Imprinted Polymers Prepared with Stoichiometric Template−Monomer Complexes: Efficient Binding of Ampicillin from Aqueous Solutions

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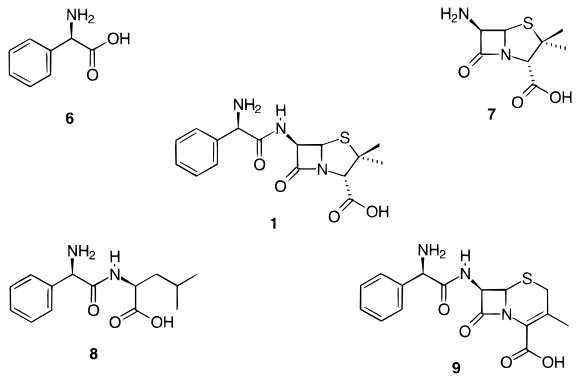
ABSTRACT: Two new functional monomers for molecular imprinting, 5-(4‴-vinyl)benzyloxy-1,3-bis[2′-(3″,3″,4″,4″-tetramethyl-2″,5″-dioxaborolanyl)phenylcarbomoyl]benzene (**3**) and 2-(4-vinylphenyloxy)-3,5,6-trichlorobenzoquinone (**4**), designed to interact with carboxylate and amino groups, respectively, were synthesized. NMR titrations confirmed the interactions of **3** with tertabutylammonium acetate and ampicillin carboxylate in d_6-DMSO and of **4** with the free amino group of ampicillin in the same solvent. Polymers were prepared, using DMSO or THF as porogen, imprinted with ampicillin carboxylate using **3** and **4** present in the polymerization mixture in only stoichiometric amounts. The polymer made in DMSO was shown to bind ampicillin from aqueous buffer at pH 8.0 with two populations of binding sites, the first characterized by $K_d = (3.0 \pm 0.3) \times 10^{-5}$ mol/L at a capacity of 5.8 ± 0.3 μmol/g and the second by $K_d = (9.6 \pm 1.1) \times 10^{-4}$ mol/L and 48 ± 3.4 μmol/g.

Introduction

The development of new synthetic materials with highly selective binding properties for particular target molecules can be seen as the key to unlocking a host of new technologies. In pursuit of this goal, a great deal of effort has been expended in recent years on the synthesis and characterization of synthetic host molecules designed to interact specifically with individual amino acids, peptides, monosaccharides, nucleotides, and nucleosides among others.[1] An enormous amount of synthetic expertise has been expended in constructing the (often asymmetric) framework of some of these supramolecular hosts. A fundamentally different approach to the preparation of synthetic receptors is offered by the process of molecular imprinting[2] which overcomes these difficulties by holding individual recognition elements in place by virtue of their interactions with a template, while they are incorporated into a rigid macromolecular scaffolding formed by a web of growing polymer chains. This allows the connecting pathways between the individual recognition elements which make up a receptor site to be of virtually any length through the network. Thus, the preparation of polymeric receptors bearing functional groups positioned in the precise spatial arrangement to interact with complementary functionality of the target is greatly facilitated by this methodology which opens the door on a wealth of new application areas.

Noncovalent imprinting[3] is arguably the most versatile embodiment of the molecular imprinting technique. This traditionally relies on the use of relatively simple functional monomers such as acrylic and methacrylic acids, acrylamide,[4,5] itaconic acid,[6] vinylpyridine,[7] aminoethyl methacrylamide,[8] diethylaminoethyl methacrylate,[9] and others[10] which coordinate to the template by polar associations and by the formation of hydrogen bonds. These monomers often have to be used in considerable excess to ensure that a sufficiently high degree of complexation with functional groups of the template has occurred for effective imprinting. As a result, functionality within the polymer is not incorporated exclusively into the recognition sites, which inevitably leads to increased nonspecific binding and therefore a loss of selectivity.[11] This can be overcome through covalent approaches where the polymer-bound functionality is only revealed by chemical cleavage[12,13] or by conventional covalent imprinting methods using ketals,[14] boronate esters,[15,16] or metal complexes[17,18] or by the use of monomers which exhibit very strong noncovalent associations with features of the template, such that they can be used in stoichiometric amounts. The latter stoichiometric noncovalent interactions can be achieved either by exploiting ion-pair associations in polar solvents[19] or by the introduction of new functional monomers with superior association constants.[20]

Scheme 1. Structures of Ampicillin (1), D-Phenylglycine (6), 6-Aminopenicillanic Acid (7), D-Phenylglycyl-L-leucine (8), and Cephalexin (9)



In this study we have set out to demonstrate the feasibility of preparing highly selective imprinted polymers using stoichiometric complexes of a mixture of electrochemically neutral functional monomers and a hydrophilic template, designed to be used in water. Ampicillin (**1**) (Scheme 1) was chosen as the target for this investigation since it is representative of an important class of compounds, namely the penicillin antibiotics, with many commercially available structural analogues which can be used to probe the specificity of the polymer. It was also judged to be an attractive

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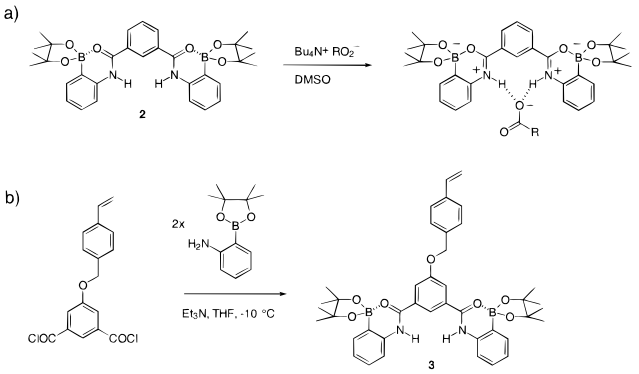
Scheme 2. (A) Boron-Containing Receptor of Hughes and Smith[25] (2) and Its Complexation with Acetate Anion; (B) Synthesis of 3, a Polymerizable Derivative of 2



generic target because the molecule carries functional groups, namely carboxyl and amino, found in many bioactive compounds, e.g., other antibiotics, amino acids, peptides, nucleotides, and alkaloids, which may be imprinted in the same way.

