Binding of Nucleotide Bases by Imprinted Polymers

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ABSTRACT: Molecular imprinting technology was used to create polymeric receptors for the DNA and RNA bases. The binding affinity and selectivity of ethylene glycol dimethacrylate (EGDMA) methacrylic acid (MAA) imprinted copolymers were evaluated chromatographically. Specific binding was found for adenine, cytosine, and guanosine derivatives. These bases contain a 2-aminopyridine substructure previously found important for the binding and specificity of polymers imprinted with 9-ethyladenine. Thymine and uracil derivatives, which do not contain the 2-aminopyridine substructure, exhibited little specific binding to the imprinted polymers. The magnitude of the binding affinity for each of the nucleoside derivatives to its own imprinted polymer follows the order A > G > C > T, U. This differs from the order of binding between butyric acid and the RNA base derivatives in solution (G > C > A > U: Lancelot, G. *J. Am. Chem. Soc.* **1977**, *99*, 7037) or between control polymers that contain randomly distributed carboxylic acid groups and the DNA and RNA bases (C ≥ A > G > T, U).

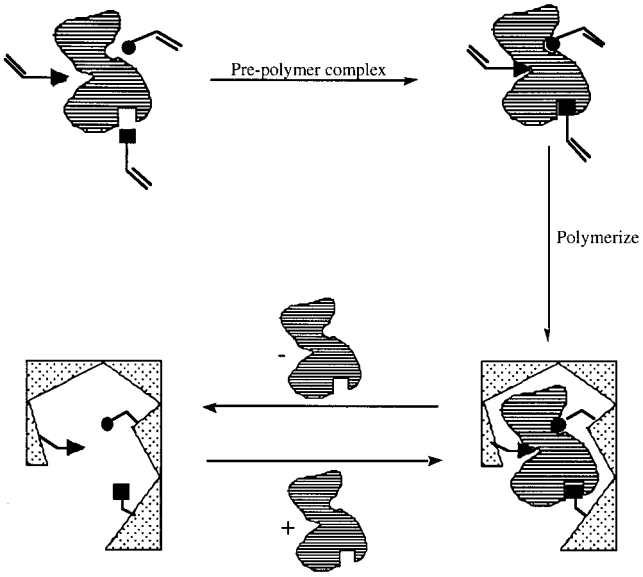
Introduction

The literature provides many examples of abiotic synthetic receptors for each of the common nucleotides.[1−5] These artificial small molecule receptors provide excellent binding constants in the range 11 000−120 000 M^{-1} in chloroform. Many of these receptors also show selectivity in nucleoside binding. However, fashioning rationally designed synthetic receptors is often a complicated and difficult process, in both design and execution. Although this approach has shown rewarding results, the method of molecular imprinting[6−8] holds the advantage of providing rapid (within hours) and facile preparation of specific polymeric receptors.

The strategy for the method of molecular imprinting is outlined in Scheme 1. Functionalized monomers are bound either covalently or noncovalently to a print molecule or template. The resulting prepolymer complex is copolymerized with an excess of cross-linking monomer in the presence of an equal volume of inert solvent (porogen) and a free radical initiator. Thermal- or photochemical-initiated polymerization results in a highly cross-linked insoluble polymer. Removal of the template by extraction or hydrolysis leaves sites in the polymer containing functionalized monomers. These sites are complementary in size and shape[9] to the template molecule, resembling the "lock and key" paradigm of enzymes. Moreover, the functional groups of the polymer are positioned to converge on the template molecule.

We have previously reported that network polymers, which contain strong binding sites for adenine, can be prepared by copolymerization of methacrylic acid and cross-linking monomers in the presence of the template molecule 9-ethyladenine (9-EA).[10,11] The effects of polymerization and binding variables, such as choice of porogen and temperature, were investigated for the 9-EA imprinted polymers, and complete separation of adenine from a mixture of adenine, guanine, cytosine, uracil, and thymine was demonstrated. Furthermore, quantitative structure−binding relationship studies determined which substructural elements of 9-EA were primarily responsible for binding and selectivity of 9-EA to its imprinted polymer. Evaluation of the binding and selectivity of all the nucleotide bases and some derivatives by imprinted polymers is presented here.

