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A Route to Water-Soluble Molecularly Templated Nanoparticles Using Click Chemistry and Alkyne-Functionalized Hyperbranched Polyglycerol

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Abstract. Cored polymeric nanoparticles were prepared using an adenosine template and allylated hyperbranched polyglycerols (HPGs). The HPGs contained a single alkyne that was capable of 1,3-dipolar cycloaddition with a decaazido adenosine template to facilitate the rapid synthesis of HPGs containing a large number of allyl end-groups. Ring closing metathesis (RCM) mediated cross-linking at high dilution using a Hoveyda–Grubbs catalyst produced single polymer nanoparticles containing a single template. These particles were rendered water-soluble through treatment with osmium tetroxide and NMO co-oxidant. After hydrolytic removal of the template, the nanoparticles were tested for their ability to bind a variety of nucleosides and nucleobases and showed a preference for purine bases over pyrimidine bases. This is the first reported use of hyperbranched polymers for the synthesis of monomolecularly imprinted polymers (mMIPs) as well as the first time monomolecular imprinting has been applied to a homogeneous aqueous system.

INTRODUCTION

Monomolecular imprinting represents a type of covalent or semicovalent molecular imprinting[1] in which a single template produces a single molecular imprint in a polymer.[2] In its current implementation the monomolecular approach is distinct from conventional molecular imprinting,[3] because the imprinting occurs during a post polymerization intramolecular cross-linking reaction. Removal of the template produces polymers that rebind guests in a manner that is defined by the template.[4] Unlike conventional imprinted polymers they form homogeneous solutions, have a fairly well-defined molecular weight distribution, and have nanoscale dimensions without the need for post synthetic processing. Most significantly, they contain a single binding site per polymer so unacceptable levels of heterogeneity in the imprints could be followed by a fractionation of the polymeric nanoparticles.

Previous examples of monomolecular imprinting have focused on organic soluble polymers, which utilized Fréchet-type dendrimers as hosts.[4,5] The limited water solubility of these hosts precludes their use in aqueous environments such as the interstitial fluid of multicellular organisms or in cell cultures. Polymers containing reporter groups for small molecule detection have already been synthesized using monomolecular imprinting[5b,6] and conventional imprinting approaches.[7] Thus, water-soluble monomolecular imprinted polymers (mMIPs) could facilitate the production of molecular probes capable of monitoring small molecules in cell culture or in vivo.[8]

Here we present a route to water-soluble imprinted polymers using hyperbranched polyglycerols (HPGs) and an adenosine template. Allylated HPGs were synthesized in two steps by the anionic ring opening of

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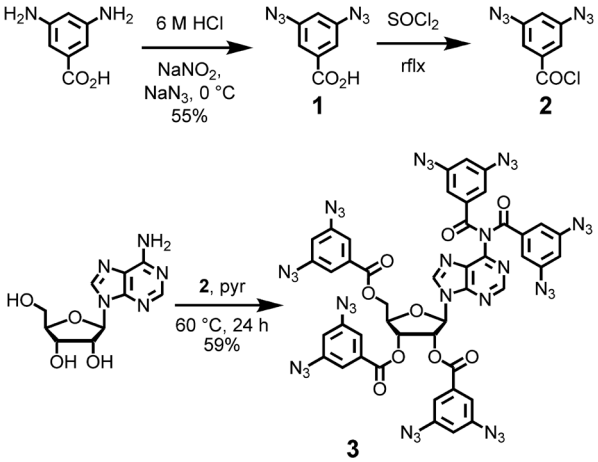
glycidol[9] followed by allylation, thus avoiding multistep dendrimer synthesis. Allylated HPGs have been cross-linked using the ring closing metathesis (RCM) reaction and subsequently dihydroxylated to produce fully water-soluble nanoparticles.[10] Adenosine was chosen as the first target for aqueous mMIPs because of its chemical structure and biological relevance. Its nucleobase, adenine, is known to function as a signaling molecule in G protein coupled receptors[11] and adenine-based templates have already been investigated for the production of molecularly imprinted polymers.[7a,12] Chemically, adenosine is known to form up to five ester/amide bonds when treated with acid chlorides,[13] allowing the synthesis of a complex core for polymer grafting and subsequent hydrolytic template removal. There are also a large number of related nucleosides and nucleobases available to test the binding selectivity of such a mMIP.



Scheme 1. Synthesis of the adenosine core.

