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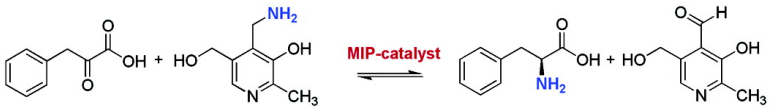

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A Molecularly Imprinted Polymer-Based Synthetic Transaminase

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Abstract: The design, synthesis, and evaluation of a molecularly imprinted polymer transaminase mimic is described. Methacrylic acid−ethylene glycol dimethacrylate copolymers were synthesized using, as a template, a transition state analogue (TSA) for the reaction of phenylpyruvic acid and pyridoxamine to yield phenylalanine and pyridoxal. Polymer suitability was established on the basis of ^{1}H NMR studies of template−functional monomer interactions. Polymer recognition characteristics were examined in a series of HPLC studies using the polymers as chromatographic stationary phases. Selectivity for the TSA, relative to substrates and products, was observed in both aqueous and nonpolar media. In the latter case (chloroform/AcOH, 96:4), an enantioseparation factor (α) of 2.1 was obtained, and frontal chromatographic studies revealed the presence of 11.9 $\pm$ 0.2 μmol g^{-1} (dry weight) of enantioselective sites. Polymers imprinted with the L-form of the oxazine-based TSA induced a 15-fold enhancement of the apparent reaction rate (app. V_{max} 2.5 $\times$ 10^{-7} mol s^{-1}; app. K_m 8.2 $\times$ 10^{-3} M) and enantioselective production of phenylalanine (32 $\pm$ 4% ee) for reactions conducted in an aqueous buffer system. Substrate selectivity was evident, and a turnover number (k_{cat}) of 0.1 s^{-1} was determined. This is the first example of the catalysis of sigmatropic shifts in aqueous media by molecularly imprinted polymers.

Introduction

Removal of the α-amino groups is the first step in the catabolism of most L-amino acids.[1] In animal species, the L-amino acids are deaminated and converted to the corresponding α-keto acids in a process involving the transfer of the amino group to α-ketoglutarate. This reaction is an example of transamination, a process of central importance in biology, which is catalyzed by a family of pyridoxal phosphate-dependent enzymes known as the transaminases, or aminotransferases. These cofactor-dependent enzymes facilitate the 1,3-prototropic shift necessary for the reaction. Accordingly, the reverse reaction, from pyridoxamine and an α-keto acid, yields an amino acid and pyridoxal, which is a key step in amino acid biosynthesis, Figure 1.

Pyridoxal phosphate, the coenzyme form of vitamin B_6, is the prosthetic group responsible for catalysis of the transamination reaction and is also involved in a range of mechanistically related transformations of amino acids including racemization, decarboxylation, aldol-type condensations, and retro-condensations.[2] On account of the unique reactivities of pyridoxal phosphate and pyridoxamine phosphate in biology, many attempts have been made to develop enzyme mimics using these cofactors, in particular for transamination.[3] The seminal work on transaminase mimics by Breslow and co-workers led to a system demonstrating up to an 80-fold rate enhancement of the conversion of pyruvic acid to alanine using pyridoxamine derivatives with different basic side chains.[3b] Furthermore, the inherent chirality and hydrophobic binding pockets of functionalized cyclodextrins have been used to produce transaminases.[4] By covalent attachment of pyridoxamine, modified analogues, and various basic groups to cyclodextrins, rate enhancements for enantio- and substrate-selective transaminations were obtained. More recently, a water-soluble polyethyleneimine (PEI) polymer with incorporated pyridoxamine moieties has been described. These polymers displayed a large increase in the observed rate of the transamination reaction between pyruvic acid and pyridoxamine (~2300-fold increase at pH 7.0).[5] General acid−base catalysis and a hydrophobic environment within the polymer matrix were believed to be the main contributors to the rate enhancement, which also seemed to be dependent on the hydrophobicity of the substrates. Other reports of transaminase mimics include catalytic antibodies by

