Electronic supporting information for:

Self-immolative dendron hydrogels

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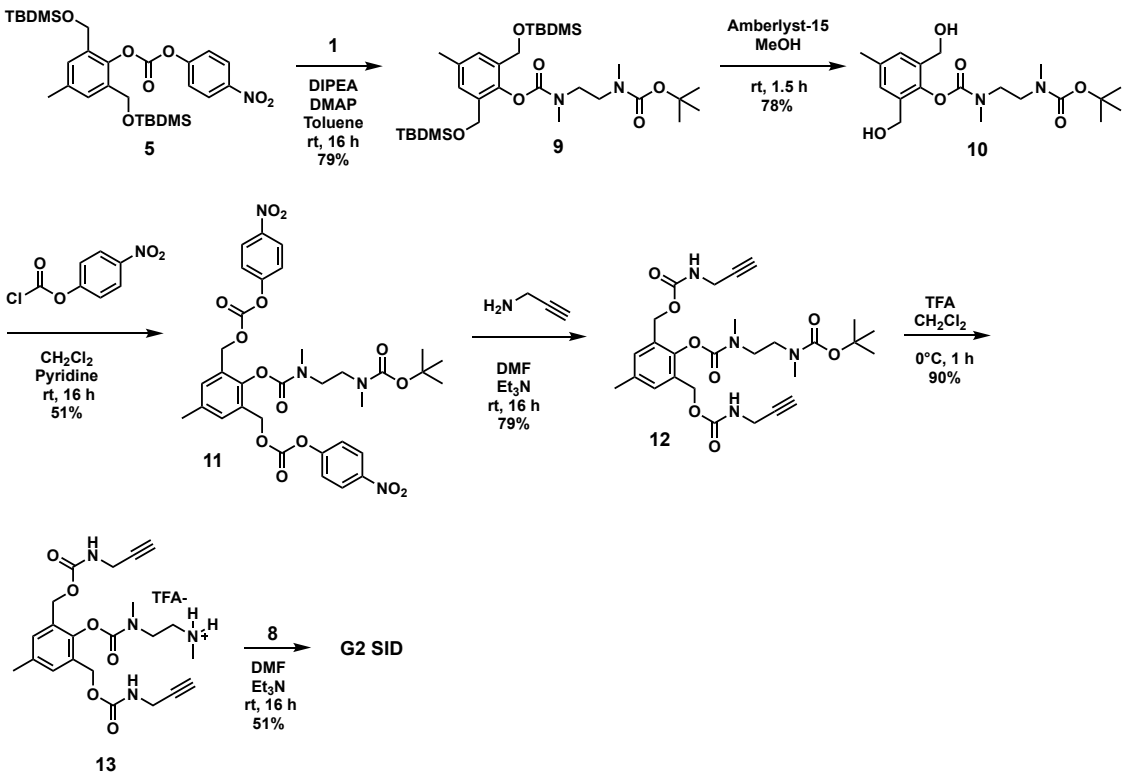
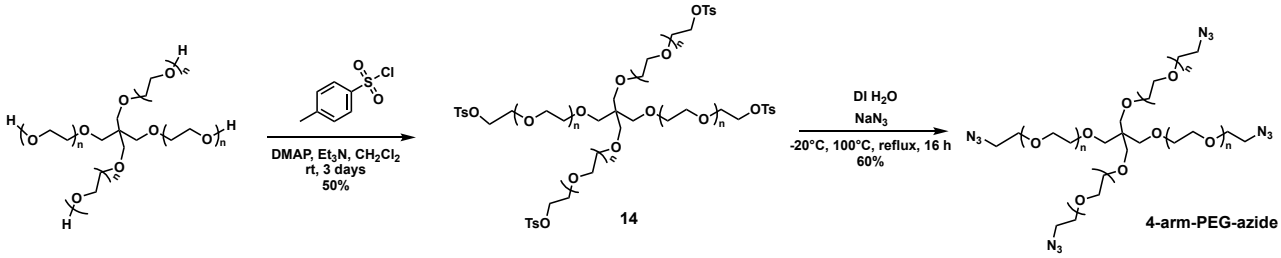
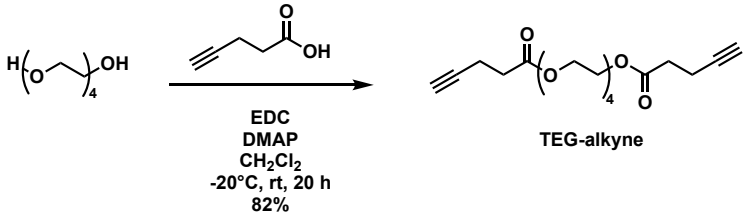
Table of contents:



Scheme S1. Synthesis of **G2 SID**.



Scheme S2. Synthesis of **4-arm-PEG-azide**.



Scheme S3. Synthesis of **TEG-alkyne**

Experimental procedures

General Materials. Compounds **1**,[1] compound **2**,[2] and compound **5**[3] were synthesized as previously reported. All reactions were carried out using flame dried glassware under an inert atmosphere of nitrogen unless otherwise stated. Pyridine, Et_3N, Na_2HPO_4, and TFA were purchased from Caledon Laboratory Chemicals (Georgetown, ON, Canada). *N,N′*-dimethylethylenediamine, 4-nitrophenyl chloroformate, and 2-nitrobenzyl alcohol were purchased from TCI Chemicals (Tokyo, Japan). 2,6-Bis(hydroxymethyl)-*p*-cresol, TEG, DMSO, sodium azide, and DIPEA were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). DMAP was purchased from AK Scientific (Union City, CA, USA). Amberlyst-15 and 4-pentynoic acid were purchased from Alfa Aesar. 4-arm-PEG was purchased from JenKem Technology. *N*-Ethyl-*N′*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was purchased from Advanced ChemTech. NaCl was purchased from Fisher Scientific. KH_2PO_4 and KCl were purchased from EMD Millipore. All chemicals were used as received unless otherwise noted. Under a nitrogen atmosphere, toluene was distilled over sodium, while Et_3N and CH_2Cl_2 were distilled over CaH_2. Phosphate buffered saline was prepared to contain 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and KH_2PO_4 at a pH of 7.4 in either deionized H_2O or D_2O.

General Procedures. Dialysis was performed using Spectra/Por 6 dialysis tubing from Spectrum Laboratories (Rancho Dominguez, CA, USA) with a molecular weight cutoff (MWCO) of 3.5 kg/mol. Column chromatography was performed using silica gel (0.040−0.063 mm particle size, 230−430 mesh). Thin layer chromatography (TLC) was carried out using Siliaplate silica gel F254 plates (20 cm × 20 cm, 250 μm). NMR spectroscopy was conducted on a Bruker AvIII HD 400 MHz Spectrometer (^{1}H 400.09 MHz, ^{13}C 100.5 MHz). The ^{1}H and ^{13}C chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane and were calibrated against $CHCl_3$ (7.26 ppm) and $CDCl_3$ (77 ppm) respectively. Coupling constants (J) are expressed in Hertz (Hz). FTIR spectra were recorded using a PerkinElmer Spectrum Two FTIR spectrometer with an attenuated total reflectance (ATR) attachment and a single reflection diamond. FTIR spectra of the hydrogels were obtained after lyophilization of the purified hydrogels. High-resolution mass spectrometry (HRMS) was conducted on a Synapt high definition mass spectrometer using electrospray (ESI) ionization. Samples were irradiated in an ultraviolet (UV) box (wavelength: 365-370 nm, intensity: 10 mW/cm^2).

Synthesis of Compound 3. Compound **1**[1] (1.85 g, 9.84 mmol, 1.0 equiv), compound **2**[2] (3.13 g, 9.84 mmol, 1.0 equiv), DIPEA (3.43 mL, 19.68 mmol, 2 equiv) and DMAP (1.20 g, 9.84 mmol, 1.0 equiv) were combined with stirring in dry toluene (90 mL). The reaction mixture was stirred at room temperature overnight. The solvent was removed, and the crude residue was dissolved in CH_2Cl_2 (100 mL), washed with saturated Na_2CO_3 (3 x 100 mL), and dried over $MgSO_4$. The crude product was purified via column chromatography with a gradient from 100% hexane to 50:50 hexane/ethyl acetate as the eluent yielding 1.54 g of a yellow oil. Yield = 43%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 – 8.02 (m, 1H), 7.70 – 7.43 (m, 3H), 5.53 (s, 2H), 3.54 – 3.32 (m, 4H), 3.15 – 2.82 (m, 6H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.0, 163.1, 155.4, 147.4, 133.6, 132.9, 129.17, 128.6, 128.5, 126.0, 125.0, 124.8, 122.5, 122.1, 115.5, 79.6, 63.9, 63.7, 47.2, 46.9, 46.7, 46.4, 46.9, 45.5, 35.3, 35.0, 34.7, 34.4, 28.3. FTIR: 2957, 1687, 1524, 1399 cm^{-1}. MS (*m/z*): calcd for $C_{17}H_{25}N_3O_6$, 366.1660; found, 366.1655 $[M]^+$.

Synthesis of Compound 4. Compound **3** (180 mg, 0.493 mmol, 1.0 equiv) was dissolved with stirring in dry CH_2Cl_2 (0.63 mL) at 0 °C. TFA (0.75 mL, 9.86 mmol, 20 equiv) was added dropwise and the mixture was stirred for 1 hour. The solution was concentrated to afford 180 mg of a yellow oil. Yield = 96 %. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.35 – 8.24 (m, 2H), 8.09 (d, J = 8.1 Hz, 1H), 7.71 – 7.50 (m, 3H), 5.50 (s, 2H), 3.65 (t, J = 3.7 Hz, 2H), 3.36 (br s, 2H), 3.00 (s, 3H), 2.85 (t, J = 2.9 Hz, 3H).^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.4 (q, J = 160), 158.5, 147.1, 134.3, 131.1, 130.4, 129.8, 129.3, 128.9, 125.2, 125.2, 122.5, 122.4, 114.8 (q, J = 115), 65.6, 49.3, 48.9, 47.8, 46.5, 46.4, 45.7, 45.3, 35.5, 35.0, 34.1, 34.0. MS (*m/z*): calcd for $C_{12}H_{17}N_3O_4$, 268.1292; found, 268.1293 $[M]^+$.

Synthesis of Compound 6. Compound **4** (170 mg, 0.636 mmol, 1.0 equiv), compound **5**[3] (357 mg, 0.636 mmol, 1.0 equiv), DIPEA (0.22 mL, 1.27 mmol, 2.0 equiv), and DMAP (77.7 mg, 0.636 mmol, 1.0 equiv) were combined in dry toluene (30 mL) and stirred at room temperature overnight. The solvent was removed, and the crude residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated Na_2CO_3 (3 x 50 mL), and dried over $MgSO_4$. The product was purified via column chromatography with a 75% hexane/ethyl acetate solvent system yielding 310 mg of a yellow oil. Yield = 74 %. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 – 8.05 (m, 1H), 7.71 – 7.35 (m, 3H), 7.17 (s, 2H), 5.57 – 5.51 (m, 2H), 4.65 – 4.55 (m, 4H), 3.68 – 3.44 (m, 4H), 3.19 – 2.97 (m,

6H), 2.3– 2.31 (m, 3H), 0.91 (s, 18H), 0.05 (s, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.7, 155.5, 155.3, 154.0, 153.7, 147.8, 147.5, 147.4, 143.0, 142.8, 135.2, 133.6, 133.4, 133.2, 133.0, 132.8, 132.5, 132.3, 129.6, 129.2, 128.7, 128.6, 128.4, 127.1, 127.0, 124.8, 64.0, 63.9, 60.4, 60.2, 60.1, 47.9, 47.7, 47.4, 47.0, 47.0, 46.9, 46.3, 35.8, 35.7, 35.5, 35.3, 35.2, 35.1, 34.7, 34.6, 34.4, 25.8, 21.2, 18.3, -5.4. FTIR: 2910, 1715, 1527, 1400, 1118 cm^{-1}. MS (*m/z*): calcd for $C_{34}H_{55}N_3O_8Si_2$, 690.3600; found, 690.3812 $[M]^+$.

Synthesis of Compound 7. Compound **6** (300 mg, 0.456 mmol, 1.0 equiv) and 1.53 g of Amberlyst-15 resin were combined with stirring in methanol (5 mL). The reaction was left to stir at room temperature for 1.5 hours. Amberlyst-15 was removed via filtration and the filtrate was concentrated to afford 249 mg of a brown oil. Yield = 83%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 – 8.03 (m, 1H), 7.65 – 7.41 (m, 3H), 7.23 –7.17 (m, 2H), 5.58 – 5.48 (m, 2H), 4.54 – 4.43 (m, 4H), 3.73 – 3.44 (m, 4H), 3.24 – 2.96 (m, 6H), 2.40 – 2.30 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.2, 156.0, 155.7, 147.7, 147.5, 145.1, 144.8, 136.2, 133.6, 133.5, 133.2, 132.8, 132.3, 130.5, 130.1, 129.9, 129.2, 129.0, 128.8, 128.5, 124.8, 64.3, 64.1, 60.6, 60.4, 58.2, 50.6, 47.7, 47.3, 47.1, 46.9, 46.7, 45.9, 35.9, 35.7, 35.2, 35.0, 34.9, 34.3, 20.8. FTIR: 3400, 2939, 1691, 1527, 1399 cm^{-1}. MS (*m/z*): calcd for $C_{22}H_{27}N_3O_8$, 462.1871; found, 462.2117 $[M]^+$.

Synthesis of Compound 8. Compound **7** (240 mg, 0.520 mmol, 1.0 equiv), 4-nitrophenyl chloroformate (630 mg, 3.13 mmol, 6.0 equiv) and dry pyridine (380 mg, 4.80 mmol, 9.2 equiv) were combined with stirring in dry CH_2Cl_2 (30 mL). The reaction was stirred at room temperature overnight. The solvent was removed, and the crude residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated NH_4Cl (3 x 50 mL) and Na_2CO_3 (3 x 50 mL), and dried over $MgSO_4$. The product was purified by column chromatography with a 50% hexane/ethyl acetate solvent system and then precipitated in pentane to afford 240 mg of a white solid. Yield = 59%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.29 – 8.22 (m, 4H), 8.09 – 8.00 (m, 1H), 7.63 – 7.29 (m, 9H), 5.54 – 5.48 (m, 2H), 5.27 – 5.15 (m, 4H), 3.73 – 3.46 (m, 4H), 3.24 – 2.92 (m, 6H), 2.38 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.5, 154.2, 154.0, 152.3, 146.0, 145.4, 136.4, 133.6, 132.0, 131.7, 129.0, 128.6, 127.9, 125.3, 124.9, 121.8, 66.0, 64.1, 64.0, 47.7, 47.5, 47.1, 45.7, 46.1, 35.9, 35.6, 35.3, 34.9, 34.4, 20.8. FTIR: 2946, 1761, 1520, 1347 cm^{-1}. MS (*m/z*): calcd for $C_{36}H_{33}N_5O_{16}$, 792.2001; found, 792.1949 $[M]^+$.