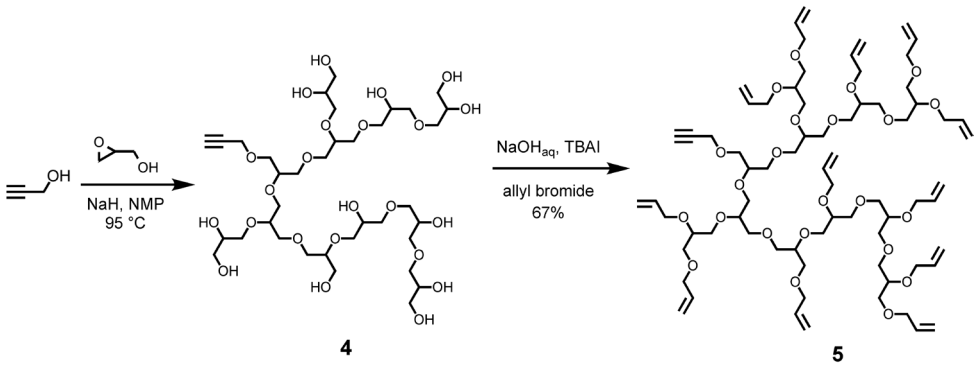
RESULTS AND DISCUSSION

Synthesis of Adenosine Cored Hyperbranched Polyglycerols

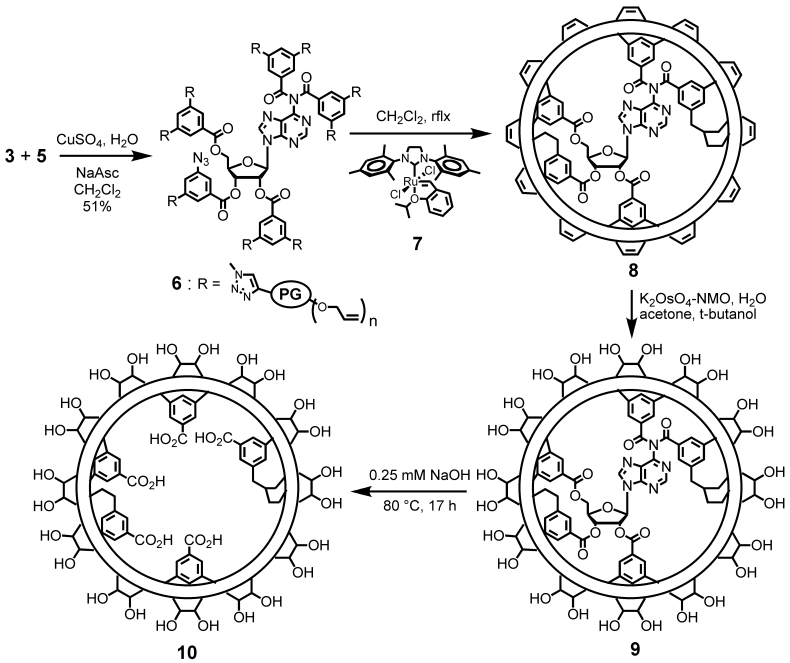
To synthesize hyperbranched polyglycerols with a removable adenosine core, we took advantage of the highly orthogonal 1,3-dipolar cycloaddition of azides and alkynes. This variant of the click reaction has been used in the synthesis of a variety of complex polymeric architectures[14] and specifically for making polyglycerol dendrimers.[15] The synthesis of the adenosine core, **3,** is outlined in Scheme 1. Diazide **1** was prepared via a Sandmeyer reaction of 3,5-diaminobenzoic acid in the presence of sodium azide. Treatment of **1** with thionyl chloride generated acid chloride **2**, which was not isolated but reacted (8 equiv) with adenosine to produce **3**, which could be purified chromatographically. ^{1}H and ^{13}C NMR and mass spectrometry confirmed the structure and purity of **3**.

Allylated HPGs containing a single propargyl group were synthesized from polymer **4**,[16] using the standard allylation procedure for the synthesis of polyglycerol dendrimers[17] (Scheme 2). This procedure produced polymers with low polydispersity (PDI = 1.3, 1.15 after preparative SEC fractionation) without the need for any protecting group chemistry. The major series of peaks (*m*/*z*) observed by MALDI corresponded to the fully allylated polymer containing a single propargyl group. Molecular weight analysis based on analytical SEC indicated an apparent $M_{n(ps)}$ of 5400 compared to an expected M_n of 2000, assuming complete conversion of the hydroxyl groups. This overestimation is surprising considering the highly branched structure of the polymers, however, molecular weight distributions of low molecular weight HPGs obtained with SEC using polyglycerol standards are known to be overestimated by 2- to 5-fold.[9] Molecular weights obtained using ^{1}H NMR and MALDI indicate an M_n of 3900 and 2060, respectively.

