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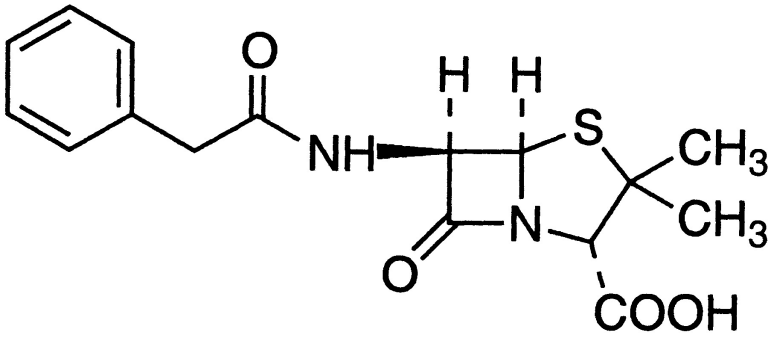


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# Synthesis and Screening of a Molecularly Imprinted Polymer Library Targeted for Penicillin G

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A library of molecularly imprinted polymers (MIPs) was synthesized by radical bulk polymerization using the $\beta$-lactam antibiotic penicillin G as the template. Diversity of the library was obtained by combining various functionalized monomers and cross-linkers and by varying the stoichiometry and the concentration of the components in the prepolymerization mixtures. The library was screened for selectivity to penicillin G by a radioligand binding assay and was compared to a corresponding control library. The best MIP candidate, showing the highest selectivity for penicillin G, was prepared from methacrylic acid and trimethylolpropane trimethacrylate as the functionalized monomer and cross-linker, respectively. Cross-reactivity studies with other $\beta$-lactam antibiotics showed a low cross-reactivity of penicillin V (15%), ampicillin (16%), and amoxicillin (19%). Nafcillin and oxacillin showed less cross-reactivity (<1%). Cross-reaction with a cephalosporin antibiotic (cephapirin) and structurally nonrelated antibiotics (chloramphenicol, tetracycline, dapsone, and erythromycin) was less than 0.01%.

## Introduction

Molecular imprinting is attracting a wide interest as a viable method for the production of nanostructured materials capable of molecular recognition. MIPs[1] have found application as stationary phases for chiral separations and solid-phase extractions, as in vitro antibody mimics, as recognition elements in sensors, and as catalysts of chemical reactions.[2−13] The recognition sites are tailor-made in situ by self-assembly of functionalized monomers and templates followed by copolymerization with cross-linkers to form a polymer network. The templates are subsequently extracted from the imprinted polymer, leaving recognition sites complementary in the positioning of functional groups and in shape. The sites recognize and rebind the template molecule upon reexposure.

In this paper, the preparation and screening of a MIP library targeted against penicillin G (**7**) is described. Antibiotics previously imprinted and reported include penicillin V, oxacillin, ampicillin, chloramphenicol, cyclosporin A, erythromycin A, and oleandomycin.[14−18] Since the introduction of penicillin in 1941, antibiotics have been used effectively to fight infectious diseases, both in humans and in animals. In veterinary medicine, antibiotics are administered for therapeutic and prophylactic treatment of mastitis and respiratory and enteric diseases.[19] The compounds have also been applied as food additives for growth promotion of livestock.[20] A risk with widespread use of antibiotics is the development of antibiotic resistant bacterial strains.[21−24] Such strains might pose a threat to public health, and concerns have been expressed that our future ability to fight infectious diseases will be jeopardized with continued unrestricted use of antibiotics. Other problems associated with the presence of antibiotic residues in meat and milk relate to the risk of allergic reactions in hypersensitive individuals and inhibition of starter cultures in the dairy industry.[25]

In an effort to address the problems associated with antibiotic residues in food, legislative authorities have prohibited or restricted the use of antibiotic growth promoters and the prophylactic use of certain antibiotics in some countries.[26] Maximum permitted levels of antibiotics (MRLs) have been defined by the European Commission and the FDA. To be able to control the observance of prohibitions and restrictions, efficient methods for the detection of antibiotics in foodstuff are needed. Current methods for their analysis include microbial inhibition tests, enzymatic assays, enzyme-linked immunoassays, and various chromatographic methods.[27−32] Several of the tests used for routine screening of milk samples cannot discriminate between different $\beta$-lactam antibiotics. The aim of the present paper was to develop synthetic recognition elements selective for penicillin G.

