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Photocleavable nanocarriers

# Photoresponsive Crosslinked Hyperbranched Polyglycerols as Smart Nanocarriers for Guest Binding and Controlled Release

*Ewelina Burakowska, Steven C. Zimmerman,* and Rainer Haag**

***A** convenient methodology for the synthesis of photolabile crosslinked hyperbranched polyglycerol nanocapsules is presented. These nanocarriers selectively and efficiently bind ionic guest molecules. The stability of the host–guest complexes formed depends on the counterion of the guest molecules. Moreover, the control over guest binding can be achieved by modification of the polymer building blocks, in particular the outer shell. In addition, photo-triggered degradation of the nanocarrier leads to efficient release of encapsulated guest molecules. This approach, using photolabile dendritic nanocarriers to bind and release guest molecules, is of particular relevance for biomedical applications where selective guest binding and controlled release are crucial.*

**Keywords:**
- dendrimers
- encapsulation
- hyperbranched polymers
- nanocarriers
- supramolecular chemistry

## 1. Introduction

The ability to control guest binding and their release from defined nanoscale carrier systems is an important issue for the preparation of functional and responsive targeting materials. In the last few decades many strategies to encapsulate and deliver active agents in a controlled fashion have been developed.[1–5] Polymers that are responsive to an external stimulus such as temperature, pH, magnetic or electrical field, light, or enzymatic action have been proposed as triggered delivery systems.[6–10] Dendritic polymers are of particular interest because of their ability to bind small molecules internally whereas their multiple end-groups can control solubility and target specific sites.

Many successful pH-cleavable dendritic nanocarriers have been reported.[11–19] However, there are far fewer investigations of light-responsive dendritic hosts. Shabat and coworkers have reported dendrons that release their end-groups through a photo-triggered self-immolative process initiated by a single photocleavage at a focal point.[20] These systems release the covalently bound units after being irradiated with ultraviolet light in a process that can deliver multiple drug molecules (pro-drug approach). Smith et al. reported reversible DNA binding by multivalent dendrons, which release the DNA upon a photoinitiated degradation leading to a charge reversal of the multivalency.[21] Kim et al. reported the self-assembly of amide dendrons with a photoresponsive focal functionality, which release entrapped molecules upon exposure to UV light.[22] These examples clearly demonstrate the significant potential of phototunable dendritic systems as nanomaterials for delivery.

Our previous studies focused on the ability of crosslinked hyperbranched polyglycerols (HPGs) to bind metal ions and extract them from aqueous to organic media.[23] We observed significant selectivity in metal ion binding, which could be achieved by specific cation recognition. Inspired by these results and in an attempt to achieve controlled release of the guest molecules we directed our efforts to the building of photoactivated nanocapsules that can release their cargo based on external stimuli. In this regard, we modified our previously reported closed-shell polymers[23] by introduction of *o*-nitrobenzyl groups on the surface of HPG (Scheme 1). Moreover, by the varying the building blocks (allylated hexa(ethylene glycol) versus 1-hexene) we studied the effect

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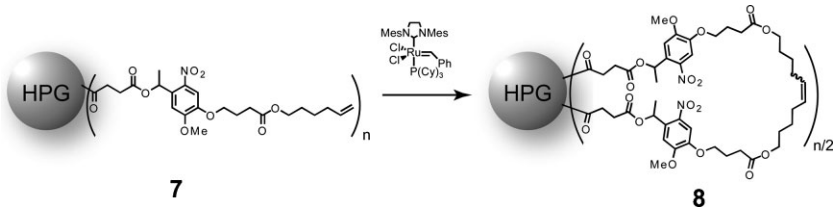


7 8

**Scheme 1.** Nanotransporter with a 1-hexene outer-shell before (**7**) and after RCM (**8**).

of the shell architecture on the potential of the final nanotransporters to bind small molecules.

## 2. Results and Discussion

The synthesis of the crosslinked HPGs with photocleavable linkers began with acetovanillone, which was alkylated with ethyl 4-bromobutanoate in the presence of potassium carbonate (see Supporting Information, Scheme S1). Treatment with nitric acid followed by ester hydrolysis and ketone reduction yielded the photolinker **3**,[24] which was further modified by coupling it to hex-5-enyl 4-methylbenzenesulfonate and succinic anhydride. Photolinker **6** was next coupled to 5 kDa HPG. After this step, size-exclusion chromatography (SEC) purification was performed to separate uncoupled linker (Supporting Information, Figure S1). Functionalization of the HPG core with the photolinker was found to be approximately 50% and could not be improved by increasing the reaction time or by increasing the amount of coupling agent. Finally, intramolecular crosslinking using a ring-closing metathesis reaction (RCM)[25] formed the stimuli-responsive nanocarrier **8**. $^{1}$H NMR spectroscopy analysis showed nearly complete conversion with the use of 8 mol% of Grubbs' 2nd generation catalyst. Structural characterization of each product was carried out using $^{1}$H and $^{13}$C NMR spectroscopy, electrospray ionization mass spectrometry (ESI-MS), and SEC. The latter technique suggests that the dendrimers are monomeric and do not aggregate significantly.

