Supplementary Information

Thiol-methylsulfone-based hydrogels for cell encapsulation: reactivity optimization of aryl-methylsulfone substrate for fine-tunable gelation rate and improved stability

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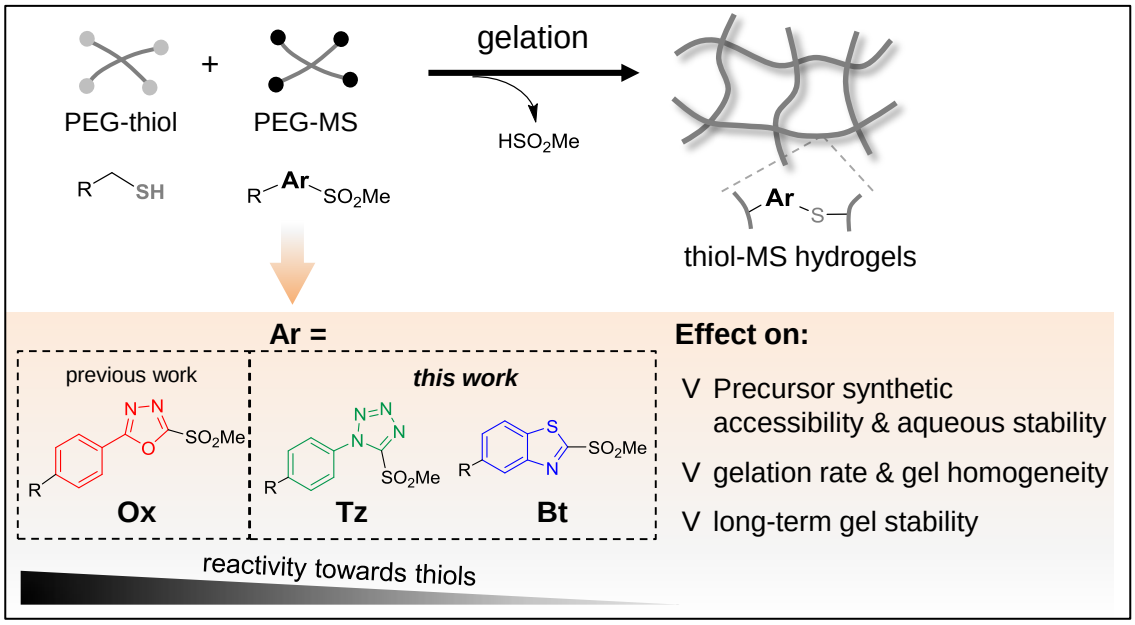

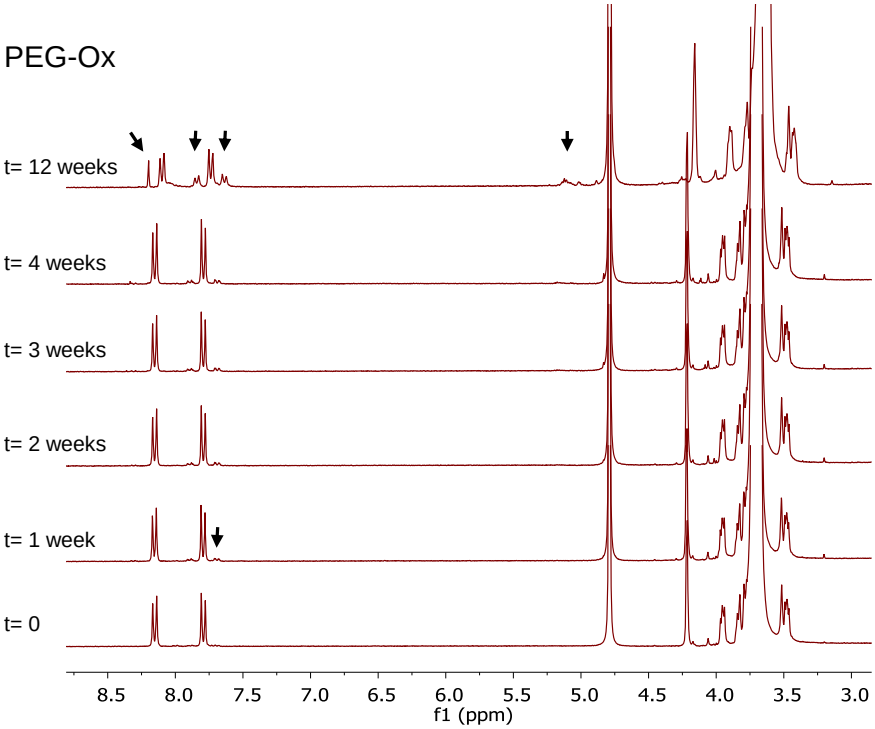
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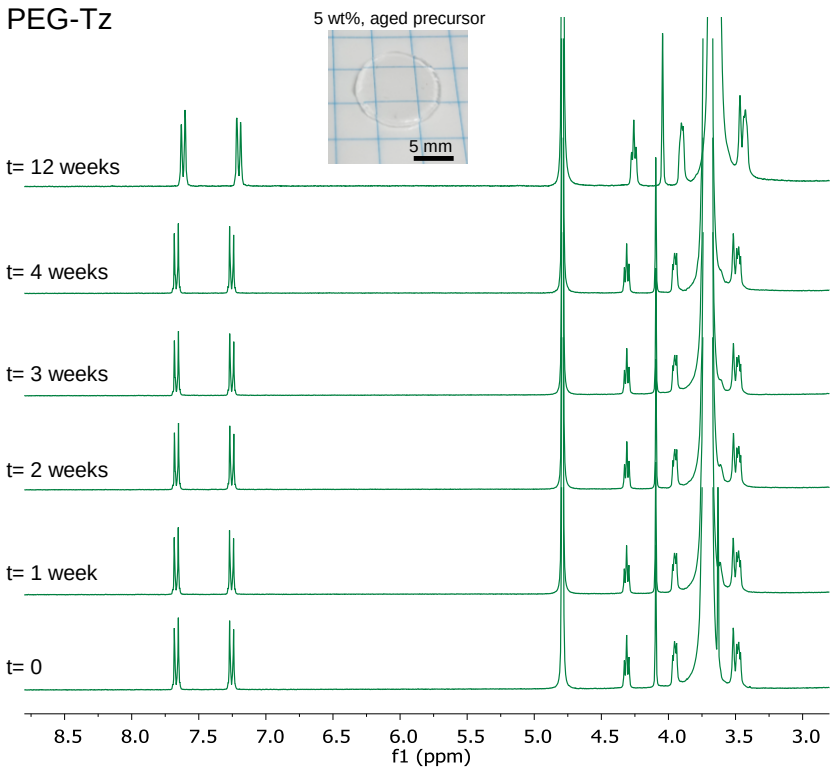
f1 (ppm)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f1 (ppm)

