Supporting information

Strain-Promoted Crosslinking of PEG-based Hydrogels via Copper-Free Cycloaddition

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General methods and materials

Chemicals and solvents were purchased from Sigma-Aldrich and Acros and were used without further purification. All reactions were performed in anhydrous conditions under an atmosphere of Argon. Flash chromatography was performed on silica gel (Sorbent Technologies Inc., 70-230 mesh).

^{1}H and ^{13}C NMR spectra were acquired using a Varian NMRS 500 and Varian NMRS 300. FTIR spectra were acquired using a FTIR-ATR MIRacle 10 SHIMADZU spectrometer.

ESI MS was performed on a Waters Synapt HDMS quadrupole/time-of-flight (Q/ToF) instrument. The sprayed solution was prepared by dissolving sample in chloroform/methanol (1/1) containing 1% NaTFA (1 mg/mL in methanol) solution. The following ESI parameters were selected: ESI capillary voltage, 3.5 kV; sample cone voltage, 35 V; extraction cone voltage, 3.2 V; desolvation gas flow, 500 L/h (N2); trap collision energy (CE), 6 eV; transfer CE, 4 eV; trap gas flow, 1.5 mL/min (Ar); sample flow rate, 5 μL/min; source temperature, 80 °C; desolvation temperature, 150 °C.

MALDI-TOF mass spectra were acquired with a Bruker Ultraflex-III TOF/TOF mass spectrometer (Bruker Daltonics, Inc., Billerica, MA) equipped with a Nd:YAG laser (355 nm). All spectra were measured in positive reflection mode. The instrument was calibrated using external polystyrene or PMMA standards at the molecular weight under consideration. A solution of trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]-malononitrile (DCTB, Santa Cruz Biotechnology, Inc., >99%) which was used as matrix was prepared in $CHCl_3$ at a concentration of 20 mg/mL. Solutions of sodium trifluoroacetate or silver trifluoroacetate, which were used as cationizing salt, were prepared in $MeOH/CHCl_3$ (v/v = 1/3) at a concentration of 10 mg/mL. All the samples were dissolved in $CHCl_3$. Matrix and cationizing salt were mixed in a ratio of 10/1 (v/v). The sample preparation involved depositing 0.5 μ L of matrix and salt mixture on the wells of a 384-well ground-steel plate, allowing the samples to air-dry, depositing 0.5 μ L of sample on the matrix spot, and adding another 0.5 μ L of matrix and salt mixture on the dry sample spot. After evaporation of solvent, the target plate was inserted into the MALDI source. The laser was adjusted and attenuated to minimize undesired polymer fragmentation and to optimize the peak intensity.

Experimental section

The synthesis of 4-dibenzocyclooctynol is based on methods decribed previously.[1-3]

2,3:6,7-Dibenzo-9-oxabicyclo[3.3.l]nona-2,6-diene (1).
A 250 mL flask was flame dried and charged with argon. Phenylacetaldehyde (18.52 g, 0.154 mol) and 100 mL of chloroform (anhydrous) were then added via syringe. The reaction flask was cooled in an ice bath. Trimethylsilyl iodide (25 mL, 37.5 g, 0.188 mol) was added to the solution and the reaction was allowed to stand at 5 °C for 7 days. The reaction was monitored by TLC. After 7 days, sodium thiosulfate (1.0 M, 160 mL) and chloroform (200 mL) were added, and the mixture was stirred until the iodine color was discharged. The organic phase was separated, dried (sodium sulfate), and concentrated in vacuum. Chromatography on silica gel eluting with chloroform yielded 6.1 g of the crystalline ether compound (35%).
^{1}H NMR (300 MHz, $CDC1_3$) δ= 7.09 (m, 8H), 5.30(d, 2H, J=5.9 Hz, C*H*), 3.55(dd, 2H, J= 6.3, 16.2 Hz, CH_2), 2.75(d, 2H, J=16.4 Hz, CH_2); ^{13}C NMR (300 MHz, $CDC1_3$) δ=137.98, 131.79, 129.28, 127.02, 126.16, 125.35, 69.75, 36.31. ESI MS m/z 245.1334 [M+Na^+]; calcd for $C_{16}H_{14}NaO^+$: 245.0942

3-Hydroxy-2',3',2'',3''-tetramethox1y,-2 :5,6-dibenzocyclocta- 1,5,7-triene (2).
2,3:6,7-Dibenzo-9-oxabicyclo[3.3.l]nona-2,6-diene 1 (2.00 g, 5.84 mmol) in anhydrous THF (60 mL) was placed into a three-necked round bottom flask and cooled in an ice bath under argon. n-butyl lithium (4.92 mL, 2.5 M, 12.4 mmol) was added slowly via syringe. The reaction mixture was stirred at room temperature under argon for 4 h. The reaction was quenched by careful addition of water and extracted with 2 x 50 mL $CHCl_3$. The combined organic phases were washed with 30 mL of brine, dried over Na_2SO_4, concentrated under vacuum and purified by column chromatography on silica gel $CHC1_3$ to yield 1.83 g of 3-hydroxy-2',3',2'',3''-tetramethox1y,-2 :5,6-dibenzocyclocta- 1,5,7-triene (90%).
^{1}H NMR (300 MHz, $CDCl_3$): δ=7.48 (m, 1 H), 7.10–7.30 (m, 7H), 6.86 (q, 2H, J=2.7, 12.0 Hz, C*H*), 5.31 (q, 1H, J=6.1, 10.0 Hz, C*H*OH), 3.45 (m, 2H, CH_2); ^{13}C NMR (300 MHz, $CDCl_3$): d=140.9, 136.9, 136.3, 134.6, 131.8, 131.7, 130.3, 129.9, 129.3, 128.8, 127.3, 127.2, 126.1, 125.9, 74.7, 42.7. ESI MS m/z 245.1277 [M+Na^+]; calcd for $C_{16}H_{14}NaO^+$: 245.0942.

