

*Supporting Information*

**Covalently functionalized amide-crosslinked hydrogels from primary amines  
and polyethylene glycol acyltrifluoroborates (PEG-KATs)**

Dominik Schauenburg<sup>†</sup>, Alberto Osuna Gálvez<sup>†</sup> and Jeffrey W. Bode<sup>\*,†</sup>

<sup>†</sup>Laboratorium für Organische Chemie, Department of Chemistry and Applied  
Biosciences, ETH Zürich, 8093 Zürich, Switzerland

[bode@org.chem.ethz.ch](mailto:bode@org.chem.ethz.ch)

**Table of Contents**

<b>General Information</b>	<b>S3</b>
<b>Reagent and Buffer preparation</b>	<b>S4</b>
<b>General procedure for hydrogel formation</b>	<b>S4</b>
<b>Variation the wt % of PEG</b>	<b>S4</b>
<b>Variation of the amine cross-linker</b>	<b>S6</b>
<b>Latent gel formation from premixed components</b>	<b>S7</b>
<b>Immobilization of dyes in KAT-hydrogels</b>	<b>S8</b>
<b>pH sensing gels</b>	<b>S9</b>
<b>Biotinylation of hydrogels</b>	<b>S10</b>
<b>Preparation of Starting Materials</b>	<b>S11</b>
<b>Spectral Data</b>	<b>S15</b>
<b>References</b>	<b>S23</b>

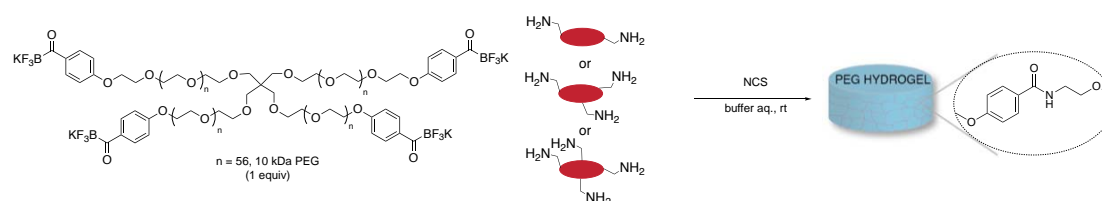
## General Information

All reagents, including the amine-containing molecules in substrate table 1, were purchased from ABCR, Acros, Fluka, Fluorochem, JenKem Technology USA, Sigma-Aldrich, TCI or Merck Millipore and used without further purification, with the following exceptions: The 4-arm PEG was dried by azeotropic distillation using toluene and *N*-chlorosuccinimide (NCS) was recrystallized by following a reported procedure.<sup>1</sup> Thin layer chromatography (TLC) was performed on aluminum-backed plates pre-coated with silica gel (Merck, Silica Gel 60 F254), which were visualized by fluorescence quenching under UV light or by staining (using ninhydrin or KMnO<sub>4</sub> solutions). Flash column chromatography was performed on Silicycle Silica Flash F60 (230–400 Mesh) using a forced flow of air at 0.5–1.0 bar. HPLC (high performance liquid chromatography) was performed on JASCO analytical and preparative instruments. Unless otherwise stated, analytical and preparative reversephase HPLC were performed using Shiseido C18 UG120 5 µm (4.6 mm I.D. × 250 mm) and YMC C18 (20 mm I.D. × 250 mm) columns with flow rates 1.0 mL/min and 10 mL/min respectively, using MQ-H<sub>2</sub>O with 0.1% TFA (eluent A) and HPLC grade CH<sub>3</sub>CN with 0.1% TFA (eluent B). Dynamic rheometry experiments to determine the gelation kinetics were performed on an Anton Paar MCR 301 instrument with a 20 mm diameter parallel plate geometry (PP20) using following settings: T = 24 °C, gap d = 0.37 mm, frequency f = 5 Hz, amplitude a = 5 %. UV/Vis spectroscopy studies of hydrogels were performed on micro-plate reader; Thermo Scientific Varioskan (Typ 3001), using a fixed wavelength of 280 nm and scanning from 305 nm to 600 nm in 2 nm steps. NMR spectra were measured on VARIAN Mercury 300 MHz, 75 MHz, Bruker Avance 400 MHz, 100 MHz or Bruker AV-II 600 MHz, 150 MHz. Chemical shifts are expressed in parts per million (ppm) and are referenced to CDCl<sub>3</sub> 7.26 ppm, 77.0 ppm; CD<sub>2</sub>Cl<sub>2</sub> 5.32 ppm, 53.38 ppm. Coupling constants are reported as Hertz (Hz). Splitting patterns are indicated as follows: br, broad; br s, broad singlet; s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; sx, sextet; sp, septet; dd, doublet of doublets; dt, doublet of triplets; td, triplet of doublets; qd, quartet of doublets; ddd, doublet of doublet of doublets; dddd, doublet of doublet of doublet of doublets; tdd, triplet of doublet of doublets; m, multiplet. High-resolution mass spectra were measured on a Bruker Daltonics maXis ESI-QTOF or Bruker solarix ESI/MALDI-FTICR by the mass spectrometry service of the Laboratorium für Organische Chemie at the ETH Zürich.

**Reagent preparation.** The functionalized 4-arm PEG KAT (10 kDa) was prepared in one step from commercially available 4-arm PEG (azeotropic dried) and potassium 4-fluorobenzoyltrifluoroborate using previous reported method.<sup>2</sup> All amine cross-linkers were commercially available.

**Buffer preparation.** Aqueous citric acid buffer (pH 3.0, 25 mM) was prepared by dissolving citric acid monohydrate (4.48 g, 21.35 mmol) and trisodium citrate dihydrate (1.07 g, 3.64 mmol) in deionized water (1 L); the final pH was adjusted using NaOH and HCl (aqueous, 0.1 M) solutions.