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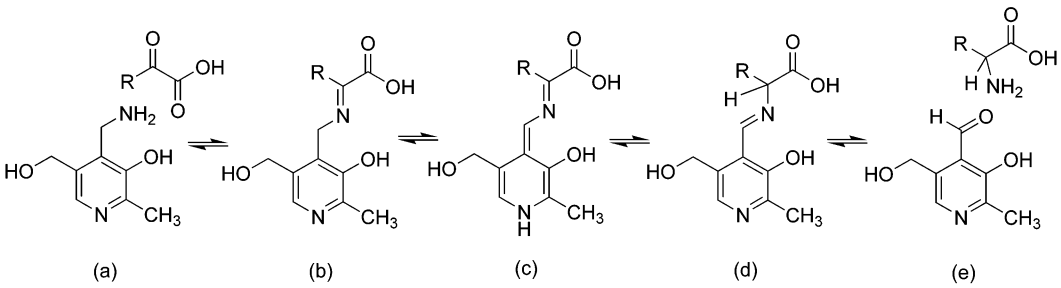

Figure 1. α-Amino acid synthesis by transamination: (a) pyridoxamine and α-keto acid, (b) ketimine, (c) conjugated quinone-like intermediate, (d) aldimine, and (e) pyridoxal and amino acid.

Schultz et al.,[6] polypeptide−pyridoxamine chimeras by Imperali and Roy,[7] and a pyridoxamine-modified adipocyte lipid binding protein (ALBP).[8]

Molecular imprinting[9] is a technique for the synthesis of polymeric materials with predetermined ligand selectivity and was perceived as an alternative approach for the development of a synthetic transaminase. The underlying strategy entails the use of functionalized monomers which can bind reversibly to a template structure; the resultant complex is subsequently incorporated into a network polymer by copolymerization in the presence of an excess of a cross-linking monomer and an inert solvent (porogen). The removal of the template leaves sites of complementary shape and functionality, which are capable of selectively rebinding the template. Molecularly imprinted polymers (MIPs) with antibody-like recognition characteristics have been prepared for a large number of compound classes, and these have been studied with respect to an increasing range of application areas.[10] One area of particular interest, which to this point in time has received relatively little attention, is their use in the development of tools for organic synthesis.[11] MIPs have been employed as noncovalent protecting groups for stereoselective synthesis,[12] and, by analogy to the preparation of catalytic antibodies, MIPs have been synthesized using template substances with structures mimicking the transition states of reactions to produce polymers with catalytic function. Some of the reactions thus far addressed with the technique include: dehydrofluorination,[13] various hydrolyses,[14] and the Diels−Alder[15] and aldol[16] reactions. While success has been obtained with the application of molecularly imprinted polymers to a number of hydrolytic reactions,[14] the range and catalytic efficiencies of other reaction types have been limited. The inherent thermal and chemical stability of these materials renders MIPs suitable for use under conditions not conducive to biological macromolecules.[17] The flexibility offered by molecular imprinting in terms of choice of template, together with the significance of the transamination reaction, suggested the use of molecular imprinting of TSAs for the development of a synthetic transaminase. Previously, attempts have been made to produce transaminase-related activities using substrate-imprinted MIPs, although with modest results.[18] In this paper, we report the synthesis and evaluation of a MIP prepared using a transition state analogue (TSA) for the reaction of phenylpyruvic acid and pyridoxamine to yield phenylalanine and pyridoxal, the first example of such an approach to this class of reaction, and the first example of the catalysis of a sigmatropic shift reaction in aqueous media.

Results and Discussion

Design and Synthesis of Transition State Analogues. The reaction between pyridoxamine and phenylpyruvic acid to yield phenylalanine and pyridoxal was selected for use in this study. The choice of TSA for use was designed based upon transition state structures derived from semiempirical molecular orbital calculations (MINDO/3) performed by Andrews et al., Figure 2a.[19] Their study described the mechanism of the pyridoxal-phosphate-dependent enzyme γ-aminobutyric acid (GABA) transaminase and led to the development of inhibitors for this enzyme.[20] The calculations indicated that in the transition state the Schiff base is coplanar with the protonated pyridine ring and an intramolecular hydrogen bond is present between the phenolic hydroxyl and the Schiff base nitrogen. Protonation of the pyridine nitrogen ($pK_a \sim 5$), instead of the more basic imine ($pK_a \sim 11$, only suggested to be initially protonated), originates

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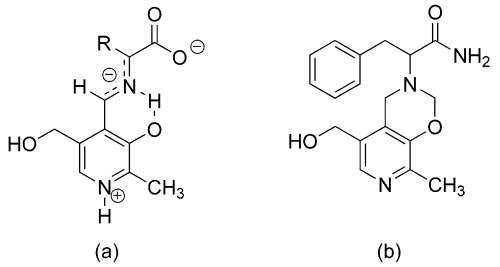

Figure 2. General structure of the proposed transition state (a), and the transition state analogue (b).

