Synthesis of Pyridine Acrylates and Acrylamides and Their Corresponding Pyridinium Ions as Versatile Cross-Linkers for Tunable Hydrogels

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In memoriam Alan R. Katritzky

Abstract: A small library of cross-linkers for hydrogels was synthesized. The cross-linkers consisted of 2,6- and 3,5-diacylpyridine or 2,4,6-triacylpyridine as the core unit, which were tethered via ethylene glycol, amino ethanol, and 1,n-diamine spacers to terminal acrylate or acrylamide moieties. Esterification and amide formation of the terminal acryl units were found to be dependent on the ratio of NH/O in the spacer, the constitution pattern of the pyridine ring, and the total number of acryl groups. Thus, esters generally gave higher yields than amides decreasing with increasing number of NH in the spacer and with increasing number of acryl units. In the case of 3,5-diacylpyridine derivatives, these trends were less prominent as compared to the 2,6-diacylpyridine series, indicating that steric hindrance and unfavorable hydrogen bonding interaction of the spacers might influence the observed reactivity differences. The 3,5-diacylpyridines were converted to the *N*-methylpyridinium salts and selected members of both neutral and cationic 3,5-diacylpyridinium derivatives were submitted to hydrogelations with synthetic polymer poly(1-glycidylpiperazine) via aza-Michael addition and thiolated natural hyaluronan via thio-Michael reaction, respectively. Rheological properties of the resulting hydrogels were studied, revealing that both spacer type as well as charge affected elastic moduli and degree of swelling.

Key words: acylation, alkylation, biomimetic synthesis, polyanions, pyridines, cross-linkers, hydrogels

Synthetic and hybrid hydrogels are promising materials for tissue engineering. In contrast to their animal derived counterparts such as collagen, they possess a well-defined molecular structure, their properties can be adjusted in a reproducible way, for example, by using cross-linkers; they are cost-effective and provide no infection risk.[1] However, a major disadvantage is the low tendency for cell attachment, which can be circumvented by modification (with small RGD peptides) or introduction of charged moieties. While several research groups have studied charge effects on the properties of the hydrogels employing cationic (or anionic) comonomers, charged cross-linkers have rarely been applied for such purpose.[2] Pyridine and pyridinium ions have been extensively used as subunits of polyelectrolytes and polymeric hydrogels because they can be easily combined with biopolymers such as peptides, enzymes, or even whole organisms leading to promising applications such as decontamination of chemical and biological warfare agents[3] or polymeric supports for microbial biofilms.[4] Furthermore, pyridine and pyridinium ions have been successfully used as electron-transporting layers in organic light emitting devices (OLEDs).[5–7] In most cases the pyridinium unit is either part of the polymer backbone (main chain polymer)[4–8] or incorporated in the side chain[3] of homopolymers or block copolymers resulting in a relatively high concentration of pyridine units within the polymer. Copolymers in which the heteroaromatic cross-linkers are present in relatively low concentrations are only rarely explored.[9] We have recently prepared pyridine-derived cross-linkers, which were tethered via ester or amide spacers to acryl units and used them for the preparation of novel biocompatible hydrogels derived from synthetic poly(1-glycidylpiperazine)[10] and thiolated hyaluronan.[11] It turned out that both the type of connecting unit (ester versus amide) between pyridine and spacer as well as the presence or absence of a charge on the pyridine had some influence on the gelation rate, solubility of the hydrogels and the rheological properties. However, in order to perform more detailed studies to understand structure–property relationships and to get access to hydrogels suitable for biological studies, we required a dedicated library of cross-linkers **1** and **2** with different spacers varying in length and solubility, and 2,6- or 3,5-diacylpyridines as the central heterocyclic unit as shown in Figure 1. In order to increase the number of cross-linkable groups, 2,4,6-triacylpyridines were studied as well. Esters and amides were chosen as connecting units between spacers and polymerizable terminal groups to take advantage of commercially available acrylate building blocks and easily accessible synthetic groups.

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Furthermore, esters and amides should provide sufficient solubility of the target molecules in the gelation solvent, which is anticipated to be advantageous for the hydrogelation by hetero-Michael reactions.[12–15] The results are discussed below.

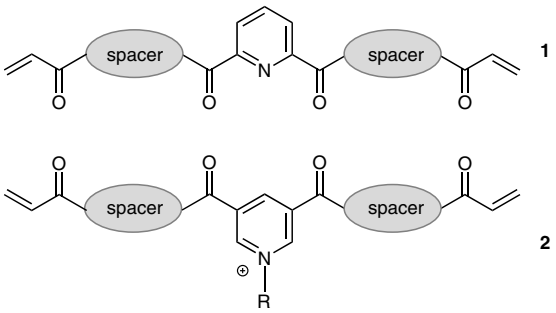
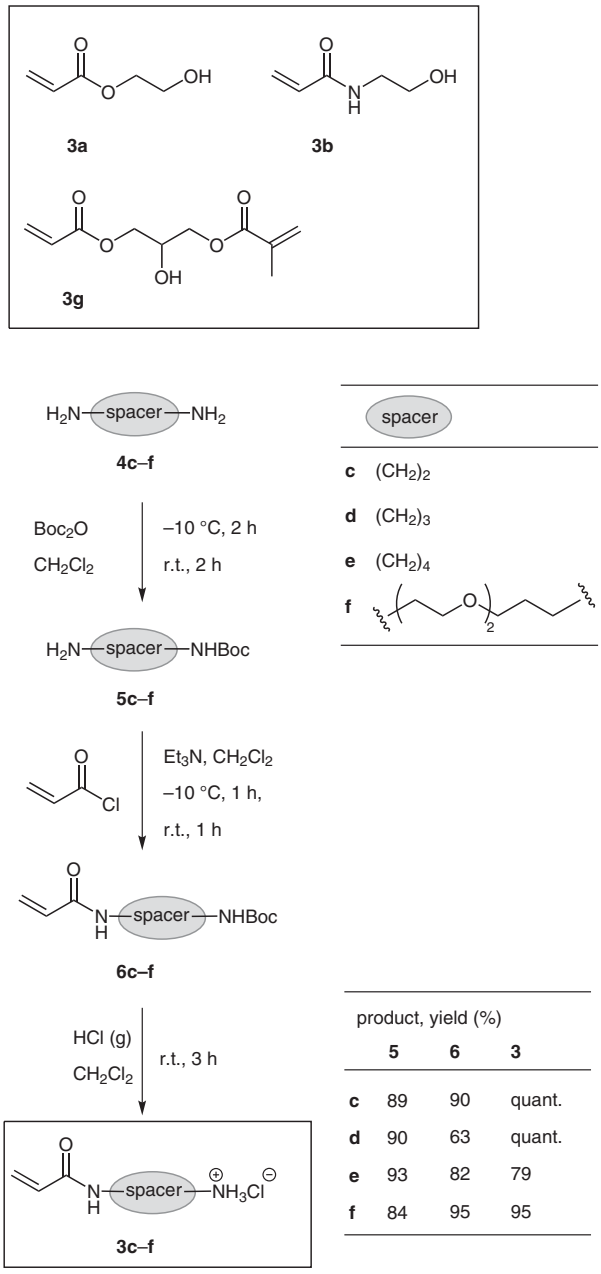


Figure 1 Graphical representation of cross-linkers **1** and **2**

In order to attach polymerizable groups to the pyridine core, the following building blocks were selected (Scheme 1): the ester-based commercially available compounds 2-hydroxyethyl acrylate (**3a**), *N*-hydroxyethyl acrylamide (**3b**), and 3-(acryloyloxy)-2-hydroxypropyl methacrylate (AHM) (**3g**), which enabled an increase of cross-linkable groups through its branched structure. Furthermore, the amide-based compounds **3c**–**f** were chosen, incorporating longer alkyl and the water soluble triethylene glycol spacers, respectively. The amine hydrochlorides **3c**–**f** were prepared as shown in Scheme 1. Boc-protection of the diamines **4c**–**f** followed by acylation with acryloyl chloride were carried out according to a modified procedure by Feast and Hamachi to give the *N*-*tert*-butoxycarbonyl-*N*′-acryloyl-1,2-diaminoalkanes **6c**–**f**.[16,17] The protected amines **6c**–**f** were deprotected with dry gaseous hydrogen chloride to assure that the final amino hydrochlorides **3c**–**f** did not contain any water prior to acylation.[17]

As shown in Scheme 2, the pyridine dicarboxylic acid **7** was activated with thionyl chloride and reacted with the acrylic building blocks **3a**–**c**,**g** via two different methods. The reactions with the well soluble acrylates **3a**,**b**,**g** were carried out in CH_2Cl_2 by employing stoichiometric amounts of Et_3N for a faster conversion (method A).[18,19] The crude products could be purified by column chromatography on silica gel and the desired acryl cross-linkers **8a**,**b**,**g** were obtained in yields of 21–93%. In contrast, for cross-linker **8c** method A turned out to be not suitable because Et_3NHCl, which is formed during the reaction, has similar solubility to **8c** and could not be removed via column chromatography (CH_2Cl_2–MeOH) but only by extensive repetitive recrystallization (CH_2Cl_2–acetone) with loss of material (5–10%). Furthermore, the amine **3c** was only partially soluble in CH_2Cl_2, leading to decreased reactivity towards the acid chloride of **7** but increased the tendency towards polymerization. Therefore, alternative reaction conditions were examined: NaH in DMF was found to be suitable (method B). Although NaCl could be