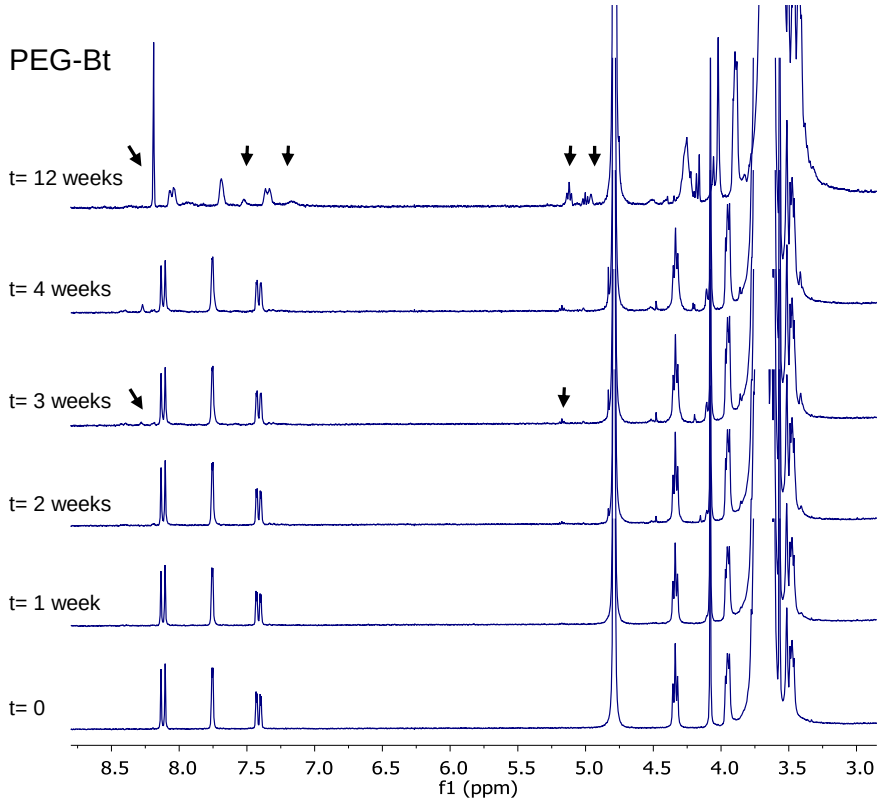


Figure S1. Stability study of aqueous solutions of the different PEG-MS macromers over 12 weeks of storage at room temperature, as measured by ^{1}H NMR. Conditions: 1 mM (20 mg mL^{-1}) PEG-MS solution in D_2O at 25°C. The PEG-MS macromer solutions were prepared in D_2O and ^{1}H spectra were recorded at increasing aging times t= 0, 1, 2, 3, 4 and 12 weeks. During the aging period, samples were stored at room temperature and exposed to normal light conditions in the laboratory. Black arrows indicate the appearance of new signals during ageing time. PEG-Ox and PEG-Bt presented changes in the aromatic (7.20- 8.20 ppm) and aliphatic (4.8-5.20 ppm) regions. At t= 12 weeks, the original aromatic signals decreased ca 35% for PEG-Ox and 33% for PEG-Bt; and new aromatic signals were observed. In addition, new aliphatic signals appeared at ca 5.00 ppm. In contrast, PEG-Tz did not evidence changes in the spectra over 12 weeks of ageing time, indicating high stability of the precursor solution. The aged PEG-Tz precursor can still be used for preparation of a thiol-MS gel at 5 wt% concentration, as shown in the picture.

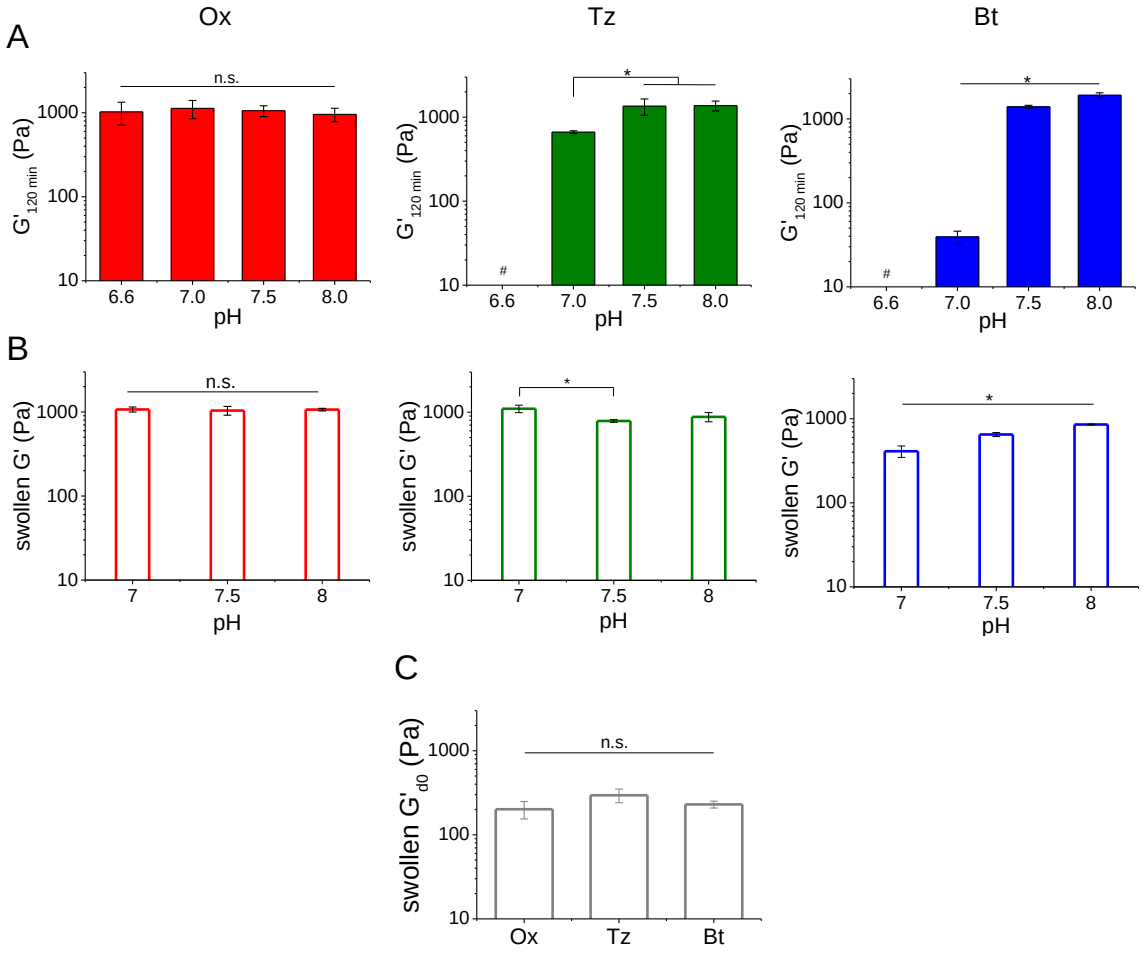


Figure S2. Effect of MS reactivity of the different thiol-MS shear gels. **A)** Final $G'_{120\ min}$ (before swelling) after 2 h curing. **B)** Final $G'_{120\ min}$ after 2 h curing and 24 h swelling in the given buffer. Gel composition A-B: 5 wt% polymer concentration, 20 mM HEPES at the indicated pH, 25°C. **C)** Post-swell G'_{d0} of the different acellular thiol-MS gels, after 45 min curing and 24 h swelling. Gel composition: 4 wt% polymer, 0.06 wt% (1 mM) cyclo(RGDfC), 0.6 wt % (3.5 mM) VPM peptide, 20 mM HEPES pH 8.0. Data are mean ± SD (n= 3); *p < 0.05, one-way ANOVA with Tukey post-hoc test, n.s. means not significant. The # symbol indicates that no gel was formed under that specific condition.