### General procedure for hydrogel formation

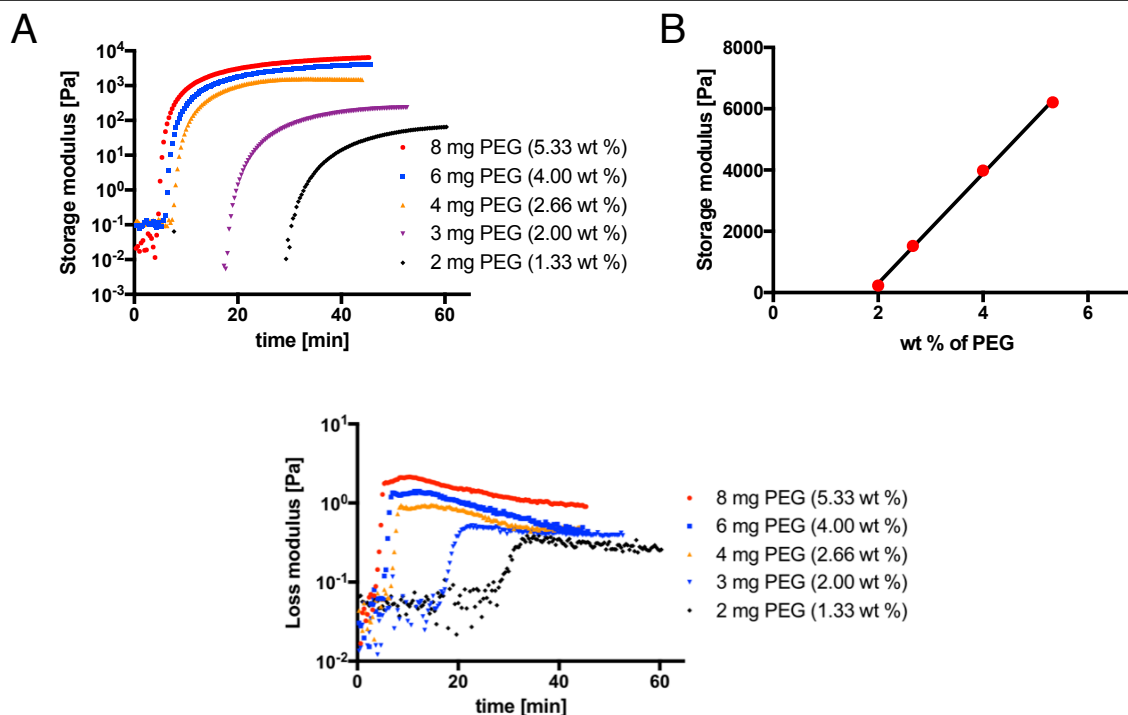
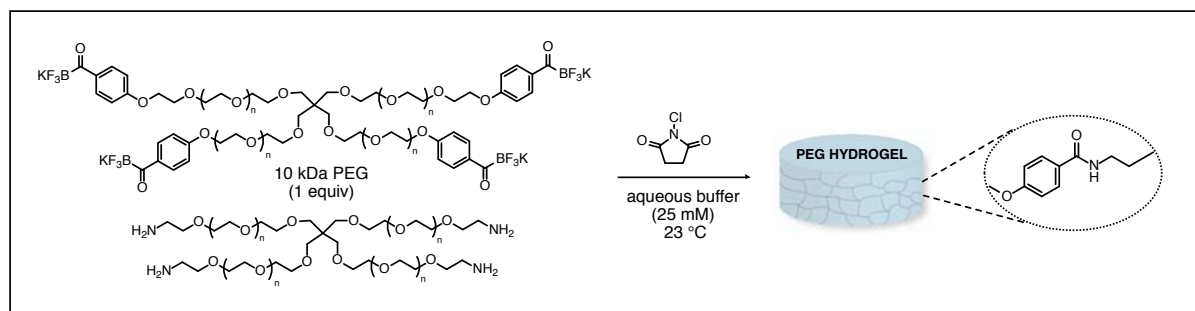


4-arm PEG-KAT (10 kDa, 4.0 wt %, 6.0 mg, 0.6  $\mu$ mol, 1.0 equiv), dissolved in 75  $\mu$ L of buffer (pH 3-6, 25 mM) and the amine-linker dissolved in 75  $\mu$ L were mixed and 140  $\mu$ L of the final solution was placed between the discs for measurement of rheometry experiments. For bidentate amine-linkers 2.0 equiv (1.2  $\mu$ mol), for tridentate amine-linkers 1.3 equiv (0.8  $\mu$ mol), for tetradentate amine-linkers 1.0 equiv (1.0  $\mu$ mol) were used. NCS was added as a solution (1.0 M in MeCN, 0 - 20 equiv) after the PEG-amine solution was placed on the plate of the rheometer. For each pH condition and amine-linker, the gelation experiment was repeated three times.

### Variation the wt % of PEG

We evaluated the gelation time and the storage modulus for different PEG concentration in the KAT-hydrogels. A 4-arm PEG-KAT and a 4-arm PEG-NH<sub>2</sub> (both 10 kDa) were mixed in a stoichiometric ratio (at pH 3) and the total amount of PEG was determined. We found that the storage modulus is

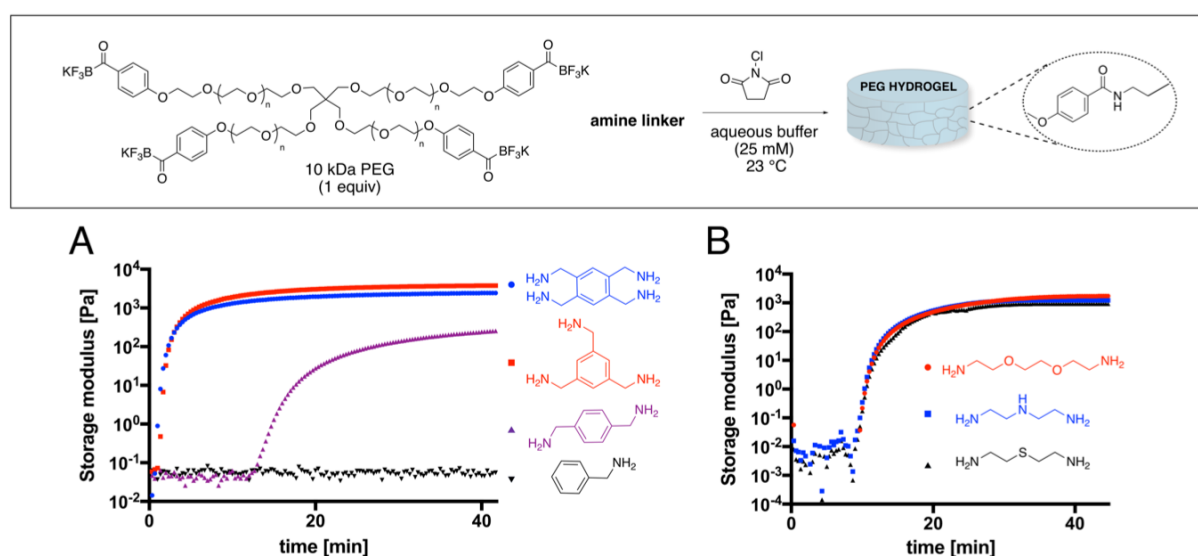
linearly correlated to the amount of PEG in the gel (Figure 4 B). It increases from 0.2 kPa using 2 wt % of PEG to 1.5 kPa (2.66 wt %), 4 kPa (4 wt %) to 6 kPa using 5.33 wt % of PEG. This behavior demonstrates the capability of our method to generate stiff gels despite the use of low PEG content. Additionally, we were able to prepare KAT hydrogels with a total polymer amount of just 1 wt %. To our knowledge not many methods for preparation of chemically cross-linked hydrogels are able to tolerate such low solid content.

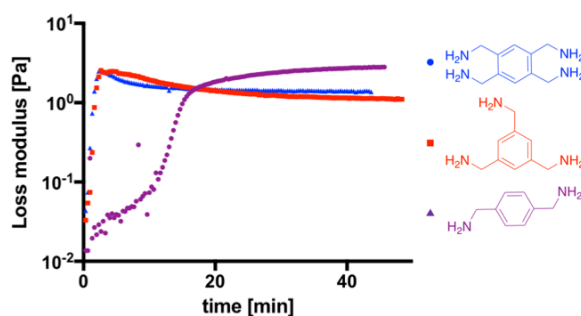


Hydrogel formation between 4-arm PEG-KAT and 4-arm PEG-NH<sub>2</sub>. Gelation dependence on wt % of PEG, by dynamic rheology (A). Linear correlation between storage modulus [Pa] and wt % of PEG in the gels (B). Performed with PEG-KAT (1.0 equiv) PEG-amine (1.0 equiv) and NCS (8 equiv) citric acid buffer (25 mM) pH 3 (150  $\mu$ L) at rt.

## Variation of the amine crosslinker

We also monitored KAT hydrogel formation using NCS as an in situ activating agent for different benzylamine cross-linkers and 4-arm PEG-KAT (4 wt %) at pH 3 by dynamic rheology. No significant difference in gelation time was observed using a tris- or tetrakis aminomethylbenzene compound. Gelation began immediate after mixing the components with no significant induction period. The storage modulus was about 1.5 times higher using the tri-amine (3.8 kDa, compared to 2.5 kDa) compared to the tetra-amine. We believe this is due to the formation of more branched networks when the tri- or tetra-amine-linkers were used compared to the corresponding di-amine compound. If linear 1,4-bis(aminomethyl)benzene is used instead, an induction period of around 15 min is observed and the storage modulus is 0.2-0.3 kPa, much lower than in the other two examples. As expected, no hydrogel formation was observed using benzylamine. We compared gelation behavior of different di-amine compounds and noticed that the plots of storage modulus as function of gelation time look similar. The induction period, even at pH 3, is longer using linear methylene amines compared to tri- or tetra-amines. All three linkers show a gelation point of 12 min. Although this induction period is comparable to the one observed for 1,4-bis(aminomethyl)benzene (Figure 5 A), the storage modulus obtained is approximately 10-fold higher. These results show that hydrogel formation using amine containing molecules and 4-arm PEG-KAT is strongly dependent on the denticity of the amine cross-linker employed and not on the structure of the molecule itself.