Synthesis of G1 SID. Compound **8** (200 mg, 0.253 mmol, 1.0 equiv), propargylamine (0.065 mL, 1.01 mmol, 4.0 equiv), DIPEA (0.18 mL, 1.01 mmol, 4.0 equiv), and DMAP (30.9 mg, 0.253 mmol, 1.0 equiv) were combined with stirring in dry toluene (30 mL). The reaction was stirred at room temperature overnight. The solvent was removed, and the crude residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated NH_4Cl (3 x 50 mL) and Na_2CO_3 (3 x 50 mL), and then dried over $MgSO_4$ to afford 150 mg of a yellow solid. Yield =75%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 – 8.03 (m, 1H), 7.70 – 7.30 (m, 3H), 7.24 – 7.14 (m, 2H), 5.54 (s, 2H), 5.14 – 4.92 (m, 4H), 4.00 – 3.46 (m, 8H), 3.26 – 2.95 (m, 6H), 2.38 – 2.31 (m, 3H), 2.21 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.7, 133.6, 131.3, 130.5, 129.3, 129.0, 128.8, 128.2, 125.3, 124.9, 79.7, 71.5, 64.2, 62.5, 46.9, 35.1, 30.7, 29.7, 20.8. FTIR: 3294, 2935, 2119, 1699, 1525, 1405 cm^{-1}. MS (*m/z*): calcd for $C_{30}H_{33}N_5O_{10}$, 622.2144; found, 622.1957 $[M]^-$.

Synthesis of Compound 9. Compound **5**{Fomina, 2012 #2347} (1.25 g, 2.22 mmol, 1.0 equiv), compound **1**[1] (0.63 g, 3.33 mmol, 1.5 equiv), DIPEA (0.86 g, 6.66 mmol, 3.0 equiv), and DMAP (0.27 g, 2.22 mmol, 1.0 equiv) were combined in dry toluene (60 mL). The reaction was stirred at room temperature overnight. The solvent was removed, and the crude residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated NH_4Cl (3 x 80 mL) and Na_2CO_3 (3 x 80 mL), and dried over $MgSO_4$. The product was purified via column chromatography with a 75% hexane/ethyl acetate solvent system to afford 1.07 g of a colourless oil. Yield = 79%. Spectral characterization data agreed with those previously reported.[4]

Synthesis of Compound 10. Compound **9** (1.05 g, 1.72 mmol, 1.0 equiv) and Amberlyst-15 were combined in methanol (20 mL) and the reaction was stirred at room temperature for 1.5 hours. Amberlyst-15 was removed via filtration and the filtrate was concentrated to afford 0.51 g of a brown oil. Yield = 78%. Spectral characterization data agreed with those previously reported.[4]

Synthesis of Compound 11. Compound **10** (800 mg, 1.73 mmol, 1.0 equiv), 4-nitrophenyl chloroformate (2.10 g, 10.42 mmol, 6.0 equiv), and dry pyridine (1.26 g, 16.0 mmol, 9.2 equiv) were combined in dry CH_2Cl_2 (70 mL). The reaction was stirred at room temperature overnight. The solvent was removed, and the crude residue was dissolved in CH_2Cl_2 (70 mL), washed with saturated NH_4Cl (3 x 70 mL) and Na_2CO_3 (3 x 70 mL), and dried over $MgSO_4$. The product was purified by column chromatography with a gradient from 85% hexane to 100% ethyl acetate as

the eluent yielding 629 mg of a white powder. Yield = 51%. Spectral characterization data agreed with those previously reported.[4]

Synthesis of Compound 12. Compound **11** (500 mg, 0.702 mmol, 1.0 equiv), propargylamine (0.18 mL, 7.81 mmol, 4.0 equiv), and Et_3N (1.09 mL, 7.81 mmol, 4.0 equiv) were combined in dry DMF (30 mL). The reaction was stirred at room temperature overnight. The crude product was dissolved in CH_2Cl_2 (50 mL), washed with saturated NH_4Cl (3 x 50 mL) and Na_2CO_3 (3 x 50 mL), and dried over $MgSO_4$. The product was purified by column chromatography with a 40% hexane/ethyl acetate solvent system to afford 256 mg of a yellow powder. Yield = 79%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.25 – 8.7.14 (m, 2H), 5.70 – 5.16 (m, 2H), 5.03 (s, 4H), 3.99 – 3.87 (m, 4H), 3.17 – 2.88 (m, 6H), 2.31 (s, 3H), 2.21 (s, 2H), 1.51 – 1.41 (m, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.8, 154.2, 154.0, 146.8, 135.7, 131.3, 130.8, 129.2, 79.9, 79.7, 71.4, 62.6, 62.4, 62.2, 47.7, 47.2, 45.8, 46.2, 35.6, 35.3, 35.2, 34.8, 30.7, 29.9, 28.4, 20.8. FTIR: 3298, 2956, 2119, 1701 cm^{-1}. MS (*m/z*): calcd for $C_{27}H_{36}N_4O_8$, 567.2425; found, 567.2403 $[M + Na]^+$.

Synthesis of Compound 13. Compound **12** (200 mg, 0.37 mmol, 1.0 equiv) was dissolved with stirring in dry CH_2Cl_2 (0.60 mL) at 0 °C. TFA (0.75 mL, 7.34 mmol, 20 equiv) was added dropwise and the mixture was stirred for 1 hour. The solution was concentrated to afford 148 mg of a yellow oil. Yield = 90%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.25 – 7.16 (m, 2H), 5.33 – 4.93 (m, 4H), 4.02 – 3.82 (m, 4H), 3.80 – 3.22 (m, 4H), 3.20 – 2.73 (m, 6H), 2.35 (s, 3H), 2.29 – 2.18 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.7 (q, J = 160.8), 156.9, 156.2, 155.1, 145.8, 136.7, 132.9, 132.2, 131.9, 129.1, 125.1, 122.5, 115.6 (q, J = 115.33 Hz), 79.2, 71.9, 71.6, 63.0, 62.8, 62.4, 45.0, 47.3, 46.2, 45.8, 35.9, 33.6, 33.4, 33.1, 31.2, 30.8, 20.7. FTIR: 3304, 2879, 2130, 1699 cm^{-1}. MS (*m/z*): calcd for $C_{22}H_{28}N_4O_6$, 445.2082; found, 445.2034 $[M]^+$.

Synthesis of G2 SID. Compound **13** (140 mg, 0.315 mmol, 6.0 equiv), compound **11** (41.6 mg, 0.05 mmol, 1.0 equiv), and Et_3N (0.56 mL, 4 mmol, 80 equiv) were combined in dry DMF (10 mL). The reaction was stirred at room temperature overnight. The crude product was dissolved in CH_2Cl_2 (50 mL), washed with saturated NH_4Cl (3 x 50 mL) and Na_2CO_3 (3 x 50 mL), and dried over $MgSO_4$. The product was purified via column chromatography with a gradient from 15% hexane:ethyl acetate to 80% ethyl acetate:CH_2Cl_2 as the eluent yielding 37 mg of a yellow powder. Yield = 54%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.11 – 7.99 (m, 1H), 7.66 – 7.42 (m, 3H),

7.24 – 7.10 (m, 6H), 5.62 – 5.45 (m, 4H), 5.10 – 4.93 (m, 10H), 3.96 – 3.79 (m, 8H), 3.67 – 3.38 (m, 12H), 3.17 – 2.89 (m, 18H), 2.36 – 2.14 (m, 13H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.1, 155.8, 155.4, 153.8, 147.4, 146.2, 145.5, 135.7, 133.6, 133.0, 132.4, 131.3, 130.4, 129.6, 129.3, 128.8, 128.5, 124.8, 79.8, 71.4, 64.0, 62.4, 62.1, 47.3, 46.9, 46.7, 46.0, 35.6, 35.3, 35.1, 34.8, 34.5, 31.3, 30.7, 20.8. FTIR: 3290, 2939, 2246, 1700, 1520, 1397 cm^{-1}. MS (*m/z*): calcd for $C_{68}H_{79}N_{11}O_{22}$, 1424.5293; found, 1424.5241 $[M + Na]^+$.

Synthesis of Compound 14. 4-arm-PEG (2.00 g, 1.00 mmol, 1.0 equiv), 4-toluenesulfonyl chloride (1.53 g, 8.00 mmol, 8.0 equiv), Et_3N (0.81 g, 8.00 mmol, 8.0 equiv), and DMAP (0.12 g, 1 mmol, 1.0 equiv) were combined in dry CH_2Cl_2 (10 mL). The reaction was stirred at room temperature for 3 days. 1M HCl (50 mL) was added and the reaction mixture was stirred at room temperature for 2 hours. The resulting solution was extracted with CH_2Cl_2 (3 x 50 mL), dried over $MgSO_4$, and then precipitated in diethyl ether to afford 1.31 g of an oil. Yield = 50%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74 (d, J = 7.7 Hz, 8H), 7.29 (d, J = 7.7 Hz, 8H), 4.10 (t, J = 4.1 Hz, 8H), 3.65 – 3.47 (m, 172H), 2.39 (s, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 144.3, 132.4, 129.4, 127.5, 70.5, 70.1, 69.8, 68.8, 68.2, 21.2 cm^{-1}. M_n = 2814 kg/mol, M_w = 2941 kg/mol, $Đ$ = 1.05.

Synthesis of 4-arm-PEG-azide. Compound **14** (0.50 g, 0.191 mmol, 1.0 equiv) and sodium azide (0.50 g, 7.64 mmol, 40 equiv) were combined with stirring in deionized water (10 mL) at room temperature. The reaction mixture was heated at reflux (100 °C) overnight. The resulting solution was extracted with CH_2Cl_2 (3 x 50 mL) and dried over $MgSO_4$. The crude product was purified by dialysis against deionized H_2O (500 mL), changing the solution 3 times over 24 hours, using a 3.5 kg/mol MWCO membrane. The resulting solution was freeze-dried to afford 0.25 g of an oil. Yield = 60%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.71 – 3.33 (m, 180H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 70.6, 70.2, 69.7, 60.3, 45.2. FTIR: 2864, 2103 cm^{-1}.

Synthesis of TEG-alkyne. TEG (500 mg, 2.57 mmol, 1.0 equiv), 4-pentynoic acid (610 mg, 6.22 mmol, 2.4 equiv), and DMAP (83.0 mg, 0.679 mmol, 0.26 equiv) were combined in dry CH_2Cl_2 (5 mL) at -20 °C. A solution of EDC hydrochloride (960 mg, 6.18 mmol, 2.4 equiv) in CH_2Cl_2 (2 mL) was added dropwise and the mixture was stirred at room temperature for 20 hours. The resulting solution was washed with 1 M NaOH (2 x 50 mL), deionized H_2O (2 x 50 mL) and NH_4Cl (2 x 50 mL), and dried over $MgSO_4$ to afford 746 mg of an oil. Yield = 82%. ^{1}H NMR

($CDCl_3$, 400 MHz): δ 4.26 (t, J = 4.3 Hz, 4H), 3.73 – 3.68 (t, J = 3.7 Hz, 4H), 3.68 – 3.64 (m, 8 H), 2.61 – 2.55 (m, 4H), 2.54 – 2.48 (m, 4H), 1.98 (t, J = 2.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 82.2, 72.3, 70.3, 68.9, 68.8, 63.4, 33.0, 14.0 cm^{-1}. FTIR: 3272, 2885, 2123, 1731 cm^{-1}.

Degradation of SIDs. **G1 SID** (10 mg) and **G2 SID** (11 mg) were dissolved in a 1.25:1 or 3.5:1 solution of DMSO-d_6:deuterated PBS, respectively. The SIDs were then exposed to 0.5 hr of UV irradiation (wavelength: 365-370 nm, intensity: 10 mW cm^{-2}) and then incubated at 37 °C. SID degradation was monitored by ^1H NMR spectroscopy using the DMSO signal as the internal standard. SID degradation was quantified by integration of the singlet at ~ 2.6 ppm relative to DMSO-d_6.

Synthesis of the Hydrogels. Hydrogels were prepared using 10, 15, and 25% (w/v) of **4-arm-PEG-azide** in 4:1 DMF:H_2O, with the specific formulations provided in Table S1. The amount of **TEG-alkyne**, **G1 SID**, and **G2 SID** were determined based on maintaining a 1:1 molar ratio of azide:alkyne functional group was used in each case. First, the alkyne and azide were dissolved in DMF (240 μL). Sodium ascorbate and $CuSO_4$ were each dissolved separately in 30 μL of H_2O and vortexed. The sodium ascorbate solution was then added to the DMF solution and mixed by vortexing, followed by the $CuSO_4$ solution. The reaction mixture was bubbled with a stream of N_2 gas and vented for one hour and then left under nitrogen overnight. The resulting gels were immersed in a 0.1 M EDTA solution (5 mL), changing the solution 3 times over 24 hours to remove copper. The gels were then immersed in deionized H_2O (5 mL), changing the solution 3 times over 24 hours. The gels were then lyophilized. To examine the effect of pH, the same procedure as detailed above was performed except that the H_2O was replaced with either 10 mM pH 7.4 or 5 phosphate solution.

Table S1. Formulations of the **TEG-alkyne**, **G1 SID**, and **G2 SID** hydrogels in a DMF:H_2O (240:60 μL).

TEG-alkyne hydrogels				
	4-arm-PEG-azide	**TEG-alkyne**	**$CuSO_4$**	**sodium ascorbate**
mass (mg)				
10% (w/v)	30	8.9	1.8	2.3
15% (w/v)	45	13	2.8	3.4
25% (w/v)	75	22	4.6	5.7
G1 SID hydrogels				
	4-arm-PEG-azide	**G1 SID**	**$CuSO_4$**	**sodium ascorbate**
mass (mg)				
10% (w/v)	30	14	1.8	2.3
15% (w/v)	45	22	2.8	3.4
25% (w/v)	75	36	4.6	5.7
G2 SID hydrogels				
	4-arm-PEG-azide	**G2 SID**	**$CuSO_4$**	**sodium ascorbate**
mass (mg)				
10% (w/v)	30	16	1.8	2.3
15% (w/v)	45	24	2.8	3.4
25% (w/v)	75	40	4.6	5.7

Analysis for trace copper. **G1 SID** (50 mg) and **G2 SID** (45 mg) SID hydrogels (15% w/v) were prepared as described above, lyophilized, and analyzed for the presence of copper by inductively coupled plasma – mass spectrometry (ICP-MS) (Agilent 7700 Series) with helium gas as the plasma. The method detection and reporting limit was 1.26 and 3.78 μg/g (ppm), respectively.

Measurement of Gel Content and Equilibrium Water Content (EWC). Gel content and equilibrium water content were measured in triplicate. After gelation as described above, the initial mass (m_i) of each hydrogel was recorded and the theoretical mass (m_t) of polymers involved in crosslinking was calculated as m_i x the m/v % in the formulation. The hydrogels were then immersed in 0.1M EDTA, followed by water as described above. After the third deionized H_2O treatment, the swollen mass (m_s) was recorded to determine the EWC. The hydrogels were

then lyophilized and their dry masses (m_d) were measured. The gel content and EWC were calculated using equations (1) and (2), respectively.

$$\text{Gel content} = \frac{m_d}{m_t}\ x\ 100\% \quad (1)$$

$$\text{EWC} = \frac{m_s - m_d}{m_s}\ x\ 100\% \quad (2)$$

Table S2. Properties of control and SID hydrogels prepared using either pH 5 or pH 7.4 phosphate solution (10 mM) in place of H_2O for the hydrogel formation. The **4-arm PEG-azide** concentration was 15% (m/v) in each case, with the ratios of other reagents the same as in Table S1.

