Enantioselective Ester Hydrolysis Catalyzed by Imprinted Polymers. 2[§,||]

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Highly cross-linked network polymers prepared by molecular imprinting catalyzed enantioselectively the hydrolysis of *N-tert*-butoxycarbonyl phenylalanine-*p*-nitrophenyl ester (BOCPheONP). The templates were designed to allow incorporation of the key catalytic elements, found in the proteolytic enzyme chymotrypsin, into the polymer active sites. Three model systems were evaluated. These were constructed from a chiral phosphonate analogue of phenylalanine (series A, C) or L-phenylalanine (series B) attached by a labile ester linkage to an imidazole-containing vinyl monomer. Free radical copolymerization of the template with methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) gave a highly cross-linked network polymer. The templates could be liberated from the polymers by hydrolysis, giving catalytically active sites envisaged to contain an enantioselective binding site, a site complementary to a transition state like structure (series A, C), and a hydroxyl, imidazole, and carboxylic acid group at hydrogen bond distance. As predicted, the enantiomer of BOCPheONP complementary to the configuration of the template was preferentially hydrolyzed with D-selectivity for the series A polymers ($k_D/k_L = 1.9$) and L-selectivity for the series B polymers ($k_L/k_D = 1.2$). The maximum rate enhancement, when compared with a control polymer, prepared using a benzoyl-substituted imidazole monomer as template, was 2.5, and comparing with the imidazole monomer in solution, a maximum rate enhancement of 10 was observed. The catalytic activity was higher for polymers subjected to the nucleophilic treatment. This was explained by a higher site density and flexibility of the polymer matrix caused by this treatment. In a comparison of template rebinding to polymers imprinted with a template containing either a carboxylate (planar ground state structure) or a phosphonate (tetrahedral transition state like structure) functionality, it was observed that imprinted polymers are able to discriminate between a transition state like and a ground state structure for transesterification. However the influence of transition state stabilization on the observed rate enhancements remains obscure. Only at acidic pH's was catalysis observed, whereas at basic pH's the polymers inhibit the reaction. At a later stage, the catalytic activity of the polymers for nonactivated D- and L-phenylalanine ethyl esters was investigated. A rate enhancement of up to 3 was observed when compared to the blank. Most important, however, the polymers imprinted with a D template preferentially hydrolyzed the D-ethyl ester and exhibited saturation kinetics.

Introduction

The ability of enzymes to catalyze reactions with high stereoselectivity and specificity has led to several applications of enzymes in the chemical and pharmaceutical industry.[1,2] For instance lipases have been successfully used for stereoselective acylation and deacylation of racemates.[3] The development of catalytic antibodies has added the possibility of tailor-making catalysts for a given reaction.[1,4] Examples of catalytic antibodies exhibiting lipase activity with either *R* or *S* specificity have been described.[4] Attempts to mimic hydrolytic enzymes using either synthetic low molecular,[1,5] polymeric,[6,7] or imprinted polymeric[8,9] model systems have been less successful. Recently however, promising rate enhancements were reported for the hydrolysis of nonactivated esters using imprinted polymers as heterogeneous catalysts.[10] Still, only a few attempts have been made to achieve stereoselective hydrolysis using molecularly imprinted polymers.[8c,d,11] The problems in achieving stereoselectivity is related to the common use of nondirectional hydrophobic binding forces as well as the lack of design

§ Dedicated to Professor Günter Wulff on the occasion of his 65th birthday.

|| For part 1, see ref 11.

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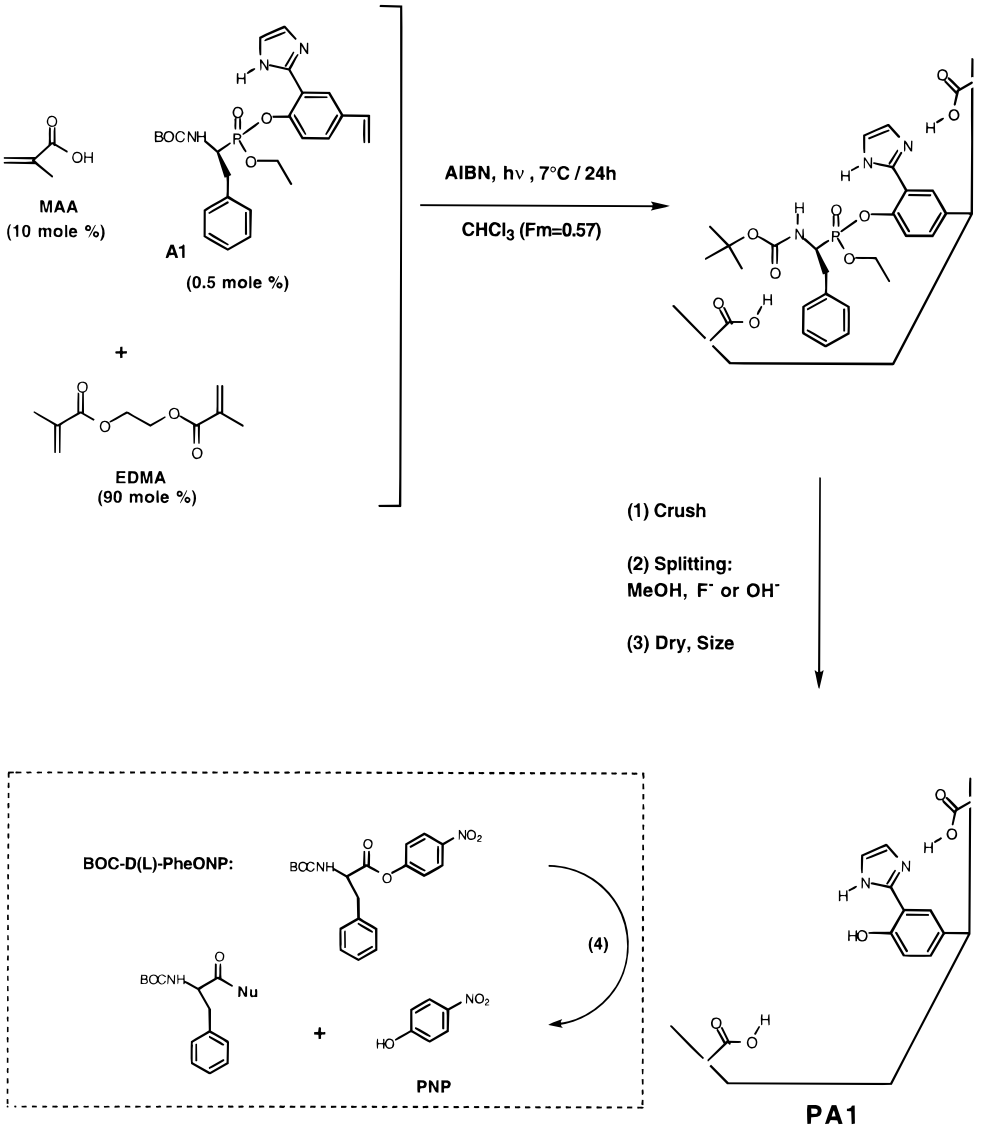
Scheme 1



elements in the preparation of the catalyst.[6,7] A stereospecific binding site is more readily achieved using directed binding forces such as hydrogen bonds.[1]

Our goal was to use the binding energy and selectivity, observed using imprinted polymers,[12] to achieve stereoselective esterolytic catalysis.[11] By allowing a network polymer to form in the presence of a suitable template molecule and subsequently liberating the template, a polymer containing selective binding sites can be prepared. Focusing on the mimic of chymotrypsin, we have synthesized several templates designed to provide an active site incorporating the elements believed to be responsible for the catalytic action of chymotrypsin:[13,14] (1) a stereoselective binding site, (2) a site able to stabilize a tetrahedral intermediate, and (3) a nucleophile in proximity of the reactive carbonyl group as well as an imidazole and a carboxyl group at hydrogen bond distance to the nucleophile. We have earlier reported predictable enantioselective ester hydrolysis of *tert*-butoxycarbonyl phenylalanine-*p*-nitrophenyl ester (BOCPheONP) using these polymers (see Scheme 1).[11]

This paper describes the synthesis of an extended series of template monomers, their incorporation into highly cross-linked methacrylate polymers, removal of the template from the polymers, and finally the evaluation of the esterase activity of monomers and polymers. At a later stage, D- and L-phenylalanine ethyl ester was used as substrates to investigate the polymers ability to catalyze the hydrolysis of nonactivated esters.

Experimental Section

General Procedures. Unless otherwise noted materials were obtained from Aldrich and used without further purification. Standard laboratory reagents and solvents were purified according to standard procedures and the reactions carried out under inert atmosphere (N_2). The NMR spectra were recorded with a 300 MHz (GE, QE-300) instrument. ^{13}C NMR spectra were obtained at 75.4 MHz. Chemical shifts are reported in δ units utilizing TMS as an internal reference for ^{1}H NMR and the solvent signal for ^{13}C NMR. The FTIR spectra were

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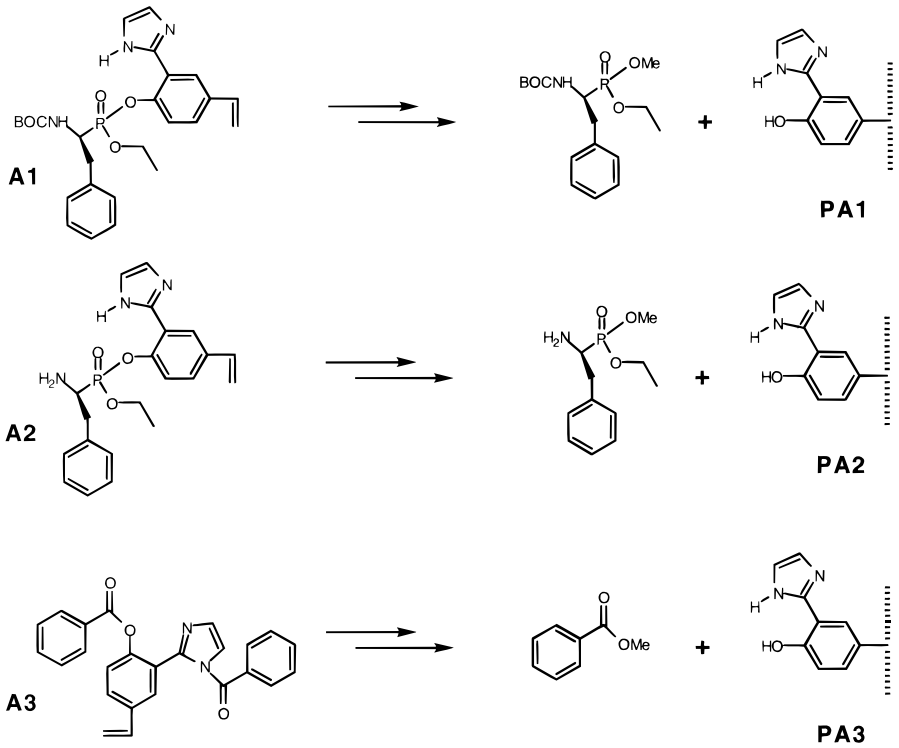
Scheme 2



recorded on a Bomem Michelson FTIR spectrometer, and the solid state ^{13}C NMR spectra were kindly recorded by Professor Thomas Hjertberg and Dr. Staffan Schantz at Chalmers Institute of Technology, Sweden.