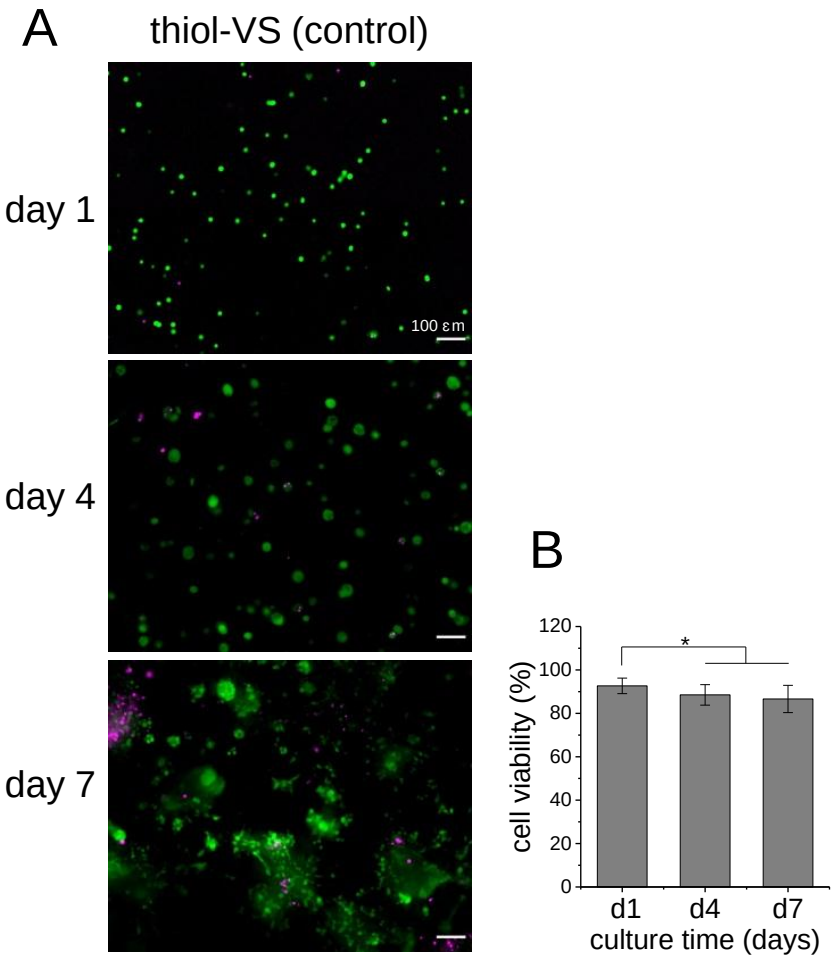


Figure S3. Encapsulation of L929 fibroblasts in control thiol-VS gels. **A)** Fluorescence images and **B)** viability quantification, showing post-encapsulation survival at 1, 4 and 7 days. Live (green)/ dead (magenta) assay was performed (scale bar = 100 μm). Gel composition: 5 wt% polymer, 0.07 wt% (1.3 mM) cyclo(RGDfC) and 0.7 wt % (4.3 mM) VPM, in 20 mM HEPES pH 8.0. In B, data are mean ± SD (n= 3); *$p<0.05$, one-way ANOVA with Tukey post-hoc test. All cell-laden thiol-VS gels prepared this way remained stable for at least 16 days under cell culture conditions (n=3).

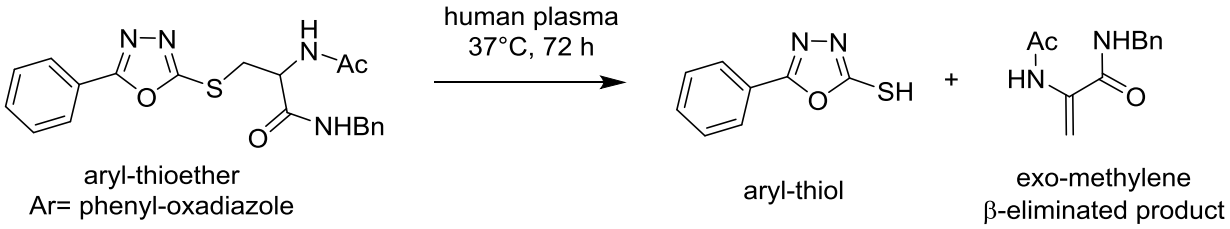


Figure S4. Reported cleavage of Ox-based aryl-thioether adduct into aryl-thiol and exo-methyl-eliminated product, upon incubation in human plasma.[1]

Supplementary Experimental Section

Materials and general methods

Chemicals and solvents were purchased from Fluka Chemie AG (Taufkirchen, DE), Merck KGaA (Darmstadt, DE), ABCR (Karlsruhe, DE), AcrosOrganics (Geel, BE), TCI (Eschborn, DE) and Sigma-Aldrich Chemie GmbH (Steinheim, DE). Solvents had p.a. purity and were used as purchased unless specified. 4-(5-(methylsulfonyl)-1,3,4-oxadiazol-2-yl)aniline was purchased from Ark Pharm (U.S.A.). 2-amino-N-(4-(5-(methylsulfonyl)-1,3,4-oxadiazol-2-yl)phenyl)acetamide (**Ox-5**) and the macromer 4-arm (20 kDa) PEG-Ox were synthesized according to our reported procedure.[2] 4-arm (20 kDa), polyethylenglycol (PEG) polymers functionalized with thiol (PEG-SH) and succinimidyl carboxymethyl ester (PEG-NHS) were purchased from Jenkem (U.S.A.) Buffer solutions were freshly prepared. 10 mM HEPES buffers (pH 8.0; 7.5, 7.0 and 6.6) were used, unless otherwise stated.

Thin layer chromatography (TLC) plates (ALUGRAM® SIL G/UV254) and silica gel for column chromatography (60 Å pore size, 63-200 µm particle size) were obtained from Macherey-Nagel, (Düren, DE). TLC plates were observed under 254 or 365 nm light. HPLC analysis and purification of the compounds were performed with a HPLC JASCO 4000 (Japan) equipped with a diode array, UV/Vis detector and fraction collector. Reprosil C18 columns were used for semi-preparative (250 × 25 mm) and analytical (250 × 5 mm) runs. Solvent gradients using a combination of the following eluents were used: solvent A (MilliQ water + 0.1% TFA) and solvent B (95% ACN/5% MilliQ water + 0.1% TFA), with runs typically over 40 min duration. Automated flash chromatography, using a Biotage® Selekt system equipped with UV/Vis detector and fraction collector, was performed for the purification of some intermediates. Purification of modified polymers was performed by dialysis against acetone and water, using a Spectra/Por 3 dialysis tubing (molecular weight cut-off MWCO= 3.5 kDa) from Spectrum Inc, and solvent was renewed at least 4 times.

Deuterated solvents were obtained from Deutero GmbH Germany (Kastellaun, DE). Solution ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 25 °C on a Bruker Avance 300 MHz or on a Bruker Avance III UltraShield 500 MHz. The latter was equipped with a He-cooled 5 mm TCI-CryoProbe, a proton-optimized triple resonance NMR ó water cooling unit (CP TCI 500S2, H-C/N-D-05 Z). Unless otherwise stated, all measurements were taken at 298 K, and the solvent residual peak (S.R.P.) was employed as

