, 171.707,171.844 (succ.-CO).



15

HO-G3-NHBoc 16: To BnO-G3-NHBoc **15** (136 mg, 0.0088 mmol) in THF (10 mL) in a Parr tube was added 5% palladium on charcoal (100 mg), and the flask was evacuated and filled with 50 psi of H_2. The mixture was shaken for 6h, and then filtered. The filtrate was dried. A flash chromatography (10% methanol in ethyl acetate) afforded HO-G3-NHBoc **16** as a colorless solid (99 mg, 90%). ^{1}H NMR ($CDCl_3$) δ^{1H} (300 MHz): 1.379, and 1.407 (270H, b, Boc-CH_3), 2.691 (56H, b, succ.-CH_2), 2.867 (56H, b, succ.-CH_2), 2.929-3.010 (60H, b, DOPA-CH_2), 4.233 and 4.305 (116H, b, OCH_2CH_2O), 4.481 and 4.929 (30H, b, DOPA-CH), 5.122 and 5.314 (30H, b, DOPA-NH), 6.484 (16H, d, *J*= 4.5 Hz, exterior 6-ArH), 6.594 (16H, d, *J*= 5.4 Hz, exterior 2-ArH), 6.698 (16H, b, exterior 5-ArH), 6.952-7.048 (42H, b, interior-ArH); ^{13}C NMR ($CDCl_3$) δ^{13C} (75 MHz): 28.281 (Boc-CH_3), 28.624 (succ.-CH_2), 37.467 (DOPA-CH_2), 54.246 and 54.597 (DOPA-CH), 62.411, and 62.838 (-OCH_2), 80.220 (Boc-C), 115.342, 116.120, 121.249, 123.339,

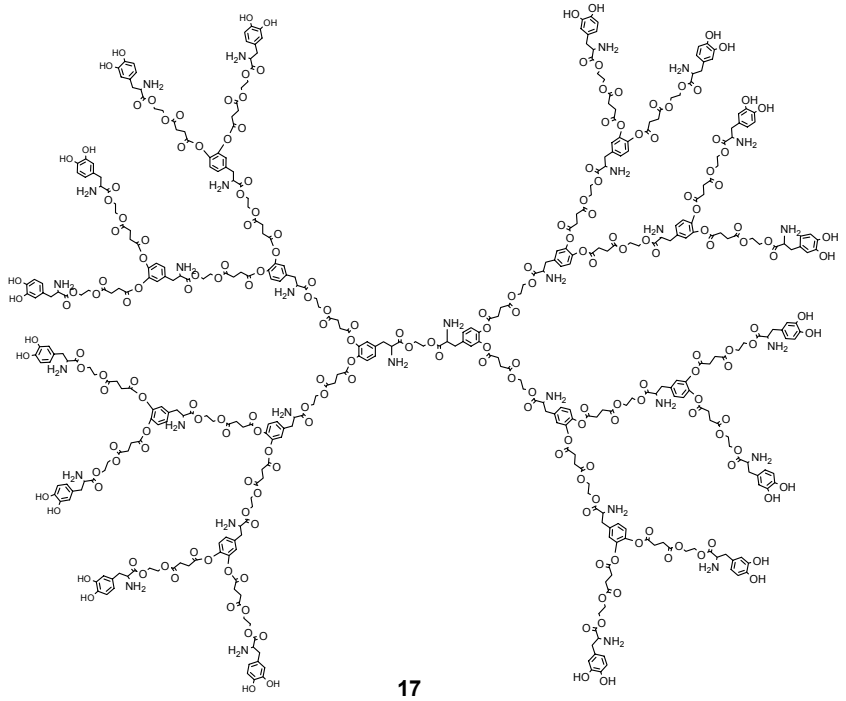
124.193, and 127.833(Ar-CH), 135.227, 140.888, 141.812, 143.361, 144.147 (Ar-C), 155.424 (Boc-CO), 170.059, 170.502 (DOPA-CO), 172.203 (succ.-CO);



16

HO-G3-NH$_2$ 17: To HO-G3-NHBoc **16** (88 mg, 0.007 mmol) a solution of 4 M HCl in dioxane (2 mL) was added. The reaction mixture was stirred for 5 minutes. The chlorate of **16** was precipitated. The mixture was dried and the residue was washed with DCM (2×2 mL). Drying afforded HO-G3-NH$_2$ **17** as a colorless powder (61 mg, 82%). ^{1}H NMR (CD$_3$OD) δ^1H (300 MHz):2.608 (56H, b, succ.-CH$_2$), 2.777-2.789 (56H, b, succ.-CH$_2$), 2.858-2.953 (32H, m, exterior DOPA-CH$_2$), 3.017-3.026 (28H, b, interior and core DOPA-CH$_2$), 4.167-4.219 (116H, b, OCH$_2$CH$_2$O), 4.305- 4.381 (30H, b, DOPA-CH), 6.468 (16H, d, J = 4.8 Hz, exterior 6-ArH), 6.569 (16H, s, exterior 2-ArH), 6.692 (16H, d, J = 4.5 Hz, exterior 5-ArH), 6.912 (6H, b, core-ArH), 7.007-7.069 (36H, b, interior-ArH); ^{13}C NMR (CD$_3$OD) δ^{13}C (75 MHz): 28.669 (succ.-CH$_2$), 35.010 (DOPA-CH$_2$), 54.101 (DOPA-CH), 62.776, 64.501, 66.729 (OCH$_2$CH$_2$O), 116.570, 116.997, 121.827, 126.069 (Ar-CH), 143.917, 144.519 (Ar-C), 169.119, 169.539, 172.553, (DOPA-CO),

174.079 (succ.-CO); MALDI-TOF-MS found $[M+H]^+$ 9474.0; $C_{440}H_{500}N_{30}O_{204}$ requires 9472.8.



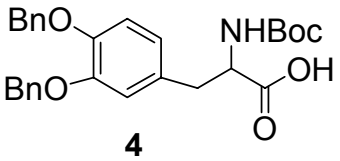
17

HO-G3-NH$_2$ (The 3rd generation dendritic L-DOPA)

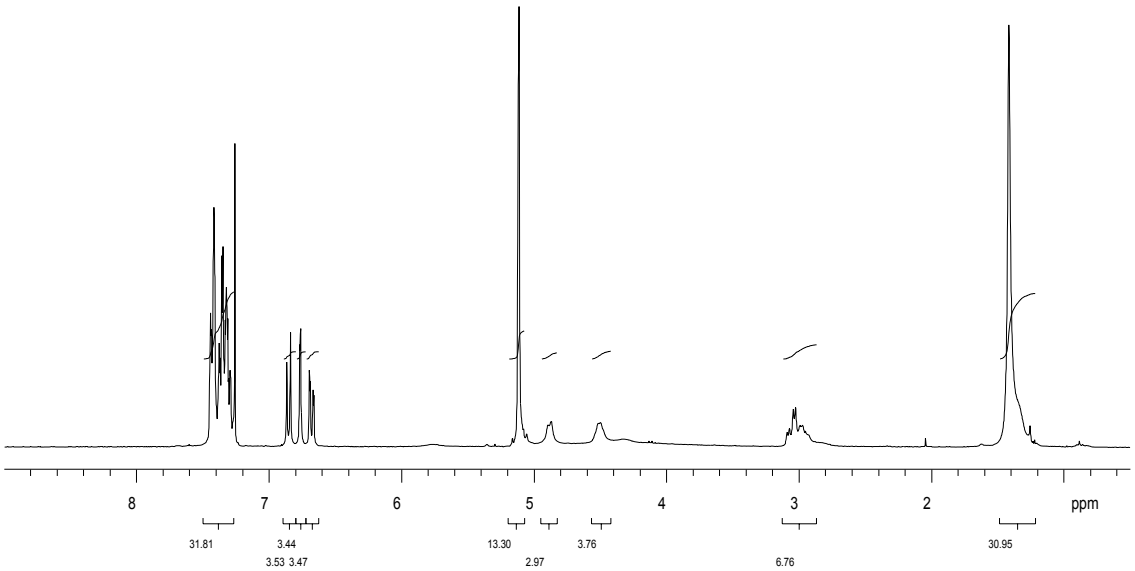
2. Gel electrophoresis Measurements

The 5% stacking gel was made in glacial acetic acid / KOH solution (final concentrations 0.75% and 120 mM, respectively, pH 5.9). Ammonium persulfate and TEMED concentration was 0.7% and 0.06%, respectively. Resolving gel was made in glacial acetic acid / KOH solution (final concentrations 13.25% and 30 mM, respectively, pH 2.9). The concentrations of persulfate were similar to that of stacking gel. TEMED concentration was increased to 0.6%. Under these conditions, polymerization took about 30-60 minutes. Electrode buffer was 0.16% acetic acid containing 0.6% β-alanine. Loading buffer was 40% sucrose and 2% methylene blue. Samples were mixed with equal volumes of loading buffer for application onto the gel (5μL sample plus 5μL loading buffer). Electrophoresis was performed in the cold (4 $^{\circ}$C), at 200V, for 75 min. After separation, the gel was placed in 0.25 M bicarbonate buffer for about 5 minutes at room temperature. It was then transferred to a fresh solution of bicarbonate buffer (containing 0.5 M glutaraldehyde) and incubated at 4 $^{\circ}$C for 1 hour. After fixation, the gel was rinsed with deionized water and placed in Coomassie Blue stain (0.2% dye made in 50% methanol/ 10% acetic acid) in the cold (4 $^{\circ}$C) overnight. Destaining was carried out in 10% methanol/10% acetic acid solution in the cold.