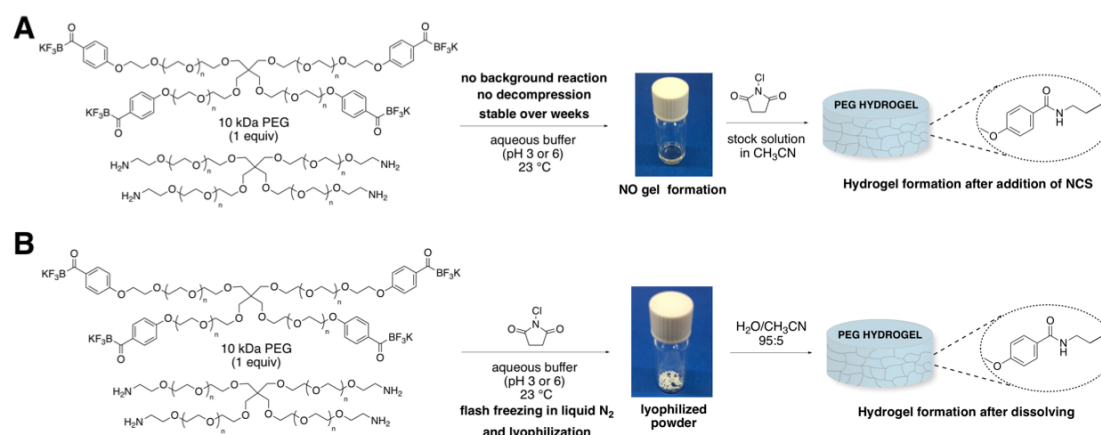




Hydrogel formation between 4-arm PEG-KAT and different amine-linker monitored by dynamic rheology. Performed with PEG-KAT (6 mg, 0.6  $\mu\text{mol}$ , 4 wt.%), benzylamine linker and NCS (8 equiv) in citric acid buffer (25 mM) pH 3 (150  $\mu\text{L}$ ) at rt (A). Performed with PEG-KAT (6 mg, 0.6  $\mu\text{mol}$ , 4 wt.%), diamine linker and NCS (8 equiv) in citric acid buffer (25 mM) pH 3 (150  $\mu\text{L}$ ) at rt (B).

### Latent gel formation from premixed components

- A) 4-arm PEG-KAT (10 kDa, 2.0 wt %, 2.0 mg, 0.2  $\mu\text{mol}$ , 1.0 equiv), and 4-Arm PEG-NH<sub>2</sub> (10 kDa, 2.0 wt %, 2.0 mg, 0.2  $\mu\text{mol}$ , 1.0 equiv) were dissolved in citric acid buffer (25.0 mM) pH 3.0 or pH 6.0 (100  $\mu\text{L}$ ). The hydrogel precursors do not undergo amide bond formation or decompose. After 1 day, 7 days or 21 days NCS was added as a solution (1.0 M in CH<sub>3</sub>CN, 8.0 equiv, 2.0 equiv per amine group). Hydrogel formation was observed after addition of NCS, gelation rate was similar to those observed when NCS was added after mixing up the components.
- B) 4-arm PEG-KAT (10 kDa, 2.0 wt %, 2.0 mg, 0.2  $\mu\text{mol}$ , 1.0 equiv), and 4-Arm PEG-NH<sub>2</sub> (10 kDa, 2.0 wt %, 2.0 mg, 0.2  $\mu\text{mol}$ , 1.0 equiv) were dissolved in citric acid buffer (25.0 mM) pH 3.0 or pH 6.0 (100  $\mu\text{L}$ ). NCS was added as a solution (1.0 M in CH<sub>3</sub>CN, 8.0 equiv, 2.0 equiv per amine group) to the polymer solution. The mixture was flash frozen in liquid nitrogen, before N-chlorination took place and lyophilized. The resulting colorless powder can be stored. After addition of H<sub>2</sub>O/CH<sub>3</sub>CN 95:5 (v/v), (40-200  $\mu\text{L}$ , 2-10 wt % of PEG in the gel), hydrogel formation was observed.



## Immobilization of dyes in KAT-hydrogels

### Sulforhodamine B –UV/VIS studies

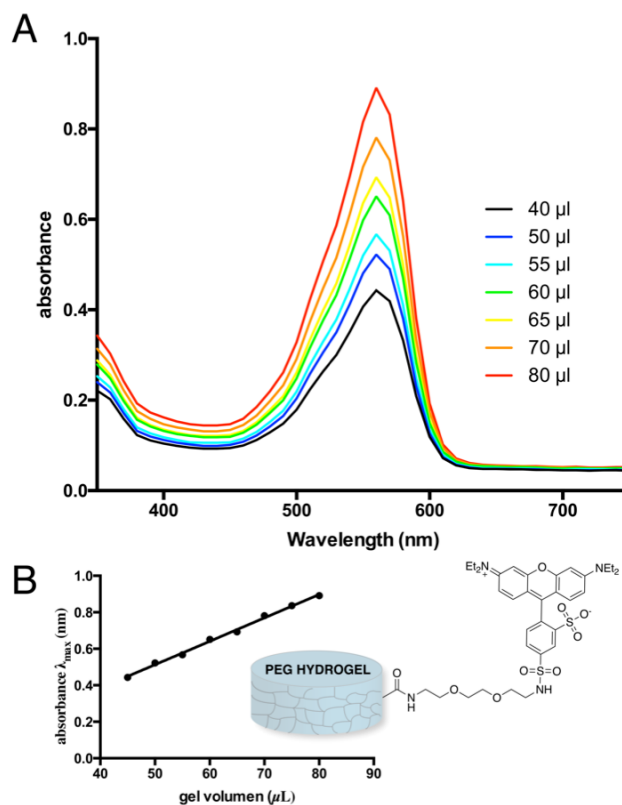
Hydrogels were prepared according to the general procedure using 4-arm PEG KAT and 4-arm PEG-NH<sub>2</sub> (both 10 kDa) in citric acid buffer pH 3. After combining the two solutions sulforhodamine B was added (0.1 M in CH<sub>3</sub>CN, final concentration 0.5-1.0 mM) followed by addition of NCS.

When (small) amine containing molecules, were covalent immobilized in the KAT hydrogels, an excess of KAT functional groups (4-arm PEG-KAT), compared to multi-arm amine was used. The total amount of KAT and amine (multi-arm cross linker plus immobilized amine) were in a stoichiometric ratio.

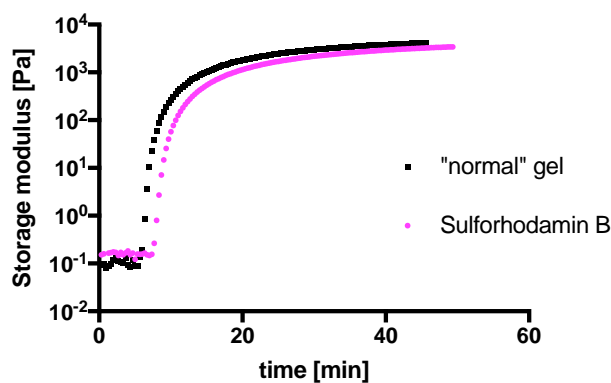
This solution was disposed on to a 96 well plate (40-120 µL per well). The concentration of sulforhodamine B in each gel (using a fixed gel volume of 60 µL) or the amount of polymer (using 0.75 mM sulforhodamine B), was varies in different 96 wells.