Scheme 1. Outline of the Molecular Imprinting Strategy



Results

In a manner similar to polymers imprinted with 9-EA, organic soluble alkylated derivatives of cytosine, thymine, and uracil were subjected to the imprinting process. It should be noted that no suitable guanine derivative was found that was sufficiently soluble in chloroform or acetonitrile for these experiments. Figure 1 shows the structures of the pyrimidine base derivatives imprinted in addition to 9-EA, a purine base.

The general formulation for these imprinted polymers consisted of nucleotide base derivative (1.0 mol %), methacrylic acid (MAA, 12.0 mol %), ethylene glycol dimethacrylate (EGDMA, 86.0 mol %), AIBN (1.0 mol %), and acetonitrile (50/50% v/v monomers/porogen). The polymers were photopolymerized below ambient temperature, followed by Soxhlet extraction to remove the template. Spectrophotometric quantification showed

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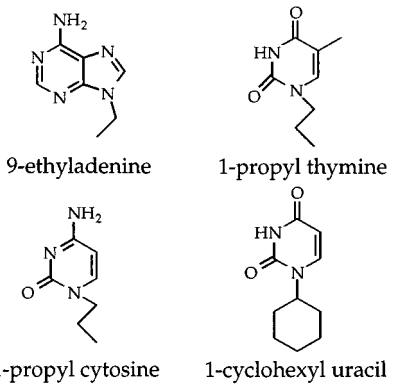


Figure 1. Organic solvent soluble derivatives of DNA bases.

Table 1. Capacity Factor (k') Values of DNA Base Derivatives[a] on Imprinted Polymers

	substrates[b]			
polymers[c]	9-EA	1-PrC	1-PrT	1-ChU
P(9-EA)	**39.7**	0.5	0.6	0.8
P(1-PrC)	1.0	**0.9**	0.5	0.6
P(1-PrT)	0.8	0.3	**0.4**	0.5
P(1-ChU)	0.7	0.2	0.4	**0.6**
P(BA)	1.2	0.6	0.5	0.6

[a] Mobile phase = 92.5:5:2.5 ACN/AcOH/H_2O, UV detection at_λ = 260, flow rate = 1.0 mL/min at room temperature. Injections were 10 μL of 1.0 mM solutions. [b] Substrate abbreviations: 9-EA = 9-ethyladenine, 1-PrC = 1-propylcytosine, 1-PrT = 1-propylthymine, 1-ChU = 1-cyclohexyluracil, BA = benzylamine. [c] P(9-EA) = polymer imprinted with 9-ethyladenine. Other polymers (P) imprinted using the nucleoside base shown in parentheses as template.

all substrates to be recovered in greater than 95% yield. Binding affinity and selectivity of the polymers were evaluated chromatographically. As a control, an imprinted polymer was prepared with a "generic" template, benzylamine (previously reported as a control template[10,11]), to produce a polymer with the same loading of carboxylic acid functional groups but randomly distributed in the polymer. Table 1 shows the capacity factors,[12] a measure of binding affinity, for each base as substrate on each of the imprinted polymers using acetonitrile/acetic acid/water

(92.5:5:2.5) as mobile phase.

The large capacity factor (shown in bold type) for 9-EA as substrate on the column made with 9-EA imprinted polymer (P(9-EA)) indicates strong binding compared to the other bases tested. 1-Propylcytosine (1-PrC) exhibited weak binding and little, if any, specificity on the column prepared with P(1-PrC) over the other substrates. The polymers imprinted with thymine and uracil derivatives show poor binding and no selectivity for their own templates. This is not surprising since the inability of carboxylic acid receptors to bind thymine or uracil appears to be rather general.[2,13] It is informative to note that both adenine and cytosine derivatives contain the 2-aminopyridine substructure previously found to be important for polymer imprinting with methacrylic acid as the functional monomer.[11] However, the thymine and uracil derivatives do not contain this moiety and their imprinted polymers exhibit little or no affinity. Moreover, the control polymer shows a slightly greater affinity for all substrates than the thymine or uracil imprinted polymers. Not only does this attest to the poor affinity by P(1PrT) and P(1ChU), but also it suggests that benzylamine is not an unbiased template for generating control polymers. Control polymers should fit two criteria: first, capacity factors for a substrate should be less than or equal to that measured for the polymer imprinted with that substrate; second, all control polymers should give roughly the same k' values to be considered unbiased. Accordingly, several different control polymers were tested in the analyses that follow.

