Supporting information

Multipurpose heterofunctional dendritic scaffolds as crosslinkers towards functional soft hydrogels and implant adhesives in bone fracture applications

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**Experimental**

**Nomenclature**

- **Ac**  Acetonide
- **Acet**  Acetylene
- **CuAAC**  Copper catalyzed Azide Alkyne Cycloaddition
- **CuSO₄**  Copper sulphate
- **CHCl₃**  Chloroform
- **DCC**  Dicyclohexylcarbodiimide
- **DCM**  Dichloromethane
- **DMAP**  Dimethylaminopyridine
- **DMSO**  Dimethylsulphoxide
- **DOPA**  3,4-dihydroxyphenylalanine
- **ene**  carbon-carbon double bound
- **COOH**  carboxylic acid
- **EtOAc**  Ethylacetate
- **Hep**  Heptane
- **MeOH**  Methanol
- **NaAsc**  Sodium ascorbate
- **Na₂CO₃**  Sodium carbonate
- **NaHSO₄**  Sodium bisulphate
NMR  Nuclear magnetic resonance
MALDI-TOF  Matrix-assisted laser desorption/ionization time of flight
MgSO₄  Magnesium sulfate
p-TSA  Para toluene sulfonic acid monohydrate
TEA  Triethylamine
THF  Tetrahydrofuran
TMP  Trimethylolpropane
PEG  Polyethylene glycol
RT  Room temperature

Materials

All chemicals were purchased from Sigma Aldrich and were used as received unless otherwise noted. Trimethylolpropane (TMP) was kindly donated by Perstorp. Irgacure 2959 and Irgacure 184 were purchased from Ciba Chemicals, 4-pentynoic acid 98% from GFS Chemicals Inc, 90/0 E glass fibers style 106, 25 g m² are from Porcher industries, 32-64 D 60 Å silica gel from ICN. All bone specimens were obtained from bovine femur.
Synthesis

15

Synthesis of acetylene anhydride (15): As previously published

16

Synthesis of acetylene functional DOPA (16): Dopamine (20.0 g, 105 mmol) was dissolved in 100 mL DMSO and TEA (12.8 g, 127 mmol). The acetylene anhydride 15 (27.9 g, 94.9 mmol) was added slowly to the mixture and the reaction was left overnight at room temperature. The solution was dissolved in 3L of diethyl ether and extracted with 300 mL NaHSO₄ four times. The product was dried with MgSO₄ and the ether evaporated. The product was then dissolved in 1L of DCM and further extracted with NaHCO₃ (4 x 200 mL) then dried with MgSO₄ once again and the solvent evaporated. ³H NMR (400 MHz, MeOD):

δ ppm 7.93 (s, 1H, -NCOCH₂-), 6.63-6.68 (m, 2H, -CCH(OH)-), 6.51 (dd, 1H, J=10.4, -C(OH)CHCH-), 4.68 (d, 2H, J=2.8, -OCH₂C-), 3.29-3.33 (m, 2H, -CH₂CH₂NH-), 2.88 (t, 1H, J=4.8, -CH₂CCH), 2.59 (t, 4H, J=14.4, -COCH₂CH₂COO-), 2.46 (t, 2H, J=13.6, -CCH₂CH₂NH-). ¹³C NMR (100 MHz, MeOD): δ ppm 174.10, 173.52, 146.33, 144.85, 132.18, 121.18, 116.96, 116.48, 78.87, 76.32, 53.03, 42.51, 36.03, 31.42, 30.32
Synthesis of protected Trizma® (17): As previously published

![Chemical structure of protected Trizma®](image)

17

Synthesis of azide anhydride (18): As previously published

![Chemical structure of azide anhydride](image)

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Synthesis of anhydride functional acetonide protected Trizma®-azide (2): As previously published

![Chemical structure of anhydride functional acetonide protected Trizma®-azide](image)

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Synthesis of di-thiol PEG6k (14): As previously published

![Chemical structure of di-thiol PEG6k](image)

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Synthesis of α-D- mannose pentaacetate (19). As previously published  

Synthesis of acetylene functional α-D- mannose acetate (20). α-D- mannose pentaacetate (19) and propargyl alcohol (0.273 g, 48.8 mmol) was dissolved in DCM (20 mL), placed in an ice bath and BF$_3$Et$_2$O (2.04 mL, 16.3 mmol) was added stepwise. After 1 hour the reaction was left to reach room temperature overnight after which it was checked by NMR. The crude product was purified by extraction in ice-water (20 mL), saturated NaHCO$_3$(20 mL), ice water (20 mL) and dried in NaSO$_4$, filtered and concentrated. The product was obtained as a yellow oil. Yield: 97% (1.52 g). $^1$H NMR (400 MHz, CDCl$_3$): δ ppm 1.98 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.15 (s, 3H, Ac), 2.47 (t, J = 2.4 Hz, 1H, -CH), 3.99-4.30 (m, 6H, H-5, H-6, H-4, -CH$_2$CCH), 5.02 (d, 1H, 1.6 Hz, H-1), 5.26-5.35 (m, 2H, H-2, H-3) $^{13}$C-NMR (CDCl$_3$, 100 MHz): δ ppm 20.79, 20.82, 20.88, 21.00, 55.07, 62.43, 66.11, 69.03, 69.09, 69.46, 75.72, 78.03, 96.35, 169.81, 169.97, 170.08, 170.77
Synthesis of acetylene functional mannose (21). Acetylene functional α-D- mannose acetate (20) (7.85 g, 20.3 mmol) was dissolved in methanol (150 mL) and NaOMe (3.29 g, 61.0 mmol) in methanol (150 mL) was added to the mixture. The reaction was monitored with TLC and was finished in 2h. Dowex 50W-X2 was added to decrease the pH, the mixture was filtered and concentrated. The product was obtained as a yellow solid. Yield: 79% (3.50g). $^1$H NMR (400 MHz, D$_2$O): δ ppm 2.86 (t, J = 2.4 Hz, 1H), 3.63-
3.97 (m, 6H, H-2, H-3, H-4, H-5, H-6), 4.24-4.35 (m, 2H, -CH₂CCH), 5.00 (s, 1H, H-1). ¹³C-NMR (D₂O, 100 MHz): δ ppm 54.01, 60.27, 66.07, 69.39, 66.90, 72.59, 75.60, 78.20, 98.19.