## Results and Discussion

Only a few reports discussing the rational design of MIPs have appeared.[33−35] The majority of MIPs rather have been prepared by intuition and trial and error. Because a large number of parameters can be varied and optimized, a combinatorial library approach seemed to be a viable strategy in our search of optimal MIP materials selective for the target molecule, penicillin G (**7**). To speed up the process, a high-

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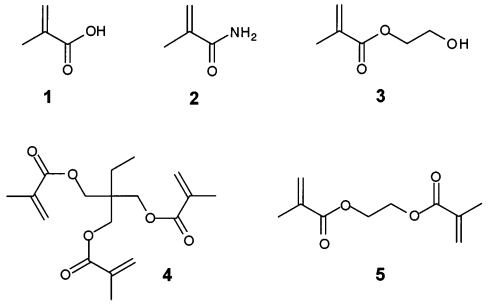
**Chart 1.** Structure of Functionalized Monomers and Cross-Linkers




throughput synthesis and screening method involving in situ synthesis of monolithic MIP libraries on the bottom of HPLC vials (often referred to as mini-MIPs) was applied initially.[36−38] This approach was, however, abandoned since our system proved to be unsuitable to automation due to solubility difficulties and instability of the template. Monolithic polymers, of a scale large enough to allow visual inspection during preparation and polymerization, were synthesized instead. After polymerization, the polymers were ground and fractionated by sieving. Particles of the size range 25−50 $\mu$m were collected and extracted to remove penicillin G used as the template. This large-scale approach was considerably more time- and labor-consuming than the small-scale high-throughput synthesis approach of mini-MIPs but in our hands turned out to be more reliable for the chosen target molecule.

Penicillin G was administered therapeutically as a salt, since the free acid (benzylpenicillinic acid) was unstable and was inactivated by moisture/small amounts of water.[39] For this reason, the sodium salt was used as the template in this study. Acetonitrile has previously been used successfully in noncovalent molecular imprinting of a broad range of templates and was the solvent of choice here. Penicillin G was not soluble in pure acetonitrile but could be dissolved in the presence of MAA (**1**) (Chart 1). Several other functionalized monomers [i.e., MAM (**2**), HEMA (**3**), 4-vinylpyridine, 2-(diethylamino)ethyl methacrylate, and (*ar*-vinylbenzyl)trimethylammonium chloride] were tested, but their presence did not increase the solubility of penicillin G in acetonitrile. MAA was therefore included in all of the MIPs of the library described in this study. MAA is by far the most widely used functionalized monomer in noncovalent molecular imprinting and has been applied successfully with a broad range of templates. The ratio between the components of the polymerization mixture and the order of their addition was critical for the solubility of penicillin G, and many combinations tested resulted in either precipitation during the self-assembly step or incomplete polymerization (detailed in the Experimental Section).

MAA was used either as the sole functionalized monomer or in combination with MAM (**2**) or HEMA (**3**). The functionalized monomers were copolymerized with either TRIM (**4**) or EDMA (**5**) as the cross-linking agent (Table 1).

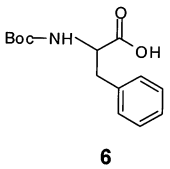
A control library was synthesized by substituting Boc-L-Phe-OH for penicillin G (**6**, Chart 2). In many reports, the CPs have been prepared without any template. We chose to include a nonrelated template to maintain, as far as possible, similar physical characteristics of the polymer network (e.g., surface area, porosity, distribution of functional groups). This was to ensure that the observed higher binding capacity of the MIPs relative to the CPs was due to a true imprinting effect and not caused by differences in intrinsic physical characteristics of the polymers.

