Supplementary Material (ESI) for Chemical Communications

Synthesis of Well-Defined Hydrogel Networks Using Click Chemistry

Michael Malkoch,[1] Robert Vestberg,[1] Nalini Gupta,[1] Laetitia Mespouille,[2,3] Philippe Dubois,[2] Andrew F. Mason,[3] James L. Hedrick,[3*] Qi Liao,[4] Curtis W. Frank,[4] Kevin Kingsbury,[5] and Craig J. Hawker[1*]

[1] Materials Research Laboratory (MRL), University of California, Santa Barbara, CA 93106, USA

[2] Laboratory of Polymeric and Composite Materials (LPCM), University of Mons-Hainaut, Place du Parc 20, B-7000 Mons, Belgium.

[3] IBM Almaden Research Center, 650 Harry Road, San Jose, CA 95120, USA

[4] Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA

[5] Department of Chemistry and Biochemistry, California Polytechnic State University, San Luis Obispo CA 93407, USA.

E-mail: hawker@mrl.ucsb.edu, hedrick@almaden.ibm.com

Supplementary Material (ESI) for Chemical Communications

Experimental Section

General Methods. Analytical TLC was performed on commercial Merck plates coated with silica gel GF254 (0.24 mm thick). Silica gel for flash chromatography was Merck Kieselgel 60 (230-400 mesh, ASTM). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) measurements were performed on a Bruker AC 400 spectrometer at room temperature. Size exclusion chromatography (SEC) was carried out at room temperature on a Waters chromatograph connected to six Waters Styragel columns (five HR-5 and one HMW-20) using THF as eluant (flow rate: 1 mL/min). A Waters 410 differential refractometer and a 996 photodiode array detector were employed. The molecular weights of the polymers were calculated relative to linear polystyrene standards. MALDI-TOF mass spectrometry was performed on a PerSeptive Biosystems Voyager DE mass spectrometer operating in linear mode, using dithranol in combination with silver trifluoroacetate as matrix. X-ray detection and quantification of atomic percentages employed a Philips XL30 ESEM FEG, fitted with a 500 μm gaseous secondary electron detector. Specimen chamber pressure ranged from 4.8-6.7 mbar, while the temperature was kept at 274-278 K using a Peltier cooled stage, allowing controlled hydration of the specimen. Measurements of uniaxial extension were carried out using an Instron Model 5844 tensile testing apparatus. The hydrogel pieces were cut into 0.7 × 3.0 cm long strips using parallel razor blades, and its thickness was measured with a caliper. The sample was then fixed by grips and kept moist with an ultrasonic air humidifier during the whole testing process. The initial grip distance was set to be 5.0 mm and a constant stretch rate of 0.01 mm/sec was applied to the sample until its failure (breakage). Testworks software recorded the load and extension data, which were used to calculate the true stress and strain, from which the

average values for Young's modulus (E), maximum strength, and elongation at break of water-saturated hydrogels could be determined.

Materials. The anhydrides of 4-pentyonic acid[1] and acetonide-2,2-bis(hydroxymethyl) propionic acid[2] were synthesized as described earlier. All reagents were purchased from Aldrich and were used without any further purifications. Poly(ethylene glycol), PEG-$(OH)_2$, (M_n = 3.4 K, 6 K, 8 K and 10 K) and PEG-(diacrylate)$_2$, (M_n = 3.4 K, and 14 K) were purchased from either Nektar pharmaceuticals or Aldrich and used without any further purification.