Synthesis of the ene-Ac-OH (22). Protected Trizma® (31.1 g, 0.193 mol) (17), TEA (29.2 g, 0.288 mol) was dissolved in DCM (1500 mL) and placed in an ice bath. The 4-pentanoic anhydride (24.6 g, 0.135 mol) was added drop wise at 0°C and the reaction was followed by NMR until complete conservation of the anhydride. The mixture was washed with NaHSO₄, Na₂CO₃, and Brine, dried in MgSO₄, and evaporated to dryness. The product was obtained as a white solid. Yield: 90% (29.6 g). ¹H NMR (400 MHz, CDCl₃): δ ppm 1.39-1.48 (m, 6H, -CH₃), 2.34-2.42 (m, 4H, -CCH₂CH₂⁻), 3.64 (d, J = 6.476, 2H, -CH₂OH), 3.79-3.85 (m, 4H, -OCH₂⁻), 5.01-5.11 (m, 2H, -CCH₂), 5.78-5.84 (m, 1H, -CH⁻), 6.32 (s, 1H, -NH⁻). ¹³C-NMR (CDCl₃, 100 MHz): δ ppm 19.06, 27.90, 29.60, 36.21, 54.99, 64.25, 64.37, 98.84, 115.93, 136.43, 173.89
Synthesis of acid functional acetonide protected Trizma®-ene (23). Ene-Ac-OH (22) (13.4 g, 0.055 mol), DMAP (1.35 g, 0.011 mol) was dissolved in DCM (50 mL) and placed in an ice bath. Succinic anhydride (6.61 g, 0.066 mol) was added stepwise and the reaction was left to reach RT overnight and checked by NMR. The mixture was washed with NaHSO₄, and Brine, dried in MgSO₄, filtered and
evaporated until dry. The product was obtained as a white solid. Yield: 90 % (17.0g). $^1$H NMR (400 MHz, CDCl$_3$): $\delta_{\text{ppm}}$ 1.38 (s, 3H, -CH$_3$), 1.45 (s, 3H, -CH$_3$), 2.23-2.35 (m, 4H, -CH$_2$CONH- and -CHCH$_2$-), 2.60-2.66 (m, 4H, -CH$_2$COO-), 3.75 (d, $J = 12$ Hz, 2H, -CH$_2$O-), 4.17 (d, $J = 12$ Hz, 2H, -CH$_2$O-), 4.46 (s, 2H, -CH$_2$OOC-), 4.96-5.06 (m, 2H, -CHCH$_2$), 5.73-5.83 (m, 1H, -CHCH$_2$), 6.10 (s, 1H, -NH-). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta_{\text{ppm}}$ 23.15, 23.73, 28.78, 28.89, 29.40, 36.01, 53.10, 62.27, 63.43, 98.77, 115.59, 136.65, 172.24, 176.02.
Synthesis of anhydride functional acetonide protected Trizma®-ene (1). The acetonide protected Trizma-ene (23) (15.0 g, 43.7 mmol) was dissolved in DCM (25 mL) and placed in an ice bath. DCC (4.51 g, 21.8 mmol) was dissolved in DCM (10 mL) and added stepwise to the reaction. The reaction was left to react RT overnight, filtered and the filtrate was evaporated to dryness. The product was obtained as yellow sticky oil. Yield: 76% (11.0 g). $^1$H NMR (400 MHz, CDCl$_3$): $\delta_{ppm}$ 1.35 (s, 6H, -CH$_3$), 1.42 (s, 6H, -CH$_3$), 2.18-2.32 (m, 4H, -CH$_2$CONH- and -CHCH$_2$-), 2.61-2.66 (m, 4H, -CH$_2$COO-), 2.73-2.76 (m, 4H, -CH$_2$COOCO-), 3.75 (d, J = 12 Hz, 4H, -CH$_2$O-), 4.11 (d, J = 12 Hz, 4H, -CH$_2$O-), 4.47 (s, 4H, -CH$_2$OOC-), 4.93-5.04 (m, 4H, -CHCH$_2$), 5.71-5.82 (m, 2H, -CHCH$_2$), 5.88 (s, 2H, -NH-). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_{ppm}$ 23.17, 23.57, 28.24, 29.23, 30.01, 35.86, 53.37, 62.34, 63.59, 98.56, 115.32, 136.76, 167.91, 171.32, 172.84.
Synthesis of HFD (ei)-G1-e-(Ac)3-i-(ene)3 (3). TMP (500 mg, 3.73 mmol), DMAP (273 mg, 2.23 mmol) and pyridine (1.15 g, 14.5 mmol) was dissolved in DCM (5 mL) and placed in an ice bath. The anhydride (1) (9.00 g, 11.5 mmol) was dissolved in DCM (20 mL) and added stepwise to the reaction mixture and the reaction was left to reach room temperature overnight. After night the completion of the reaction was confirmed by NMR and MALDI-TOF and the anhydride was quenched with water (20 mL) overnight. The organic phase was washed with NaHSO₄, NaCO₃, and brine, dried with MgSO₄ and the product was purified by flash chromatography starting from Hep and eluting the product by a gradually increasing EtoAc concentration. The concentrated product was obtained as sticky yellow oil. Yield: 94 % (3.90 g).