Water-soluble molecularly templated nanoparticles were then synthesized through a four-part sequence: click reaction, cross-linking, dihydroxylation, and hydrolytic coring (Scheme 3). The click reaction between HPG **5** and core **3** was performed following the procedure of Lee and coworkers[18] to produce **6** as shown in Scheme 3. Analytical SEC traces of the crude reaction



Scheme 2. Synthesis of the propargyl containing hyperbranched allylated polymer.



Scheme 3. Outline of the steps used for the synthesis of cross-linked water-soluble nanoparticles. (i) Polymer grafting. (ii) Cross-linking. (iii) Dihydroxylation. (iv) Core removal.

mixture and HPG **5** are shown in Fig. 1a. The crude material shows no high molecular weight shoulder, indicating that almost no intermolecular cross-linking took place. This could arise from alkene-azide cycloaddition, which has been observed in electron poor olefins.[19] The greater number and accessibility of the alkene groups suggests a strong preference for the alkyne-azide cycloaddition. The large increase in hydrodynamic volume ($M_{n(ps)}$ increased from 5,400 to 14,200) allowed for efficient purification by preparative SEC.

HPG **6** was treated with Hoveyda–Grubbs catalyst **7** to cross-link the allyl end-groups as described previously for Fréchet-type dendrimers,[3–6] and, more recently, Haag-type dendrimers and HPGs.[10] SEC analysis of the resulting polymer indicates a decrease in the hydrodynamic volume, as evidenced by an increase in retention time ($M_{n(ps)}$ of 10,000 cross-linked vs. 14,200 uncross-linked), (Fig. 1b). In the RCM cross-linking of dendrimers distinct peaks in the MALDI spectrum correspond to different numbers of cross-links providing quantitative analysis of the cross-linking distribution.[5,10] In the present case the distribution of masses for the HPGs and the different numbers of cross-links produced a broad spectrum rather than distinct peaks. As a result, the percentage of cross-linking, defined as cross-linked allyl groups/total allyl groups, was determined by ^{1}H NMR integration. Cross-link density was highly dependent on the amount of catalyst added, with

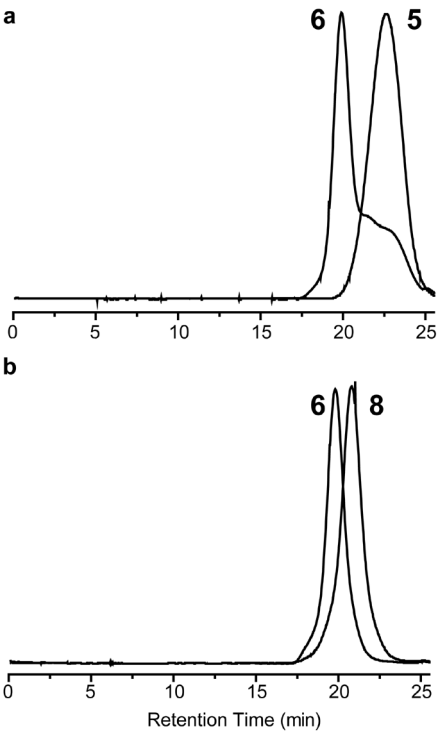


Fig. 1. (a) SEC traces of propargyl core hyperbranched polymer **5** and the crude reaction mixture from the grafting of the hyperbranched polymer **5** to the adenosine core **3**. (b) SEC trace of the final purified polymer before (**6**) and after (**8**) treatment with catalyst **7**.

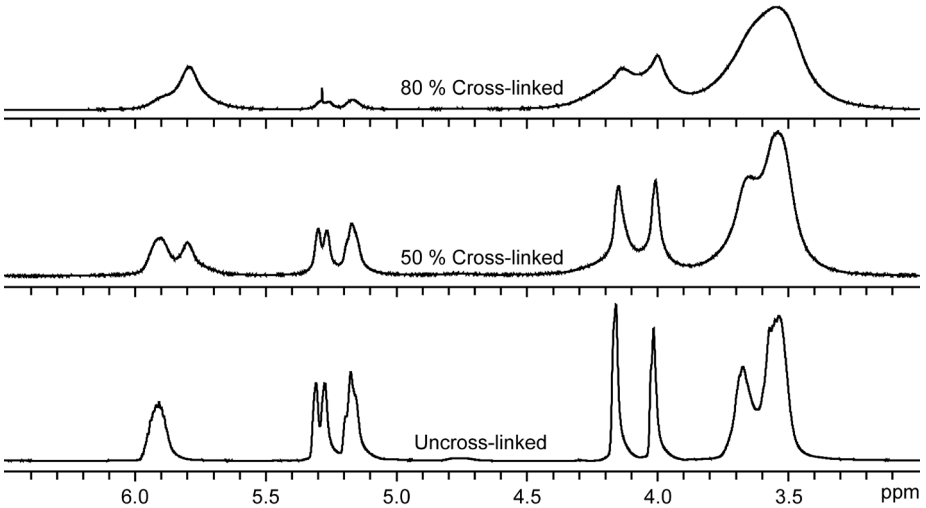


Fig. 2. NMR spectra of the uncross-linked polymer, 50% cross-linked polymer, and 80% cross-linked polymer showing the increase in line broadening observed with increasing percent cross-linking. Line width at half height at 4.0 ppm: uncross-linked polymer 11.9 Hz 50% cross-linked polymer 33.5 Hz.[21]

80% cross-linking achieved using only 2 mol% catalyst per allyl group.