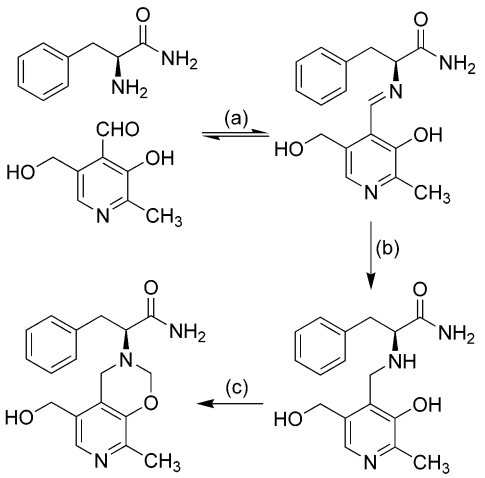

Figure 3. L-TSA synthesis: (a) methanol, room temperature, 30 min; (b) methanol, $NaBH_4$, 0 °C; (c) methanol, paraformaldehyde, cat. KOH, reflux, 2 h.

from the resonance stabilization of the conjugated carbanion during the tautomerization step. Collectively, these findings suggested that oxazine-based compounds should serve as TSAs as they allow for the heteroatom orientation and coplanarity to be maintained.[19] In terms of the reaction chosen for the present study, 2-(5-hydroxymethyl-8-methyl-3,4-dihydropyrido[4,3-e]-1,3-oxazin-3-yl)phenylalaninamide was proposed as a suitable TSA, Figure 2b. The amide was selected for use in this case, in favor of the corresponding carboxylic acid, to avoid a possible lactonization.

L-2-(5-Hydroxymethyl-8-methyl-3,4-dihydropyrido[4,3-e]-1,3-oxazin-3-yl)phenylalaninamide (L-TSA) and its optical antipode (D-TSA) were readily obtained in a two-pot procedure, Figure 3. Condensation of L- or D-phenylalaninamide and pyridoxal, followed by $NaBH_4$ reduction of the resultant imine, afforded the corresponding secondary amine in 76% yield after purification on silica. Oxazine ring formation was achieved by a Mannich cyclization using paraformaldehyde and furnished the desired products in acceptable yield (56%) after purification.

^{1}H NMR Analysis of TSA−Functional Monomer Interactions. Many previous studies have illustrated the general utility of methacrylic acid (MAA)−ethylene glycol dimethacrylate (EGDMA) copolymers for molecular imprinting using noncovalent interactions.[9] The relative resilience of these polymers to extremes of chemical environment and temperature suggests their suitability for applications in organic synthesis. Spectroscopic studies have previously been shown to be useful for the study of template−functional monomer complexation.[21] ^{1}H NMR studies were undertaken to establish the suitability of this type of polymer in conjunction with the TSA templates used in this study. d_3-Acetic acid was used as an analogue for the functional monomer (MAA), and complex formation with L-TSA in $CDCl_3$ was studied in a titration experiment yielding a saturation isotherm, Figure 4a. Interestingly, only the two nonequivalent amide protons displayed any significant changes in chemical shifts. Apparent dissociation constants (app. K_{diss}) for complexation of these protons with acetic acid were determined to be 93 ± 10 and 125 ± 15 mM, respectively. The similarity of these values is indicative of them being involved in the same or similar interactions with the functional monomer analogue. Changes in the chemical shifts of the methylene and methine protons adjacent to the tertiary amine and that of the pyridinyl ring were barely discernible (<0.02 ppm), which was attributed to the relative remoteness of these protons from the potential sites for electrostatic interactions (cf. direct interaction with the amide protons). Nonetheless, these spectral changes indicate that interactions between the monomer analogue and the amines do take place.

Job plot analysis[22] revealed a 1:1 complex stoichiometry between the amide proton(s) of the template and acetic acid, Figure 4b. It is important to note that this stoichiometry pertains only to the interaction between the functional monomer analogue and the protons of the amide nitrogen; interactions at other sites, for example, the hydroxyl and tertiary amine, were discernible using the NMR experiments described, although the extent of the chemical shifts involved precluded the calculation of meaningful dissociation constants and examination of interaction stoichiometries. It was anticipated that a combination of shape complementarity afforded by the polymer matrix in conjunction with the interaction of the functional monomer with the heteroatom-bearing functionalities should provide the basis for selective TSA−polymer interactions.