**¹H NMR** (400 MHz, CDCl₃): δ ppm 0.82 t, J = 7.4 Hz, 3H, -CH₃), 1.35 (s, -CH₂C₃H₃, 9H), 1.40-1.43 (m, 11H, -CCH₃ and -CH₂CH₃), 2.19-2.33 (m, 12H, -NHCOC₃H₂CH₂-), 2.59 (s, 12H, -COCH₂CH₂CO-), 3.73 (d, J = 12.0 Hz, 6H, -CCH₂O-, 3.96 (s, 6H, -OCH₂CCH₂CH₃), 4.19 (d, J = 12.0 Hz, 6H, -CCH₂O-), 4.44 (s, 6H, -NHCH₂OCCO-), 4.94-5.03 (m, 6H, -CHCH₂), 5.72-5.82 (m, 3H, -CHCH₃), 5.96 (s, 3H, -NH-)

**¹³C NMR** (CDCl₃) δ ppm 7.07, 22.62, 23.10, 23.57, 28.62, 28.64, 29.19, 35.83, 40.49, 50.69, 62.10, 63.42, 63.68, 98.42, 115.26, 136.66,
171.75, 172.00, 172.74 MALDI-TOF: Calculated [M⁺Na⁺] = 1132.54 m/z, found [M⁺Na⁺] = 1132.42 m/z.