A ^{1}H NMR of the cross-linked polymer showed substantial line broadening commonly observed in cross-linked dendrimers (Fig. 2).[20,21] Previously this was attributed to either restricted internal motion or the production of a large number of isomers.[20] In addition, the T_1 of the vinylic protons on the cross-linked polymers decreased with increasing cross-link density.[22] Because both of these observations are associated with decreased chain mobility,[23] and the uncross-linked polymer is a distribution of molecular weights and isomers, the line broadening associated with the polymer cross-linking is likely a result of the decrease in polymer flexibility.

Attempts to hydrolytically remove the adenosine template and produce an organic soluble imprint from **8** led to insoluble material or gelation. Polyallyl HPGs have been reported to be unstable over time as a result of intermolecular cross-linking of the surface olefins.[10,17] It is likely the same is occurring with **8** during the hydrolysis step. Thus the alkene groups were oxidized to 1,2-diols using catalytic osmium tetroxide and NMO to produce water-soluble nanoparticles.[10] ^{1}H NMR analysis indicated that >95% of the alkenes were oxidized to 1,2 diols. This polymer did not show any sign of gelation or precipitation on storage, but a higher molecular weight shoulder was present in the SEC of **9** (Fig. 3a), indicating that a limited number of intermolecular cross-links occurred during the oxidation reaction.

After oxidation, the water-soluble cross-linked HPGs **9** were treated with an aqueous solution of base at elevated temperature to remove the adenosine template. The SEC trace of the polymer obtained showed

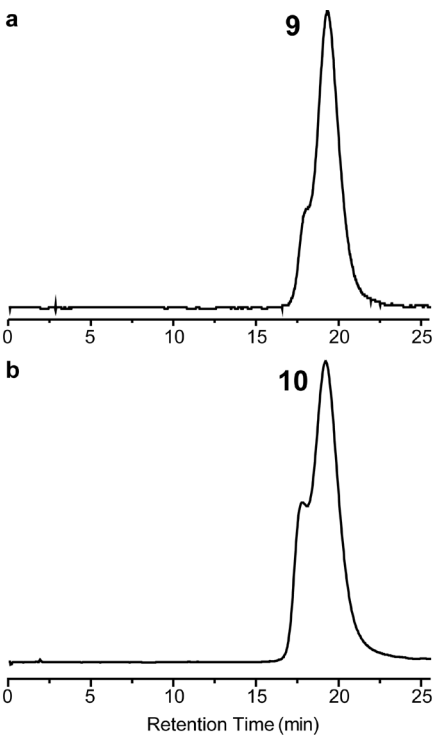


Fig. 3. SEC traces of (a) the dihydroxylated polymer **(9)** and (b) the final cored polymer **(10)**.

no low molecular weight peaks, indicating that at least one cross-link formed between each of the HPG segments in **6** during the RCM step (Fig. 3b). The ^{1}H NMR of the resulting material showed a slight decrease in line broadening around the aromatic region, however, no definitive conclusions about the template removal could be made. Likewise, MALDI, the standard method to assess