Hydrogel	**Gel content (%)**	**EWC (%)**
G1 SID (pH 7.4)	93 ± 17	90 ± 2
G1 SID (pH 5)	100 ± 2	87 ± 2
G2 SID (pH 7.4)	100 ± 6	89 ± 2
G2 SID (pH 5)	97 ± 11	92 ± 0.4

Scanning electron microscopy. **G1 SID** and **G2 SID** hydrogels were prepared as described above, but instead of lyophilizing the gels from deionized H_2O, they were gradually transitioned to 100% ethanol in 20% increments over a period of 3 days. The ethanol was then removed using a Samdri PVT-3B critical point dryer. Samples were mounted on stubs covered in carbon tape and coated with osmium using a SPI Supplies, OC-60A plasma coater. SEM was performed using a Zeiss LEO 1530 instrument, operating at 2.0 kV and a working distance of 7 mm.

Measurement of Compressive Moduli under Unconfined Compression. Cylindrical samples with diameters of ~4 mm and heights of ~6 mm for the SID gels (n=3) were prepared in 1 mL syringes as described above and equilibrated in PBS (15% w/v). Gels (n = 3) were irradiated with UV light (wavelength: 365-370 nm, intensity: 10 mW/cm^2) for four hours for four days and incubated at 37 ℃. The gels were then incubated for an additional two days. Non-irradiated gels (n=3) were also incubated for a total of six days at 37 ℃. Before compression, the dimensions of the swollen hydrogels were accurately measured using calipers. The compressive moduli were then determined using a UniVert system (CellScale, Waterloo, ON, Canada) equipped with a 0.5

N load cell. During the measurement, the samples were immersed in a 37 ℃ PBS bath, preloaded at 0.1 N and compressed to a total strain of 20% at a rate of 0.6% s^{-1}. The compression moduli were calculated from the slope of the linear region of the stress-strain curve between 10 and 15% strain.

Degradation of SID Hydrogels. First-generation (11.3 mg) and second-generation (12.1 mg) SID hydrogels (15% w/v) were immersed in a 1.00 mL of deuterated PBS and 10.0 μL of acetonitrile was added as an internal standard. The immersed gels were then exposed to 0.5 h of UV irradiation (wavelength: 365-370 nm, intensity: 10 mW cm^{-2}) at t = 0. The irradiation was repeated again after 7 days for the **G1 SID** hydrogel and after 1 day and 45 days for the **G2 SID** hydrogel. The samples were incubated at 37 ℃. Gel degradation was monitored by ^{1}H NMR spectroscopy with degradation quantified based on the percentage of solubilized PEG, based on integration of the peak at 3.75 – 3.65 ppm corresponding to the protons on PEG, compared to the integration of the acetonitrile internal standard peak at 2.09 ppm.

In vitro cytotoxicity studies. C2C12 mouse myoblast cells were cultured in medium consisting of 500 mL of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 mL of penicillin− streptomycin (1000 units/mL), 5 mL of L-glutamine (200 mM), and 50 mL of fetal bovine serum, at 37 °C, in an incubator with 5% CO_2. They were then seeded in a Nunclon 96-well U bottom transparent polystyrol plate to obtain approximately 10000 cells/well in 100 μL of culture medium. The cells were then incubated for 24 h prior to performing the assay. For the leaching assays, samples of dry hydrogel were immersed in culture media (50 mg dry hydrogel per 3 mL of media and incubated overnight to enable the leaching of potentially toxic species from the hydrogels over a period of 24 h. The growth medium was then aspirated from the cells and replaced with either solutions of sodium dodecyl sulfate (SDS) in the cell culture medium at concentrations of 0.2, 0.15, 0.10, or 0.05 mg/mL, which were used as positive controls; serial 2-fold dilutions of the leachate in culture medium; serial 2-fold dilutions of the polymers in culture medium; or fresh medium as a negative control (6 wells per concentration). The cells were then incubated at 37 °C (5% CO_2) for 24 h. The medium was again aspirated and replaced with 110 μL of fresh medium containing 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT). After 4 h of incubation (37 °C, 5% CO_2), the medium was carefully aspirated, and the purple crystals were dissolved by the addition of 50 μL of

spectroscopic grade dimethyl sulfoxide (DMSO). After shaking (1 s, 2 mm amp, 654 rpm), the absorbance of the wells at 540 nm was read using a Tecan M1000-Pro plate reader. The absorbance of wells prepared in the same way, but without cells, was subtracted as a background, and the cell viability was calculated relative to wells containing cells that were exposed only to the culture medium. No (0%) cell viability was detected for cells exposed to the highest concentrations of SDS, confirming the sensitivity of the assay.

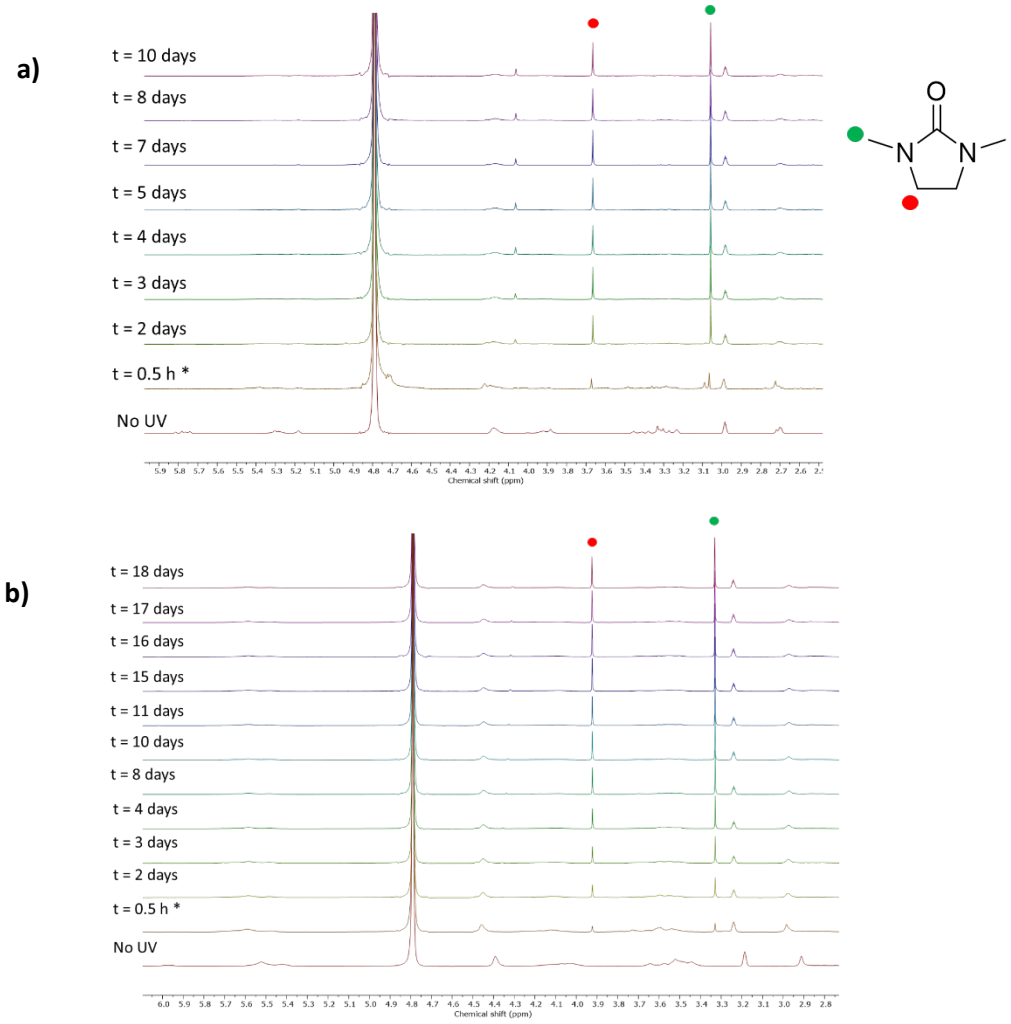


Figure S1. ^{1}H NMR spectra acquired at different time points during the degradation of (a) **G1 SID** and (b) **G2 SID** in PBS-d:DMSO-d_6 (1:1.25 for **G1 SID** and 1:3.5 for **G2 SID**) (400 MHz). *denotes an irradiation exposure of 0.5 h. Red and green markers correspond to methylene and methyl protons, respectively, in the 1,3-dimethyl-2-imidazolidinone degradation product.

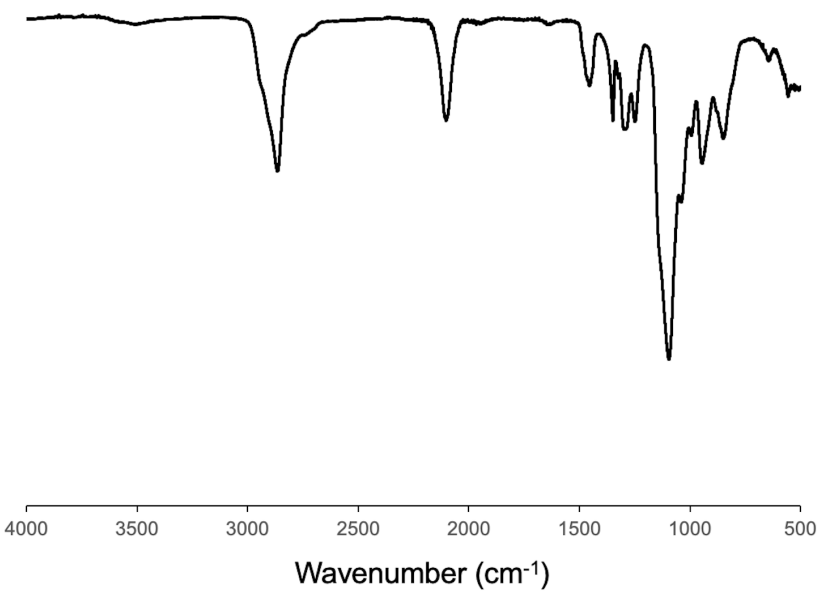


Figure S2. FTIR spectrum of the **4-arm-PEG-azide**.

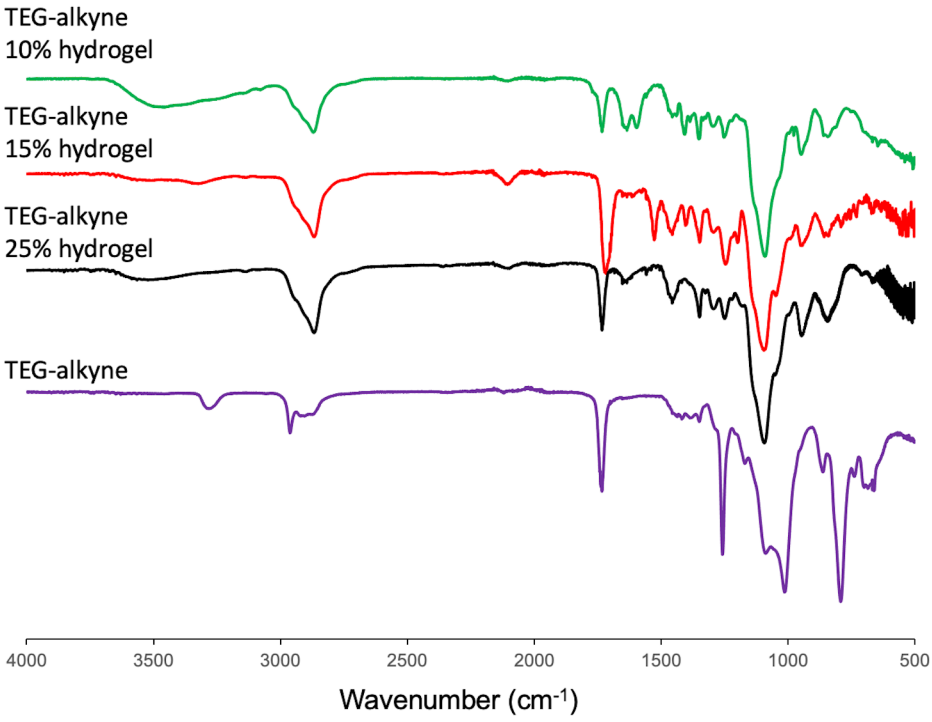


Figure S3. FTIR spectra of the **TEG-alkyne** hydrogels.

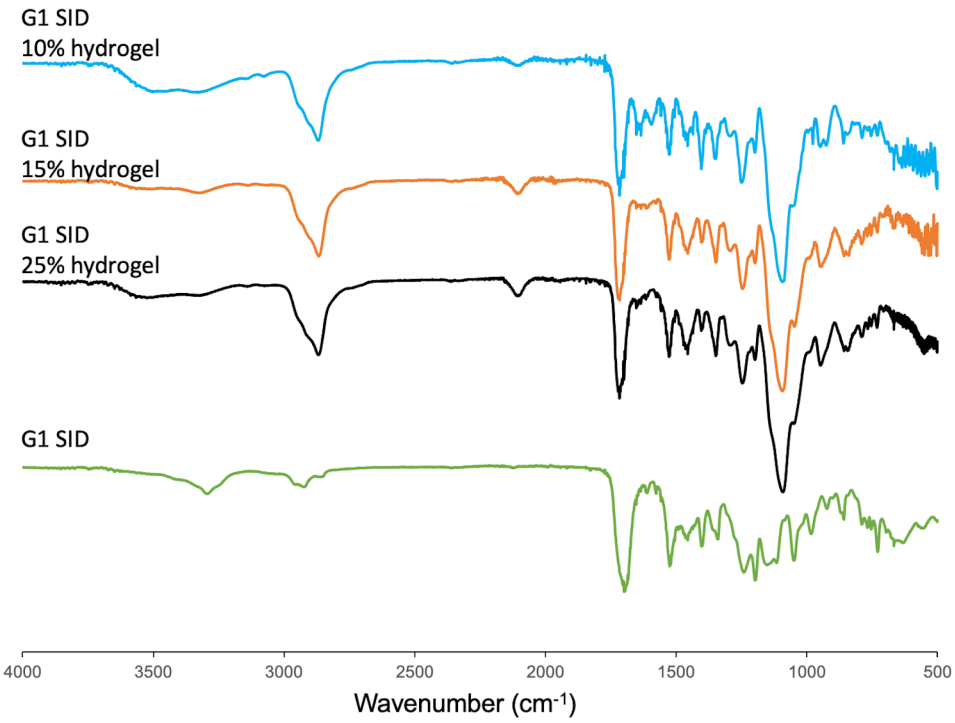


Figure S4. FTIR spectra of the **G1 SID** hydrogels.

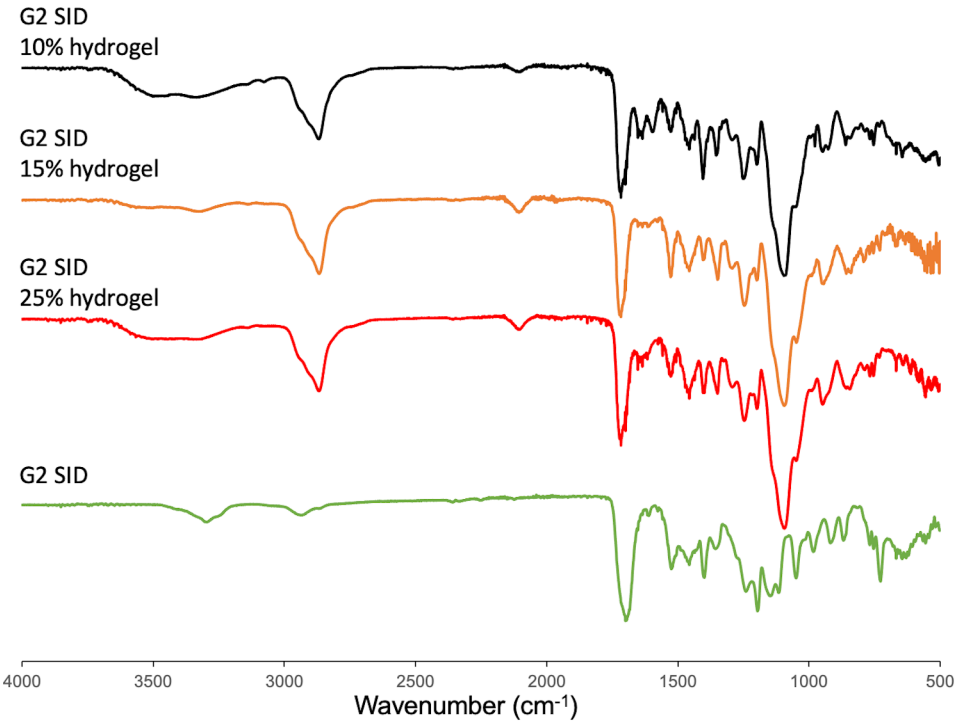


Figure S5. FTIR spectra of the **G2 SID** hydrogels.