**Table 1.** Composition of MIP and CP Libraries, $K_{MIP}$, and $K_{MIP}/K_{CP}$[a,b]

| | monomer and cross-linker (mmol) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MIP | MAA (**1**) | MAM (2) | HEMA (**3**) | TRIM (**4**) | EDMA (**5**) | mL of acetonitrile | $K_{MIP}$ | $K_{MIP}/K_{CP}$ |
| 1 | 8 | | | 8 | | 5.2 | 0.64 | 10.0 |
| 2 | 10 | | | 10 | | 7.5 | 0.49 | 9.4 |
| 3 | 10 | | | 15 | | 7.5 | 1.27 | 12.9 |
| 4 | 10 | | | 20 | | 7.5 | 0.75 | 7.6 |
| 5 | 10 | | | 30 | | 7.5 | 1.94 | 11.9 |
| 6 | 10 | | | 40 | | 8 | 1.86 | 35.3 |
| 7 | 10 | | | 50 | | 7.5 | 1.78 | 27.9 |
| 8 | 16 | | | 16 | | 6.1 | 0.52 | 6.8 |
| 9 | 24 | | | 24 | | 6 | 0.56 | 13.5 |
| 10 | 24 | | | 24 | | 12 | 1.56 | 20.8 |
| 11 | 32 | | | 32 | | 12 | 0.54 | 12.9 |
| 12 | 32 | | | 32 | | 20 | 1.50 | 17.3 |
| 13 | 10 | | | | 60 | 10.2 | 2.03 | 18.3 |
| 14 | 14 | | | | 70 | 8 | 1.17 | 7.2 |
| 15 | 14 | | | | 70 | 10 | 1.56 | 6.7 |
| 16 | 14 | | | | 70 | 14 | 1.86 | 21.4 |
| 17 | 14 | | | | 70 | 18 | 0.35 | 11.4 |
| 18 | 10 | 2 | | 20 | | 10.7 | 0.23 | 3.11 |
| 19 | 10 | 4 | | 20 | | 10.7 | 0.23 | 2.11 |
| 20 | 10 | 6 | | 20 | | 10.7 | 0.64 | 8.49 |
| 21 | 10 | 6 | | 48 | | 33.6 | 0.96 | 7.04 |
| 22 | 10 | 8 | | 20 | | 10.7 | 0.20 | 1.37 |
| 23 | 10 | 10 | | 20 | | 10 | 0.19 | 1.17 |
| 24 | 10 | 10 | | 60 | | 30 | 0.92 | 9.33 |
| 25 | 10 | 10 | | 80 | | 40 | 0.49 | 3.99 |
| 26 | 10 | 2 | | | 100 | 30 | 0.08 | 0.87 |
| 27 | 10 | 4 | | | 100 | 30 | 0.15 | 1.99 |
| 28 | 10 | 6 | | | 100 | 30 | 0.10 | 0.80 |
| 29 | 10 | 8 | | | 100 | 30 | 0.12 | 1.64 |
| 30 | 10 | 10 | | | 40 | 20 | 0.49 | 3.99 |
| 31 | 10 | 10 | | | 80 | 24 | 0.15 | 3.59 |
| 32 | 10 | 10 | | | 120 | 35 | 0.49 | 2.79 |
| 33 | 10 | 11.6 | | | 100 | 30 | 0.14 | 1.81 |
| 34 | 10 | | 5 | 15 | | 7.2 | 0.23 | 3.7 |
| 35 | 10 | | 6 | 12.4 | | 5.7 | 0.32 | 6 |
| 36 | 10 | | 6 | 16 | | 7.7 | 0.39 | 6.1 |
| 37 | 10 | | 6 | 28 | | 13.6 | 0.69 | 13.2 |
| 38 | 10 | | 6 | 32 | | 15.5 | 0.59 | 7.8 |
| 39 | 10 | | 8 | 18 | | 8.7 | 0.32 | 10.2 |
| 40 | 10 | | 10 | 20 | | 10.5 | 0.67 | 4.1 |
| 41 | 10.4 | | 6.4 | 24 | | 11.6 | 0.64 | 8.5 |
| 42 | 11.6 | | 6 | 20 | | 9.6 | 0.64 | 4.3 |
| 43 | 10 | | 2 | | 36 | 10.2 | 0.45 | 7.0 |
| 44 | 10 | | 2 | | 48 | 13.6 | 0.41 | 6.4 |
| 45 | 10 | | 2 | | 50 | 7.1 | 0.52 | 6.8 |
| 46 | 10 | | 2 | | 60 | 17 | 0.56 | 6.5 |
| 47 | 11 | | 2 | | 72 | 20.4 | 0.54 | 7.15 |
| 48 | 11 | | 2 | | 84 | 23.8 | 0.47 | 5.41 |
| 49 | 14 | | 4 | | 90 | 25.5 | 0.61 | 8.1 |
| 50 | 14 | | 8 | | 110 | 31.2 | 0.47 | 5.4 |
| 51 | 14 | | 10 | | 120 | 33.9 | 0.45 | 6.0 |
| 52 | 14 | | 12 | | 130 | 36.8 | 0.43 | 4.9 |

[a] MIPs were prepared using 1 mmol of penicillin G; CPs were prepared with 1 mmol of Boc-L-Phe-OH. [b] The partition coefficients ($K_{MIP}$ and $K_{CP}$) were calculated as $K$ = amount bound/amount free.

**Chart 2.** Structure of Boc-Phe-OH




**6**

The binding of penicillin G to the MIP and the CP libraries was screened in a batch-wise radioligand binding assay with $^3$H-labeled penicillin G of a concentration in the range of the MRL of penicillin G in milk. The partition coefficients, $K_{MIP}$ and $K_{CP}$ (showing the partition of penicillin G between the polymer [MIP or CP] and the solvent), were calculated (Table 1). The relative partition coefficient ($K_{MIP}/K_{CP}$) was used to identify the best MIP candidate.