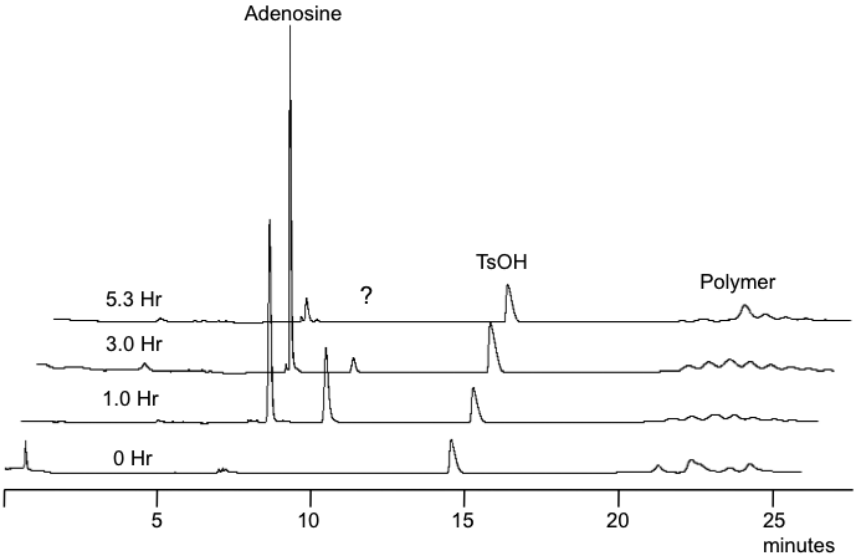


Fig. 4. Time-dependent HPLC curves from the crude reaction mixture of the template hydrolysis reaction over the course of 5 h. The peak at 14 min is the tosic acid internal standard. The appearance of adenosine at 7 min is accompanied by a second, unknown peak at 10 min.

the extent of coring, was also inconclusive as no single mass peak was observed in either the starting material or product spectrum. Instead analytic HPLC was used to observe the production of adenosine during the course of the reaction (Fig. 4). Within the first hour of the reaction the amount of adenosine observed exceeded that expected by 2-fold, assuming a polymer M_n = 20,000. This peak slowly disappeared during the reaction presumably due to further decomposition of adenosine.[24] The reaction was stopped when the adenosine peak had nearly disappeared and the final templated polymer **10** was purified by preparative SEC and extensive dialysis. HPLC analysis of the purified product indicated that adenosine was present in only trace quantities.

Binding Studies

The potential binding and selectivity profile of **10** was determined using equilibrium dialysis. Ligands were dialyzed against a polymer solution or buffer and the absorbance ratios of the dialyzed solutions were compared. The results are shown in Table 1. The polymer shows no binding for the template adenosine (entries 9–11), but ex-

Table 1. Equilibrium dialysis binding studies

entry	ligand	host	[ligand][a]	[host][a]	AbsRatio[b]	pH
1	adenine	10	1.25	1.25	0.61	6.9
2	adenine	10	0.50	0.50	0.71	4.0
3	adenine	10	0.50	0.50	0.66	10.0
4	adenine	9	1.25	1.25	0.57	6.9
5	hypoxanthine	10	1.25	1.25	1.02	6.9
6	hypoxanthine	9	0.50	0.50	0.95	6.9
7	chloropurine	10	1.00	0.50	0.53	6.9
8	uracil	10	1.00	0.50	1.10	6.9
9	adenosine	10	1.25	1.25	0.98	6.9
10	adenosine	10	0.50	0.50	0.97	4.0
11	adenosine	10	0.50	0.50	1.09	10.0
12	adenosine	9	0.50	0.50	0.95	6.9
13	deoxyguanosine	10	1.00	0.50	0.69	6.9
14	deoxyguanosine	10	1.00	0.50	1.02	6.9
15	deoxyuridine	10	1.00	0.50	1.18	6.9
16	deoxycytidine	10	1.00	0.50	1.06	6.9

[a]Concentrations listed assume the ligand and host occupy the total volume of the dialysis chamber. [b]Absorbance ratio is the ratio of the absorbance of the ligand after dialyzing against a polymer solution divided by the absorbance of the ligand after dialyzing against a buffer solution.

hibits a preference for binding purine bases (entries 1, 7, 13). Uncored polymer **9** showed no significant difference to the cored host (entries 6 and 12). Thus shape selectivity is probably not as important as hydrophobic encapsulation inside the hydrophobic polymer backbone. The extent of binding was also not affected by pH (entries 1–3 and 10–12), implying that the acid groups of the polymer's core are not critical for the observed binding. Thus, although some binding selectivity is observed it does not appear to be a result of a specific imprinted site.

When the polymer concentration was reduced to 0.25 mM the templated polymer **10** did not bind any of the tested ligands while uncored polymer **9** bound to hydrophobic guests such as adenine and 6-chloropurine (AbsRatio < 0.8). The affinity of the uncored polymer to hydrophobic guests could result from a hydrophobic interaction between the guest and template or the template maintaining a hydrophobic cavity in the interior of the cross-linked polymer. Host–guest studies on PAMAM dendrimers have shown that the size of the dendrimer core can affect its ability to encapsulate guest molecules.[25] If the cross-linked polymer is still somewhat flexible then removal of the template may result in a collapse of the binding pocket in response to the aqueous environment, which could limit its ability to act as a unimolecular micelle.[26]

SUMMARY AND CONCLUSION

We have demonstrated the synthesis of highly cross-linked hyperbranched polymers utilizing click chemistry and our standard RCM protocol. The use of the highly orthogonal click reaction allowed the allylation and polymer grafting to proceed in two steps without protective group chemistry. The polymers could be cross-linked using standard metathesis conditions and exhibited a decrease in size and increase in rigidity. The polymers were dihydroxylated and, thus, the first examples of water-soluble mMIPs.