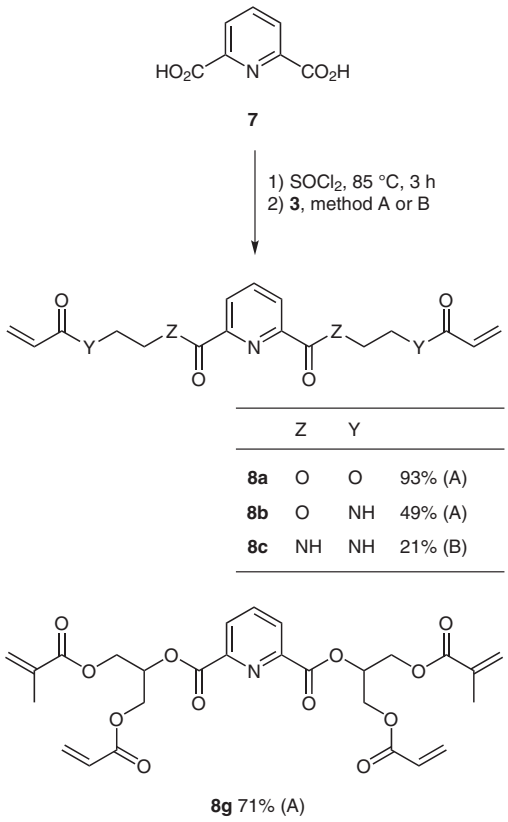


product, yield (%)	5	6	3
c	89	90	quant.
d	90	63	quant.
e	93	82	79
f	84	95	95

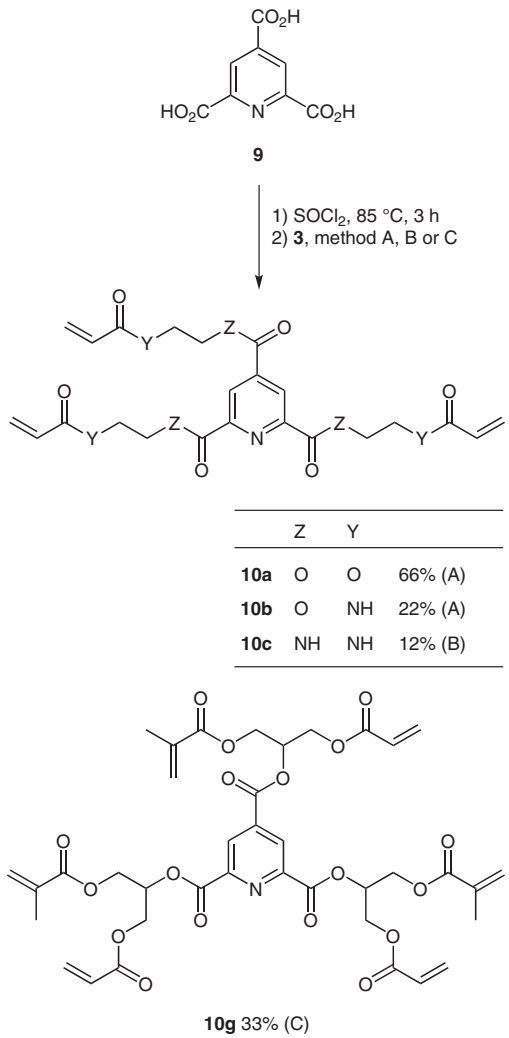
Scheme 1 Synthesis of compounds **3**

easily separated by filtration, the obtained yield of **8c** (21%) was low, presumably due to the increased formation of hydrogen bonds with higher occurrence of amines and amides in **3** and thus decreased accessibility of the acid chlorides towards OH or NH_2 nucleophiles. The employed amine **3c** could usually be recovered after the reactions.

In general, similar trends were found upon conversion of pyridine-2,4,6-tricarboxylic acid (**9**) to the corresponding acrylic derivatives **10a**–**c**,**g** (Scheme 3) although direct comparison with **8a**–**c**,**g** revealed decreased yields for the tris-functionalized pyridines **10a**–**c**,**g**, presumably due to competition between the different arms. A decrease of yields was also observed upon increasing the number of



Scheme 2 Synthesis of pyridine acrylates and acrylamides **8**. *Reagents and conditions*: Method A: **3**, Et_3N, CH_2Cl_2, 0 °C to r.t., 2 h. Method B: **3**, NaH, DMF, 0 °C to r.t., 16 h.



Scheme 3 Synthesis of pyridine acrylates and acrylamides **10**. *Reagents and conditions*: Method A: **3**, Et_3N, CH_2Cl_2, 0 °C to r.t., 2 h. Method B: **3**, NaH, DMF, 0 °C to r.t., 16 h. Method C: **3**, Py, CH_2Cl_2, 0 °C to r.t., 2 h.

polymerizable groups, for example, two in **8a** (93%) versus four in ester **8g** (71%).

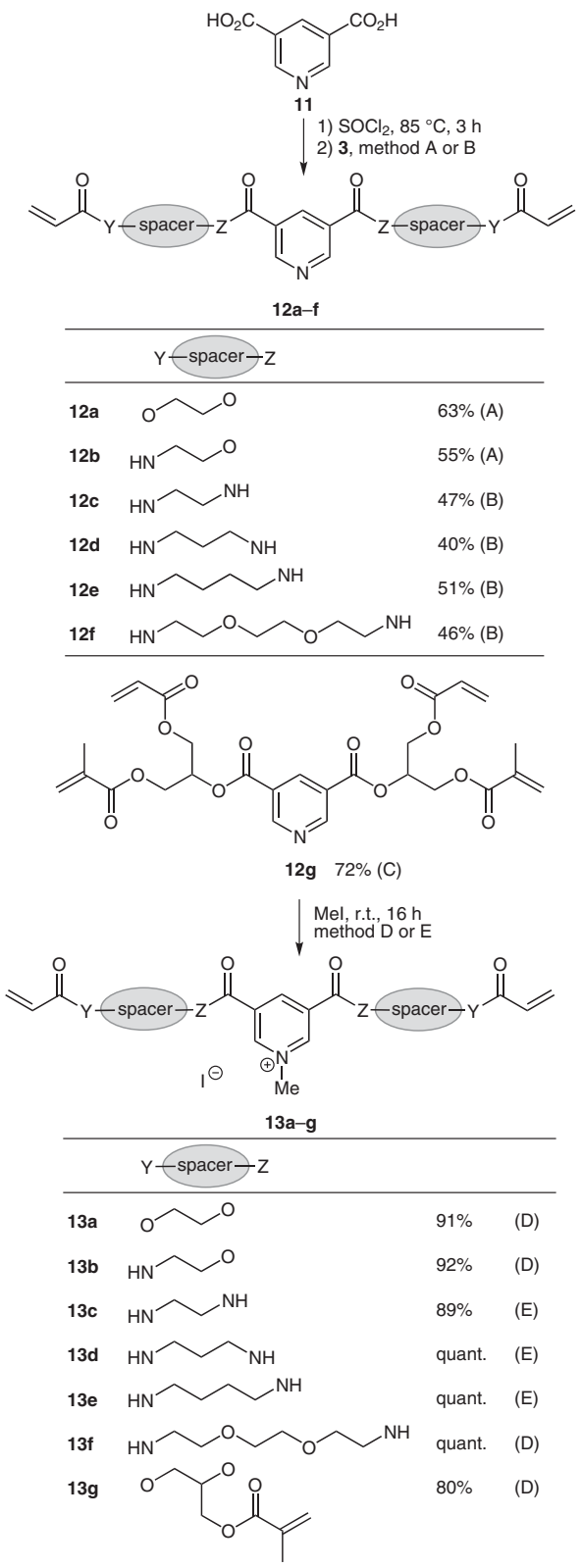
We tried to address the low yields by using different reaction conditions for selected compounds (Table S1, Supporting Information).[20,21] The use of DCC led to slower conversions (low solubility of the acids **7** and **9** in organic solvents) with even lower yields. Furthermore, pyridine or 2,6-lutidine were used as bases but caused the formation of more by-products and were only advantageous in case of the more bulky acrylate AHM **3g** (method C). All changes in conditions led to more extensive workups and purifications compared to the route via $SOCl_2$ and Et_3N (method A) or NaH and DMF (method B). In addition, both series **8a**–**c** and **10a**–**c** showed decreased yields with increasing number of NH groups again suggesting that hydrogen bonds between different arms interfere with efficient acylation.

Next, pyridine-3,5-dicarboxylic acid (**11**) was converted via the corresponding acid chloride to the 3,5-disubstituted derivatives **12a**–**f** and **12g** (Scheme 4) bearing two and four acrylic units, respectively, via methods A, B, and C in 40–72% yield. In the case of **12c**–**e** longer alkyl spacers were introduced as well as triethylene glycol in **12f** for improved water solubility. Although compounds **12a**–**c** also showed decreased yields with increasing amount of NH groups in the spacer, the effect was not as pronounced in this 3,5-disubstituted pyridine series as compared to the 2,6- and 2,4,6-substituted pyridines **8a**–**c** and **10a**–**c** suggesting that hydrogen bonds are less prominent.

While acrylate **8a** was a stable solid, compounds **8g**, **10a**,**g**, and **12a**,**g** were colorless, temperature-sensitive oils. To avoid radical polymerizations (especially in the case of AHM **3g** derivatives) and thus increase the stability, 1 wt% of 4-methoxyphenol (MEHQ) was added as inhibitor to the purified products and the compounds were stored under dry nitrogen or in solution below 4 °C. With the acrylamide building blocks **3b**–**f** more stable products were obtained as solids (**8b**,**c**, **10b**,**c**, **12b**–**e**) or as a viscous oil (**12f**), which all showed a better water solubility than the acrylates.

While 2,6-disubstituted pyridines **8a**–**c**,**g** could not be N-methylated with MeI in MeCN in agreement with literature precedence[22,23] (see Supporting Information for additional experiments), the corresponding 3,5-disubstituted pyridines **12a**–**g** were converted to the pyridinium salts **13a**–**g** with MeI in either MeCN (method D) or DMF

(method E) at room temperature without any event (Scheme 4).

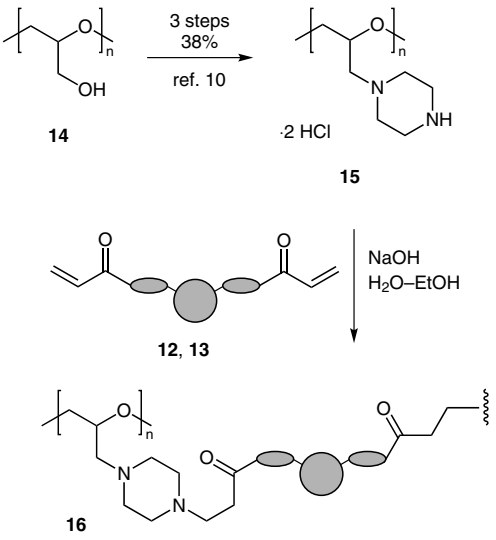


Scheme 4 Synthesis of pyridine acrylates and acrylamides **12**. *Reagents and conditions*: Method A: **3**, Et_3N, CH_2Cl_2, 0 °C to r.t., 2 h. Method B: **3**, NaH, DMF, 0 °C to r.t., 16 h. Method D: MeI, MeCN, r.t., 16 h. Method E: MeI, DMF, r.t., 16 h.

Purification of the ionic compounds was possible by flash chromatography (**13a**), extraction with water (**13b**), or washing with acetonitrile (**13c**), or diethyl ether (**13d–g**). It was observed that **13g** was especially sensitive against silica gel. Thus, it could only be roughly purified, despite the fact that its R_f of 0.15 (hexanes–EtOAc, 4: 1) should permit flash chromatography. In general, the N-methylated products **13a–g** showed a higher stability against radical polymerizations than their neutral precursors **12a–g** and were better soluble in water, which facilitated the subsequent hydrogelations.