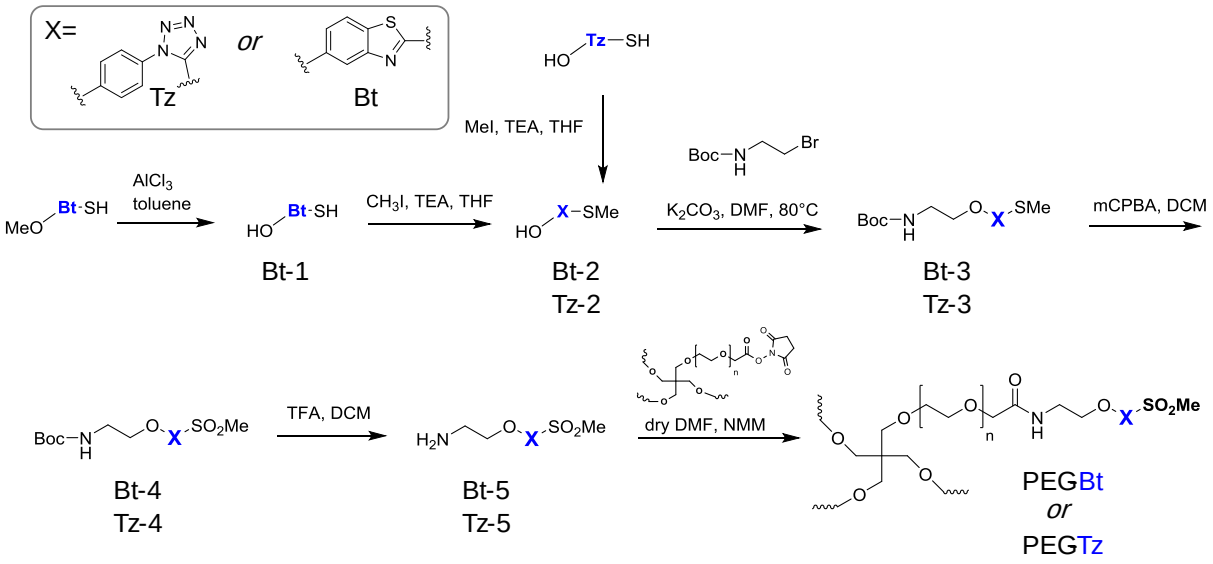
internal reference. The chemical shifts (ŭare) informed in parts per million and the coupling constants in Hz. The following abbreviations are used: *s*-singlet, *d*-doublet, *t*-triplet, *q*-quartet, *m*-multiplet. The degree of substitution of PEG polymer was calculated by end-group determination. To this purpose, the integral of the signal corresponding to the PEG backbone (3.70-3.40 ppm) was set to 440 H and compared with the integral of the protons corresponding to the bound molecule (the aromatic protons at ŭ =8.0-7.0 ppm and the methylene at ŭ =4.2 ppm). Functionalization degrees of >90% and yields of >80% were obtained in all cases. Data was analyzed and plotted with MestReNova.

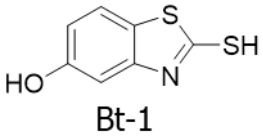
Electrospray ionization mass spectrometry (ESI-MS) was recorded with a 1260 Infinity Liquid Chromatography/Mass Selective Detector (LC/MSD) (Agilent Technologies, DE) and Quadrupole Time-of-Flight (Q-TOF) with a 6545 Accurate-Mass Quadrupole Time-of-Flight (LC/Q-TOF-MS) (Agilent Technologies, DE) using electrospray ionization. Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry was recorded with an AB Sciex 4800 (Sciex-Company, DE) in linear mode in the mass range from 4000-40000 Da. For sample preparation, dithranol (1,8,9-anthracenetriol) was used as matrix and acetonitrile, MilliQ water and THF were used as solvents. Formic acid was added to improve ionization. About 4800 single shots were accumulated for one spectrum for each sample.

The molar mass of PEG-MS macromers was characterized by gel permeation chromatography (GPC). The GPC system comprised a Waters 515 HPLC Pump (Waters, Milford, U.S.A.), three GRAM PSS (Mainz, DE) columns in series (GRAM 30, GRAM 100, GRAM 100), a Waters 2410 refractive index detector, a Waters 2487 UV detector (operating ə= 260 nm). 4-arm PEG standard kit of molar mass 2, 5, 10, 20 and 40 kDa (Jenkem USA) was used for calibration and DMF containing 1g L^{-1} LiBr was used as eluent. Runs were performed at T= 60 °C, flow= 1 mL min^{-1}, polymer concentration= 2.1 mg mL^{-1} in DMF.

Chemical Synthesis

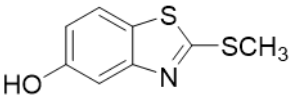
Synthetic pathway to benzothiazole (Bt) methylsulfonyl and tetrazole (Tz) methylsulfonyl derivatives





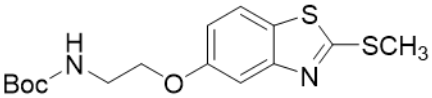
Bt-1

*2-mercaptobenzo[d]thiazol-5-ol (**Bt-1**).* A protocol previously reported by Barbas and coworkers[1] was followed, with some modifications. To a mixture of 5-methoxybenzo[d]thiazole-2-thiol (1.0 g, 5 mmol, 1 eq.) in toluene (40 mL), anhydrous $AlCl_3$ (2.0 g, 10 mmol, 2 eq.) was added at room temperature. The reaction mixture was stirred at reflux for 3 h (mixture color turned dark green). When consumption of starting reagent was observed by TLC, the reaction mixture was cooled to 0°C and 1 M HCl (15 mL) was added (mixture color turned red). The suspension was then filtered and the precipitate was collected, which was further dissolved using 1 M NaOH. The mixture was again filtered and the filtrate was collected (a clear solution was observed). The solution was acidified using acetic acid (pH 5-6), a precipitate developed that was collected by centrifugation and freeze dried. The residue was purified using column chromatography (30% EtOAc / hexane), to afford a pale grey solid (0.72 g, yield= 79%). Spectroscopic characterization data matched reported values in the literature. ESI-MS+: 367.0 (2M+H). ^{1}H-NMR (300 MHz, DMSO-d_6, ŭ[ppm]) = 7.10 (1H, d, *J*= 8.3 Hz, -CH Ar), 6.68 (1H, d, *J*= 2.3 Hz, -CH Ar), 6.40 (1H, dd, *J*= 8.3, 2.4 Hz, -CH Ar). ^{13}C-NMR (75 MHz, DMSO-d_6, ŭ[ppm]) = 190.27, 157.15, 142.54, 122.23, 118.89, 112.92, 99.04.



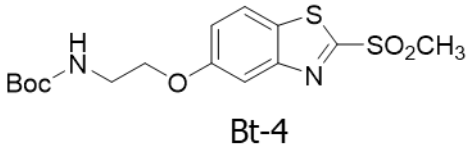
Bt-2

*2-(methylthio)benzo[d]thiazol-5-ol (**Bt-2**).* A protocol previously reported was followed,[1] with some modifications. Compound **Bt-1** (0.72 g, 3.9 mmol, 1 eq.) was dissolved in THF (30 mL) at 0°C, followed by addition of TEA (0.7 mL, 4.7 mmol, 1.2 eq.) and methyl iodide (0.27 mL, 4.3 mmol, 1.1 eq). The reaction mixture was stirred at room temperature for 4 h. Then, water (15 mL) was added to the mixture and the product was extracted with EtOAc twice. The combined organic layer was washed with water (4x) and brine, dried over magnesium sulfate, filtered to remove solids and evaporated. The pure product was obtained as a white solid (0.66 g, yield= 85%). Spectroscopic characterization data matched reported values in the literature. ESI-MS+: 198.0.0 (M+H). ^{1}H-NMR (300 MHz, DMSO-d_6, ŭ[ppm]) = 9.67 (1H, s, -OH), 7.74 (1H, d, *J*= 8.7 Hz, -CH Ar), 7.18 (1H, d, *J*= 2.3 Hz, -CH Ar), 6.84 (1H, dd, *J*= 8.7, 2.4 Hz, -CH Ar), 2.75 (3H, s, -SMe). ^{13}C-NMR (75 MHz, DMSO-d_6, ŭ[ppm]) = 168.43, 156.58, 154.32, 124.45, 121.87, 113.81, 106.91, 15.48.