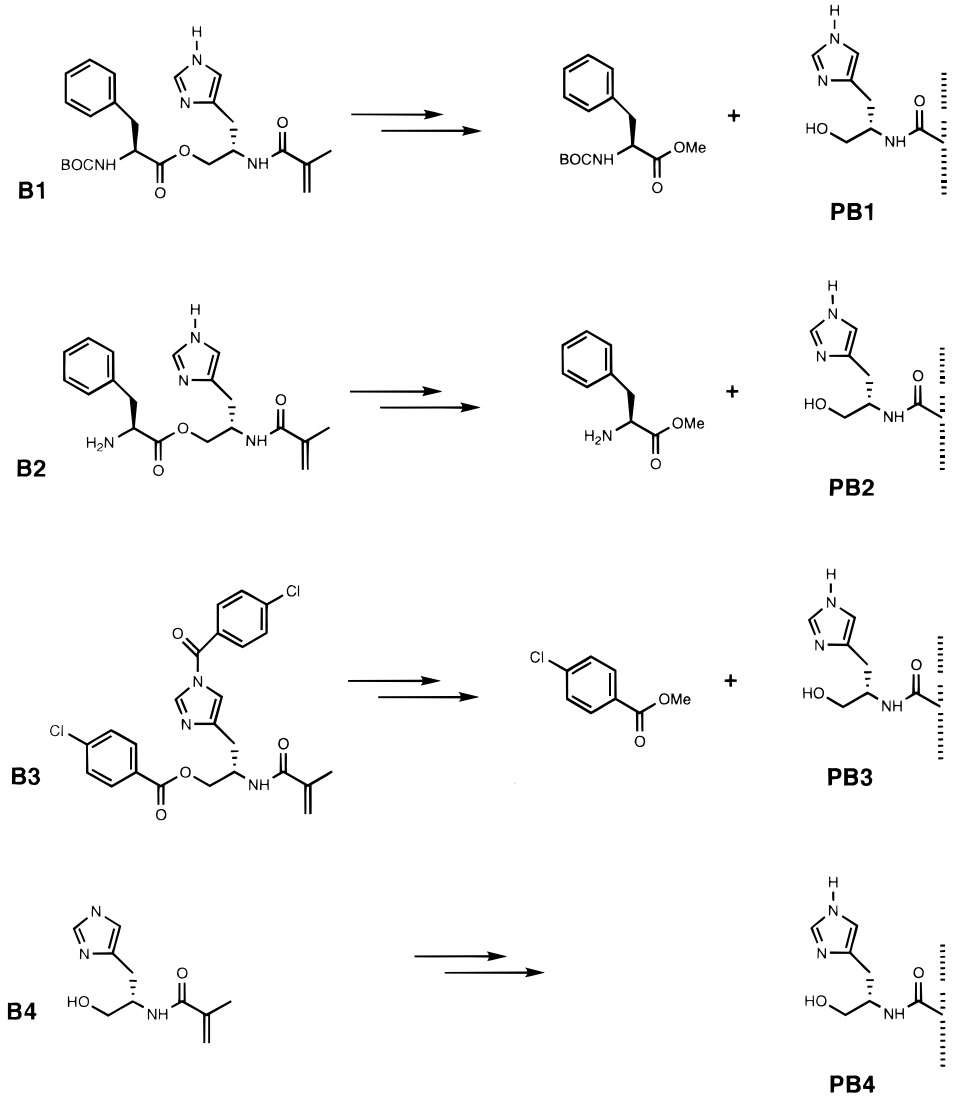
Monomer Synthesis (Series A) (Scheme 6). 2-(2′-Hydroxy-5′-bromophenyl)imidazole (5). *p*-Bromosalicylaldehyde (10 g, 0.05 mol) and glyoxal (trimertetrahydrate) (10.5 g, 0.05 mol) in 200 mL of glacial acetic acid were brought to reflux under nitrogen, and then ammonium acetate (36 g, 0.05 mol) was added followed by continued reflux for 4 h. After cooling to room temperature the dark brown solution was poured into 2 L of water, giving a dark brown precipitate. After filtration on Celite, the deep red filtrate was made basic with aqueous ammonia, and a yellow precipitate was formed. Filtration and drying gave 7.9 g (66%) of **5** as a light brown solid. Purification on a silica column failed, but active carbon may be applied however with some loss of product. The purity was sufficient for the following reaction. TLC: $R_f = 0.5$ (silica plates, $CHCl_3$/MeOH: 9/1), mp 240−242 °C. ^1H NMR (DMSO-d_6 + 3 drops CD_3COOD): δ 0.10 (bs, OH, NH), 6.82 (d, J = 8.7 Hz, 1H, H3′), 7.13 (s, 2H, H4,5), 7.23 (d, J = 8.5, 1H,H4′), 8.03 (s, 1H, H6′). ^{13}C NMR (DMSO-d_6 + 3 drops CD_3COOD): δ 110.68 (C5′), 115.97 (C1′), 119.48 (C3′), 122.32 (C4,5), 126.78 (C6′), 132.64 (C4′), 145.12 (C2′), 156.19 (C2). HRMS calcd for $C_9H_7N_2OBr$: 237.9742. Found: 237.9723.

***N*-Benzoyl-2-(2′-benzoxy-5′-bromophenyl)imidazole (6)**. To **5** (1 g, 4.2 mmol) and triethylamine (1.2 mL, 8.4 mmol) in dry THF was added benzoyl chloride (1.0 mL, 8.4 mmol), and the mixture stirred at room temperature overnight. After filtration of the salt the filtrate was evaporated to dryness, giving crude **6** as a yellow solid, which was recrystallized by dissolving the solid in 8 mL of benzene and adding 100 mL of hexane; 1.37 g (67%) of light yellow crystals precipitated. Mp: 132−134 °C. ^1H NMR ($CDCl_3$): δ 7.05−7.14 (m, 2H), 7.22−7.28 (m, 5H), 7.48−7.56 (m, 5H), 7.78−7.86 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 118.75, 121.02, 123.94, 126.66, 128.20, 128.25, 128.29, 129.28, 129.89, 130,37, 130,83, 133.08, 133.56, 133.78, 133.82, 143.86, 147.02, 163.73, 166.55. HRMS calcd for $C_{23}H_{15}BrN_2O_3$: 446.0266. Found: 446.0249.

***N*-Benzoyl-2-(2′-benzoxy-5′-ethenylphenyl)imidazole (A3)**. Palladium(tetrakistriphenylphosphine) (0.052 g, 0.045 mmol) was added to a solution of **6** (1.0 g, 2.24 mmol), vinyl-(tri-*n*-butyl)tin (0.65 mL, 2.24 mmol), and a trace of 2,6-di-*tert*-butyl-4-methylphenol in toluene (15 mL) under nitrogen, and the solution was brought to reflux resulting in a color change from yellow to red. The reaction was followed on GC (methylsilica column, T_c = 180 °C) by measuring the consumption of vinyl(tributyl)tin (R_f = 0.8) and the appearance of product peaks at k' = 1.38 and 1.70. After 5 h all of the reagent had been consumed. After filtration the solvent was evaporated at room temperature and the oil dissolved in 10 mL of acetonitrile. This was then washed with hexane and then evaporated giving 1.00 g of an oil that crystallized slowly. Recrystallization from benzene/hexane gave 0.47 g (53%) of light yellow crystals. Mp: 124−127 °C. ^1H NMR ($CDCl_3$): δ 5.25 (d, J = 10.9 Hz, 1H, vinyl), 5.74 (d, J = 17.6 Hz, 1H, vinyl), 6.68 (dd, J = 10.9, 17.7 Hz, 1H, vinyl), 7.02−7.11 (m, 2H, Ph), 7.19−7.27 (m, 5H, Ph), 7.42−7.53 (m, 5H, Ph), 7.65−7.80 (m, 3H, Ph). ^{13}C NMR ($CDCl_3$): δ 113.67, 119.94, 121.47, 124.07, 126.54, 126.99, 127.15, 127.20, 127.24, 127.64, 127.85, 128.56, 129.16, 129.78, 132.44, 132.61, 133.97, 134.11, 134.60, 143.60, 146.26, 162.73, 165.47. HRMS calcd for $C_{25}H_{18}N_2O_3$: 394.1317. Found: 394.1309.

2-(2′-Hydroxy-5′-ethenylphenyl)imidazole (7). To a solution of **A3** (0.59 g, 1.49 mmol) in ethanol (3 mL) was added sodium dissolved in ethanol (3 mg/mL). A yellow color appeared, and a strongly UV-active product spot appeared immediately on TLC (silica plates, $CHCl_3$/MeOH: 9:1, R_f = 0.6). Ethanol was evaporated and the resulting oil dissolved in aqueous NaOH (25 mL, 0.2 M). This was extracted with hexane. To the aqueous phase was added 1 M HCl until a yellow precipitate was formed at pH 10−11. The precipitate went back into solution at pH below 4−5. The pH was adjusted to 7−8 followed by extraction of the product into ethyl acetate. Drying on $MgSO_4$ and evaporation gave crude **7** as a brown solid. This was purified by column chromatography (silica, 70 g using $CHCl_3$/MeOH, 9:1, as eluent), giving 0.20 g (72%) of pure **7** as a light beige solid. HPLC: RP-18 column using MeOH/[phosphate buffer 0.05 M, pH 2.5]: 1:2 (v/v) as eluent gave a single peak ($k' \simeq 1.5$). Mp: 131−134 °C. ^1H NMR ($CDCl_3$ + 3 drops CD_3OD): δ 5.10 (bs, 2H, NH,OH), 5.12 (d, J = 10.9 Hz, 1H, vinyl), 5.65 (d, J = 17.5 Hz, 1H, vinyl), 6.56 (dd, J = 10.9, 17.6 Hz, 1H, vinyl), 6.96 (d, J = 8.5 Hz, 1H, H3, Ph), 7.08 (s, 2H, H4,5, imidazole), 7.30 (d, J = 8.5 Hz, 1H, H4,

Scheme 3



Ph), 7.77 (s, 1H, H6,Ph). ^{13}C NMR ($CDCl_3$ + 3 drops CD_3OD): δ 111.09 (vinyl), 113.54 (C1′), 116.84 (C3′), 121.02 (C4,5), 121.99 (C6′), 127.36 (C4′), 128.84 (C5′), 135.94 (vinyl), 146.15 (C2′), 156.03 (C2). HRMS calcd for $C_{11}H_{10}N_2O$: 186.0793. Found: 186.0794.

Template Synthesis (Series A) (Scheme 5). *S*- and *R*-1-amino-2-phenylethylphosphonate (**1S** and **1R**) were synthesized following the procedures by Oleksyszyn et al.[15] and Kafarski et al.[16] The diastereomers were separated by converting into tartrate salts and fractional crystallization.[17] The amino group was protected using bis-*tert*-butoxycarboxylic anhydride (**2S**) and converted to the diethyl ester (**3S**) using triethylorthoformate and then to the monoester (**4S**) by alkaline hydrolysis.

***S*-Ethyl, 2-(2′-Imidazolyl)-4-ethenylphenyl (1-(*N*-*tert*-Butoxycarbonylamino)-2-phenyl)ethylphosphonate (A1).** **4S** (197 mg, 0.6 mmol), **7** (112 mg, 0.6 mmol), triethylamine (167 μL, 1.2 mmol), and Castros BOP reagent[18] (benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate) (Sigma) (265 mg, 0.6 mmol) in CH_2Cl_2 (10 mL) were stirred at room temperature under nitrogen. The reaction was followed on HPLC (RP-18 column, eluent: MeOH/[potassium phosphate buffer, 0.05 M, pH 3]: 2:1 (v/v)) due to product decomposition. After 15 h ca. 50% conversion was observed. Additional BOP reagent (221 mg, 0.5 mmol) and triethylamine (70 μL, 0.5 mmol) were therefore added, and the reaction was allowed to proceed for a total of 24 h, at which point over 80% conversion was observed. Two adjacent spots on TLC (silica plates, eluent: $CHCl_3$/MeOH: 9:1, R_f = 0.5) as well as on HPLC (k' = 1.6) indicated the presence of diastereomers. Brine (10 mL) was added and the product extracted into ethyl acetate. The organic phase was washed with 1 M HCl, saturated $NaHCO_3$, and brine, dried on $MgSO_4$, and evaporated, giving crude product as a yellow oil. Rapid purification was done by flash chromatography on silica gel (eluent: $CHCl_3$/MeOH, 9:1), giving 270 mg (91%) of **A1** as an oil that solidified to a light yellow foam upon further evacuation. The hygroscopic product was stored dry under nitrogen in freezer. $[\alpha]^{23}_D$ = +4.33° [*c* 2, CH_2Cl_2]. ^{1}H NMR ($CDCl_3$ + 1 drop CD_3OD): δ 0.93, 1.14, 1.15 (3t, J = 7.0 Hz, 3H, $-CH_2CH_3$, $I_{1/2/3}$ = 40/30/30), 1.24, 1.26 (2s, 9H, $-C(CH_3)_3$, $I_{1/2}$ = 63/37), 2.80−2.88 (m, 1H, $-CH_2-$Ph), 3.07−3.14 (m, 1H, $-CH_2$Ph), 3.70−3.88 (m, 2H, $-CH_2CH_3$), 4.03−4.09 (m, 1H, −CH<), 4.38−4.45 (m, 1H, −NH−), 5.24, 5.25 (2d, J = 10.9 Hz, 1H, vinyl, $I_{1/2}$ = 63/38), 5.74 (d, J = 17.6 Hz, 1H, vinyl), 6.66 (dd, J = 17.5, 10.9 Hz, 1H, vinyl), 7.11−7.47 (m, 10H, PhIm), 7.99 (s, 1H, H1−Im). ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD): δ 15.66 15.94 (2d, J = 5.0,5.4 Hz, $-O-CH_2-CH_3$), 27.88 ($C(CH_3)_3$), 34.38, 34.45 ($-CH_2-$Ph), 47−50 (d, C−P), 64.45, 64.55 (2d, J = 29.7, 29.1 Hz, $-O-CH_2-$), 80.43 ($-C(CH_3)_3$), 114.61, 114.78 (vinyl),

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(16) Kafarski, P.; Lejczak, B.; Szewcyk, J. *Can. J. Chem*. **1983**, *61*, 2425.

(17) Prepared by reaction of tartaric acid and benzoyl chloride as described by: Butler, C. L.; Cretcher, L. *J. Am. Chem. Soc.* **1933**, *55*, 2605.