Preliminary cross-linking experiments revealed that acrylic esters either did not form hydrogels with the polymers or the hydrogels were too unstable as compared to the corresponding acrylamides in agreement with earlier observations.[10,11] Therefore, from the synthesized library cross-linkers **12d–f** and **13d–f** were chosen for the hydrogelation experiments.

In order to test the reactivity of the different cross-linkers **12d–f** and **13d–f**, they were reacted with the side-chain amino-functionalized polymer poly(1-glycidylpiperazine) (**15**), as described previously for cross-linkers **12c** and **13c** (Scheme 5).[10]



Scheme 5 Reaction of cross-linkers **12d–f** and **13d–f** with poly(1-glycidylpiperazine) (**15**)

Via the aza-Michael reaction, the multifunctional polymer **15** and the bifunctional cross-linkers **12d–f** and **13d–f** form polymer networks **16**, which are highly hydrophilic due to the polyelectrolyte used. The cross-linking reaction was performed in water–ethanol (1:1 v/v) because of the limited solubility of most cross-linkers in pure water. A ratio of cross-linker-bound acrylamide groups and polymer-bound amino groups of 0.8 was maintained for all gel experiments. Corresponding gelling times are given in the Supporting Information. The hydrogels synthesized with the acrylamide-based cross-linkers **12d–f** and **13d–f** are hydrolytically stable and were characterized by shear rheology (Figure 2) and by the determination of their equilib-

rium degree of swelling (EDS) (Figure 3). Since the synthetic hydrogels described here show a linear viscoelastic response only at deformations below 5%, the determination of their *E*-moduli is not possible by linear regression of the linear part of their stress-strain curves. Therefore, their elastic shear moduli *G*′ were determined by shear rheology. The *E*-moduli and *G*-moduli are connected by the Poisson's ratio, the *G*-moduli usually being equal to one third of the *E*-moduli for isotropic incompressible hydrogels.[24]

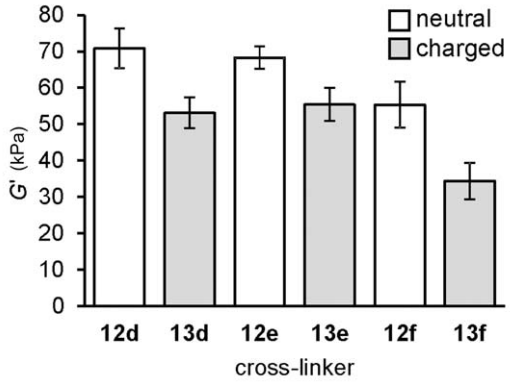


Figure 2 Elastic shear moduli *G*′ of the hydrogels **16** formed by polymer **15** and the respective cross-linkers. The gels were swollen to equilibrium in PBS before measurements.

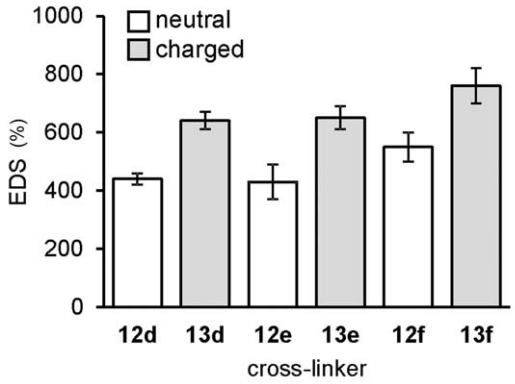


Figure 3 Equilibrium degrees of swelling EDS in PBS of the hydrogels **16** formed by polymer **15** and the respective cross-linkers

The *G*′-values are all in the same order of magnitude around 70 kPa for the neutral and 50 kPa for the charged cross-linkers. A tendency for a decrease of the elastic moduli with the spacer length can be observed. We correlate this observation with the presumably increasing mesh size of the gels. Upon comparison of pairs of neutral and charged cross-linker the neutral one always displayed a higher elastic shear modulus compared to the charged one. A typical example is **12d** versus **13d**. A possible explanation for this effect is that the charge of the cross-linker also leads to higher EDS due to higher hydration, thereby altering the mechanical properties of the gels.

We observed that the EDS is similar for all gels tested and is between 400 and 550% for the neutral cross-linkers and between 600 and 750% for the charged cross-linkers. That means for pairs of neutral and charged cross-linkers the charged ones always display the higher EDS as compared to the corresponding neutral counterparts. The charge of the cross-linker leads to a greater water uptake of the gels due to its interaction with the dipolar water molecules. It should be noted that **12f**-based gels exhibit a greater EDS than the other gels because of the increased hydrophilicity of the spacers.

Following our previously described procedures,[11] stable hydrogels **17** were obtained from thiolated hyaluronan (HA-SH) and cross-linkers **12** and **13** (Figure 4). To analyze the influence of spacer length cross-linkers with varying alkyl spacers **12d–f** and **13d–f** were first employed in the same thio-Michael addition with HA-SH (degree of thiolation: 40%).

In contrast to the synthetic hydrogels described above, the HA-SA-based hydrogels exhibit a linear viscoelastic response up to 10% deformation (Table S2, Supporting Information), enabling the determination of their *E*-moduli by compression testing.

While **12d** and **13d** lead to stable hydrogels with *E*-moduli of 1.6 kPa and 1.1 kPa, respectively, the poor solubility of **12e** and **13e** in water or mixtures of ethanol and water prevented the formation of HA hydrogels. In contrast the excellent solubility of **12f** and **13f** led to stable HA hydrogels with *E*-moduli of 1.1 kPa and 1.3 kPa (Figure 5). All four hydrogels withstood repetitive compressive stress of

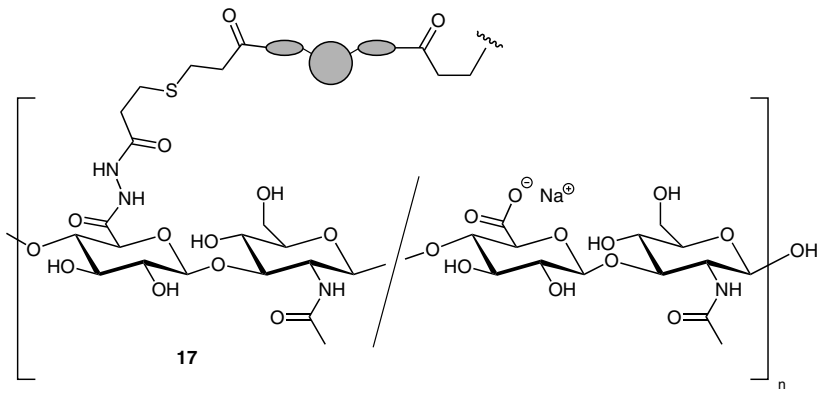


Figure 4 Statistically thiolated hyaluronan (40%) attached to a cross-linker via thio-Michael addition

up to 15% and showed a large linear elastic range. Interestingly, here we observe that as linker length of the cross-linkers increases, the *E*-moduli of hydrogels cross-linked with neutral or charged linker, respectively, display similar values, indicating that the positive charge on the cross-linker only partially compensates the negative charge on the polyanionic hydrogel.

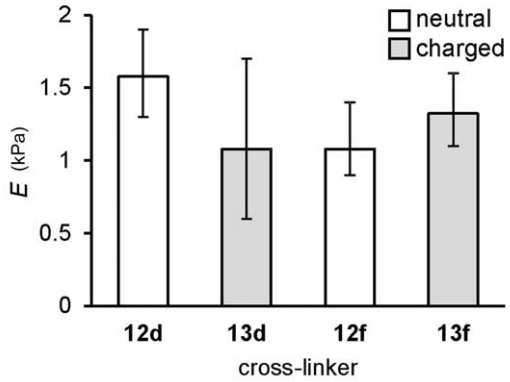


Figure 5 *E*-Moduli of the hydrogels **17** formed with the respective cross-linkers

The EDS values of the gels (Figure 6) range between 200 and 250% and again are slightly higher for positively charged cross-linkers **13d**,**f** compared to the neutral cross-linkers **12d**,**f**.

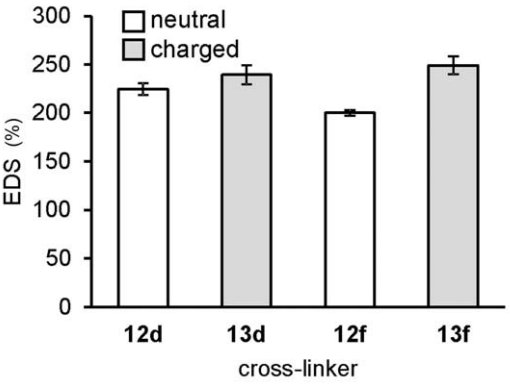


Figure 6 Equilibrium degrees of swelling of the hydrogels **17** with different cross-linkers

In conclusion, we have synthesized three series of neutral cross-linkers containing 2,6-, 3,5-diacylpyridine, and 2,4,6-triacylpyridine as the central cores, which were tethered to terminal acrylates or acrylamides via ethylene glycol, amino ethanol, and 1,n-diamine spacers. Acrylates were generally obtained in higher yields than acrylamides. In addition, yields were affected not only by the position of the side chains at the pyridine core but also by the number of polymerizable groups and the number of NH groups in the spacer suggesting that a combination of steric hindrance and unfavorable aggregation due to hydrogen bonding might lead to reduced yields.

The 3,5-diacylpyridines were N-methylated and selected members of both neutral and cationic 3,5-diacylpyridines were converted to hydrogels with neutral poly(1-glycidylpiperazine) and polyanionic hyaluronan. Rheological studies revealed that elastic shear moduli *G*′ decreased for neutral polymers with increasing water solubility of the spacers, while *E*-moduli of softer polyanionic hydrogels changed only little. Swelling ratios increased with water solubility and presence of cationic cross-linkers in both neutral and polyanionic polymers.