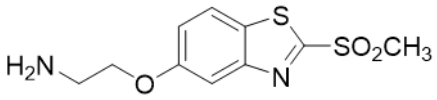
Bt-3

*tert-butyl (2-((2-(methylthio)benzo[d]thiazol-5-yl)oxy) ethyl)carbamate (**Bt-3**).* Compound **Bt-2** (0.30 g, 1.5 mmol, 1 eq.) was dissolved in DMF (20 mL), followed by the addition of 2-(Boc-amino)ethyl bromide (0.67 g, 3 mmol, 2 eq.) and potassium carbonate (0.68 g, 5 mmol, 3.3 eq.) at room temperature. The reaction mixture was stirred at 80°C overnight. The mixture was allowed to reach room temperature, filtered to remove solids and evaporated to reduce volume. The residue was diluted with EtOAc, washed with water and brine, dried over magnesium sulfate, filtered to remove solids and evaporated. The residue was purified by preparative HPLC (method: 20B to 95B, 280 nm). The pure product was obtained as a white powder (0.32 g, yield= 62%). ESI-MS+: 341.0 (M+H). ^{1}H-NMR (300 MHz, DMSO-d_6, ŭ[ppm]) = 7.86 (1H, d, *J*= 8.8 Hz, -CH Ar), 7.40 (1H, d, *J*= 2.5 Hz, -CH Ar), 7.05-7.01 (1H, m, -NH amide), 6.98 (1H, dd, *J*= 8.8, 2.5 Hz, -CH Ar), 4.03 (2H, t, *J*= 5.8 Hz, -CH_2O), 3.31 (2H, q, *J*= 5.8 Hz, -CH_2NH), 2.77 (3H, s, -SMe), 1.38 (9H, s, -tBu Boc). ^{13}C-NMR (75 MHz, DMSO-d_6, ŭ[ppm]) = 169.17, 157.85, 155.80, 154.26, 126.28, 122.16, 113.93, 105.31, 77.92, 66.82, 45.84, 28.31, 15.61.



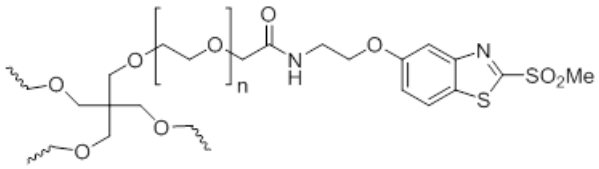
Bt-4

*tert-butyl (2-((2-(methylsulfonyl)benzo[d]thiazol-5-yl)oxy)ethyl)carbamate (**Bt-4**).* Compound **Bt-3** (0.1 g, 0.3 mmol, 1 eq.) was dissolved in dry DCM (10 mL) and cooled down to 0°C with an ice bath, followed by addition of mCPBA (0.25 g, 1.4 mmol, 5 eq.) as a solid. The reaction mixture was stirred overnight at room temperature. The course of the reaction was monitored by mass spec and ^{1}H NMR analysis of an aliquot of the reacting crude. The reaction mixture was then filtered to remove solids and evaporated. The residue was purified by preparative HPLC (method: 20B to 95B, 280 nm) to afford the pure product as a pale yellow solid (0.093 g, yield= 85%). ESI-MS+: 317.0 (2M-SO_2Me+Na). ^{1}H-NMR (300 MHz, acetone-d_6, ŭ[ppm]) = 8.15 (1H, d, *J*= 9.0 Hz, -CH Ar), 7.73 (1H, d, *J*= 2.5 Hz, -CH Ar), 7.33 (1H, dd, *J*= 9.0, 2.5 Hz, -CH Ar), 6.33-6.17 (1H, m, -NH amide), 4.23 (2H, t, *J*= 5.7 Hz, -CH_2O), 3.54 (2H, q, *J*= 5.8 Hz, -CH_2NH), 3.48 (3H, s, -SO_2Me), 1.41 (9H, s, -tBu Boc). ^{13}C-NMR (75 MHz, acetone-d_6, ŭ [ppm]) = 169.05, 160.13, 154.96, 154.78, 129.46, 124.20, 120.14, 107.94, 78.91, 68.30, 42.54, 40.52, 28.57.

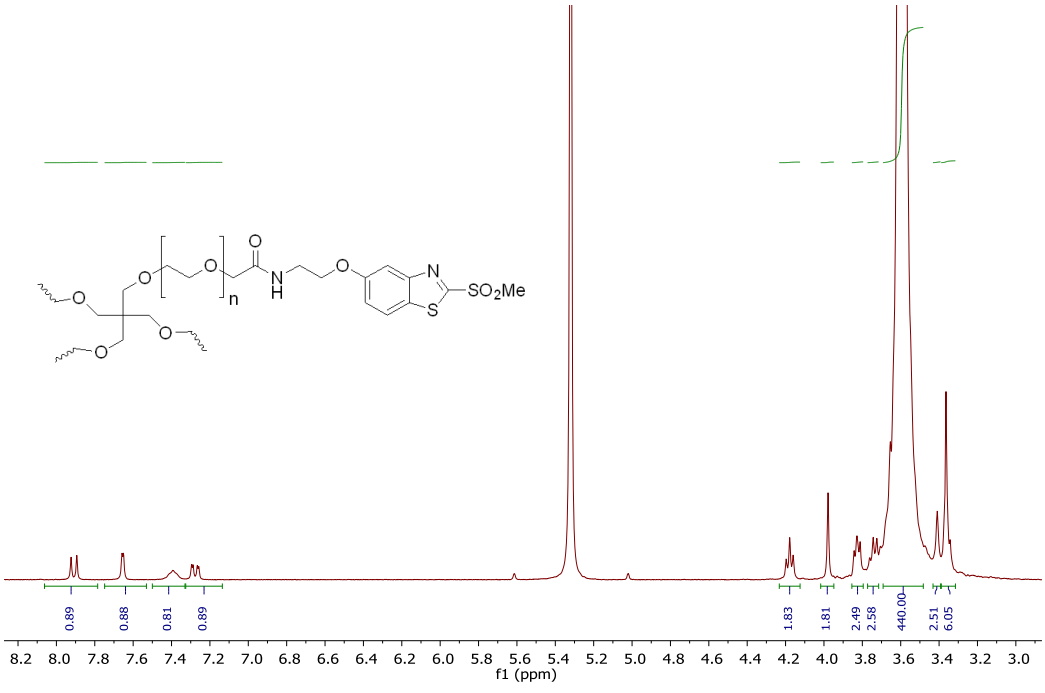


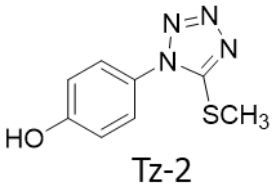
Bt-5

*2-((2-(methylsulfonyl)benzo[d]thiazol-5-yl)oxy)ethan-1-amine (**Bt-5**).* Compound **Bt-4** (0.073 g, 0.2 mmol, 1 eq.) was dissolved in DCM/TFA 1:1 (2 mL), purged under nitrogen and stirred at room temperature. When consumption of starting reagent was observed (ca. 30-45 min as checked by TLC), the solvent was evaporated under nitrogen stream and the residue was purified by preparative HPLC (method: 20B to 95 B, 280 nm). The pure product was obtained as a pale yellow solid (0.04 g, yield= 75%) and was used for the next reaction immediately. ESI-MS+: 284.0 (M+H). ^{1}H-NMR (300 MHz, acetone-d_6, ŭ[ppm]) = 8.17 (1H, d, *J*= 9.0 Hz, -CH Ar), 7.76 (1H, d, *J*= 2.5 Hz, -CH Ar), 7.36 (1H, dd, *J*= 9.0, 2.5 Hz,-CH Ar), 4.63 (2H, t, *J*= 5.0 Hz,-CH_2O), 4.39 (2H, t, *J*= 5.0 Hz,-CH_2NH_2), 3.48 (3H, s, -SO_2Me). ^{13}C-NMR (75 MHz, acetone-d_6, ŭ[ppm]) = 169.36, 159.29, 154.77, 130.11, 124.37, 119.87, 108.33, 66.15, 47.70, 42.56.