The photolytic degradation of the polymer was first studied with UV–Vis spectroscopy. For this purpose the chloroform solution of **8** was irradiated with UV light and the absorbance spectra were recorded every few seconds (Figure 1). Significant changes in absorbance at 268 and 350 nm have been observed up to 180 s irradiation. After this time degradation of the system reached a plateau.

The ability of polymer **8** to encapsulate small molecules was studied with the hydrophilic dye rose Bengal. This dye is a useful guest because its absorbance and fluorescence offer a convenient analytical tool to measure its concentration in solution. Moreover, by simple cation exchange the selectivity studies on the dye binding can be performed, and more importantly, the absorbance of rose Bengal does not cover the absorbance region of the photolinker so that photobleaching of the dye can be avoided. Encapsulation studies were performed using a solid uptake procedure. Thus, a 0.1 mM chloroform solution of polymer **8** was stirred for 12 h with the rose Bengal sodium salt. After that the suspension was filtered through a microfilter and the uptake of the dye was calculated from the UV–Vis absorption spectra. All dye molecules observed in solution can be assumed to be directly associated with the nanotransporter. The measured dye/polymer ratio was found to be 10:1, which indicates that on average ten rose Bengal sodium salt dye molecules were bound to one polymer molecule. The host–guest stability was evaluated qualitatively by extracting the organic polymer–dye solution with water for a few minutes. After phase separation the UV absorption of the organic phase was measured again. No dye was observed to migrate to the aqueous phase, which indicates high stability of the polymer–dye complex.

With the light-triggered cleavage of the polymer and the guest encapsulation separately established and optimized, attention was turned to the photoinitiated guest release from the nanocarrier. Because the exposure of the chloroform solution of the polymer–dye complex to the light in the whole UV–Vis range resulted in rose Bengal photobleaching, 350-nm monochromatic light was applied for the photo-cleavage experiments. Under this condition the dye was stable and further studies could be performed. Polymer–dye disassembly was monitored as a function of UV irradiation time (Figure 2). The solution of nanotransporter with encapsulated rose Bengal sodium salt guest molecules after treatment with light was extracted with water. The amount of released dye was

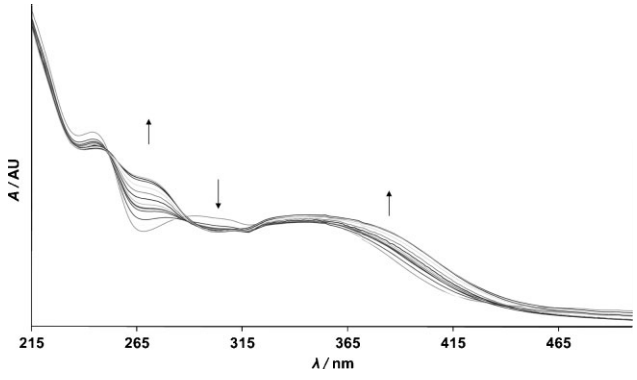


**Figure 1.** UV spectra of photosystem **8** after different irradiation times (0–300 s) with UV light.

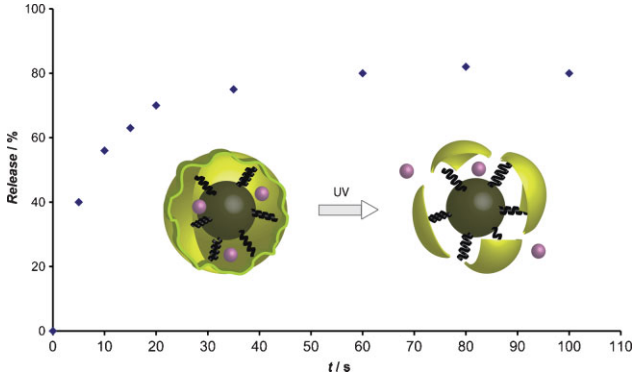


**Figure 2.** Release of rose Bengal sodium salt from polymer **8** by UV irradiation ($\lambda = 350$ nm).

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**Table 1.** Study of the host–guest stability and UV-triggered guest release from photosystem **8**.