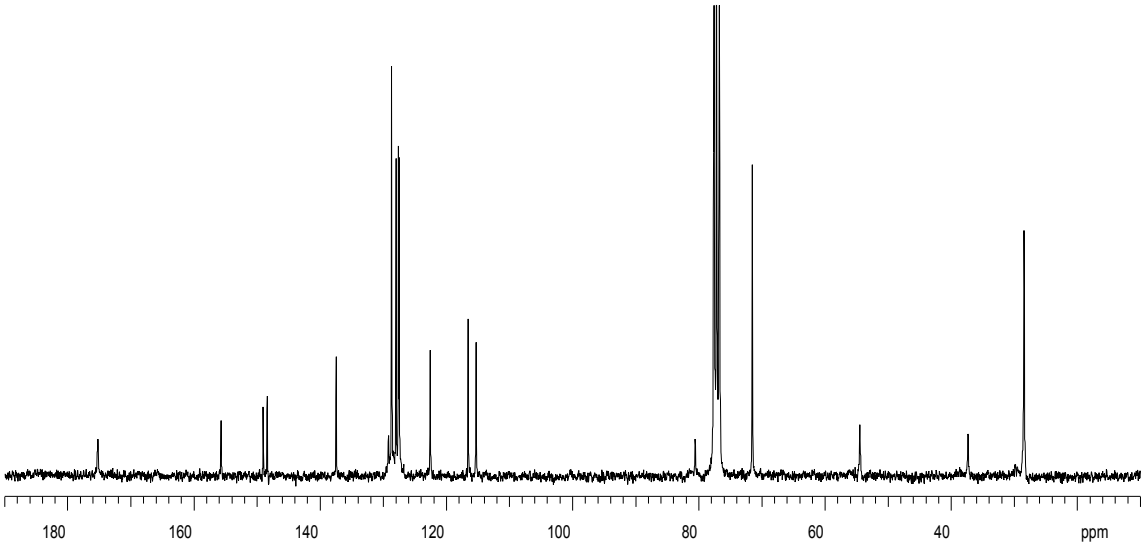
3. NMR Spectra of Important Intermediates and Final Products

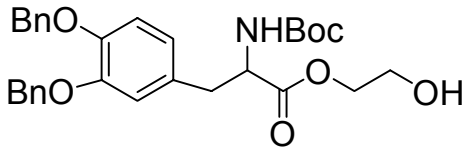


^{1}H NMR



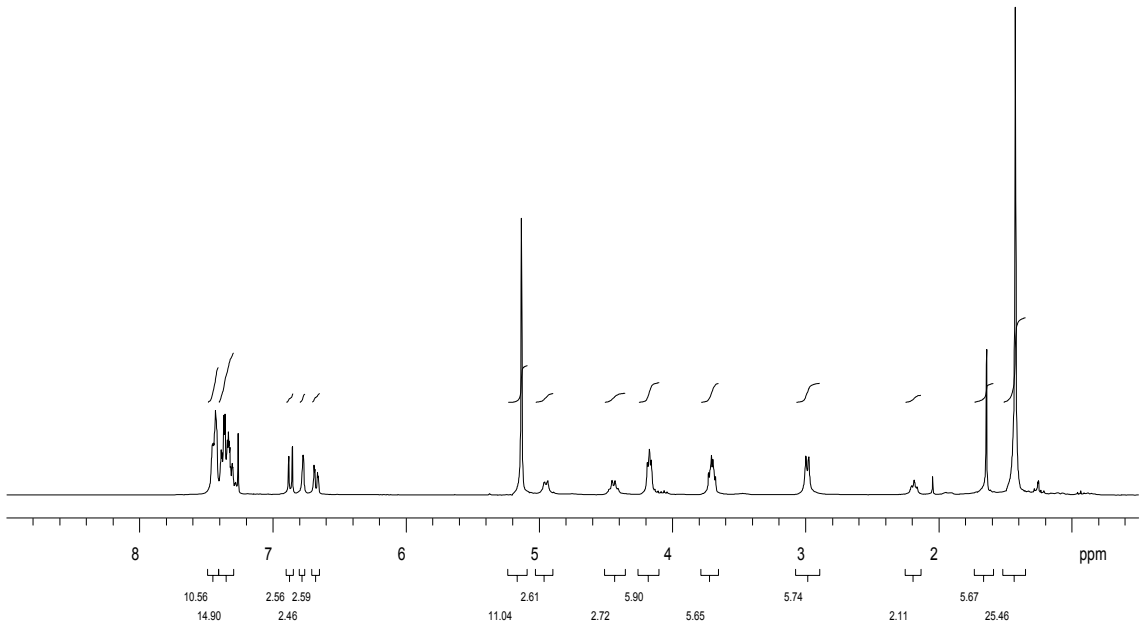
^{13}C NMR





5

^{1}H NMR



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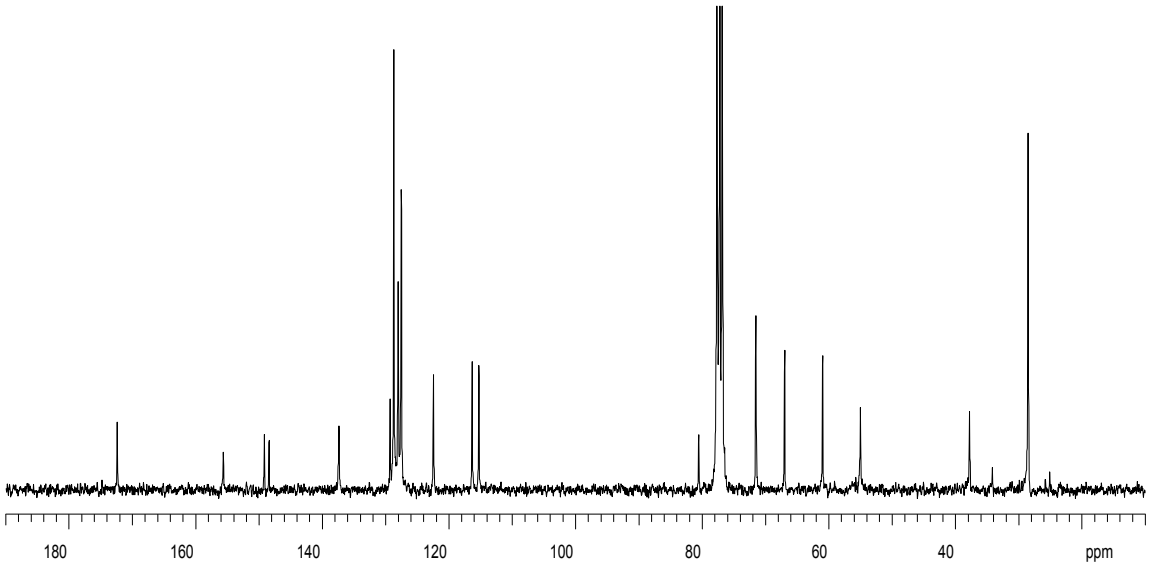
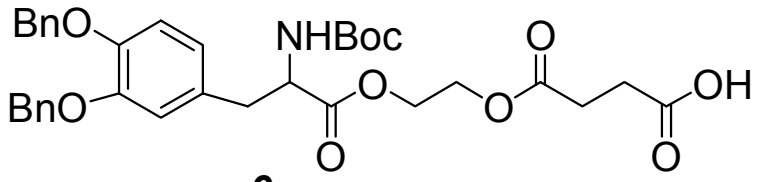
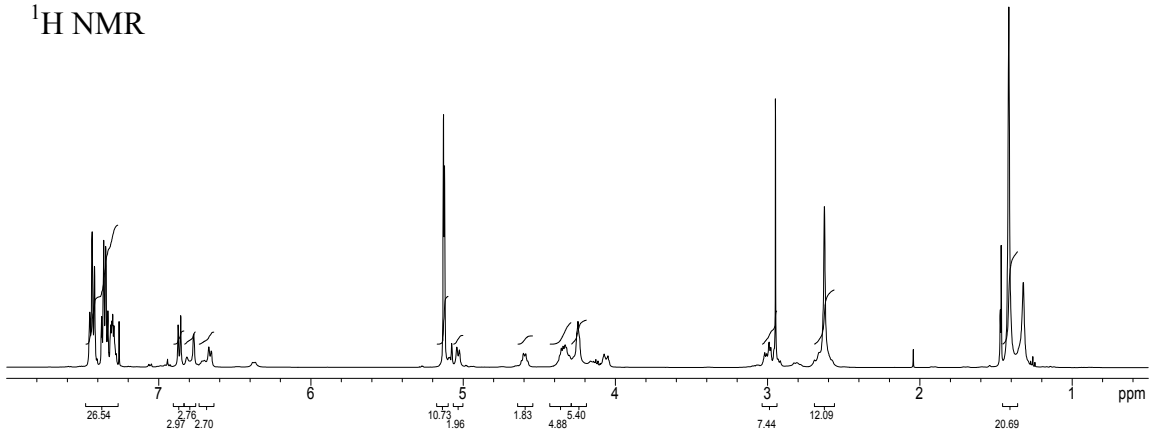