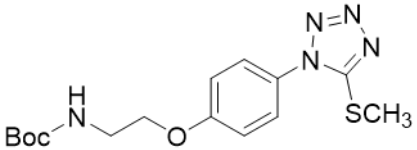
*Synthesis of **4-arm PEG-Bt**.* Freshly prepared compound **Bt-5** (40 mg, 147 μmol, 10 eq.) and *N*-methylmorpholine (58 μL, 53 μmol, 3.6 eq.) were dissolved in dry DMF (3 mL), purged with nitrogen and stirred for 15 min. 20kDa, 4-arm PEG-NHS (294 mg, 14.7 μmol, 1 eq.) was dissolved in dry DMF (2 mL) and added to above solution under nitrogen stream. The mixture was stirred overnight at room temperature under inert atmosphere, then dialyzed in acetone and water, and freeze-dried. A pale yellow solid polymer was obtained and characterized by ^{1}H NMR in DCM-d_2. Functionalization degree was calculated as 90% and yield was 80%. The polymer prepared this way proved stable as solid upon >6 months storage at -20°C (evidenced by no changes in ^{1}H-NMR spectrum). ^{1}H-NMR (300 MHz, DCM-d_2, δ [ppm]) = 7.91 (t, *J*= 9.0 Hz, -CH Ar), 7.65 (t, *J*= 2.4 Hz, -CH Ar), 7.39 (m, -NH amide), 7.28 (dd, *J*= 9.0, 2.5 Hz, -CH Ar), 4.18 (t, *J*= 5.5 Hz, -CH_2O), 3.98 (s, -OCH_2C=O PEG), 3.86-3.31 (m, PEG core, -CH_2NH and -SO_2Me group). Substitution degree = 90%.





Tz-2

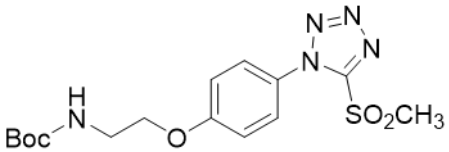
*4-(5-(methylthio)-1H-tetrazol-1-yl)phenol (**Tz-2**).* A protocol previously reported by Barbas and coworkers[1] was followed, with some modifications. 4-(5-mercapto-1H-tetrazol-1-yl)phenol (3.00 g, 15.4 mmol, 1 eq.) was dissolved in THF (65 mL) and the solution was cooled to -10°C using a bath of dry ice in acetone. To this solution, TEA (2.58 mL, 18.5 mmol, 1.2 eq.) and methyliodide (0.96 mL, 15.4 mmol, 1eq.) were added, and the reaction mixture was stirred for 3 h at -10°C. The course of the reaction was monitored every 30 min by TLC (50% EtOAc/hexane). When all the starting reactant was consumed (ca 3 h), the reaction mixture was quenched by adding 1 M HCl and allowed to reach room temperature. The organic layer was extracted with EtOAc and then with brine, dried over magnesium sulfate, filtered to remove solids and evaporated. The solid residue was then purified by flash chromatography (45% EtOAc in hexane) and the expected product was obtained in high purity as a white solid (2.91 g, yield= 74%), and used directly for the next step. The expected product was obtained in high purity as a white solid (0.63 g, yield= 61%) and used directly for the next step. Spectroscopic characterization data matched reported values in the literature. ESI-MS+: 209.0 (M+H), 439.2 (2M+Na). ^{1}H-NMR (300 MHz, acetone-d_6, ŭ[ppm]) = 9.13 (1H, s, -OH); 7.46 (2H, dd, J_1= 8.5 Hz, J_2= 1.5 Hz, -CH Ar;); 7.08 (2H, dd, J_1= 8.5 Hz, J_2= 1.5 Hz, -CH Ar); 2.79 (3H, s, -SMe). ^{13}C-NMR (125 MHz, acetone-d_6, ŭ[ppm]) = 160.00, 155.97, 126.97, 126.27, 117.12, 15.29.



Tz-3

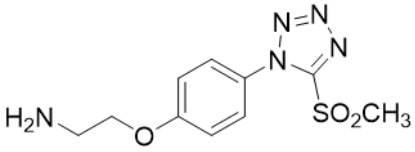
*tert-butyl (2-(4-(5-(methylthio)-1H-tetrazol-1-yl)phenoxy)ethyl)carbamate (**Tz-3**).* Compound **Tz-2** (2.90 g, 13.9 mmol, 1 eq.) was dissolved in DMF (100 mL), followed by the addition of 2-(Boc-amino)ethyl bromide (4.68 g, 20.9 mmol, 1.50 eq.) and K_2CO_3 (6.35 g, 46.0 mmol, 3.30 eq.) at room temperature. The reaction mixture was stirred at 80°C overnight. Then, another equivalent of 2-(Boc-amino)ethyl bromide was added and the reaction was stirred at 80°C overnight. Reaction completion was confirmed by ^{1}H NMR spectroscopy. The mixture was allowed to reach room temperature, filtered to remove solids, and evaporated to reduce volume. The residue was diluted with EtOAc, washed with brine and water, dried over magnesium sulfate, filtered to remove solids and evaporated. Further purification was performed by flash chromatography (36% EtOAc in hexane). The pure product was obtained

as a white powder (3.28 g, yield = 67%). ESI-MS+: 352.2 (M+H). ^{1}H-NMR (300 MHz, acetone-d_6, ŭ[ppm]) = 7.60-7.52 (2H, m, -CH Ar); 7.24-7.17 (2H, m, -CH Ar); 6.26 (1H, m, -NH amide), 4.18 (2H, t, *J*= 5.7 Hz, -CH2O), 3.55-3.47 (2H, m, -CH2NH), 2.79 (3H, s, -SMe), 1.41 (3H, s, -tBu Boc). ^{13}C-NMR (75 MHz, acetone-d_6, ŭ[ppm]) = 161.15, 156.73, 156.01, 127.38, 126.87, 116.38, 78.89, 68.23, 40.49, 28.60, 15.38.



Tz-4

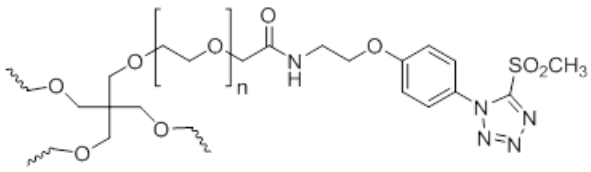
*tert-butyl (2-(4-(5-(methylsulfonyl)-1H-tetrazol-1-yl)phenoxy)ethyl)carbamate (**Tz-4**).* Compound **Tz-3** (3.00 g, 8.54 mmol, 1 eq.) was dissolved in dry DCM (200 mL) and cooled down to 0°C with an ice bath, followed by addition of mCPBA (7.36 g, 42.7 mmol, 5 eq.) as a solid. The reaction mixture was stirred overnight at room temperature. The course of the reaction was monitored by mass and ^{1}H NMR spectroscopy of an aliquot of the reaction mixture, since TLC and analytical HPLC proved not useful because of the similarity in the elution of starting reactant and product (i.e. they both had similar Rf or Rt in a variety of eluents). The reaction mixture was then filtered to remove solids and evaporated. The residue was subjected to flash chromatography (gradient 20-50% EtOAc in hexane) to afford the pure product as a colorless transparent sticky solid (2.89 g, yield = 88%). ESI-MS+: 422.0 (M+K), 687.2 (2M-SO_2Me). ^{1}H-NMR (300 MHz, acetone-d_6, ŭ[ppm]) = 7.76-7.59 (2H, m, -CH Ar); 7.29-7-12 (2H, m, -CH Ar); 6.27 (1H, m, -NH amide), 4.19 (2H, t, *J*= 5.6 Hz, -CH2O), 3.62 (3H, s, -SO_2Me), 3.52 (2H, q, *J*= 5.6 Hz, -CH2N), 1.41 (3H, s, -tBu Boc). ^{13}C-NMR (75 MHz, acetone-d_6, ŭ [ppm]) = 161.89, 156.86, 155.38, 128.29, 126.88, 116.14, 115.91, 78.99, 68.25, 44.20, 40.54, 28.56.