Although the polymers exhibited binding selectivity, it could not be attributed to binding through the nanocavity left behind by template removal. This may be attributed to the large conformational change in the polymer structure resulting from the dihydroxylation step as well as the change in solvent conditions from cross-linking to binding. Future work will focus on aqueous cross-linking conditions[27] and more rigidly cross-linked architectures. We are also exploring more hydrophobic templates that may provide a larger hydrophobic surface for shape selectivity in future mMIPs.

EXPERIMENTAL

General

All solvents and reagents were of reagent quality, and used without further purification except where noted. Toluene preparative SEC was carried out on Bio-Beads S-X1 Beads gel permeation 200–400 mesh (Bio-Rad Laboratories), which has exclusion limits from 400 to 14,000. Aqueous preparative SEC was carried out on Bio-Gel p-10 gel, fine (Bio-Rad Laboratories), exclusion limits 1500–20,000. Analytical SEC chromatograms were acquired using a Viscotek Model 300 TDA with 3 columns: 2 Viscogel I-MBLMW-3078 and Viscogel IMBHMW-3078, using 0.05M LiBr in DMF as eluting solvent, flow rate 1mL/min, at 50 ºC. Dialysis was performed using Spectrumlabs Spectra/por Membrane MWCO: 3500 dialyzed against deionized water. Equilibrium dialysis performed with Harvard Apparatus 2 chamber 100 µL Micro-Equilibrium Dialyzer using a cellulose acetate membrane MWCO: 2000.

NMR spectra were recorded on a Varian Unity 500 M, 500 VXR, or Inova M narrow bore spectrometer MHz spectrometer and were performed in chloroform-*d* unless otherwise specified. The ^{1}H NMR spectra in chloroform-*d* and DMSO-d_6 were referenced to the residual protio peak at 7.26 and 2.49 ppm, respectively. In cases where H_2O was used as the solvent, acetone was added as the internal standard and referenced to 2.22 ppm. The ^{13}C NMR spectra in chloroform-*d* and DMSO-d_6 were referenced to the carbon peak of the solvent at 77.467 and 40.277 ppm, respectively. Coupling constants are reported in Hertz (Hz).

High and low resolution mass spectra were obtained by the Mass Spectrometry Laboratory, School of Chemical Science, University of Illinois, by ESI on a Waters Micromass Q-Tof or by EI on a Micromass 70 VSE. MALDI-TOF mass spectra were acquired on an Applied Biosystems Voyager-DE STR. High performance liquid chromatography (HPLC) was performed on a Dynamax SD-200 system with a UV detector set to 259 nm using an Alltech Denali C-18 column (250 10 mm) with a dual solvent system of 0.1% TFA/H_2O (Solvent A) and 0.1 % TFA/MeCN (Solvent B). IR data were obtained on a Perkin Elmer Spectrum BX FT-IR. Slow addition of reagents was performed using a Sage Instruments syringe pump Model 341A or 341B.

3,5-Diazidobenzoic acid (1). A solution of 5.0 g (33 mmol) of 3,5-diaminobenzoic acid and 6.7 g (100 mmol) of NaN_3 in 40 mL of 6.0 M aqueous HCl (0.24 mol) was cooled on ice. This was kept in the dark but open to air as 10 g (140 mmol) of sodium nitrite was slowly added to the reaction mixture over 2 h. The solution bubbled vigorously with each addition and produced a large amount of foam. The solution was allowed to stir an additional 30 min. The solution was filtered and the red solid was washed with water. The solid was dissolved in 100 mL of a 5% (w/w) aqueous sodium bicarbonate solution and washed three times with 25 mL of methylene chloride. The aqueous layer was isolated, then acidified with 1.5 M HCl and the red solid was again filtered, washed with water, and dried overnight. Obtained: 3.4 g, 55%, of a red clay. ^{1}H NMR (DMSO): δ 13.52 (b, 1 H), 7.31 (d, J = 2, 2 H), 7.01 (t, J = 2 Hz, 1 H). ^{13}C NMR (DMSO): δ 166.4, 142.3, 134.6, 116.6, 114.6. MS (EI). Calcd 204.0396. Found 204.0396. IR (nujol): 2128, 1708 cm^{-1}.