| | Guest loading [mol/mol][a] | Host–guest stability[b] | Guest release [%][c] |
|---|---|---|---|
| Rose bengal Sodium salt | 10 | bdl | 80 |
| Rose bengal Cesium salt | 4 | 0.67 | 75 |

bdl = below detection limit. [a] Number of dye molecules encapsulated per one polymer molecule as determined by UV–Vis spectroscopy. [b] Expressed as the distribution constant $K_D$ of the dye between the chloroform solution of photosystem **8** and water ($K_D = [Dye]_{org}/[Dye]_{aq}$). [c] Calculated from the difference in the UV–Vis absorption in chloroform before and after irradiation.

**Table 2.** Study of the host–guest stability and UV-triggered guest release from photosystem **10**.

| | Guest loading [mol/mol][a] | Host–guest stability[b] | Guest release [%][c] |
|---|---|---|---|
| Rose bengal Sodium salt | 7 | bdl | No release |
| Rose bengal Cesium salt | 2 | 9.0 | ≈30 |

bdl = below detection limit. [a] Number of dye molecules encapsulated per one polymer molecule as determined by UV–Vis spectroscopy. [b] Expressed as the distribution constant $K_D$ of the dye between the chloroform solution of photosystem **10** and water ($K_D = [Dye]_{org}/[Dye]_{aq}$). [c] Calculated from the difference in the UV–Vis absorption in chloroform before and after irradiation.

calculated from the difference in the UV–Vis absorption in chloroform before and after irradiation. Approximately 80% of dye release could be observed after only 60 s of irradiation. Longer exposures to UV light did not result in further release.

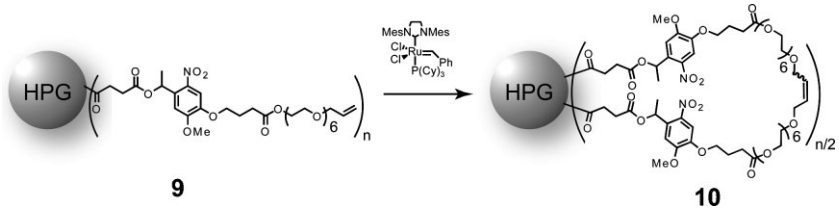
The counterion effect on the stability of the host–guest complex was examined by comparing the encapsulation and release studies of the rose Bengal cesium and sodium salts. The results showed the uptake of approximately four rose Bengal cesium salt guest molecules per polymer, which is lower than that found for the sodium salt. Moreover, the host–guest stability was weaker for rose Bengal cesium salt (Table 1).

To examine the effect of the crosslinked shell on the guest encapsulation and the host–guest complex stability, polymer **10** was prepared from **9**, which contains allylated hexa(ethylene glycol) end-groups instead of the hexene outer shell previously used (Scheme 2). Introduction of polyether chains that mimic crown ethers into the nanotransport system might be expected to provide additional guest binding and assure higher transport capacities. The synthetic pathway presented in the Supporting Information (Scheme S2) led to architecture **10**, which has been further tested for its ability to encapsulate and release guest molecules, analogous to the behavior observed for **8**. We observed (Table 2) high host–guest complex stabilities for both guest molecules (rose Bengal sodium and cesium salt). Nevertheless the transport loading of rose Bengal sodium salt was lower than that observed with polymer **8**. The ability of polymer **10** to complex rose Bengal cesium salt was even lower. Only two guest molecules could be hosted by one polymer molecule. Release studies performed on this polymer showed that very limited quantities of the dye molecules, or none, were present in the aqueous phase. This suggests that either photocleavage of the polymer did not take place or that the guest molecules were so strongly complexed to the polymer shell that they could not be extracted to the aqueous phase. If the last hypothesis is true it should be possible to separate the dye by dialyzing it out of the polymer. Indeed after 24 h of dialysis in chloroform and concentration of the solvent the rose Bengal dye was isolated. The very high stability of the dye–polymer complexes observed for polymer **10** evidences a significant contribution of the crown shell to the stabilization of the dye molecules and indicates the location of guests in the corona-shell of the polymers.

## 3. Conclusions

Herein we demonstrated an efficient route to photodegradable crosslinked HPG nanocapsules. These systems show high capacity and selectivity in guest encapsulation, which could be achieved by the variation of the counterion of ionic guest molecules. Furthermore, the modification of the building blocks, in particular the outer shell, allows control of the host–guest complex stability. In general, the presence of the hexa(ethylene glycol) outer-shell instead of the hexene shell increased the stability of the formed host–guest complexes but leads to difficulties in guest release. However, the introduction of the hexene shell assures a high release of guest molecules from the nanocapsules of up to 80%. The stability of the formed host–guest complexes depends on the counterion of the guest molecules. For example, rose Bengal sodium salt guest molecules form very stable complexes with the hosts while cesium counterions do not maintain the stability. Consequently, the easy tunability of these nanocapsules allows us to gain control over guest binding and release.