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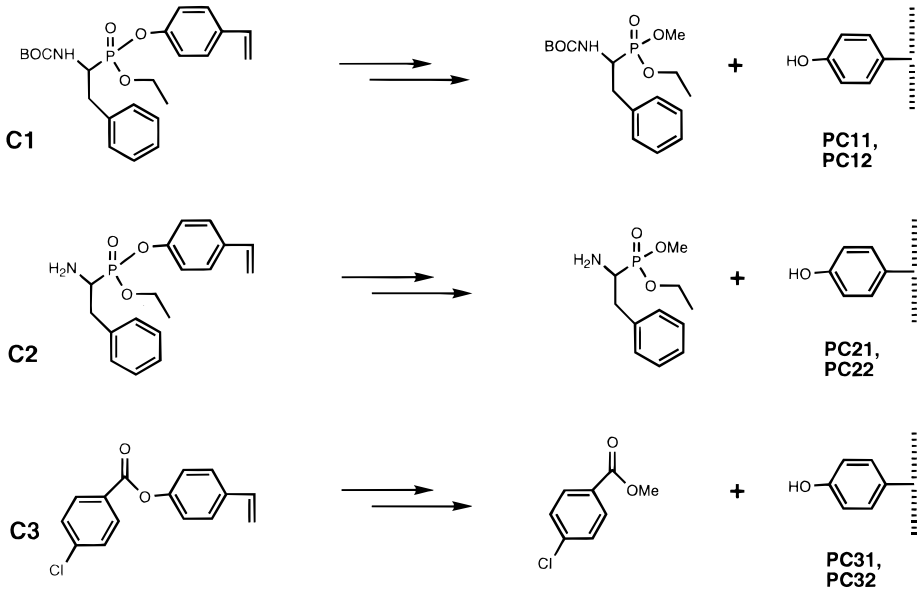
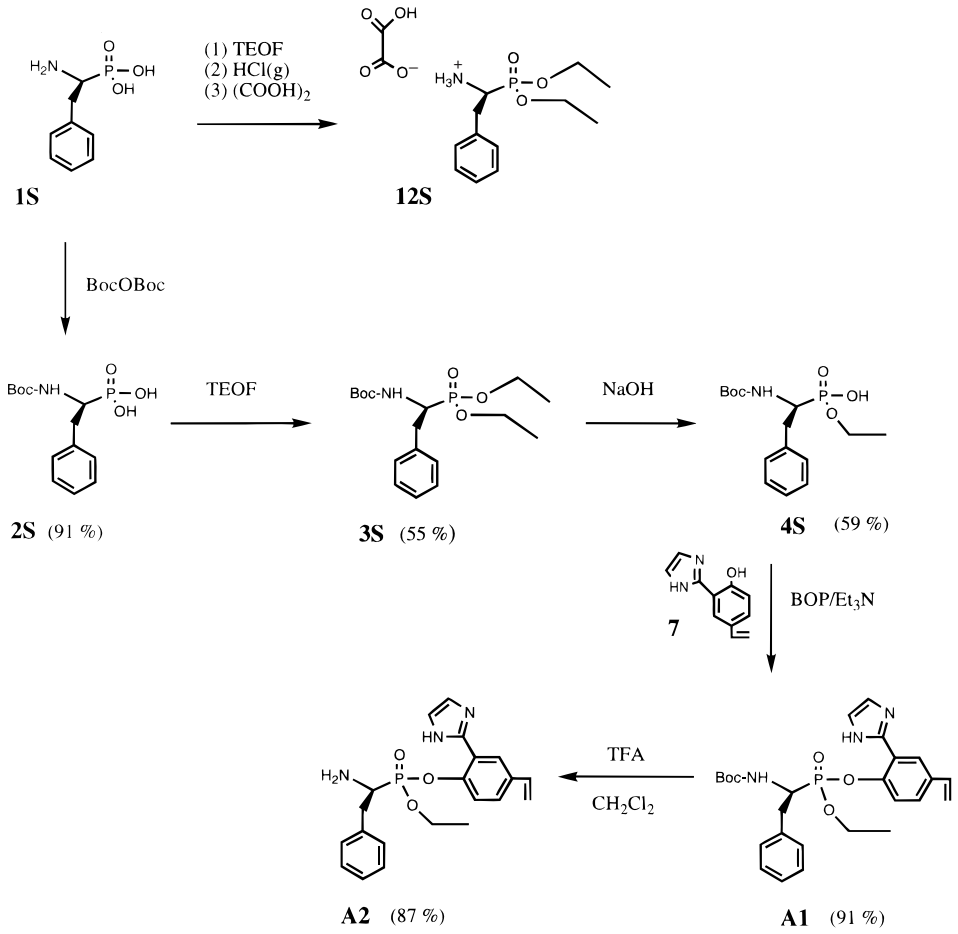
Scheme 4



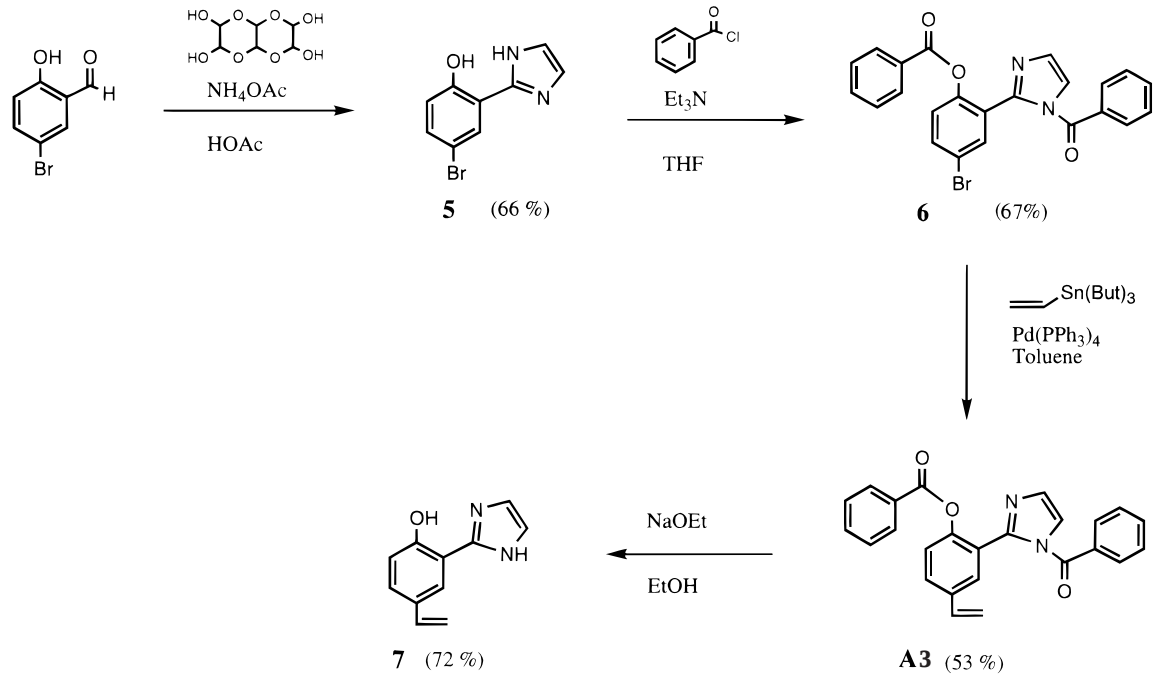
Scheme 5



121.00, 121.03, 121.07, 126.75, 126.94, 127.11, 127.26, 127.71, 128.30, 128.85, 128.93, 135.01, 135.05 (−Ph, −PhIm), 135.90 (vinyl), 142.04 (−CO−). HRMS calcd for $C_{26}H_{32}N_3O_5P$: 497.2080. Found: 497.2065. Anal. Calcd for $C_{26}H_{32}N_3O_5P$: C 62.7; H 6.5; N 8.4; P 6.2. Found: C 60.6, H 6.4, N 8.3; P 6.5.

***S*-Ethyl, 2-(2′-Imidazolyl)-4-ethenylphenyl 1-Amino-2-phenylethylphosphonate (A2).** **A1** (200 mg, 0.4 mmol) was dissolved in an ice cold mixture of freshly distilled trifluoroacetic acid (TFA, distilled in the presence of TFA anhydride 0.05%) and CH_2Cl_2 (1:1, v/v) and stirred for 30 min at room temperature. The TFA and CH_2Cl_2 were evaporated and the resulting oil taken down four times in anhydrous CH_2Cl_2 and one time in ether, giving 210 mg (87%) of the TFA salt of **A2** as a light brown solid. This can be converted to the free base by rapid extraction in cold CH_2Cl_2/$NaHCO_3$(aq) (saturated). For the TFA salt: $[\alpha]^{23}_D = +4.35°$ [*c* 2, CH_2Cl_2]. TLC ($CHCl_3$/MeOH, 9:1) R_f = 0.5 (two close spots colored purple by ninhydrin). ^{1}H NMR ($CDCl_3$ + 1 drop CD_3OD): δ 0.83, 1.05,

Scheme 6



1.19, 1.27, (3t+1m, J = 7.0, 6.8, 7.0 Hz, 3H, −O−CH_2−CH_3, $I_{1/2/3}$ = 50/32/18), 3.21−3.33 (m, 2H, −O−CH_2−), 3.55−3.80 (2m, 2H, −CH_2−Ph), 3.95−4.20 (m, 1H, −CH−P), 5.35 (d, J = 10.9 Hz, 1H, vinyl), 5.81 (d, J = 17.6 Hz, 1H, vinyl), 5.8−7.0 (bs, 5H, exchangeable), 6.66 (dd, J = 10.9, 17.6 Hz, 1H, vinyl), 7.22−7.87 (m, 10H, Ph, Im). ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD): δ 15.39, 15.46, 15.52, 15.59 (−O−CH_2−CH_3), 33.88, 33.93, 33.95, (−CH_2−Ph), 48−50 (d, C−P), 65.69, 65.78, 65.90 (−O−CH_2−), 108.65, 113.12, 115.98, 116.03, 116.75, 116.85, 117.17, 118.22, 120.06, 121.93, 122.81, 124.78, 127.76, 127.89, 127.94, 128.62, 128.90, 128.96, 129.11, 129.13, 129.28, 130.28, 130.86, 131.14, 133.29, 133.51, 133.63, 133.71, 134.67, 136.43, 136.73, 139.96, 140.00 (aromatic), 144.81, 144.95 (−CO−), 155.18, 160.38, 160.85 (CF_3CO). FAB-MS calcd for $C_{21}H_{24}N_3O_3P$: 398.1633, Found: 398. Anal. Calcd for $C_{25}H_{26}F_6N_3O_7P$ + $6H_2O$: C 42, H 3.66, N 5.87, F 15.94, P 4.33. Found: C 41.98, H 3.98, N 5.24, F 15.02, P 4.11.

Monomer Synthesis (Series B) (Scheme 7). *S*-2-Amino-3-(5′-imidazolyl)propanol + 2HCl (**9**) was prepared from L-histidine methyl ester·2HCl (**8**), which was prepared as described by Davis.[19] The carboxylic group was reduced using sodium borohydride and the product isolated as the hydrochloride salt.[20]