Table 2. Capacity Factor (k') of Nucleotide Base Derivatives[a]

	substrates			
polymers	9-EA	1-PrC	1-PrT	1-ChU
P(9-EA)	**54.8**	3.9	0.3	0.4
P(1-PrC)	2.2	**13.1**	0.2	0.4
P(1-PrT)	2.1	2.1	**0.2**	0.4
P(1-ChU)	1.9	3.2	0.2	**0.4**
P(BzA)	1.7	2.4	0.2	0.3
P(AA)	1.1	1.2	0.1	0.2
P(NT)	1.3	1.3	0.1	0.2

[a] Mobile phase = 95:5 MeCN/H_2O, UV detection at λ = 260 nm, flow = 1 mL/min.

Binding measurements of the imprinted polymers were repeated using a solvent system with lower eluting strength (95:5:acetonitrile/water) in an effort to elicit better binding and selectivity behavior. These results are summarized in Table 2.

The general trends for substrate selectivity utilizing the new mobile phase were the same; however, there were differences in binding strength (magnitude of the capacity factor) and the degree of selectivity. For example, greater affinity and specificity is seen for polymers imprinted with 9-EA and 1-PrC. These imprinted polymers show the greatest self-recognition (shown in bold type in the table above). This is particularly significant in the case of P(1-PrC), which now exhibits a larger capacity factor for 1-PrC than for any of the other nucleotide derivatives, including 9-EA. The derivatives of thymine and uracil continue to show poor binding and no selectivity for their imprinted polymers. Three new EGDMA/MMA control polymers were tested: P(BzA), imprinted with benzoic acid; P(AA), imprinted with acetic acid; and P(NT), a polymer made without any template at all. P(BzA) seems to have some bias not exhibited by P(AA) or P(NT), as evidenced by the larger and more varied capacity factors. Both P(AA) and P(NT) give roughly the same values. This consistency indicates a lack of bias that makes these polymers better choices for use as control polymers.

The binding and selectivity of *nucleosides* were investigated using organic soluble derivatives of all five nucleosides, spcifically those with a fully acetylated ribose unit (Figure 2). These derivatives allowed a comparison of polymer complements of *all* the DNA and RNA bases including guanine derivatives. In addition, the influence on selectivity of ribose substituents on the bases can be studied.

The nucleoside derivatives were imprinted in the same fashion as the nucleotide base derivatives. The nucleoside template (0.7 mol %) was dissolved in a mixture of MAA (12.0 mol %), EGDMA (86.3 mol %), AIBN (1.0 mol %), and the porogen acetonitrile (40/60% v/v monomers/porogen). The chromatographic studies were performed in two different solvent systems, and the results are summarized in Tables 3 and 4.

In agreement with the preceding cases, the polymers imprinted with adenosine and cytidine derivatives

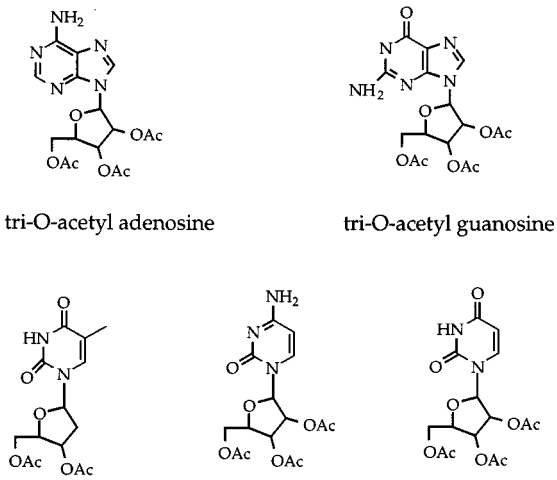


Figure 2. Nucleosides with acetylated sugar units used for imprinting

Table 3. Capacity Factor (k') Values of Nucleoside Derivatives[a]

