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Enantioselective Ester Hydrolysis Catalyzed by Imprinted Polymers

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Abstract: *The first imprinted polymers with enantioselective catalytic activity are reported.*

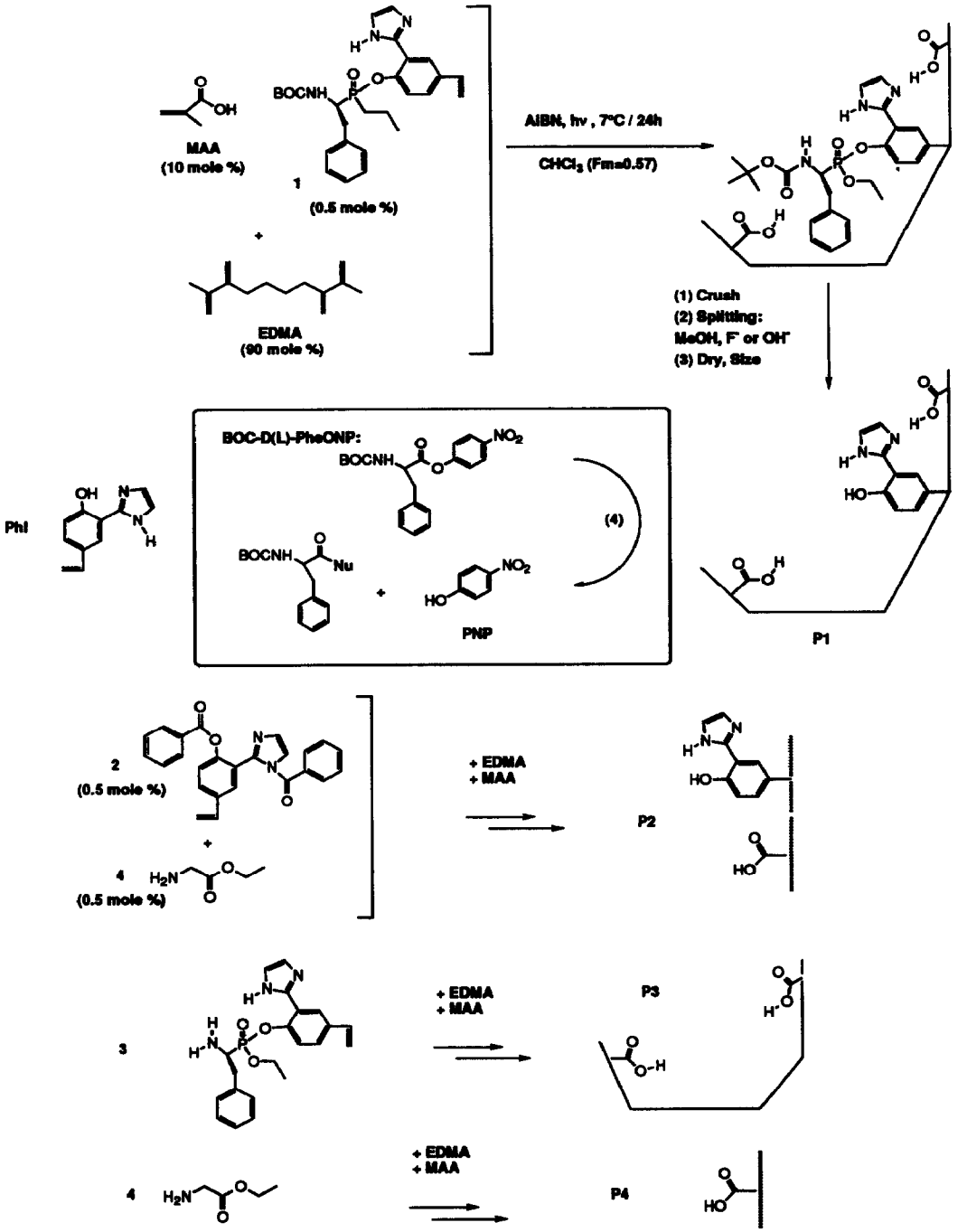
We report a successful application of molecular imprinting[1,2] to the "designed" synthesis of a stereoselective[3] esterolylic catalyst.[4] By allowing a network polymer to form in the presence of a template molecule and subsequently liberating the template, a polymer containing selective binding sites can be prepared. This technique, called Molecular Imprinting, has been used to prepare highly crosslinked network polymers that are selective for enantiomers of carbohydrates[5] and amino acids,[2] as well as β-blockers,[6] and nucleotide bases.[7] In these examples, molecular recognition arises from a combination of covalent, ion-pairing and hydrogen bonds between the functional monomers and the template molecule.