All chemicals for cross-linker synthesis were purchased from Sigma-Aldrich or Alfa-Aesar. Acryloyl chloride, CH_2Cl_2, and Et_3N were freshly distilled before use. Pyridine-2,4,6-tricarboxylic acid and pyridine-3,5-dicarboxylic acid were prepared by oxidation of 2,4,6-lutidine and 3,5-lutidine, respectively, according to literature.[25] Melting points were measured on a Stuart SMP10 instrument. ^1H and ^{13}C NMR spectra were recorded on with Bruker Avance 300 and Avance 500 spectrometers at 300 or 500 MHz (^1H) and 75 or 125 MHz (^{13}C). Standard abbreviations were used to denote the multiplicities of the peaks. NMR assignments were based on COSY, HMBC, and HSQC spectra. IR spectra were recorded on Bruker MKII Golden Gate Single Reflection Diamant spectrometer by ATR method. Mass spectra were recorded on a Bruker micrOTOF-Q spectrometer under positive electrospray ionization (ESI^+) conditions. The assignments for the mass signals are given for $[M]^+$ or adducts, $[M]^+$ being the molecular mass of the positively charged molecule without anion. Elemental analyses were carried out with Carlo Erba Strumentazione Elemental Analyzer model 1106. Column chromatography was performed using silica gel 60 (Fluka, mesh 40–63 μm) with hexanes (bp 30–75 °C). Reactions were performed using standard Schlenk type conditions under inert atmosphere.

Bis[1-(acryloyloxy)-3-(methacryloyloxy)propan-2-yl]pyridine-2,6-dicarboxylate (8g); Typical Procedure

To a solution of 3-(acryloyloxy)-2-hydroxypropyl methacrylate (**3g**; AHM, 3.94 g, 8.98 mmol) and Et_3N (2.6 mL, 1.9 g, 18.9 mmol) in anhydrous CH_2Cl_2 (20 mL) was slowly added a solution of pyridine-2,6-dicarbonyl dichloride (1.83 g, 8.98 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C. The slightly turbid mixture was stirred at r.t. for 16 h, filtered through Celite, and concentrated in vacuo under addition of MEHQ as polymerization inhibitor. The crude product was purified by flash chromatography (hexanes–EtOAc, 3:1 → 2:1) to yield **8g** (3.56 g, 6.36 mmol, 71%) as a clear colorless oil; R_f = 0.21 (hexanes–EtOAc, 2:1).

FT-IR (ATR): 2931 (w), 2184 (w), 1962 (w), 1720 (s), 1293 (m), 1236 (m), 1162 cm^{-1} (s).

^1H NMR (300 MHz, $CDCl_3$): δ = 1.93 (s, 6 H, CH_3), 4.41–4.69 (m, 8 H, OCH_2), 5.59–5.62 (m, 2 H, *cis*-3′-H), 5.65–5.73 (m, 2 H, OCH), 5.85–5.91 (m, 2 H, *cis*-3″-H), 6.08–6.21 (m, 4 H, *trans*-3′-H, 2″-H), 6.40–6.49 (m, 2 H, *trans*-3″-H), 7.99–8.04 (m, 1 H, 4-H), 8.23–8.26 (m, 2 H, 3-H, 5-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.2 (CH_3), 62.4 (OCH_2), 71.0 (OCH), 126.6 (C-3′), 127.6 (C-2″), 128.2 (C-3, C-5), 131.9 (C-3″), 135.6 (C-2′), 138.3 (C-4), 148.1 (C-2, C-6), 163.3 (Ar-C=O), 165.6 (C-1″), 166.7 (C-1′).

MS (ESI): *m*/*z* = 582.2 $[M + Na]^+$, 560.2 $[M + H]^+$, 197.1 $[C_{10}H_{13}O_4$ (AHM − OH)$]^+$.

HRMS (ESI): *m*/*z* calcd for $C_{27}H_{29}NO_{12}$: 582.1582; found: 582.1586 $[M + Na]^+$.

Bis[2-(acryloyloxy)ethyl]pyridine-2,6-dicarboxylate (8a)

An analogous procedure as described for **8g** was used. The product was purified by washing with H_2O and by flash chromatography

(CH_2Cl_2–EtOAc, 5:1; R_f = 0.28); yield: 3.03 g (93%); colorless solid; mp 77 °C.

FT-IR (ATR): 3088 (w), 2958 (w), 1730 (s), 1715 (s), 1400 (m), 1289 (m), 1240 (s), 1178 cm^{-1} (s).

^1H NMR (300 MHz, $CDCl_3$): δ = 4.54–4.57 (m, 4 H, 1′-H), 4.67–4.71 (m, 4 H, 2′-H), 5.87 (dd, ^3J = 10.4 Hz, ^2J = 1.5 Hz, 2 H, *trans*-C*H*H=CH), 6.15 (dd, ^3J = 17.3 Hz, ^3J = 10.4 Hz, 2 H, C*H*=CH_2), 6.45 (dd, ^3J = 17.3 Hz, ^2J = 1.5 Hz, 2 H, *cis*-C*H*H=CH), 8.05 (dd, ^3J = 8.3 Hz, ^3J = 7.5 Hz, 1 H, 4-H), 8.28–8.31 (m, 2 H, 3-H, 5-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 62.1 (C-2′), 63.7 (C-1′), 127.9 (*C*H=CH_2), 128.2 (C-3, C-5), 131.5 (*C*H_2=CH), 138.4 (C-4), 148.2 (C-2, C-6), 164.2 (vinyl-C=O), 165.9 (Ar-C=O).

MS (ESI): *m*/*z* = 386.1 $[M + Na]^+$, 364.1 $[M + H]^+$, 349.2, 301.1, 209.1, 149.0, 99.0.

HRMS (ESI): *m*/*z* calcd for $C_{17}H_{17}NO_8$: 386.0846; found: 386.0854 $[M + Na]^+$.

Anal. Calcd for $C_{17}H_{17}NO_8$ (+ 0.03 equiv MEHQ): C, 56.32; H, 4.74; N, 3.81. Found: C, 56.37; H, 4.77; N, 3.75.

Bis(2-acrylamidoethyl)pyridine-2,6-dicarboxylate (8b)
An analogous procedure as described for **8g** was used. The product was purified by flash chromatography [EtOAc–acetone, 5:1 (R_f = 0.17) → 4:1 → 3:1]; yield: 1.59 g (49%); colorless solid; mp 154 °C.

FT-IR (ATR): 3258 (s), 3062 (w), 2963 (w), 2925 (w), 2877 (w), 1740 (m), 1551 (s), 1242 cm^{-1} (s).

^1H NMR (300 MHz, DMSO-d_6): δ = 3.54 (q, ^3J = 5.6 Hz, 4 H, 2′-H), 4.39 (t, ^3J = 5.6 Hz, 4 H, 1′-H), 5.60 (dd, ^3J = 9.9 Hz, ^2J = 2.4 Hz, 2 H, *trans*-C*H*H=CH), 6.09 (dd, ^3J = 17.1 Hz, ^2J = 2.4 Hz, 2 H, *cis*-C*H*H=CH), 6.22 (dd, ^3J = 17.1 Hz, ^3J = 9.9 Hz, 2 H, C*H*=CH_2), 8.21 (dd, ^3J = 9.2 Hz, ^3J = 5.9 Hz, 1 H, 4-H), 8.27–8.3 (m, 2 H, 3-H, 5-H), 8.38 (t, ^3J = 5.5 Hz, 2 H, NH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 37.7 (C-2′), 64.1 (C-1′), 125.5 (*C*H_2=CH), 128.2 (C-3, C-5), 131.4 (*C*H=CH_2), 139.1 (C-4), 147.8 (C-2, C-6), 163.9 (vinyl-C=O), 165.0 (Ar-C=O).

MS (ESI): *m*/*z* = 384.1 $[M + Na]^+$, 362.1 $[M + H]^+$, 287.1, 98.1.

HRMS (ESI): *m*/*z* calcd for $C_{17}H_{19}N_3O_6$: 384.1166; found: 384.1165 $[M + Na]^+$.

Anal. Calcd for $C_{17}H_{19}N_3O_6$: C, 56.51; H, 5.30; N, 11.63. Found: C, 56.29; H, 5.35; N, 11.59.

Tris[2-(acryloyloxy)ethyl]pyridine-2,4,6-tricarboxylate (10a)
An analogous procedure as described for **8g** was used. The product was purified by flash chromatography [hexanes–EtOAc, 2:1 → 1:1 (R_f = 0.24)]; yield: 3.00 g (66%); colorless oil.

FT-IR (ATR): 2985 (w), 1719 (s), 1408 (m), 1227 (s), 1183 (s) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 4.55–4.58 (m, 6 H, 2′-H), 4.68–4.74 (m, 6 H, 1′-H), 5.88 [dd, ^3J = 10.4 Hz, ^2J = 1.5 Hz, 2 H, *trans*-C*H*H=CH (C-2, C-6)], 5.90 [dd, ^3J = 10.4 Hz, ^2J = 1.4 Hz, 1 H, *trans*-C*H*H=CH (C-4)], 6.16 [dd, ^3J = 17.3 Hz, ^3J = 10.4 Hz, 2 H, C*H*=CH_2 (C-2, C-6)], 6.17 [dd, ^3J = 17.3 Hz, ^3J = 10.4 Hz, 1 H, C*H*=CH_2 (C-4)], 6.45 [dd, ^3J = 17.3 Hz, ^2J = 1.5 Hz, 2 H, *cis*-C*H*H=CH (C-2, C-6)], 6.47 [dd, ^3J = 17.3 Hz, ^2J = 1.4 Hz, 1 H, *cis*-C*H*H=CH (C-4)], 8.78 (s, 3-H, 5-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 61.8 [C-2′ (C-4)], 62.0 [C-2′ (C-2, C-6)], 64.0 [C-1′ (C-2, C-6)], 64.2 [C-1′ (C-4)], 127.4 (C-3, C-5), 127.7 [*C*H=CH_2 (C-4)], 127.8 [*C*H=CH_2 (C-2, C-6)], 131.6 [*C*H_2=CH (C-2, C-6)], 131.8 [*C*H_2=CH (C-4)], 139.9 (C-4), 149.3 (C-2, C-6), 163.3 [vinyl-C=O (C-4)], 163.6 [vinyl-C=O (C-2, C-6)], 165.7 [Ar-C=O (C-4)], 168.8 [Ar-C=O (C-2, C-6)].

MS (ESI): *m*/*z* = 528.1 $[M + Na]^+$, 506.1 $[M + H]^+$, 444.1, 400.1.

HRMS (ESI): *m*/*z* calcd for $C_{23}H_{23}NO_{12}$: 528.1112; found: 528.1106 $[M + Na]^+$.

Tris(2-acrylamidoethyl)pyridine-2,4,6-tricarboxylate (10b)
An analogous procedure as described for **8g** was used. The product was purified by flash chromatography (EtOAc–acetone, 5:1; R_f = 0.08); yield: 993 mg (22%); colorless solid; mp 135 °C.

FT-IR (ATR): 3300 (w), 3056 (w), 1750 (m), 1728 (m), 1654 (s), 1542 (m), 1210 (s), 1170 (m), 1025 cm^{-1} (m).