Figure S6. Representative images of **G2 SID** hydrogels prepared from a) 10, b) 15, and c) 25% (w/v) of polymer showing the poor properties of the 10% formulation.

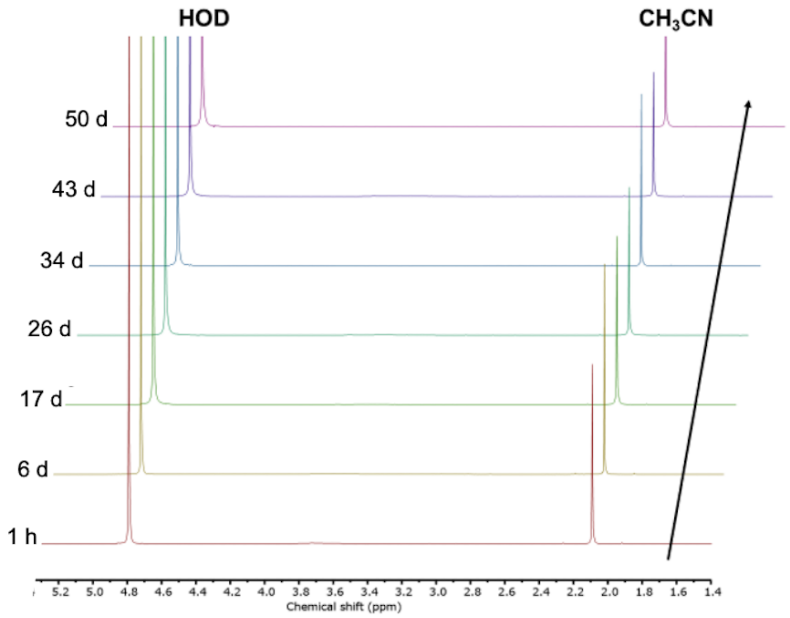


Figure S7. ^{1}H NMR spectra of non-irradiated **G1 SID** hydrogels in PBS-d:CH_3CN (100:1) (400 MHz). No degradation was observed.

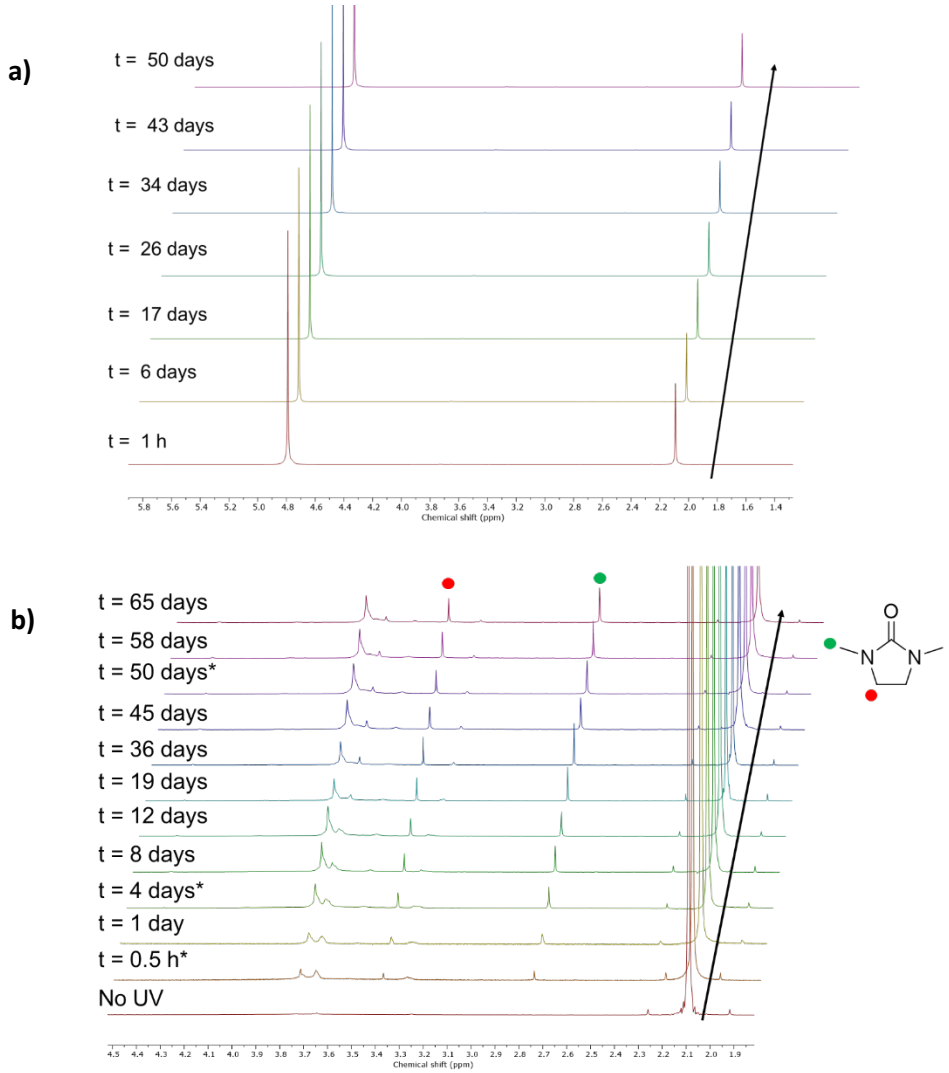


Figure S8. ^{1}H NMR spectra of (a) non-irradiated, (b) irradiated **G2 SID** hydrogels in PBS-d:CH_3CN (100:1) (400 MHz). *denotes an irradiation exposure of 0.5 h followed by 1 h incubation at 37 °C. Red and green markers correspond to methylene and methyl protons, respectively, in the 1,3-dimethyl-2-imidazolidinone degradation product.

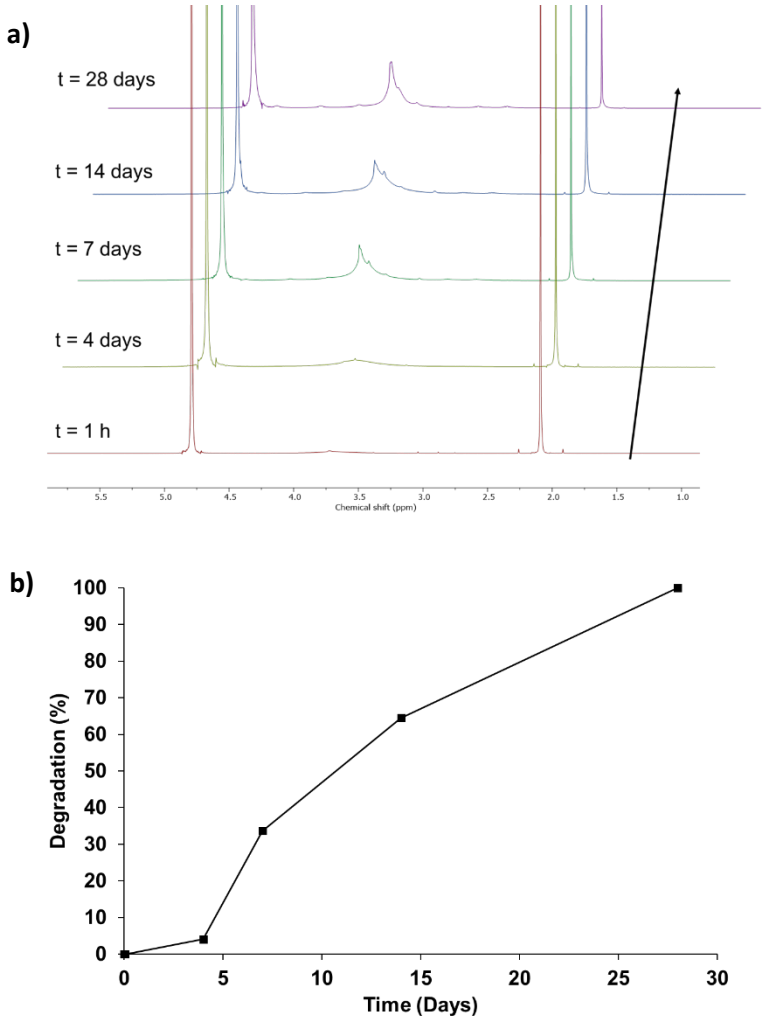


Figure S9. (a) ^{1}H NMR (400 MHz), (b) degradation profile of non-irradiated TEG-alkyne in deuterated PBS: acetonitrile (100:1) incubated at 37 ℃.

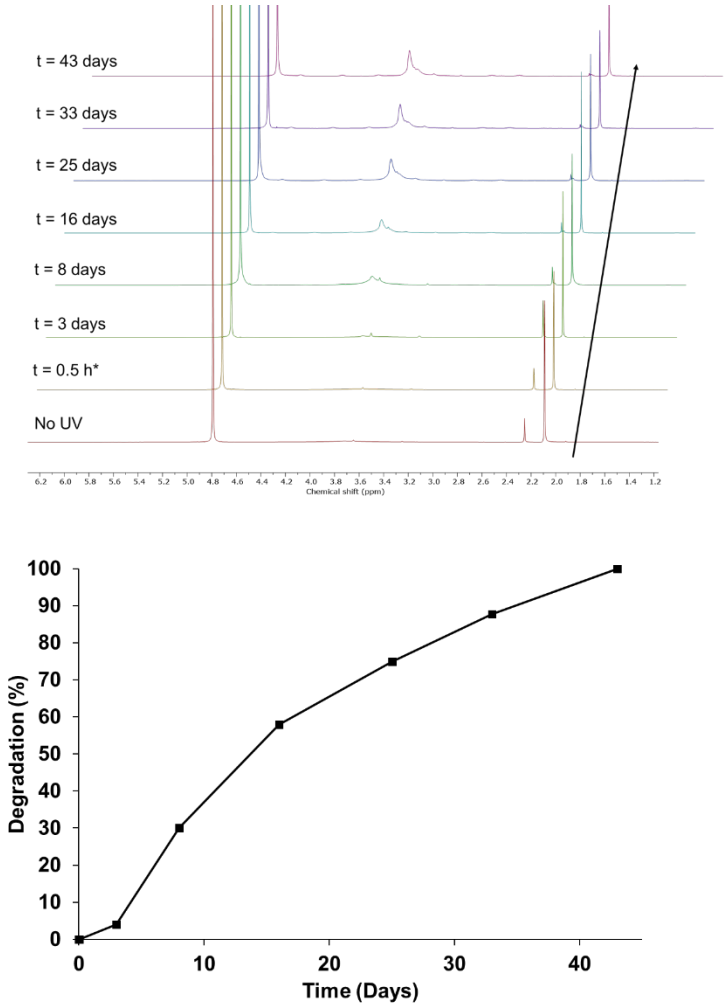


Figure S10. (a) ^{1}H NMR (D_2O, 400 MHz), (b) degradation profile of UV-light irradiated TEG-alkyne in deuterated PBS: acetonitrile (100:1) incubated at 37℃. *denotes to 0.5 h of UV-light exposure.

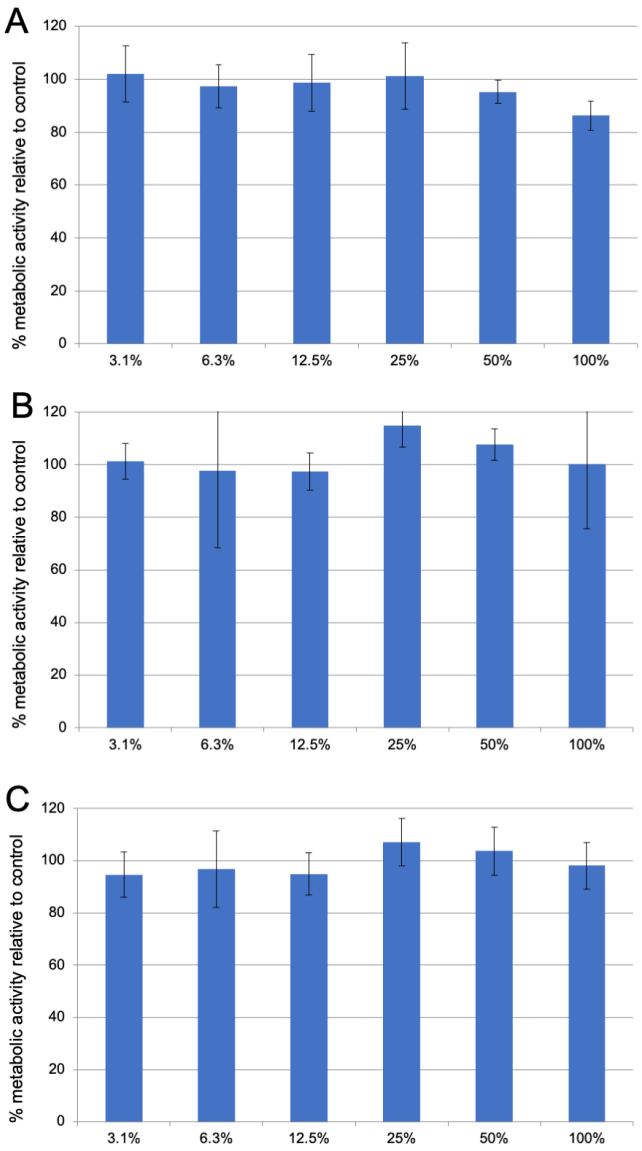


Figure S11. Metabolic activities measured by MTT assays on C2C12 cells incubated in varying dilutions of culture media that was exposed to the hydrogels (100% corresponds to no dilution of the leachate-containing media, whereas 50% corresponds to 2x dilution and so on): A) **TEG-alkyne**; B) **G1 SID**; C) **G2 SID**. 100% metabolic activity corresponds to control cells incubated in media that was not exposed to hydrogels. Error bars correspond to standard deviations of 6 measurements.