11,12-Dibromo-5,6,11,12-tetrahydro-dibenzo[a,e]cycloocten-5-ol (3).
Bromine (0.51 mL, 10 mmol) was added dropwise to a stirred solution of **2** (2.22 g, 10 mmol) in $CHCl_3$ (50 mL). After stirring the mixture for 0.5 h, TLC analysis indicated completion of the reaction. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography over silica gel (2:1/1:2, v/v, hexanes/CH_2Cl_2) to yield **3** as a light-yellow oil (60%).
^{1}H NMR (500 MHz, $CDCl_3$): δ=7.70 – 7.68 (2 H, aromatics), 7.39–6.88 (6 H, aromatics), 5.88 (d, 1H, J=5.4 Hz, C*H*Br), 5.47 (dd, 1H, J=3.6, 15.9 Hz, C*H*OH), 5.30 (d, 1H, J=5.4 Hz, C*H*Br), 3.60 (dd, 1H, J=3.7, 16.1 Hz, CH_2), 2.87 (dd, 1H, J=3.7, 16.1 Hz, CH_2); ^{13}C NMR (500 MHz, $CDCl_3$): δ=142.2, 138.9, 138.2, 135.0, 134.3, 132.5, 132.3, 131.1, 128.8, 127.2, 124.7, 122.3, 80.3, 70.6, 60.1 36.0. ESI MS m/z 402.9749 [M+Na^+]; calcd for $C_{16}H_{14}Br_2NaO^+$:402.9309.

5,6-Dihydro-11,12-didehydro-dibenzo[a,e]cycloocten-5-ol (4).
Lithium diisopropylamide in tetrahydrofuran (2.0 M; 8.0 mL, 16 mmol) was added dropwise to a stirred solution of **3** (1.53 g, 4.0 mmol) in tetrahydrofuran (40mL) under an atmosphere of argon. The reaction mixture was stirred for 0.5 h, after which it was quenched by the dropwise addition of water (0.5 mL). The solvents were removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (hexanes/CH_2Cl_2 2:1/0:1, v/v) to yield **4** as a white amorphous solid (0.52 g,

60%).
^{1}H NMR (500 MHz, $CDCl_3$): δ=7.77 (1 H, aromatics), 7.44–7.23 (7 H, aromatics), 4.66 (dd, J=2.1, 14.7 Hz, 1H, C*H*OH), 3.10 (dd, J=2.1, 14.8 Hz, 1H, CH_2), 2.94 (dd, J=2.1, 14.8 Hz, 1H, CH_2); ^{13}C NMR (500 MHz, $CDCl_3$): δ=155.5, 151.6, 129.6, 128.0, 127.9, 126.9, 126.8, 126.1, 124.7, 124.0, 123.7, 121.2, 112.9, 110.6, 75.2, 48.7. ESI MS m/z 243.1162 [M+Na^+]; calcd for $C_{16}H_{12}NaO^+$:243.0786.

Carbonic acid, 5,6-dihydro-11,12-didehydro-dibenzo[a,e]cycloocten-5-yl ester, 4-nitrophenyl ester (5)
4-Nitrophenyl chloroformate (0.4 g, 2 mmol) and pyridine (0.4 mL, 5 mmol) were added to a solution of 3 (0.22 g, 1 mmol) in CH_2Cl_2 (30 mL). After being stirred for 4 h at room temperature, the mixture was washed with brine (2×10 mL) and the organic layer was dried ($MgSO_4$). The solvents were evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate, 10:1, v/v) to afford 5 (0.32 g, 82%).
^{1}H NMR (500 MHz, $CDCl_3$): δ=8.28–8.14 (2 H, aromatics), 7.64–7.63 (2 H, aromatics), 7.45–7.23 (8 H, aromatics), 5.61 (dd, J=3.9, 15.3 Hz, 1H, C*H*OH), 3.37 (dd, 1H, J=3.9, 15.3 Hz, CH_2), 2.05 (dd, 1H, J=3.9, 15.3 Hz, CH_2); ^{I3}C NMR (500 MHz, $CDCl_3$): δ=155.5, 151.7, 150.1, 149.7, 129.9, 128.4, 128.3, 127.7, 127.5, 126.6, 126.2, 125.4, 125.0, 123.6, 123.5, 121.8, 121.7, 121.3, 113.3, 109.6, 81.6, 45.8. ESI MS m/z 408.1450 [M+Na^+]; calcd for $C_{23}H_{15}NNaO_5^+$:408.0848.