^1H NMR (500 MHz, DMSO-d_6): δ = 3.57–3.62 (m, 6 H, 2′-H), 4.43–4.46 (m, 6 H, 1′-H), 5.61 [dd, ^3J = 10.1 Hz, ^2J = 2.1 Hz, 2 H, *trans*-C*H*H=CH (C-2, C-6)], 5.63 [dd, ^3J = 10.1 Hz, ^2J = 2.2 Hz, 1 H, *trans*-C*H*H=CH (C-4)], 6.10 [dd, ^3J = 17.1 Hz, ^2J = 2.2 Hz, 2 H, *cis*-C*H*H=CH (C-2, C-6)], 6.11 (dd, ^3J = 17.1 Hz, ^2J = 2.2 Hz, 1 H, *cis*-C*H*H=CH (C-4)], 6.23 [dd, ^3J = 17.1 Hz, ^3J = 10.1 Hz, 2 H, C*H*=CH_2 (C-2, C-6)], 6.23 (dd, ^3J = 17.1 Hz, ^3J = 10.1 Hz, 1 H, C*H*=CH_2 (C-4)], 8.43 [t, ^3J = 5.7 Hz, 2 H, NH (C-2, C-6)], 8.46 [t, ^3J = 5.8 Hz, 1 H, NH (C-4)], 8.60 (s, 2 H, 3-H, 5-H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 37.5 [C-2′ (C-4)], 37.6 [C-2′ (C-2, C-6)], 64.5 [C-1′ (C-2, C-6)], 65.0 [C-1′ (C-4)], 125.5 [*C*H_2=CH (C-2, C-6)], 125.6 [*C*H_2=CH (C-4)], 126.6 (C-3, C-5), 131.4 (*C*H=CH_2), 139.9 (C-4), 149.0 (C-2, C-6), 163.2 [vinyl-C=O (C-2, C-6)], 163.3 [vinyl-C=O (C-4)], 165.0 [Ar-C=O (C-2, C-6)], 165.1 [Ar-C=O (C-4)].

MS (ESI): *m*/*z* = 525.2 $[M + Na]^+$, 503.2 $[M + H]^+$, 428.1, 406.1, 331.1.

HRMS (ESI): *m*/*z* calcd for $C_{23}H_{26}N_4O_9$: 525.1592; found: 525.1606 $[M + Na]^+$.

Tris[1-(acryloyloxy)-3-(methacryloyloxy)propan-2-yl]pyridine-2,4,6-tricarboxylate (10g)
An analogous procedure as described for **8g** was used. The product was purified by flash chromatography [hexanes–EtOAc, 3:1 → 2:1 (R_f = 0.24)]; yield: 2.37 g (33%); colorless oil.

FT-IR (ATR): 2960 (w), 1718 (s), 1635 (m), 1235 (m), 1155 (s), 1064 (m), 946 (m), 808 cm^{-1} (m).

^1H NMR (300 MHz, $CDCl_3$): δ = 1.94 (s, 9 H, CH_3), 4.38–4.66 (m, 12 H, OCH_2), 5.59–5.64 (m, 3 H, *cis*-3′-H), 5.65–5.74 (m, 3 H, OCH), 5.82–5.92 (m, 3 H, *cis*-3″-H), 6.08–6.21 (m, 6 H, *trans*-3′-H, 2″-H), 6.41–6.49 (m, 3 H, *trans*-3″-H), 8.70 (s, 2 H, 3-H, 5-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.3 (CH_3), 61.4 (OCH_2), 70.4 (OCH), 125.3 (C-3′), 125.7 (C-2″), 126.8 (C-3, C-5), 131.0 (C-3″), 134.7 (C-2′), 138.8 (C-4), 148.4 (C-2, C-6), 161.7 (4-C=O), 164.6 (2-C=O, 6-C=O), 165.7 (C-1″), 166.4 (C-1′).

MS (ESI): *m*/*z* = 822.2 $[M + Na]^+$, 626.2 [M − AHM + H + Na]$^+$, 596.2, 419.6, 237.1, 197.1.

HRMS (ESI): *m*/*z* calcd for $C_{38}H_{41}NO_{18}$: 822.2216; found: 822.2208 $[M + Na]^+$.

Bis[1-(acryloyloxy)-3-(methacryloyloxy)propan-2-yl]pyridine-3,5-dicarboxylate (12g)
An analogous procedure as described for **8g** was used. The product was purified by flash chromatography (hexanes–EtOAc, 4:1; R_f = 0.15); yield: 3.62 g (72%); colorless oil.

FT-IR (ATR): 2960 (w), 2196 (w), 1966 (w), 1636 (w), 1453 (w), 1409 (w), 1294 (m), 1234 (m), 1159 (s), 1102 (m), 809 (w), 745 cm^{-1} (w).

^1H NMR (300 MHz, $CDCl_3$): δ = 1.93–1.95 (m, 6 H, CH_3), 4.41–4.63 (m, 8 H, OCH_2), 5.60–5.64 (m, 2 H, *cis*-3′-H), 5.66–6.73 (m, 2 H, OCH), 5.88–5.94 (2 H, *cis*-3″-H), 6.10–6.21 (4 H, *trans*-3′-H, 2″-H), 6.41–6.5 (2 H, *trans*-3″-H), 8.83–8.85 (m, 1 H, 4-H), 9.36–9.37 (m, 2 H, 3-H, 5-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.2 (CH_3), 62.4 (OCH_2), 70.8 (OCH), 125.6 (C-3′), 126.6 (C-2″), 127.4 (C-3, C-5), 132.1 (C-3″), 135.5 (C-2′), 138.4 (C-4), 154.5 (C-2, C-6), 163.4 (aryl-C=O), 165.6 (C-1″), 166.8 (C-1′).

MS (ESI): m/z = 582.2 [M + Na]$^+$, 560.2 [M + H]$^+$, 451.2, 334.1, 237.1.

HRMS (ESI): m/z calcd for $C_{27}H_{29}NO_{12}$ 560.1763; found: 560.1764 [M + Na]$^+$.

N^3,N^5-Bis(2-acrylamidoethyl)pyridine-3,5-dicarboxamide (12c); Typical Procedure

A suspension of *N*-(2-aminoethyl)acrylamide hydrochloride (**3c**; 8.27 g, 54.9 mmol) and NaH (2.89 g, 121 mmol) in anhydrous DMF (120 mL) was stirred for 10 min at r.t. To this mixture was added a solution of pyridine-3,5-dicarbonyl dichloride (5.60 g, 27.4 mmol) in anhydrous DMF (25 mL) dropwise at 0 °C. The mixture was stirred at r.t. for 16 h, filtered through silica gel, and concentrated in vacuo. To the brown residue was added MeOH (30 mL) and the resulting suspension filtered again. The colorless residue consisted of the pure product. The filtrate could be recrystallized further to give an additional amount of the product. Product **12c** (4.92 g, 12.9 mmol, 47%) was obtained as a colorless solid; mp 295 °C (dec.); R_f = 0.16 (CH_2Cl_2–MeOH, 10:1).

FT-IR (ATR): 3258 (w), 2945 (w), 1638 (m), 1533 (s), 1236 (m), 671 cm^{-1} (m).

^{1}H NMR (500 MHz, D_2O): δ = 3.55–3.58 (m, 4 H, 2′-H), 3.62–3.65 (m, 4 H, 1′-H), 5.75 (dd, ^3J = 10.1 Hz, ^2J = 1.3 Hz, 2 H, *trans*-C*H*H=CH), 6.16 (dd, ^3J = 17.1 Hz, ^2J = 1.3 Hz, 2 H, *cis*-C*H*H=CH), 6.26 (dd, ^3J = 17.1 Hz, ^3J = 10.1 Hz, 2 H, C*H*=CH_2), 9.14 (t, ^4J = 1.9 Hz, 1 H, 4-H), 9.28 (d, ^4J = 1.9 Hz, 2 H, 2-H, 6-H).

^{13}C NMR (125 MHz, D_2O + TFA): δ = 38.4 (C-2′), 39.8 (C-1′), 127.4 (*C*H=CH_2), 129.9 (*C*H_2=CH), 133.5 (C-3, C-5), 142.5 (C-4), 143.3 (C-2, C-6), 163.9 (vinyl-C=O), 170.0 (aryl-C=O).

MS (ESI): m/z = 382.2 [M + Na]$^+$, 360.2 [M + H]$^+$.

HRMS (ESI): m/z calcd for $C_{17}H_{21}N_5O_4$: 382.1486; found: 382.1473 [M + Na]$^+$.

Anal. Calcd for $C_{17}H_{21}N_5O_4$: C, 56.82; H, 5.89; N, 19.49. Found: C, 56.30; H, 5.93; N, 19.33.

Spectral data were in accordance with the literature data.[11]

N^2,N^6-Bis(2-acrylamidoethyl)pyridine-2,6-dicarboxamide (8c)

An analogous procedure as described for **12c** was used. The product was purified by recrystallization from EtOH; yield: 678 mg (21%); colorless solid; mp 280 °C (dec.); R_f = 0.52 (CH_2Cl_2–MeOH, 10:1).

FT-IR (ATR): 3257 (m), 3075 (m), 2940 (w), 1651 (s), 1542 (s), 1404 (m), 1253 (m), 949 (m), 808 (m), 714 (m), 681 cm^{-1} (m).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.41–3.52 (m, 8 H, CH_2), 5.61 (dd, ^3J = 9.8 Hz, ^2J = 2.5 Hz, 2 H, *trans*-C*H*H=CH), 6.10 (dd, ^3J = 17.1 Hz, ^2J = 2.5 Hz, 2 H, *cis*-C*H*H=CH), 6.23 (dd, ^3J = 17.1 Hz, ^3J = 9.8 Hz, 2 H, C*H*=CH_2), 8.14–8.23 (m, 3 H, 3-H, 4-H, 5-H), 8.40 (t, ^3J = 5.6 Hz, 2 H, 2′-NH), 9.47 (t, ^3J = 5.6 Hz, 2 H, 1′-NH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 37.4 (C-2′), 38.1 (C-1′), 123.2 (*C*H=CH_2), 124.4 (*C*H_2=CH), 130.7 (C-3, C-5), 138.6 (C-4), 147.6 (C-2, C-6), 162.4 (vinyl-C=O), 164.3 (aryl-C=O).

MS (ESI): m/z = 382.2 [M + Na]$^+$, 360.2 [M + H]$^+$.

HRMS (ESI): m/z calcd for $C_{17}H_{21}N_5O_4$: 382.1486; found: 382.1492 [M + Na]$^+$.