Polymer Synthesis and Characterization. Highly cross-linked molecularly imprinted polymers were synthesized using L-TSA as template (**P(L)**), MAA as functional monomer, and EGDMA as cross-linker in a relative ratio of 1:5:20 (template: MAA:EGDMA). Polymerizations were performed at 65 °C using azo-bis(isobutyronitrile) (AIBN) as the initiator and chloroform as the porogen. The concentration of template used in the polymer synthesis was comparable to that used in the earlier NMR studies, 92 and 30 mM, respectively. Extrapolation from the nonlinear regression-fitted saturation curves indicated that approximately 75% complexation of the template is achieved at the concentrations used in the polymer synthesis, in terms of interactions with the amide and bridging (N−CH_2−O) methylene of the oxazine ring. **P(L)** was obtained as particles (25−63 μm) in better than 80% yields after manual grinding, sieving, and repeated sedimentation to remove fine particles. Three reference polymers were synthesized using the same procedure as described above, although either D-TSA (**P(D)**) or the anti-asthma drug theophylline (**P(T)**) was used as template, or there was no template (**P(B)**). Residual template was removed by exhaustive washing using a protocol previously

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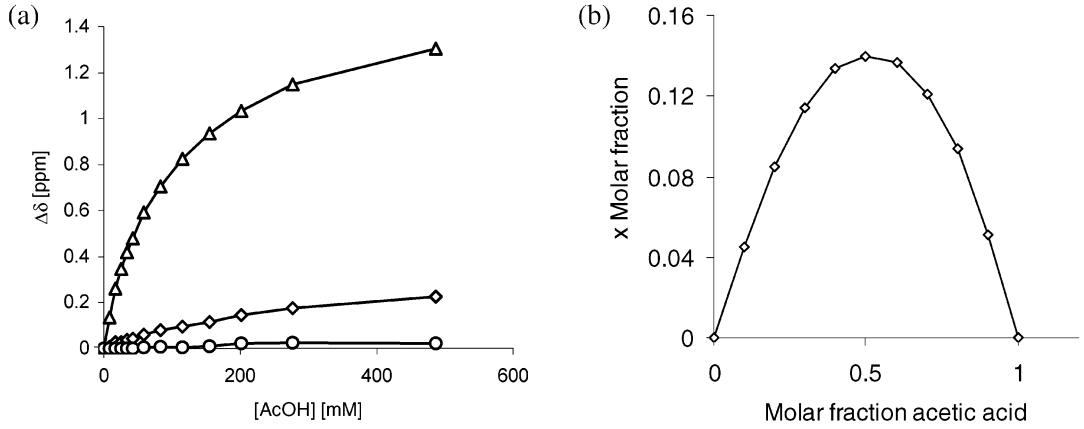

Figure 4. (a) Saturation plot for the ^{1}H NMR titration: −Δ− amide proton (shift from δ 5.64), −◇− amide proton (δ 6.37), −○− oxazine N−CH_2−O methylene (δ 4.93). (b) Job plot of the interaction between amide proton δ 5.64 and acetic acid.

Table 1. Polymer−Ligand Recognition in Aqueous and Organic Media

		capacity factors k' [a]				
polymer	mobile phase	pyridoxamine	pyridoxal	L-TSA	D-TSA	phenylpyruvic acid
P(L)	MeOH/buffer[b] 1:1	2.82	0.16	3.51	3.29	0.01
P(L)	$CHCl_3$/AcOH[c]	n.a.[d]	n.a.[d]	10.14	4.84	0.47
P(B)	MeOH/buffer[b] 1:1	1.66	0.03	3.22	3.23	0.05
P(B)	$CHCl_3$/AcOH[c]	n.a.[d]	n.a.[d]	0.75	0.75	0.53

[a] $k' = (V_a - V_0)/V_0$, where V_a is the retention volume for the analyte and V_0 is the retention volume for a noninteracting void marker (acetone). All values are the result of a minimum of three experiments with an uncertainty of ±0.02. Flow rate (1.0 mL min^{-1}), injection volume (30 μL), sample concentrations (1.0 mM). [b] 0.1 M acetate, pH 7.0. [c] $CHCl_3$/AcOH 96:4, v/v. [d] Analyte not soluble in the mobile phase.