We believe that these smart light-responsive materials may provide an effective tool for biomedical applications where




9 10

**Scheme 2.** Nanotransporter with a hexa(ethylene glycol) outer-shell before (**9**) and after RCM (**10**).

selective guest binding and controlled release are crucial. In order to promote the solubilization of the nonpolar guest molecules our current efforts are focused on the synthesis of water-soluble, light-responsive nanocapsules.

## 4. Experimental Section

*General:* All solvents and reagents were of reagent quality, purchased commercially, and used without further purification, except as noted below. The following solvents were freshly distilled prior to use: dichloromethane (DCM) and chloroform ($CHCl_3$) from calcium hydride, and tetrahydrofuran (THF) from sodium. *N*,*N*-Dimethylformamide (DMF) was dried and stored over 4 Å molecular sieves. Water was deionized and distilled in a Milipore-Q system. HPG 5 kDa was prepared according to a published procedure and analyzed by NMR spectroscopy, SEC, and matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF MS). [26–28] The polydispersity index (PDI) values of the polymers used are in the range previously reported. [26] The rose Bengal sodium salt was of ACS grade and rose Bengal cesium salt was prepared by cation exchange. An aqueous solution of rose Bengal sodium salt was acidified with 1 M HCl to pH 2 and extracted with chloroform. The organic layer was washed three times with water and the product was concentrated under reduced pressure. Product (0.2 g) was mixed with 1 mL of 11% cesium hydroxide solution. After 1 h of stirring, product was lyophilized overnight to afford 0.22 g of rose Bengal cesium salt as a pink solid.

Analytical SEC was performed on a PSS Agilent 1100 system with a Suprema (10 μm) column (8.0 × 300 mm) using DMF as eluent. Preparative SEC was performed on a PSS SDV (10 μm) column (300 × 20 mm) using THF as eluent. A refractive index RI 1100 detector was used for all analyses. The system was calibrated by narrow polystyrene standards (MW range: 200 to $4 \times 10^6$ Da). UV–Vis measurements were performed on a Scinco S-3100 PDA UV–Vis spectrophotometer at 25 ± 0.1 °C with wavelength from 190 to 850 nm. Thin-layer chromatography (TLC) was performed on 0.2-mm silica 60-coated plastic sheets (EM Science) with potassium permanganate stain. Flash chromatography was performed on Merck 40–63-μm silica gel. Solvent ratios for the purification of compounds by flash chromatography are reported as percent volume (v/v). $^1$H and $^{13}$C NMR spectra were recorded on a Varian Unity 400 spectrometer ($^1$H, 400 MHz; $^{13}$C, 100 MHz). All mass spectra were obtained in the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, Urbana-Champaign. Mass spectra were measured by the technique of field desorption (FD) and field ionization (FI) on a Finnigan-MAT 731 spectrometer, electron impact (EI) on a VG 70-VSE spectrometer, fast atom bombardment (FAB) on a VG ZAB-SE spectrometer, and ESI on a Quattro II.

Due to the complexity of structure, the photosystems **7** and **9** are named HPG–photolinker followed by the word HEX (for hexene) or HEG (for hexa(ethylene glycol)). The photosystems **9** and **10** are additionally designated with the word crosslinked.

*UV-light irradiation:* Samples were irradiated in 10-mm quartz cuvettes for different times using a 75 W Xenon arc lamp (intensity approximately 9 mW cm$^{-2}$). A narrow band UV filter (350 nm) was used.

*Guest-solubilization experiments:* A chloroform solution (10 mL) of the respective polymer **7**–**10** at a concentration within 0.1–10 wt% was stirred for 12 h at room temperature with an excess of each guest molecule. Next, the mixture was centrifuged for 20 min (15 000 rpm) and additionally filtered via 0.45-μm nylon filters to remove any trace of unsolubilized dye. The clear polymer solutions with encapsulated guests were then analyzed by means of UV–Vis spectroscopy by measuring the characteristic absorption of guests in solution of the polymer. Obtained absorptions were compared with calibration curve of the guest molecules, which allowed the determination of the concentration of the guest molecules in a solution.