(2*R*,3*R*,4*R*,5*R*)-2-(6-(3,5-Diazido-*N*-(3,5-diazidobenzoyl)benzamido)-9*H*-purin-9-yl)-5-((3,5-diazido-benzoyl-

oxy)methyl)tetrahydrofuran-3,4-diyl bis(3,5-diazidobenzoate) (3). In 15 mL of thionyl chloride was dissolved 1.36 g (6.68 mmol) **1**. The suspension was refluxed in an 80 ºC oil bath for 1 h and became a homogeneous solution. The solution was cooled to 45 ºC and the thionyl chloride was removed under vacuum. To the black solid residue was added 230 mg (0.84 mmol) of adenosine and 20 mL of pyridine, which was distilled from CaH_2 directly into the reaction flask. The mixture was heated to 65 ºC in an oil bath for 18 h. Pyridine was removed under vacuum and the resulting residue was purified by column chromatography on silica gel eluting with 1% MeOH in CH_2Cl_2. Solvent removal afforded 590 mg (59%) of **3** as an off white solid: ^{1}H NMR: δ 8.66 (s, 1 H), 8.33 (s, 1 H), 7.48 (d, J = 2 Hz, 2 H), 7.36 (d, J = 2 Hz, 2 H), 7.31 (d, J = 2.5 Hz, 2 H), 7.22 (d, J = 2 Hz, 4 H), 6.83 (t, J = 2 Hz, 1 H), 6.81 (t, J = 2 Hz, 1 H), 6.79 (t, J = 2 Hz, 1 H), 6.50 (d, J = 4.5 Hz, 1 H), 6.44 (t, J = 5.5 Hz, 1 H), 6.26 (t, J = 5 Hz, 1 H), 4.86 (m, 2 H), 4.74 (m 1 H). ^{13}C NMR: δ 170.3, 164.6, 163.9, 163.7, 153.0, 152.9, 151.1, 144.8, 142.9, 142.8, 142.8, 142.6, 136.7, 132.5, 131.6, 131.3, 128.4, 116.7, 116.5, 116.0, 114.9, 114.8, 114.3, 114.1, 87.5, 80.5, 74.3, 72.0, 64.2. MS (ESI + H^+). Calcd 1198.2496. Found 1198.2483.

Allylation of HPG (5). To a 250 mL two-neck flask with condenser and septum was added 34 g (810 mmol) NaOH in 50 mL H_2O, 10 g (14 mmol hydroxyl groups) HPG, and 4.3 g (14 mmol) tetrabutylammonium iodide. This suspension was heated to 45 ºC and 55 mL (680 mmol) allyl chloride was added to the suspension over an 18-h period. The white suspension was dissolved in 200 mL CH_2Cl_2 and 100 mL H_2O and the organic layer was isolated. The aqueous layer was twice extracted with an additional 50 mL CH_2Cl_2. A small amount of methanol was used to prevent emulsification. The organic extracts were dried over Na_2SO_4 and then gravity filtered. The solvent was removed under reduced pressure, then dissolved in 20 mL ethyl acetate. This solution was passed through a short silica plug and eluted with an additional 200 mL ethyl acetate. The polymer was further fractionated by preparative SEC chromatography to provide 8.9 g (57%) of **5** as a clear liquid: ^{1}H NMR δ 5.90 (bm, 34 H), 5.28–5.24 (d, 35 H), 5.17–5.13 (bm, 36 H), 4.13 (s, 42 H), 3.99 (d, 32 H), 3.65–3.50 (b 173 H), 2.47 (b, 1 H). SEC: $M_{n(ps)}$ = 5390 g/mol, M_w/M_n = 1.13. MS (MALDI): Major peak = 2058.99 g/mol, peak separation 114.16 g/mol, M_n = 2344 g/mol M_w/M_n = 1.23 g/mol.

1,3-Dipolar cycloaddition to adenosine template (6). To a solution of 4 g (2 mmol) of allylated HPG **5** (M_n = 2000) in 12 mL of CH_2Cl_2 was added 12 mL H_2O. To this emulsion was added 200 mg (0.167 mmol) **3**. A Cu(I) solution was prepared by dissolving 420 mg (1.8 mmol) $CuSO_4$ and 660 mg (3.34 mmol) sodium ascorbate in 4 mL H_2O. This black solution was added to the reaction flask in a single portion. After 6 h, an additional 750 mg (0.37 mmol) of **5** was added along with 500 mg (2 mmol) $CuSO_4$ and 800 mg (4 mmol) sodium ascorbate. The solution was filtered and then added to a 250 mL separatory funnel. The organic layer was isolated and the aqueous layer was extracted 3 times with 25 mL portions of CH_2Cl_2. The organic layers were dried over Na_2SO_4 and filtered before solvent was removed under reduced pressure. The residue was passed through a preparative SEC column collecting fractions that had a slight yellow color. Fractions were analyzed by analytical SEC and those that did not have any starting material were combined. Solvent removal produced 1.37 g (51%) (based on adenosine core) of **6** as an orange liquid. ^{1}H NMR δ 8.67–7.96 (bm, 15 H), 5.88 (b, 250 H), 5.27–5.13 (bm, 470 H), 4.90–4.80 (s, 20 H), 4.12 (s, 270 H), (s 220 H), 3.9–3.25 (b, 1270 H). SEC: $M_{n(ps)}$ = 15,500 g/mol, M_n/M_w = 1.20. MS (MALDI): Major peak = 20,300 g/mol, M_n = 20,900 g/mol.