Synthesis of HFD(ei)-G1-e-(OH)₆-i-(ene)₃ (4). HFD(ei)-G1-e-(Ac)₃-i-(ene)₃ (3) (3.00 g, 2.70 mmol) was dissolved in THF (60 mL) and pTSA (3.47 g, 18.2 mmol) was dissolved in an ionized water (60 mL) and
added to the reaction mixture. The reaction was monitored by TLC and MALDI ToF until completion. TEA (1.84 g, 18.2 mmol) was added and the mixture was diluted in THF and extracted in 75% brine solution, evaporated and freeze dried. The product was purified by flash chromatography starting from EtoAc and gradually increasing to 10:90 MeOH:EtoAc. The product was obtained as a colorless oil. Yield: 76 % (2.04 g) ¹H NMR (400 MHz, MeOD): δ ppm 1.02 (t, J = 7.4 Hz, 3H, -CH₃), 1.61 (q, J = 7.4 Hz, 2H, –CH₂CH₃), 2.42-2.46 (m, 12H, -NHCOCH₂CH₂-), 2.76 (s, 12H, -COCH₂CH₂CO-), 3.82-3.89 (m, 12H, -CH₂OH), 4.17 (s, 6H, -OCH₂CCH₂CH₃), 4.45 (s, 6H, -NHCCH₂OCO-), 5.08-5.20 (m, 6H, -CHCH₂), 5.90-6.01 (m, 3H, -CHCH₃), 7.03 (s, 3H, -NH-) ¹³C NMR (MeOD) δ ppm 7.71, 23.90, 28.38, 30.82, 36.65, 42.06, 47.73, 62.11, 62.37, 63.63, 64.98, 115.83, 138.25, 173.61, 173.67, 176.18 MALDI-TOF: Calculated [M⁺+Na]= 1012.45 m/z, Found [M⁺+Na]= 1012.38 m/z.
Synthesis of HFD(ei)-G1-e-(azide)₆-i-(ene)₃ (5). HFD(ei)-G1-e-(OH)₆-i-(ene)₃ (4) (1.60 g, 1.61 mmol), DMAP (0.237 g, 1.94 mmol) and pyridine (4 mL) was dissolved in DCM (40 mL) and placed in an ice bath. Azide anhydride 18 (4.45 g, 15.0 mmol) was added stepwise and the mixture was left to react overnight. The reaction was followed by MALDI-TOF and NMR until full substitution of all hydroxyl groups. The organic phase was then washed with NaHSO₄, NaCO₃, and brine, dried with MgSO₄ and the crude product was purified with flash chromatography starting from Hep and eluting the product by a gradually increasing EtoAc concentration. The product was obtained as sticky yellow oil. Yield: 74 % (2.19 g) ¹H NMR (400 MHz, CDCl₃): δ ppm 0.84 (t, J = 7.5 Hz, 3H, -CH₃), 1.21-1.65 (m, 38H, N(CH₂)₃CH₂CH₃ and -CH₂CH₃), 2.20-2.33 (m, 24H, -NHCOC₂H₄CH₂-, -N(CH₂)₃CH₂CH₂CH₂CH₂-), 2.60 (s, 12H, -COCH₂CH₃CO-), 3.24 (t, J = 6.8 Hz, 12H, -CH₂N₃), 3.99 (s, 6H, CH₃CH₂CCH₂OCO-), 4.37-4.39 (m, 18H, -NHCC₂H₄-), 4.95-5.04 (m, 6H, -CHCH₂), 5.72-5.82 (m, 3H, -CHCH₃), 6.00 (s, 3H, -NH-) ¹³C NMR (CDCl₃) δ ppm 7.17, 22.72, 24.15, 26.03, 28.36, 28.66, 29.21, 33.65, 35.97, 40.55, 51.00, 58.01, 62.24, 62.65, 63.85, 115.42, 136.64, 171.68, 171.77, 172.46, 172.79 MALDI-TOF: Calculated [M⁺+Na⁺] = 1846.90 m/z Found [M⁺+Na⁺]= 1847.03 m/z.
Synthesis of HFD(ei)-G1-e-(Ac)3-i-(azide)3 (6). TMP (396 mg, 2.95 mmol), DMAP (216 mg, 1.77 mmol) and pyridine (910 mg, 11.5 mmol) was dissolved in DCM (5 mL) and placed in an ice bath. The anhydride functional acetonide protected Trizma®-azide (2) (9.00 g, 11.5 mmol) was dissolved in DCM (20 mL) and added stepwise to the reaction mixture and the reaction was left to reach room temperature overnight. After night the reaction was checked by NMR and MALDI-TOF, the anhydride was quenched with water (20 mL). The organic phase was washed with NaHSO₄, NaCO₃, brine and dried with MgSO₄. The product was purified with flash chromatography with a gradually increased concentration of EtoAc in Hep. The concentrated product was obtained as yellow oil. Yield: 84 % (3.18 g). ¹H-NMR (CDCl₃, 400 MHz) δ ppm 0.86 (t, J = 7.4 Hz, 3H, -CH₃), 1.23-1.27 (m, 2H, CH₃CH₂-) 1.39 (s, 12H, -CCH₃), 1.46 (s, 12H, -CCH₃), 1.55-1.66 (m, 18H, -CH₂CH₂CH₂CH₂CH₂-), 2.16 (t, J = 7.2 Hz, 6H, -CH₂CONH-), 2.62 (s, 12H, -CH₂CH₂COO-), 3.25 (t, J = 6.8 Hz, 6H, N₃CH₂-), 3.76 (d, J=12 Hz, 6H, -CH₂OCCH₃), 4.00 (s, 6H, -OCH₂CCH₂CH₂), 4.23 (d, J= 12 Hz, 6H, -CH₂OCCH₃), 4.46 (s, 6H, -CH₂CNH-), 5.88 (s, 3H, -NH-). ¹³C-NMR (CDCl₃, 100 MHz) δ ppm 7.06, 22.60, 23.29, 23.38, 24.71, 25.94, 28.35, 28.61, 36.51, 40.49, 50.95, 52.69, 62.04, 63.51, 63.69, 98.43, 171.77, 172.06, 173.12. MALDI-TOF: Calculated [M⁺+Na⁺] = 1303.64 m/z, found [M⁺+Na⁺]= 1303.70 m/z.
Synthesis of HFD(ei)-G1-e-(OH)_6-i-(azide)_3 (7). HFD(ei)-G1-e-(Ac)_3-i-(azide)_3 (6) (2.00 g, 1.6 mmol) dissolved in distilled water (17 mL) and pTSA (2.00 g, 10.5 mmol) dissolved in THF (17 mL) were mixed and the deprotection was followed with MADLI-TOF until completion. TEA (1.06 g, 10.5 mmol) was added, the reaction mixture was diluted in THF (1 L), extracted in brine:H_2O (3: 1), concentrated and freeze dried. The product was purified with flash chromatography, eluting the product with a gradually increased concentration of MeOH in EtoAc. The purified product was obtained as colorless oil. Yield: 64 % (1.1 g). ^1H-NMR (MeOD, 400 MHz) δ ppm 0.92 (t, J=7.4 Hz, 3H, -CH_3), 1.29-1.68 (m, 20H, CH_3C_H_2, -CH_2CH_2CH_2N_3-), 2.26 (t, J=7.4 Hz, 6H, -CH_2CONH-), 2.67 (s, 12H, -CH_2CH_2COO-), 3.32 (m, 2H, N_3CH_2-), 3.73-3.79 (m, 12H, -CH_2OH), 4.08 (s, 6H, -OCH_2CH_2CH_3), 4.35 (s, 2H, -CH_2CCH_2OH), 7.91 (s, 1H, -NH-). ^13C-NMR (MeOD, 100 MHz) δ ppm 7.75, 24.02, 26.47, 27.36, 29.70, 29.90, 29.93, 37.31, 42.20, 52.35, 62.20, 62.39, 63.79, 65.10, 173.77, 173.82, 176.94. MALDI-TOF: Calculated [M_w+Na^+] = 1183.55 m/z, found [M_w+Na^+] = 1183.44 m/z.
Synthesis of HFD(ei)-G1-e-(ene)\textsubscript{6}-i-(azide)\textsubscript{3} (8). HFD(ei)-G1-e-(OH)\textsubscript{6}-i-(azide)\textsubscript{3} (7) (0.900 g, 0.775 mmol), DMAP (0.114 g, 0.930 mmol) and pyridine (0.442 g, 5.58 mmol) were dissolved in DCM (18 mL) and placed in an ice bath. 4-pentenoic anhydride (1.02 g, 5.58 mmol) was added and the reaction was left to react overnight and checked by MALDI-TOF until completion. The residual anhydride was quenched with H\textsubscript{2}O (5 mL) overnight. The reaction mixture was diluted in DCM (300 mL) and extracted with NaHSO\textsubscript{4}, NaCO\textsubscript{3} and brine, dried with MgSO\textsubscript{4}, filtered and evaporated. Finally the product was purified with flash chromatography starting from Hep and eluting the product by a gradually increasing EtoAc.
concentration. The product was obtained as transparent oil. Yield 40\% (0.5 g). $^1$H-NMR (CDCl$_3$, 400 MHz) 
$\delta_{\text{ppm}}$ 0.88 (t, $J$=7.4 Hz, 3H, $\text{-CH}_3$), 1.24-1.49 (m, 8H, $\text{CH}_3\text{CH}_2$- and $\text{-CH}_2\text{CH}_2\text{CH}_2\text{N}_3$), 1.57-1.66 (m, 12H, $\text{-CH}_2\text{CH}_2\text{CH}_2\text{N}_3$), 2.16 (t, $J$=7.4 Hz, 6H, $\text{-CH}_2\text{CONH}$-), 2.36-2.46 (m, 24H, $\text{CH}_2\text{CHCH}_2\text{CH}_2$-), 2.63 (s, 12H, $\text{-CH}_2\text{CH}_2\text{COO}$-), 3.27 (t, $J$=6.8 Hz, 6H, $\text{N}_3\text{CH}_2$-), 4.03 (s, 6H, $\text{-OCH}_2\text{CCH}_2\text{CH}_3$), 4.43 (s, 18H, $\text{-NHCCCH}_2\text{COO}$-), 5.00-5.08 (m, 12H, $\text{CH}_2\text{CH}$-), 5.75-5.85 (m, 6H, $\text{CH}_2\text{CH}$-), 5.97 (s, 3H, $\text{-NH}$-). $^{13}$C-NMR (CDCl$_3$, 400 MHz) 
$\delta_{\text{ppm}}$ 7.47, 23.01, 25.04, 26.33, 28.72, 28.82, 28.93, 33.40, 36.90, 40.85, 51.32, 58.33, 62.69, 63.00, 64.14, 115.92, 136.45, 171.96, 172.05, 172.77, 173.12. MALDI-TOF: Calculated [M$_{\omega}$Na$^+$] = 1675.80 m/z, found [M$_{\omega}$Na$^+$] = 1675.73 m/z.
Synthesis of HFD(ei)-G1-e-(ene)₆-i-(DOPA)₃ (9). HFD(ei)-G1-e-(ene)₆-i-(azide)₃ (8) (0.15 g, 0.091 mmol) and acetylene functional DOPA (16) (0.16 g, 0.544 mmol) was dissolved in THF (4 mL), sodium ascorbate (0.08 g, 0.408 mmol) dissolved in deionized water (2 mL) and copper sulphate (0.02 g, 0.082 mmol) dissolved in water (0.5 mL) was added to the reaction. The reaction was checked by MADLI-TOF until completion. Thereafter the mixture was filtered, washed with THF and freeze dried. The product was purified with flash chromatography eluting the product with THF which was concentrated and decantated in cold diethyl ether (20 mL). The product was obtained as a transparent solid. Yield 33 % (75 mg). ¹H-NMR (MeOD, 400 MHz) δ ppm 0.89 (t, J= 7.5 Hz, 3H, -CH₃), 1.27-1.35 (m, 6H, -CH₂CH₂CH₂), 1.48 (q, J=7.5 Hz, 2H, CH₃CH₂-), 1.57-1.65 (m, 6H, -NHCOCH₂CH₂-), 1.86-1.93 (m, 6H, -NCH₂CH₂CH₂-), 2.19 (t, J=7.3 Hz, 6H, -CH₂CH₂NCOCH₂CH₂CO-), 2.31-2.48 (m, 36H, CH₂CH₂CH₂, -CH₂CH₂NCOCH₂CH₂CO-), 2.57-2.63 (m, 18H, -OCOCH₂CH₂CO-, -NCOCH₂CH₂CO-), 3.27-3.35 (m, 6H, -CH₂NH-), 4.04 (s, 6H, -CH₂CH₂CH₂), 4.36-4.42 (m, 24H, -NHCC₂- and -NCH₂-), 4.96-5.06 (m, 12H, CH₂-), 5.18 (s, 6H, -CH₂CN-), 5.77-5.87 (m, 6H, CH₂CH₂-), 6.50-6.52 (m, 3H, -HOCCHCH₂-), 6.63-6.69 (m, 6H, HOCCH-), 7.97 (s, 3H, -CCHN-). MALDI-TOF: Calculated [M⁺Na⁺] = 2549.13 m/z, found [M⁺Na⁺] = 2550.67 m/z.
Synthesis of HFD(ei)-G1-e-(DOPA)$_6$-i-(ene)$_3$ (10). HFD(ei)-G1-e-(azide)$_6$-i-(ene)$_3$ (5) (0.15 g, 0.082 mmol) and acetylene functional DOPA (16) (0.288 g, 0.986 mmol) was dissolved in THF (2.5 mL). Thereafter sodium ascorbate (0.04 g, 0.740 mmol) and copper sulphate (0.02 g, 0.073 mmol) dissolved
in deionized water (2 mL and 0.5 mL respectively) was added to the reaction. The reaction was checked by MADLI-TOF until completion. The mixture was concentrated and freeze dried. The product was purified with flash chromatography eluting in 80/20 (THF/MeOH), concentrated and precipitated in diethyl ether (40 mL) from methanol and passed through a neutral activated Al₂O₃ column. The product was obtained as a brown solid. Yield 7% (20 mg).