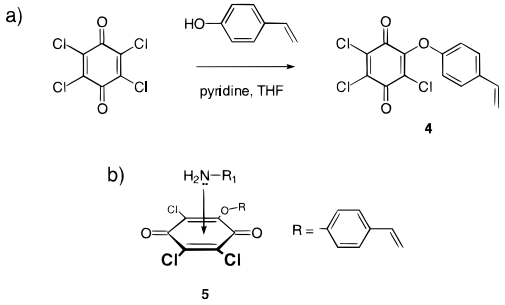
Results and Discussion

The nature of noncovalent imprinting usually dictates that a single functional monomer is used in the preparation of molecular imprints. Nonproductive associations between monomers would normally prevent the use of, for example, an acidic and basic monomer together in the same polymerization mixture. Some synergy however is possible with methacrylic acid and vinylpyridine mixtures,[21] and the use of a mixture of acrylamide and vinylpyridine has recently been reported;[22] but generally speaking, imprinted polymers featuring two functional monomers are usually prepared by a combination of covalent and noncovalent methods.[12,23] The success of mixed noncovalent systems depends on finding monomers that interact strongly with their target functional groups but not with each other.

Monomer Synthesis and Binding Studies. In considering the suitability of functional monomers to complex the carboxyl group, the neutral urea- and amide-based receptors of Hughes and Smith[24,25] showed considerable promise. These receptors employ internal Lewis acid coordination between boron and the carbonyl oxygen to polarize the urea or amide bonds, thus increasing their affinity for oxygen anions. Receptors of this type were shown to bind tetrabutylammonium acetate in DMSO with association constants of between 2×10^3 and 6×10^4 M^{-1}. While the highest affinities were exhibited by the urea-based receptors, these were also reported as being relatively unstable, and so the bis(boronate−amide) receptor **2** (Scheme 2) was chosen to form the basis of our polymerizable carboxylate receptor **3**.

A receptor monomer was prepared (Scheme 2), incorporating a vinylbenzyl ether as the polymerizable entity, using essentially the same synthetic protocol reported for the synthesis of **2**. We did not expect that introduction of the polymerizable functionality would dramatically alter the binding properties of **3**, but in order to confirm the formation of complexes with tetrabutylammonium acetate, **3** was tested in NMR titration experiments in d_6-DMSO (Figure la). These experiments

Scheme 3. (A) Synthesis of the Polymerizable Quinone (4); (B) Proposed Complex (5) between 4 and a Primary Amine



confirmed that a stoichiometric 1:1 complex was indeed formed, although the measured association constant of 1.4×10^2 M^{-1} was somewhat lower than that reported by Smith. Similar results were obtained for ampicillin carboxylate, $K_a = 2.8 \times 10^2$ M^{-1}, and for acetate in d_3-acetonitrile (not shown).[26] It is not entirely clear at present why the association constant between acetate and **3** should be about an order of magnitude lower than that reported for the analogous complex with **2**. Mesomeric donation by the ether will increase electron density at the ortho and para positions but is not expected to strongly influence the amides at the meta positions. This leaves the small inductive effect of oxygen which may be responsible for reducing electron density at the carbonyl oxygen. Other analogues of **2** could be envisaged that do not rely on an ether to introduce the polymerizable functionality, and these will be considered for future work.

As a suitable candidate for complexing the amine group, the polymerizable chlorinated quinone **4** was proposed. This was synthesized in one step by the reaction of *p*-vinylphenol with *p*-chloranil (Scheme 3a). It is known that *p*-chloranil readily forms n−π complexes with relatively strong electron pair donors,[27] including amines,[28] and it was hoped that **4** would exhibit the same behavior (Scheme 3b). Electron-deficient quinones such as **4** have two coordination sites, one on each face of the planar molecule, with the potential to form 2:1 complexes. However, at stoichiometric ratios the 1:1 complex **5** would be expected to predominate. The complexation between **4** and ampicillin was investigated by NMR titration in d_6-DMSO. As the "host" molecule has no ^{1}H NMR signals close to the point of complexation, the titration was carried out using a constant concentration of "guest" by following the shift of the hydrogen geminal to the amino group of ampicillin (Figure 1b).[29] The titration experiment confirmed the formation of a 2:1 complex in d_6-DMSO as expected, with a remarkably strong association constant estimated[30] to exceed 3×10^4 M^{-1}.

Polymer Synthesis. Having prepared and characterized two new polymerizable monomers, we proceeded with the preparation of imprinted polymers. The tetrabutylammonium salt of the template was prepared and stirred overnight with **4** in the polymerization solvent before the addition of the remaining monomers and initiator. This procedure was adopted as a precaution; while complex formation appeared to be rapid in DMSO, slow complexation between **4** and amines was observed in some other solvents (e.g., THF). DMSO and THF were used as porogens, and imprinted and nonimprinted polymers were prepared by thermal polymerization at 65 °C, as given in Table 1. The template loading in