General Synthesis of acetylated 3.4 K Poly(ethylene glycol) (2a). To a solution of 3.4 K PEG-$(OH)_2$, **1a**, (10.0 g, 2.94 mmol), 4-dimethylaminopyridine (0.5 equiv/OH group) (359 mg, 2.94 mmol), and pyridine (5 equiv./OH group) (2.30 g, 29.4 mmol) in CH_2Cl_2 (30 mL) was added 4-pentynoic anhydride (2.5 equiv/OH) (2.62 g, 1.47 mmol). The reaction was allowed to stir for 12 hours at room temperature and monitored by ^{13}C NMR (completion determined by the presence of a resonance for the carbonyl of excess anhydride starting material at 167 ppm), concentrated, and added dropwise to ca 500 mL of diethyl ether. The polymer, **2**, was precipitated twice in diethyl ether, filtered and dried (9.73 g, 92.7%). ^1H NMR ($CDCl_3$): δ 1.98 (t, *J* = 5.2, $CH_2CH_2C{\equiv}C\mathbf{H}$), 2.48-2.61 (m, $C\mathbf{H}_2C\mathbf{H}_2C{\equiv}CH$), 3.43-3.81 (m, $OC\mathbf{H}_2C\mathbf{H}_2O$), 4.25 (t, *J* = 9.6, $OCH_2C\mathbf{H}_2OOC$). ^{13}C NMR ($CDCl_3$): δ 14.26 (s, $CH_2\mathbf{C}H_2C{\equiv}C$), 33.19 (s, $\mathbf{C}H_2CH_2C{\equiv}CH$), 63.79 (s, $COO\mathbf{C}H_2CH_2O$), 69.02 (s, $COOCH_2\mathbf{C}H_2O$), 69.04 (s, $CH_2CH_2C{\equiv}\mathbf{C}H$), 70.51 (s, $O\mathbf{C}H_2\mathbf{C}H_2O$), 82.39 (s, $CH_2CH_2\mathbf{C}{\equiv}CH$), 171.69 (s, $OO\mathbf{C}CH_2$). GPC: M_n = 3,400 a.m.u.; polydispersity index 1.02.

6K PEG-(Acetylene)$_2$ (2b). This polymer was prepared from a 6K PEG according to the general procedure described above (Yield 91.8 %). GPC: M_n = 6,000 a.m.u.; polydispersity index 1.03.

8K PEG-(Acetylene)$_2$ (2c). This polymer was prepared from a 8K PEG according to the general procedure described above (Yield 93.4 %). GPC: M_n = 8,000 a.m.u.; polydispersity index 1.04.

10K PEG-(Acetylene)$_2$ (2d). This polymer was prepared from a 10K PEG according to the general procedure described above (Yield 92.6 %). GPC: M_n = 10,000 a.m.u.; polydispersity index 1.02.

Bis(Acetonide-2,2-bis(hydroxymethyl)propionate)-tetra(ethylene glycol). To a stirred solution of tetraethylene glycol, (5.00 g, 25.7 mmol), 4-dimethylaminopyridine (1.24 g, 10.2 mmol), and pyridine (3.04 g, 38.5 mmol) in 200 mL of CH_2Cl_2 was added the anhydride of acetonide-2,2-bis(hydroxymethyl)propionic acid (12.7 g, 38.5 mmol). The reaction was stirred at RT for 24 hrs, filtered and the organic phase concentrated. The crude product was purified by column chromatography on silica gel eluting with hexane, gradually increasing the polarity to 20:80 hexane:ethyl acetate to give the product as a colorless oil (11.0 g, 91.2%). ^{1}H NMR ($CDCl_3$): δ 1.18 (s, C**H**$_3$CCOO, 6H), 1.35 (s, CC**H**$_3$, 6H), 1.39 (s, CC**H**$_3$, 6H), 3.61-3.66 (m, OC**H**$_2$C**H**$_2$O, 12H), 3.67 (d, *J* = 11.6, CC**H**$_2$OCCH$_3$, 4H), 4.16 (d, *J* = 11.6, CC**H**$_2$OCCH$_3$, 4H), 4.26 (t, *J* = 9.6, COOC**H**$_2$CH$_2$O, 4H). ^{13}C NMR ($CDCl_3$): δ 18.57 (s, OCC**C**H$_3$, 2C), 22.92 (s, OC**C**H$_3$,

2C), 24.17 (s, OC**C**H_3, 2C), 41.68 (s, OOC**C**CH_3, 2C), 63.76 (s, CH_2**C**H_2OOC, 2C), 65.83 (s, C**C**H_2OC, 4C), 68.91 (s, O**C**H_2CH_2OOC, 2C), 70.49 (s, OCH_2**C**H_2O, 2C), 70.54 (s, O**C**H_2CH_2O, 2C), 97.94 (s, CH_2O**C**CH_3, 2C), 174.03 (s, OO**C**CCH_3, 2C).