**¹HNMR (MeOD, 400 MHz)** δ ppm 0.89 (t, J= 7.5 Hz, 3H, -CH₃), 1.26-1.36 (m, 12H, -CH₂CH₂CH₂CH₂CH₂-), 1.46-1.52 (m, 2H, CH₃CH₂-), 1.61-1.69 (m, 12H, -NCH₂CH₂CH₂CH₂), 1.86-1.94 (m, 12H, -NCH₂CH₂-), 2.28-2.37 (m, 24H, CH₂CHCH₂CH₂- and -NHCH₂CH₂-) 2.48 (t, 12H, J = 6.8 Hz, -NCH₂CH₂CH₂CH₂CH₂-), 2.59-2.65 (m, 6H, -COCH₂CH₂CO-), 3.31 (m, 12H, -CH₂NH-), 4.05 (s, 6H, -CH₂CCH₂CH₃-), 4.37-4.43 (m, 24H, -NHCH₂- and -NCH₂-), 4.90-5.08 (m, 6H, CH₂-), 5.20 (s, 12H, -CH₂CN-), 5.78-5.88 (m, 3H, CH₂CH₂-), 6.49-6.54 (m, 6H, HOCCHCH₂-), 6.65-6.70 (m, 12H, HOCCH-), 8.00 (s, 6H, -CCHN-) **MALDI-TOF**: Calculated [M⁺Na⁺]=3593.56 m/z, found [M⁺Na⁺]= 3594.67 m/z.