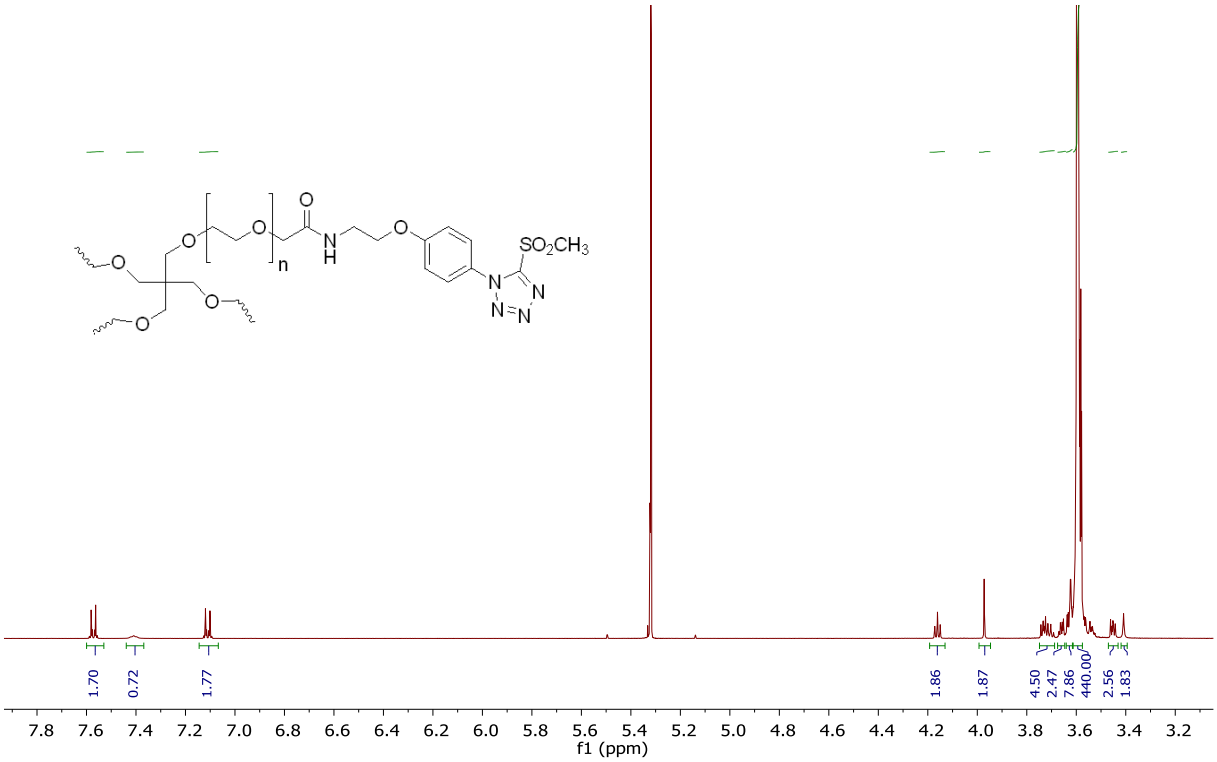
Tz-5

*2-(4-(5-(methylsulfonyl)-1H-tetrazol-1-yl)phenoxy)ethan-1-amine (**Tz-5**).* Compound **Tz-4** (0.100 g, 0.26 mmol, 1 eq.) was dissolved in DCM (1 mL). After addition of a DCM/TFA 1:1 mixture (2 mL) the reaction solution was purged under nitrogen and stirred at room temperature. When consumption of starting reagent was observed (ca. 30-45 min as checked by TLC in 50% EtOAc/hexane), the solvent was evaporated under nitrogen stream and the residue was

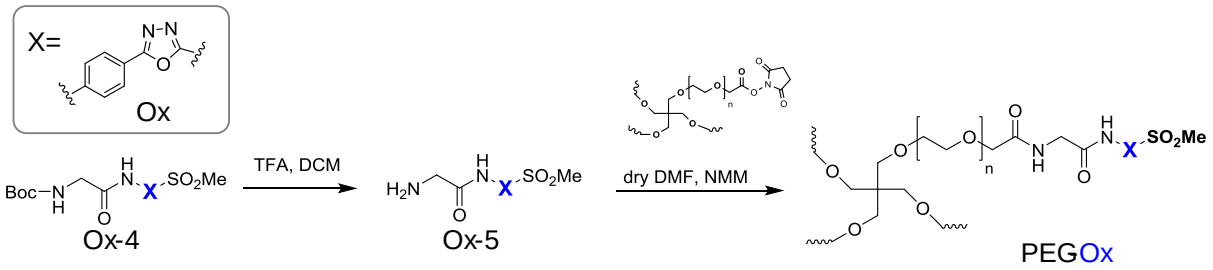
purified by preparative HPLC (method: 20B to 95 B, 280 nm; r.t.: 12 min). The pure product was obtained as a colorless transparent sticky solid (0.065 g, yield = 80%) and was used for the next reaction immediately. ESI-MS+: 284.0 (M+H). ^{1}H-NMR (300 MHz, acetone-d_6, ŭ [ppm]) = 7.72-7.67 (2H, m, -CH Ar); 7.27-7.22 (2H, m, -CH Ar); 4.56 (2H, t, *J*= 5.0 Hz, -CH_2O), 4.35 (2H, t, *J*= 5.0 Hz, -CH_2NH_2), 3.63 (3H, s, -SO_2Me). ^{13}C-NMR (75 MHz, acetone-d_6, ŭ[ppm]) = 161.05; 155.40; 128.40; 127.51; 116.06; 66.07; 47.60; 44.23.

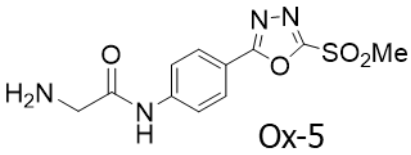


*Synthesis of **4-arm PEG-Tz**:* Freshly prepared compound **Tz-5** (0.092 g, 325 ămol, 10 eq.) and N-methylmorpholine (12.8 µL, 117 ămol, 3.6 eq.) were dissolved in dry DMF (2 mL), purged with nitrogen and stirred for 15 min. 20kDa, 4-arm PEG-NHS (0.65 g, 32.5 ămol, 1 eq.) was dissolved in dry DMF (3 mL) and added to above solution under nitrogen stream. The mixture was stirred overnight at room temperature under inert atmosphere, then dialyzed in acetone and water, and freeze-dried. A white solid polymer was obtained and characterized by ^{1}H NMR in DCM-d_2. Functionalization degree was calculated as >95% and yield was 93%. The polymer prepared this way proved stable as solid upon >6 months storage at -20°C (evidenced by no changes in ^{1}H-NMR spectrum). ^{1}H-NMR (500 MHz, DCM-d_2, ŭ[ppm]) = 7.60-7.54 (m, -CH Ar), 7.41 (pseudo-t, -NH), 7.14-7.07 (m, -CH Ar), 4.16 (t, *J*= 5.5 Hz, -CH_2O), 3.97 (s, -OCH_2C=O PEG); 3.74-3.41 (m, PEG core, -CH_2NH and ïSO_2Me group). Substitution degree = 90%.