Bis(2,2-bis(hydroxymethyl)propionate)-tetra(ethylene glycol) (3). To a stirred solution of bis(acetonide-2,2-bis(hydroxymethyl)propionate)-tetra(ethylene glycol) (8.00 g, 15.8 mmol) in 300 ml of MeOH was added DOWEX, 50X2 resin (5.0 g). The reaction was allowed to stir at 50 °C for 8 h, the resin was filtered, and the organic phase concentrated to give the product, **3**, as a colorless oil (6.14 g, 91.1%). ^{1}H NMR ($CDCl_3$): δ 1.11 (s, C**H**$_3$CCOO, 6H), 3.61-3.66 (m, OC**H**$_2$C**H**$_2$O, 8H), 3.70-3.71 (m, OC**H**$_2CH_2$OOC and CC**H**$_2$OH, 8H), 3.83 (d, *J* = 11.2, CC**H**$_2$OH, 4H), 4.33 (t, *J* = 9.2, COOC**H**$_2CH_2$O, 4H). ^{13}C NMR ($CDCl_3$): δ 17.14 (s, OCC**C**H_3, 2C), 49.66 (s, OOC**C**CH_3, 2C), 63.81 (s, CH_2**C**H_2OOC, 2C), 67.35 (s, C**C**H_2OH, 4C), 68.79 (s, O**C**H_2CH_2OOC, 2C), 70.31 (s, OCH_2**C**H_2O, 2C), 70.46 (s, O**C**H_2CH_2O, 2C), 175.65 (s, OO**C**CCH_3, 2C).

Bis(2,2-bis(mesyloxymethyl)propionate)-tetra(ethylene glycol). To a stirred solution of bis(2,2-bis(hydroxymethyl)propionate)-tetra(ethylene glycol) **3** (4.00 g, 9.38 mmol) and triethylamine (7.65 g, 75.1 mmol) in 300 ml of dry THF at 0°C, mesyl chloride (8.60 g, 75.1 mmol) was added droppwise. The reaction was allowed to warm to room temperature and stirred for an additional 3 h. The solids were filtered, the organic phase concentrated, and the crude product purified by column chromatography eluting with hexane:ethyl acetate 50:50, gradually increasing the polarity to ethyl acetate to give the product as a colorless oil (6.15 g, 88.7%). ^{1}H NMR ($CDCl_3$): δ 1.30 (s, C**H**$_3$CCOO, 6H), 3.04 (s, C**H**$_3SO_2CH_2$, 12H), 3.59 (s, OC**H**$_2$C**H**$_2$O, 8H), 3.67 (t, *J* = 9.6, OC**H**$_2CH_2$OOC,

4H), 4.27-4.30 (m, COOC**H**$_2$CH$_2$O and CC**H**$_2$OSO$_2$CH$_3$, 8H), 3.83 (d, *J* = 10.0, CC**H**$_2$OSO$_2$CH$_3$, 4H). ^{13}C NMR ($CDCl_3$): δ 17.44 (s, OCC**C**H$_3$, 2C), 37.09 (s, OSO$_2$**C**H$_3$, 4C), 46.55 (s, OOC**C**CH$_3$, 2C), 64.64 (s, CH$_2$**C**H$_2$OOC, 2C), 68.54 (s, OCH$_2$CH$_2$OOC, 2C), 69.14 (s, OCH$_2$CH$_2$OOC, 2C), 70.38 (s, O**C**H$_2$**C**H$_2$O, 4C), 171.06 (s, OO**C**CCH$_3$, 2C).