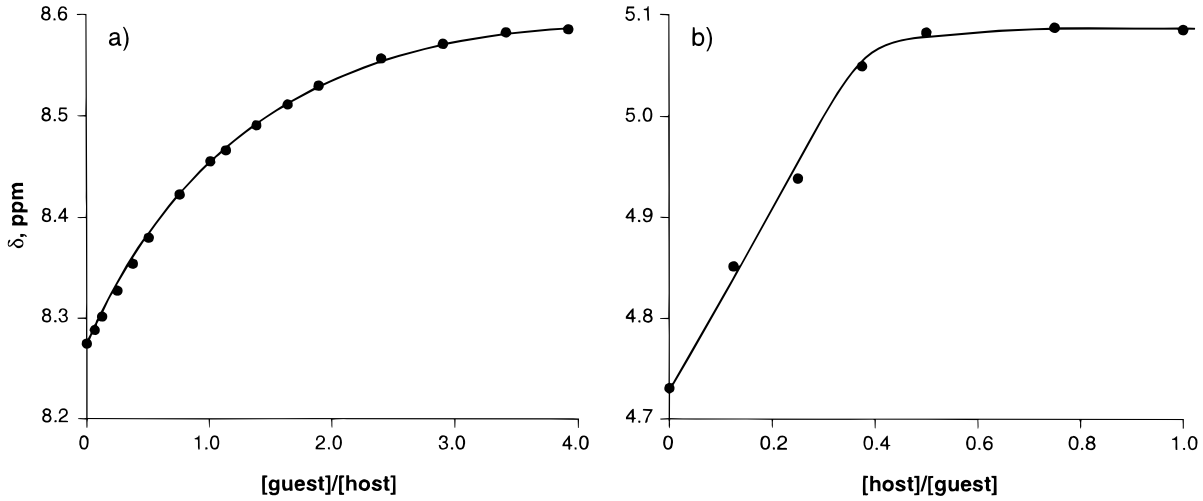
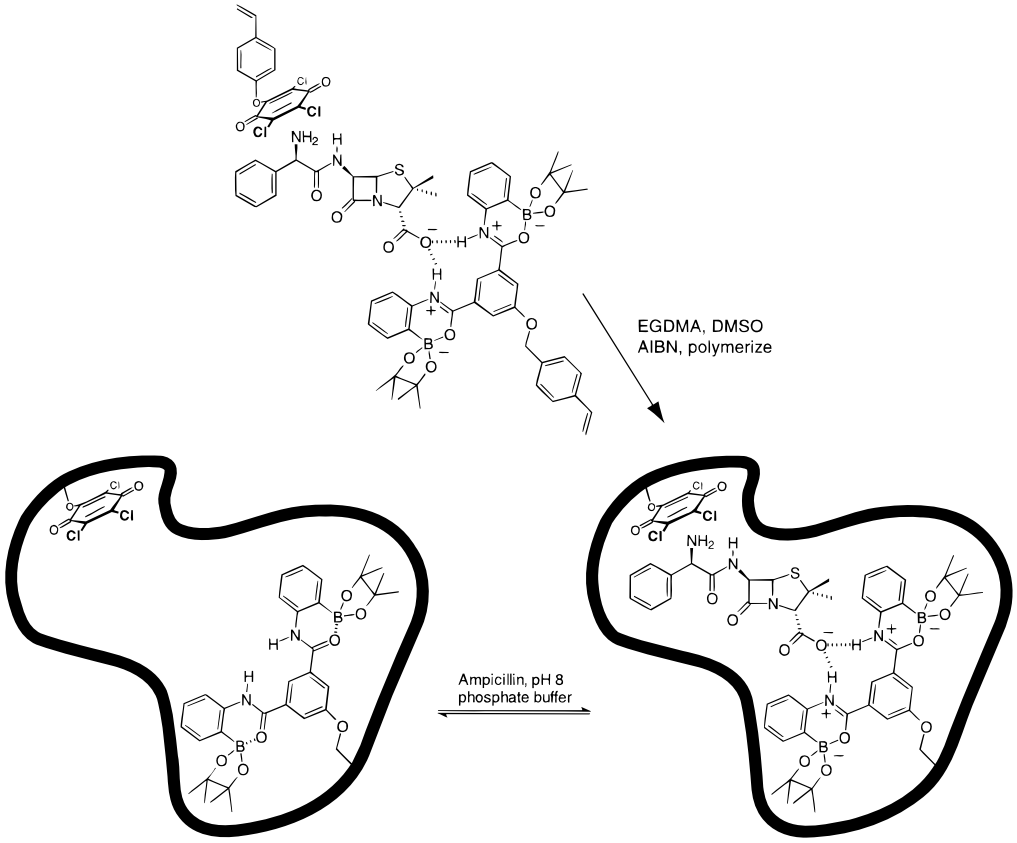


Figure 1. (a) NMR titration showing complexation between the receptor monomer **3** with tetrabutylammonium acetate in d_6-DMSO, obtained by following the chemical shift of the C2 proton of the host monomer **3** ($\delta_0 = 8.27$ ppm). (b) Titration of ampicillin with the quinone monomer **4** in d_6-DMSO showing the chemical shift of the proton geminal to the amino group ($\delta_0 = 4.73$ ppm).

Scheme 4. Schematic Representation of the Proposed Imprinting Mechanism of Ampicillin via a Stoichiometric Termolecular Complex with 3 and 4



imprinted polymers **P1** and **P2** was 1 mol %. Nonimprinted polymers **PN1** and **PN2** were prepared with the same monomers and solvents but in the absence of template. Control imprinted polymers **P3** and **P4** were also prepared using each of the functional monomers separately. In all cases only stoichiometric amounts, 1 mol %, of **3** and **4** were used.

Template removal was carried out by extraction of the polymer with acetonitrile. Under these conditions it is expected that the tetrabutylammonium counterion is removed from the polymer along with the ampicillin. Template removal was estimated from the UV absorbance at 203 nm of the water-soluble fraction from the polymer washings after removal of the solvent. This spectral feature is due to the aromatic ring of ampicillin and should be present, even if (as expected) some template decomposition had occurred. The same extracts from the nonimprinted polymer showed a very low absorbance at this frequency, confirming that the band was indeed due to the presence of the template. On this basis the removal of template from **P1** was estimated to be 92% (Table 1). The surface area of **P1**, measured by nitrogen porosimetry, was 338 m^2/g, while that of the corresponding nonimprinted polymer **PN1** was 55 m^2/g. This discrepancy may be due to the strong complex formed between template and monomers which

Table 1. Composition and Characterization of Imprinted and Nonimprinted Polymers

polymer[a]	template	porogen	functional monomers[b]	BET surf. area, m^2/g	template removal, %	binding parameters for ampicillin	
						K_d, μM	capacity, μmol/g
P1[37]	ampicillin$^-$Bu$_4$N$^+$	DMSO	**3**, **4**	338	92	30 ± 3	5.8 ± 0.3
						960 ± 110	42 ± 3.5
PN1		DMSO	**3**, **4**	55			
P2	ampicillin$^-$Bu$_4$N$^+$	THF	**3**, **4**	375	ND	ND	ND
PN2		THF	**3**, **4**	<5			
P3	ampicillin$^-$Bu$_4$N$^+$	DMSO	**3**	519	74	3000 ± 900	82 ± 17
P4	ampicillin$^-$Bu$_4$N$^+$	DMSO	**4**	345	60	110 ± 8	12 ± 0.5
						1200 ± 506	60 ± 17

[a] Polymerization mixtures contained 98 mol % (**P1/PN1** and **P2/PN2**) or 99 mol % (**P3** and **P4**) of EGDMA. [b] Both functional monomers were used at the level of 1 mol %, equimolar with template. ND = not determined.