Cross-linking of allylated HPG (7). In 200 mL CH_2Cl_2 was dissolved 400 mg (0.88 mmol allyl groups) polymer **6**. To this was added 22 mg (0.018 mmol) Hoveyda–Grubbs second-generation catalyst. The flask was equipped with a condenser and refluxed for 24 h. Ethyl vinyl ether, 10 mL (100 mmol), was then added and the solution was allowed to reflux 1 h. The solvent was then removed under reduced pressure and the thin brown film was taken up in toluene. The polymer was passed through an SEC column and the dark fast-moving band collected. Solvent removal produced 400 mg crude polymer that was carried through without further purification and never dried to avoid gelation. ^{1}H NMR ($CDCl_3$, 500 MHz): δ ($CHCl_3$ = 7.26) 6.0–5.6 (b, 5 H), 5.35–5.1 (b, 1 H), 4.5–3.2 (b, 36 H). SEC: $M_{n(ps)}$ = 12600 g/mol, M_n/M_w = 1.18. MS (MALDI): Major peak = 19,000 g/mol, M_n = 19,500 g/mol.

Dihydroxylation of cross-linked polymer (9). In a 20 mL scintillation vial was dissolved 400 mg (1.7 mmol olefins) cross-linked polymer **7** in 10 mL acetone. To this solution was added 1 mL of an aqueous NMO solution (50% w/w) (4 mmol), 2 mL *tert*-BuOH, and 8 mg $K_2OsO_4 \cdot H_2O$ and the reaction was stirred open to air overnight. The solution was diluted with 5 mL H_2O, a nitrogen stream was used to concentrate it, and 5 mL H_2O was added. The solution was allowed to stir 1 d. The solution was then pumped dry and purified by preparative SEC, yielding 360 mg. ^{1}H NMR (D_2O, 500 MHz): δ (acetone = 2.22) 4.1–3.56 (b) SEC: $M_{n(ps)}$ = 20,500 g/mol, M_n/M_w = 1.45. MS (MALDI): Major peak = 22,000 g/mol, M_n = 22,000 g/mol.

Core removal of cross-linked polymer (10). To a 25 mL aqueous solution of 250 mg (6.3 mmol) NaOH was added 250 mg (0.013 mmol) water-soluble cross-linked polymer **9** and 135 mg (0.710 mmol) $TsOH \cdot H_2O$ as an internal standard for HPLC analysis. The solution was heated in a 75 ºC oil bath. Samples were removed for HPLC analysis after 1, 3, 5, and 17 h. After 17 h the reaction mixture was gravity filtered, then purified by preparative SEC and dialyzed against deionized water. Drying under vacuum yielded 208 mg of a brown film, 83% mass recovery. ^{1}H NMR (D_2O, 500 MHz): δ (Acetone = 2.22) 8.9–8.3 (b, 3 H), 4.1–3.5 (b, 184 H) SEC: $M_{n(ps)}$ = 22,500 g/mol, M_n/M_w = 1.43. MS (MALDI): Major peak = 21,700 g/mol, M_n = 22,000 g/mol.

Example Procedure for Equilibrium Dialysis

A cellulose acetate membrane was washed with Millipore water and carefully dried, then placed inside a dialysis chamber. To the top half of the dialysis chamber was added 100 µL of a stock 1 mM adenine solution in a 15 mM, pH 10 sodium acetate buffer. The top half of the chamber was then screwed down tightly. This procedure was repeated with a second dialy-

sis chamber. To the bottom half of one of the dialysis chambers were added 40 μL of a 2.5 mM solution of **10**, 59 μL Millipore water, and 1 μL 360 mM pH 10 sodium acetate buffer. To the other dialyzer were added 99 μL Millipore water and 1 μL sodium acetate buffer. The bottom chambers were then screwed down tightly and the entire dialyzer was wrapped in parafilm. The dialyzers were then placed in a shaker at room temperature for 8 h to equilibrate. The contents of the top chamber were then removed and analyzed by UV-Vis spectroscopy. A separate reference was prepared by diluting 100 μL of the 1 mM stock adenine solution in 99 uL Millipore water and 1 μL buffer to ensure the solutions had reached equilibrium. A summary of the results is shown in Table 1.

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