Within the sublibrary of MIPs prepared with MAA as the sole functionalized monomer (*MIP 1*−*17* in Table 1), *MIP 6* showed the highest selectivity. This MIP was prepared with TRIM as the cross-linker and molar ratios of template−MAA−TRIM of 1:10:40. TRIM has previously been shown to produce MIPs of high selectivity and load capacity.[40] The ratios of the components of the MIPs in that report were typically in a range comparable to those of *MIP 1* of the library described here. With MIPs imprinted with penicillin G, it was found that a higher degree of cross-linking and a lower amount of porogen were more favorable. The two studies can, however, not be directly compared since the nature of both the templates (amino acid derivatives/peptides vs pencillin G) and the porogens (chloroform vs acetonitrile) is different. It is known that polymers prepared using chloroform as the porogen in general have lower surface area and pore volume than those prepared in acetonitrile.[41] This can be the reason that lower amounts of acetonitrile were found to give MIPs of higher selectivity in the present study.

Among the MIPs prepared with both MAA and MAM as the functionalized monomers (*MIP 18*−*33* in Table 1), *MIP 24* showed the highest selective binding of penicillin G. The molar ratio of template−MAA−MAM−TRIM was 1:10:10:60. MAM has previously been used successfully as the sole functionalized monomer for the preparation of MIPs selective for amino acid derivatives and phenytoin.[42,43] Those studies concluded that MAM forms stronger hydrogen bonds than MAA with the template. In our case, however, we could not use MAM as the sole functionalized monomer since MAA was required to dissolve the template. The best *ter*-polymer prepared with MAA and HEMA as the functionalized monomers was *MIP 37*. It was prepared in molar ratios of 1:10:6:28 of template−MAA−HEMA−TRIM.

MAA may not only have a beneficial effect on the solubility of penicillin G in acetonitrile but can also be responsible for degrading the template due to its instability against acids. To ascertain that penicillin G was not degraded to any significant extent in the polymerization mixture and that intact molecules were imprinted, stability tests were carried out on some of the formulations. Mixtures of penicillin G, MAA, TRIM, and acetonitrile (in proportions corresponding to those in the MIPs) were incubated and analyzed by reversed-phase HPLC. In the test mixture corresponding to *MIP 11*, which is the MIP with the highest ratio of MAA:template, 5% of penicillin G had degraded after 1.5 h. Analysis of the test mixture corresponding to *MIP 9*, which had the highest ratio of MAA:acetonitrile, revealed that 6% had degraded after 4.5 h. During the polymerization, the mixtures started to gel immediately and the mixtures were not fluid at all after 15 min. It is reasonable to assume that the degradation is even slower in the solid phase than in the solution test mixtures and that the majority of molecules being imprinted is intact. *MIP 6*, which turned out to be the best penicillin G selective MIP candidate in the screening, was selected for competition studies with a number of compounds representing different classes of antibiotics. $EC_{50}$ values were determined in competitive binding experiments using various concentrations of nonlabeled (cold) antibiotics competing with a fixed concentration of radioactively labeled (hot) penicillin G. The cross-reactivity of each antibiotic, i.e., the ratio of the $EC_{50}$ value of penicillin G to that of the competitor, is shown in Table 2. The largest competition was seen with penicillin V (**8**, Chart 3), amoxicillin (**9**), and ampicillin (**10**), with cross-reactivities of 15, 19, and 16%, respectively. These compounds show structural similarities to penicillin G in that they are all $\beta$-lactam antibiotics and are derivatives of 6-aminopenicillanic acid (**13**). The cross-reactivity of 6-aminopenicillanic acid was 4%. Nafcillin (**11**) and oxacillin (**12**) also belong to the same class of antibiotics but show less cross-reaction (<1%). Cephapirin (**14**) is also a $\beta$-lactam antibiotic but differs from the others included in this study in that it belongs to the cephalosporin antibiotics and is derived from 7-aminocephalosporanic acid. No cross-reactivity could be detected with cephapirin within the concentration range studied. Antibiotics structurally nonrelated to penicillin G were also investigated. These included chloramphenicol (**15**), tetracycline (**16**), dapsone (**17**), and erythromycin (**18**). None of these showed any cross-reactivity within the concentration range studied.