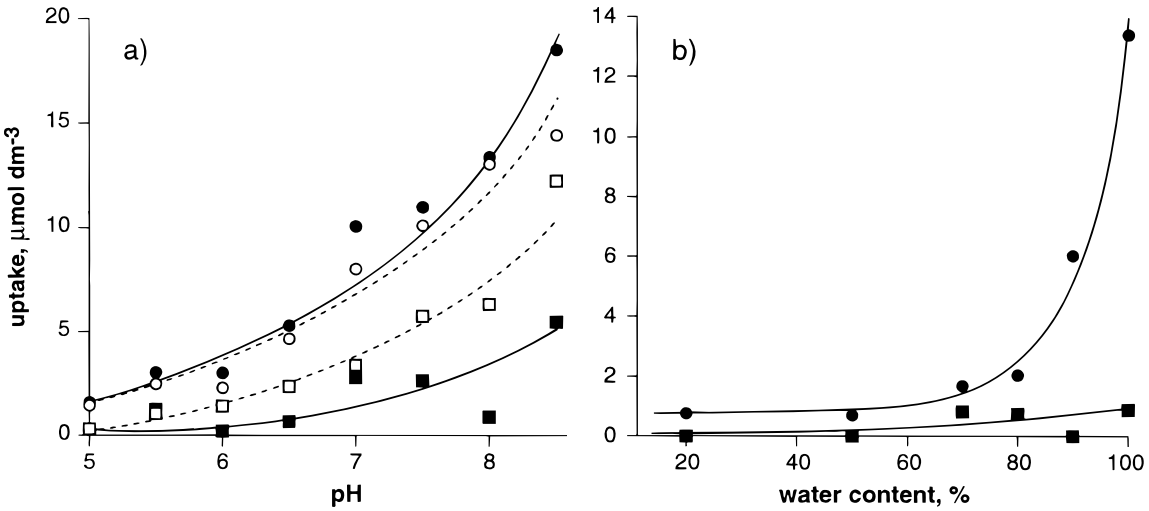


Figure 2. Uptake of ampicillin by imprinted polymers. (a) From aqueous buffer over the pH range 5.0−8.5 for **P1** (closed circles), **P2** (open circles), **PN1** (closed squares), and **PN2** (open squares). (b) As a function of acetonitrile content at pH 8.0 for **P1** (closed circles) and **PN1** (closed squares).

acts as an additional cross-linker. Such behavior is more commonly seen in covalently imprinted materials where the presence of the template inevitably influences the polymerization reaction, and marked differences in porosity between imprinted materials and controls can be seen.[12]

Binding Studies. The binding characteristics of the polymers were initially determined by batch adsorption experiments with solutions of ampicillin in aqueous buffer at a range of pH. Binding was determined after 24 h by HPLC analysis (with UV detection) of the supernatant after filtration of the polymer particles from the suspensions, and the results are shown in Figure 2a. Binding to all polymers shows a near exponential rise with increasing pH. This study also showed that **P1** and **P2** have a remarkably similar affinity for **1** over the whole pH range investigated; however, the binding to the nonimprinted polymer was rather high in the case of **PN2**, prepared in THF. This material was also effectively nonporous and therefore not a suitable control (Table 1), and so the following discussions will focus solely on the polymers prepared in DMSO, **P1** and **PN1**.

Uptake of **1** by **P1** increased as the pH was raised (Figure 2a). The binding to **PN1** remained relatively low until pH 8.5, when a significant increase in the nonspecific binding was seen. At higher pH ampicillin is rapidly destroyed by hydrolysis. This established an optimal pH for binding of 8.0. Binding at slightly alkaline pH can be explained by the fact that the fully deprotonated form of the template was used in the preparation of imprinted polymer; therefore, it is reasonable to expect that ampicillin must be present in this form to rebind to the polymer.[31]

Having established an optimum pH of 8.0, binding of **1** was determined in various acetonitrile/aqueous buffer mixtures (Figure 2b). Clearly solvent effects are very important, as shown by the substantial reduction in binding on the addition of 20% acetonitrile (Figure 2b). Similar behavior has been reported for acrylamide- and methacrylic acid-based imprinted polymers,[5,32] where templating is due primarily to hydrogen bonding in relatively nonpolar organic solvents; the explanation normally proffered is that hydrogen bonding is favored by nonpolar solvents and a "hydrophobic" mechanism operates in water, but neither is particularly favored by aqueous−organic mixtures. However, it is unlikely that all of the loss of binding energy shown by our materials in mixed solvents is due to the destabilization of the functional group interactions since both complexes (of amine with **4** and carboxylate with **3**) can be characterized in acetonitrile (or acetonitrile/water mixtures in the case of **4**) as well as in DMSO.

To further probe the recognition properties of the polymers, binding studies were undertaken with a number of structural analogues of ampicillin. Each analogue was chosen on the basis of common structural features and possession of a free amino and carboxyl group. The two constituent parts of ampicillin, namely D-phenylglycine (**6**) and 6-aminopenicillanic acid (**7**), were selected, as was the dipeptide (**8**) and the analogous cephalosporin antibiotic, cephalexin (**9**).[33] All binding experiments were performed under the same conditions (50 μM ligand concentration, 50 mM phosphate

Table 2. Binding of Ampicillin and Structural Analogues to Imprinted Polymer P1 and Nonimprinted Polymer PN1

	uptake, %[a]	
ligand	P1	PN1
ampicillin (**1**)	26.8	1.8
D-phenylglycine (**6**)	12.4	6.4
6-aminopenicillanic acid (**7**)	5.2	0.8
D-phenylglycyl-L-leucine (**8**)	15.1	3.7
cephalexin (**9**)	15.1	<0.5

[a] From 50 μM solutions in 50 mM aqueous phosphate buffer, pH 8.0, with 5 mg/mL polymer at 20 °C.