**Synthesis of HFD(ei)-G1-e-(ene)₆-i-(COOH)₃ (11).** HFD(ei)-G1-e-(ene)₆-i-(azide)₃ (8) (0.15 g, 0.091 mol) was dissolved in THF (1.5 mL), 4-pentynoic acid (0.09 g; 0.871 mmol) dissolved in deionized water (300 μL), sodium ascorbate (0.19 g, 0.998 mmol) and copper sulphate (0.05 g, 0.200 mmol) dissolved in
deionized water (1.5 mL and 0.5 mL respectively) was added to the reaction mixture and the reaction was followed by MALDI-TOF until completion. The THF was evaporated and the crude mixture was freeze dried. The product was purified by flash chromatography starting from THF and eluting the product by a gradually increasing MeOH concentration, after which it was concentrated and decantated in ether from methanol. The product was obtained as a white solid. Yield: 5% (9 mg). $^1$HNMR (MeOD, 400 MHz) $\delta$ ppm 0.90 (t, J=7.5 Hz, 3H, -CH$_3$), 1.28-1.35 (m, 6H, -CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$-), 1.47-1.52 (m, 2H, CH$_3$CH$_2$-), 1.58-1.66 (m, 6H, -NHOCH$_2$CH$_2$-), 1.86-1.94 (m, 6H, -NCH$_2$CH$_2$CH$_2$-), 2.18 (t, J = 7.3 Hz, 6H, -CNHCOCH$_2$-), 2.32-2.47 (m, 30H, CH$_2$CHCH$_2$CH$_2$- and -CH$_2$COOH), 2.65 (s, 12H, -OCOCH$_2$CH$_2$CO-), 2.97 (t, J=7.4 Hz, 6H, COOHCH$_2$CH$_2$-), 4.05 (s, 6H, -CH$_2$CCH$_2$CH$_3$), 4.35-4.42 (m, 24H, -NHCC- and -NC-), 4.97-5.07 (m, 12H, CH$_2$-), 5.78-5.88 (m, 6H, CH$_2$CH-), 7.77 (s, 3H, -CCHN-). MALDI-TOF: Calculated [M$_w$+Na$^+$] = 1969.91 m/z, found [M$_w$+Na$^+$] = 1970.14 m/z, calculated [M$_w$+2Na$^+$-H$^+$] = 1991.89 m/z, found [M$_w$+2Na$^+$-H$^+$] = 1992.14 m/z, calculated [M$_w$+3Na$^+$-2H$^+$] = 2013.87 m/z, found [M$_w$+3Na$^+$-2H$^+$] = 2014.13 m/z, calculated [M$_w$+4Na$^+$-3H$^+$] = 2035.85 m/z, found [M$_w$+4Na$^+$-3H$^+$] = 2036.12 m/z.
Synthesis of HFD(ei)-G1-e-(COOH)6-i-(ene)3 (12). HFD(ei)-G1-e-(azide)6-i-(ene)3 (5) (0.15 g, 0.082 mmol) was dissolved in THF (1.5 mL), 4-pentynoic acid (0.10 g, 1.084 mmol) dissolved in deionized water (5 mL), sodium ascorbate (0.24 g, 1.232 mmol) dissolved in deionized water (1.5 mL) and copper sulphate (0.06 g, 0.246 mmol) was added to the reaction mixture and the reaction was followed by MALDI-TOF until completion. The THF was evaporated and the crude mixture was freeze dried. The product was purified from copper using flash chromatography in THF with increasing concentration of methanol and after the concentrated product was dissolved in methanol and precipitated in diethyl ether. The product was obtained as a brown solid. Yield: 26 % (52 mg).