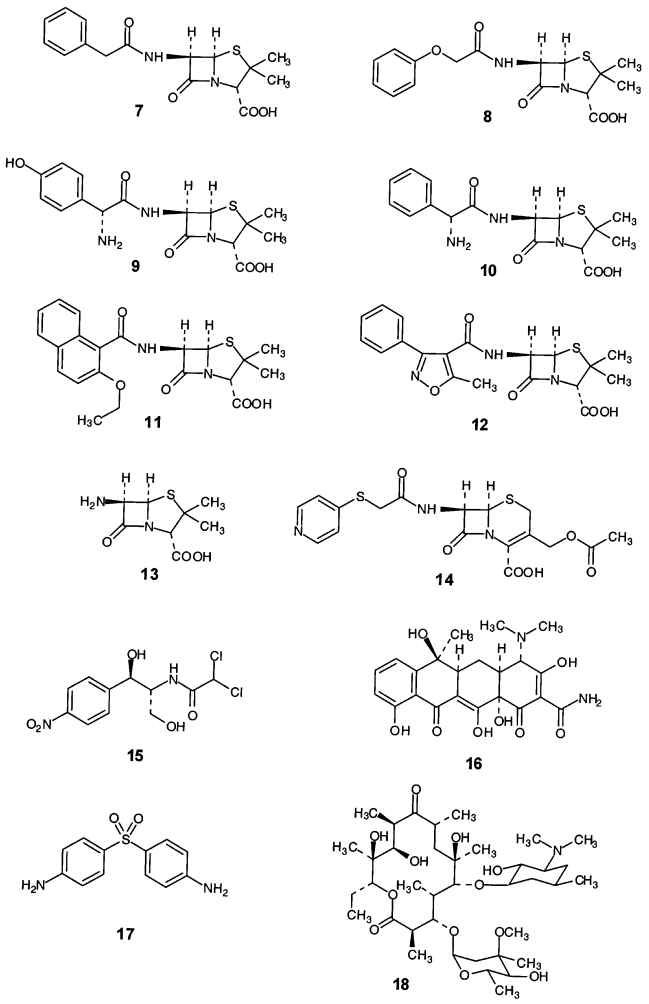
**Table 2.** $EC_{50}$ and Cross-Reactivity of Antibiotics Competing with Penicillin G for the Binding to *MIP 6*

| antibiotic | $EC_{50}$ (nM) | cross-reactivity (%) |
|---|---|---|
| penicillin G (**7**) | 60 | 100 |
| amoxicillin (**9**) | 313 | 19 |
| ampicillin (**10**) | 370 | 16 |
| penicillin V (**8**) | 403 | 15 |
| oxacillin (**12**) | 15 270 | <1 |
| nafcillin (**11**) | 196 300 | <0.1 |
| 6-aminopenicillanic acid (**13**) | 1612 | 4 |
| cephapirin (**14**) | NC | <0.01 |
| chloramphenicol (**15**) | NC | <0.01 |
| tetracycline (**16**) | NC | <0.01 |
| dapsone (**17**) | NC | <0.01 |
| erythromycin (**18**) | NC | <0.01 |

[a] NC means that no competition was found within the concentration range investigated.

MIPs relying on hydrogen bonds for the molecular recognition ability often lose their selective recognition in aqueous media. The competitive binding assay of *MIP 6* shows that selective recognition was retained in acetonitrile containing 1% of water. Because the final aim of this work is to apply the MIPs to milk assays, the molecular recognition properties in aqueous media are currently being investigated. Milk samples will be treated with acetonitrile to precipitate the proteins, and assays will be performed on the supernatant to assess the usefulness of these MIPs toward the final aim of the project.

**Chart 3.** Structure of Antibiotics




The synthesis of MIPs is a process involving a large number of variables. We envisioned that a chemometric approach should be a helpful tool to identify parameters of importance and to optimize the synthesis protocols. Multivariate data analysis of a *poly*(MAA−*co*−TRIM) library has therefore been carried out.[44]

## Conclusions

A *poly*(MAA−*co*−TRIM) MIP selective for penicillin G was selected from a MIP library by screening. Competitive binding studies showed that the cross-reactivity of $\beta$-lactams derived from 6-aminopenicillanic acid is higher with derivatives showing structural similarities to penicillin G (i.e., penicillin V, amoxicillin, and ampicillin) than with derivatives differing more in structure (i.e., nafcillin and oxacillin). A cephalosporin $\beta$-lactam (cephapirin) and antibiotics from other classes (chloramphenicol, tetracycline, dapsone, and erythromycin) did not compete at all with penicillin G for the binding to the MIP.

## Experimental Section

**Materials.** Penicillin G sodium salt, penicillin V, amoxicillin, ampicillin, nafcillin sodium salt monohydrate, oxacillin sodium salt monohydrate, tetracycline, and cephapirin sodium salt were purchased from Sigma (St. Louis, MO). (−)-Erythromycin hydrate, chloramphenicol, dapsone, MAA, HEMA, MAM, EDMA, TRIM, and ABCHC were from Aldrich (Milwaukee, WI). AIBN was purchased from Acros (Geel, Belgium). Boc-L-Phe-OH was from Advanced ChemTech (Louisville, KY). Scintillation liquid Ecoscint A was obtained from National Diagnostics (Hessle Hull, England). [Phenyl-4(*n*)-$^3$H]benzylpenicillin was from Amersham Pharmacia (Uppsala, Sweden). Organic solvents (pa grade) were from Merck (Darmstadt, Germany). Monomers and cross-linkers were purified by chromatography using either active carbon (particle size 0.25−1 mm) from Kebo Lab (Spånga, Sweden) or inhibitor remover from Aldrich.