Hydrogels with covalently immobilized sulforhodamine B were washed in aqueous buffer (citric acid pH 3.0 or potassium phosphate pH 7.2) for 24 h and the supernatant fluid was analyzed using UV/VIS spectroscopy. No significant leaching of sulforhodamine B was observed.





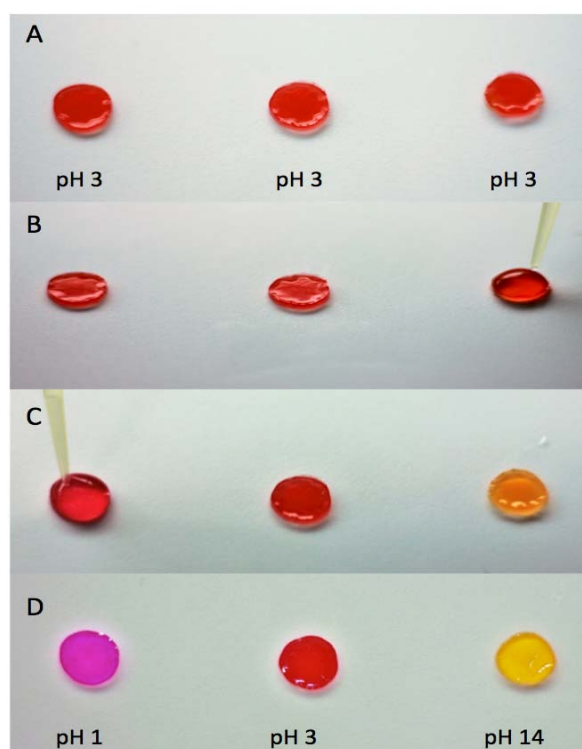
Immobilizing of sulforhodamine B in PEG-KAT hydrogels. UV-Vis spectra of KAT-hydrogels with covalently immobilized sulforhodamine B, variation of the gel volume (A) Linear correlation of absorbance maximum (at 565 nm) by variation of gel volume (B).



Hydrogel formation between 4-arm PEG-KAT and 4-arm PEG-NH<sub>2</sub>, monitored by dynamic rheology. Performed with PEG-KAT (1.0 equiv) PEG-NH<sub>2</sub> and sulforhodamine B amine (0.5mM, together 1.0 equiv) and NCS (8 equiv) citric acid buffer (25 mM) pH 3 (150  $\mu\text{L}$ ) at rt.

### pH sensing gels

Hydrogels were prepared according to the general procedure using 4-arm PEG KAT and 4-arm PEG-NH<sub>2</sub> (both 10 kDA) in citric acid buffer pH 3 before addition of NCS methyl red-NH<sub>2</sub> was added (0.1 M in MeCN/H<sub>2</sub>O (1:1) final concentration 2.5 mM). Gels could be acidified using aqueous HCl (1.0 M, pH 1.0) or basified using aqueous NaOH (1.0 M, pH 14.0), as indicated by a simultaneous change in color, in a few seconds, (from red to yellow using NaOH and red to pink using HCl). UV/VIS–studies of the gels were done at pH 1.0, 3.0 or 14.0 using same method previous described.



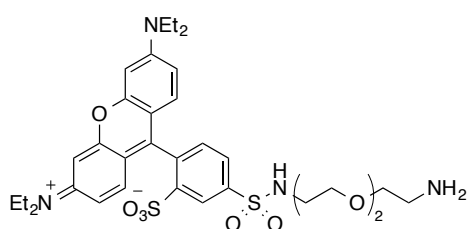
pH sensing hydrogels – A) gels prepared in citric acid buffer pH 3.0; B) adding one drop of NaOH (1M in H<sub>2</sub>O, pH 14.0) to the right gel – color change was observed immediately from red to yellow; C) adding one drop of HCl (1M in H<sub>2</sub>O, pH 1.0) to the left gel – color change was observed immediately from red to pink; D) gels 1 min after acidifying or basifying.

### Biotinylation of hydrogels

Hydrogels were prepared according to the general procedure using 4-arm PEG KAT and 4-arm PEG-NH<sub>2</sub> (both 10 kDA) in citric acid buffer pH 3.0. After combining the two solutions, biotin-NH<sub>2</sub> or biotin-NH-Boc was added (0.01 M in CH<sub>3</sub>CN, final concentration 0.1 μmol biotin per hydrogel) followed by addition of NCS. After gelation time hydrogels were washed in aqueous buffer (potassium phosphate,

25 mM, pH 7.2) two times for 12 h. After the washing step hydrogels were incubated with HRP-streptavidin conjugate (Bio-Rad, concentration concentration 1 mg/mL), diluted with millipore water by a thousand-fold. The hydrogels were incubated for 12 h using 2 mL of HRP- Streptavidin solution. After incubation hydrogels were washed again two times for 12 h using aqueous buffer (potassium phosphate, 25 mM, pH 7.2). The hydrogels were incubated with a colorimetric hydrogen peroxide indicator.

**5-(*N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl)sulfamoyl)-2-(6-(diethylamino)-3-(diethyliminio)-3*H*-xanthen-9-yl)benzenesulfonate (**S1**)<sup>1</sup>**



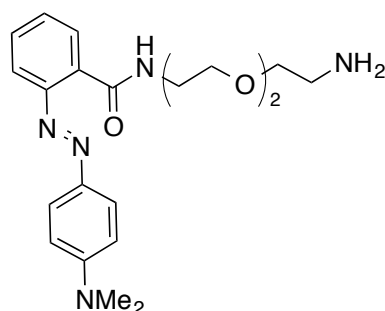
To a solution of *tert*-butyl *N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl carbamate (372.0 mg, 1.50 mmol, 1.0 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added 2-(6-(diethylamino)-3-(diethyliminio)-3*H*-xanthen-9-yl)-5-chlorosulfonylbenzenesulfonate (988 mg, 1.65 mmol, 1.1 equiv) portionwise. The mixture was stirred at rt for 1.0 h and quenched with excess aqueous citric acid (10% m/v). The phases were separated and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 3.0 mL). The collected organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The residue was purified by flash column chromatography (eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH 100:6 + 1% AcOH). The fractions were collected, washed with sat aqueous NaHCO<sub>3</sub> and evaporated to give Boc-protected **S1**.

Boc-**S1** was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2.0 mL) and trifluoroacetic acid (0.5 mL) was added. The mixture was stirred at rt for 1 h. The volatiles were evaporated in vacuo to give **S1** trifluoroacetate salt (650 mg, 54% over two steps). For characterization purposes, the product was further purified by preparative reverse-phase HPLC (YMC C18 column, A/B eluent gradient: 20-95% for 28 min, *t<sub>R</sub>* = 24.8 min)

<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.43 (d, *J* = 2.0 Hz, 1H), 8.04 (t, *J* = 5.9 Hz, 1H), 7.97 – 7.92 (m, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.04 (dd, *J* = 9.6, 2.4 Hz, 2H), 6.99 – 6.92 (m, 4H), 3.69 – 3.51 (m, 14H), 3.02 (dq, *J* = 33.4, 5.7 Hz, 6H), 1.21 (t, *J* = 7.0 Hz, 12H) ppm; <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ 157.3, 157.1, 155.0, 147.8, 141.6, 133.0, 132.6, 130.7, 126.5, 125.6, 113.6, 113.4, 95.4, 69.6, 69.5, 69.0,