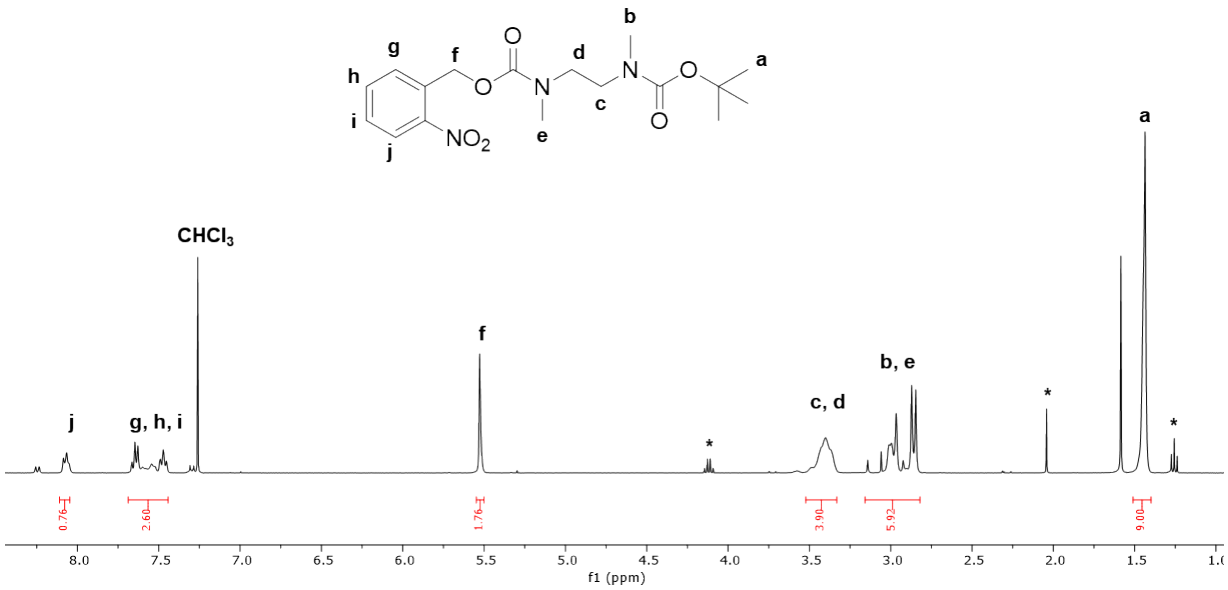


Figure S12. ^{1}H NMR spectrum of compound **3** (400 MHz, $CDCl_3$). Asterisks correspond to ethyl acetate. Rotational isomers about the carbamate bond are observed.

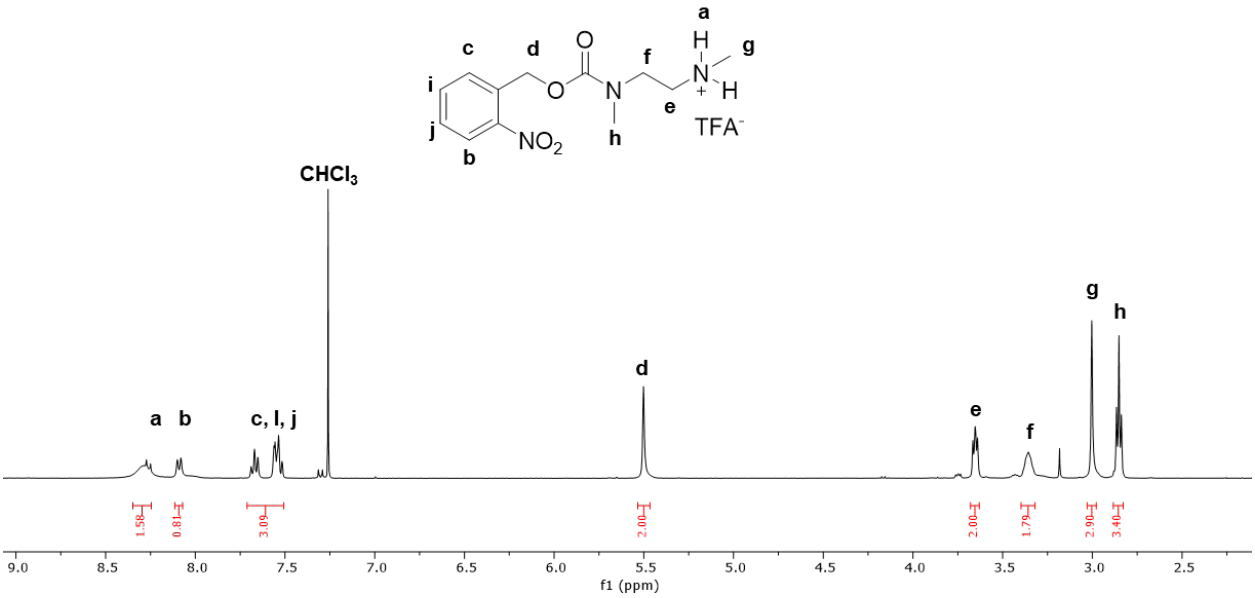


Figure S13. ^{1}H NMR spectrum of compound **4** (400 MHz, $CDCl_3$). Rotational isomers about the carbamate bond are observed.

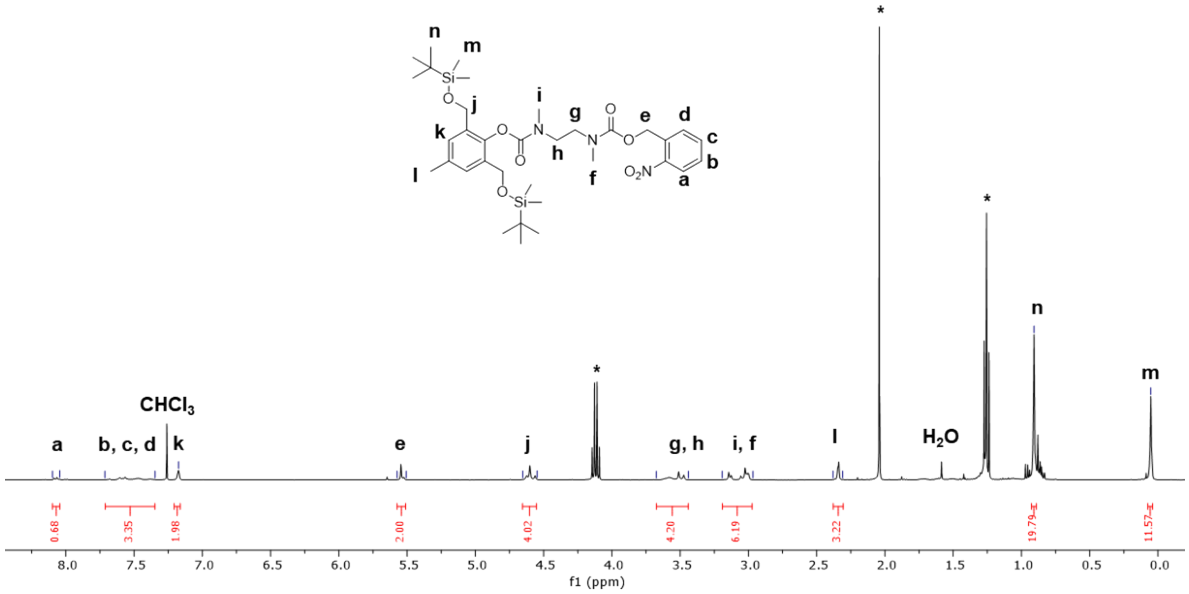


Figure S14. ^{1}H NMR spectrum of compound **6** (400 MHz, $CDCl_3$)**.** Asterisks correspond to ethyl acetate. Rotational isomers about the carbamate bonds are observed.

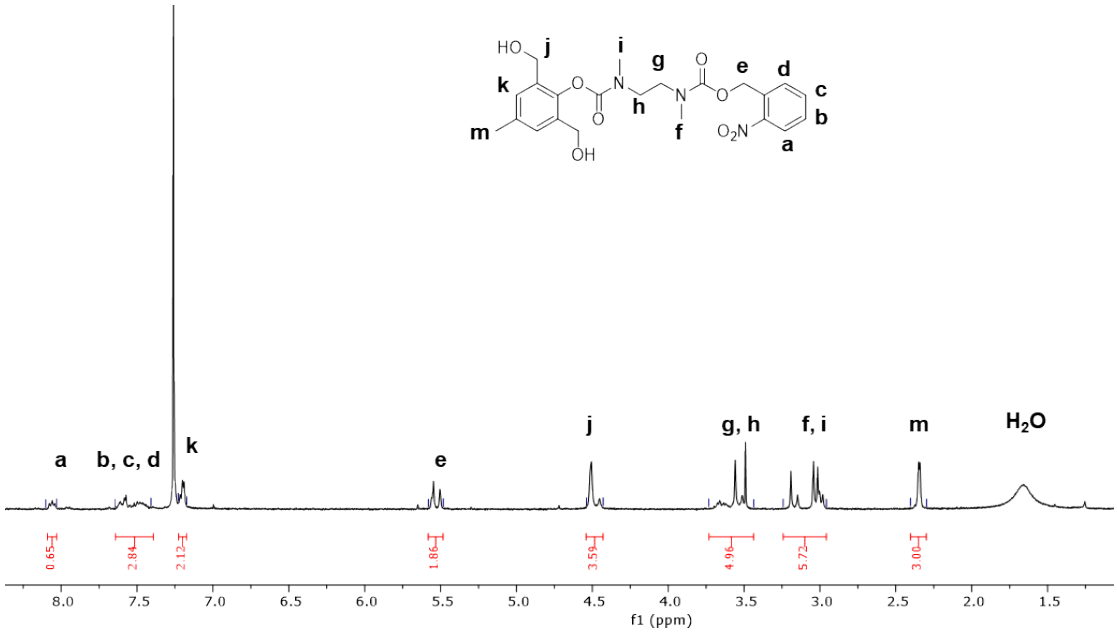


Figure S15. ^{1}H NMR spectrum of compound **7** (400 MHz, $CDCl_3$). Rotational isomers about the carbamate bonds are observed.

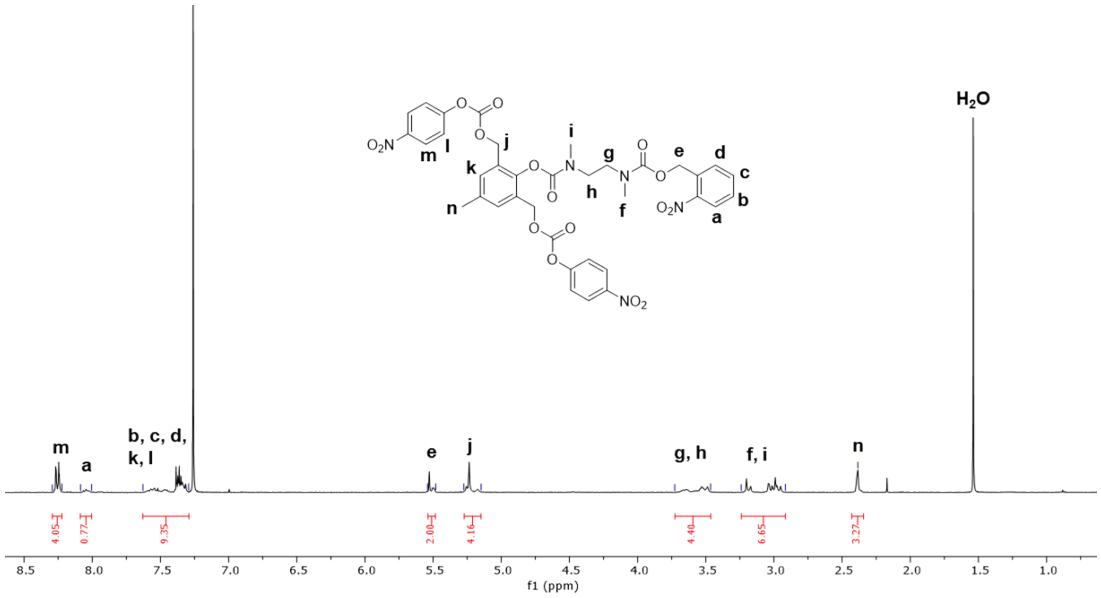


Figure S16. ^{1}H NMR spectrum of compound **8** (400 MHz, $CDCl_3$). Rotational isomers about the carbamate bonds are observed.

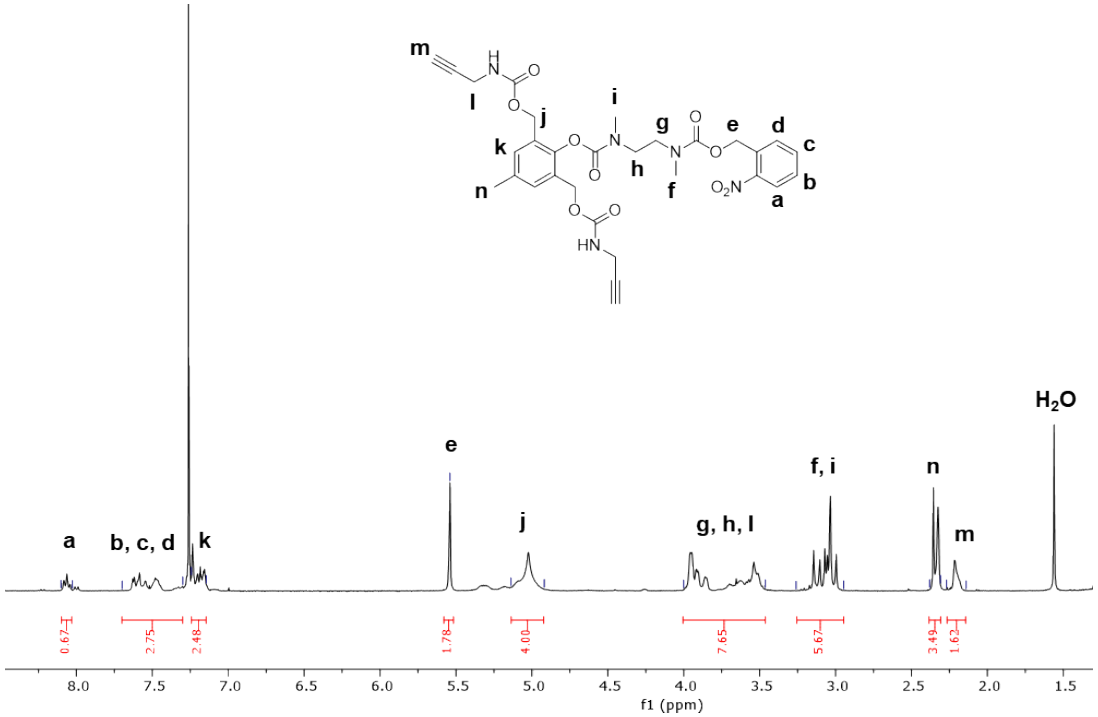


Figure S17. ^{1}H NMR spectrum of G1 SID (400 MHz, $CDCl_3$). Rotational isomers about the carbamate bonds are observed.