**Synthesis of MIP Libraries.** In a typical procedure, 1 mmol of penicillin G was weighed into a screw-cap borosilicate glass tube and suspended by sonication in a fraction of the total volume of acetonitrile. A fraction of the MAA, enough to dissolve penicillin G, was added. The remaining volume of acetonitrile was then added followed by the remaining MAA and any additional functionalized monomer (as specified in Table 1). Cross-linker (as specified in Table 1) and initiator (2,2′-azobisisobutyronitrile or ABCHC, 0.01 equiv of the total amount of functionalized monomer(s) and cross-linker) were finally added. During these steps, care had to be taken to not precipitate penicillin G by adding the components in the order described above. The amounts of the components are specified in Table 1. The prepolymerization mixture was cooled on ice and purged with a stream of nitrogen for 10 min. The copolymerization was performed at 350 nm for 24 h at 4 °C in a Rayonet photochemical minireactor model RMR-600 (Branford, CT). The bulk polymers were ground in a Retsch Ultra Centrifugal Mill model ZM 100 (Haan, Germany). The ground particles were wet-sieved in water using sieves from Retsch. Size fractions of 25−50 $\mu$m were collected. To extract penicillin G from the polymer network, the particles were incubated with MeOH−HOAc (1:4, v/v) (3 × 1 h + 16 h), MeOH (4 × 5 min + 1 h), $CH_3CN$ (2 × 30 min + 16 h), and MeOH (4 × 5 min). The first incubation (with MeOH−HOAc) was done in an ultrasonic bath, and the rest were done on a rotator. The particles were finally dried in a vacuum overnight.

**Synthesis of a CP Library.** A library of CPs was prepared following the same procedure as described for the MIP library but substituting Boc-L-Phe-OH for penicillin G as the template. For each MIP prepared, a corresponding CP was prepared.

**Screening of the Libraries for Binding to Penicillin G.** The binding of penicillin G to the MIP and control libraries was screened by a batch-wise radioactive assay. MIPs (1 mg) or CPs (1 mg) were incubated for 16 h on a shaking table with 1 pmol of [phenyl-4(*n*)-$^3$H]benzylpenicillin in a total volume of 1 mL of $CH_3CN$ in microcentrifuge tubes. After centrifugation (5 min, 14 000 rpm), 500 $\mu$L of the supernatant was withdrawn and added to 10 mL of Ecoscint A scintillation liquid. Radioactivity was measured by liquid scintillation counting using a Rackbeta 1219 counter (LKB Wallac, Turku, Finland).

**Radioligand Competitive Binding Assay of MIP 6.** The competition between penicillin G and the competitors (penicillin V, ampicillin, amoxicillin, oxacillin, nafcillin,

6-aminopenicillanic acid, cephapirin, chloramphenicol, tetracycline, dapsone, and erythromycin) was determined in radioligand competitive binding assays. A 0.1 mg amount of *MIP 6* was incubated for 16 h on a shaking table with 1 pmol of [phenyl-4(*n*)-$^3$H]benzylpenicillin and 1 mL of various concentrations of the competitors (1 nM−1 mM) in $CH_3CN-H_2O$ (99:1) in microcentrifuge tubes. After centrifugation (5 min, 14 000 rpm), 500 $\mu$L of the supernatant was withdrawn and added to 10 mL of Ecoscint A scintillation liquid. Radioactivity was measured by liquid scintillation counting. Data were analyzed with GraphPad Prism software (Graphpad Software, Inc., San Diego) to determine $EC_{50}$ values. The cross-reactivity was calculated as the ratio of the $EC_{50}$ value of penicillin G to that of the competitor.

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## References and Notes

(1) The following abbreviations are used. ABCHC, 1,1′-azobis(cyclohexanecarbonitrile); AIBN, 2,2′-azobis(2-methylpropionitrile); Boc, *tert*-butyloxycarbonyl; CP, control polymer; $EC_{50}$, effective concentration 50%; EDMA, ethylene glycol dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; HOAc, acetic acid; HPLC, high-performance liquid chromatography; MAA, methacrylic acid; MAM, methacrylamide; MeOH, methanol; MIP, molecularly imprinted polymer; MRL, maximum residue level; Phe, phenylalanine; TRIM, trimethylolpropane trimethacrylate.

(2) Kempe, M.; Mosbach, K. Molecular imprinting used for chiral separations. *J. Chromatogr. A* **1995**, *694*, 3−13.

(3) Kempe, M. Molecular imprints as stationary phases. In *Encyclopedia of Separation Science*; Wilson, I. D., Adlard, T. R., Poole, C. F., Cook, M., Eds.; Academic Press: London, U.K., 2000; pp 2387−2397.