	substrate[b]				
polymer[c]	TOAA	TOAG	TOAC	DOAT	TOAU
P(TOAA)	**6.1**	0.2	0.5	0.2	0.1
P(TOAG)	0.4	**0.3**	0.5	0.2	0.1
P(TOAC)	0.3	0.1	**0.7**	0.1	0.0
P(DOAT)	0.3	0.1	0.4	**0.1**	0.0
P(TOAU)	0.2	0.1	0.4	0.2	**0.0**
P(BzA)	0.3	0.1	0.3	0.1	0.0
P(AA)	0.1	0.0	0.1	0.0	0.0
P(NT)	0.2	0.0	0.1	0.0	0.0

[a] Mobile phase = 95:5 acetonitrile/water, λ = 260 nm. [b] Substrate abbreviations: TOAA = tri-*O*-acetyladenosine, TOAG = tri-*O*-acetylguanosine, TOAC = tri-*O*-acetylcytidine, DOAT = di-*O*-acetylthymidine, TOAU = tri-*O*-acetyluridine, BzA = benzoic acid, AA = acetic acid, NT = no template. [c] P(TOAA) = polymer made with tri-*O*-acetyladenosine. Other polymers (P) made using the substrate shown in parentheses as template.

showed selectivity for their own substrates. Guanosine derivatives showed slight selectivity over thymidine and uridine, but not over those of adenine or cytosine. The thymine and uracil derivatives continued to show little or no binding or specificity (Table 3). In this case, none of the control polymers exhibited any significant measure of binding affinity. *The specificity for self-recognition in imprinted polymers is most dramatically revealed in solvents of relatively low polarity. For example, when a less polar solvent system, pure acetonitrile, was employed to amplify the specificity of the polymers, adenine, cytosine, and guanosine imprinted polymers all exhibited high selectivity* (Table 4).

This solvent did not elicit any selectivity for the thymidine and uridine derivatives. The guanosine derivative, however, showed very good specificity as well as a relatively large affinity (bold type). Guanosine also has the 2-aminopyridine moiety in its structure, which appears to be responsible for the good binding characteristics of molecules containing this unit with methacrylic acid functionalized imprinted polymers.[11] The control polymers parallel their performance in solvents with greater eluting strength (Table 2). The capacity factors for P(BzA) are too large relative to the other polymers to consider it an unbiased generic template. P(AA) and P(NT) appear to both serve well as control polymers, with P(NT) exhibiting the lowest k' values and the least amount of bias (differences in k' values) of all controls tested.

Discussion

Several factors are postulated to influence the selective binding of DNA and RNA bases by imprinted polymers that utilize the carboxylic acid group as the functional monomer. These include the inherent ability of the carboxylic acid groups to bind the base, hydrophobic/hydrophilic interactions with the polymer matrix, shape selectivity, and the preorganization of the carboxylic acid groups within the polymer. The inherent ability of carboxylic acid groups to bind DNA bases is demonstrated by the order of binding strength between butyric acid and the bases in chloroform.[13] The order of association constants for this homogeneous case is G > C > A > U. Similarly, both imprinted and control polymers exhibit chemoselectivity favoring binding to adenine, guanosine, and cytosine over thymine or uracil. This is in agreement with a number of other carboxylic acid based receptors that also show low affinities for thymine and uracil derivatives.[2,13] However, the order of selectivity for the purine and pyrimidine bases on these imprinted polymers is not that found in homogeneous solution.

Structural elements common to adenine, guanosine, and cytosine include an exocyclic amine α to a nitrogen embedded in the purine or pyrimidine ring. This substructure was shown to be important for the binding and selectivity of 9-EA imprinted polymers.[11] To investigate the origins of specific binding by 9-EA imprinted polymers, a quantitative structure−binding relationship (QSBR) was investigated by imprinting a number of molecules that mimic substructures of 9-EA. It was found that structures that have two nitrogens positioned α or β to each other, one being part of an aromatic ring, appear to be mainly responsible for the binding and specificity of P(9-EA) for 9-EA.[11] Nature uses this 2-aminopyridine substructure in Watson−Crick binding modes found between nucleic acids in double helical DNA. It is likely that the imprinted polymers provide similar donor−acceptor binding interactions such as those found in native DNA (Figure 3a-c).