developed for similar polymer systems.[23] BET-surface area analysis showed comparable surface areas for the TSA-imprinted and nonimprinted polymers (**P(L)** 336 m^2/g, **P(B)** 379 m^2/g). FT-IR studies indicated the presence of the expected functionalities. To confirm that the complexation of template by functional monomer survives the polymerization process, a polymerization mixture containing L-TSA was prepared in an NMR tube and a proton spectrum was acquired. The shifts induced by the functional monomer reflected those observed using acetic acid. Polymerization was initiated and spectra recorded at regular intervals over 60 min. The chemical shifts induced in the ^{1}H NMR spectra of L-TSA by the functional monomer, MAA, were clearly retained throughout the first 30 min of the polymerization reaction. Sample anisotropy resulted in severe line broadening as the reaction mixture approached the gel phase, and complete solidification was apparent after 1 h.

Polymer−Ligand Recognition Studies. The recognition characteristics of the polymers were examined to establish the presence of sites selective for the template in the imprinted polymers. Polymers were packed into HPLC columns and used as stationary phases in a series of studies that employed two distinctly different eluent systems, Table 1. In a nonpolar eluent system, chloroform/acetic acid (96:4, v/v), significant differences in capacity factors (k') were observed for the enantiomers on the **P(L)** with a separation factor (α) of 2.1. When pure chloroform was used as eluent, the TSAs failed to elute from the column. The observed enantioselectivity corresponds to a difference in Gibbs' free energy of binding between the enantiomers of 1.8 kJ/mol at 293 K, as calculated using: $\Delta G = -RT \ln \alpha$.[24] Importantly, binding of the TSAs to the nonimprinted **P(B)** was minimal, k' 0.75, further supporting the observation that **P(L)** contains sites selective for the template. Significantly weaker recognition was observed for **P(L)** when using a polar eluent (methanol/0.1 M sodium acetate (aq); 1:1, v/v; pH 7.0 − the buffer used for subsequent studies of the influence of polymers on transamination reaction rates) as reflected in the markedly lower capacity factors, although some enantioselectivity was still evident, $\alpha = 1.1$ ($\Delta G = 0.2$ kJ mol^{-1} at 293 K). Thus, the more polar environment is detrimental with respect to the electrostatic interactions between the carboxylate residues of the polymer and the analyte. Binding to the nonimprinted reference polymer, **P(B)**, was stronger in the aqueous buffer than in chloroform/acetic acid. This is indicative of the prevalence of nonspecific binding arising from hydrophobic interactions with the polymer matrix. Collectively, the recognition behavior supports the presence of sites selective for the template and that the basis for selectivity lies in interactions between the carboxyl groups of the polymer and the template, as implicated by the NMR studies discussed earlier. Importantly, the D-TSA-imprinted polymer **P(D)** behaved identically, although with a reversed enantioselectivity.

Polymer recognition of the substrates, pyridoxamine and phenylpyruvic acid, and one of the products, pyridoxal, was similarly examined. Difficulty in the detection of phenylalanine prevented its use in this experiment. In studies in the aqueous buffer, pyridoxamine was the only analyte to show any significant retention on **P(L)**, which was attributed to its greater structural similarity to the template and the strong electrostatic interactions available through the amine moiety. Pyridoxal,

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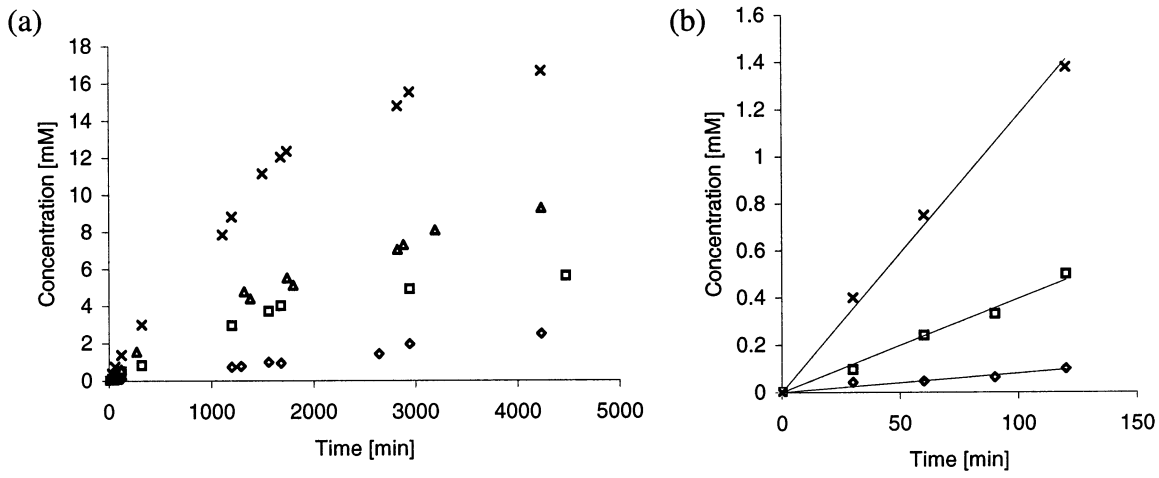