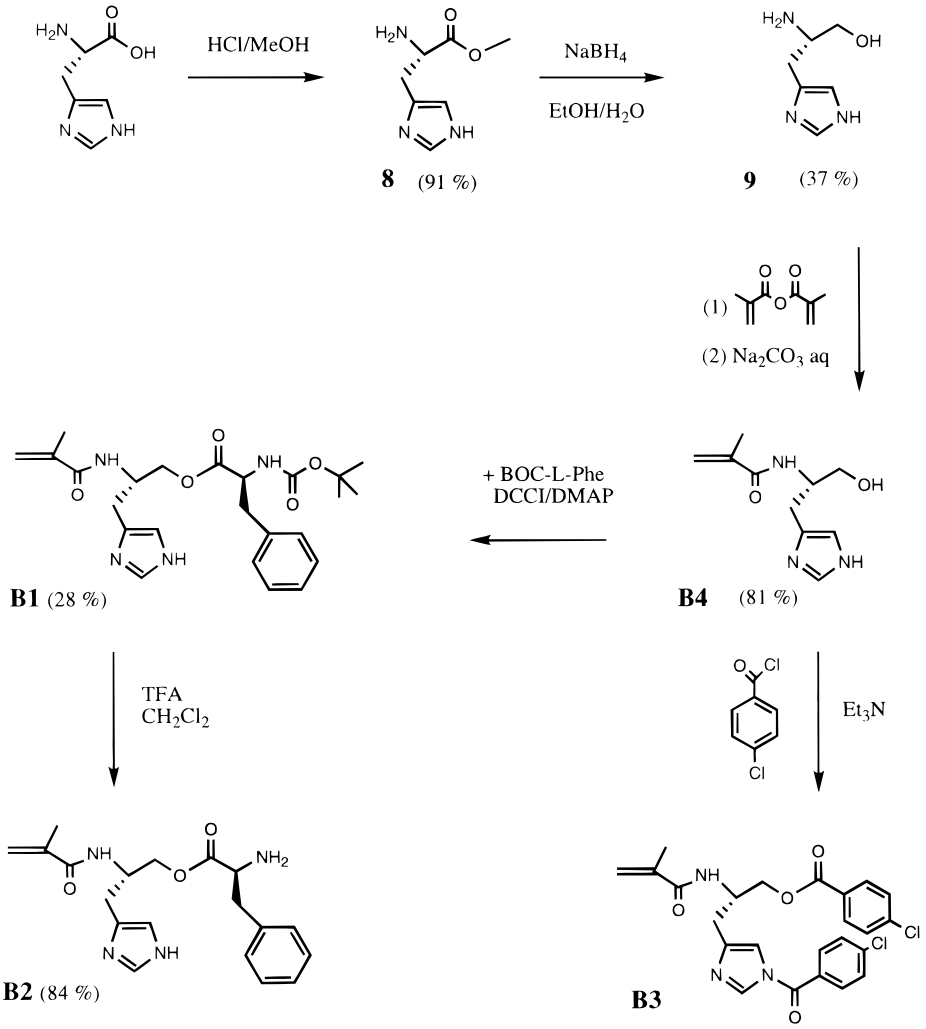
***S*-2-(*N*-Methacryloylamino)-3-(5′-imidazolyl)propanol-(*N*-methacryloyl-L-histidinol) (B4)**. To **9** (1.3 g, 6.1 mmol) in NaOH(aq) (20 mL, 1 M) was added dropwise a solution of methacryloyl anhydride (0.9 mL, 16.1 mmol) in dioxane (5 mL). After 1 h stirring, additional anhydride (0.5 equiv, 3.05 mmol) and NaOH (6 mL, 1 M) were added. The reaction could be followed on TLC (silica plates, eluent: $CHCl_3$/MeOH/H_2O/HOAc, 6:4:1:1) as the disappearance of the ninhydrin-active spot of **9** (R_f = 0.3) and the appearance of a UV-active spot (R_f = 0.6). After 7 h, the pH was adjusted to 2−3 and the solution extracted with ether. Then the pH was adjusted to 7 and the solution lyophilized. The product was dissolved in ethanol, and the salts were removed by filtration. Evaporation gave crude **B4** as an oil that was purified by column chromatography on silica (eluent: MeOH). This gave 1.03 g (81%) of **B4** as a clear oil that crystallized slowly to a hygroscopic white solid. This can be recrystallized from THF/MeOH. Mp: 144−148 °C (polymerization). $[\alpha]^{23}_D$ = −16.9° [c 2, MeOH]. ^{1}H NMR (CD_3OD + 1 drop D_2O): δ 1.88, 1.90, 1.92 (3s, 3H, −CH_3, $I_{1/2/3}$ = 66/15/19), 2.85 (m, 2H, −CH_2−Im), 3.53−3.63 (m, 2H, −CH_2OH), 4.20−4.22 (m, 1H, −CH<), 5.33 (s, 1H, zH−vinyl), 5.62 (s, 1H, eH−vinyl), 6.87 (s, 1H, H4−Im), 7.63 (s, 1H, H2−Im). ^{13}C NMR (CD_3OD + D_2O): δ 18.85 (−CH_3), 28.97 (−CH_2−Im), 52.84 (−CH<), 64.08 (−CH_2OH), 118.54 (H_2C=), 120.66 (C4−Im), 134.93 (C5−Im), 135.97 (=C(CH_3)−CO), 141.19 (C2−Im), 171.42 (−CO−). HRMS calcd for $C_{10}H_{15}N_3O_2$: 209.1164. Found: 209.1150.

Template Synthesis (Series B). ***S*-*O*-(*N*-*tert*-Butoxycarbonyl-L-phenylalanyl)-2-(*N*-methacryloylamino)-3-(5′-imidazolyl)propanol (B1)**. *tert*-Butoxycarbonyl-L-phenylalanine (133 mg, 0.5 mmol), dicyclohexylcarbodiimide (DCCI) (124 mg, 0.6 mmol), **B4** (105 mg, 0.5 mmol), and (dimethylamino)pyridine (DMAP) (12 mg, 0.1 mmol) were dissolved in order in DMF (5 mL) and allowed to react overnight. After 15 min a precipitate was formed. After 12 h, additional DCCI was added (42 mg, 0.2 mmol) and the reaction allowed to proceed for an additional 6 h. EtOAc (50 mL) was added followed by extraction with 1 M $NaHCO_3$, drying of the organic phase ($MgSO_4$), and evaporation, giving 0.244 g of crude product as an oil. Purification by column chromatography (silica, eluent: $CHCl_3$/MeOH, 2:1), removing unreacted DCCI adduct of BOC-L-Phe, gave 64 mg (28%) of **B1** as a clear oil. $[\alpha]^{23}_D$ = −2.37° [c 2, $CHCl_3$]. ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H, −C(CH_3)$_3$), 1.97 (s, 3H, CH_3−C=), 2.85 (m, 2H, −CH_2−Ph), 3.08 (m, 2H, −CH_2−Im), 4.10 (m, 2H, −CH_2−O−), 4.48 (m, 2H, −CH<), 5.23 (d, J = 7.4 Hz, 1H, −NH−), 5.36 (s, 1H, H_2C=), 5.78 (s, 1H, H_2C=), 6.83 (s, 1H, H5−Im), 7.17−7.35 (m, 5H, Ph), 7.42 (bs, 1H, H1−Im), 7.57 (s, 1H, H2−Im). ^{13}C NMR ($CDCl_3$): δ 18.50 (H_3C−C=), 47.97 (−CH_2−Im), 54.73 (−CH<His), 64.80 (−CH_2−O−), 64.84 (−CH<Phe), 80.08 (−C(CH_3)$_3$), 120.34 (C4−Im), 127.00 (C4−Ph), 128.48 (H_2C=), 128.56 (C3−Ph), 129.12 (C2−Ph), 129.28 (C1 Ph), 135.08 (=CCH_3CO), 135.92 (C5−Im), 139.38 (C2−Im), 155.33 (−CO− BOC), 168.26 (−CO− amide), 171.98 (−CO− ester). HRMS calcd for $C_{24}H_{32}N_4O_5$: 456.2372. Found: 456.2357.

***S*-*O*-(L-Phenylalanyl)-2-(*N*-methacryloylamino)-3-(5′-imidazolyl)propanol (B2)**. **B1** (25 mg, 55 μmol) was dissolved in ice cold $CHCl_3$/TFA, 1:1 (v/v) (4 mL), and left to react at room temperature for 30 min. After evaporation the resulting oil was taken down twice in $CHCl_3$. Addition of ether resulted in a white precipitate. The ether was removed with pasteur pipet and the solid dried, 27 mg (84%). The free base could be obtained by extraction into EtOAc with 1 M $NaHCO_3$. Drying ($MgSO_4$) and evaporation gave the free base as an oil,

(19) Davis, N. C. *J. Biol. Chem*. **1956**, *223*, 935.
(20) Bentley, K. W.; Crease, E. H. *Org. Prep. Proc. Int.* **1973**, *5*, 5.

Scheme 7



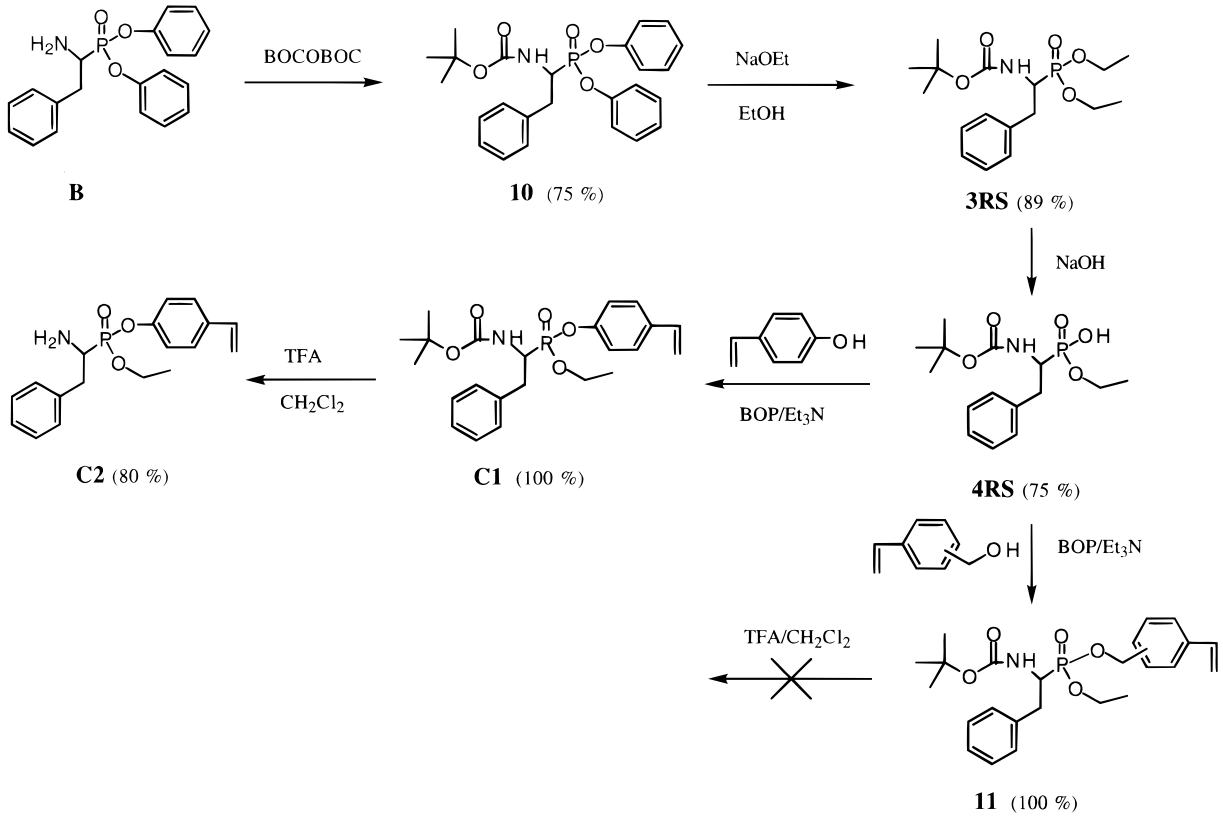
which gave a purple ninhydrin reaction on TLC (silica plates, eluent: $CHCl_3$/MeOH/HOAc/H_2O, 6:4:1:1, R_f = 0.6). Characterization of the TFA salt of **B2**: $[\alpha]^{23}_D = -14.7°$ [*c* 1.35,$CHCl_3$/MeOH, 3:1]. ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, $-CH_3$), 2.86−3.06 (m, 2H, $-CH_2Ph$), 3.23 (m, 2H, $-CH_2-Im$), 4.00−4.45 (m, 9H, $-CH_2-O-$, $-CH<$, $-NH_3+$, ImH2+), 5.29 (s, 1H, $H_2C=$), 5.60 (s, 1H, $H_2C=$), 7.15−7.32 (m, 6H, Ph, H4−Im), 8.37 (s, 1H, H2−Im). ^{13}C NMR ($CDCl_3$): δ 14.89 ($-CH_3$), 25.45 ($-CH_2-$Im), 36.25 ($-CH_2-$Ph), 48.73 (−CH<His), 65.79 ($-CH_2-$O−), 66.68 (−CH<Phe), 116.74, 120.80, 127.85, 128.94, 129.11, 130.46, 132.28, 133.34, 138.66 (Ph, Im, vinyl), 167.79 (−CO−NH−), 169.48 (−COO−). FAB-MS calcd for $C_{19}H_{24}N_4O_3$: 356. Found: 356.2.

Template Synthesis (Series C) (Scheme 8). Diphenyl (1-(*N-tert*-Butoxycarbonylamino)-2-phenyl)ethylphosphonate (10). A solution of **B** (3.4 g, 9.6 mmol) (obtained as intermediate in the synthesis of **1R** and **1S**) and BOCOBOC (2.30 g, 10.5 mmol) in THF (50 mL) was refluxed overnight. The reaction was followed on TLC (silica plates, eluent: $CHCl_3$/MeOH, 9:1) with a product spot at R_f = 0.8. Additional BOCOBOC was added (0.44 g, 2 mmol) and the reaction allowed to continue for an additional 5 h. After filtration and evaporation the residue was dissolved in $CHCl_3$ (50 mL) and washed with 1 M HCl, 1 M $NaHCO_3$, and brine. The organic phase was dried ($MgSO_4$) and evaporated, giving 5.3 g of crude **10** as an oil that solidified overnight to a light yellow solid. This was recrystallized in hexane, giving 3.25 g (75%) of **10** as a white solid. Mp: 100−103 °C. ^{1}H NMR ($CDCl_3$): δ 1.12, 1.27, 1.50 (3s, 9H, $-C(CH_3)_3$, $I_{1/2/3}$: 17/70/13), 2.80−3.10 (m, 1H, $-CH_2-$Ph), 3.30−3.45 (m, 1H, $-CH_2-$Ph), 4.35−4.60, 4.60−4.88 (2m, 1H, −CH<, $I_{1/2}$ = 24/76), 5.19, 5.42 (2d, J = 10 Hz, −NH−, $I_{1/2}$ = 76/24), 7.05−7.38 (m, 15H, −Ph). ^{13}C NMR ($CDCl_3$): δ 27.27, 27.62, 28.00 ($-C(CH_3)_3$), 35.97, 36.03 ($-CH_2-$Ph), 48.53 (d, J = 156 Hz, −C−P), 79.91 ($-C(CH_3)_3$), 120.33, 120.39, 120.50, 120.55, (C2,6-PhO), 125.10, 125.27 (C4−PhO), 126.7, 128.29, 129.26, 129.53, 129.69, (C2,6-Bzl), 135.91, 136.10 (C1−Bzl), 149.87, 150.00, 150.19, 150.32 (C1−PhO), 154.70, 154.80 (CO−). HRMS calcd for $C_{25}H_{38}NO_5P$: 453.1705. Found: 453.1687.