*Guest-release experiments:* A chloroform solution (2 mL) of the respective polymer **8** or **10** with a determined amount of encapsulated guest molecule was irradiated in a quartz cuvette for different time periods. After each time period the chloroform solution of the polymer was extracted with 2 mL of distilled water. The release profile was obtained by calculating the amount of guest present in the water phase using the equation

$$\text{Release percentage}\,(\%) = 100\% - (100\% - A_t) \quad (1)$$

where $A_t$ is the percent of rose Bengal in aqueous phase at a measured time period.

*Hex-5-enyl-4-methylbenzenesulfonate (**4**):* To a solution of 11.9 mL (0.1 mol) of hex-5-en-1-ol in 50 mL of pyridine cooled to 0 °C with an ice/water bath was added slowly 22.9 g (0.12 mmol) of toluene-4-sulfonyl chloride. The mixture was allowed to warm to room temperature and stirred until the reaction was complete as indicated by TLC. Next the reaction mixture was diluted with diethyl ether (35 mL) and washed successively with water, 1 M HCl, $NaHCO_3$, and brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The product was purified by column chromatography with 50% $Et_2O$/50% hexane to afford 17.4 g (70%) of **4** as a colorless oil. $^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C, $\delta$): 7.80 (d, $J$ = 8.46 Hz, 2H; Ar–H), 7.49 (d, $J$ = 8 Hz, 2H; Ar H), 5.76 (m, 1H; CH=$CH_2$), 4.97 (m, 2H; $CH_2$=CH), 4.03 (t, $J$ = 6.32, 2H; $CH_2$–O), 2.42 (s, 3H; Ar–$CH_3$), 1.96 (q, $J$ = 7.03 Hz, 2H; CH–$CH_2$), 1.59 (m, 2H; $CH_2$–$CH_2$), 1.35 (q, $J$ = 7.48 Hz, 2H; $CH_2$–$CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$, $\delta$): 144.7 (C–Ar), 138.0 (CH=), 132.5 (C–Ar), 130.0 (C–Ar), 127.4 (C–Ar), 114.9 ($CH_2$=), 70.6 ($CH_2$–O), 32.2 ($CH_2$–CH), 27.5 ($CH_2$), 23.9 ($CH_2$), 21.0 ($CH_3$).

*Hex-5-enyl 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)-butanoate (**5**):* To 30 mL of DMF 3 g (10.02 mmol) of 4-(4-(1-hydroxyethyl)-2-methoxy-6-nitrophenoxy) butanoic acid (**3**) and 1.2 g (20.7 mmol) of KF were added. The suspension was stirred for 10 min, and then a solution of 3.06 g (12.02 mmol) of hex-5-enyl-4-methylbenzenesulfonate (**4**) in 10 mL of DMF was added in one portion. The reaction mixture was then heated at 50 °C for 48 h. The reaction was stopped by adding 50 mL of EtOAc. The organic layer was extracted with water, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was loaded onto a silica gel column and eluted with 90% $CHCl_3$/10% MeOH. The product was dried under vacuum overnight to afford 2.8 g (74%) of **5** as pale yellow oil. $^1$H NMR

(400 MHz, $CDCl_3$, 25 °C, $\delta$): 7.52 (s, 1H; Ar–H), 7.27 (s, 1H; Ar–H), 5.81 (m, 1H; CH=$CH_2$), 5.55 (q, $J$ = 6.26 Hz, 1H; Ar–CH–$CH_3$), 5.02 (m, 2H; $CH_2$=CH), 4.10 (m, 4H, $CH_2$–O), 3.94 (s, 3H; $CH_3$), 2.52 (t, $J$ = 7.24 Hz, 2H; O=C–$CH_2$), 2.39 (s, 1H; OH), 2.15 (q, $J$ = 6.75 Hz, 2H; $CH_2$–$CH_2$), 2.09 (m, 2H; CH–$CH_2$), 1.67 (m, 2H; $CH_2$–$CH_2$), 1.52 (d, $J$ = 6.26 Hz, 3H; $CH_3$), 1.42 (q, $J$ = 7.48 Hz, 2H; $CH_2$–$CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta$): 173.2 (C=O), 154.3 (C–Ar), 147.0 (C–Ar), 139.6 (C–Ar), 138.4 (CH=), 137.2 (C–Ar), 115.0 ($CH_2$=), 109.2 (C–Ar), 108.9 (C–Ar), 68.4 ($CH_2$–O), 65.8 (CH), 64.7 ($CH_3$), 56.5 ($CH_2$–O), 33.4 ($CH_2$), 30.7 ($CH_2$–CO), 28.1 ($CH_3$), 25.3 ($CH_2$), 24.5 ($CH_2$).