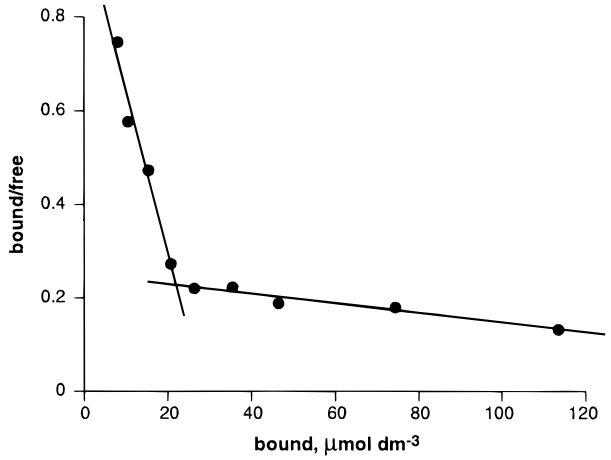


Figure 3. Scatchard plot for ampicillin binding to **P1**, corrected for nonspecific binding.

buffer, pH 8.0), and the results are presented in Table 2. All ligands were less strongly bound to the polymer than **1**, with **7** showing the lowest affinity. D-Phenylglycine (**6**) showed moderate binding of 12.4%, but about half this amount was also bound to **PN1**. The dipeptide (**8**) and cephalexin (**9**), the closest structural analogue of **1**, both showed the highest uptake of any of the compounds tested, but the figure of 15.1% uptake is approximately half (56%) of that of ampicillin. In the case of the peptide, however, a higher uptake by the nonimprinted polymer was seen. Significant interaction with **PN1** is probably due to the relatively hydrophobic nature of ligands **6** and **8**.

Finally, the equilibrium binding of **1** to polymer **P1** was determined at 20 °C over a range of ampicillin concentration from 20 μM to 1 mM, and a Scatchard graph[34] (Figure 3) was constructed in order to estimate the values of binding constants and site densities. As has previously been observed with imprinted polymers prepared by the noncovalent method,[35,36] the Scatchard plot was biphasic, implying the presence of two distinct populations of binding sites: the first with $K_d = (3.0 \pm 0.3) \times 10^{-5}$ mol/L and capacity of 5.8 ± 0.3 μmol/g and the second characterized by the values of $K_d = (9.6 \pm 1.1) \times 10^{-4}$ mol/L and 42 ± 3.4 μmol/g (Table 1).[37] The template loading of **P1** is 49 μmol/g, which is in fairly good agreement with the measured capacities.[38] Evidence that the two functional monomers are both contributing to recognition is given by the Scatchard analyses of polymers **P3** and **P4**. Binding to these polymers was uncorrected as nonimprinted controls were not prepared at these compositions. In the case of imprinted polymer **P3**, bearing the carboxylate receptor monomer **3** only, the value of K_d was determined to be 3.0 ± 0.9 mM, similar to the value determined for **3** binding to carboxylates in DMSO, although the high capacity of this polymer suggests much of the binding is nonspecific in nature. Polymer **P4**, prepared by imprinting with only the quinone monomer **4**, is much more interesting. This also shows a biphasic Scatchard plot with the high affinity sites being characterized by a K_d of $(1.1 \pm 0.1) \times 10^{-4}$ mol/L at a capacity of 12 ± 0.5 μmol/g, which may imply that binding in some sites is due to two interactions with **4**. Given that **4** has two potential binding sites and that there may be other donor sites within the template (e.g., aromatic ring, carbonyl oxygen), this may be possible. The potential of this monomer for imprinting templates with a range of structural features is currently being investigated in our laboratory.

Binding parameters for other polymers in purely aqueous media have been reported for morphine,[39] yohimbine,[40] and *S*-propranolol,[32] all relatively hydrophobic templates, imprinted by various noncovalent methods. Among these, the lowest dissociation constants and highest overall capacity was observed with an *S*-propranolol-imprinted polymer[32] ($K_d = 4.0$ nM, $C_{ap} = 0.63$ μmol/g; $K_d = 4.1$ μM, $C_{ap} = 28$ μmol/g). However it must be stressed that ampicillin (log $P = -2.68$[41]) is a markedly more hydrophilic ligand than propranolol (log $P = 0.73$[42]). In this context the measured capacity of 5.8 μmol/g for the "good" sites is quite respectable, although it falls somewhat short of the estimated capacity of around 30 μmol/g (calculated on the basis on the affinities of the individual monomers for the template and the template removal). It seems likely therefore that some disruption of complexes occurs on polymerization even when high affinity monomers are used. It is not possible with the present system to investigate the effect of preparing the polymer by photochemical initiation at low temperature, which may improve recognition, because **4** is strongly absorbing in the UV/vis spectrum. Clearly there is great potential to further improve the current system and to extend the preparation of stoichiometrically imprinted polymers to a wide range of hydrophilic templates of biological importance. Aspects of this work are currently being addressed in our laboratory.

Experimental Section

Materials and Methods. 5-Hydroxydimethylphthalate, benzeneboronic acid, pinacol, *p*-chloranil, tetrabutylammonium hydroxide (1 M in methanol), oxalyl chloride, D-phenylglycine, and 6-aminopenicillanic acid were obtained from Aldrich Chemical Co. (Dorset, UK); 4-vinylbenzyl chloride was purchased from Acros Organics (Loughborough, UK); ampicillin and cephalexin were supplied by Fluka (Dorset, UK); and D-phenylglycyl-L-leucine was purchased from Bachem (Saffron Waldon, UK). FT-NMR spectra were obtained on a JEOL EX-270 at either 270 MHz (^{1}H) or 67.9 MHz (^{13}C) using deuterium solvent lock. NMR peak assignments, where given, are based on DEPT and/or COSY spectra and published data. FT-IR spectra were recorded on a Perkin-Elmer series 1600 by diffuse reflectance from samples dispersed in KBr. UV/vis spectra were recorded on a Perkin-Elmer Lambda 15 spectrophotometer. Polymers were ground to an average particle size of approximately 30 μm in a Fritsch Pulverisette grinding mill, equipped with an agate mortar.