Figure 5. Comparison of the phenylalanine production. (a) Time course studies: −×− **P(L)**, −Δ− **P(T)**, −□− **P(B)**, −◇− solvent reaction. (b) Initial reaction velocities: −×− **P(L)**, −□− **P(B)**, −◇− solvent reaction. In all cases, substrate concentrations were as follows: pyridoxamine (22.5 mM) and phenylpyruvic acid (22.5 mM), polymer 100.0 mg (except solution reaction) in a total volume of 1.0 mL.

although relatively poorly retained, also displayed preferential binding to **P(L)**. Neither phenylpyruvic acid nor pyridoxal demonstrated significant levels of binding to the polymers studied. The enantioselectivity of **P(L)** in both nonpolar and aqueous media was most encouraging, as the function of MIPs in aqueous media is a significant challenge for their use as biomimetic systems.

Frontal chromatography[25] was used to determine the strength of the polymer−TSA interactions in the aqueous buffer used for the reaction studies described later. Enantioselectivity was evidenced by the differences in the apparent dissociation constants observed for the interaction of **P(L)** with the L- and D-TSAs (app. $K_{diss} = 1.5 \pm 0.1$ and 2.0 ± 0.1 mM, respectively) and in terms of the binding site populations (B_t) available for the analytes, 28.2 ± 0.1 and 16.3 ± 0.1 μmol/g (dry weight), respectively. Thus, it can be concluded that **P(L)** possesses 11.9 ± 0.2 μmol/g (dry weight) of high fidelity sites with selectivity for the L-TSA. As anticipated, and reflecting the zonal chromatographic studies, binding of the L-TSA to **P(B)** was weaker, app. $K_{diss} = 1.9 \pm 0.1$ mM ($B_t = 35.9 \pm 0.1$ μmol/g). It is noteworthy that the apparent dissociation constants obtained for L-TSA binding to **P(L)** are approximately 2 orders of magnitude lower than those found for complexation of L-TSA by acetic acid in the same solvent. This reflects the greater energetic penalty (translational and rotational free energy) that must be paid for formation of a complex comprised of a greater number of monomer/template components.[26]

Evaluation of Polymer Catalysis of Transamination. The influence of the polymers on the transamination reaction of phenylpyruvic acid and pyridoxamine, to afford phenylalanine and pyridoxal, was studied in an aqueous buffer (methanol/0.1 M sodium acetate, pH 7.0; 1:1) using an HPLC-based assay. Studies of the influence of the polymers **P(L)**, **P(B)**, and **P(T)** on reaction rates were performed using the solution reaction (in the absence of polymer) as reference, Figure 5a. The latter polymer, **P(T)**, was included in the study to provide an assessment of the extent of any possible influence of polymer morphology or cavity functionalization (localization of functional monomer residues in the resultant polymer) on reaction rates due to the imprinting process. The choice of reaction medium was based upon a compromise between the feasibility of analyzing reaction kinetics with the assay employed, substrate solubility, and a desire to illustrate the utility of these polymers in aqueous media. The reaction solvent influences the position of equilibrium, which is rapidly shifted in favor of Schiff base formation in a less polar solvent, or at higher pH. Indeed, reactions run in less polar media, pure methanol, were some 15 times faster than those described here in the buffer.

Table 2. Kinetic Data for the Production of Phenylalanine

polymer	inhibitor	app. V_{max} (mol s^{-1})	app. K_m (M)
MeOH/buffer		1.7×10^{-8}	
P(L)		2.5×10^{-7}	8.2×10^{-3}
P(B)		1.4×10^{-7}	2.3×10^{-2}
P(L)	L-TSA[a]	1.7×10^{-7}	2.9×10^{-2}
P(L)	D-TSA[a]	2.0×10^{-7}	2.3×10^{-2}
P(B)	L-TSA[a]	1.3×10^{-7}	2.3×10^{-2}

[a] Inhibitor concentration 0.50 mM.