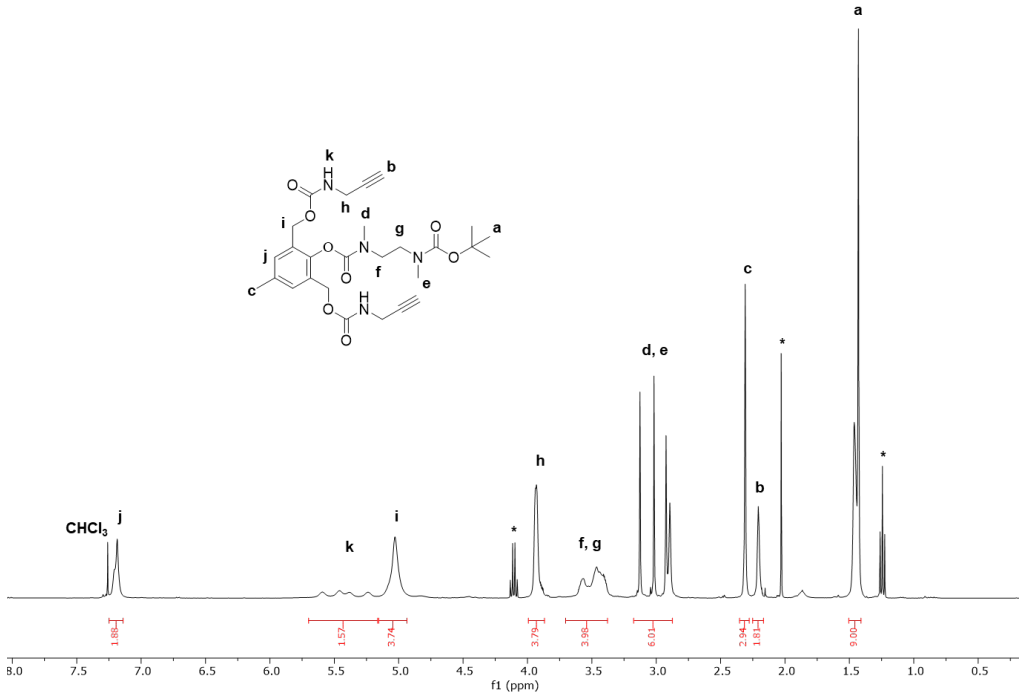


Figure S18. ^{1}H NMR spectrum of compound **12** (400 MHz, $CDCl_3$). Asterisks correspond to ethyl acetate. Rotational isomers about the carbamate bonds are observed.

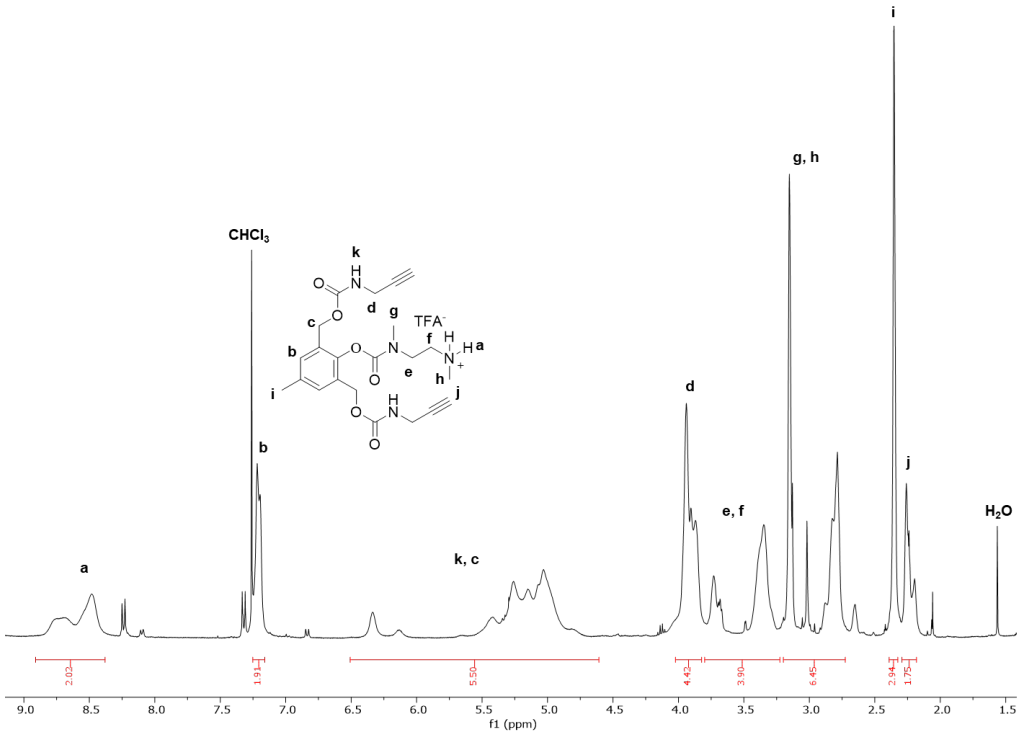


Figure S19. ^{1}H NMR spectrum of compound **13** (400 MHz, $CDCl_3$). Rotational isomers about the carbamate bonds are observed.

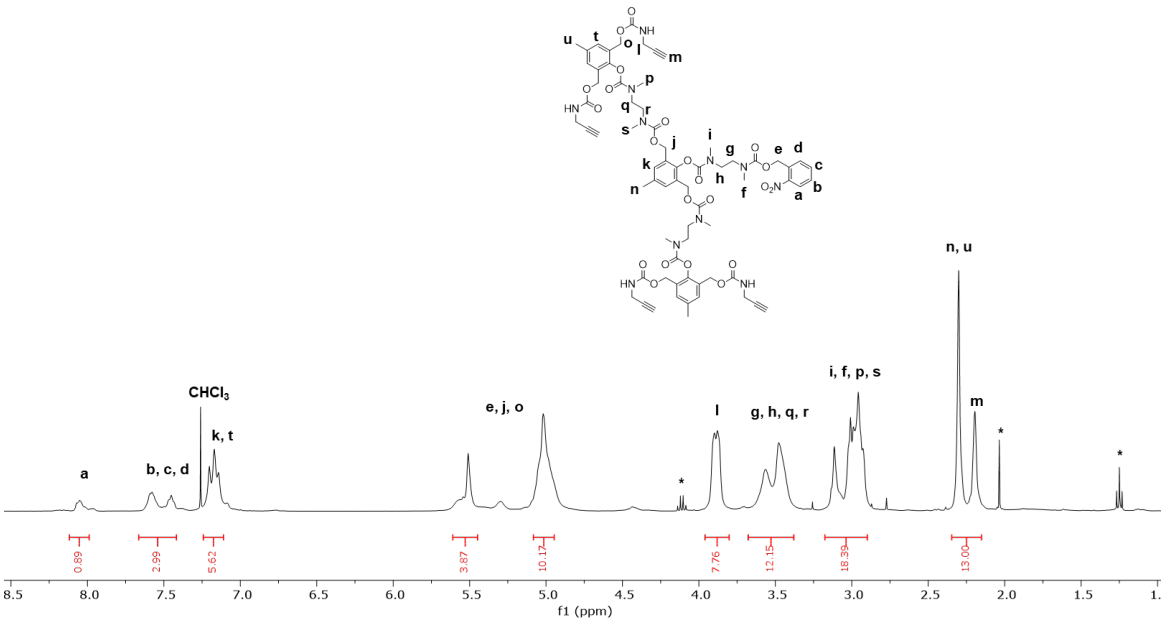


Figure S20. ^{1}H NMR spectrum of G2 SID (400 MHz, $CDCl_3$). Asterisks correspond to ethyl acetate. Rotational isomers about the carbamate bonds are observed.

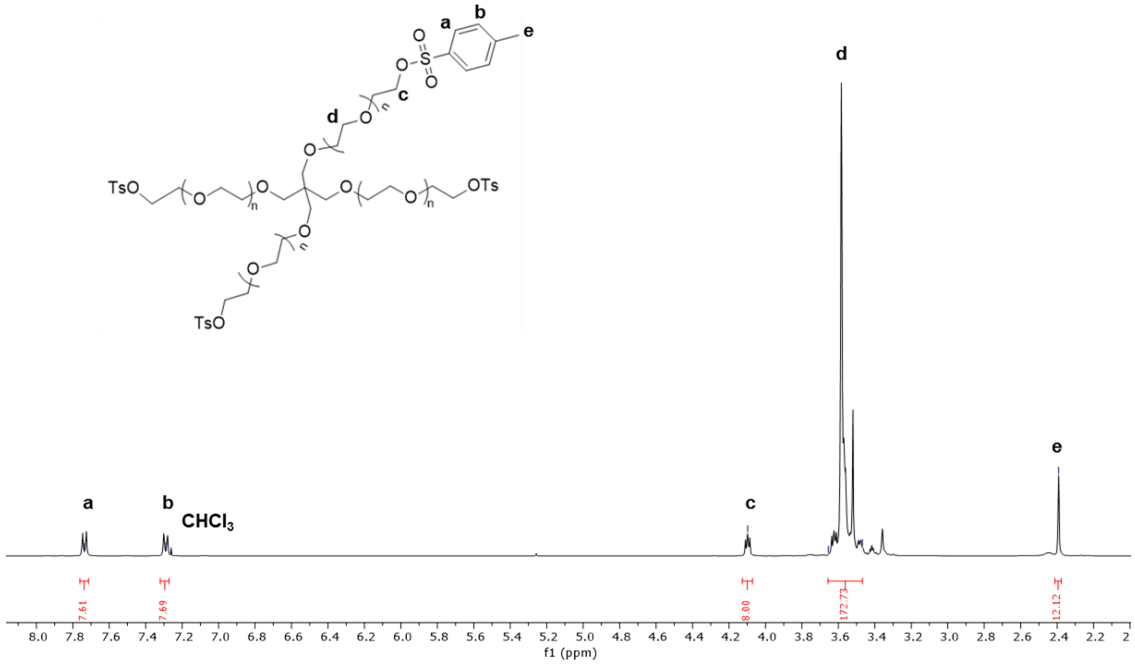


Figure S21. ^{1}H NMR spectrum of compound **14** (400 MHz, $CDCl_3$).

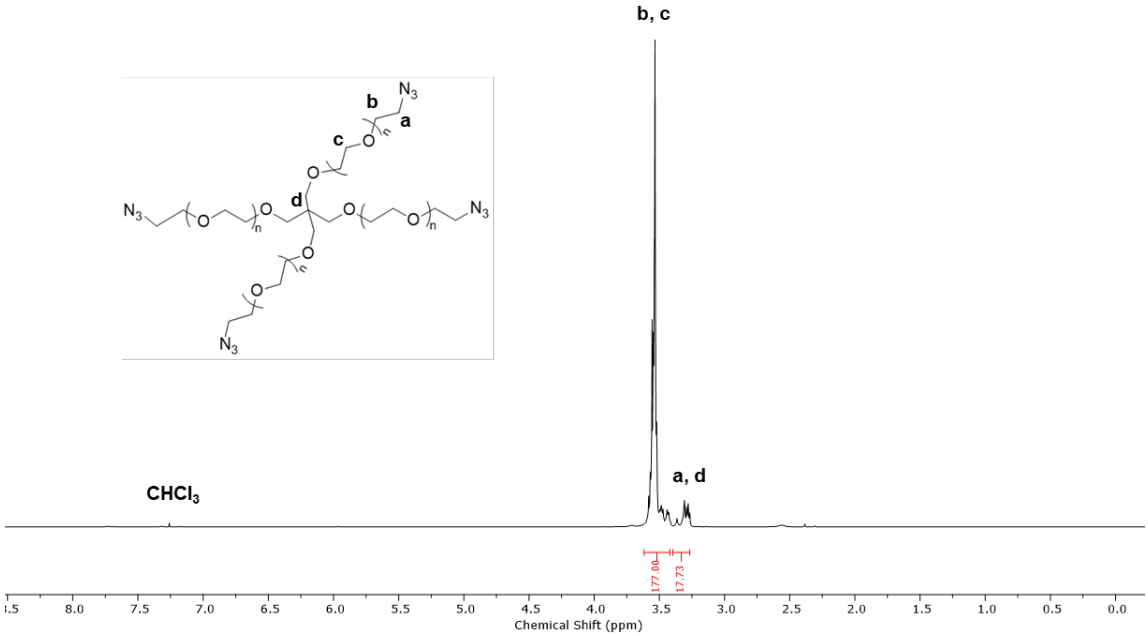


Figure S22. ^{1}H NMR spectrum of 4-arm-PEG-azide (400 MHz, $CDCl_3$).

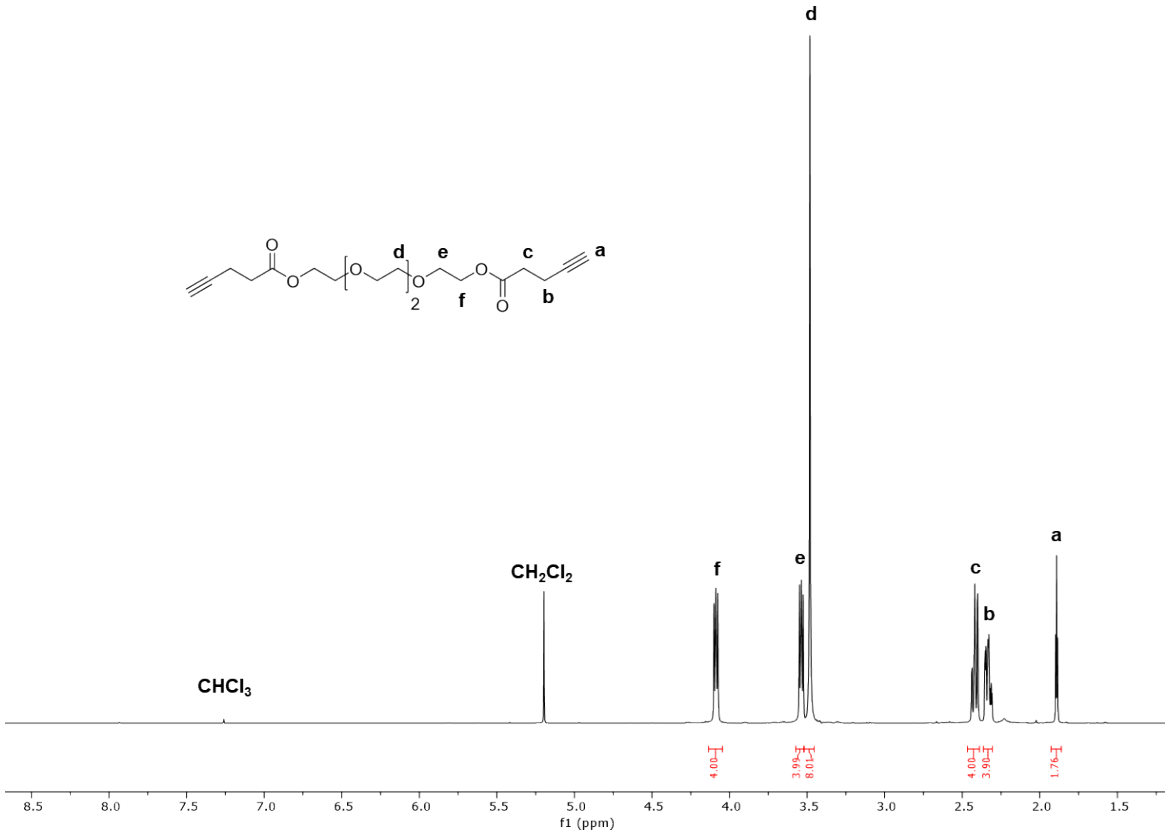


Figure S23. ^{1}H NMR spectrum of TEG-alkyne (400 MHz, $CDCl_3$)

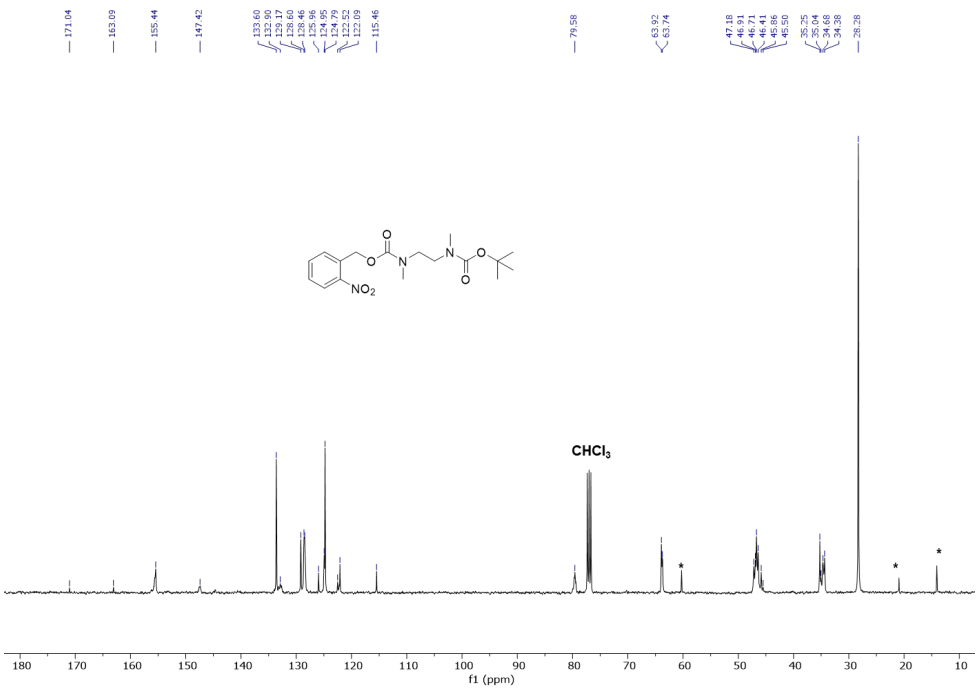


Figure S24. ^{13}C NMR spectrum of compound **3** (100 MHz, $CDCl_3$). Asterisks correspond to ethyl acetate. Rotational isomers about the carbamate bonds are observed.