N^2,N^4,N^6-Tris(2-acrylamidoethyl)pyridine-2,4,6-tricarboxamide (10c)

An analogous procedure as described for **12c** was used. The product was purified by flash chromatography (CH_2Cl_2–MeOH, 10:1; R_f = 0.29); yield: 538 mg (12%); colorless solid; mp 270 °C (dec.).

FT-IR (ATR): 2922 (s), 2852 (s), 1711 (m), 1623 (s), 1537 (s), 1247 (s), 765 (m), 685 cm^{-1} (s).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.34–3.51 (m, 12 H, CH_2), 5.59 [dd, ^3J = 9.9 Hz, ^2J = 2.3 Hz, 2 H, *trans*-C*H*H=CH (C-2, C-6)], 5.60 [dd, ^3J = 10.0 Hz, ^2J = 2.5 Hz, 1 H, *trans*-C*H*H=CH (C-4)], 6.09 [dd, ^3J = 17.1 Hz, ^2J = 2.5 Hz, 1 H, *cis*-C*H*H=CH (C-4)], 6.10 [dd, ^3J = 17.1 Hz, ^2J = 2.3 Hz, 2 H, *cis*-C*H*H=CH (C-2, C-6)], 6.22 [dd, ^3J = 17.1 Hz, ^3J = 9.9 Hz, 2 H, C*H*=CH_2 (C-2, C-6)], 6.26 [dd, ^3J = 17.1 Hz, ^3J = 10.0 Hz, 1 H, C*H*=CH_2 (C-4)], 8.31 [t, ^3J = 5.4 Hz, 1 H, 2′-NH (C-4)], 8.52 [t, ^3J = 5.7 Hz, 2 H, 2′-NH (C-2, C-6)], 8.60 (s, 2 H, 3-H, 5-H), 9.26 [t, ^3J = 5.1 Hz, 1 H, 1′-NH (C-4)], 9.68 [t, ^3J = 5.9 Hz, 2 H, 1′-NH (C-2, C-6)].

^{13}C NMR (75 MHz, DMSO-d_6): δ = 37.90 (C-2′), 38.50 (C-1′), 121.70 (*C*H=CH_2), 125.10 (*C*H_2=CH), 131.70 (C-3, C-5), 144.50 (C-4), 149.50 (C-2, C-6), 162.90 [vinyl-C=O (C-2, C-6)], 163.60 [vinyl-C=O (C-4)], 164.80 [aryl-C=O (C-4)), 165.00 (aryl-C=O (C-2, C-6)].

MS (ESI): m/z = 522.2 [M + Na]$^+$, 500.2 [M + H]$^+$, 102.1.

HRMS (ESI): m/z calcd for $C_{23}H_{29}N_7O_6$: 522.2072; found: 522.2069 [M + Na]$^+$.

N^3,N^5-Bis(3-acrylamidopropyl)pyridine-3,5-dicarboxamide (12d)

An analogous procedure as described for **12c** was used. The product was purified by flash chromatography (CH_2Cl_2–MeOH, 10:1; R_f = 0.20); yield: 1.39 g (40%); colorless solid; mp 199 °C.

FT-IR (ATR): 3291 (m), 3247 (m), 3069 (w), 3026 (w), 2961 (w), 2924 (w), 2867 (w), 1635 (s), 1537 (s), 1407 (m), 1359 (m), 1328 (m), 1299 (m), 1241 (m), 1130 (m), 951 (m), 707 (s), 685 cm^{-1} (s).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.73 (quint, ^3J = 7.0 Hz, 4 H, 2′-H), 3.21 (q, ^3J = 6.6 Hz, 4 H, 3′-H), 3.32 (q, ^3J = 6.6 Hz, 4 H, 1′-H), 5.58 (dd, ^3J = 10.2 Hz, ^2J = 2.2 Hz, 2 H, *trans*-C*H*H=CH), 6.02 (dd, ^3J = 17.1 Hz, ^2J = 2.2 Hz, 2 H, *cis*-C*H*H=CH), 6.22 (dd, ^3J = 17.1 Hz, ^3J = 10.2 Hz, 2 H, C*H*=CH_2), 8.15 (t, ^3J = 5.2 Hz, 2 H, 3′-NH), 8.58 (t, ^4J = 2.1 Hz, 1 H, 4-H), 8.81 (t, ^3J = 5.5 Hz, 2 H, 1′-NH), 9.09 (d, ^4J = 2.1 Hz, 2 H, 2-H, 6-H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 28.9 (C-2′), 36.4 (C-1′), 37.2 (C-3′), 124.9 (*C*H_2=CH), 129.6 (C-3, C-5), 131.7 (*C*H=CH_2), 133.7 (C-4), 150.1 (C-2, C-6), 164.2 (aryl-C=O), 164.6 (vinyl-C=O).

MS (ESI): m/z = 410.2 [M + Na]$^+$, 388.2 [M + H]$^+$.

HRMS (ESI): m/z calcd for $C_{19}H_{25}N_5O_4$: 410.1799; found: 410.1802 [M + Na]$^+$.

Anal. Calcd for $C_{19}H_{25}N_5O_4$: C, 58.90; H, 6.50; N, 18.08. Found: C, 59.01; H, 6.58; N, 17.86.

N^3,N^5-Bis(4-acrylamidobutyl)pyridine-3,5-dicarboxamide (12e)

An analogous procedure as described for **12c** was used. The product was purified by flash chromatography (CH_2Cl_2–MeOH, 10:1; R_f = 0.14); yield: 1.90 g (51%); colorless solid; mp 191 °C.

FT-IR (ATR): 3298 (m), 3061 (w), 2954 (w), 2870 (w), 1654 (m), 1625 (s), 1530 (s), 1476 (m), 1318 (m), 1304 (m), 1287 (m), 1233 (m), 982 (m), 954 (m), 656 cm^{-1} (s).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.47–1.58 (m, 8 H, 2′-H, 3′-H), 3.16 (q, ^3J = 6.4 Hz, 4 H, 4′-H), 3.30 (q, ^3J = 6.4 Hz, 4 H, 1′-H), 5.56 (dd, ^3J = 10.1 Hz, ^2J = 2.2 Hz, 2 H, *trans*-C*H*H=CH), 6.06 (dd, ^3J = 17.1 Hz, ^2J = 2.2 Hz, 2 H, *cis*-C*H*H=CH), 6.20 (dd, ^3J = 17.1 Hz, ^3J = 10.1 Hz, 2 H, C*H*=CH_2), 8.10 (t, ^3J = 5.3 Hz, 2H, 4′-NH), 8.57 (t, ^4J = 2.1 Hz, 1 H, 4-H), 8.80 (t, ^3J = 5.5 Hz, 2 H, 1′-NH), 9.08 (t, ^4J = 2.1 Hz, 2 H, 2-H, 6-H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 26.5 (C-2′), 26.6 (C-3′), 38.2 (C-1′), 39.0 (C-4′), 124.9 (*C*H_2=CH), 129.8 (C-3, C-5), 131.8 (*C*H=CH_2), 133.8 (C-4), 150.2 (C-2, C-6), 164.2 (aryl-C=O), 164.5 (vinyl-C=O).

MS (ESI): m/z = 438.2 [M + Na]$^+$, 416.2 [M + H]$^+$, 393.3, 284.1.

HRMS (ESI): m/z calcd for $C_{21}H_{29}N_5O_4$: 438.2112; found: 438.2134 [M + Na]$^+$.

Anal. Calcd for $C_{21}H_{29}N_5O_4$: C, 60.71; H, 7.04; N, 16.86. Found: C, 60.76; H, 7.40; N, 15.08.

N^3,N^5-Bis{2-[2-(2-acrylamidoethoxy)ethoxy]ethyl}pyridine-3,5-dicarboxamide (12f)

An analogous procedure as described for **12c** was used. Purified by flash chromatography (CH_2Cl_2–MeOH 10:1; R_f = 0.35); yield: 2.21 g (46%); pale yellow oil.

FT-IR (ATR): 3289 (w), 3076 (w), 2870 (w), 1652 (s), 1627 (s), 1543 (s), 1291 (m), 1245 (m), 1102 (m), 705 cm^{-1} (w).

^1H NMR (300 MHz, DMSO-d_6): δ = 3.27 (q, ^3J = 5.7 Hz, 4 H, 6′-H), 3.43–3.48 (m, 8 H, 1′-H, 5′-H), 3.52–3.58 (m, 12 H, 2′-H, 3′-H, 4′-H), 5.56 (dd, ^3J = 10.0 Hz, ^2J = 2.3 Hz, 2 H, *trans*-C*H*H=CH), 6.07 (dd, ^3J = 17.1 Hz, ^2J = 2.3 Hz, 2 H, *cis*-C*H*H=CH), 6.24 (dd, ^3J = 17.1 Hz, ^3J = 10.0 Hz, 2 H, C*H*=CH_2), 8.17 (t, ^3J = 5.2 Hz, 2 H, 6′-NH), 8.60 (t, ^4J = 2.1 Hz, 1 H, 4-H), 8.88 (t, ^3J = 5.4 Hz, 2 H, 1′-NH), 9.10 (d, ^4J = 2.1 Hz, 2 H, 2-H, 6-H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 38.5 (C-6′), 39.3 (C-1′), 68.7 (C-5′), 69.0 (C-2′), 69.5 (C-3′, C-4′), 125.0 (*C*H_2=CH), 129.5 (C-3, C-5), 131.6 (*C*H=CH_2), 133.9 (C-4), 150.3 (C-2, C-6), 164.4 (aryl-C=O), 164.6 (vinyl-C=O).

MS (ESI): *m*/*z* = 558.3 $[M + Na]^+$, 536.3 $[M + H]^+$, 439.2 [M − H_2C=CHCONHC_2H_4 + 2 H]$^+$, 377.2, 342.2, 290.6, 280.1 [M − H_2C=CHCONH − H_2C=CHCONH$(C_2H_4O)_2C_2H_4]^+$, 227.0, 218.1, 185.1, 98.1 [H_2C=CHCONH$C_2H_4]^+$.

HRMS (ESI): *m*/*z* calcd for $C_{25}H_{37}N_5O_8$: 558.2534; found: 558.2530 $[M + Na]^+$.

3,5-Bis[(3-acrylamidopropyl)carbamoyl]-1-methylpyridin-1-ium Iodide (13d); Typical Procedure

N^3,N^5-Bis(3-acrylamidopropyl)pyridine-3,5-dicarboxamide (**12d**; 200 mg, 515 μmol) was treated with MeI (0.14 mL, 2.07 mmol) in DMF (5 mL) at r.t. for 16 h. The mixture was evaporated and the residue washed with Et_2O (10 mL) to give **13d** (273 mg, 515 μmol, quant) as an orange oil; R_f = 0.13 (CH_2Cl_2–MeOH, 5:1).