Diethyl (1-(*N-tert*-Butoxycarbonylamino)-2-phenyl)-ethylphosphonate (3RS). 10 (5.18 g, 11.3 mmol) dissolved in ethanol (30 mL) was added to a solution of sodium (1.3 g, 57 μmol) in ethanol (30 mL). The reaction was allowed to proceed at room temperature for 2 h. Then ether was added to 500 mL and extraction done with 1 M NaOH and brine. After drying ($MgSO_4$) and evaporation **3RS** was obtained as a light yellow oil (3.6 g, 89%). ^{1}H NMR ($CDCl_3$): δ 1.18, 1.31 (m, 15H, $-C(CH_3)_3$, $-CH_2-CH_3$), 2.85−2.93 (m, 1H, $-CH_2-$Ph), 3.15−3.28 (m, 1H, $-CH_2-$Ph), 4.05−4.20 (m, 4H, $-CH_2-CH_3$), 4.25−4.45 (m, 1H, −CH<), 4.74 (d, J = 10.1 Hz, −NH−), 7.15−7.31 (m, 5H, −Ph). ^{13}C NMR ($CDCl_3$): δ 16.25, 16.32, 16.38 ($-CH_2-CH_3$), 28.08 ($-C(CH_3)_3$), 36.06, 36.12 ($-CH_2-$Ph), 47.72 (d, J = 156.1 Hz, C−P), 62.31, 62.62, 62.71 ($-O-CH_2-$), 79.81 ($-C(CH_3)_3$), 126.57 (C4−Ph), 128.26 (C3,5-Ph), 129.20 (C2,6-Ph), 136.59, 136.77 (C1−Ph), 154.87, 154.96 (−CO-). HRMS calcd for $C_{17}H_{28}NO_5P$: 357.1705. Found: 357.1722.

H, Ethyl (1-(*N-tert*-Butoxycarbonylamino)-2-phenyl)-ethylphosphonate (4RS). A solution of **3RS** (3.6 g, 10 mmol) in 2 M NaOH (15 mL) and ethanol (30 mL) was heated at 70 °C overnight. Water was added and the solution extracted with ether. The aqueous phase was acidified to pH 1.2 and the

Scheme 8



product extracted into EtOAc. After drying ($MgSO_4$) and evaporation, 2.48 g (75%) of **4RS** was obtained as a white solid. Mp: 130−132 °C. ^{1}H NMR ($CDCl_3$): δ 1.20−1.35 (m, 12H, $-CH_3$), 2.77−2.89 (m, 1H, $-CH_2-$Ph), 3.17−3.29 (m, 1H, $-CH_2-$Ph), 4.08−4.21 (m, 2H, $-CH_2-CH_3$), 4.30−4.42 (bm, 1H, −CH<), 5.17, 5.95 (2bd, J = 9.0 Hz, 1H, −NH−, $I_{1/2}$ = 2.4). ^{13}C NMR ($CDCl_3$): δ 16.11, 16.19, 16.28 ($-CH_2CH_3$), 27.75, 27.86, 28.03 ($-C(CH_3)_3$), 35.64, 35.69, 35.85, 35.89, 35.92 ($-CH_2-$Ph), 47.89, 50.53 (2D, J = 158.1, 155.8 Hz, C−P), 62.29, 62.34, 62.52, 62.61 ($-O-CH_2-CH_3$), 79.73, 80.85, ($-C(CH_3)_3$), 126.43 (C4−Ph), 128.17 (C3,5-Ph), 129.13 (C2,6-Ph), 136.60, 136.79, 137.16 (C1−Ph), 155.08, 155.17, 155.97 (−CO−). HRMS calcd for $C_{15}H_{24}NO_5P$: 329.1392. Found: 329.1383.

Ethyl, 4-Ethenylphenyl (1-(*N-tert*-Butoxycarbonylamino)-2-phenyl)ethylphosphonate (C1). 4-Vinylphenol was prepared in 50.8% yield as described by Overberger et al.[21] by decarboxylation and sublimation of *p*-hydroxycinnamic acid. **4RS** (0.66 g, 2 mmol), *p*-vinylphenol (0.24 g, 2 mmol), triethylamine (0.56 mL, 4 mmol), and Castros BOP reagent[18] (Sigma) (0.88 g, 2 mmol) were dissolved successively in CH_2Cl_2 (15 mL). The reaction was followed on HPLC (RP-18 column, eluent: MeOH/[potassium phosphate buffer, 0.05 M, pH 3], 4:3 (v/v)). After 20 h an additional 0.5 equiv of BOP and triethylamine were added, and the reaction was left overnight, leading to high conversion according to the HPLC analysis. Brine (50 mL) was added and the product extracted into EtOAc. The organic phase was washed with 1 M HCl, $NaHCO_3$ (saturated), and brine. After evaporation 0.81 g of a dark brown solid was obtained. The product was purified by column chromatography (silica, eluent: $CHCl_3$/MeOH, 9:1), giving 0.88 g (100%) of **C1** as a red green oil. ^{1}H NMR ($CDCl_3$): δ 1.15−1.30 (m,3H, $-CH_2-CH_3$), 1.28, 1.32 (2s, 9H, $-C(CH_3)_3$), $I_{1/2}$ = 50/50), 2.88−3.00 (m, 1H, $-CH_2-$Ph), 3.24−3.35 (m, 1H, $-CH_2-$Ph), 4.15−4.27 (m, 2H, $-O-CH_2-CH_3$), 4.50−4.60 (m, 1H, −CH<), 5.05−5.15 (m, 1H, −NH−), 5.20, 5.22 (2d, J = 11.0, 10.9 Hz, 1H, vinyl, $I_{1/2}$ = 56/44), 5.65, 5.17 (2d, J = 17.6 Hz, 1H, vinyl, $I_{1/2}$ = 42/58), 6.65, 6.66 (2dd, J = 11.0, 10.8, 17.5 Hz, 1H, vinyl), 7.13−7.43 (m, 9H, Ph). ^{13}C NMR ($CDCl_3$): δ 15.91, 15.98 ($-CH_3$), 27.75, 27.78 ($-C(CH_3)_3$), 35.66, 35.73, 35.79 ($-CH_2-$Ph), 47.74, 48.03 (2d, J = 158.1, 157.4 Hz, C−P), 63.11, 63.21 (2d, J = 22.1 Hz, $-O-CH_2-$), 79.47, 79.57 ($-C(CH_3)_3$), 113.34, 113.45, 113.52 (vinyl), 120.07, 120.12, 120.23, 120.28 (C2,6-*p*-vinylphenol), 127.99 (C3,5-Ph), 128.97 (C2,6-Ph), 134.09, 134.27 (C4-*p*-vinylphenol), 135.38 (C1−Ph), 135.98, 136.17 (vinyl), 149.32, 149.36, 149.44 (C1-*p*-vinylphenol), 154.49, 154.52, 154.59, 154.61 (−CO−). HRMS calcd for $C_{23}H_{30}NO_5P$: 431.1861. Found: 431.1853.

***S*-Ethyl, 4-Ethenylphenyl (1-(*N-tert*-Butoxycarbonylamino)-2-phenyl)ethylphosphonate (C1S) (BOP Procedure)**. Prepared as described for **C1** but starting from **4S**. Yield: 37 mg (57%). $[\alpha]^{23}_D$ = +10.2° [*c* 2, $CHCl_3$]. ^{1}H NMR ($CDCl_3$): δ 1.13−1.26 (m, 3H, $-CH_2CH_3$), 1.28, 1.32 (2s, 9H, $-C(CH_3)_3$, $I_{1/2}$ = 43/57), 2.85−3.00 (m, 1H, $-CH_2-$Ph), 3.25−3.34 (m, 1H, $-CH_2-$Ph), 4.16−4.27 (m, 2H, $-O-CH_2-$), 4.45−4.58 (m, 1H, −CH<), 4.83−4.91 (m, 1H, −NH−), 5.21, 5.22 (2d, J = 10.9 Hz, 1H, vinyl, $I_{1/2}$ = 30/70), 5.66, 5.67 (2d, J = 17.6 Hz, 1H, vinyl, $I_{1/2}$ = 42/58), 7.13−7.42 (m, 9H, Ph+*p*-vinylphenol).

***S*-Ethyl, 4-Ethenylphenyl (1-(*N-tert*-Butoxycarbonylamino)-2-phenyl)ethylphosphonate (C1S) (BOPCl Procedure)**.[22] **4S** (50 mg, 0.15 mmol), BOPCl (Aldrich) (bis(2-oxo-3-oxazolidinyl)phosphinic chloride) (42 mg, 0.17 mmol), triethylamine (0.046 mL, 0.33 mmol), and *p*-vinylphenol (18 mg, 0.15 mmol) were dissolved successively in CH_2Cl_2 (1 mL) and stirred at room temperature overnight. The reaction was followed by TLC (silica plates, $CHCl_3$/MeOH, 9:1). After addition of an additional 1 equiv of triethylamine and BOPCl and again stirring overnight a high conversion was observed. The mixture was washed with 0.5 M $NaHCO_3$, 0.1 M HCl, and H_2O and the organic phase dried (K_2CO_3) and evaporated. This gave 52 mg of an oil. This was purified by column chromatog-

(21) Overberger, C. G.; Salamone, J. C.; Yaroslavsky, S. *J. Am. Chem. Soc.* **1967**, *89*, 6231.

(22) Diago-Meseguer, J.; Palomo-Coll, L.; Fernandez-Lizarbe, J. R.; Zugaza-Bilbao, A. *Synthesis* **1980**, 547.

raphy (silica, eluent: $CHCl_3$/MeOH, 9:1), giving 51 mg (80%) of a light yellow oil. $[\alpha]^{23}_D = +2.0°$ [*c* 3.5, $CDCl_3$]. ^{1}H NMR ($CDCl_3$): δ 1.13−1.27 (m, 3H, $-CH_2CH_3$), 1.28, 1.32 (2s, 9H, $-C(CH_3)_3$, $I_{1/2}$ = 35/65), 2.88−2.93 (m, 1H, $-CH_2-$Ph), 3.25−3.34 (m, 1H, $-CH_2$Ph), 4.16−4.24 (m, 2H, $-O-CH_2-$), 4.45−4.60 (m, 1H, −CH<), 4.75−4.82 (m, 1H, −NH−), 5.22 (d, *J* = 10.9 Hz, 1H, vinyl), 5.67, 5.68 (2d, *J* = 17.5 Hz, 1H, vinyl, $I_{1/2}$ = 33/67), 6.67 (dd, *J* = 17.6, 10.9 Hz, 1H, vinyl), 7.12−7.38 (m, 9H, Ph+*p*-vinylphenol). ^{13}C NMR ($CDCl_3$): δ 16.21, 16.28 ($-CH_2CH_3$), 28.08 ($-C(CH_3)_3$), 36.05, 36.11, 36.19 ($-CH_2-$Ph), 47.98 (2d, *J* = 158 Hz, C−P), 63.41, 63.51 (2d, *J* = 16.0 Hz, $-O-CH_2-$), 79.9, 80.0 ($-C(CH_3)_3$), 113.67, 113.76 (vinyl), 120.40, 120.53, 120.58 (C2,6-*p*-vinylphenol), 126.69 (C4−Ph), 127.31, 127.43 (C3,5-*p*-vinylphenol), 128.31 (C3,5-Ph), 129.27 (C2,6-Ph), 134.61 (C4-*p*-vinylphenol), 135.68 (C1−Ph), 136.20, 136.38 (vinyl), 149.58, 149.70 (C1-*p*-vinylphenol), 154.73, 154.83 (−CO−). HRMS calcd for $C_{23}H_{30}NO_5P$: 431.1861. Found: 431.1848.