66.7, 45.2, 42.4, 40.03, 38.6, 12.5 ppm; **ESI-HRMS** calculated for  $C_{33}H_{45}N_4O_8S_2$   $[M + H]^+$  689.2673, found 689.2677

**Methyl red-amine linker (N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-((4-(dimethylamino)phenyl) diazenyl)benzamide) (S2)**

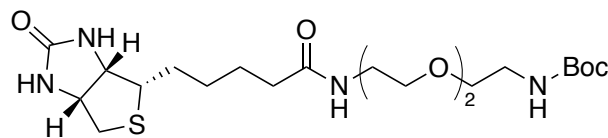


In a round-bottom flask, methyl red, 2-(N,N-dimethyl-4-aminophenyl) azobenzenecarboxylic acid (100 mg, 0.37 mmol, 1.0 equiv) was dissolved in 3.0 mL of  $CH_2Cl_2$ . N,N-Dicyclohexylcarbodiimide (76 mg, 0.37 mmol, 1.0 equiv) and 1-hydroxybenzotriazol (50 mg, 0.37 mmol, 1.0 equiv) were added. To the activated carboxylic acid *tert*-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (100 mg, 0.4 mmol, 1.1 equiv) was added

and the mixture stirred over night (approx. 12h) at rt. The next morning the colorless precipitate was removed by filtration and the filtrate was washed with sat aqueous  $Na_2CO_3$  (3 x 2.0 mL) and brine (1 x 3.0 mL). The organic extracts were collected, dried over  $MgSO_4$ , filtered and 3 mL of trifluoroacetic acid were added. The mixture was stirred for 2 h at rt before quenching with sat. aq.  $Na_2CO_3$  (until pH 8). The aqueous solution was extracted with  $CH_2Cl_2$  (3 x 3.0 mL), and the combined organic fractions were collected, dried over  $MgSO_4$ , filtered and evaporated. The crude material was purified by column chromatography on silica gel (eluting with  $CH_2Cl_2/MeOH/NEt_3 = 95:5:1$ ) to give methyl red-amine linker S2 as a dark red oil (65 mg, 44% yield over two steps).

**$^1H$  NMR** (500 MHz, Methylene Chloride- $d_2$ )  $\delta$  9.53 (s, 1H), 8.28 (dd,  $J = 7.8, 1.6$  Hz, 1H), 7.86 – 7.81 (m, 3H), 7.59 – 7.54 (m, 1H), 7.50 (td,  $J = 7.5, 1.3$  Hz, 1H), 6.85 – 6.81 (m, 2H), 3.79 – 3.67 (m, 6H), 3.62 (tq,  $J = 5.3, 3.7, 2.7$  Hz, 4H), 3.16 (s, 6H), 3.14 (d,  $J = 5.2$  Hz, 2H) ppm;  **$^{13}C$  NMR** (151 MHz, Methylene Chloride- $d_2$ )  $\delta$  154.1, 150.9, 143.6, 132.2, 131.6, 123.0, 129.3, 126.6, 116.6, 112.3, 71.0, 70.8, 70.7, 67.3, 40.7, 40.4, 40.1, 1.3 ppm **ESI-HRMS** calculated for  $C_{21}H_{30}N_5O_3$   $[M + H]^+$  400.2343, found 400.2343

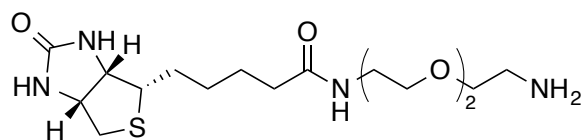
***tert*-Butyl *N*-(2-(2-(2-(5-((3*aS*,4*S*,6*aR*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)ethoxy)ethoxy)ethyl)carbamate (**S3**)**



A round-bottom flask was charged with D-biotin (1.7 g, 7.0 mmol, 1.0 equiv) and HCTU (3.2 g, 7.7 mmol, 1.1 equiv). DMF (25 mL) and *N,N*-

diisopropylethylamine (1.5 mL, 8.4 mmol, 1.2 equiv) were added and the suspension stirred at rt for 5 min. A solution of *N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (1.9 g, 7.7 mmol, 1.1 equiv) in DMF (2 mL) was added and the mixture stirred at rt overnight. The resulting solution was diluted with CH<sub>2</sub>Cl<sub>2</sub> (10 mL), washed with aqueous LiCl (15 mL, 5%) and H<sub>2</sub>O/brine, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 10 mL). The organic extracts were collected, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The residue was purified by flash column chromatography (MeOH/CH<sub>2</sub>Cl<sub>2</sub> 1:10). The fractions were combined, evaporated and dried in vacuo to give **S3** as a white solid (2.2 g, 66%) For characterization purposes, the product was further purified by preparative reverse-phase HPLC (YMC C18 column, A/B eluent gradient: 5-95% for 28 min, *t<sub>R</sub>* = 16.3 min)

**<sup>1</sup>H NMR** (500 MHz, Methanol-*d*<sub>4</sub>) δ 4.50 (m 1H), 4.31 (dd, *J* = 7.9, 4.4 Hz, 1H), 3.62 (s, 4H), 3.54 (dt, *J* = 15.7, 5.5 Hz, 4H), 3.37 (t, *J* = 5.5 Hz, 2H), 3.33 (s, 1H), 3.26 – 3.18 (m, 3H), 2.93 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.71 (dd, *J* = 12.7, 0.8 Hz, 1H), 2.25 – 2.20 (m, 2H), 1.79 – 1.56 (m, 4H), 1.44 (s, 10H) ppm;  
**<sup>13</sup>C NMR** (126 MHz, Methanol-*d*<sub>4</sub>) δ 174.7, 157.0, 78.7, 69.9, 69.8, 69.7, 69.2, 62.0, 60.3, 55.6, 48.1, 47.9, 47.8, 47.6, 47.4, 47.3, 47.1, 39.8, 39.6, 38.9, 35.3, 28.4, 28.1, 27.4, 25.5 ppm **ESI-HRMS** calculated for C<sub>21</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>S [M + Na]<sup>+</sup> 497.2404, found 497.2409.

**Biotin amine linker (*N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide) (S4)**

For the Boc deprotection biotin-NH-Boc (50 mg, 0.10 mmol, 1.0 equiv) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) and trifluoroacetic acid (1.0 mL) was added. The mixture was stirred for 2 h at rt. The