*4-(1-(4-(4-(Hex-5-enyloxy)-4-oxobutoxy)-5-methoxy-2-nitrophenyl)ethoxy)-4-oxobutanoic acid (**6**):* Hex-5-enyl 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy) butanoate (**5**) (2 g, 5.24 mmol) was dissolved in 100 mL of dichloromethane (DCM). To this solution were added 0.52 g (5.24 mmol) of succinic acid anhydride, 0.8 mL (6.30 mmol) of triethylamine (TEA), and 15 mg (0.13 mmol) of dimethylaminopyridine (DMAP). The reaction mixture was heated to 50 °C and stirred at this elevated temperature for 12 h. The reaction mixture was then washed three times with water, dried over sodium sulfate, and filtered. The filtrate was concentrated under reduced pressure and dried under vacuum overnight to afford 2.4 g of **6** as pale yellow oil. $^1$H NMR (400 MHz, $CDCl_3$, 25 °C, $\delta$): 7.57 (s, 1H; Ar–H), 7.0 (s, 1H; Ar–H), 6.48 (q, $J$ = 6.41 Hz, 1H; Ar–CH–$CH_3$), 5.83 (m, 1H; CH=$CH_2$), 5.03 (m, 2H; $CH_2$=CH), 4.12 (m, 4H, $CH_2$–O), 3.95 (s, 3H; $CH_3$), 2.67 (bs, 4H; $CH_2$), 2.53 (t, $J$ = 7.24 Hz, 2H; O=C–$CH_2$), 2.17 (q, $J$ = 6.73 Hz, 2H; $CH_2$–$CH_2$), 2.10 (m, 2H; CH–$CH_2$), 1.71 (m, 2H; $CH_2$), 1.61 (d, $J$ = 6.49 Hz, 3H; $CH_3$), 1.44 (q, $J$ = 7.48 Hz, 2H; $CH_2$–$CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta$): 173.2 (C=O), 171.1 (C=O), 154.3 (C–Ar), 147.4 (C–Ar), 139.8 (C–Ar), 138.5 (CH=), 133.3 (C–Ar), 115.1 ($CH_2$=), 109.3 (C–Ar), 108.4 (C–Ar), 69.2 (CH), 68.5 ($CH_2$–O), 64.8 ($CH_3$), 56.5 ($CH_2$–O), 33.4 ($CH_2$), 30.8 ($CH_2$–CO), 29.2 ($CH_2$–CO), 28.7 ($CH_2$–CO), 28.2 ($CH_3$), 25.4 ($CH_2$), 24.5 ($CH_2$). HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{23}H_{31}NO_{10}Na$, 504.1840; found, 504.1860.

*3,6,9,12,15,18-Hexaoxaheneicos-20-en-1-ol, 1-(4-methylbenzenesulfonate) (**4a**):* To a solution of 2.3 g (7.14 mmol) of 3,6,9,12,15,18-hexaoxahenicos-20-en-1-ol and 1.35 mL (8.6 mmol) of TEA in 50 mL of DCM was added slowly 1.64 g (8.6 mmol) of toluene-4-sulfonyl chloride. The mixture was stirred at room temperature until the reaction was complete as indicated by TLC. Next the reaction mixture was diluted with an additional 30 mL of DCM and washed successively with water, 1 M HCl, $NaHCO_3$, and brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The product was purified by column chromatography with 95% $CHCl_3$/5% MeOH to afford 3.1 g (92%) of **4a** as a colorless oil. $^1$H NMR (400 MHz, [D6]DMSO-$d_6$, 25 °C, $\delta$): 7.78 (d, $J$ = 8.34 Hz, 2H; Ar–H), 7.48 (d, $J$ = 8.09 Hz, 2H; Ar–H), 5.92 (m, 1H; CH=$CH_2$), 5.27 (m, 2H; $CH_2$=CH), 4.13 (m, 2H; $CH_2$–O), 3.93 (t,t, $J$ = 1.56, 1.56, 2H; CH–$CH_2$), 3.59 (m, 2H; $CH_2$–O), 3.53 (bm, ≈16H; $CH_2$–O), 3.45 (bs, ≈4H; $CH_2$–O); MS (FD *m/z*): $[M + H]^+$ calcd for $C_{22}H_{36}NO_9S$, 477.2; found, 477.3.