FT-IR (ATR): 3256 (m), 3061 (w), 2931 (w), 1651 (s), 1543 (s), 1436 (m), 1407 (m), 1386 (m), 1285 (s), 1237 (s), 1096 (m), 984 (m), 959 (m), 806 (w), 664 (s), 606 cm^{-1} (m).

^1H NMR (500 MHz, DMSO-d_6): δ = 1.76 (quint, ^3J = 7.0 Hz, 4 H, 2′-H), 3.24 (q, ^3J = 6.5 Hz, 4 H, 3′-H), 3.38 (q, ^3J = 6.5 Hz, 4 H, 1′-H), 4.45 (s, 3 H, CH_3), 5.60 (dd, ^3J = 10.2 Hz, ^2J = 2.1 Hz, 2 H, *trans*-C*H*H=CH), 6.09 (dd, ^3J = 17.1 Hz, ^2J = 2.1 Hz, 2 H, *cis*-C*H*H=CH), 6.24 (dd, ^3J = 17.1 Hz, ^3J = 10.1 Hz, 2 H, C*H*=CH_2), 8.17 (t, ^3J = 5.3 Hz, 2 H, 3′-NH), 9.14 (t, ^3J = 5.4 Hz, 2 H, 1′-NH), 9.25 (t, ^4J = 1.4 Hz, 1 H, 4-H), 9.49 (d, ^4J = 1.4 Hz, 2 H, 2-H, 6-H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 28.7 (C-2′), 36.4 (C-1′), 37.7 (C-3′), 48.5 (CH_3), 125.1 (*C*H_2=CH), 131.7 (*C*H=CH_2), 133.1 (C-3, C-5), 140.6 (C-4), 146.9 (C-2, C-6), 161.0 (aryl-C=O), 164.7 (vinyl-C=O).

MS (ESI +): *m*/*z* = 402.2 $[M]^+$; (ESI −): *m*/*z* = 446.2, 436.2, 418.2, 400.2, 126.9 $[I]^-$.

HRMS (ESI +): *m*/*z* calcd for $C_{20}H_{28}N_5O_4$: 402.2136; found: 402.2137 $[M]^+$; (ESI –): *m*/*z* calcd for I^- 126.9039; found: 126.9025 $[I]^-$.

3,5-Bis[(4-acrylamidobutyl)carbamoyl]-1-methylpyridin-1-ium Iodide (13e)

An analogous procedure as described for **13d** was used. Solvent: DMF. Purified by washing with Et_2O; yield: 287 mg (quant); orange solid; mp 69 °C; R_f = 0.17 (CH_2Cl_2–MeOH, 5:1).

FT-IR (ATR): 3276 (m), 3067 (m), 2932 (m), 2867 (w), 1644 (s), 1616 (s), 1535 (s), 1432 (m), 1405 (m), 1308 (m), 1290 (m), 1224 (m), 1192 (m), 978 (m), 960 (m), 805 (w), 674 cm^{-1} (m).

^1H NMR (500 MHz, DMSO-d_6): δ = 1.49–1.61 (m, 8 H, 2′-H, 3′-H), 3.18 (q, ^3J = 6.4 Hz, 4 H, 4′-H), 3.36 (q, ^3J = 6.4 Hz, 4 H, 1′-H), 4.44 (s, 3 H, CH_3), 5.58 (dd, ^3J = 10.2 Hz, ^2J = 2.2 Hz, 2 H, *trans*-C*H*H=CH), 6.07 (dd, ^3J = 17.1 Hz, ^2J = 2.2 Hz, 2 H, *cis*-C*H*H=CH), 6.22 (dd, ^3J = 17.1 Hz, ^3J = 10.2 Hz, 2 H, C*H*=CH_2), 8.13 (t, ^3J = 5.7 Hz, 2 H, 4′-NH), 9.13 (t, ^3J = 5.6 Hz, 2 H, 1′-NH), 9.24 (t, ^4J = 1.3 Hz, 1 H, 4-H), 9.49 (d, ^4J = 1.3 Hz, 2 H, 2-H, 6-H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 26.2 (C-2′), 26.5 (C-3′), 38.1 (C-1′), 39.4 (C-4′), 48.5 (CH_3), 124.9 (*C*H_2=CH), 131.8 (*C*H=CH_2), 133.2 (C-3, C-5), 140.6 (C-4), 146.8 (C-2, C-6), 160.9 (aryl-C=O), 164.5 (vinyl-C=O).

MS (ESI): *m*/*z* = 430.3 $[M]^+$, 416.2 [M − CH_3 + H]$^+$, 393.3, 325.3, 311.3.

HRMS (ESI): *m*/*z* calcd for $C_{22}H_{32}N_5O_4$: 430.2449; found: 430.2459 $[M]^+$.

3,5-Bis({2-[2-(2-acrylamidoethoxy)ethoxy]ethyl}carbamoyl)-1-methylpyridin-1-ium Iodide (13f)

An analogous procedure as described for **13d** was used. Solvent: DMF. Purified by washing with Et_2O; yield: 349 mg (quant); orange oil; R_f = 0.15 (CH_2Cl_2–MeOH, 10:1).

FT-IR (ATR): 3261 (w), 3065 (w), 2868 (w), 1655 (s), 1624 (m), 1539 (s), 1288 (m), 1238 (m), 1095 (s), 986 (m), 960 (m), 806 (w), 669 cm^{-1} (m).

^1H NMR (500 MHz, D_2O): δ = 3.46 (t, ^3J = 5.4 Hz, 4 H, 6′-H), 3.67 (t, ^3J = 5.4 Hz, 4 H, 5′-H), 3.69 (t, ^3J = 5.2 Hz, 4 H, 1′-H), 3.71–3.74 (m, 8 H, 3′-H, 4′-H), 3.79 (t, ^3J = 5.2 Hz, 4 H, 2′-H), 4.55 (s, 3 H, CH_3), 5.75 (dd, ^3J = 10.1 Hz, ^2J = 1.5 Hz, 2 H, *trans*-C*H*H=CH), 6.17 (dd, ^3J = 17.2 Hz, ^2J = 1.5 Hz, 2 H, *cis*-C*H*H=CH), 6.25 (dd, ^3J = 17.2 Hz, ^3J = 10.1 Hz, 2 H, C*H*=CH_2), 9.23 (t, ^4J = 1.6 Hz, 1 H, 4-H), 9.42 (d, ^4J = 1.6 Hz, 2 H, 3-H, 5-H).

^{13}C NMR (125 MHz, D_2O): δ = 38.9 (C-6′), 39.9 (C-1′), 49.1 (CH_3), 68.5 (C-5′), 68.7 (C-2′), 69.4 (C-3′, C-4′), 127.3 (*C*H_2=CH), 129.8 (*C*H=CH_2), 134.2 (C-3, C-5), 141.5 (C-4), 146.8 (C-2, C-6), 163.1 (vinyl-C=O), 168.5 (aryl-C=O).

MS (ESI): *m*/*z* = 550.3 $[M]^+$, 453.2, 391.2, 350.2 [M − H_2C=CHCONH$(C_2H_4O)_2C_2H_4$NH + H]$^+$, 344.2, 322.2, 297.6, 280.1, 251.1 [M − H_2C=CHCONHC_2H_4 − H_2C=CHCONH$(C_2H_4O)_2C_2H_4]^+$, 232.1, 218.1, 189.1, 166.1, 122.1, 98.1.

HRMS (ESI): *m*/*z* calcd for $C_{26}H_{40}N_5O_8$: 550.2871; found: 550.2875 $[M]^+$.

3,5-Bis({[1-(acryloyloxy)-3-(methacryloyloxy)propan-2-yl]oxy}carbonyl)-1-methylpyridin-1-ium Iodide (13g)

An analogous procedure as described for **13d** was used. Solvent: MeCN. Purified by washing with Et_2O; yield: 289 mg (80%); orange oil; R_f = 0.56 (CH_2Cl_2–MeOH, 20:1, partial decomposition on silica gel).

FT-IR (ATR): 3323 (w), 2959 (w), 2556 (w), 1968 (w), 1716 (s), 1634 (w), 1408 (m), 1294 (m), 1247 (s), 1156 (s), 1066 (m), 1032 (m), 955 (m), 810 (m), 742 (w), 658 (m), 634 (m), 538 cm^{-1} (m).

^1H NMR (300 MHz, $CDCl_3$): δ = 1.92–1.94 (m, 6 H, CH_3), 4.24–4.65 (m, 8 H, OCH_2), 4.84–4.85 (m, 3 H, CH_3N), 5.60–5.65 (m, 2 H, *cis*-3′-H), 5.67–5.72 (m, 2 H, OCH), 5.86–5.93 (m, 2 H, *cis*-3″-H), 6.10–6.19 (m, 4 H, *trans*-3′-H, 2″-H), 6.40–6.48 (m, 2 H, *trans*-3″-H), 9.22–9.24 (m, 1 H, 4-H), 9.83–9.95 (m, 2 H, 2-H, 6-H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.4 (CH_3C), 51.2 (CH_3N), 62.1 (OCH_2), 68.3 (OCH), 126.4 (C-3′), 127.2 (C-2″), 127.8 (C-3, C-5), 131.8 (C-3″), 132.5 (C-2, C-6), 135.3 (C-2′), 135.5 (C-4), 159.8 (aryl-C=O), 166.0 (C-1″), 166.8 (C-1′).

MS (ESI): *m*/*z* = 574.2 $[M]^+$, 538.2, 392.1.

HRMS (ESI): *m*/*z* calcd for $C_{28}H_{32}NO_{12}$: 574.1919; found: 574.1916 $[M]^+$.

Hydrogel Formation with Poly(1-glycidylpiperazine)

Hydrogel Preparation: The following is a typical experiment leading to a hydrogel formed from PEPP **15** and cross-linker **8c** with a ratio of cross-linker acrylamide groups and polymer bound amino groups of 0.8. The preparation of all the other gels was performed

accordingly. For the preparation of a polymer solution of PEPP **15**, the polymer was dissolved in H_2O–EtOH (1:1) to yield a total polymer concentration of 50% (w/w). The pH value was adjusted to 9.0 using a 4 M aq NaOH–EtOH mixture (1:1). The resulting solution was diluted with H_2O–EtOH (1:1) until a polymer concentration of 20% (w/w) was reached. For the preparation of the cross-linker solution of **6c**, the cross-linker **2c** (62 mg, 345 μmol of acrylamide groups, 0.8 equiv) was dissolved in H_2O–EtOH (442 mg, 1:1). To this solution, 465 mg of the above prepared polymer solution was added, containing polymer **15** (93 mg, 432 μmol of amino groups, 1 equiv). The total solid content of the mixture was 16% (w/w). The mixture was left under gentle agitation at r.t. After curing the gel for 3 days, the gel was washed with H_2O–EtOH (1:1, 3 × mL), H_2O (3 × mL), each washing step taking 24 h. Finally, the gel was swollen to equilibrium in PBS buffer.