\(^1\)HNMR (MeOD, 400 MHz) \(\delta_{ppm}\) 0.89 (t, \(J = 6.3\) Hz, 3H, -CH\(_3\)), 1.26-1.36 (m, 12H, -CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)), 1.46-1.52 (m, 2H, CH\(_3\)CH\(_2\)), 1.61-1.69 (m, 12H, -NCH\(_2\)CH\(_2\)CH\(_2\)H), 1.86-1.94 (m, 12H, -NCH\(_2\)CH\(_2\)), 2.15-2.18 (m, 6H, -NHCOCH\(_2\)), 2.27-2.39 (m, 24H, CH\(_2\)CHCH\(_2\)CH\(_2\)CO\(-\), -CH\(_2\)COOH), 2.65 (m, 6H, -OCOCH\(_2\)CH\(_2\)CO\(-\)), 2.97 (t, \(J = 7.6\) Hz, 12H, -CH\(_2\)CH\(_2\)COOH), 3.55-4.55 (m, 36H, -CH\(_2\)CH\(_2\)CH\(_3\), -NHCH\(_2\), -NCH\(_2\)), 4.95-5.07 (m, 6H, CH\(_2\)), 5.77-5.87 (m, 3H, CH\(_2\)H), 7.76 (s, 6H, -CCHN-), 8.48 (s, 3H, -NH-). MALDI-TOF: Calculated [M\(_{\text{w}}+\text{Na}^+\)]\(^{+}\) = 2435.12 m/z, found [M\(_{\text{w}}+\text{Na}^+\)]\(^{+}\) = 2435.47 m/z
Synthesis of HFD (ei)-G1-e-(Mannose)e-i-(ene)₃ (13). HFD(ei)-G1-e-(azide)e-i-(ene)₃ (5) (0.160 g, 0.087 mmol) was dissolved in THF (5 mL), acetylene mannose (21) (0.126 g, 0.579 mmol) dissolved in deionized water (0.5 mL), sodium ascorbate (0.031 g, 0.156 mmol) dissolved in deionized water (0.5 mL) and copper sulphate (0.017 g, 0.073 mmol) dissolved in deionized water (0.5 mL) was added to the reaction mixture and the reaction was followed by MALDI-TOF until completion. The THF was evaporated and the crude mixture was freeze dried. The product was purified by size exclusion chromatography using a Sephadex column and freeze dried. The product was obtained as a yellow powder. Yield: 60% (180 mg). \(^1\)HNMR (D₂O, 400 MHz) δ ppm: 0.77 (m, 3H, -CH₃), 1.22 (s (broad peak), 12H, -CH₂CH₂CH₂CH₂CH₂-), 1.36 (s(broad peak), 2H, CH₃CH₂-), 1.47-1.58 (m, 12H, -NCH₂CH₂CH₂CH₂), 1.75-1.88 (m, 12H, -NCH₂CH₂-), 2.24-2.31 (m, 24H, CH₂CHCH₂CH₂- and NCH₂CH₂CH₂CH₂CH₂-), 2.64 (s (broad peak), 12H, COCH₂CH₂CO-), 3.58-3.97 (m, 48H, H-2, H-3, H-4, H-5, H-6 and -OCH₂CH₂CH₂), 4.27-4.90 (m, 42H, -NHCH₂-, -NCH₂CH₂ and triazol-CH₂-mannose), 4.85-4.90 (m, 12H, CH₂CH- and H-1), 5.64-5.77 (m, 3H, CH₂CH-), 8.00 (s, 6H, -CCHN-) MALDI-TOF: Calculated [Mₓ+Na⁺]=3156.37 m/z, found [Mₓ+Na⁺]= 3156.86 m/z
Figure S1. MALDI-ToF results for HFD(ei)-G1-e-(Ac)$_3$-i-(ene)$_3$ 3 and HFD(ei)-G1-e-(OH)$_6$-i-(ene)$_3$ 4
Figure S2. MALDI-ToF results for HFD(ei)-G1-e-(Ac)$_3$-$i$-(azide)$_3$ 6 and HFD(ei)-G1-e-(OH)$_6$-$i$-(azide)$_3$ 7

Figure S3. MALDI-ToF results for postfunctionalization of scaffold 5 using acetylene functional mannose
Figure S4. MALDI-ToF results for postfunctionalization of scaffolds 5 and 8 using acetylene functional DOPA 16 or 4-pentynoic acid.

Instrumentation

MALDI-TOF MS: Bruker UltraFlex MALDI-TOF MS with SCOUT-MTP Ion Source (Bruker Daltonics, Bremen) prepared with a gridless ion source, N$_2$-laser (337nm), and reflector design was used. The intensity of the laser was set to the lowest possible to acquire high resolution spectra of the product and all spectra were acquired using a reflector-positive mode. The instrument was calibrated using SpheriCal™ calibrants purchased from Polymer Factory Sweden AB. The received spectra were analyzed with FlexAnalysis Bruker Daltonics, Bremen, version 2.2.

$^1$H NMR and $^{13}$C NMR: Bruker Avance 400 MHz NMR instrument was used. Proton NMR spectra’s were acquired with a spectral window of 20 ppm, an acquisition time of 4 seconds and a relaxation
delay of 1 second. $^{13}$C NMR spectra were acquired with a spectral window of 240 ppm, an acquisition time of 0.7 seconds and a relaxation delay of 2 seconds.

**Shear strength:** The mechanical shear strength test was performed using Instron 5566 with a load cell of 10 kN or a static testing machine 5567 with a 30kN load cell at a cross head speed of 10mm/min.

**UV sources:** The thiol-ene crosslinking of the hydrogels were preformed Black ray lamp of 100W with an intensity of 9 mW cm$^{-2}$ for 10 min. The thiol-ene crosslinking of the FRAP system was induced by UV light using a Fusion UV Curing System Model F300 equipped with an Hg-bulb. The dose was measured using UVICURE Plus (320-390 nm) from EIT Inc., Sterling, VA, USA. A dose of 0.36 J cm$^{-2}$ was used in the first layer and 1.09 J cm$^{-2}$ in the second.