Ethyl, 4-Ethenylphenyl 1-Amino-2-phenylethylphosphonate (C2). **C1** (0.35 g, 0.8 mmol) was dissolved under ice cooling in TFA/CH_2Cl_2 (1:1, v/v) (5 mL) and then stirred for 30 min at room temperature. The residue obtained after evaporation was taken down in ether four times, giving 0.39 g of a red oil. This was dissolved in CH_2Cl_2 (25 mL) and washed with $NaHCO_3$ (saturated) (2 × 25 mL). The organic phase was dried ($MgSO_4$) and evaporated, giving 0.21 g (80%) of **C2** as a yellow oil pure on TLC (silica, $CHCl_3$/MeOH, 9:1, R_f = 0.5). This was stored dry in a freezer. The product can also be isolated as the more stable oxalate salt by slowly adding the free base as an etheral solution to an ether solution containing dissolved anhydrous oxalic acid.[23] ^{1}H NMR ($CDCl_3$): δ 1.29 (2t, *J* = 7.1 Hz, 3H, $I_{1/2}$ = 50/50), 2.71−2.84 (m, 1H, $-CH_2-$Ph), 3.28−3.35 (m, 1H, $-CH_2-$Ph), 3.39−3.48 (m, 1H, −CH<), 4.15−4.30 (m, 2H, O−CH_2-), 5.23 (d, *J* = 11 Hz, vinyl), 7.18−7.42 (m, 9H, Ph+*p*-vinylphenol). ^{13}C NMR ($CDCl_3$): δ 16.27, 16.34 ($-CH_3$), 37.55, 37.62 ($-CH_2-$Ph), 50.24, 50.31 (2d, *J* = 153.1 Hz, C−P), 63.18, 63.24 ($-O-CH_2-$), 113.62 (vinyl), 120.48 (C2,6-*p*-vinylphenol), 126.71 (C4−Ph), 127.32 (C3,5-*p*-vinylphenol), 128.49 (C3,5-Ph), 129.10, 129.12 (C2,6-Ph), 134.33 (C4-*p*-vinylphenol), 135.59 (C1−Ph), 137.30, 137.50 (vinyl), 149.95, 150.03 (C1-*p*-vinylphenol). HRMS calcd for $C_{18}H_{22}NO_3P$: 331.1337. Found: 331.1316.

Ethyl, 3(4)-Ethenylbenzyl-(1-(*N*-*tert*-Butoxycarbonylamino)-2-phenyl)ethylphosphonate (11). **4RS** (0.33 g, 1 mmol), 3(4)-vinylbenzyl alcohol (0.13 g, 1 mmol) (prepared as described in the Supporting Information), BOP (0.44 g, 1 mmol), and triethylamine (0.21 mL, 2 mmol) were dissolved successively in CH_2Cl_2 and stirred at room temperature. The reaction was followed on HPLC (RP18, eluent: MeOH/potassium phosphate buffer 0.05 M, pH 3], 1:1 (v/v), *k′*(product) = 6.9). After 24 h an additional 0.5 equiv of BOP and triethylamine were added and left again overnight. High conversion was obtained according to HPLC. The color changed from light yellow to a red color. Ethyl acetate (50 mL) was added and the solution washed with brine, 1 M HCl, $NaHCO_3$, and finally brine. After drying ($MgSO_4$) and evaporation 0.56 g of a red oil was obtained. The product was purified by column chromatography (silica, eluent: $CHCl_3$/MeOH, 9:1). This gave 0.40 g (100%) of **11** as an orange oil. ^{1}H NMR ($CDCl_3$): δ 1.15−1.44 (m, 12H, $-CH_3$), 1.82−2.88 (m,1H, $-CH_2-$Ph), 3.15−3.25 (m, 1H, $-CH_2-$Ph), 4.02−4.18 (m, 2H, $-O-CH_2-$), 4.37−4.46 (m, 1H, −CH<), 4.85 (d, *J* = 10 Hz, −NH−), 5.03−5.15 (m, 2H, $-O-CH_2-$Ph), 5.27 (d, *J* = 10.9 Hz, 1H, vinyl), 5.75, 5.76 (2d, *J* = 17.7 Hz, 1H, vinyl), 6.70 (dd, *J* = 10.9, 17.6 Hz, 1H, vinyl), 7.20−7.50 (m, 9H, Ph). ^{13}C NMR ($CDCl_3$): δ 15.92, 15.99, 16.04 ($-CH_2-CH_3$), 27.81 ($-C(CH_3)_3$), 35.69, 35.73 ($-CH_2-$Ph), 47.60 (d, *J* = 156.1 Hz, C−P), 62.32, 62.47, 62.55 ($-O-CH_2-CH_3$), 67.19, 67.27 ($-O-CH_2-$Ph), 79.53 ($-C(CH_3)_3$), 114.13 (vinyl), 125.43, 125.49, 125.92, 126.06, 126.34, 126.99, 127.05, 127.99, 128.48, 128.92, 135.94, 136.02, 136.24, 136.42, 137.54 (aromatic+vinyl), 154.62, 154.70 (−CO−). HRMS calcd for $C_{24}H_{32}NO_5P$: 445.2018. Found: 445.2037.

(23) Kowalik, J.; Kupezyk-Subotkowska, L.; Mastalerz, P. *Synthesis* **1981**, 57.

Table 1. Imprinted Polymer Series A and B and the Amount of Template Removed[a]

		removal (%)			
polymer	template	$CHCl_3$/MeOH (1)	MeOH Δ (2)	CsF (3)	OH^- (4)
PA1	**A1**	11	23	98	89
PA2	**A2**	57	75	98	97
PA3	**A3** + GlyOEt	18 (60)[b]	25 (73)[b]		
PA4	GlyOEt	34	70		
PB1	**B1**		9		
PB2	**B2**		12		
PB3	**B3** + GlyOEt		9		
PB4	**B4** + GlyOEt				

[a] The polymers were prepared using the templates shown in Schemes 2 and 3 by free radical polymerization for 24 h using AIBN (1 mol %) as photochemical initiator at 7 °C. The monomer mixture consisted of the template (0.5 mol %), MAA (10 mol %), and EDMA (90 mol %) in $CHCl_3$ (F_m = volume porogen/(volume porogen + volume monomers) = 0.56). In polymers **PA3**, **PA4**, **PB3**, and **PB4** an equimolar amount of glycine ethyl ester (GlyOEt) (0.5 mol %) was added to the template. The template splitting after the various treatments is calculated from the amount of template monomer originally added. After treatment 1 and 2 this was determined with ^{1}H NMR analysis of the extracts, while after treatment 3 and 4 it was determined from elemental analysis on phosphorus. The phosphorus analysis gave the following results. **PA1**: After treatment 1 and 2 (MeOH Δ) 0.010%, 3 (CsF) 0.0015%, 4 (OH^-) 0.0020%. **PA2**: 1 and 2 (MeOH Δ) 0.032%, 3 (CsF) 0.0015%, 4 (OH^-) 0.0092%. [b] Value referring to splitting of GlyOEt.

4-Ethenylphenyl-4-chlorobenzoate (C3). To a solution of *p*-vinylphenol (1.2 g, 10 mmol) and triethylamine (1.5 mL, 11 mmol) in ice cold THF was added *p*-chlorobenzoyl chloride (1.4 mL, 11 mmol) under stirring. The reaction was followed on TLC (silica, eluent: CH_2Cl_2/hexane, R_f = 0.7). After completion, purification was done by aqueous washes with 1 M HCl, saturated $NaHCO_3$, and brine. After drying ($MgSO_4$) and evaporation 1.9 g (74%) of product was obtained as a white solid. Mp: 88−90 °C. ^{1}H NMR ($CDCl_3$): δ 5.25, (d, *J* = 10.9 Hz, 1H, vinyl), 5.72 (d, *J* = 17.6 Hz, 1H, vinyl), 6.71 (dd, *J* = 17.6, 10.9 Hz, 1H, vinyl), 7.14 (d, *J* = 8.6 Hz, 2H, H3′,5′), 7.44,7.45 (2d, *J* = 8.6 Hz, 4H, H2′,6′,3,5), 8.11 (d, *J* = 8.6 Hz, 2H, H2,6). ^{13}C NMR ($CDCl_3$): δ 114.10 (vinyl), 121.58 (C2′,6′), 127.18, 127.84, 128.85, 131.44, 135.45, 135.75, 140.04, 150.18, 164.16. HRMS calcd for $C_{15}H_{11}ClO_2$: 258.0447. Found: 258.0442.

Molecular Imprinting of L-Phenylalanine Ethyl Ester (13) and *S*-Diethyl-1-Amino-2-Phenylethylphosphonate (12S). The templates were converted to the free amine by extraction into ethyl acetate in bicarbonate, and 0.8 mmol of the oils was dissolved in 4.6 mL of methylene chloride. To this was added 3.1 mL (16.5 mmol) of EDMA, 0.28 mL (3.3 mmol) of MAA, and 0.034 g (0.2 mmol) of AIBN. The homogeneous solutions were then transferred to polymerization tubes and subjected to three freeze−thaw−degas cycles before sealing. They were then photopolymerized as described below at 15 °C for 20 h. The tubes were turned 180° after 10 min, 40 min, and 10 h to achieve an even exposure. After polymerization the transparent polymers were crushed and Soxhlet extracted in MeOH for 24 h followed by sieving (25−38 μm) and packing into HPLC columns (10 cm × 0.5 cm). The chromatographic performance was then investigated at room temperature using HPLC at 1 mL/min and MeCN/H_2O/HOAc, 96.25:1.25:2.5, as eluent and detection at 267 nm if not otherwise mentioned. The recovery of template after Soxhlet extraction was determined using L-phenylalanine anilide as internal standard on RP-18 column with eluent: MeOH/3% HOAc, 1:1 (v/v).

Imprinted Polymers: Series A and B. Polymer Preparation. The polymers were prepared using the templates indicated in Table 1 and shown in Schemes 1−3. In a typical preparation (**PA1**) template A1 (50 mg, 0.1 mmol) was dissolved in $CHCl_3$ (3.76 mL) and MAA (0.17 mL, 2 mmol), and EDMA (3.39 mL, 18 mmol) and the initiator AIBN (33 mg,

Table 2. Imprinted Polymer Series C and the Amount of Template Removed[a]

					splitting (%)				
		monomers (mol %)			CsF (1)	MeOH/NaOH(aq) (2)			
polymer	templ	VIm	MAA	EDMA	NMR 40 h	HPLC 17 h	NMR 3.5 h	NMR 17 h	Anal. 17 h
PC11	C1		12	87		19	78	100	>60
PC12	C1	6	6	87	72	10		100	
PC21	C2		12	87			56	100	
PC22	C2	6	6	87	51			89	
PC31	C3		12	87		69	93	100	
PC32	C3	6	6	87	55	68		100	

[a] The polymers were prepared as described in the Experimental Section by free radical polymerization of the indicated monomer mixtures in the presence of template (1 mol %) using benzene (F_m = 0.56) as porogen and AIBN (1 mol %) as photochemical initiator. VIm = vinylimidazole, MAA = methacrylic acid, EDMA = ethylene glycol dimethacrylate. Template splitting was carried out by treatment with CsF in MeOH (20 mg/mL) at 60 °C (treatment 1) or MeOH/10 M NaOH, 4:1 (v/v), (treatment 2) overnight. The splitting was followed by ^{1}H NMR, HPLC, or elemental analysis on phosphorus (Anal.) as described in the Experimental Section and was calculated from the amount of template added as monomer.

0.2 mmol) in 1 mL of $CHCl_3$ were added followed by transfer of the mixture to a thick-walled glass tube. Oxygen was removed by two freeze−thaw cycles (or by helium bubbling) and the tube sealed under vacuum. The polymerization was then started by placing the tubes at ca. 10 cm distance from a medium-pressure mercury vapor lamp (Canrad Hanovia, 550 W, 33 W in λ range 320−400 nm) and allowed to proceed at 7 °C. The tubes were turned at regular intervals for symmetric exposure and left for 24 h. In the preparation of **PA2** the poor stability of template **A2** made it necessary to synthesize it just prior to polymerization. It was then rapidly converted to the free base form by cold extraction into $CHCl_3$ with 1 M $NaHCO_3$. After drying ($MgSO_4$) a slightly colored oil was obtained, appearing pure on ^{1}H NMR and TLC. This was taken directly into the polymerization, where in order to save time the degassing was done by He bubbling. Blank samples lacking initiator were used in order to check stability of the templates by TLC ($CHCl_3$/MeOH, 9:1) under the polymerization conditions.

Workup and Template Splitting. The polymers were crushed and passed through a 250 μm sieve, and the splitting of templates was attempted in order as follows: (1) The polymers were placed in flasks and shaken with 100 mL of $CHCl_3$/MeOH, 1:1 (v/v), for 5 h followed by filtration into Soxhlet extraction timbles. The filtrate was evaporated and saved for analysis. (2) Soxhlet extraction was performed in MeOH overnight. The extract was evaporated and saved for analysis. Then either of the following procedures were followed: (3) To 0.5 g of polymer was added 5 mL of a solution of CsF (32 mg/mL) in MeOH, and the mixture shaken at room temperature and then heated at 60 °C on a hot plate for 48 h. The supernatant was removed (saved for analysis), the polymers were washed with MeOH and dried, and the MeOH wash was combined with the supernatant, evaporated, and saved for analysis. (4) To 0.5 g of polymer was added 10 mL of Na_2CO_3 (0.5 M)/MeOH, 1:1 (v/v), and the mixtures were shaken overnight. The supernatant was removed and the polymers were washed and dried as described under (3). (5) To 0.5 g of polymer was added 10 mL of NaOH (1 M)/MeOH, 1:1 (v/v), and the mixtures were shaken overnight at either room temperature or 60 °C. The supernatant was removed and the polymers were washed and dried as described under (3). All wash solutions were combined, evaporated, and saved for analysis. The analysis was done by TLC, ^{1}H NMR, and elemental analysis on phosphorus.