Hydrogel Characterization: The gelling time was monitored by the test tube inverting method, which defines gelling to have occurred when the mixture does not flow under the influence of gravity for 1 min.[26] The equilibrium degree of swelling is defined as the ratio of the mass of the swollen hydrogel and the dry mass of the hydrogel. The masses of the swollen hydrogels were determined by weighing the hydrogels, which were obtained following the preparation procedure described later. The dry masses of the hydrogels were obtained by washing the gels with H_2O (3 × mL) (each time for 24 h) and subsequent drying in vacuum at 60 °C. The storage shear moduli G' of the swollen gels were measured at 23 °C on a Physica MCR 301 (Anton Paar) using a parallel plate geometry with a diameter of 20 mm and a normal force of 0.2 N (gap approx. 1 mm) at a frequency of 1 Hz and an amplitude of 0.5%. Samples were swollen to equilibrium in PBS buffer before starting the measurements. PBS was used to close the air gaps of the solvent trap so that the inside of the trap was hermetically sealed.

Hydrogel Formation with Thiolated Hyaluronan

HA hydrogels were generally prepared using 40% thiolated hyaluronan (HA-SH) **17** and various acrylate containing cross-linkers. The HA-SH was synthesized as described by Prestwich.[27] using high molecular weight hyaluronan (Sigma-Aldrich). The degree of thiolation of the HA-SH used was measured by Ellman's assay.[28] All solutions for the hydrogel formation were degassed in an ultrasonic bath for 15 min to avoid disulfide bond formation.

Hydrogel Formation: A 40 mg/ml solution of HA-SH **17** in PBS (Gibco) was prepared and the pH was adjusted to 9.0. The cross-linkers were dissolved in a 50/50 (v/v) mixture of PBS/EtOH in appropriate concentrations. The HA-SH solution was mixed with the cross-linker solution in a 70:30 ratio, giving a final HA-SH concentration of 2.8% in the hydrogel. This mixture was vortexed for 3 s to achieve a homogeneous mixture, afterwards the solution was centrifuged at 1500 rpm for 1 min to remove air bubbles. After this, the mixture was immediately filled in cylindrical wells (r = 3 mm, h = 3 mm), which were sealed with a glass slide. This allowed the gels to form via thio-Michael addition during 24 h at 37 °C. Subsequently, the gels were allowed to swell in PBS for 48 h at 37 °C in order to reach equilibrium.

Hydrogel Characterization: The swelling ratio was measured by dividing the wet weight of the hydrogels after the swelling process by the dry weight of the hydrogels which was determined by drying of the swollen hydrogel at 90 °C for 24 h. All experiments were performed in triplicate. Errors represent standard deviations. The MTS Nano Bionix Testing System was used to measure the mechanical properties of the swollen hyaluronan hydrogels, using a parallel plate geometry in uniaxial compression mode. The instrument measures the force as a function of the applied strain. The analysis of the E-modulus was performed in the linear regime between 0 and 5% compression using a linear fit. The hydrogels were kept wet during the measurement to avoid barrel shape formation during compression. All measurements were carried out in triplicate. Error bars represent standard deviations of three independent sets of experiments.

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References

(1) (a) Kopeček, J.; Yang, J. *Angew. Chem. Int. Ed.* **2012**, *51*, 7396; *Angew. Chem.* **2012**, *124*, 7512. (b) van Vlierberghe, S.; Dubruel, P.; Schacht, E. *Biomacromolecules* **2011**, *12*, 1387. (c) Bashur, C. A.; Venkataraman, L.; Ramamurthi, A. *Tissue Eng. Part B Rev.* **2012**, *18*, 203.

(2) (a) Chen, Y. M.; Ogawa, R.; Kakugo, A.; Osada, Y.; Gong, J. P. *Soft Matter* **2009**, *5*, 1804. (b) Schneider, G. B.; English, A.; Abraham, M.; Zaharias, R.; Stanford, C.; Keller, J. *Biomaterials* **2004**, *25*, 3023. (c) Li, H.; Lai, F. *Anal. Bioanal. Chem.* **2011**, *399*, 1233. (d) Tan, F.; Xu, X.; Deng, T.; Yin, M.; Zhang, X.; Wang, J. *Biomed. Mater.* **2012**, *7*, 55009. (e) Schillemans, J. P.; Hennink, W. E.; van Nostrum, C. F. *Eur. J. Pharm. Biopharm.* **2010**, *76*, 329. (f) Smith, M. H.; Lyon, L. A. *Macromolecules* **2011**, *44*, 8154. (g) Chen, Y. M.; Gong, J. P.; Tanaka, M.; Yasuda, K.; Yamamoto, S.; Shimomura, M.; Osada, Y. *J. Biomed. Mater. Res.* **2009**, *88*, 74.

(3) Amitai, G.; Murata, H.; Andersen, J. D.; Koepsel, R. R.; Russell, A. J. *Biomaterials* **2010**, *31*, 4417.

(4) Krishnan, S.; Finlay, J. A.; Hexemer, A.; Wang, N.; Ober, C. K.; Kramer, E. J.; Callow, M. E.; Callow, J. A.; Fischer, D. *Polym. Prepr.* **2005**, *46*, 1248.

(5) Hughes, G.; Bryce, M. R. *J. Mater. Chem.* **2005**, *15*, 94.

(6) Wang, Y. Z.; Epstein, A. J. *Acc. Chem. Res.* **1999**, *32*, 217.

(7) Yoshida, M. *Chem. Record* **2010**, *10*, 230.

(8) Stavrouli, N.; Aubry, T.; Tsitsilianis, C. *Polymer* **2008**, *49*, 1249.

(9) Wang, Q.; Mynar, J. L.; Yoshida, M.; Lee, E.; Lee, M.; Okuro, K.; Kinbara, K.; Aida, T. *Nature* **2010**, *463*, 339.

(10) Southan, A.; Mateescu, M.; Hagel, V.; Bach, M.; Schuh, C.; Kleinhans, C.; Kluger, P. J.; Tussetschläger, S.; Nuss, I.; Haraszti, T.; Wegner, S. V.; Spatz, J. P.; Boehm, H.; Laschat, S.; Tovar, G. E. M. *Macromol. Chem. Phys.* **2013**, *214*, 1865.

(11) Hagel, V.; Mateescu, M.; Southan, A.; Wegner, S. V.; Nuss, I.; Haraszti, T.; Kleinhans, C.; Schuh, C.; Spatz, J. P.; Kluger, P. J.; Bach, M.; Tussetschläger, S.; Tovar, G. E. M.; Laschat, S.; Boehm, H. *Nature Sci. Rep.* **2013**, *3*, 2043.

(12) Hennink, W.; van Nostrum, C. *Adv. Drug Deliver. Rev.* **2002**, *54*, 13.

(13) Shu, X. Z.; Liu, Y.; Palumbo, F. S.; Luo, Y.; Prestwich, G. D. *Biomaterials* **2004**, *25*, 1339.

(14) Schultz, K. M.; Baldwin, A. D.; Kiick, K. L.; Furst, E. M. *Macromolecules* **2009**, *42*, 5310.

(15) (a) Rulev, A. Y. *Russ. Chem. Rev.* **2011**, *80*, 197. (b) Rizzi, S. C.; Hubbell, J. A. *Biomacromolecules* **2005**, *6*, 1226.

(16) Hobson, L. J.; Feast, W. *Polymer* **1999**, *40*, 1279.

(17) Kimura, S.; Ikeda, M.; Shimizu, Y.; Matsumoto, S.; Komatsu, H.; Tamaru, S.-i.; Takigawa, T.; Hamachi, I. *Macromol. Biosci.* **2008**, *8*, 1019.

(18) Chuchuryukin, A. V.; Chase, P. A.; Dijkstra, H. P.; Suijkerbuijk, B. M. J. M.; Mills, A. M.; Spek, A. L.; van

Klink, G. P. M.; van Koten, G. *Adv. Synth. Catal.* **2005**, *347*, 447.
(19) Okawa, T.; Osakada, N.; Eguchi, S.; Kakehi, A. *Tetrahedron* **1997**, *53*, 16061.
(20) Cynamon, M. H.; Gimi, R.; Gyenes, F.; Sharpe, C. A.; Bergmann, K. E.; Han, H. J.; Gregor, L. B.; Rapolu, R.; Luciano, G.; Welch, J. T. *J. Med. Chem.* **1995**, *38*, 3902.
(21) Neises, B.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 522; *Angew. Chem.* **1978**, *90*, 556.
(22) (a) Katritzky, A. R.; Ramsden, C. A.; Joule, J. A.; Zhdankin, V. V. In *Handbook of Heterocyclic Chemistry*; Katritzky, A. R.; Ramsden, C. A.; Joule, J. A.; Zhdankin, V. V., Eds.; Elsevier: Oxford, **2010**, 242–382. (b) Wang, X.-B.; Dacres, J. E.; Yang, X.; Broadus, K. M.; Lis, L.; Wang, L.-S.; Kass, S. R. *J. Am. Chem. Soc.* **2003**, *125*, 296.
(23) Berg, U.; Gallo, R.; Klatte, G.; Metzger, J. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1350.
(24) Vanderhooft, J. L.; Alcoutlabi, M.; Magda, J. J.; Prestwich, G. D. *Macromol. Biosci.* **2009**, *9*, 20.
(25) (a) Ghosh, S. K.; Bharadwaj, P. K. *J. Chem. Sci.* **2005**, *117*, 23. (b) Shi, Q.; Zhang, S.; Wang, Q.; Ma, H.; Yang, G.; Sun, W.-H. *J. Mol. Struct.* **2007**, *837*, 185.
(26) Jeong, B.; Bae, Y. H.; Kim, S. W. *Macromolecules* **1999**, *32*, 7064.
(27) Shu, X. Z.; Liu, Y.; Luo, Y.; Roberts, M. C.; Prestwich, G. D. *Biomacromolecules* **2002**, *3*, 1304.
(28) (a) Ellman, G. L. *Arch. Biochem. Biophys.* **1958**, *74*, 443. (b) Thannhauser, T. W.; Konishi, Y.; Scheraga, H. A. *Methods Enzymol.* **1987**, *143*, 115.