HPLC Conditions. Analyses were performed using Gilson 305/306 pumps fitted with a UV detector (detection at 192 nm) and autosampler (Milton Roy) with a 20 μL injection loop. Ampicillin and cephalexin were analyzed by reversed phase chromatography on a 250 × 4.6 mm RP-8, deactivated column (Shandon, 5 μm Hypersil BDS) by isocratic elution with 10% acetonitrile, 90% 10 mM KH_2PO_4, pH 5.2, 1 mL/min. 6-Aminopenicillanic acid was analyzed on the same column by elution

with 100% 10 mM KH_2PO_4 at 1 mL/min. D-Phenylglycine was analyzed using the RP-8 column (as above) in series with a 100 × 4.6 mm Spherisorb RP-6 column (Phase Sep S5 C6) eluting with 100% 10 mM KH_2PO_4 at 0.75 mL/min. d-Phenylglycyl-L-leucine was analyzed on a 250 × 4.6 mm, 5 μm RP-18 (Hichrom Ltd., ODS2) column by isocratic elution with 40% acetonitrile, 60% water (containing 0.1% trifluoroacetic acid). Retention times were as follows: ampicillin, 7.2 min; cephalexin, 7.8 min; 6-aminopenicillanic acid, 11.0 min; D-phenylglycine, 8.6 min; D-phenylglycyl-L-leucine, 6.2 min.

5-(4-Vinylbenzyloxy)isophthalic Acid. 5-Hydroxydimethylisophthalate (25.25 g, 0.12 mol), 4-vinylbenzyl chloride (18.3 g, 0. 12 mol), K_2CO_3 (17 g), and KI (trace) in DMF (100 mL) was heated to 90−100 °C for 1 h. The cooled reaction mixture was added to water (500 mL) and extracted with diethyl ether (3 × 150 mL). The combined organics were washed with KOH solution and water before being dried ($MgSO_4$), evaporated, and finally dried under high vacuum. The crude residue was crystallized from hexane to give 5-(4-vinylbenzyloxy)dimethylisophthalate as colorless crystals in 60% yield; mp 71−74 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) 3.94 (s, 6H, CO_2CH_3), 5.13 (s, 2H, $-CH_2O-$), 5.27 (dd, 1H, $J_{cis} = 10.9$ Hz, $J_{gem} = 0.7$ Hz, *cis*-CH=CH_2), 5.77 (dd, 1H $J_{trans} \approx 17.6$ Hz, $J_{gem} = 0.7$ Hz, *trans*-CH=CH_2), 6.73 (dd, 1H $J_{trans} \approx 17.6$ Hz, $J_{cis} = 10.9$ Hz, $-CH=CH_2$), 7.40, 7.45 (aromatic AB system, 4H, $J_{A,B} = 8.6$ Hz), 7.83, (d, 2H, $J \approx 1.4$ Hz, H4, H6), 8.29 (t, 1H, $J \approx 1.4$ Hz, H2). ^{13}C NMR ($CDCl_3$): δ (ppm) 52.4 (p, CO_2CH_3), 70.14 (s, $-CH_2O-$), 114.27 (s, $-CH=CH_2$), 120.09, 123.16, 216.43, 127.73 (4 × t), 131.73, 135.45 (2 × q), 136.26 (t, $-CH=CH_2$), 137.52, 158.65 (2 × q), 166.04 (C=O). The dimethyl ester (5 g, 15.3 mmol) was heated with a solution of NaOH in aqueous methanol for 30 min. The cooled solution was diluted with water and extracted with diethyl ether. The aqueous portion was acidified with HCl and the resultant precipitate collected by filtration and washed with water. Recrystallization twice from ethanol gave the title compound as colorless crystals; yield 62%, mp 240 °C, decomposition. FT-IR (KBr): 2870 (broad, −OH), 1698 (carbonyl), 1595 (aryl), cm^{-1}. ^{1}H NMR (d_6-DMSO): δ (ppm) 5.22 (s, 2H, $-CH_2O-$), 5.25 (d, 1H, $J_{cis} = 11$ Hz, *cis*-CH=CH_2), 5.84 (d, 1H $J_{trans} = 18$ Hz, *trans*-CH=CH_2), 6.73 (dd, 1H $J_{trans} = 18$ Hz, $J_{cis} = 11$ Hz, $-CH=CH_2$), 7.43, 7.49 (aromatic AB system, 4H, $J_{A,B} = 8$ Hz), 7.72, (s, 2H, H4, H6), 8.07 (s, 1H, H2), 13.32 (broad s, 2H, $-CO_2H$). ^{13}C NMR (d_6-DMSO): δ (ppm) 69.40 (s, -CH_2O-), 114.58 (s, $-CH=CH_2$), 119.50, 122.46, 126.27, 127.91(4 × t), 132.63, 136.16 (2 × q) 136.26 (t, $-CH=CH_2$), 136.80, 158.42 (2 × q), 166.38 (C=O).

(2-Nitrophenyl)boronic Acid. This compound was prepared by nitration of benzeneboronic acid, according to the published procedures.[43,44]

(2-Aminophenyl)boronic Acid. This material was prepared from the nitro compound by catalytic transfer hydrogenenation.[45] (2-Nitrophenyl)boronic acid (4.94 g, 29.6 mmol) was dissolved in 50 mL of methanol and stirred at room temperature under a blanket of nitrogen. The hydrogenation catalyst, 10% palladium on carbon (2.5 g), was suspended in this solution. Ammonium formate (11.20 g, 177.5 mmol) was added in small portions over a period of 1.5 h. After the addition was complete, the reaction mixture was gently refluxed at 55−60 °C for 2.5 h. The cooled reaction mixture was filtered through Celite to remove the catalyst. The solvent was removed from the yellow filtrate to yield a dark yellow oil which solidified on standing. The residue was triturated with 30 mL of ethyl acetate, filtered, and washed with two further portions of ethyl acetate to obtain the product, 0.95 g, in 23% yield; analysis results were in agreement with the published data[24,44] for (2-aminophenyl)boronic acid.