Dibenzylcyclooctyne Polyethylene glycol (6). Polyethylene glycol bisamine (6.0 k, 100 mg, 0.017 mmol) was dissolved in 20 mL anhydrous CH_2Cl_2, then 6.8 uL TEA was added. Under argon **5** (25 mg, 0.067 mmol) was added. After 24 h the solvent was removed under vacuum and water was added to dissolve the product and dialysis against water with cut off MW 1000. The product was then lyophillized to afford **6** as white powder (80 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.51–7.23 (16H, aromatics), 5.51 (2H, dd, J=3.9, 15.3 Hz, C*H*OH), 3.70-3.60 (~550H, s, OCH_2CH_2O), 3.40 (4H, d, J=6.4 Hz, CH_2NH), 3.20 (dd, 2H, J=3.9, 15.3 Hz, CH_2), 2.91 (dd, 2H, J=3.9, 15.3 Hz, CH_2)

Triarm PEG azide (7). Glycerol ethoxylate (4.67 g 4.67 mmol) was dissolved in 100 mL anhydrous CH_2Cl_2, then TEA (4.35 mL, 31.9 mmol) was added. Methanesulfonyl chloride (2.40 mL, 31.1 mmol) was then added dropwise via syringe to the solution under argon in an ice bath. After 1 h the solution was removed from the ice bath and allowed to warm to room temperature for 24 h. The salt was removed via filtration and the solution was concentrated under vacuum. Water (100 mL) was added to extract the desired compound. Sodium carbonate was added to the aqueous layer until the pH = 8. Sodium azide (1.82 g, 28.0 mmol) was added and the reaction was heated at 85 °C for 24 h. The solvent was removed under vacuum and extracted with 5×100 mL CH_2Cl_2 and dried over Na_2SO_4. The desired compound was eluted with CH_2Cl_2:methanol (15:1) on neutral Aluminum oxide to afford **7** as colorless oil (2.1g, 45%).
^{1}H NMR (500 MHz, $CDCl_3$): δ=3.70-3.63 (~84H, s, OCH_2CH_2O), 3.60-3.52 (5H, m, CH_2C*H*CH_2), 3.40 (t, 6H, J=5.0 Hz, CH_2N_3).

FTIR spectrum of the freeze dried Hydrogel and triazide

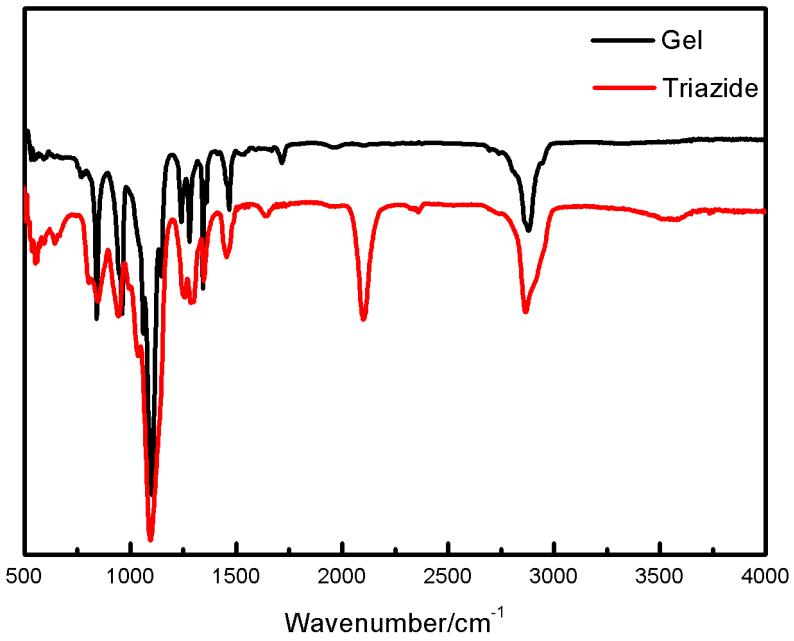

Figure S1. FTIR spectra of freeze dried hydrogels verifying the loss of azide(2095 cm^{-1})

Shear Rheology experiment. Oscillatory shear measurements were made at 24 °C with a TA Instruments ARES-G2 rheometer equipped with 8 mm parallel plates and using a frequency of 10 rad/s (1.6 Hz) and a strain amplitude of 10%. Briefly, 100 µL of the 20 wt% DIBO-PEG solution was added to the plate followed by 100 µL of a 2.56 wt% glycerol exytholate triazide solution. The oscillatory shear measurement was conducted immediately.

References:

(1) Jung, M. E.; Mossman, A. B.; Lyster, M. A. *J. Org. Chem.* **1978**, *43*, 3698.
(2) Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G.-J. *Angew. Chem. Int. Ed.* **2008**, *47*, 2253
(3) Mbua, N. E.; Guo, J.; Wolfert, M. A.; Steet, R.; Boons, G. J. *chembiochem* **2011**, *12*, 1912