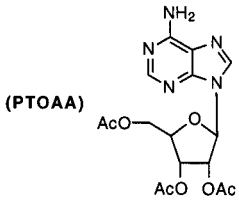
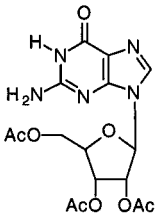
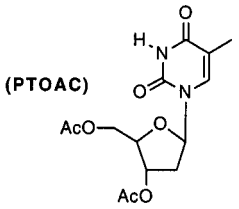
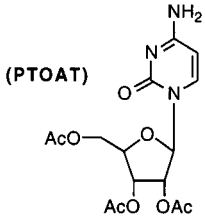
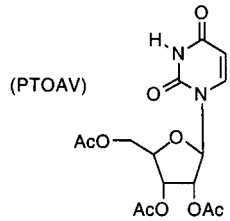
In the case of guanine, two 2-point donor−acceptor hydrogen-bonding interactions are possible with methacrylic acid functionalized polymers[13] (Figure 3b,d). The binding mode shown in Figure 3d partially mimics Watson−Crick type interactions, which have been shown to dominate in the case of 2-(dimethylamino)-6-hydroxypurine.[13] However, the two methyl groups on the exocyclic amine of 2-(dimethylamino)-6-hydroxypurine prevent interactions of the type shown in Figure 3d.

It is likely that both types of hydrogen bond interactions such as those shown in Figure 3b,d are operative between tri-*O*-acetylguanosine and the carboxylic acid functionalized polymers and may be influential for the molecular recognition exhibited by the TOAG imprinted polymers.

The chemoselectivity of the carboxylic acid groups in the polymers provides a first level of selection for adenine, guanine, and cytosine over thymine or uracil. Multiple interactions with the polymer matrix supporting the carboxylic acid functionality also appear to affect the binding selectivity of the nucleobases.

This intrinsic affinity and selectivity of the polymers can be modified by changes in the eluting strength of

Table 4. Capacity Factor (k') Values of Nucleoside Derivatives[a]

	substrate				
polymer	TOAA	TOAG	TOAC	DOAT	TOAU
 (PTOAA)	**>440**	5.2	4.2	0.0	0.0
 (PTOAG)	6.5	**50.9**	6.5	0.3	0.1
 (PTOAC)	3.6	3.5	**32.3**	0.3	0.7
 (PTOAT)	3.5	2.1	4.6	**0.2**	0.1
 (PTOAV)	3.6	1.1	4.2	0.7	**1.1**
	8.3	3.8	19.4	0.4	0.2
P(AA)	2.0	1.7	3.1	0.3	0.1
P(NT)	1.7	0.7	1.9	0.2	0.1

[a] Mobile phase = acetonitrile, λ = 260 nm, flow rate = 1 mL/min at room temperature. Injections were 10 μL of 1.0 mM solutions.

the mobile phase. Tables 1−4 show that selectivity for adenine, guanine, and cytosine derivatives can be achieved by the appropriate choice of mobile phase. This is most dramatically seen in Table 4, which shows striking selectivity for adenosine, guanosine, and cytosine derivatives by their own imprinted polymers. Furthermore the capacity factors in the table show that the order of binding affinity of each of the bases by its own imprinted polymer was A > G > C > T, U. If the effect of binding by the imprinted polymers was due *only* to the inherent binding affinity of the carboxylic acid groups, the order should parallel relative strengths of their binding to carboxylic acids in solution (G > C > A > U). Similarly, if the order of binding strength was due to the natural affinity of the carboxylic acid functionalized polymers for the bases, the order of binding affinity of the imprinted polymers would parallel the control polymers ($C \geq$ A > G > T, U). Neither was the case, owing to the additional microenvironment effects attributable to the process of *imprinting* the polymers. This must arise from multiple interactions between polymer and substrate. These factors may include a complementary shape built into the imprinted polymer for the template molecule and multiple functional group interactions for optimal interaction with the substrate molecule. Molecular imprinting utilizes these basic tenets of molecular recognition to tailor-make selective binding sites similar to those of antibodies or enzymes.