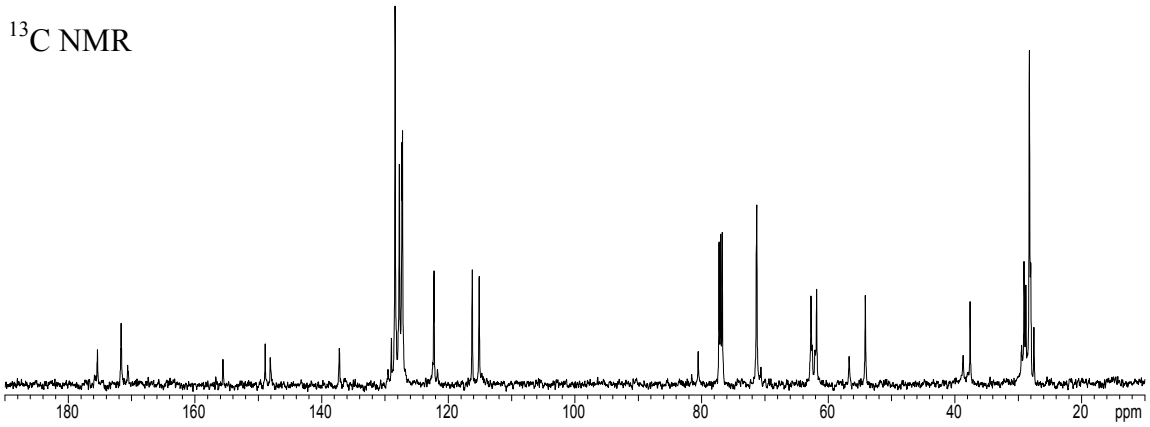


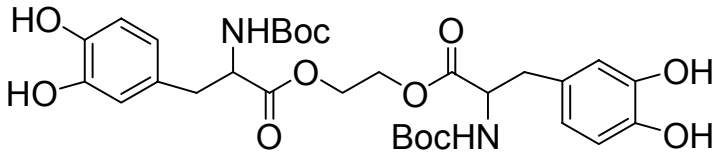
6

^{1}H NMR



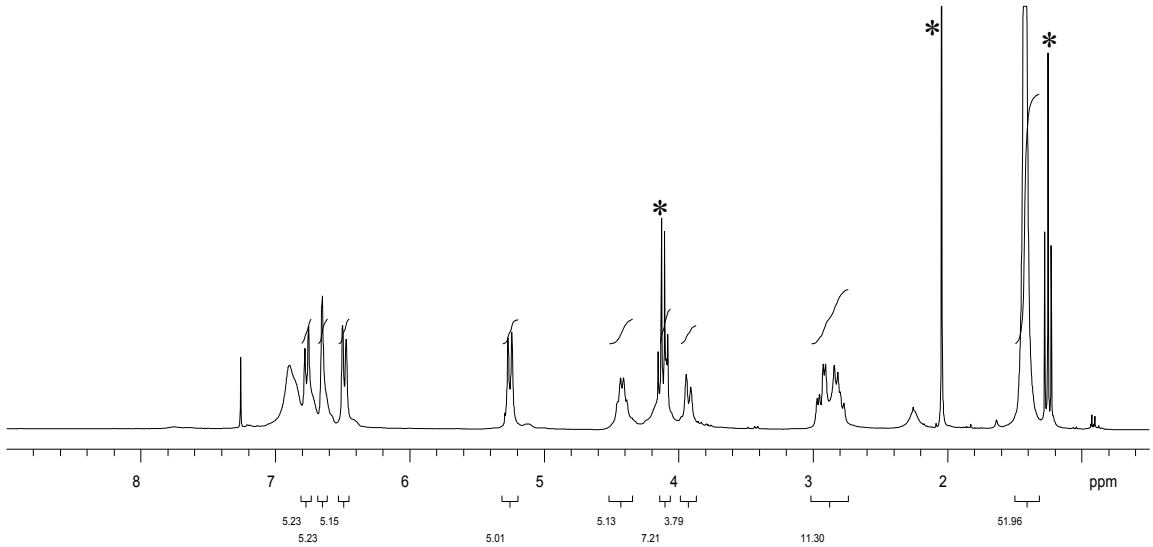
^{13}C NMR



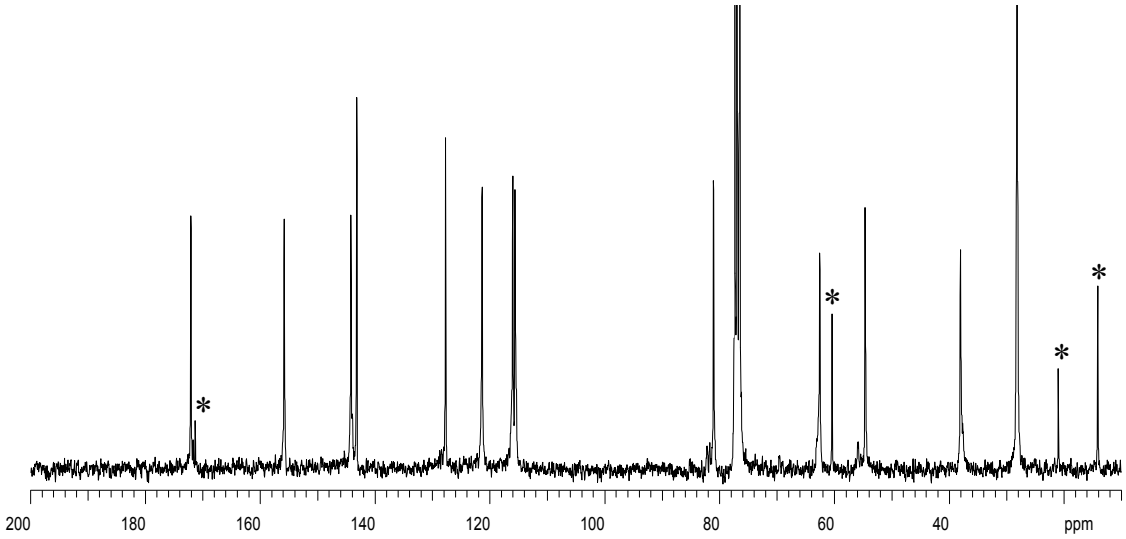


8

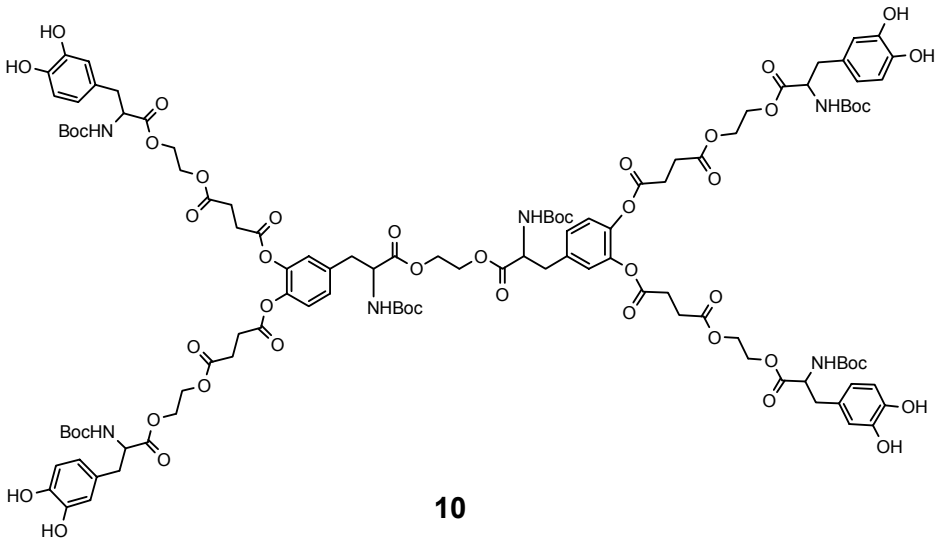
^{1}H NMR



^{13}C NMR

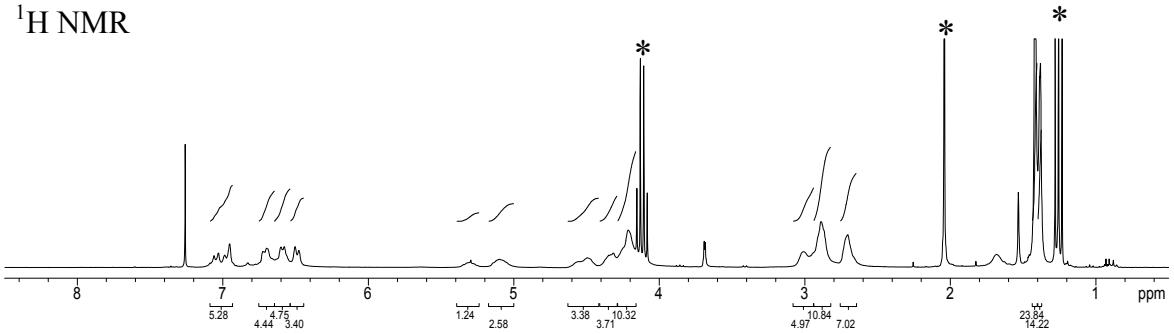