2-(3′,3′,4′,4′-Tetramethyl-2′,5′-dioxaborolanyl)benzenamine. This compound was prepared according to the procedure of Hughes and Smith.[25]

5-(4‴-Vinyl)benzyloxy-1,3-bis[2′-(3″,3″,4″,4″-tetramethyl-2″,5″-dioxaborolanyl)phenylcarbomoyl]benzene (3). 5-(4-Vinylbenzyloxy)isophthalic acid was converted to the corresponding acid chloride with oxalyl chloride. This product (1.0 g, 3.0 mmol) was reacted with 2-(3′,3′,4′,4′-tetramethyl-2′,5′-dioxaborolanyl)benzenamine (1.34 g, 6.1 mmol) according to the method of Hughes and Smith.[25] After mixing the reactants the mixture was stirred for 5 h at −10 °C and then left at room temperature overnight. The crude product, obtained as an oil after evaporation of the reaction solvent, was dissolved in ethyl acetate (20 mL) and washed with 10 mL of saturated brine. On mixing the two phases, a precipitate formed which was collected and extracted with ethyl acetate; the two layers were separated. The combined ethyl acetate fractions were dried over $MgSO_4$ and evaporated. The residue was taken up in 16 mL of ethyl acetate and stirred during the dropwise addition of 50 mL of *n*-hexane. The precipitate which formed was discarded and the solution concentrated in vacuo. Dropwise addition of further *n*-hexane, with stirring, produced a precipitate which was collected and dried over P_2O_5 in a desiccator to yield 0.65 g, 31% of the product as a pale yellow powder; mp 80 °C decomposition. FT-IR (KBr): 3364 (N−H), 2979 (C−H), 1682 (amide carbonyl), 1615 (C=C), 1581 (aryl), 1539 (amide carbonyl) cm^{-1}. ^{1}H NMR (d_6-DMSO): δ (ppm) 1.26 (s, 24H, $-CH_3$), 5.29 (d, lH, $J_{cis} = 11$ Hz, *cis*-CH=CH_2), 5.34 (s, 2H, $-CH_2O-$), 5.86 (d, 1H $J_{trans} = 18$ Hz, *trans*-CH=CH_2), 6.76 (dd, 1H $J_{trans} = 18$ Hz, $J_{cis} = 11$ Hz, $-CH=CH_2$), 7.21 (m, 2H, H5′), 7.43 (m, 2H, H4′), 7.47−7.59 (m, 6H, H3′, H2′, H6‴, H3‴, H5‴), 7.75 (d, 2H, $J = 8$ Hz, H6′), 7.97 (s, 2H, H4, H6), 8.28 (s, 1H, H2), 11.42 (broad s, 2H, $-NH$). ^{13}C NMR (d_6-DMSO): δ (ppm) 25.35 (p, $-CH_3$), 69.77 (s, $-CH_2O-$), 81.63 (q, C3″, C4″), 114.57 (s, $-CH=CH_2$), 117.75 (t, C4, C6), 118.27 (t, C3′), 119.46 (t, C2), 124.94 (t, C5′), 126.23, 127.85 (2 × t, C2‴, C6‴, C3‴, C5‴), 129.59 (t, C4′), 133.71 (t, C6′), 134.27, 135.67 (2 × q, C1, C3, C4‴), 136.12 (t, $-CH=CH_2$), 136.87 (q, C1‴), 140.38 (q, C1′), 158.56 (q, C5), 163.91 (C=O). EI-HRMS calcd for $C_{41}H_{46}O_7N_2B_2$, 700.3491; found, 700.3486 *m*/*z*.

2-(4-Vinylphenyloxy)-3,5,6-trichlorobenzoquinone (4). Tetrachloro-1,4-benzoquinone (*p*-chloranil, 2.95 g, 12 mmol) was dissolved in 80 mL of anhydrous THF in a reaction vessel under a blanket of nitrogen gas. A solution containing 4-vinylphenol[46] (1.44 g, 12 mmol) and 12 mmol of anhydrous pyridine in 10 mL of anhydrous THF was added dropwise with stirring. The reaction mixture was stirred overnight before being poured onto 200 mL of water and 200 mL of dichloromethane. The phases were separated, and the organic layer was washed twice with water, dried over $MgSO_4$, and evaporated to dryness. The crude product was chromatographed twice on silica gel with hexane/dichloromethane (70:30) to yield dark red crystals; 0.58 g (15%), mp 177−178 °C. FT-IR (KBr): 3340 (quinone C=C), 3038 (aromatic C−H), 1677 (carbonyl), 1201 (C−O) cm^{-1}. ^{1}H NMR (d_6-DMSO): δ (ppm) 5.21 (d, 1H, $J_{cis} = 11$ Hz, *cis*-CH=CH_2), 5.76 (d, 1H, $J_{trans} = 18$ Hz, *trans*-CH=CH_2), 6.70 (dd, 1H, $J_{trans} = 18$ Hz, $= 11$ Hz, $-CH=CH_2$), 7.15, 7.45 (aromatic AB system, 4H, $J_{A,B} = 9$ Hz). ^{13}C NMR (d_6-DMSO): δ (ppm) 113.8 (s, C8′), 116.2 (t, C2′, C6′), 127.6 (t, C3′, C5′), 131.0 (q, C3), 133.0 (q, C4′), 135.6 (t, C7′), 138.2 (q, C6), 139.5 (q, C5), 150.2 (q, C1′), 155.6 (q, C2), 171.4, 171.7 (2 × C=O, C1, C4). EI-HRMS calcd for $C_{14}H_7O_3Cl_3$, 327.9461; found, 327.9464 *m*/*z*.