(4) Sellergren, B. Imprinted chiral stationary phases in high-performance liquid chromatography. *J. Chromatogr. A* **2001**, *906*, 227−252.

(5) Kempe, M. Molecularly imprinted polymers in chiral separations. In *Molecularly Imprinted Polymers. Man Made Mimics of Antibodies and Their Application in Analytical Chemistry*. Sellergren, B. Ed.; Elsevier Science: Amsterdam, The Netherlands, 2001; pp 395−415.

(6) Olsen, J.; Martin, P.; Wilson, I. D. Molecular imprints as sorbents for solid-phase extraction: potential and applications. *Anal. Commun.* **1998**, *35*, 13H−14H.

(7) Stevenson, D. Molecular imprinted polymers for solid-phase extraction. *Trends Anal. Chem.* **1999**, *18*, 154−158.

(8) Sellergren, B. Noncovalent molecular imprinting: antibody-like molecular recognition in polymeric network materials. *Trends Anal. Chem.* **1997**, *16*, 310−320.

(9) Andersson, L. I. Molecular imprinting for drug bioanalysis. A review on the application of imprinted polymers to solid-phase extraction and binding assay. *J. Chromatogr. B* **2000**, *739*, 163−173.

(10) Dickert, F. L.; Hayden, O. Molecular imprinting in chemical sensing. *Trends Anal. Chem.* **1999**, *18*, 192−199.

(11) Yano, K.; Karube, I. Molecularly imprinted polymers for biosensor applications. *Trends Anal. Chem.* **1999**, *18*, 199−204.

(12) Mosbach, K. Toward the next generation of molecular imprinting with emphasis on the formation, by direct molding, of compounds with biological activity (biomimetics). *Anal. Chim. Acta* **2001**, *435*, 3−8.

(13) Wulff, G. Enzyme-like catalysis by molecularly imprinted polymers. *Chem. Rev.* **2002**, *102*, 1−28.

(14) Skudar, K.; Brüggemann, O.; Wittelsberger, A.; Ramström, O. Selective recognition and separation of $\beta$-lactam antibiotics using molecularly imprinted polymers. *Anal. Commun.* **1999**, *36*, 327−331.

(15) Lübke, C.; Lübke, M.; Whitcombe, M. J.; Vulfson, E. N. Imprinted polymers prepared with stoichiometric template-monomer complexes: efficient binding of ampicillin from aqueous solutions. *Macromolecules* **2000**, *33*, 5098−5105.

(16) Levi, R.; McNiven, S.; Piletsky, S. A.; Cheong, S.-H.; Yano, K.; Karube, I. Optical detection of chloramphenicol using molecularly imprinted polymers. *Anal. Chem.* **1997**, *69*, 2017−2021.

(17) Senholdt, M.; Siemann, M.; Mosbach, K.; Andersson, L. I. Determination of cyclosporin A and metabolites total concentration using a molecularly imprinted polymer based radioligand binding assay. *Anal. Lett.* **1997**, *30*, 1809−1821.

(18) Siemann, M.; Andersson, L. I.; Mosbach, K. Separation and detection of macrolide antibiotics by HPLC using macrolide-imprinted synthetic polymers as stationary phases. *J. Antibiot.* **1997**, *50*, 89−91.

(19) Johnston, A. M. Animals and antibiotics. *Int. J. Antimicrob. Agents* **2001**, *18*, 291−294.

(20) Boerlin, P.; Wissing, A.; Aarestrup, F. M.; Frey, J.; Nicolet, J. Antimicrobial growth promotor ban and resistance to macrolides and vancomycin in Enterococci from pigs. *J. Clin. Microbiol.* **2001**, 4193−4195.

(21) Smith, D. L.; Harris, A. D.; Johnson, J. A.; Silbergeld, E. K.; Morris, J. G., Jr. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6434−6439.

(22) Threlfall, E. J.; Ward, L. R.; Frost, J. A.; Willshaw, G. A. The emergence and spread of antibiotic resistance in food-borne bacteria. *Int. J. Food. Microbiol.* **2000**, *62*, 1−5.

(23) Heinemann, J. A. How antibiotics cause antibiotic resistance. *Drug Discovery Today* **1999**, *4*, 72−79.

(24) Aarestrup, F. M.; Wegener, H. C. The effects of antibiotic usage in food animals on the development of antimicrobial resistance of importance for humans in Campylobacter and Escherichia coli. *Microbes Infect.* **1999**, *1*, 639−644.

(25) Patriarca, G.; D'Ambrosio, C.; Schiavino, D.; Nucera, E.; Sun, J. Y.; Del Ninno, M.; Misuraca, C.; Buonomo, A. Allergy to betalactams. *Int. Arch. Allergy Immunol.* **1999**, *118*, 247−250.

(26) Hileman, B. Furor over animal antibiotic use. *Chem. Eng. News* **2001**, 47−52.