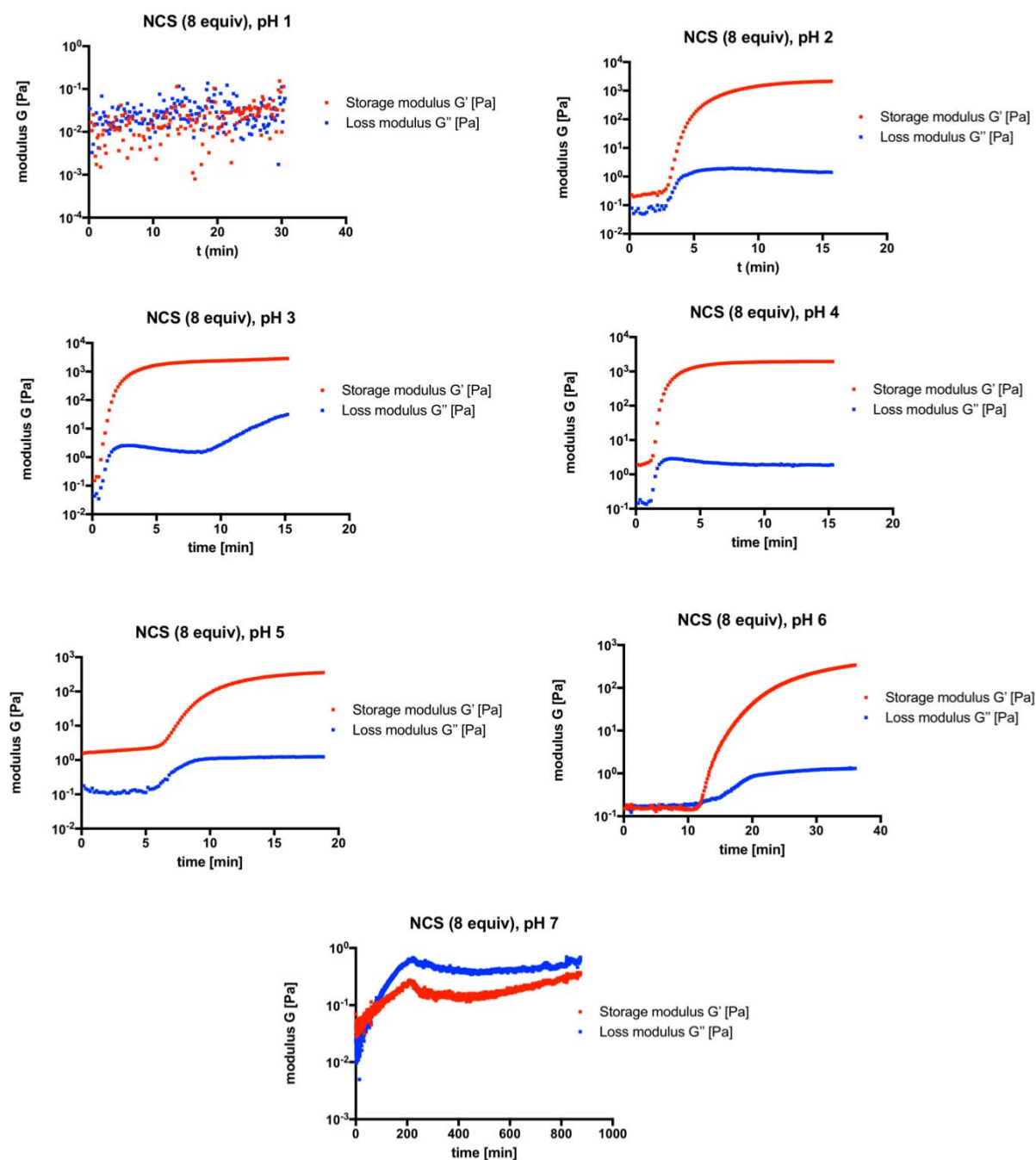
volatiles were evaporated to afford biotin-NH<sub>2</sub> (**S3**) trifluoroacetate salt, which was used without further purification as a colorless oil (46 mg, 94 %). For characterization purposes, the product was further purified by preparative reverse-phase HPLC (YMC C18 column, A/B eluent gradient: 5-95% for 28 min, *t<sub>R</sub>* = 11.2 min)

**<sup>1</sup>H NMR** (500 MHz, MeOH-*d*<sub>4</sub>) δ 4.51 (m, 1H), 4.32 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.75 – 3.65 (m, 6H), 3.57 (t, *J* = 5.7 Hz, 2H), 3.42 – 3.37 (m, 2H), 3.22 (m, 1H), 3.13 (t, *J* = 5.0 Hz, 2H), 2.94 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.72 (m, 1H), 2.23 (t, *J* = 7.4 Hz, 2H), 1.81 – 1.55 (m, 4H), 1.51 – 1.40 (m, 2H) ppm; **<sup>13</sup>C NMR** (126 MHz, Methanol-*d*<sub>4</sub>) δ 176.3, 166.1, 71.3, 71.3, 70.7, 67.9, 63.4, 61.7, 57.0, 41.0, 40.7, 40.1, 36.7, 29.7, 29.5, 26.8 ppm **ESI-HRMS** calculated for C<sub>16</sub>H<sub>31</sub>N<sub>4</sub>O<sub>4</sub>S [M + H]<sup>+</sup> 375.2061, found 375.2064

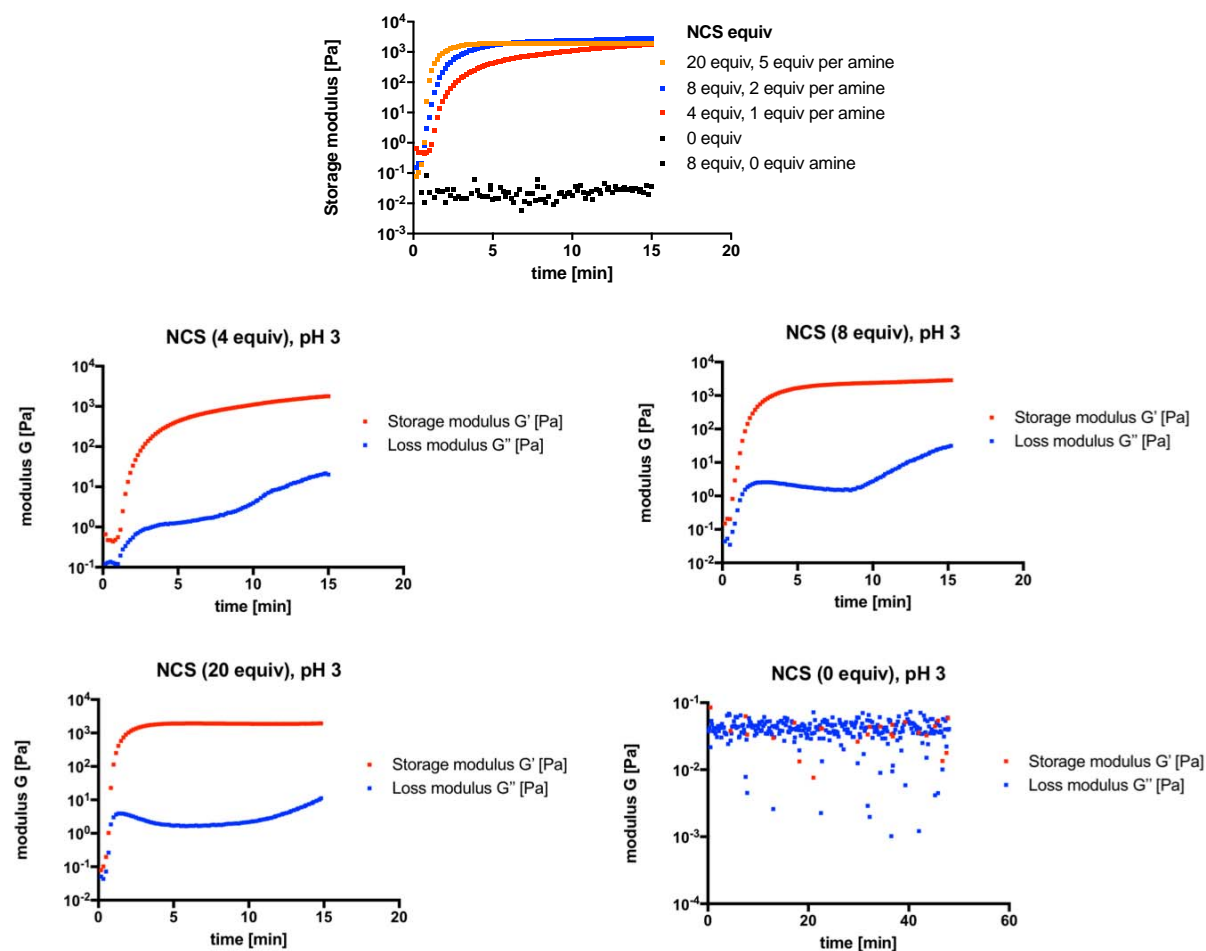
## Spectral Data

### Rheometry measurements

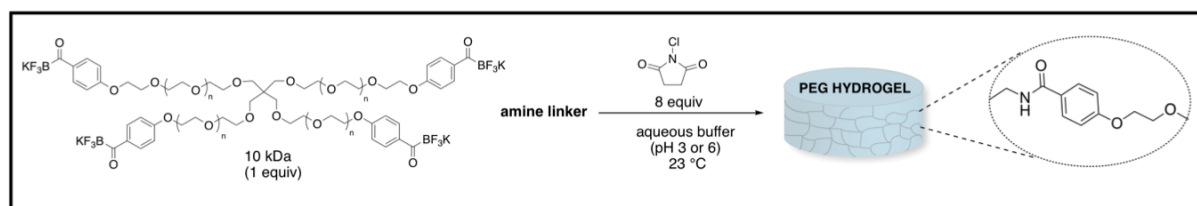
4-arm PEG-KAT (10 kDa, 1.0 equiv) and tris(2-aminoethyl)amine (1.3 equiv), NCS (8.0 equiv, 2.0 equiv per amine group), aqueous buffer pH 1.0 – 7.0