*3,6,9,12,15,18-Hexaoxaheneicos-20-enyl4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy) butanoate (**5a**):* To 30 mL of DMF 2 g (6.63 mmol) of 4-(4-(1-hydroxyethyl)-2-methoxy-6-nitrophenoxy) butanoic acid (**3**) and 0.78 g (13.26 mmol) of KF were added. The suspension was stirred for 10 min and then a solution of 3.8 g (7.96 mmol) of 3,6,9,12,15,18-hexaoxaheneicos-20-en-1-ol, 1-(4-methylbenzenesulfonate) (**4a**) in 10 mL of DMF was added in one portion. Next the reaction mixture was heated at 50 °C for 48 h. The reaction was stopped by adding 50 mL of EtOAc. The organic layer was extracted with water, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was loaded onto a silica gel column and eluted with 90% $CHCl_3$/10% MeOH. The product was dried under vacuum overnight to afford 5.3 g (70%) of **5a** as yellow oil. $^1$H NMR (400 MHz, DMSO-$d_6$, 25 °C, $\delta$): 7.52 (s, 1H; Ar–H), 7.36 (s, 1H; Ar–H), 5.92 (m, 1H; CH=$CH_2$), 5.48 (d, $J$ = 4.52 Hz, 1H; OH), 5.29 (bm, 3H; =$CH_2$, CH–OH), 4.16 (m, 2H, $CH_2$–O), 4.06 (t, $J$ = 6.49 Hz, 2H; $CH_2$–O), 3.94 (t,t, $J$ = 1.51, 1.51 Hz, 2H; CH–$CH_2$), 3.90 (s, 1H; $CH_3$–O), 3.62 (m, 2H; $CH_2$–O), 3.54 (bm, ≈22H; $CH_2$–O), 2.48 (m, 2H; O=C–$CH_2$), 1.99 (q, $J$ = 6.84 Hz, 2H; $CH_2$–$CH_2$), 1.37 (d, $J$ = 6.26 Hz, 3H; $CH_3$). HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{28}H_{45}NO_{13}Na$, 626.2791; found, 626.2785.

*4-(1-(5-Methoxy-2-nitro-4-(23-oxo-4,7,10,13,16,19,22-heptaoxahexacos-1-en-26-yloxy) phenyl)ethoxy)-4-oxobutanoic acid (**6a**):* **5a** (5 g, 8.24 mmol) was dissolved in 100 mL of DCM. To this solution were added 0.82 g (8.24 mmol) of succinic acid anhydride, 1.4 mL (10 mmol) of TEA, and 24 mg (0.21 mmol) of DMAP. The reaction mixture was heated to 50 °C and stirred at this elevated temperature for 12 h. The reaction mixture was then washed three times with water, dried over sodium sulfate, and filtered. The filtrate was concentrated under reduced pressure and dried under vacuum overnight to afford 5.7 g of **6a** as yellow oil. $^1$H NMR (400 MHz, $CDCl_3$, 25 °C, $\delta$): 7.51 (s, 1H; Ar–H), 7.0 (s, 1H; Ar–H), 6.39 (q, $J$ = 6.41 Hz, 1H; Ar–CH–$CH_3$), 5.92 (m, 1H; CH=$CH_2$), 5.27 (m, 2H; $CH_2$=CH), 4.24 (m, 2H, $CH_2$–O), 4.06 (t, $J$ = 6.20 Hz, 2H; $CH_2$–O), 3.98 (t,t, $J$ = 1.33, 1.33 Hz, 2H; CH–$CH_2$), 3.93 (s, 3H; $CH_3$), 3.71 (bm, 24H; $CH_2$–O), 2.66 (m, 6H; O=C–$CH_2$, $CH_2$–$CH_2$–C=O), 2.13 (q, $J$ = 6.73 Hz, 2H; $CH_2$–$CH_2$), 1.56 (d, $J$ = 6.49 Hz, 3H; $CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta$): 173.0 (C=O), 171.5 (C=O), 154.3 (C–Ar), 147.3 (C–Ar), 139.6 (C–Ar), 134.8 (CH=), 133.5 (C–Ar), 117.3 ($CH_2$=), 109.1 (C–Ar), 108.3 (C–Ar), 72.3 ($CH_2$–CH=), 70.6 ($CH_2$–CO), 69.5 (CH), 63.8 ($CH_3$), 56.5 ($CH_2$–O), 30.6 ($CH_2$–CO), 27.6 ($CH_2$–CO), 24.4 ($CH_2$), 22.1 ($CH_3$). HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{32}H_{49}NO_{16}Na$, 726.2944; found, 726.2950.