* peaks from ethyl acetate

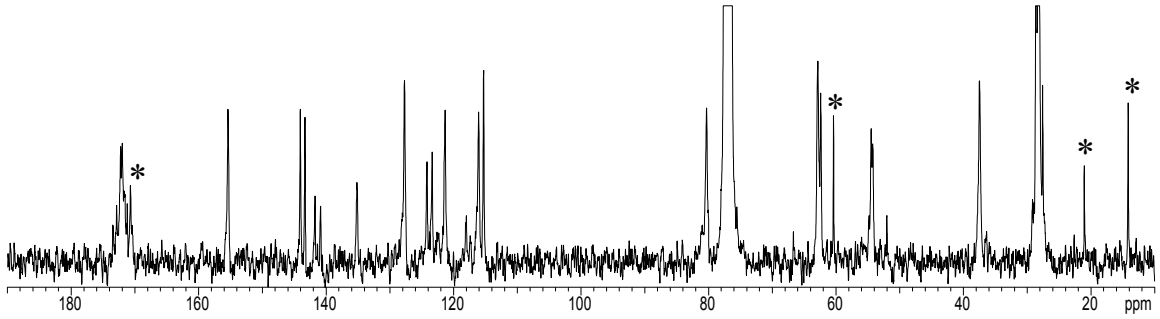


10

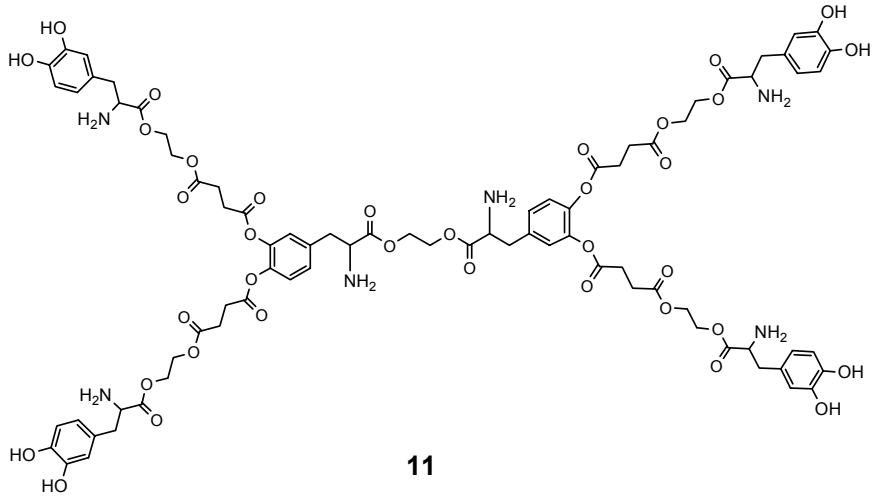
^{1}H NMR



^{13}C NMR

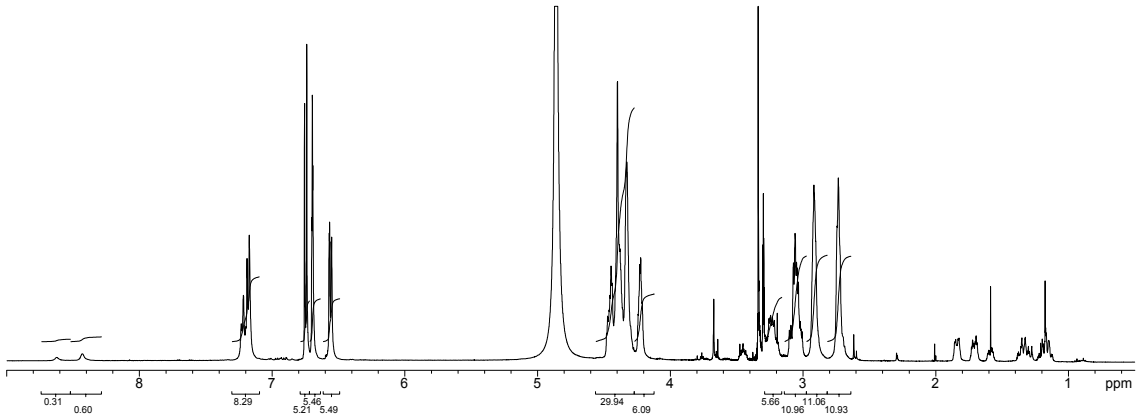


* peaks from ethyl acetate

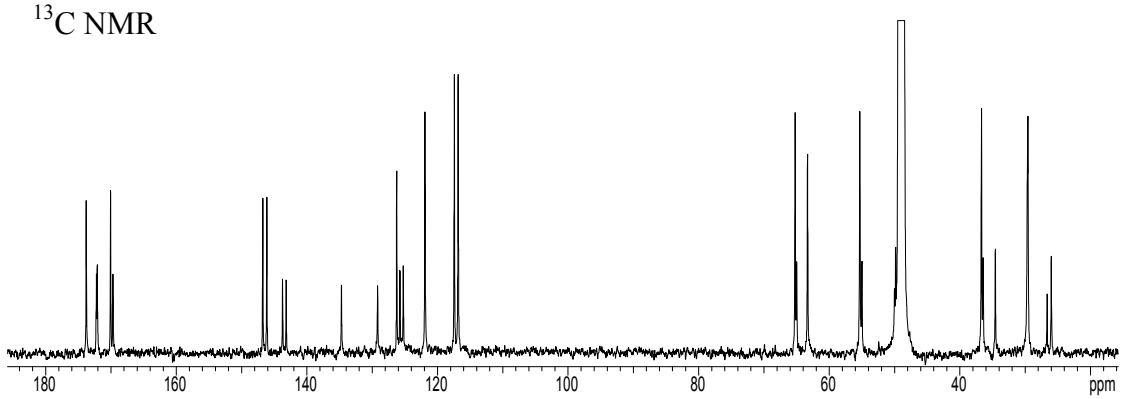


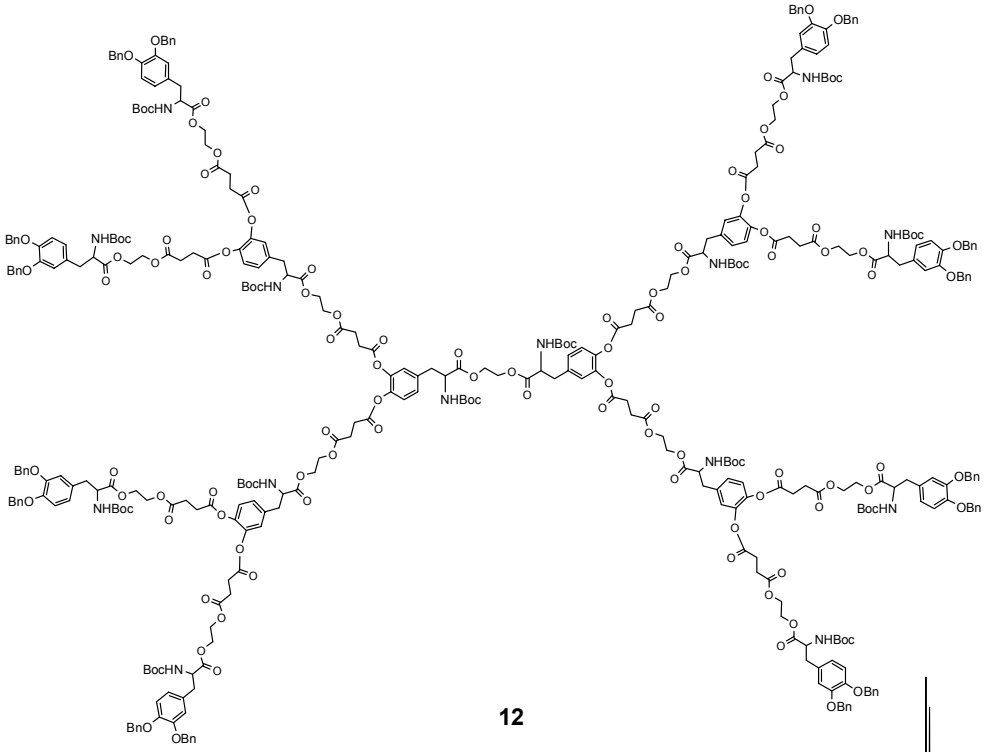
11

^{1}H NMR