4-arm PEG-KAT (10 kDa, 1.0 equiv) and tris(2-aminoethyl)amine (1.3 equiv), NCS (0 – 20.0 equiv, 0 – 5.0 equiv per amine group), aqueous buffer pH 3.0





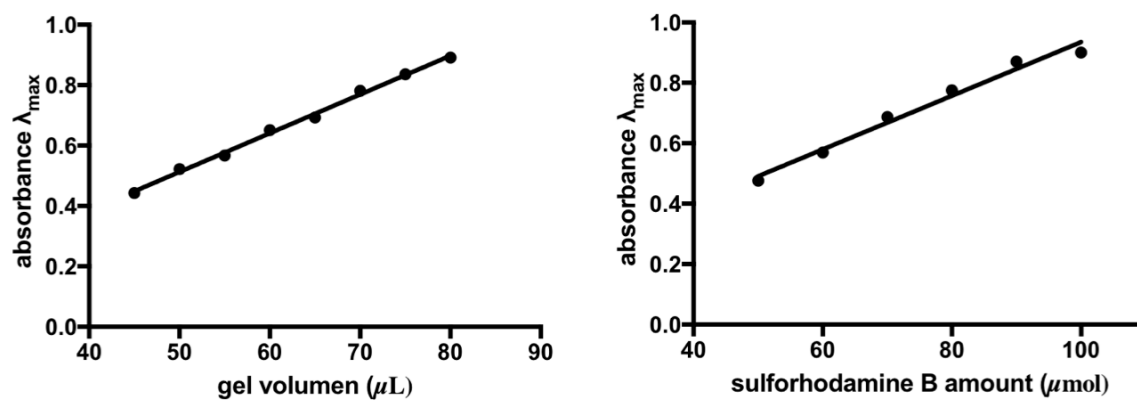


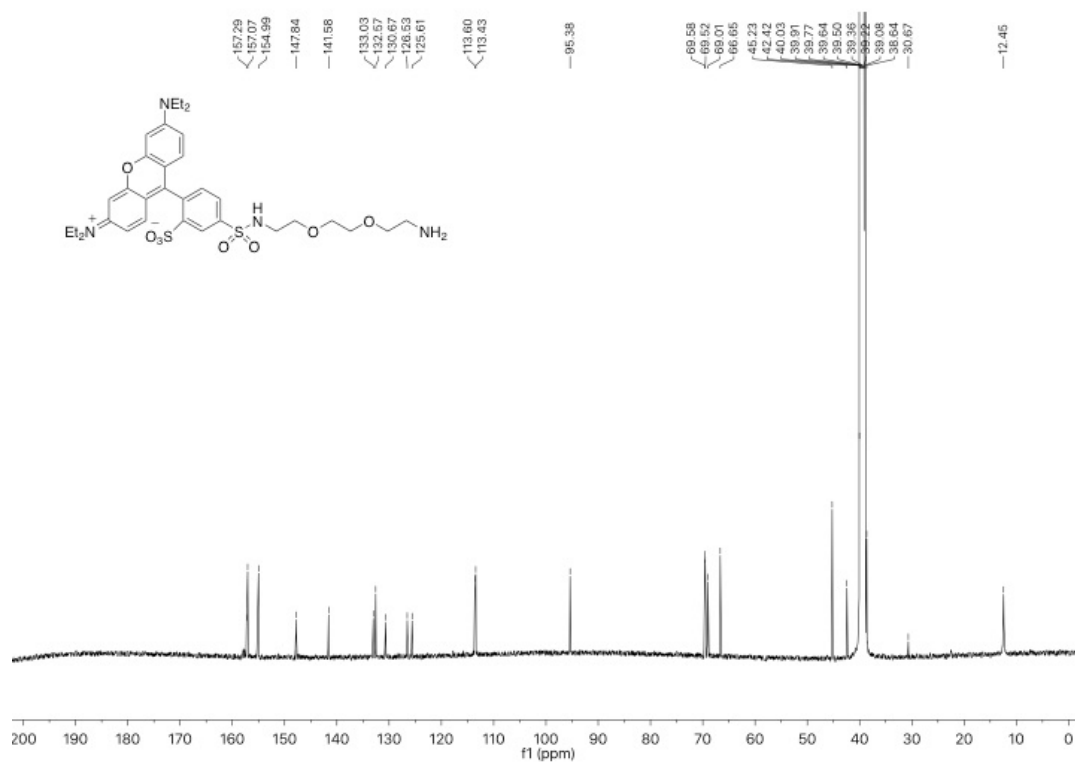
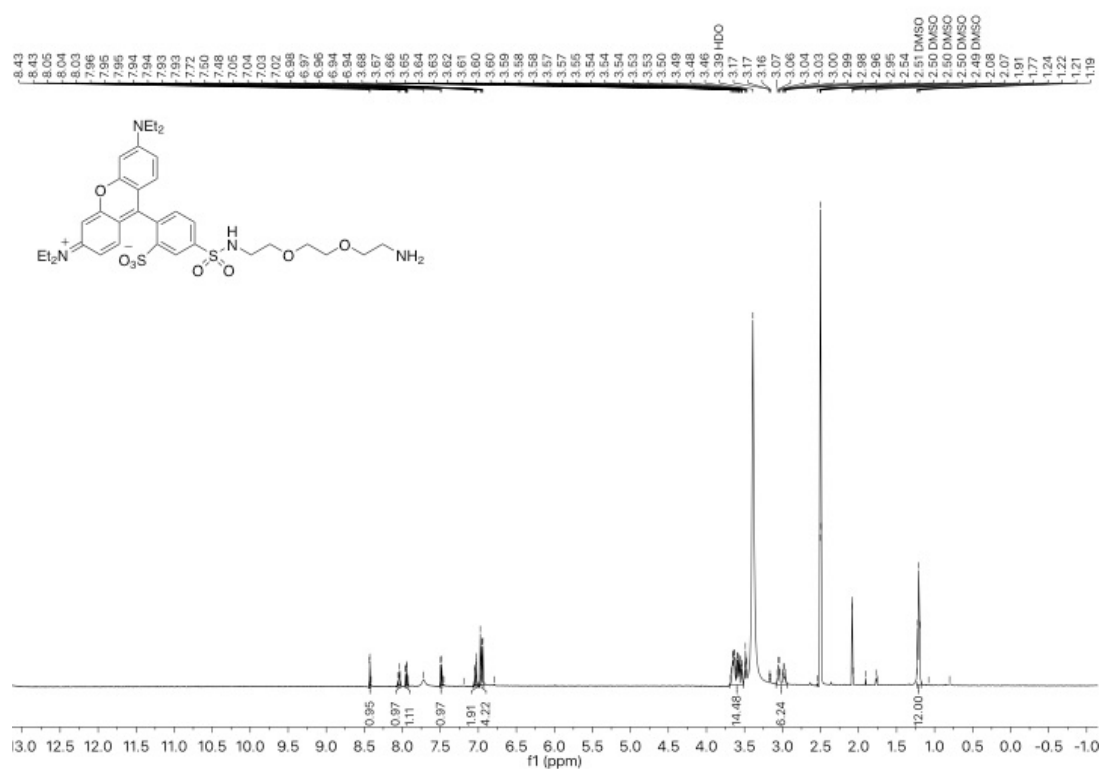
Entry	amine linker	equiv of amine linker	storage modulus of the plateau ( $G'$ [Pa])
1		2	1700
2		2	1200
3		2	930
4		2	270
5		1.33	3600
6		1.33	3800
7		1	2450
8	 poly(ethylene glycol) tetraamine (10 kDa)	1	1530
9	 polymyxin B	1	4700