Michaelis−Menten-like kinetics were observed for the reactions performed in the presence of polymers. A 5-fold enhancement of the initial reaction rate was observed with the nonimprinted reference polymer, **P(B)**, as compared to the solution reaction, and a 15-fold increase was achieved with **P(L)**, Figure 5b and Table 2. The general effect of a polymer on reaction rate is often explained as resulting from local increases in the concentration of substrate in the vicinity of the polymer surface due to nonspecific adsorption. However, when comparing the reaction rates obtained with **P(B)** and **P(L)**, the difference cannot be explained by differences in surface areas, for, as determined by BET analysis, the nonimprinted polymer, **P(B)**, has a greater (12%) surface area than that of **P(L)**. The enhancement of reaction rate observed with **P(L)** can therefore be interpreted as a result of the imprinting of the L-TSA, that is, template-induced localization of monomer functionalities. To elucidate the possible influence of a general localization of functionalities around cavities within the polymer, a polymer of the same composition but imprinted with an unrelated template, the anti-asthma drug theophylline, **P(T)**, was examined. The rate enhancement induced by **P(T)** was slightly higher (6-fold relative to the solution reaction) than that of **P(B)**,

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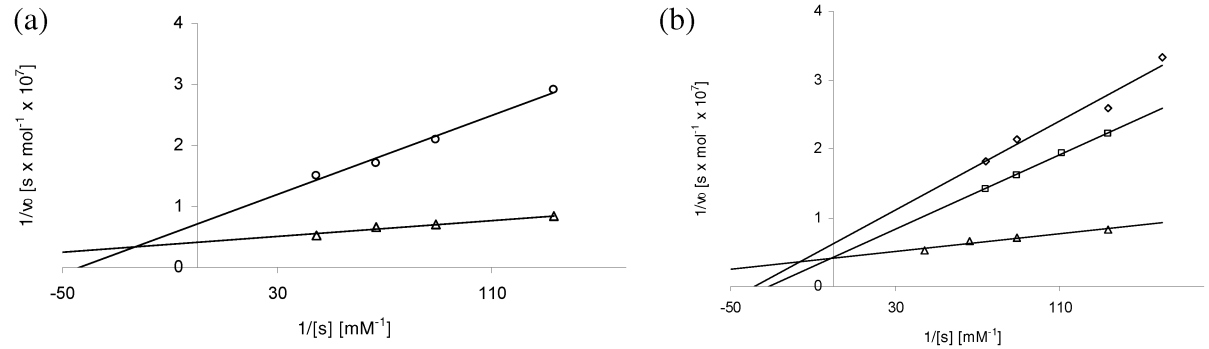

Figure 6. Lineweaver−Burk plots: (a): −Δ− **P(L)**, −○− **P(B)**, substrate concentrations: pyridoxamine and phenylpyruvic acid (each 7.5−22.5 mM). (b) Inhibition studies using **P(L)**: inhibitor concentrations −◇− 0.50 mM, −□− 0.25 mM, −Δ− 0.00 mM. Substrate concentrations in the presence of inhibitors: 6.25−13.5 mM.

although significantly lower than for **P(L)**. This result provides further support for the unique nature of **P(L)**; that is, the imprinting effect-derived selective recognition of the transition state analogue is the basis for the observed reaction rate enhancement.

The apparent reaction velocities (V_{max}) and Michaelis constants (K_m) were determined using substrate concentrations ranging from 7.5 to 22.5 mM, Figure 6 and Table 2. Unfortunately, higher concentrations were impossible on account of substrate solubilities, and lower concentrations were prohibited due to assay detection limits. A 1:1 pyridoxamine/phenylpyruvic acid stoichiometry was employed in the data presented to optimize the width of the concentration range studied. Accordingly, the kinetic parameters reflect the performance of the reaction system as a whole.