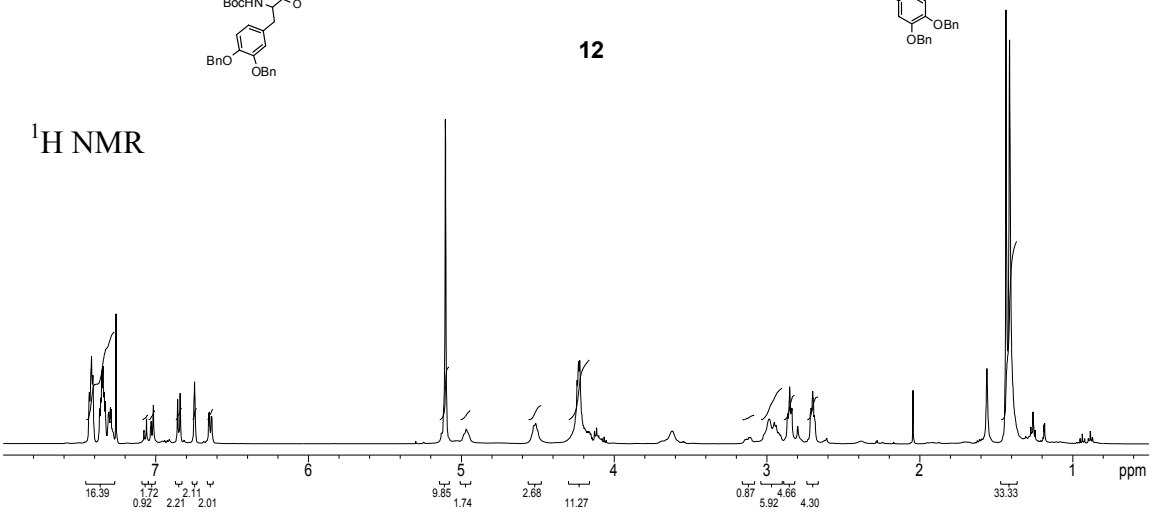
^{13}C NMR

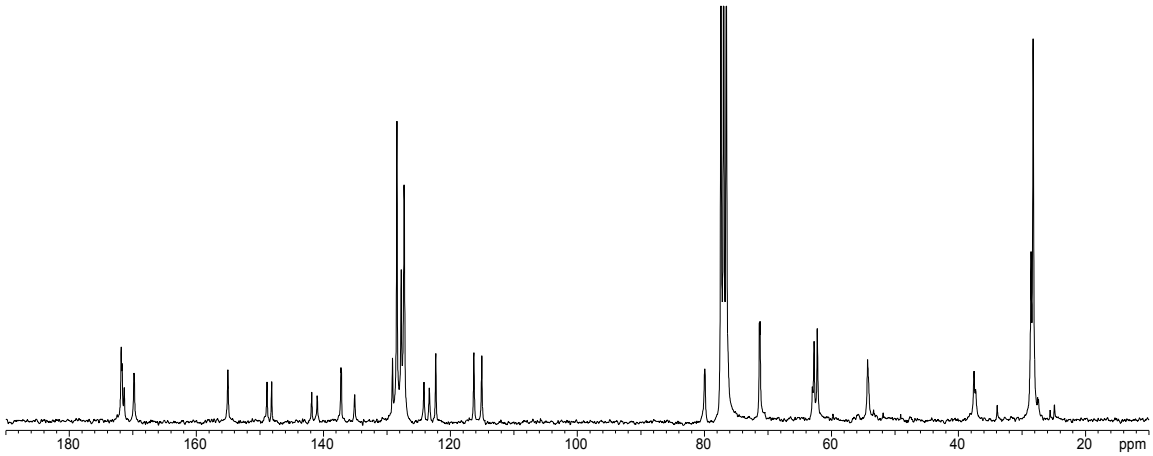


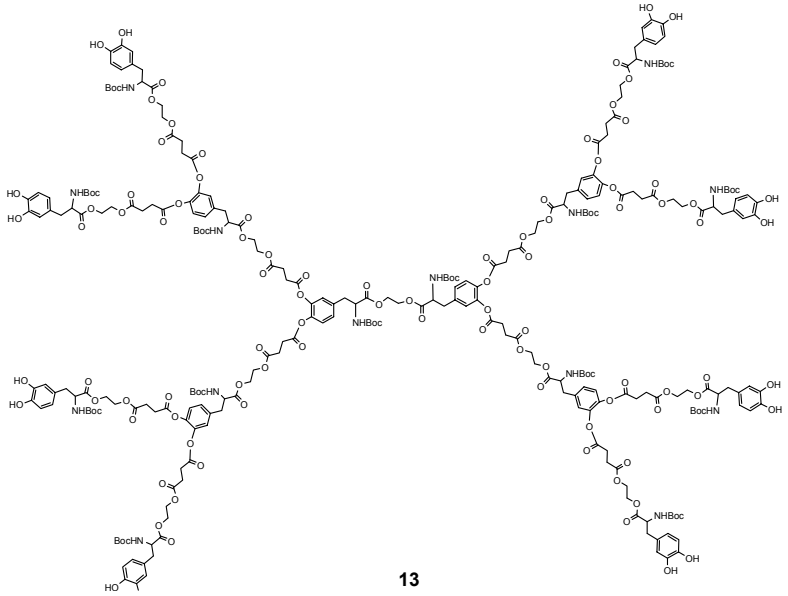


12

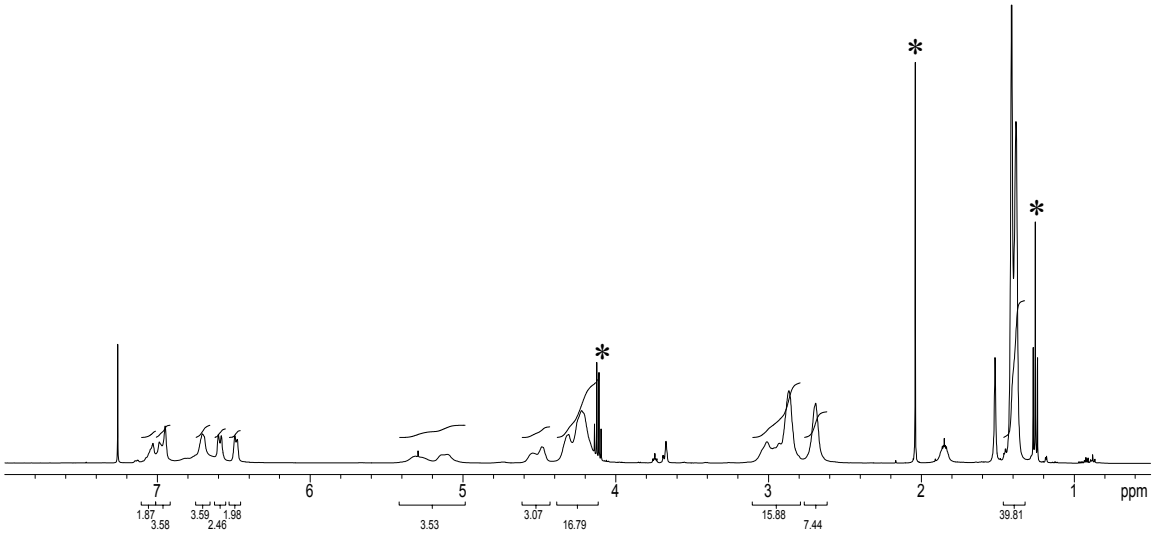
^{1}H NMR

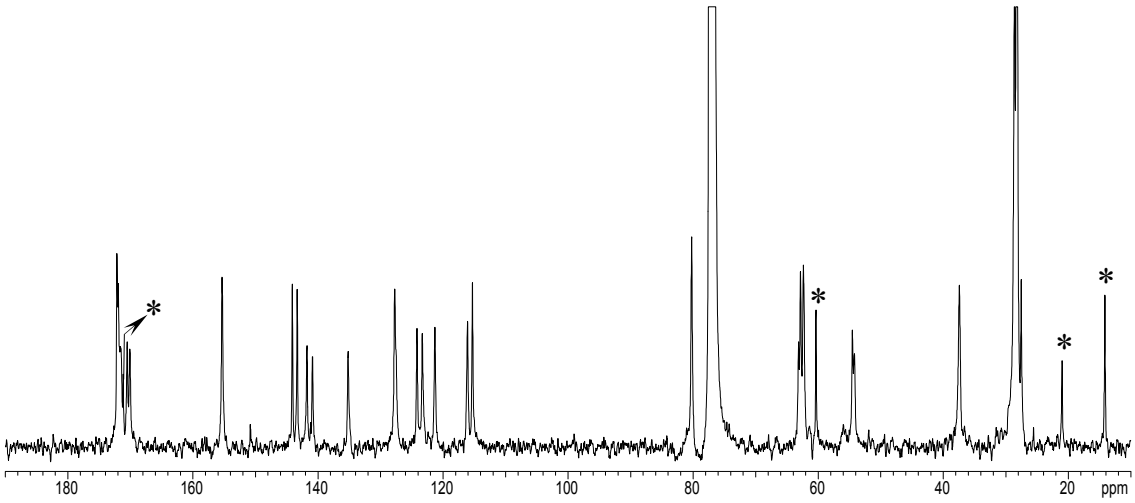

7
6
5
4
3
2
1
ppm
16.39
1.72
0.92
2.21
2.11
2.01
9.85
1.74
2.68
11.27
0.87
5.92
4.66
4.30
33.33