Analysis of Splitting Products. TLC. This was done on silica gel plates in $CHCl_3$/MeOH, 9:1, with UV and ninhydrin development.

^{1}H NMR. To the oily residues were added $CDCl_3$ and a few drops of CD_3OD for solubilization. Then 1 equiv (based on 100% splitting) of dioxane was added as internal standard, and the integrals of the template characteristic signals were compared with the dioxane signal at δ 3.7 ppm corresponding to 8H. The signals that were used in the comparison were for templates **A1** and **A2**: δ 1.25 (m, 3H, $-O-CH_2-CH_3$), 2.7−3.3 (m, 2H, $-CH_2-Ph$), 4.0−4.3 (m, 2H, $-O-CH_2-$), 4.2−4.5 (m, 1H, −CH<), 7.1−7.4 (m, 5H, −Ph). Template **A3**: δ 7.2−7.8 (m, 10H, Ph). GlyOEt: δ 1.1−1.3 (t, 3H, $-O-CH_2-CH_3$), 3.5−4.0 (m, 2H, $-CH_2-$), 4.0 (q, 2H, $-OCH_2-$). Templates **B1** and **B2**: δ 1.5 (s, 9H, tBut), 2.5−3.5 (m, 2H, $-CH_2-Ph$), 3.5−4.5 (m, 1H, −CH<), 7.0−7.5 (m, 5H, −Ph). Template **B3**: δ 7.9−8.1 (m, 2H, H2,6-Ph).

Elemental Analysis. Polymer samples were dried for 24 h in a vacuum oven at 50 °C and then submitted for P-analysis. The splitting percentage was calculated based on a total incorporation of template corresponding to 0.081% P.

Spectroscopic Characterization of Polymers. The polymers subjected to template splitting procedure (4) were characterized by IR and ^{1}H NMR spectroscopy. FTIR (KBr) of **PA1**: 3530 (br), 2977, 1739, 1637, 1468, 1394, 1261, 1155, 947, 869, 814, 753, 654, 597, 520, 468. **PB4**: 3446 (br), 2973, 1734, 1637, 1470, 1394, 1261, 1160, 949, 869, 814, 752, 653, 465.

CP-MAS ^{13}C NMR of **PA1**: δ 177, 167, 137, 127, 63, 55, 46, 24, 19 ppm. Level of unsaturation: I_{167}/I_{177}: **PA1** 10%, **PA2** 14%, **PA3** 11%.

Imprinted Polymers: Series C (Scheme 4). The polymers were prepared using the monomer mixtures shown in Table 2. Vinylimidazole was prepared according to Overberger et al.[24] In a typical preparation (**PC11**) C1 (86 mg, 0.2 mmol), benzene (3.7 mL), MAA (0.20 mL, 2.4 mmol), and EDMA (3.28 mL, 17.4 mmol) were added to a 50 mL thick-walled glass tube. After ensuring complete solubility of the template, a small sample of this mixture was removed in order to check template stability. The initiator, AIBN (33 mg, 0.2 mmol) in benzene (1 mL), was added. Degassing was done by two freeze−thaw cycles and the polymerization started by placing the polymers at a 10 cm distance from a medium-pressure mercury vapor lamp (Canrad Hanovia, 550 W, 33 W in λ range 320−400 nm) in a thermostated water bath (15 °C). The tubes were turned at regular intervals for symmetric exposure and left for 24 h. The template stability was checked by TLC ($CHCl_3$/MeOH, 9:1) on the solution samples lacking initiator. No side reactions could here be detected.

Choice of Splitting Conditions: Model Study. Base-Catalyzed Reaction. To samples of either template **C1**, benzyl ester **11**, or diethyl *p*-vinylphenylphosphate (20 μmol) was added, MeOH/[NaOH 1 M], 1:1 (1 mL), and the hydrolysis was followed by HPLC (RP-18, MeOH/[potassium phosphate buffer 0.05 M, pH 3], 4:3 (v/v)).

Acid- and TBAF-Catalyzed Reaction. To samples (20 μmol) of templates C2, C3, diethyl 1-amino-3-phenylethylphosphonate (12), and EDMA was added MeOH/[HCl 2 M], 1:1 (v/v), or 1 mL of tetrabutylammonium fluoride (TBAF) (0.5 M) in THF/MeOH, 1:1 (v/v). The reactions were followed on HPLC (RP-18, MeOH/[potassium phosphate buffer 0.05 M, pH 7.5], 4:3 (v/v)).

CsF-Catalyzed Reaction. To templates C1 or C3 (20 μmol) was added cesium fluoride (CsF) (60 μmol) in dry MeOH (4

(24) Overberger, C. G.; Vorheimer, N. *J. Am. Chem. Soc.* **1963**, *85*, 951.

mL). The reaction was followed on TLC (silica plates: $CHCl_3$/MeOH, 9:1) and HPLC (RP-18: MeOH/H_2O, 3:1).

Workup and Splitting of Template. The polymers were crushed and Soxhlet extracted in methanol for 24 h. After drying under vacuum at 50 °C for ca. 10 h they were mechanically sieved to a particle size of 38−250 μm. The fine particles were removed by repeated sedimentation in methanol. The amount of template removed was analyzed by HPLC (RP 18, MeOH/[potassium phosphate buffer 0.05 M, pH 3], 4:3 (v/v)). No template or unreacted monomer was detected in the methanol extracts.

Treatment A: Aqueous Base in MeOH. To 1.5 g of each polymer in a 25 mL screwcap vial was added 20 mL of MeOH/10% NaOH, 4:1 (v/v), followed by gentle shaking overnight. Polymers **PC31** and **PC32** immediately turned light yellow. After 17 h, the supernatant was removed and neutralized with HCl before analysis. Polymer **PC11** was subjected to further splitting treatment at elevated temperature. The splitting yield was checked by HPLC and by ^{1}H NMR analysis. Before the ^{1}H NMR analysis the polymers were washed as follows: 1 × 15 mL of MeOH/H_2O, 4:1, 5 × 15 mL of MeOH/[0.1 M HCl], 4:1 (pH acidic), 2 × 15 mL of MeOH/H_2O, 4:1 (pH neutral), 1 × 15 mL of MeOH. The wash solutions were combined with the hydrolyzate and neutralized followed by evaporation. The residues were then dissolved in 5 mL of MeOH, and salts were separated, evaporated, and dissolved in CD_3OD/D_2O containing 3-trimethylsilylpropane sulfonic acid sodium salt as internal standard.

Treatment B: Cesium Fluoride (CsF) in MeOH. To dry polymer (0.4 g) was added dry MeOH (4 mL) containing CsF (20 mg/mL). The mixtures were heated to 60 °C, and the splitting was followed on HPLC (RP-18, MeOH/H_2O, 3:1 (v/v)) and by ^{1}H NMR. In the ^{1}H NMR investigation the supernatant was removed after 40 h and the polymer washed repeatedly with MeOH. The wash solutions were pooled with the CsF fraction, evaporated, and then dissolved in 2 mL of $CDCl_3$ containing dioxane as internal standard (20 μmol). The integrals were compared: for dioxane, δ 3.7 (8H); for **PC11**−**PC22**, δ 4−4.3 (2H), 2.6−3.3 (2H); and for **PC31**,**32**, δ 7.9−8.1 (2H).

Homogeneous Catalyzed Reactions. Homogeneous reactions using the functionalized monomers **7** and **B4** as soluble catalysts were followed. To 2 mL of an MeCN/aqueous mixture in a stoppered quartz cuvette containing **7**, **B4** (0.7 mM), or no catalyst was added substrate (BOCPheONP) to a concentration of 0.025 mM. The amount of added catalyst corresponds to the theoretical amount of catalytic groups bound to the polymer. The reaction was performed at room temperature and followed spectrophotometrically using a Perkin-Elmer Lambda 4A UV−vis spectrophotometer by single-point measurements at regular time intervals. Prior to start, the spectra of the substrate, product (PNP), and the catalyst were recorded in order to choose a wavelength where absorption overlap of these species is at a minimum. Monomer **B4** showed a weakly pH-dependent $\lambda_{max} = 220$ nm ($\epsilon = 3.0$ mM^{-1} cm^{-1}). No absorbance was seen above $\lambda = 280$ nm. Monomer **7** however absorbed strongly at $\lambda = 320$ nm with a shoulder at $\lambda = 350$ nm.

Polymer-Catalyzed Reactions. Dry polymer (50 mg) was weighed into 4 mL screwcap vials. MeCN or MeCN/aqueous reaction medium was added and the mixture left for a few hours for equilibration. After gentle shaking the polymer was allowed to settle for a few minutes and the supernatant removed with a pasteur pipet by careful suction from the bottom of the vial. This procedure was repeated 2−3 times in order to remove all fine particles and ensure a pH unaffected by ionization of pendant acid groups of the polymer. Finally fresh reaction medium was added to a total volume of 2 mL. If not otherwise stated, the reaction was started by adding 20 μL of a freshly prepared solution of substrate (2.5 mM) in dry acetonitrile. This gave a final concentration of 0.025 mM. The vials were gently shaken at room temperature, and the reaction was followed by HPLC by injecting 20 μL of the supernatant after settling of the polymer. A reversed-phase column (C-18), a variable-wavelength UV detector (Waters), and an integrating recorder were used (Hewlett-Packard). The mobile phase was MeCN/[potassium phosphate buffer 0.01 M, pH 6], 65:35 (v/v), and the flow rate was 1.5 mL/min. The wavelength was chosen as the λ_{max} of PNP, which varies with pH. Thus at pH 6 detection was done at 310 nm. The peaks of the substrate BOCPheONP ($k' = 1.7$) and the product ($k' = 0.6$) were well separated. A linear response was verified by injecting various concentrations of BOCPheONP and PNP and IP for PNP determined. To get a true IP for PNP, the supernatant was analyzed after several weeks reaction, thus compensating for nonspecific absorption of PNP to the polymer. Moreover the UV spectra obtained at this point confirmed that no change in pH had occurred, which would have resulted in a change in ϵ and λ_{max}. Above pH 7 the reactions were performed in stoppered quartz cuvettes and followed by monitoring the increase in UV absorption at 400 nm due to the formation of PNP. This technique could not be used at low pH due to interference of fine particles in the spectral window used here.

Evaluation of the Rate Data. Since the catalyst is in excess of the substrate (theoretical amount of sites 0.5 mM in the PA series and 0.15 mM in PB series; substrate concentration 0.025 mM), the hydrolysis can be assumed to follow pseudo-first-order kinetics. This can be expressed in terms of HPLC peak integrals for either the substrate (IS) or the product (IP) as

$$\ln IS_t = \ln IS_o - kt \quad (1)$$

or

$$\ln(IP_{inf} - IP_t) = \ln IP_{inf} - kt \quad (2)$$

where k is the first-order rate constant, IS_t and IS_o are the substrate peak integral at time t and at the start. IP_{inf} is the product peak integral at infinte time.

Thus by plotting the natural logarithm of the peak integrals versus time for both the substrate and the product, the pseudo-first-order rate constants are obtained from the slopes of the graphs. In the case where the reactions were followed to high conversion (>3 half-lives) the given k is the average of these rates. Otherwise since $\epsilon_{(BOCPheONP)} < \epsilon_{(PNP)}$, k is most accurately obtained by following the product formation. Reproducibility was verified by repeating several runs twice and the error determined. The lines were drawn based on at least four data points, and the correlation coefficient was larger than 0.98. For the hydrolysis of phenylalanine ethyl esters the reaction was followed by the appearance of the product D(L) phenylalanine and also the by the conversion of the ester.

Results and Discussion

Catalyst Design. The templates were designed with the objective of incorporating the key catalytic elements, found in the proteolytic enzyme chymotrypsin, into the polymer active sites. After template removal we anticipated that template **A1** would provide an active site complementary to the D enantiomer of BOCPheOEt (see Scheme 1), equipped with a phenolimidazole catalytic group that would be situated in proximity to the reactive carbonyl group of a bound substrate. Complementary hydrogen bonds between carboxylic acid groups of the site and hydrogen-bonding groups of the substrate are responsible for the binding selectivity[12] and the tetrahedral phosphonate function provides a site complementary to a transition state like structure (vide supra). Note that an additional carboxylic acid group hydrogen bonded to the imidazole group[25] (Scheme 1) completes the catalytic triad of functional groups found in chymotrypsin.[13,14] Moreover due to the relative basicity of the syn and anti

(25) Lindemann, R.; Zundel, G. *J. Chem. Soc., Faraday Trans. 2* **1977**, *73*, 788.

lone pair of the carboxylic acid group, it is likely to be positioned syn to the imidazole group, providing an additional catalytic advantage.[26] In the control polymer **PA3** however (Scheme 2) the carboxylic acid groups responsible for substrate binding are expected to be randomly distributed in the polymer (ion-paired with GlyOEt), tetrahedral complementarity is absent, and with the achiral template **A3** no stereoselectivity is expected.[27] Control polymer **PA2**, equal to **PA1** except for the absence of the BOC group in the template, we believed would additionally probe the structural complementarity of the site, and the simplest control polymer, **PA4**, would reveal whether nonspecific catalysis takes place. In series B polymer catalysts however (Scheme 3) **PB1** is expected to be complementary to BOC-L-PheONP with an active site containing a chiral pendant L-histidinole group and carboxylic acid groups positioned to bind BOC-L-PheONP. **PB2** as well as **PA2** will test the spatial requirement of the substrate, and **PB3** and **PB4** the importance of the binding site as well as the influence of carboxylic acid groups neighboring the imidazole group. Finally series C (Scheme 4) was designed to investigate the importance of the imidazole group regarding its position and general role in catalysis.