The observed rate enhancements were competitively inhibited by L-TSA. It induced a decrease in V_{max} (∼30%) and an almost 4-fold increase in K_m for **P(L)**, indicative of competitive inhibition by the template for the imprinted cavities. Most importantly, D-TSA had a less pronounced effect on the reaction rate, a 3-fold decrease in K_m and a 20% reduction in V_{max}, which reflects the stereochemical integrity of the high affinity sites and implies that sites with affinity for L-TSA are responsible for the observed rate enhancements. The influence of inhibitor chirality on the performance of the polymer was further underscored by the effect on **P(B)**, with which no significant inhibition was observed at the concentrations employed. The catalytic performance of these materials is reflected in the turnover number (k_{cat}), which for **P(L)** is 0.1 s^{-1}, based upon 28 μmol of sites per gram of polymer. While a number of entropically favorable hydrolytic reactions have been catalyzed by MIPs,[11] in some cases with high catalytic efficiencies, the catalysis of the sigmatropic transamination reaction described here is unique not only by virtue of the observed catalytic efficiencies, but also the fact that the polymer system is functional in an aqueous medium.

The enantioselectivity of **P(L)** was further reinforced upon analysis of the product, phenylalanine, which was obtained in favor of the L-form, 32 ± 4% ee. Importantly, both reference polymer systems, **P(B)** and **P(T)**, furnished a racemic product. Reactions performed in the presence of the L-TSA (0.50 mM) resulted, in addition to a lower product yield, in a significantly lower product enantiomeric excess, 9 ± 4% ee. However, no change in the enantiomeric excess of the reaction product was observed when run in the presence of the D-TSA (0.50 mM). Moreover, no product enantiomeric excess was observed in reactions with **P(B)** or buffer in the presence of the L-TSA. These results highlight the enantioselectivity of the polymer and the role the recognition sites play in the observed chiral induction.

Together with the enantioselectivity of **P(L)** demonstrated in the frontal and zonal chromatographic studies of polymer−ligand recognition, these data support the existence of recognition sites selective for the L-TSA, which are due to its presence as a template during the polymerization process. This concurs with the evidence obtained from the NMR studies, which indicate specific interactions between functional monomer (analogue) and the template − the basis for the observed ligand selectivity in the resultant polymers. Furthermore, the enantioselectivity observed in product formation provides the strongest evidence in support for the origin of the observed catalytic effect being the result of the presence of sites selective for the specific enantiomer of the transition state employed in the polymerization process. The catalytic effect observed using this transition state analogue as a template is superior to those previously obtained by the molecular imprinting of substrate-based structures.[18] This supports the use of mimics of transition state structures in the synthesis of catalytic MIPs.

To further examine the selectivity of the polymers, reactions were run using an alternative substrate, pyruvic acid. The initial solution reaction rate (1.8×10^{-8} mol s^{-1}) was similar to that observed using phenylpyruvic acid (1.3×10^{-8} mol s^{-1}). Both **P(B)** and **P(L)** induced increases in initial reaction rate for pyruvic acid, 7.3×10^{-8} and 9.8×10^{-8} mol s^{-1}, respectively. The fact that **P(L)** has only a marginal effect on rate in this case, as compared to **P(B)**, can be attributed to the substrate being less well recognized by the sites selective for the transition state for the reaction due to its smaller size. To establish the robustness of the polymeric material, polymer used in assays was washed using the protocol used for polymer preparation and evaluated in subsequent assays where the polymer retained at least 90 ± 5% of the initial activity. This highlights the potential of these types of polymers for use as substitutes for enzymes in industrial applications.

Conclusion

The results presented demonstrate the use of molecular imprinting for preparing a synthetic polymer with enzyme-like activity (catalytic turnover, substrate selectivity, enantioselec-

tivity, and rate enhancement). Furthermore, this is the first report of a TSA-imprinted polymer capable of mediating a transamination reaction and is the first example of catalysis of a sigmatropic shift in aqueous media by a molecularly imprinted polymer. The versatility of the technique with respect to choice of template suggests the use of this system for the catalytic asymmetric synthesis of nonnatural amino acids and related structures. The relatively simple preparation and high mechanical, chemical, and thermal stability make MIP "artificial enzymes" interesting alternatives to their biological counterparts.

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Supporting Information Available: Synthesis and characterization of transition state analogues, including ^{1}H NMR and ^{13}C NMR spectra of the L-TSA. ^{1}H NMR studies of template−monomer analogue complexation. Polymer syntheses and polymer characterization. Zonal and frontal chromatographic procedures and data from frontal chromatographic studies. Assay protocol and reagent preparation. Data from kinetics studies on reactions with pyruvate, and reactions of phenylpyruvic acid in methanol. This material is available free of charge via the Internet at http://pubs.acs.org.

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