180
160
140
120
100
80
60
40
20
ppm

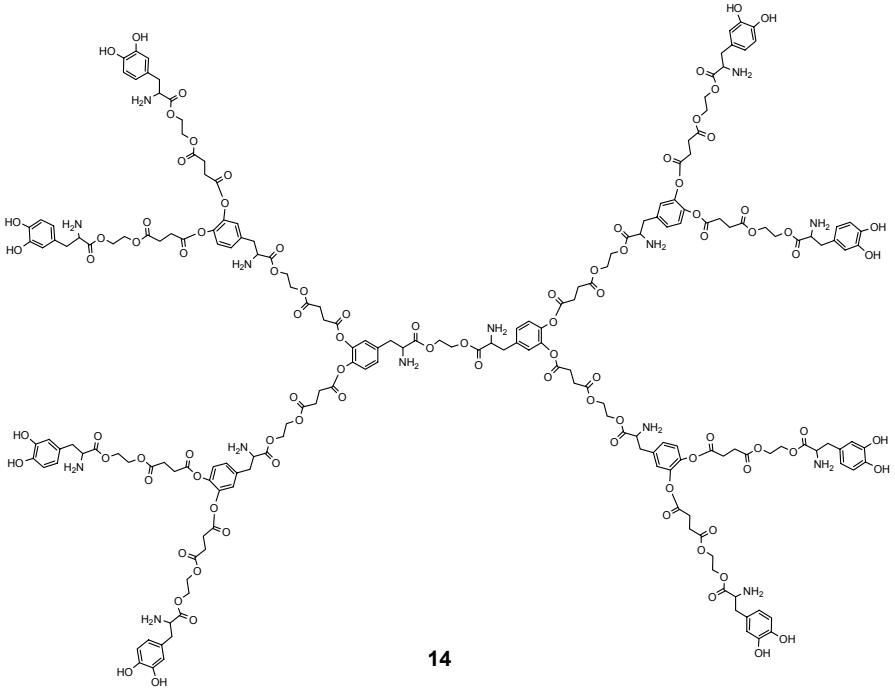


13



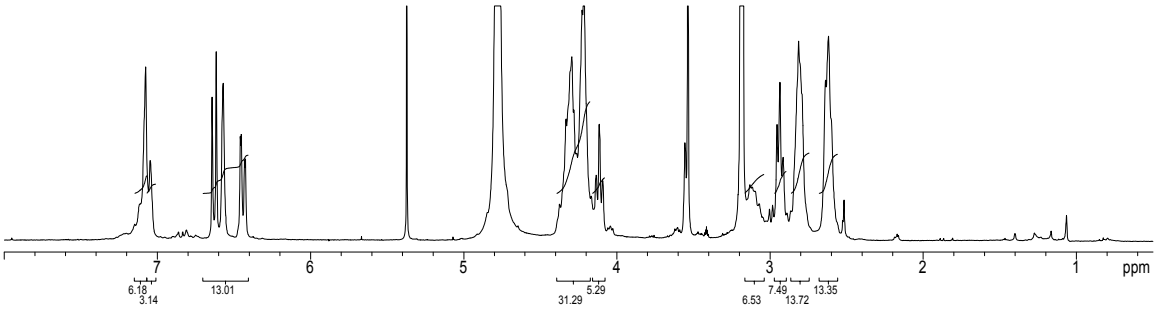


* peaks from ethyl acetate

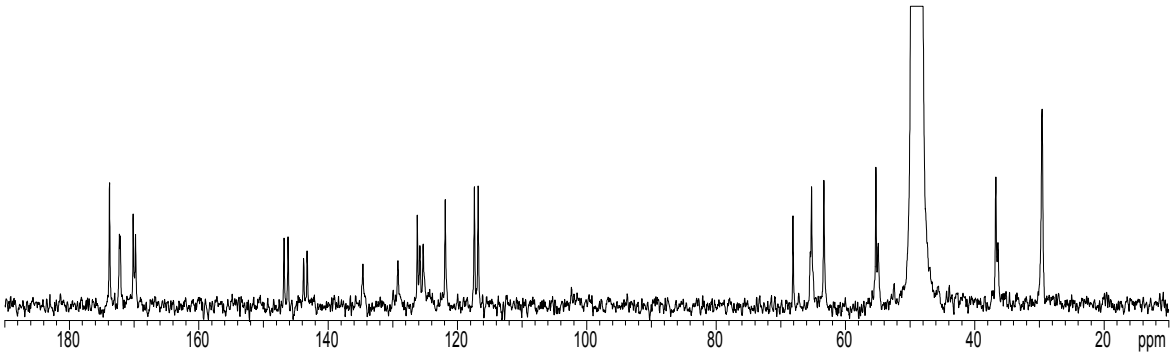


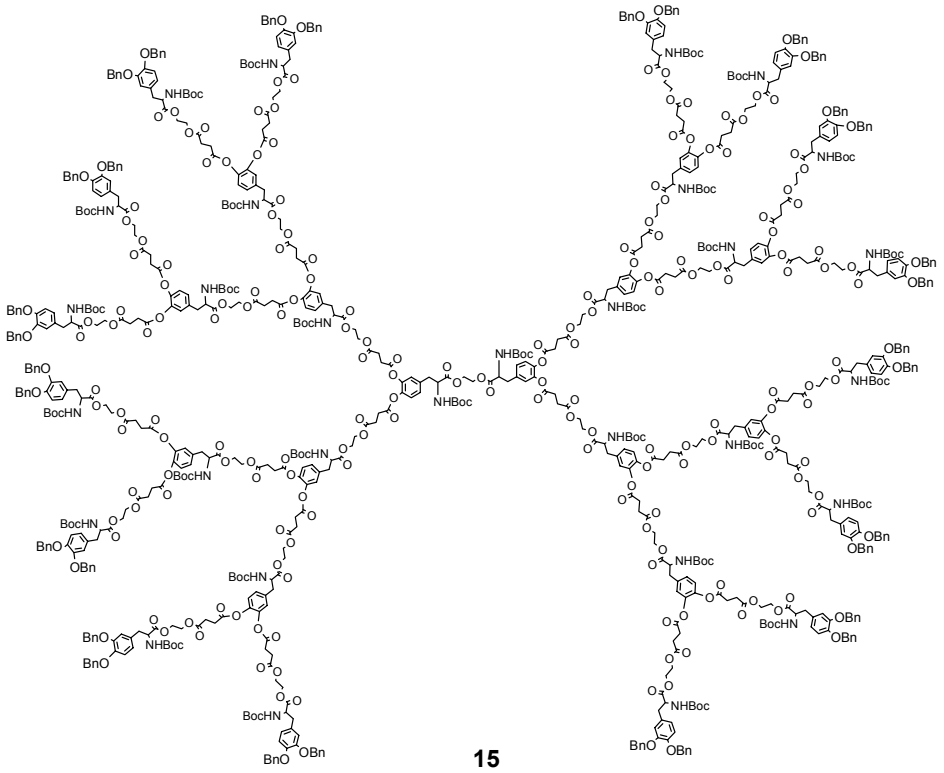
14

^{1}H NMR



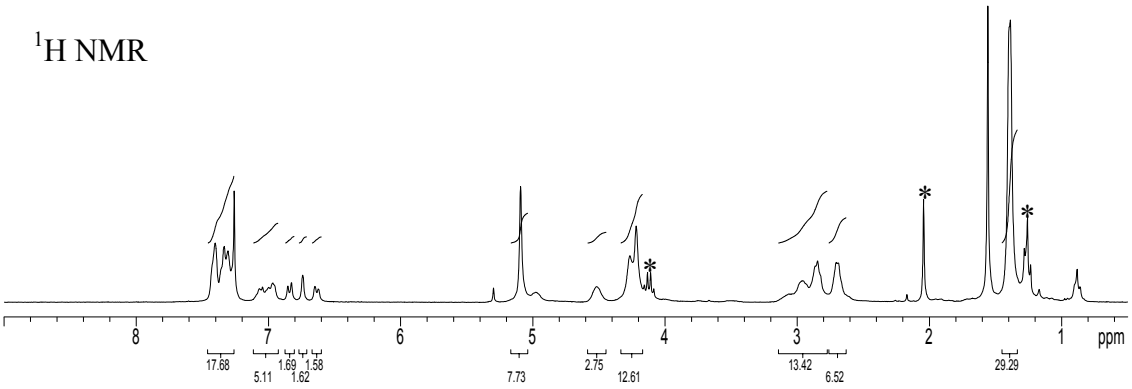
^{13}C NMR





15

^{1}H NMR



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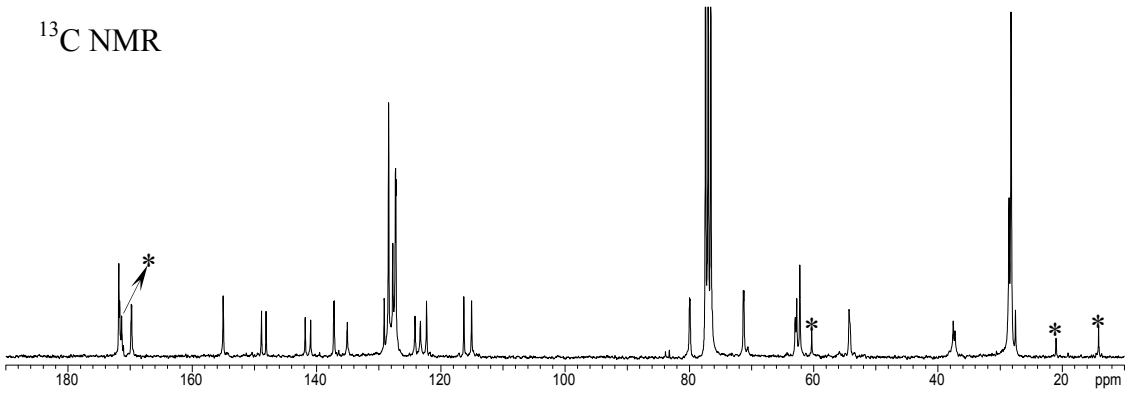
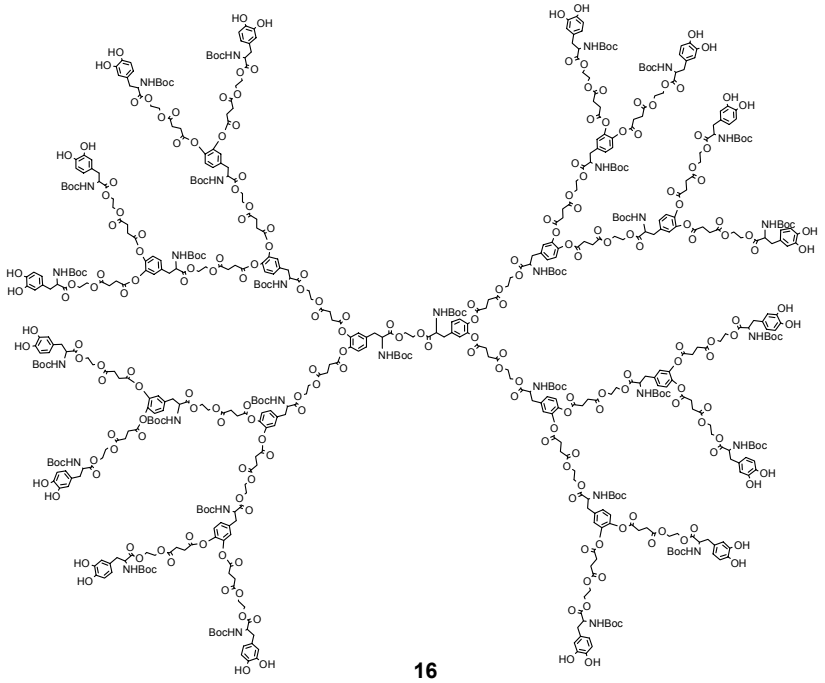
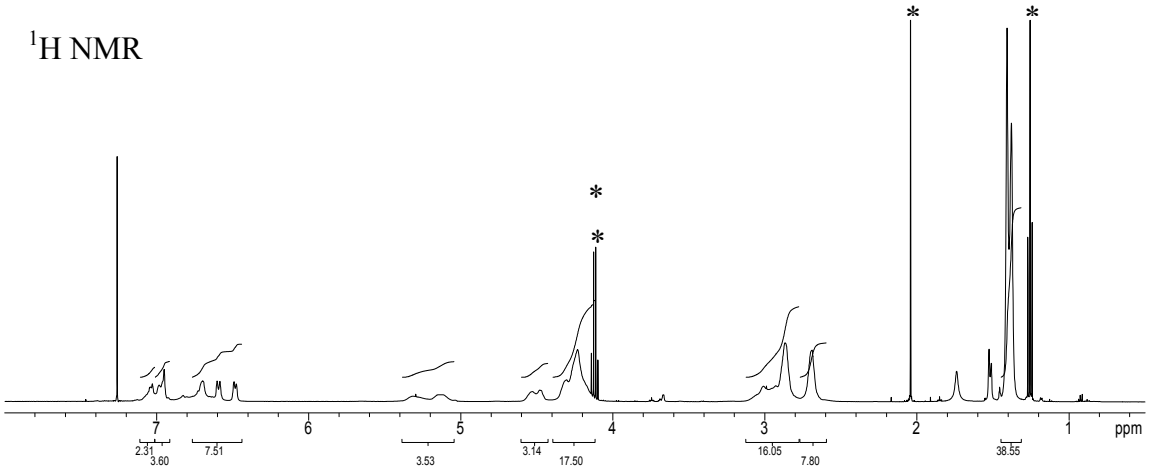