**Rheometry:** Hydrogel rheological measurements were performed using a ARES RDA-III rheometer, TA

**Matrix preparation for MALDI:**

The matrix (dihydroxybenzoic acid sinapic acid or 9-Nitroanthracene) was dissolved in THF (1 mg ml$^{-1}$). Trifluoro acetic acid (TFA) sodium salt was dissolved in THF (1 mg ml$^{-1}$). The product was dissolved in THF or methanol dependent on its solubility in a concentration of 1 mg ml$^{-1}$. In the sample preparation was 20: 5: 5 µl of matrix: salt: product mixed and 0.5 µl of this mixed solution was added to the MALDI target plate.

**Bone preparation and patch fabrication.**

Bovine femur bones received from an abattoir and split into rectangular species with dimensions of approximately 50x10x5mm were used. All samples were wet sanded with grain size of 80 until a smooth and even surface was achieved. A generic fracture was created by sawing each rod into two halves.
bone surface of the bone halves was firstly covered with a thin layer of the primer and after a thin layer of matrix, and UV cured with a dose of 0.35 J/cm². The primer was applied to the bone surface, using a solution of 0.50mg/ml in ethanol and the acid functional dendrimers was dissolved in a 1:1 solution of ethanol: water. Then after five E-glass fibers were embedded between two layers of the thiol-ene matrix and UV cured a second time in a dose of 1.05 J/cm². The doses used for HFD(ei)-G1-e-(ene)₆-i-(azide)₃ and HFD(ei)-G1-e-(ene)₆-i-(DOPA)₃ were 0.70 J/cm² the first time and 1.75J/cm² the second time. The thiol-ene mixture used consisted of 1,3,5-triallyl-1,3,5-triazine-2,4,6-trione and tris[2-(3-mercaptopropinyloxy) ethyl] mixed in a 1:1 molar ratio. A tip of a knife of Irgacure 184 was used as initiator to the thiol-ene matrix. The cured samples were submerged into a 0.9 wt% NaCl solution in deionized water to mimic the in vivo conditions. The adhesive area of the bone was measured using a digital slide caliper.

**Mechanical test**

The tensile tests were performed by insertion of two parallel metal pins throw the ends of the bone, and connecting them via a wire to the load cell. All bones were tested until a cohesive or adhesive break of the patch was observed and the maximum load (N) was measured, Table S1. The shear strength was calculated by dividing the maximal load with the adhesive area, after failure, and only the bones with adhesive failure was used in the calculations.

<table>
<thead>
<tr>
<th>HFD Scaffold</th>
<th>Primer (50 mg/mL EtOH)</th>
<th>Mean Shear Strength (MPa)*</th>
<th>Standard deviation</th>
</tr>
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<tbody>
<tr>
<td>Reference 1</td>
<td>No primer</td>
<td>0.4</td>
<td>0.3</td>
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<tr>
<td>10</td>
<td>HFD(ei)-G1-e-(DOPA)₆-i-(ene)₃</td>
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<td>1.02</td>
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<td>0.77</td>
</tr>
<tr>
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<td>HFD(ei)-G1-e-(ene)₆-i-(azide)₃</td>
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<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Formula</td>
<td>Shear Strength</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
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</tr>
<tr>
<td>9</td>
<td>HFD(ei)-G1-e-(ene)6-i-(DOPA)3</td>
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<tr>
<td>Reference 2</td>
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<tr>
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<td>HFD(ei)-G1-e-(azide)6-i-(ene)3</td>
<td>4.2</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Table S1. Overall shear strength values obtained from FRAPs with 5 layers of 90/0 E glass fibers.

**Hydrogel formation**

The hydrogels were formed by mixing the dendritic scaffolds, HFD (ei)-G1-e-(azide)6-i-(ene)3 5 or HFD (ei)-G1-e-(ene)6-i-(azide)3 8, with dithiolPEG6kDa 14 in EtOH (30 wt% dry content, equimolar concentrations of thiols and enes). From a stock solution in EtOH 0.5 wt% of Irgacure 2959 was added under darkness and the mixtures were vortexed until homogeneity. They were thereafter pipetted either into circular Teflon molds (Ø = 10 mm, depth 1 mm) and covered by a glass slide (swelling samples) or onto a flat glass plate and covered with a glass slide held by 1 mm spacers (rheometer samples). Curing was induced by irradiation for 10 min (9 mW cm⁻², 365 nm).

**General procedure of the swelling test of hydrogels.**

The cured hydrogels were dried in room temperature overnight and one additional hour at 50 °C in vacuum. They were thereafter swelled in deionized water and the weight was measured after 1h, 2h, 4h, 6h, 24h, 48h and 72 hours.

**Rheology measurements**

Hydrogel rheological measurements were performed using a ARES RDA-III rheometer (TA) with γ = 5% and ω= 10 rad s⁻¹ (linear viscoelastic regime). Hydrogels, which had been swelled for 72 h were punched out as Ø 25 mm circular discs and were positioned between the Ø 25 mm parallel plates of the instrument. The hydrogel storage modulus (G’) was measured during 240s (in these cases G = G’ since G’ > G’”). The storage modulus was additionally converted to Young’s modulus (E) according to rubber
Elasticity theory, where $G = \frac{E}{2(1+\nu)}$, assuming a Poisson’s ratio ($\nu = 0.5$) for bulk measurements of elastic hydrogels.

References