Determination of Monomer/Template Interactions by NMR Titration: Method 1. A stock solution of guest compound in d_6-DMSO was divided into a number of NMR tubes. Aliquots of host stock solution in d_6-DMSO were added to each NMR tube in increasing amounts. The concentration of guest compound was kept constant by addition of the appropriate amounts of solvent to a total volume of 700 μL. The migration of the guest peak chemical shifts were followed by ^{1}H NMR measurements (at 25 °C, with TMS as standard).

Ampicillin with 4. In this case, 500 μL of a 28 mM solution of **1** (guest) was added to each of seven NMR tubes and titrated at intervals with a 70 mM solution of **4** (host) by additions as follows (in μL): 0, 25, 50, 75, 100, 150, 200. This provided a range of host:guest ratios from 0 to 1 equiv. The NMR measurements were carried out after each tube had been standing for 6 h at 19 °C. The change in the chemical shift of the proton geminal to the amino group of ampicillin ($\delta_0 = 4.73$ ppm) was used to estimate the binding constant.

Ampicillin Carboxylate with 3. Guest stock solution (250 μL of 28 mM ampicillin tetrabutylammonium salt) was added

to eight NMR tubes and titrated with a 31.1 mM solution of **3** (host) as follows (in μL): 0, 50, 110, 145, 190, 225, 330, 450. The obtained range of host:guest ratios was from 0 to 2 equiv. The binding was estimated from the shift of the signal due to the proton geminal to the carboxylate group of the ampicillin moiety ($\delta_0 = 4.12$ ppm).

Determination of Monomer/Template Interactions by NMR Titration: Method 2. A stock solution of host **3** (800 μL, 10 mM in d_6-DMSO + TMS) was added to a 5 mm NMR tube. Aliquots of a guest stock solution (50.6 mM tetrabutylammonium acetate in d_6-DMSO) were added stepwise to the NMR tube such that the total amount of guest solution varied as follows (in μL): 0, 10, 20, 40, 60, 80, 120, 160, 180, 220, 260, 300, 380, 460, 540, 620. This provided a range of guest: host ratios from 0 to 3.9 equiv. Care was taken to avoid water absorption from the atmosphere. The migration of the host peak chemical shift due to the C2 aromatic proton of **3** ($\delta_0 =$ 8.27 ppm) was followed by ^{1}H NMR measurement at 25 °C immediately after each addition. The obtained titration curve was fitted to a 1:1 binding model. A similar measurement was also made in d_3-acetonitrile, following the same host proton signal ($\delta_0 = 8.18$ ppm).

Polymer Syntheses. Ampicillin was converted to the tetrabutylammonium salt for incorporation into imprinted polymers. Ampicillin (34.5 mg, 98.7 μmol) was placed in a 10 mL pear-shaped flask, and 200 μL of anhydrous methanol was added. One equivalent, 98.7 μL, of tetrabutylammonium hydroxide (1 M solution in methanol) was added to obtain a clear solution of the salt. Methanol was removed by rotary evaporation, and the residue dried for several hours on a high-vacuum line at room temperature. The template was dissolved in the polymerization solvent (THF or DMSO, 2 mL), and 1 mL of this solution was transferred to a test tube fitted with a ground glass joint. The quinone monomer **4** (16.1 mg, 48.8 μmol) was added and the tube stoppered and stirred overnight. The boronate monomer **3** (34.2 mg, 48.8 μmol), ethylene glycol dimethacrylate (950 mg), and AIBN (15.9 mg, 1 mol % with respect to double bonds) were added. The tube was sealed with a vacuum stopcock and degassed on a vacuum line by a sequence of freeze−pump−thaw cycles before placing in a water bath at 65 °C for 24 h. Polymer was obtained by breaking apart the solid mass formed at the bottom of the tube with a spatula and washing the fragments onto a sintered glass filter with acetonitrile. The air-dried pieces were ground and extracted for 12 h in a Soxhlet apparatus with further acetonitrile and dried in a vacuum oven at 80 °C. Nonimprinted polymer was prepared by mixing monomers, solvent, and initiator in the same proportions, but without template. Polymers were also prepared in the presence of template but omitting one of the functional monomers from the polymerization mixture. Template removal from imprinted polymers was estimated from the UV absorbance at 203 nm[47] of the aqueous-soluble fraction from the combined washings from each polymer.

Binding Studies. Polymer (5 mg) was weighed into 2 mL capacity screw-top vials, and 1 mL of ligand solution (50 μM, in 50 mM phosphate buffer, pH 8.0) was added. The vials were closed and shaken overnight at 20 °C to be certain of attaining equilibrium. Polymer suspensions were filtered through 13 mm diameter, 0.4 μm porosity, PTFE membrane filters attached to the barrel of a disposable 5 mL syringe into sample vials which were sealed with septum caps. The concentration of ligand in the supernatant was determined by HPLC analysis. Each uptake experiment was performed in duplicate.

pH Dependence. The dependence of binding on pH was determined as described above except that the pH's of stock solutions of ampicillin in 50 mM KH_2PO_4 solution were adjusted by the addition of NaOH solution before pipetting onto the polymer. Individual HPLC calibration curves were determined for each pH.

Acetonitrile:Water Mixtures. The dependence of binding on solvent composition was determined as above except samples were prepared by dilution of a 500 μM stock solution with the calculated quantities of water and acetonitrile such that the concentration of ampicillin was 50 μM, buffer was 50 mM, and the pH was 8.0. Each aqueous solvent mixture was individually calibrated by HPLC.

Determination of Polymer Binding Characteristics by the Scatchard Method. The binding of ampicillin at 20 °C in 50 mM phosphate buffer to polymers **P1**, **PN1**, **P3**, and **P4** was determined at 20, 30, 50, 100, 150, 200, 300, 500, and 1000 μM concentrations of ligand in duplicate experiments, as described above. The free ligand concentration was determined by HPLC and the bound ampicillin calculated by subtraction. For the polymer **P1** the bound concentrations were corrected for nonspecific adsorption by subtracting the amount bound to the nonimprinted polymer **PN1** at the same free ligand concentration (obtained by interpolation). The binding parameters were obtained by curve-fitting the binding isotherms for each section of the Scatchard plot using a one-site binding model (using the Grafit package, Erithacus Software). In the case of **P3** and **P4** the data were uncorrected.

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