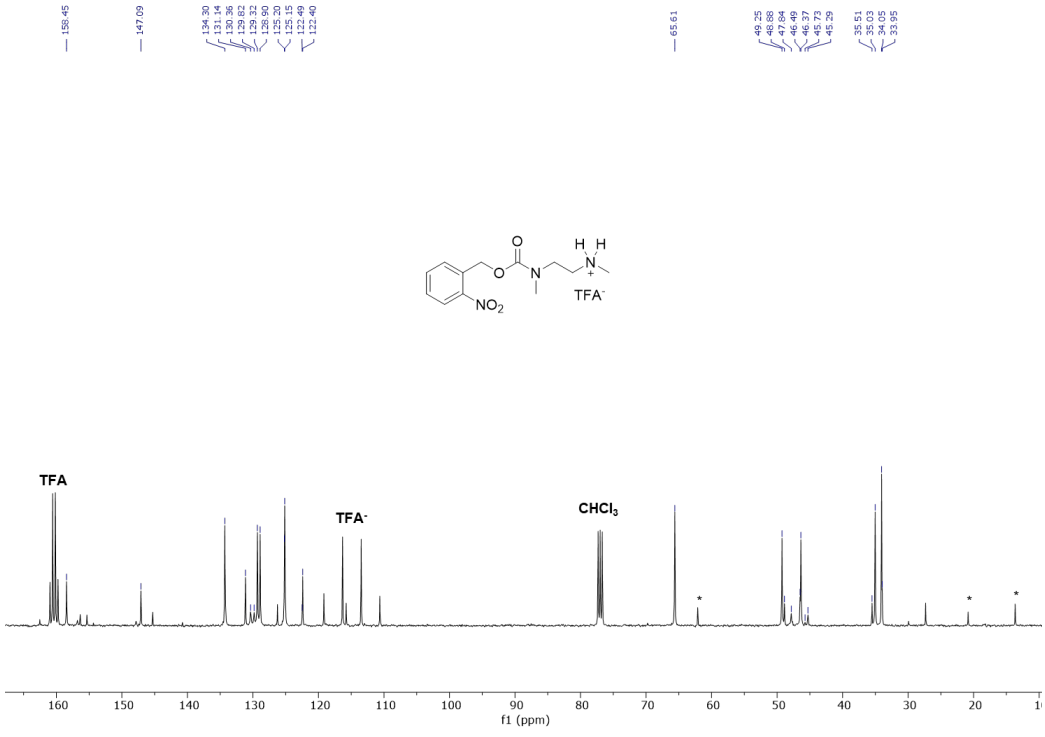


Figure S25. ^{13}C NMR spectrum of compound **4** (100 MHz, $CDCl_3$). Asterisks correspond to ethyl acetate. Rotational isomers about the carbamate bonds are observed.

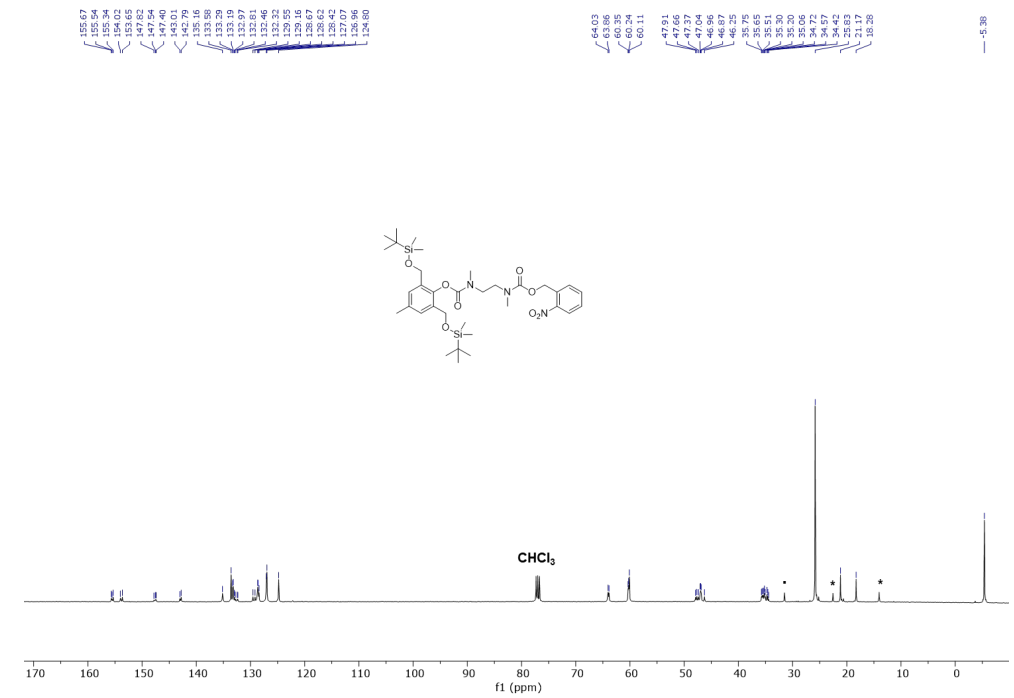


Figure S26. ^{13}C NMR spectrum of compound **6** (100 MHz, $CDCl_3$). Asterisks correspond to ethyl acetate. Rotational isomers about the carbamate bonds are observed.

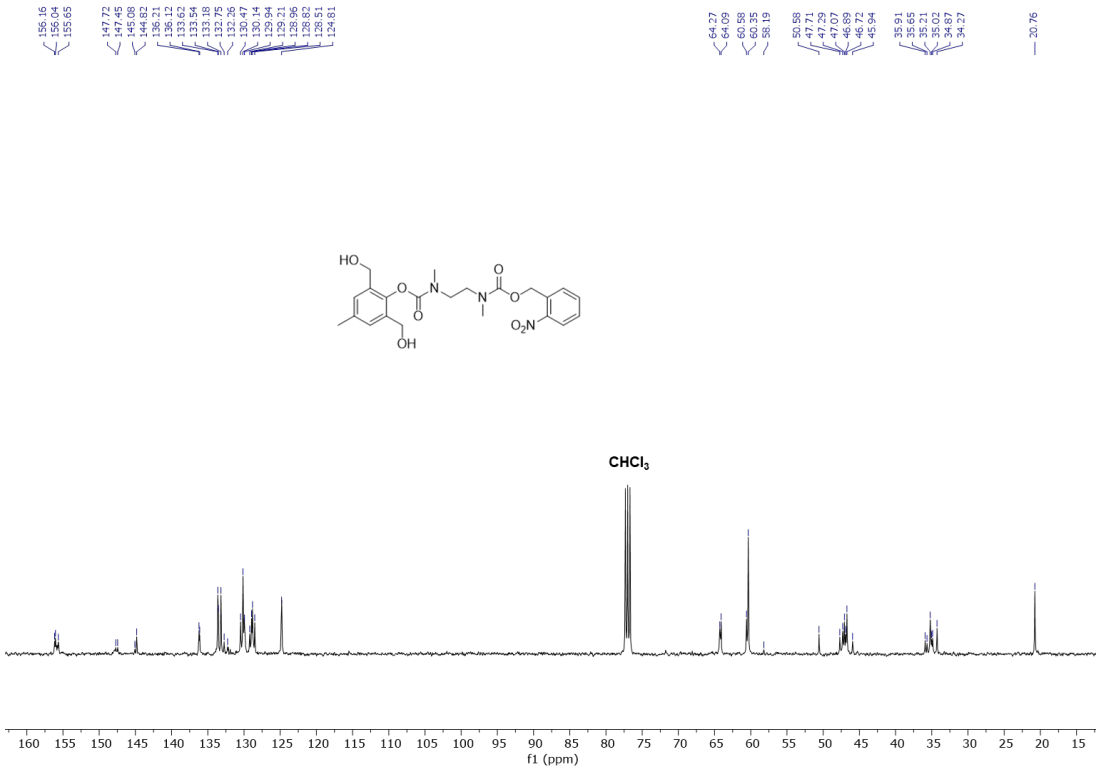


Figure S27. ^{13}C NMR spectrum of compound **7** (100 MHz, $CDCl_3$). Rotational isomers about the carbamate bonds are observed.

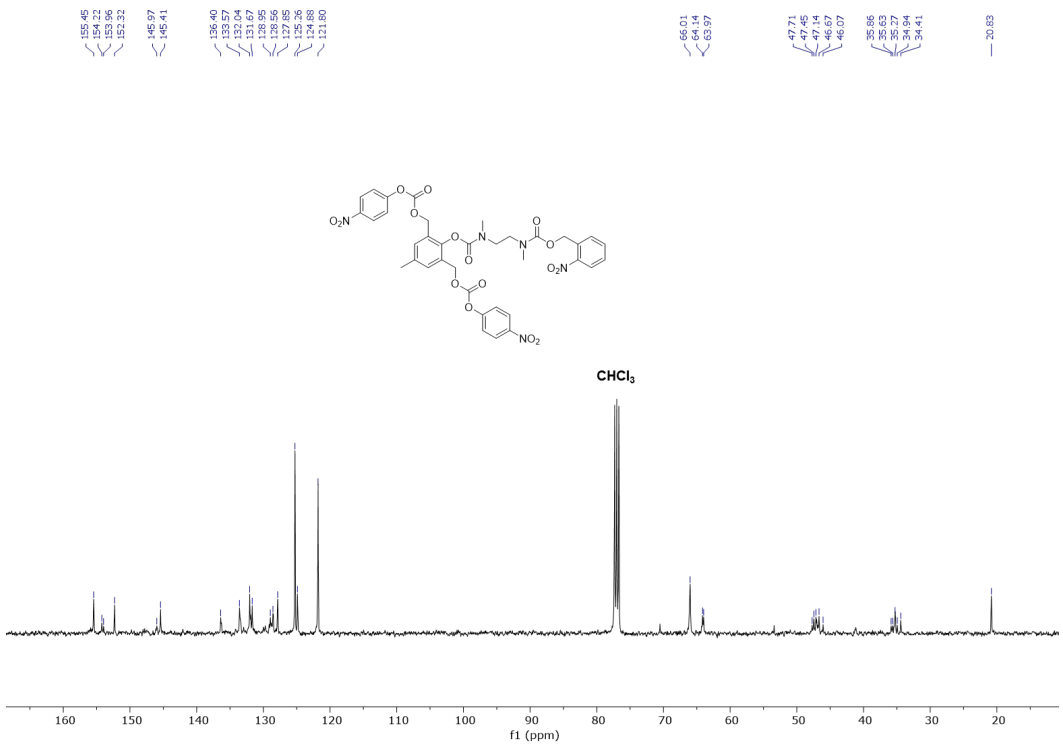


Figure S28. ^{13}C NMR spectrum of compound **8** (100 MHz, $CDCl_3$). Rotational isomers about the carbamate bonds are observed.

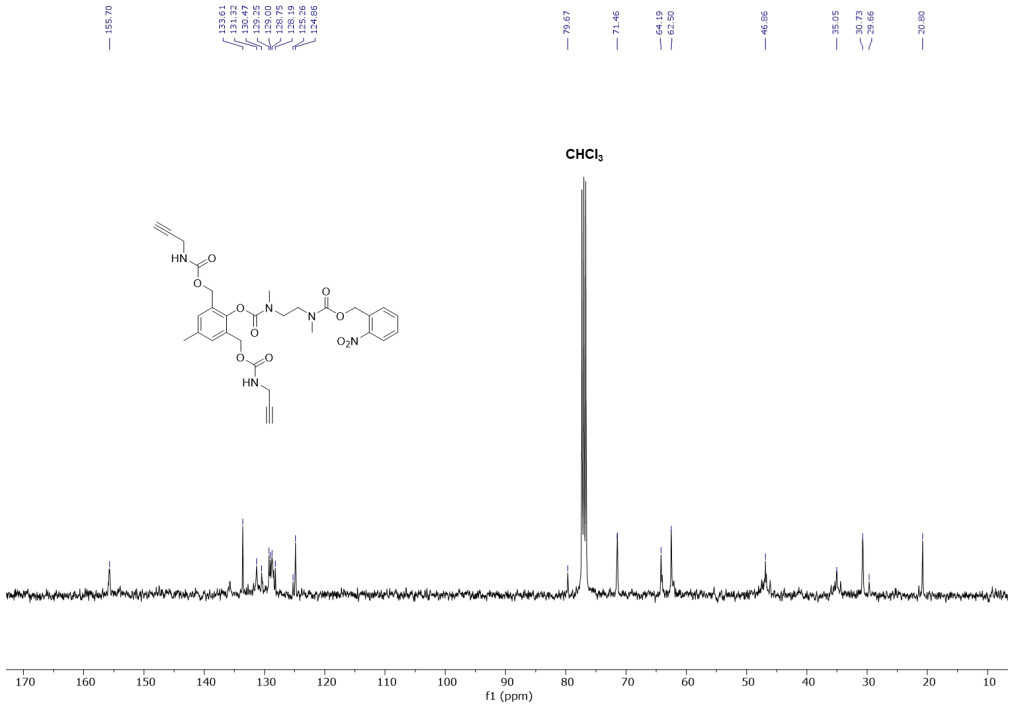


Figure S29. ^{13}C NMR spectrum of G1 SID (100 MHz, $CDCl_3$). Rotational isomers about the carbamate bonds are observed.