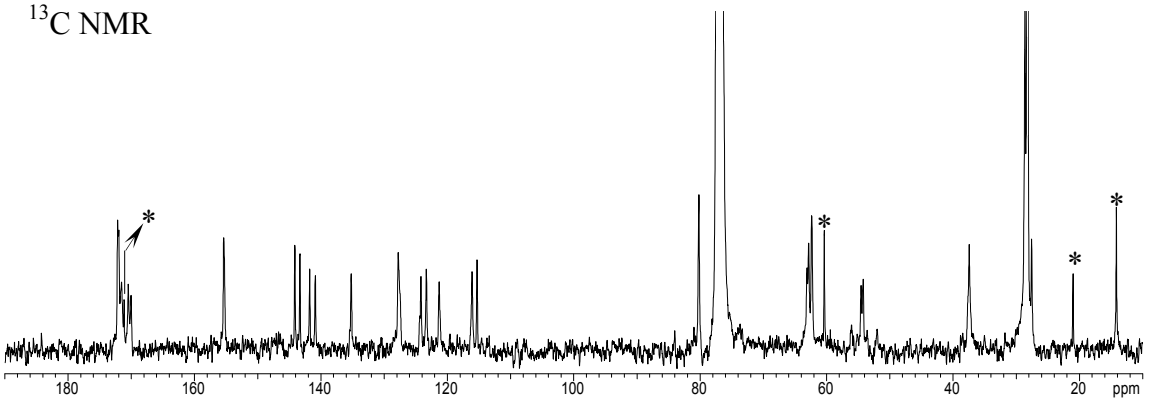


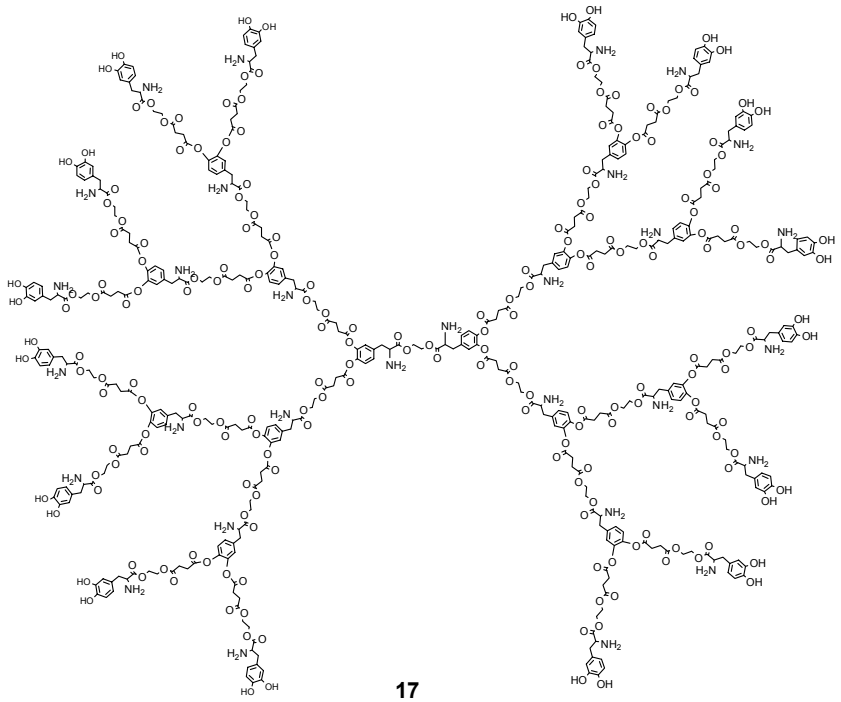
16

^{1}H NMR



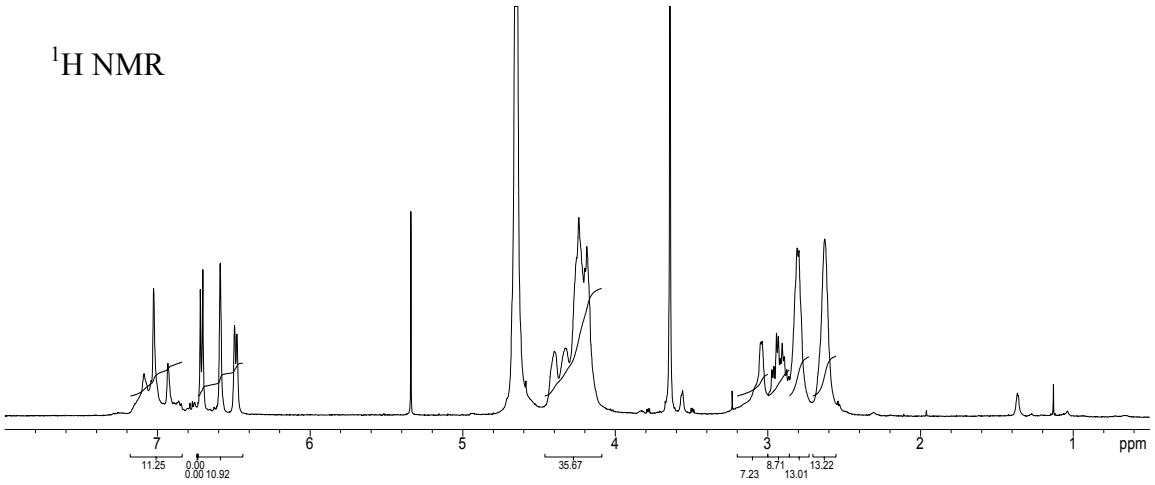
^{13}C NMR



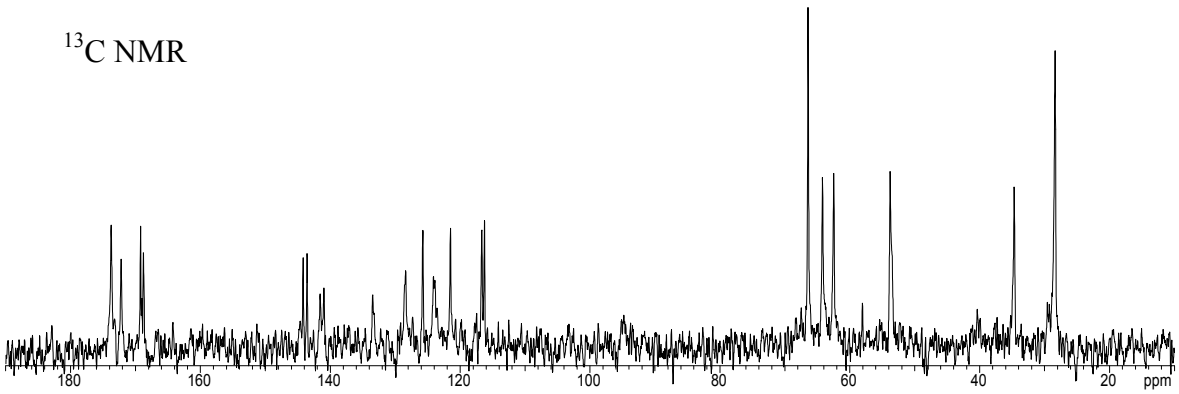


17

^{1}H NMR

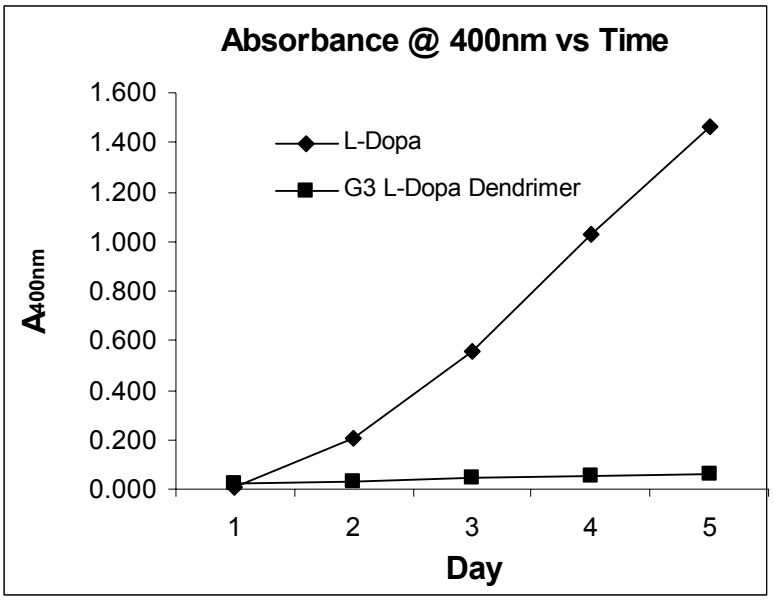


^{13}C NMR



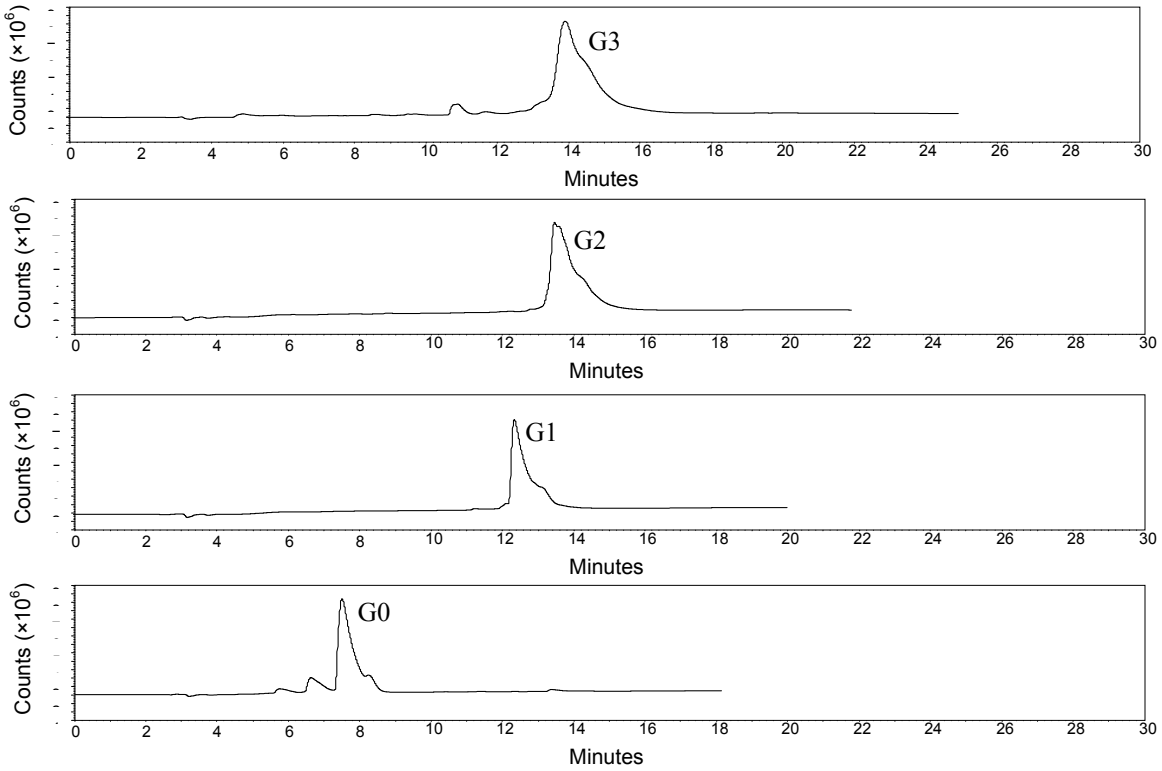
4. UV-Vis Data on the Photostability of L-DOPA and G3 L-DOPA Dendrimer

Both L-DOPA and G3 L-DOPA dendrimer were freshly dissolved in deionized water (1mg /mL) for photostability study. L-DOPA solution turned black after a day because of the photo-oxidation; however, G3 L-DOPA dendrimer solution kept clear for more than several weeks. The dark L-DOPA solution has a distinct absorption around 400 nm. The UV-Vis absorbances at 400 nm were measured for both L-DOPA and G3-L-DOPA dendrimer solutions for 5 days. The UV-Vis absorbance at 400 nm versus time (by day) was plotted shown in the following figure. It is clearly observed that the absorbance at 400nm of L-DOPA solution was increasing dramatically per day. But the absorbance at 400 nm of G3 L-DOPA dendrimer solution changed very little over the time.