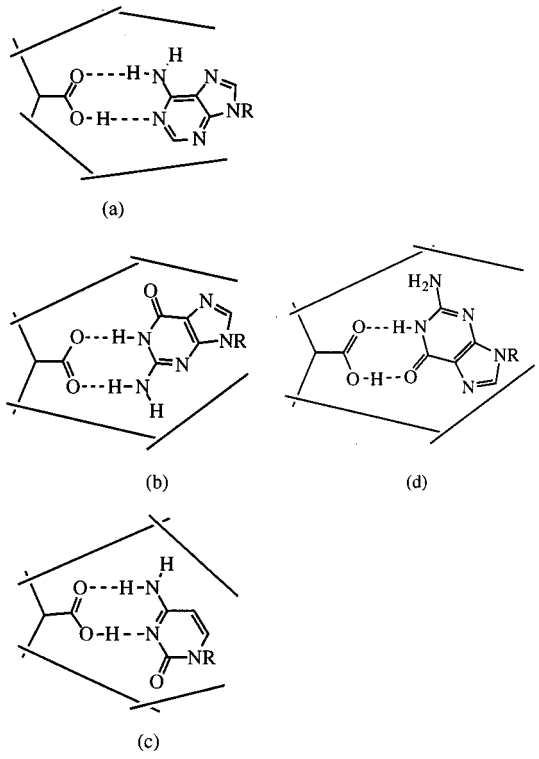


Figure 3. Proposed hydrogen bonding interactions between methacrylic acid imprinted polymers and adenine (a), guanine (b,d), and cytosine (c) derivatives.

In addition, the imprinted polymers also function as their own solid support, predisposed for applications in affinity chromatography.

Conclusion

The scope of molecularly imprinted polymers utilizing carboxylic acid functional groups to bind nucleobases has been expanded to all the DNA and RNA bases. Specific binding was found for adenine, cytosine, and guanine derivatives. These three bases have a 2-aminopyridine substructure in common. This appears to be an important component for binding and specificity of purine and pyrimidine bases to imprinted polymers with carboxylic acid functionality. In contrast, thymine and uracil derivatives, which do not contain the 2-aminopyridine moiety, showed little binding or specificity under any of the conditions examined.

Several different control polymers made with the intent to randomly distribute the carboxylic acid functional groups were evaluated. Of these, polymers imprinted with acetic acid or with no template served best, showing the least bias to bind any particular compound. The contribution of microenviromental effects to specificity in the imprinted polymers was discussed. The natural tendency for the DNA and RNA bases to bind carboxylic acid groups in homogeneous solution or to carboxylic acid functionalized control polymers can be modified by the polymer imprinting method. This we attribute to shape complementary and preorganization of multiple functional groups in the polymer binding site. Future work will assess the ability of molecularly imprinted polymers to bind specifically to defined sequences of DNA or RNA oligomers.

Experimental Section

General Information. UV spectra were recorded on a Perkin-Elmer Lambda 4A UV/vis spectrophotometer or a Hewlett-Packard 8452A diode array spectrophotometer. HPLC analyses were performed with a Millipore Waters 501 or 6000A solvent delivery system equipped with a Waters 484 tunable absorbance detector and a Hewlett-Packard 3396A integrator or a Shimadzu LC-10AS dual pump gradient solvent delivery system equipped with an SPD-10AV UV/vis detector and a Hewlett-Packard 3396A integrator.

9-Ethyladenine, 1-propylcytosine, and 1-propylthymine were all synthesized according to procedures found in ref 14. 1-Cyclohexyluracil was obtained from Aldrich Chemicals and used without further purification. Tri-*O*-acetyladenosine, tri-*O*-acetylguanosine, tri-*O*-acetylcytidine, di-*O*-acetylthymidine, and tri-*O*-acetyluridine were all obtained from Sigma Chemical Co. and used without further purification. Ethylene glycol dimethacrylate (EGDMA, Polysciences) was first washed twice with aqueous 1 M NaOH and once with aqueous saturated NaCl solution to remove inhibitor and further dried with anhydrous $MgSO_4$. The monomer was then filtered away from the solids and distilled under reduced pressure (10 mmHg, 60 °C). Methacrylic acid (MAA, Aldrich) was distilled over $CaCl_2$ (10 mmHg, 80 °C). AIBN (Fluka) was recrystallized from methanol. All solvents were obtained from commercial suppliers and purified prior to use.