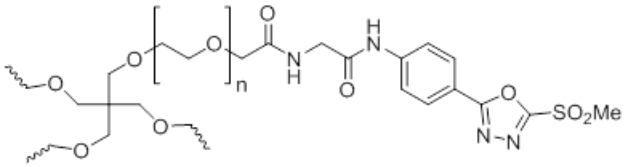


Synthesis of oxadiazole (Ox) methylsulfonyl derivatives

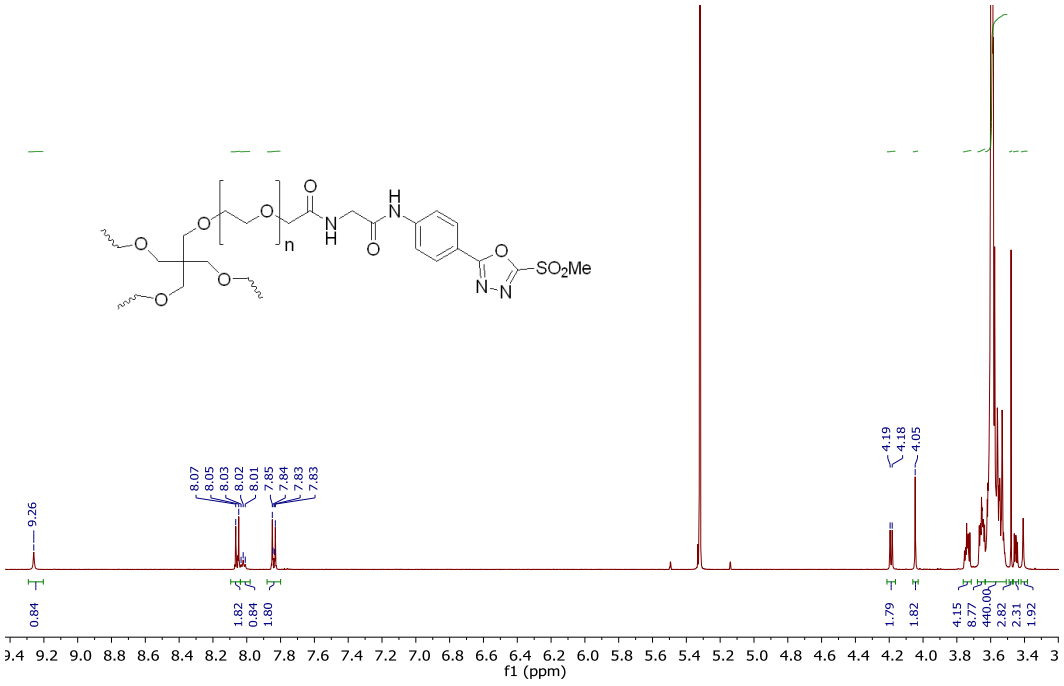




*Synthesis of 2-amino-N-(4-(5-(methylsulfonyl)-1,3,4-oxadiazol-2-yl)phenyl)acetamide (**Ox-5**).* Amine **Ox-5** was synthesized from compound **Ox-4**[2-3] by acidic cleavage of the Boc protecting group. Spectroscopic characterization data matched the reported values from the literature. ESI-MS+: 297.1 (M+H), 593.2 (2M+H), 615.2 (2M+Na). ^{1}H-NMR (300 MHz, acetone-d_6, ŭ [ppm]) = -d,8-CH0A7); (2H, 7.92 (2H, pseudo-d, -CH Ar); 4.32 (2H, s, -CH_2); 3.64 (3H, s, -SO_2Me). ^{13}C-NMR (75 MHz, acetone-d_6, ŭ [ppm]) = 166.86; 164.24; 163.15; 143



Synthesis of ***4-arm PEG-Ox****:* A reported protocol from our group was followed.[2] Freshly prepared amine **Ox-5** (170 μmol, 0.05 g) and *N*-methylmorpholine (260 μmol, 70 μL) were dissolved in dry DMF (3 mL), purged with nitrogen and stirred for 15 min. 20kDa, 4-arm PEG-NHS (0.35 g, 17.5 μmol) was dissolved in dry DMF (2 mL) and added to above solution under nitrogen stream. The mixture was stirred overnight at room temperature under inert atmosphere, then dialyzed in acetone and water, and freeze-dried. A white solid polymer was obtained and characterized by ^{1}H NMR in DCM-d_2. Functionalization degree was calculated as 90% and yield was 85%. The polymer prepared this way proved stable as solid upon >6 months storage at -20°C (evidenced by no changes in ^{1}H-NMR spectrum). ^{1}H-NMR (500 MHz, DCM-d_2, δ[ppm]) = 9.26 (s, -NH amide Ar), 8.07-8.04 (m, -CH Ar), 8.02 (t, *J*= 6.2 Hz, -NH amide), 7.86-7.82 (m, -CH Ar); 4.19 (d, *J*= 6.3 Hz, -CH_2NH); 4.05 (s, -CH_2C=O PEG); 3.80-3.40 (m, PEG core and -SO_2Me group). Substitution degree= 90%.



GPC characterization of PEG-MS macromers

Results obtained confirmed the expected molar mass at around 20,000 g mol^{-1} (see Table S1).

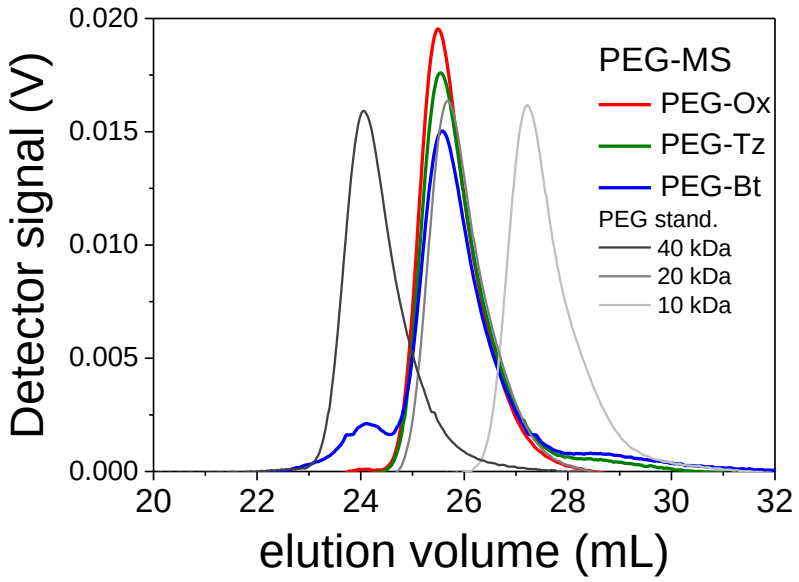


Table S1. GPC results of the different PEG-MS macromers.

	PEG-MS macromer		
	Ox	**Tz**	**Bt**
M_n [g mol^{-1}]	17735	14669	18341
M_w [g mol^{-1}]	19464	18055	21907
M_z [g mol^{-1}]	21037	20137	27395
M_p [g mol^{-1}]	21422	20981	20785
$Đ$	1.0975	1.2308	1.1944

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1. Toda, N.; Asano, S.; Barbas, C. F., Rapid, Stable, Chemoselective Labeling of Thiols with Julia–Kocienski-like Reagents: A Serum-Stable Alternative to Maleimide-Based Protein Conjugation. *Angew. Chem., Int. Ed.* **2013,** *52* (48), 12592-12596.

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