*HPG-photolinker-HEX (**7**):* To a solution of 200 mg (0.04 mmol) of 5 kDa HPG in 20 mL of DMF were added 1.27 g (2.64 mmol) of **6**, 0.51 g (2.64 mmol) of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), and 16 mg (0.13 mmol) of DMAP. The reaction was stirred at room temperature for 48 h. The DMF was removed under reduced pressure. The reaction mixture was vacuum filtered and the resulting filtrate was concentrated under reduced pressure. The product was redissolved in 20 mL of chloroform, washed three times with water, dried over sodium sulfate, and filtered. The crude product was purified by SEC on a SDV (10 μm) column (300 × 20 mm) using THF as eluent. The product was dried under vacuum to afford 500 mg of **7** as yellow viscous oil. $^1$H NMR (400 MHz, $CDCl_3$, 25 °C, $\delta$): 7.54 (s, 26H; Ar–H), 7.01 (s, 26H; Ar–H), 6.48 (m, 30H; Ar–CH–$CH_3$), 5.83 (m, 29H; CH=$CH_2$), 5.03 (m, 59H; $CH_2$=CH), 4.12 (m, 118H, $CH_2$–O), 3.96 (s, 80H; $CH_3$), 3.92 (bm, 326H; $CH_2$–O, CH–O), 2.69 (bs, 90H; $CH_2$), 2.52 (t, $J$ = 7.24 Hz, 50H; O=C–$CH_2$), 2.16 (q, $J$ = 6.73 Hz, 50H;

$CH_2-CH_2$), 2.07 (q, $J=7.15$ Hz, 50H; $CH-CH_2$), 1.67 (m, 120H; $CH_2$, $CH_3$), 1.43 (q, $J=7.48$ Hz, 50H; $CH_2-CH_2$); SEC $t_R=23.3$ min.

*HPG-photolinker-HEG (**9**):* To a solution of 200 mg (0.04 mmol) of 5 kDa HPG in 20 mL of DMF were added 1.86 g (2.64 mmol) of **6a**, 0.51 g (2.64 mmol) of EDC, and 16 mg (0.13 mmol) of DMAP. The reaction was stirred at room temperature for 48 h. The DMF was removed under reduced pressure. The reaction mixture was vacuum filtered and the resulting filtrate was concentrated under reduced pressure. The product was redissolved in 20 mL of chloroform, washed three times with water, dried over sodium sulfate, and filtered. The crude product was purified by SEC on a SDV (10 μm) column (300 × 20 mm) using THF as eluent. The product was dried under vacuum to afford 500 mg of **9** as yellow viscous oil. $^1$H NMR (400 MHz, $CDCl_3$, 25 °C, $\delta$): 7.54 (s, 26H; Ar−H), 7.02 (s, 26H; Ar−H), 6.48 (bs, 27H; Ar−CH−C), 5.95 (m, 29H; CH=C), 5.29 (m, 62H; $CH_2$=C), 4.29 (m, 60H, $CH_2-O$), 4.12 (m, ≈64H; $CH_2-O$), 4.02 (m, 60H; $C-CH_2$), 3.96 (s, 80H; $CH_3$), 3.88 (bm, ≈1000H, $CH_2-O$, CH−O), 2.73 (bm, ≈110H; $CH_2-CH_2$), 2.56 (t, $J=7.24$ Hz, 58H; $O=C-CH_2$), 2.21 (m, 50H; $CH_2-C$), 1.61 (bs, 90H; $CH_3$); SEC $t_R=22.4$ min.

*Crosslinked HPG-photolinker-general procedure for **8** and **10**:* To 0.8 L of methylene chloride was added 200 mg (0.01 mmol) of **9** and 17 mg (8 mol%) of Grubbs' 2nd generation catalyst. The mixture was stirred for 16 h. The reaction was quenched with 20 mL of ethyl vinyl ether and the mixture was filtered through a plug of silica gel. The product was eluted with 200 mL of 5% methanol/methylene chloride. The solution was concentrated in vacuum to give 188 mg (96% yield) of **10** as a light brown oil. $^1$H NMR (400 MHz, $CDCl_3$, 25 °C, $\delta$): 7.54 (s, 26H; Ar−H), 7.02 (s, 26H; Ar−H), 6.43 (bs, 27H; Ar−CH−C), 5.79 (bs, 24H; CH=), 5.0 (bs, 23H; CH=), 4.24 (bs, 62H, $CH_2-O$), 4.04 (bm, ≈190H; $CH_2-O$, $C-CH_2$, $CH_3$), 3.88 (bm, ≈1000H, $CH_2-O$, CH−O), 2.73 (bm, ≈110H; $CH_2-CH_2$), 2.56 (m, ≈70H; $O=C-CH_2$), 2.21 (m, 50H; $CH_2-C$), 1.61 (bs, ≈90H; $CH_3$); SEC $t_R=23.3$ min.

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