Bis(2,2-bis(Azidomethyl)propionate)-tetra(ethylene glycol) (4). To a stirred solution of bis(2,2-bis(mesyloxymethyl)propionate)-tetra(ethylene glycol) (1.00 g, 1.35 mmol) in 5 ml of DMSO was added sodium azide (1.73 g, 27.1 mmol) and the reaction was stirred at 80 °C for 12 h. The product mixture was purified by column chromatography eluting with hexane, gradually increasing the polarity to hexane:ethyl acetate 50:50 to give **4** as a colorless oil (620 mg, 87.7%). ^{1}H NMR ($CDCl_3$): δ 1.21 (s, C**H**$_3$CCOO, 6H), 3.48 (d, *J* = 12.4, CC**H**$_2$N$_3$, 4H), 3.59 (d, *J* = 12.4, CC**H**$_2$N$_3$, 4H), 3.62 (s, OC**H**$_2$C**H**$_2$O, 8H), 3.69 (t, *J* = 9.6, OC**H**$_2$CH$_2$OOC, 4H), 4.29 (t, *J* = 9.6, OCH$_2$C**H**$_2$OOC, 4H), ^{13}C NMR ($CDCl_3$): δ 19.23 (s, OCC**C**H$_3$, 2C), 47.60 (s, OOC**C**CH$_3$, 2C), 54.71 (s, **C**H$_2$N$_3$, 4C), 64.21 (s, **C**H$_2$CH$_2$OOC, 2C), 68.77 (s, CH$_2$**C**H$_2$OOC, 2C), 70.47 (s, OCH$_2$**C**H$_2$O, 2C), 70.52 (s, O**C**H$_2$CH$_2$O, 2C), 172.99 (s, OO**C**CCH$_3$, 2C).

Acetonide protected 1,1,1-tris(hydroxymethyl)ethane, 8. A solution of 1,1,1-tris(hydroxymethyl)ethane (5.00, 37.3 mmol), 2,2-dimethoxypropane (5.82 g, 56.0 mmol), and *p*-Toluene sulfonic acid (0.14 g, 7.42 mmol) in acetone (100 ml) was stirred at room temperature for 5 hours. Ammonium hydroxide (0.26 g, 7.42 mmol – 50% solution in ethanol) was added to the reaction and the acetone removed by evaporation. The crude product was dissolved in dichloromethane (200 ml), extracted with water three times (20

ml) and the organic phase dried with $MgSO_4$. Purification by column chromatography eluting with hexane:ethyl acetate 10:90, gradually increasing the polarity to hexane:ethyl acetate 40:60 gave **8** as a colorless oil (5.06, 78 %) ^{1}H NMR ($CDCl_3$): δ 0.79 (t, *J* = 7.6, C**H**$_3$CH$_2$C, 3H), 1.28 (q, *J* = 7.4, CH$_3$C**H**$_2$C, 2H), 1.32 (s, CC**H**$_3$, 3H), 1.38 (s, CC**H**$_3$, 3H), 2.70 (t, *J* = 5.4, CH$_2$OH, 1H), 3.54 (s, HOC**H**$_2$C, 2H), 3.65 (s, OC**H**$_2$C, 2H), 3.68 (s, OC**H**$_2$C, 2H). ^{13}C NMR ($CDCl_3$): δ 7.00 (s, **C**H$_3$CH$_2$C, 1C), 20.17 (s, CH$_3$**C**H$_2$C, 1C), 23.74 (s, OC**C**H$_3$, 1C), 27.30 (s, OC**C**H$_3$, 1C), 36.91 (s, CH$_2$**C**CH$_2$, 1C),62.51 (s, HO**C**H$_2$C, 1C), 65.15 (s, C**C**H$_2$OC, 2C), 98.16 (s, CH$_2$O**C**CH$_3$, 1C).

Bismesyloxy-tetra(ethylene glycol) (9). Mesyl chloride (47.2 g, 412 mmol) was added dropwise to a solution of triethylamine (42.0 g, 102 mmol) and tetra(ethylene glycol) (20.0 g, 194 mmol) in 1000 ml of dry THF at 0 °C. The reaction was allowed to warm to room temperature, stirred for an additional 3 h and the solids removed by filtration. The organic phase was then concentrated and the crude product purified by column chromatography eluting with hexane:ethyl acetate 20:80, gradually increasing the polarity to ethyl acetate to give the bis(mesylate), **9**, as a colorless oil (28.2 g, 89.7%). ^{1}H NMR ($CDCl_3$): δ 3.01 (s, C**H**$_3$SO$_2$OCH$_2$, 6H), 3.66-3.68 (m, OC**H**$_2$C**H**$_2$O, 8H), 3.68-3.72 (m, OC**H**$_2$CH$_2$OSO$_2$, 4H), 4.29-4.33 (m, OCH$_2$C**H**$_2$OSO$_2$, 4H). ^{13}C NMR ($CDCl_3$): δ 37.63 (s, **C**H$_3$SO$_2$OCH$_2$, 2C), 68.99 (s, OCH$_2$**C**H$_2$OSO$_2$, 2C), 69.31 (s, OCH$_2$**C**H$_2$OSO$_2$, 2C), 70.47-70.60 (m, O**C**H$_2$**C**H$_2$O, 4C).