(27) Seymour, E. H.; Jones, G. M.; McGilliard, M. L. Comparisons of on-farm screening tests for detection of antibiotic residues. *J. Dairy Sci.* **1988**, *71*, 539−544.

(28) van Eenennaam, A. L.; Cullor, J. S.; Perani, L.; Gardner, I. A.; Smith, W. L.; Dellinger, J.; Guterbock, W. M.; Jensen, L. Evaluation of milk antibiotic residue screening tests in cattle with naturally occurring clinical mastitis. *J. Dairy Sci.* **1993**, *76*, 3041−3053.

(29) Bell, C.; Rhoades, J. R.; Neaves, P.; Scannella, D. An evaluation of the IDEXX SNAP test for the detection of beta-lactam antibiotics in ex-farm raw milks. *Neth. Milk Dairy J.* **1995**, *49*, 15−25.

(30) Schenk, F. J.; Callery, P. S. Chromatographic methods of analysis of antibiotics in milk. *J. Chromatogr. A* **1998**, *812*, 99−109.

(31) Schügerl, K.; Seidel, G. Monitoring of the concentration of $\beta$-lactam antibiotics and their precursors in complex cultivation media by high-performance liquid chromatography. *J. Chromatogr. A* **1998**, *812*, 179−189.

(32) Niessen, W. M. A. Analysis of antibiotics by liquid chromatography−mass spectrometry. *J. Chromatogr. A* **1998**, *812*, 53−75.
(33) Piletsky, S. A.; Karim, K.; Piletska, E. V.; Day, C. J.; Freebairn, K. W.; Legge, C.; Turner, A. P. F. Recognition of ephedrine enantiomers by molecularly imprinted polmers designed using a computational approach. *Analyst* **2001**, *126*, 1826−1830.
(34) Chianella, I.; Lotierzo, M.; Piletsky, S. A.; Tothill, I. E.; Chen, B.; Karim, K.; Turner, A. P. F. Rational design of a polymer specific for microcystin-LR using a computational approach. *Anal. Chem.* **2002**, *74*, 1288−1293.
(35) Nicholls, I. A.; Adbo, K.; Andersson, H. S.; Andersson, P. O.; Ankarloo, J.; Hedin-Dahlström, J.; Jokela, P.; Karlsson, J. G.; Olofsson, L.; Rosengren, J.; Shoravi, S.; Svenson, J.; Wikman, S. Can we rationally design molecularly imprinted polymers? *Anal. Chim. Acta* **2001**, *435*, 9−18.
(36) Takeuchi, T.; Fukuma, D.; Matsui, J. Combinatorial molecular imprinting: an approach to synthetic polymer receptors. *Anal. Chem.* **1999**, *71*, 285−290.
(37) Lanza, F.; Sellergren, B. Method for synthesis and screening of large groups of molecularly imprinted polymers. *Anal. Chem.* **1999**, *71*, 2092−2096.
(38) Lanza, F.; Hall, A. J.; Sellergren, B. Bereczki, A.; Horvai, G.; Bayoudh, S.; Cormack, P. A. G.; Sherrington, D. C. Development of a semiautomated procedure for the synthesis and evaluation of molecularly imprinted polymers applied to the search for functional monomers for phenytoin and nifedipine. *Anal. Chim. Acta* **2001**, *435*, 91−106.
(39) *The Merck Index*, 12th ed.; Budavari, S., O'Neil, M. J., Smith, A., Heckelman, P. E., Kinneary, J. F., Eds.; Merck & Co., Inc.: Whitehouse Station, NJ, 1996.
(40) Kempe, M. Antibody mimicking polymers as chiral stationary phases in HPLC. *Anal. Chem.* **1996**, *68*, 1948−1953.
(41) Kempe, M. Chiral recognition. Studies on chiral discrimination in enzymatic peptide synthesis and noncovalent molecular imprinting. Doctoral thesis, Lund University, Studentlitteratur, Lund, 1994.
(42) Yu, C.; Mosbach, K. Molecular imprinting utilizing an amide functional group for hydrogen bonding leading to highly efficient polymers. *J. Org. Chem.* **1997**, *62*, 4057−4064.
(43) Bereczki, A.; Tolokán, A.; Horvai, G.; Horváth, V.; Lanza, F.; Hall, A. J.; Sellergren, B. Determination of phenytoin in plasma by molecularly imprinted solid-phase extraction. *J. Chromatogr. A* **2001**, *930*, 31−38.
(44) Navarro, F.; Kempe, M. Chemometric analysis of a polymer library imprinted with penicillin G. *Abstract book of 2nd International Workshop on Molecularly Imprinted Polymers (MIP2002)*; La Grande Motte, France, September 16−19, 2002; abstr. no. 41.

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