Polymer Preparation with Nucleotide Base Derivatives. A typical example is given here; all polymers were prepared in identical fashion. To a solution of 1-propylcytosine (61.2 mg, 0.4 mmol) in acetonitrile (9.3 mL) was added methacrylic acid (0.4 mL, 4.8 mmol), ethylene glycol dimethacrylate (6.6 mL, 34.8 mmol), and AIBN (66 mg, 0.4 mmol). The reaction mixture was transferred to a 50 mL thick walled tube. The tube was degassed by three freeze−thaw cycles under vacuum and then flame sealed. The polymerization was initiated photochemically by a Hanovia medium-pressure 500 W mercury arc lamp at 5 °C and allowed to proceed for 24 h. The polymerization tubes were turned 180° after the first 10 min, 20 min, and 10 h of polymerization. After crushing, the polymers were Soxhlet extracted in methanol overnight and then dried under vacuum at 50 °C. The extract was evaporated and redissolved in MeOH (10 mL) and a portion diluted 1000 times in $CHCl_3$. The splitting yield (amount of extracted template) was determined spectrophotometrically at 255 nm using a calibration curve for quantification. The substrates were recovered in greater than 95% yield. The particles were ground by mortar and pestle and sized using USA Standard Testing Sieves (ASTME-11 specification). Particles sized 25−38 mm were used to slurry pack stainless steel columns (length, 10.0 cm; i.d., 4.6 mm) to full volume (approximately 0.6 g of polymer) for chromatographic experiments.

Polymer Preparation with Nucleoside Derivatives. The following procedure is general for all polymers unless otherwise indicated. In a 50 mL thick walled tube was added a solution of tri-*O*-acetyluridine (108.6 mg, 0.29 mmol) in acetonitrile (9.3 mL) and methacrylic acid (0.4 mL, 4.8 mmol), ethylene glycol dimethacrylate (6.6 mL, 34.8 mmol), and AIBN (66 mg, 0.4 mmol). The tube was degassed by three freeze−thaw cycles under vacuum and then flame sealed. The polymerization was initiated photochemically by a standard laboratory UV light source (a Hanovia medium-pressure mercury arc lamp) at 7.3 °C and allowed to proceed for 13 h. The polymerization tubes were turned 180° after the first 15 and 30 min and then a quarter turn at 1 h and every hour afterward for 10 h. The remaining procedure follows that used for imprinting nucleotide base derivatives.

Due to the greater insolubility of tri-*O*-acetylguanosine, the formulation for the polymer was modified. In a 50 mL thick walled tube was added a solution of tri-*O*-acetylguanosine (120.1 mg, 0.2934 mmol) in acetonitrile (9.3 mL) and methacrylic acid (0.61 mL, 7.32 mmol), ethylene glycol dimethacrylate (10 mL, 52.9 mmol), and AIBN (98.4 mg, 0.66 mmol). The tube was degassed by three freeze−thaw cycles under vacuum and then flame sealed. The rest of the procedures follow those above.

Chromatographic Experiments. The polymer particles (25−38 μm size range) were slurry packed in stainless steel chromatographic columns (length, 100 mm; i.d., 4.6 mm) using

a Waters 501 or 6000A solvent delivery system. Once slurry packed, the columns were then washed on line (in addition to previous Soxhlet extraction) with acetonitrile or 7:3 acetonitrile/water until a stable baseline was obtained. HPLC analyses were performed isocratically at room temperature using a Waters 501 or 6000A solvent delivery system and a Waters 484 UV/vis tunable absorbance detector; flow rate, mobile phase, and substrate conditions are indicated in the text for each experiment. The void volume was determined by injecting a small amount of an inert substance, namely acetone, acetonitrile, or sodium nitrate; of the three, the void volume marker utilized was that with the smallest retention volume.

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