Bis(2,2-bis(acetonide)propyloxy)-tetra(ethylene glycol) (10). To a stirred solution of acetonide protected TMP (14.2 g, 81.9 mmol) and 18-c-6 (20 mg) in dry THF (100 ml) was added NaH 60 % dispersion in oil (3.3 g, 81.9 mmol). The solution was stirred for

15 min under Ar and the bis(mesylate), **9** (10.0 g, 32.7 mmol), dissolved in dry THF (10 ml) added dropwise and the reaction stirred at room temperature for 12 hours. Addition of water was followed by evaporation to dryness and the crude product purified by column chromatography eluting with hexane:ethyl acetate 20:80, gradually increasing the polarity to hexane:ethyl acetate 50:50 to give **10** as a colorless oil (14.7 g, 88.6%). ^{1}H NMR ($CDCl_3$): δ 0.77 (t, *J* = 7.6, C**H**$_3$CH_2C, 6H), 1.29 (q, *J* = 7.2, CH_3C**H**$_2$C, 4H), 1.30 (s, CC**H**$_3$, 6H), 1.35 (s, CC**H**$_3$, 6H), 3.44 (s, OC**H**$_2$C, 4H), 3.49-3.67 (m, OC**H**$_2$C**H**$_2$O and CC**H**$_2$OC, 20H). ^{13}C NMR ($CDCl_3$): δ 6.84 (s, **C**H_3CH_2C, 2C), 20.96 (s, CH_3**C**H_2C, 2C), 23.80 (s, OC**C**H_3, 2C), 26.00 (s, OC**C**H_3, 2C), 36.29 (s, CH_2**C**CH_2, 2C), 64.87 (s, C**C**H_2OC, 4C), 70.09-70.65 (m, O**C**H_2**C**H_2O and O**C**H_2C 10C), 97.49 (s, CH_2O**C**CH_3, 2C).

Bis(2,2-bis(hydroxymethyl)propyloxy)-tetra(ethylene glycol) (11). To a stirred solution of the bis(acetonide), **11** (10.0 g, 19.7 mmol), in 300 ml of MeOH was added 10 grams of DOWEX, 50X2 resin. The reaction was allowed to stir at 50 °C for 8 h, the resin was filtered, and the organic phase concentrated to give a colorless oil (7.79 g, 92.7%) which was shown to be essentially pure tetra-alcohol, **12**. ^{1}H NMR (MeOH-d_4): δ 0.83 (t, *J* = 7.6, C**H**$_3$CH_2C, 6H), 1.31 (q, *J* = 7.6, CH_3C**H**$_2$C, 4H), 3.22 (s, OC**H**$_2$C, 4H), 3.44 (s, CC**H**$_2$O, 8H), 3.53-3.63 (m, OC**H**$_2$C**H**$_2$O, 16H). ^{13}C NMR (MeOH-d_4): δ 7.92 (s, **C**H_3CH_2C, 2C), 23.19 (s, CH_3**C**H_2C, 2C), 44.77 (s, CH_2**C**CH_2, 2C), 64.20 (s, C**C**H_2OH, 4C), 68.81-70.62 (m, O**C**H_2**C**H_2O, 8C), 73.25 (s, O**C**H_2C, 2C).

Bis(2,2-bis(mesyloxymethyl)propyloxy)-tetra(ethylene glycol) (13). To a stirred solution of the tetra-alcohol, **12** (3.5 g, 8.21mmol), and triethylamine (6.7 g, 65.7 mmol)