The esterase site was designed to provide a stereoselective binding site for a tetrahedral intermediate in addition to positioning a nucleophile in proximity to the reactive carbonyl group and an imidazole and carboxyl group at hydrogen bond distance to the nucleophile.[8]

The templates[9], synthesized as described elsewhere,[10] were incorporated into the network polymers as shown in Scheme 1. Photo-initiated free radical polymerization of the monomer mixture at 5 °C gave gel-like transparent polymers[11] which were crushed then subjected to various treatments in order to remove the covalently linked template from the polymers. These consisted of (1) wash in methanol/chloroform:1/1 (v/v) for 5 h and (2) soxhlet extraction in methanol overnight. Subsequently they were treated overnight in either (3) CsF (anhydrous) in methanol at 60 °C, (4) sodium carbonate (0.5 M) / methanol : 1/1 (v/v) at room temperature, or (5) sodium hydroxide (1 M) / methanol : 1/1 (v/v). While most of templates **2**, **3** and **4** were liberated by mild treatments (1) and (2), template **1** required treatment (3) (transesterification[12]), (4) or (5) (hydrolysis) for efficient splitting.[13] Following template removal, we anticipated that **1** would provide an active site complementary to the D enantiomer of t-butoxycarbonyl-phenylalanine p-nitrophenylester (Boc-D-PheONP) (see Scheme 1), equipped with a phenol-imidazole catalytic group in proximity to the reactive carbonyl group of the substrate. In the control polymer **P2** however, the carboxylic acid groups responsible for substrate binding are expected to be randomly distributed in the polymer (ion-paired with **4**), tetrahedral complementarity is absent and with the achiral template **2** no stereoselectivity is expected.[14] Control polymer **P3**, equal to **P1** except for the absence of the Boc group in the template, we believed would probe the structural requirements of the site and the simplest control polymer, **P4**, would reveal the role of the catalytic phenol-imidazole group.

The rate of hydrolysis of D or L BocPheONP was measured in the presence of the polymers or soluble phenol-imidazole (PhI) (see Table 1 and Figure 1). The hydrolysis of the complementary substrate, Boc-D-PheONP, was fastest in the presence of **P1**. Moreover the D enantiomer was hydrolyzed faster than the L enantiomer only in the presence of **P1**. *The rate enhancement was higher for the polymers subjected to further template splitting.* This shows that the catalysis takes place in the imprinted active sites. The polymers treated with aqueous base showed the largest rate enhancements. Interestingly, this effect seems to be unrelated to the splitting yield in view of the similar splitting results obtained after treatment (3) and (4).[13] *When the hydrolytic treatment was carried out at elevated temperature, enantioselectivity was completely lost. At the same time, the rate enhancement relative to the control was unaffected.* Apparently the functional groups of the sites are still positioned for substrate binding while the stereochemical complementarity has been lost. The importance of a complementary binding site for catalysis is also seen in the control polymer **P3** (prepared from template lacking the Boc group) which is clearly less efficient than **P1** in catalyzing the hydrolysis (Table 1).

The catalysts described here are truly "designed" since enantioselectivity is predictable. This was further shown in a different model system by using the enantiomer of template **1** where instead L-selectivity was observed.[10] It is further noted from Table 1 that the reactions took place in an acidic medium. Above pH 7, the polymers inhibited the reaction and only at low pH was catalysis observed.


MAA
(10 mole %)
1
(0.5 mole %)
EDMA
(90 mole %)
AIBN, hv , 7°C / 24h
CHCl3 (Fm=0.57)
(1) Crush
(2) Splitting:
MeOH, F- or OH-
(3) Dry, Size
P1
PhI
BOC-D(L)-PheONP:
(4)
PNP
2
(0.5 mole %)
4
(0.5 mole %)
+ EDMA
+ MAA
P2
3
P3
4
P4