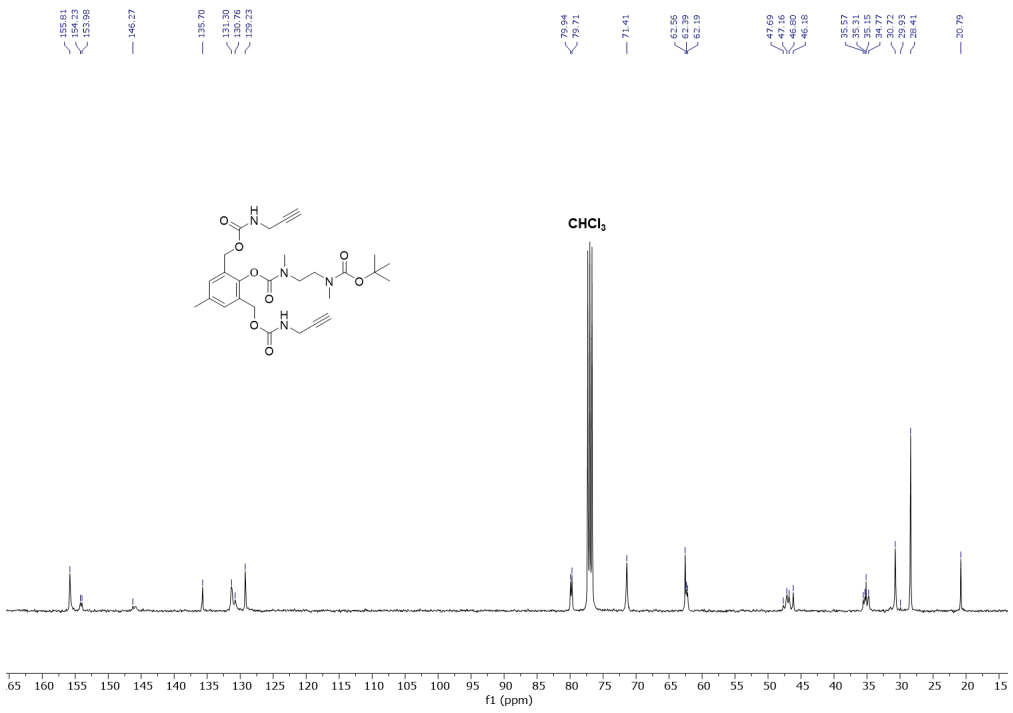


Figure S30. ^{13}C NMR spectrum of compound **12** (100 MHz, $CDCl_3$). Rotational isomers about the carbamate bond are observed.

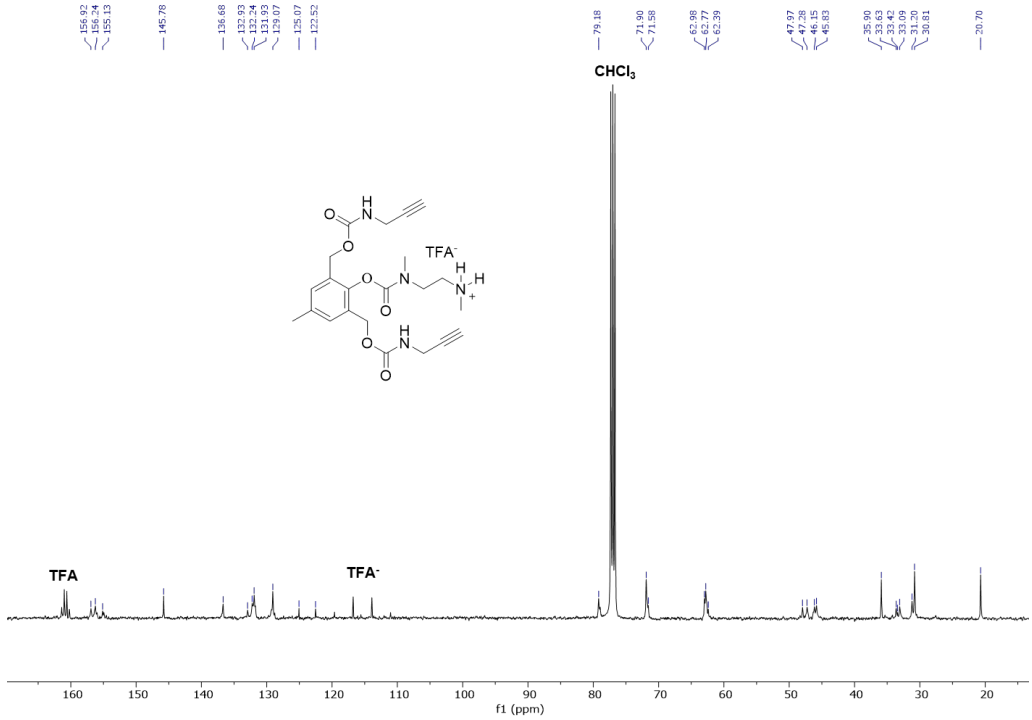


Figure S31. ^{13}C NMR spectrum of compound **13** (100 MHz, $CDCl_3$). Rotational isomers about the carbamate bond are observed.

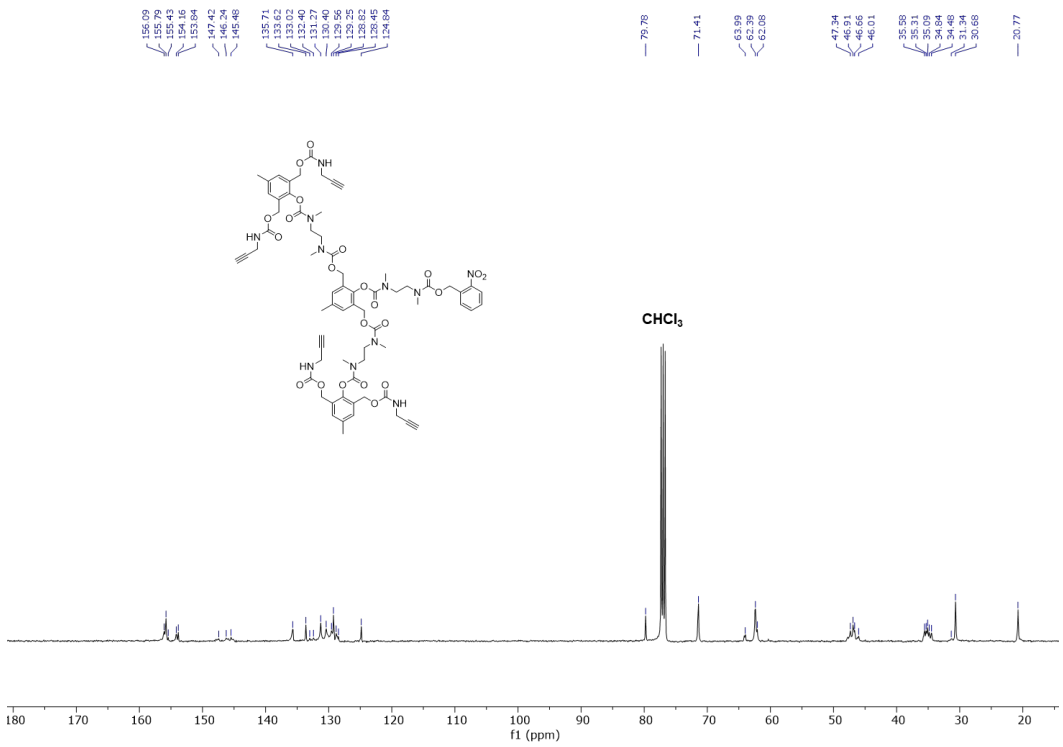


Figure S32. ^{13}C NMR spectrum of G2 SID (100 MHz, $CDCl_3$). Rotational isomers about the carbamate bonds are observed.

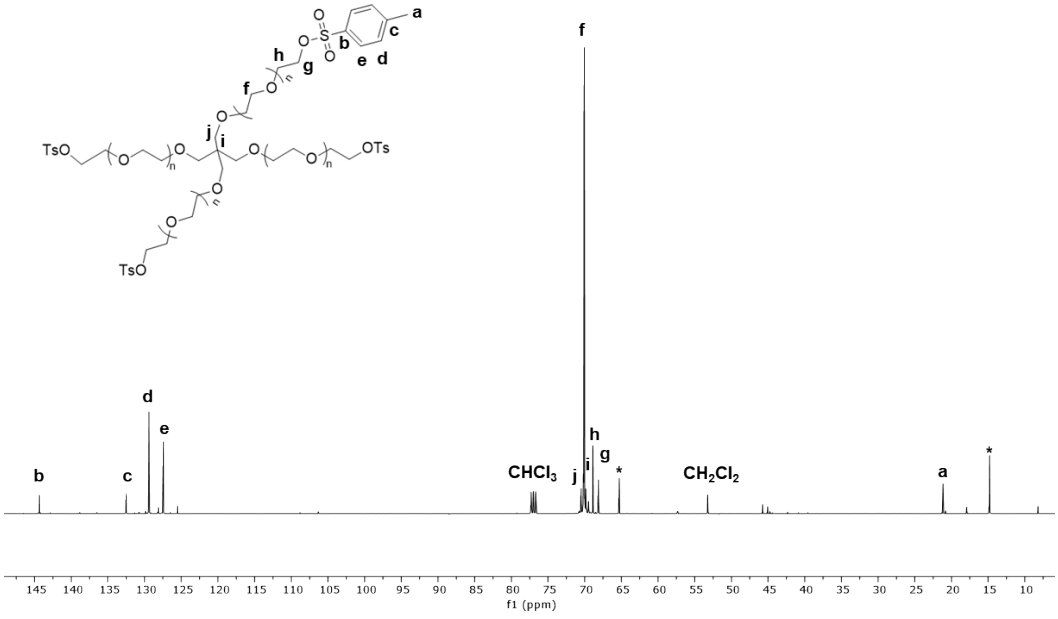


Figure S33. ^{13}C NMR spectrum of compound **14** (100 MHz, $CDCl_3$). Asterisks correspond to diethyl ether.

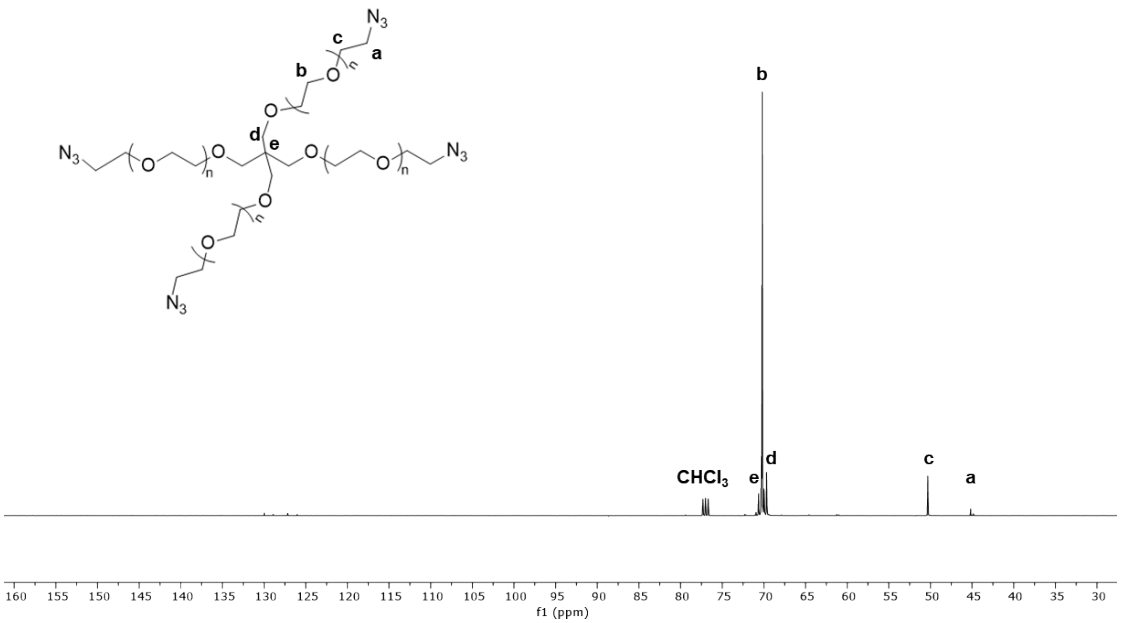


Figure S34. ^{13}C NMR spectrum of 4-arm-PEG-azide (100 MHz, $CDCl_3$).

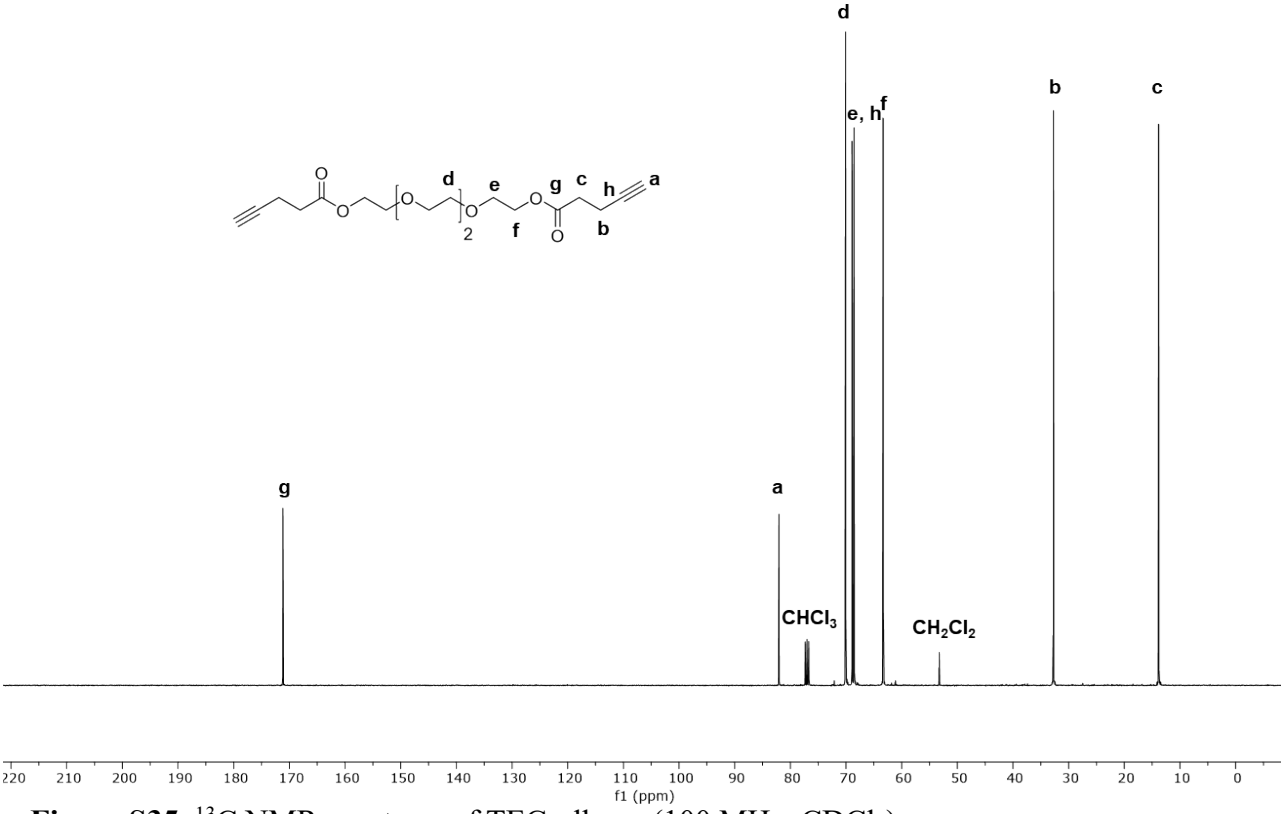


Figure S35. ^{13}C NMR spectrum of TEG-alkyne (100 MHz, $CDCl_3$).

References

1. W. Sun, H. Bandmann and T. Schrader, Chem. Eur. J., 2007, **13**, 7701.
2. T. Barra, L. Arrue, E. Urzúa and L. Ratjen, J. Phys. Org. Chem., 2019, **32**, e3935.
3. N. Fomina, C. L. McFearin and A. Almutairi, Chem. Commun., 2012, **48**, 9138.
4. R. Perry, R. Amir and D. Shabat, New J. Chem., 2007, 31, 1307.