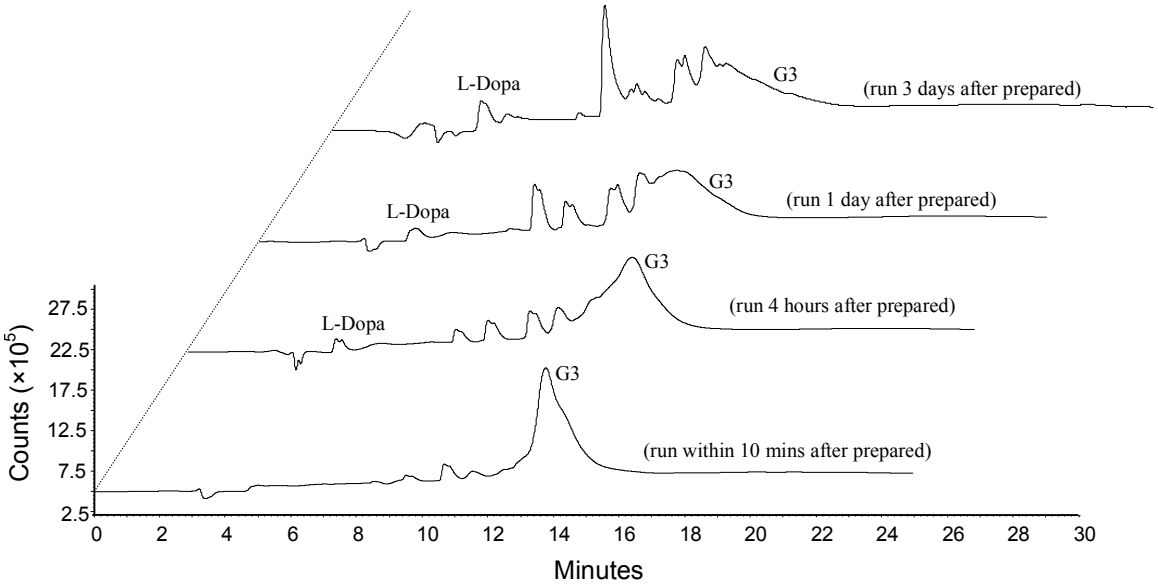
5. HPLC Chromatograms of L-DOPA Dendrimers G0-G3

All generations of L-DOPA dendrimers were analyzed by HPLC using a C18 column with water-acetonitrile bi-solvent gradient elution (starting with 19:1 ratio of water to acetonitrile and ending with 100% of acetonitrile) at a flow rate of 1.00 mL/min. The chromatogram of each generation L-DOPA dendrimer showed the final product has high purity for dendritic macromolecules.

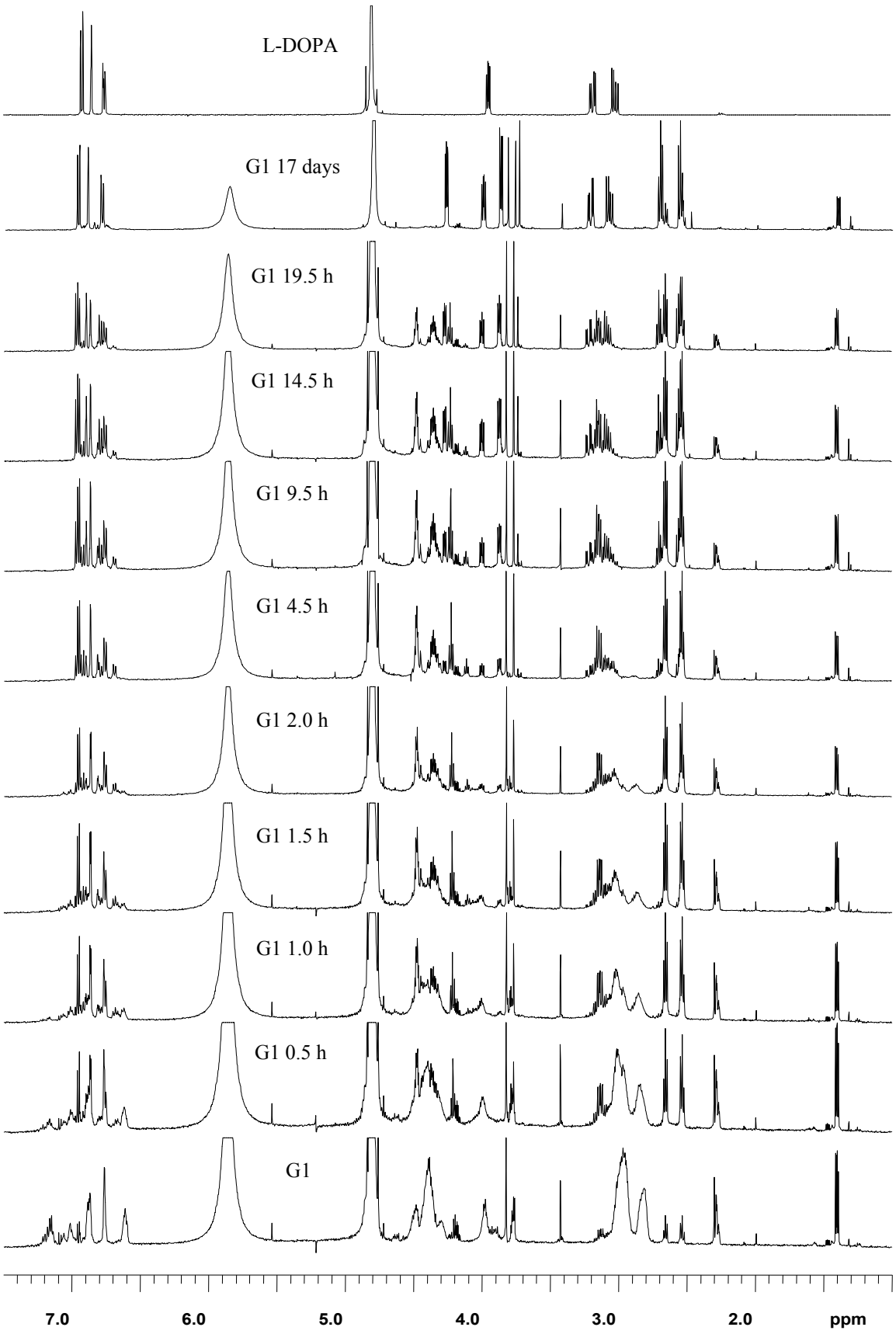


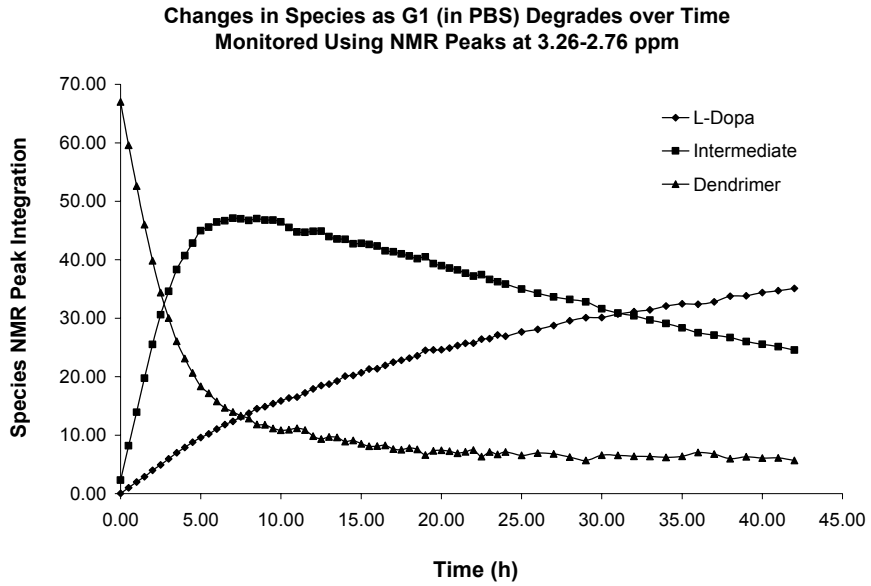
6. NMR and HPLC Studies on the Degradation of L-DOPA Dendrimers

The *in vitro* degradation (or hydrolysis) of novel dendritic L-DOPA prodrugs was studied using ^{1}H NMR and HPLC. The 3rd generation dendrimer was dissolved in deionized water and the sample was monitored by HPLC for four days. The chromatograms acquired at the different time or days were shown as follows:



The degradation of the G3 dendrimer was clearly observed by HPLC even within half a day time. The degradation pattern with multiple intermediates formed and consumed over the time period of observation indicates that the degradation may undergo sequentially as expected from the dendritic architecture. The amount of L-DOPA released is gradually increased in the course of hydrolysis, which is also consistent with the sequential degradation hypothesis. Furthermore, the degradation of G1 dendritic prodrug was monitored for a couple of weeks by ^{1}H NMR under deuterated PBS (pH = 7.5) condition at the room temperature. The ^{1}H NMR plots shown as follows demonstrated the degradation began instantly within half an hour. The NMR peak integration values of the L-DOPA released, the intermediates formed as well as the existing L-DOPA dendrimer


L-DOPA
G1 17 days
G1 19.5 h
G1 14.5 h
G1 9.5 h
G1 4.5 h
G1 2.0 h
G1 1.5 h
G1 1.0 h
G1 0.5 h
G1
7.0
6.0
5.0
4.0
3.0
2.0
ppm



were used to represent the amount of each species in the system during degradation and plotted versus time, which provided the kinetic information of the degradation. The kinetic plot is clearly shown that L-DOPA G1 dendrimer was nearly consumed within 9.5 hours. Several intermediates (but NMR resonances were overlapped) were formed and consumed gradually at different time periods, and L-DOPA amount steadily increased during the entire process and eventually became the major component of the system after 40 hours. This study proved that the L-DOPA did release from the dendrimer sequentially. Same types of NMR experiments were also performed on the G2 dendrimer in the deionized water solution as well as the PBS solution (all solutions were made using deuterated reagents). Similar degradation trends were observed as in the G1 dendrimer system. Furthermore, the degradation rate of the G2 dendrimer was much slower in the deionized water solution than in the PBS solution. Studies at different pH conditions indicated that the degradation of L-DOPA dendrimers is a base-catalyzed hydrolysis process. Further detailed quantitative measurements of the L-DOPA released from the dendritic prodrugs will be done in the future using both NMR and HPLC.