Scheme 1

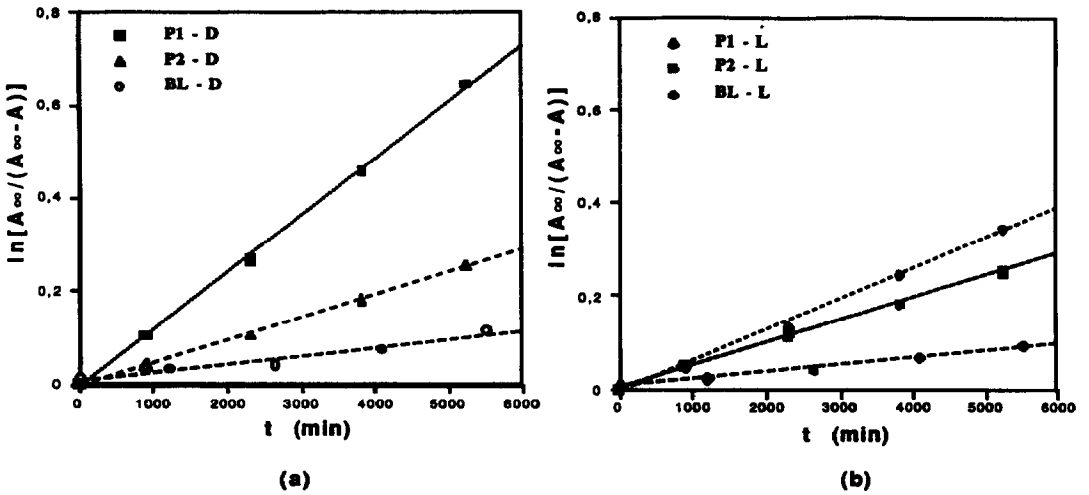


Figure 1. Evaluation of pseudo first order rate constants as described in Table 1 for the hydrolysis of D- (Figure 1a) or L- BocPheONP (0.025 mM) (Figure 1b) in acetonitrile/[potassium phosphate 0.05 M, pH 4.5]: 1/1 (v/v) catalyzed by the Na_2CO_3 aq treated (procedure 4 - 48 h) polymers (P1, P2) or with no added catalyst (BL).

Table 1. Kinetic Data from the Hydrolysis of D- or L- Boc-Phe-ONP in the Presence of Imprinted Polymers Subjected to Various Nucleophilic Treatments

SPLITTING PROCEDURE	$k_D \times 10^3$ (min-1) (a)				k_{P1}/k_{P2} (b)	k_D/k_L (c)	k_{P1}/k_{PhI} (d)
	P1	P2	P3	P4			
(1+2): MeOH	0.026	0.024	0.038	0.014	1.08	1.04	2
(3): CsF/MeOH	0.057	0.035			1.63	1.06	4
(4): Na_2CO_{3aq} 24h	0.140 (±0.001)[e]	0.080 (±0.004)[e]			1.75	1.37	10
(4): Na_2CO_{3aq} 48h	0.122	0.048			2.54	1.85	7[f]
(5): NaOH aq	0.102	0.046	0.056	0.022	2.22	1.60	6[f]
(5): NaOH aq 60°C	0.108	0.046			2.35	1.00	6[f]

The polymers were prepared and treated as described in the text. The reactions were carried out in 5ml vials containing polymer (50mg, <250μm) and solvent (2ml acetonitrile/[potassium phosphate 0.05M, pH 4.5] : 1/1) by adding a concentrated solution of D or L- BocPheONP to give a final concentration of 0.025mM. This results in an excess of the maximum available sites (0.7mM) over substrate. The vials were then shaken mechanically. At regular time intervals the shaking was stopped and the polymer allowed to settle. Analysis by HPLC (C-18 reversed phase, Mobile phase: acetonitrile/[potassium phosphate buffer, 0.01M, pH 6] : 65/35 (v/v)) was then done at regular intervals by injecting 20μl of the supernatant and monitoring by UV (310nm) the substrate peak (BocPheONP) (k'=1.7) and the product peak (p-nitrophenol) (k'=0.6).

(a) Pseudo first order rate constants obtained from the slopes of the linear plots of ln[A∞/(A∞-A)] versus time (see Fig.1) where A is the peak area of p-nitrophenol. A∞ measured after completion of the reaction corresponded to A obtained for a reference solution of p-nitrophenol (0.025 mM).

(b) Ratio of pseudofirst order rate constants using BOC-D-PheONP as substrate.

(c) Enantioselectivity of polymer P1.

(d) k_{PhI} (0.014×10^{-3} min^{-1}) was obtained from the homogeneous catalysis by PhI at a concentration corresponding to the theoretical concentration of polymer active sites.

(e) Average of two independent experiments with the error in parentheses.

(f) A different stirring technique was applied here possibly causing lower rate enhancements compared to blank.

In summary, fully predictable enantioselective catalysis of ester hydrolysis has been achieved with template imprinted polymers containing similar catalytic elements believed to be responsible for the catalytic action of chymotrypsin. Hydrogen bonding is the main driving force in the stereoselective binding step and a phenol-imidazole group responsible for the catalytic action. Although modest, the rate enhancements here reported should be considered as a first step in the development of imprinted polymers for enantioselective catalysis. Simple modifications of the template as well as mechanistic investigations of the catalysis are guide-lines for our future efforts to achieve an efficient mimic of chymotrypsin.

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13. The splitting after treatment (1+2) was **P1 23%, P2 73%, P3 75%** and **P4 70%**. After treatment (3) the splitting was: **P1 98%, P3 98%** and after treatment (4): **P1 89%** and **P3 97%**. This was determined by quantitative 1H-NMR on the extracts (1+2) or by phosphorous analysis (3 and 4).
14. Note that **1** is a better hydrogen bond acceptor than **2**, both in terms of the carbamate (1) versus the ester group (2) of the binding site and in terms of the imidazole (1) versus the bensoylimidazole (2) group of the catalytic group (see Ref. 11). Polymers imprinted with a template having a ground state structure and a template having a transition state like structure for transesterification were compared. It was shown that polymers imprinted with L-phenylalanine ethyl ester (planar carboxylate) only resolved and retained the carboxylate racemate while those imprinted with diethyl-S-1-amino-2-phenyl-ethylphosphonate (tetrahedral phosphonate) only resolved and retained the phosphonate racemate.

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