### UV/Vis measurements

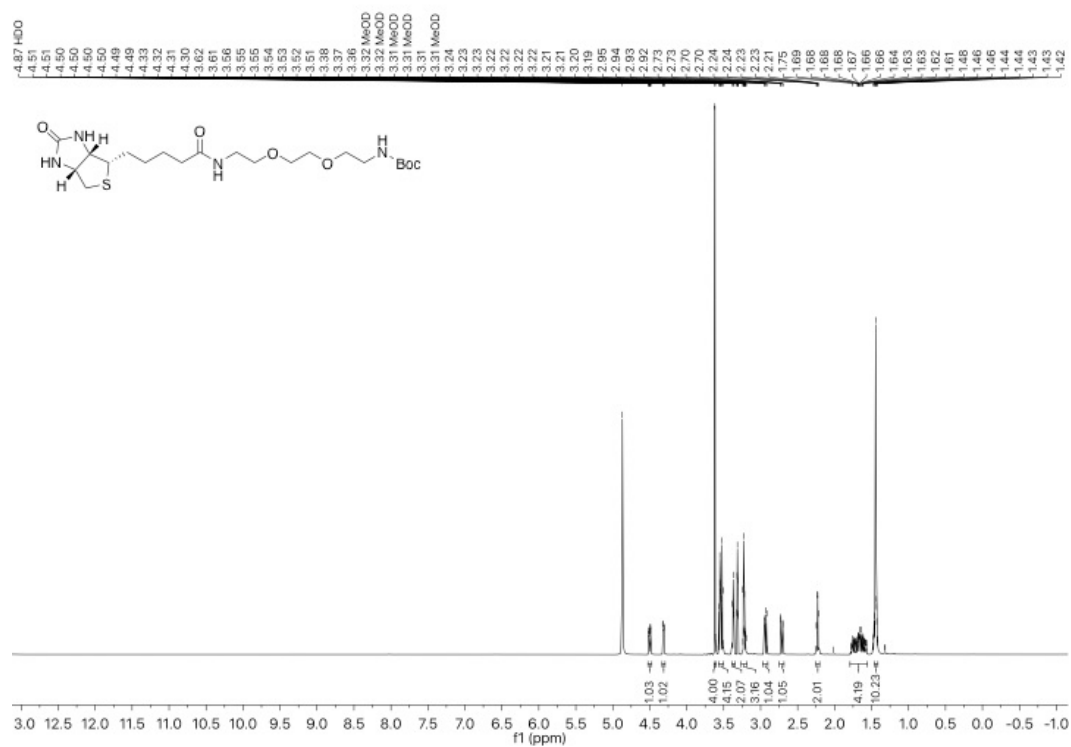
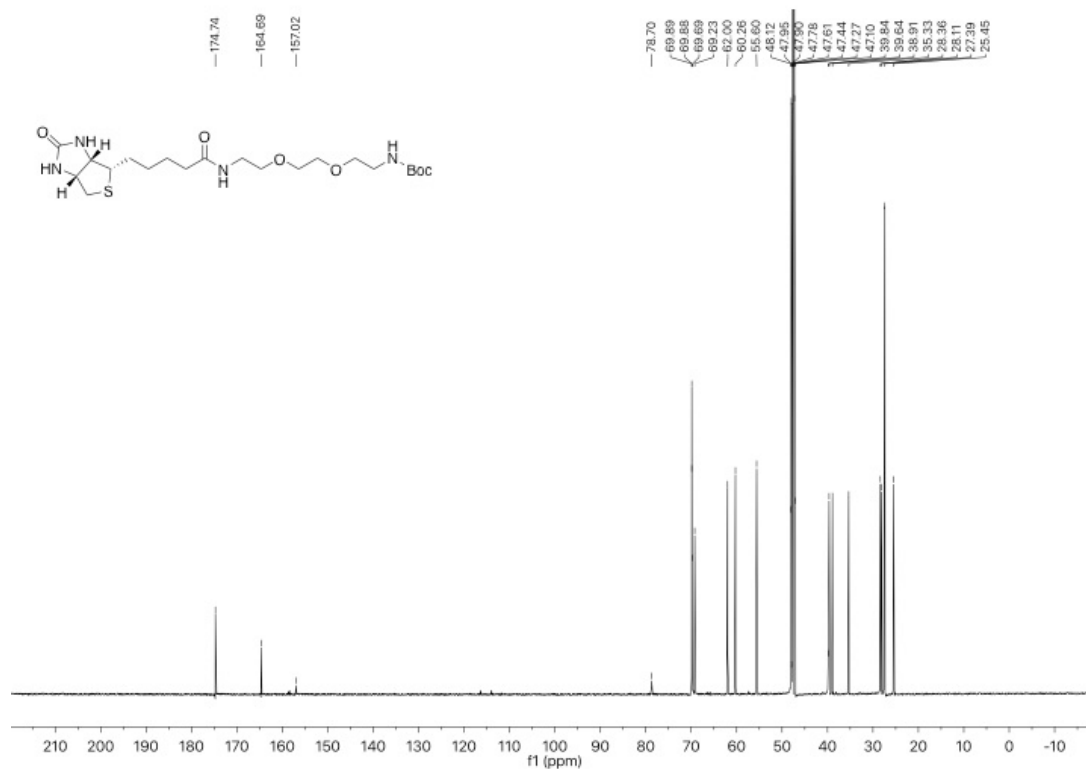
4-arm PEG-KAT (10 kDa, 1.0 equiv) and 4-arm PEG-NH<sub>2</sub> (10 kDa, 1.0 equiv), Sulforhodamine B (100  $\mu$  mol / 100  $\mu$ L gel volume) NCS (8.0 equiv, 2.0 equiv per amine group), aqueous buffer pH 3.0

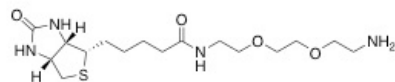
Linear correlation of  $\lambda_{\max}$  (565 nm) for increasing of gel thickness and amount of immobilized Sulforhodamine B (accordance with Beer-Lambert law)



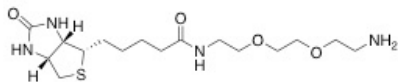




**<sup>13</sup>C NMR (126 MHz, Methanol-*d*<sub>4</sub>)****<sup>1</sup>H NMR (500 MHz, Methanol-*d*<sub>4</sub>)**



**$^{13}\text{C}$  NMR (500 MHz, Methanol- $d_4$ )**



## References

- (1) Paquette, L.; Crich, D.; Fuchs, P.; Molander, G. A., Eds. *Encyclopedia of reagents for organic synthesis*, 2nd Ed.; Wiley, Chichester, **2009**.
- (2) Mazunin, D.; Broguiere, N.; Zenobi-Wong, M.; Bode, J. W. *ACS Biomater. Sci. Eng.* **2015**, *1*, 456–462.
- (3) Sunbul, M.; Jäschke, A. *Angew. Chem. Int. Ed.* **2013**, *52*, 13401.