in 200 ml of dry THF at 0 °C, was added mesyl chloride (7.52 g, 65.7 mmol) dropwise. The reaction was allowed to warm to room temperature and stirred for an additional 3 h. The solids were then removed by filtration, the organic phase concentrated, and the crude product purified by column chromatography eluting with hexane:ethyl acetate 50:50 and gradually increasing the polarity to ethyl acetate to give **13** as a colorless oil (5.32 g, 87.7%). ^{1}H NMR ($CDCl_3$): δ 0.81 (t, J = 7.4, C**H**$_3$CH_2C, 6H), 1.38 (q, J = 7.4, CH_3C**H**$_2$C, 4H), 3.04 (s, C**H**$_3$SO_2OCH_2, 12H), 3.27 (s, OC**H**$_2$C, 4H), 3.49 (s, OC**H**$_2$C**H**$_2$O, 16H), 4.01 (s, CC**H**$_2$OSO_2CH_3, 8H). ^{13}C NMR ($CDCl_3$): δ 6.90 (s, **C**H_3CH_2C, 2C), 21.78 (s, CH_3**C**H_2C, 2C), 36.59 (s, OSO_2**C**H_3, 4C), 42.10 (s, CH_2**C**CH_2, 2C), 68.81-68.93 (m, C**C**H_2OSO_2CH_3, 4C), 70.00-70.62 (m, O**C**H_2**C**H_2O and O**C**H_2C, 10C).

Bis(2,2-bis(Azidomethyl)propyloxy)-tetra(ethylene glycol) (7). To a stirred solution of the tetramesylate, **13** (6.25 g, 8.46 mmol), in 50 ml of DMSO was added sodium azide (11.00 g, 169.2 mmol) and the reaction mixture stirred at 80 °C for 12 h. Addition of water (300 ml) followed by extraction with dichloromethane (3 x 100 ml) gave the crude product which was purified by column chromatography eluting with hexane and gradually increasing the polarity to hexane:ethyl acetate 50:50 to give **7** as a colorless oil (3.98, 89.3%). ^{1}H NMR ($CDCl_3$): δ 0.82 (t, J = 7.6, C**H**$_3$CH_2C, 6H), 1.38 (q, J = 7.6, CH_3C**H**$_2$C, 4H), 3.22-3.32 (m, OC**H**$_2$C and CC**H**$_2$N$_3$, 12H), 3.54-3.62 (m, OC**H**$_2$C**H**$_2$O, 16H). ^{13}C NMR ($CDCl_3$): δ 7.01 (s, **C**H_3CH_2C, 2C), 23.47 (s, CH_3**C**H_2C, 2C), 43.22 (s, CH_2**C**CH_2, 2C), 53.27 (s, C**C**H_2N$_3$, 4C), 70.88-71.28 (m, O**C**H_2**C**H_2O, C**C**H_2N$_3$ and O**C**H_2C, 10C).

General Synthesis of Hydrogels using Click Chemistry, 5a. To a small vial was added poly(ethylene glycol)-diacetylene (3,400 a.m.u.), **2a** (233 mg, 65.4 μmol), bis(2,2-bis(azidomethyl)propionate)-tetra(ethylene glycol), **4** (17.2 mg, 32.7 μmol), and 250 mg of ethanol. To the vial was added 250 mg of deionized H_2O containing sodium ascorbate (2.50 mg, 12.6 μmol) and the mixture was stirred to give a clear solution using a vortexer. Copper sulfate (2.50 mg, 15.7 μmol) in water (250 mg) was added and after stirring for 10 seconds, the reaction mixture was dropped in the centre of a teflon O-ring (500 μm thick and 3 cm in inner diameter) sandwiched between two glass. The solution was allowed to react for 3 h and upon separation of the plates a uniform Click gel was formed. The gel was first transferred to a pH ~7-8 EDTA water solution (5%) to extract the trapped $CuSO_4$ and ethanol and finally allowed to reach maximum water absorption in pure deionized water.

Hydrogels, **5b-d**, based on **2b-d** or the corresponding ether derivative, **7**, were prepared under similar conditions to that described above, using 2.50 mg of $CuSO_4$ and sodium ascorbate for every 250 mg of PEG-$(acetylene)_2$/tetraazide hydrogel starting materials.

References

1. H. Ihre, L. O. Padilla, J. M. J Fréchet*, J. Am. Chem. Soc.* **2001**, *123*, 5908-5917.
2. M. Malkoch, K. Schleicher, E. Drockenmuller, T. P. Russell, P. Wu, V. V. Fokin, C. J. Hawker, *Macromolecules*; **2005**; *38*; 3663-3678.