In Schemes 2−4 are seen the three groups of synthetic template targets and the result of polymerization and nucleophilic splitting of the template from the polymer. In each group the first two entries correspond to the actual mimic and the last entries to blank control polymers. A convergent synthetic strategy was chosen where the chiral phosphonate (Scheme 5), racemic phosphonate (Scheme 8), and the functional monomers (Schemes 6 and 7) were first synthesized followed by a final condensation step and deprotection.

Synthesis of the Chiral Phosphonate Moiety (4S) (Scheme 5). 1R and **1S** were synthesized in three steps as reported elsewhere.[15,16] Starting with condensation of phenylacetaldehyde, benzylcarbamate, and triphenylphoshite catalyzed by acetic acid giving diphenyl-*N*-carbobenzyloxy-1-amino-2-phenyl-ethylphosphonate (**A**), the latter was deprotected to the free amine (**B**) and then reacted with dibenzoyltartaric anhydride, giving the corresponding *N*-dibenzoyltartrate (**C**). The diastereomers of the latter could easily be resolved in two crystallization steps followed by HBr aqueous hydrolysis of both the N and PO protecting groups. The amino acids **1R** and **1S** were finally purified by either cation exchange chromatography or precipitation with propylene oxide. The latter procedure was preferable since it was less time-consuming and gave a higher yield. **1S** was N protected by BOC[28] in good yield, giving the BOC-protected P-amino acid **2S** (Scheme 5). This was O alkylated with triethylorthoformate (TEOF) to give the diethylphosphonate **3S**,[29] which was monohydrolyzed by base to the acid ester **4S**.[30] The latter was used as a common precursor for the synthesis of the chiral transition state analogue template assemblies. A more versatile synthesis of racemic **4** was developed (Scheme 8). **B** was here directly BOC protected, giving **10** followed by transesterification with sodium ethoxide, giving **3RS**, and monohydrolysis as with the chiral precursor to give **4RS**. As judged from the doubling of some ^{1}H NMR signals the above BOC-protected compounds seemed to be present as at least two stable conformers with a relative abundacy of approximately 5/2.

Synthesis of the Hydroxyimidazole monomers 7 and B4 (Schemes 6 and 7). The functionalized monomers were synthesized as shown in Schemes 6 and 7. The first attempt to synthesize the phenolimidazole monomer departed from 5-bromosalicylic acid following a procedure by Rogers and Bruice.[31] The acid was converted to the methyl ester followed by reaction with ethylenediamine to give the imidazoline. Dehydrogenation of the imidazoline to give the imidazole using Pd/C or barium manganate failed. Moreover attempts to protect the phenol hydroxy group by TBDMS, TBDPS−silyl, or MOM ether formation failed. Instead as shown in Scheme 6, 5-bromosalicylaldehyde was converted to the 2-substituted imidazole **5** by a condensation reaction with ammonium acetate and the trimer of glyoxal.[32] Direct Pd-catalyzed coupling of the vinyl group was not possible probably due to the presence of the basic nitrogen.[33] Benzoyl chloride allowed a facile protection of both the phenol and imidazole functions, which could not be achieved with other protecting groups such as acetyl, silyl (not stable), or MOM ethers (leaves imidazole unprotected). The Pd coupling[33] gave **A3,** which was deprotected to give the vinylphenol imidazole monomer **7**. In the ^{13}C NMR the signals from the C4 and C5 carbons of the imidazole ring were broadened, probably because of slow rotation around the phenylimidazole bond due to conjugation and intramolecular hydrogen bonding.[31] By adding a drop of CD_3COOD, the signal was sharpened. The histidinole monomer **B4** (Scheme 7) was synthesized starting with esterification of L-histidine, giving the methyl ester **8**. This was reduced to the alcohol by sodium borohydride, giving **9**,[34] which was finally N acylated under Schotten−Bauman conditions with methacrylic acid anhydride to give **B4.**

The Final Coupling Step. Several condensing agents were tried in the final coupling step (Schemes 5, 7, 8). In the phosphonate coupling, DCC was inefficient while Castros BOP reagent[18] gave a high yield and a fast reaction. In the coupling of the phosphonate precursor **4S** with *p*-vinylphenol (see Scheme 8 for coupling with **4R**,**S**) the use of BOP and BOPCl as condensing agents was compared. While both reactions proceeded in a high yield, the optical purity of the BOPCl reaction was lower. Apparently some racemization occurred here. The BOP reagent was therefore used in all couplings of the phosphonate precursor. The spectra of the phosphonate products **A1** and **A2** of Scheme 5 were complicated by the existence of multiple conformers, by the presence of two diastereomers due to the newly formed stereogenic

(26) Gandour, R. D. *Bioorg. Chem.* **1981**, *10*, 169.

(27) Note that **A1** is a better hydrogen bond acceptor than **A3**, both in terms of the carbamate and phosphonate (**A1**) versus the ester group (**A3**) of the binding site and in terms of the imidazole (**A1**) versus the benzoylimidazole (**A3**) group of the cataytic group (see ref 25).

(28) Of the common N-protecting groups available, the acid labile BOC group is suitable here. FMOC protection proceeded without problem, but it was not compatible with the following base treatment, and Cbz protection was not suitable due to the hydrogenation required in deprotection which would reduce the vinyl group.

(29) Bartlett, P. A.; Lamden, L. A. *Bioorg. Chem.* **1986**, *14*, 356.

(30) Yamauchi, K.; Kinoshita, M.; Imoto, M. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2528.

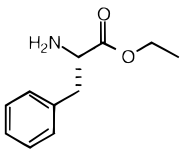
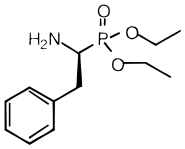
(31) Rogers, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 2463.

(32) For a similar reaction with diketones: Lombardino, J. G. *J. Heterocycl. Chem.* **1973**, *10*, 697.

(33) Krolski, M. E.; Renaldo, A. F.; Rudisill, D. E.; Stille, J. K. *J. Org. Chem.* **1988**, *53*, 1170.

(34) Andersson, L.; Ekberg, B.; Mosbach, K. *Tetrahedron. Lett.* **1985**, *26*, 3623.

Table 3. Discrimination between Chiral Phosphonate- and Carboxylate-Containing Substrates by Their Complementary Polymers[a]

TEMPLATE:	SUBSTRATE: **13D,L** k'max	α	**12R,S** k'max	α
 **13L**	2.4	2.0	0.9	1.3
 **12S**	0.4	1.1	0.8	2.3

[a] The polymers were prepared as described in the Experimental Section. In the chromatographic evaluation 25 nmol of either enantiomer was applied using as eluent MeCN/H_2O/HOAc, 96.3:1.2:2.5 (v/v %), and a flow rate of 0.4 mL/min. k'_{max} refers to the capacity factor of the most retained enantiomer which is complementary to the configuration of the template. a = separation factor = k'_{max}/k'_{min}.

center at phosphorus, and finally by CP and HP couplings. Nevertheless adequate characterization by HRMS, elemental analysis, and NMR indicated a high purity of the products. **A1** and **A2** were also hygroscopic and unstable and had to be kept cool and dry.

Coupling of the phosphonate and the L-histidinole monomer **B4** failed. Therefore the phosphonate was replaced with BOC-L-phenylalanine and the coupling with **B4** again attempted. Using DCCI as condensing agent and DMAP as catalyst, **B1** could be obtained in poor yield.

Imprinting with Carboxylate (Ground State) and Phosphonate (Transition State) Templates. The mechanism of ester hydrolysis or transesterification is believed to involve a high-energy tetrahedral oxyanion intermediate (transition state like). Rate enhancements caused by esterolytic enzymes can to a large part be ascribed to eletrostatic stabilization of this structure.[13,14] In some cases the transition state theory has been proven by a correlation between the enzymatic rate enhancement and the ratio of binding constants of stable transition state analogues and the substrate in the ground state.[14,35] As stable transition state (TS) analogues, phosphonates have been used.

To probe the ability of imprinted polymers to discriminate between a transition state like structure and a ground state structure, we therefore imprinted the simple ethylesters **13L** (L-PheOEt) and **12S** (Table 3). Note that template **12S** ressembles the tetrahedral intermediate formed upon transesterification of **13D** with ethanol (with the exception of a negatively charged oxyanion). *R*- and *S*-diethyl-1-amino-2-phenylethylphosphonate (**12R, 12S**) (oxalate salts) were synthesized as described elsewhere. (Scheme 5).[36] Although the triethylorthoformate reaction was relatively unclean, the product could be purified by precipitation as the oxalate salt. Templated polymers were prepared by photopolymerization using either **12S** or **13L** as templates, as described in the Experimental Section. The polymers were then evaluated in the chromatographic mode regarding their ability to discriminate between the enantiomers of both templates (Table 3).

The enantiomers of the templates were efficiently retained and resolved only on their complementary polymers. This shows that an imprinted polymer can be prepared that binds a tetrahedral (transition state like) structure in preference to a planar structure (ground state). The separation factors observed for the template on both polymers are very similar despite PO being a stronger hydrogen bond acceptor than CO. This may reflect the influence of an intramolecular hydrogen bond[37] which is stronger in **12** than in **13** and which in turn would reduce the extent of intermolecular hydrogen bonding to MAA. On the other hand this intramolecular hydrogen bond would limit the number of stable conformers which could lead to more well-defined recognition sites. The absence of discrimination of D- and L-**13** on the polymer imprinted with **12S** supports this argument. This polymer also showed weaker binding of its template than the **13L** imprinted polymer, possibly a result of the weaker intermolecular hydrogen bonding to MAA. The **13L** imprinted polymer resolved to some extent **12R**,**S**, indicating a larger conformational freedom for the template **13L**. Attempts to use the polymer imprinted with **12S** for the catalysis of transesterification of **13** failed.

Preparation of Esterase-Mimicking Polymers. Polymer Synthesis. The polymers were prepared by free radical photoinitiated polymerization of the monomer mixtures indicated in Tables 1 and 2 using the templates

(35) For a review see: Kraut, J. *Science* **1988**, *242*, 533−540.
(36) Kafarski, P.; Lejczak, B. *Synthesis* **1980,** 307.
(37) Gilmore, W. F.; McBride, M. A. *J. Pharm. Sci.* **1972**, 1087.

shown in Schemes 1−4. As judged by TLC, the templates **A1**−**A3** containing the phenolimidazole function were not completely stable under the polymerization conditions. The poor stability was also noted during the BOP-catalyzed synthesis of these derivatives. Since the decomposition might be catalyzed by the TLC silica gel plate, an estimate of the extent of decomposition was done with ^{1}H NMR. The stability of templates **A1** (as TFA salt and as free base) and **A3** in $CDCl_3$ both in the presence and absence of MAA was checked. For **A1 (**TFA salt) this showed a decomposition of 8% in the absence and 15% in the presence of MAA after 2.5 h. After 17 h about 20% of A1 as TFA salt and as free base had decomposed. **A3**, however, appeared stable under these conditions. The poor stability of template **A1** made it necessary to synthesize it fresh just prior to polymerization.

Template Removal: Model Study. To remove the template from the polymers, they were subjected to various nucleophilic treatments. These were chosen on the basis of initial attempts to cleave templates **B2**, **C1**−**3**, diethyl-*p*-vinylphenyl phosphate, and the benzyl ester **11** in homogeneous solution. Base treatment (MeOH/[NaOH 1 M], 1/1 (v/v)) led to quantitative hydrolysis of **B2**, **C1**, and diethyl-*p*-vinylphenyl phosphate whithin 3 h, while **11** reacted considerably slower, giving a 60% conversion after 3 h and a 90% conversion after 18 h. About 10% of **11** hydrolyzed by cleavage of the ethyl ester linkage. Note that the cross-linker EDMA was hydrolyzed under these conditions. No acid-catalyzed reaction was observed at room temperature, and only after prolonged heating did some of the **C2** react. EDMA was also stable under the acidic splitting conditions. However fluoride-catalyzed transesterification[38] was a more efficient way of cleaving the templates. As evidenced by the appearance of nonpolar products, tetrabutylammonium fluoride (TBAF) catalyzed efficiently the transesterification of **C2** to the corresponding methyl ester with complete conversion after 2 h. **C3** reacted only slowly but relatively fast at elevated temperature. Cesium fluoride (CsF) was also efficient in catalyzing the transesterification. After overnight reaction at room temperature **B2** and **C3** had been completely converted, while only 55% of **C1** was transesterified according to HPLC. This was detected as the release of *p*-vinylphenol and the absence of polar products. After 48 h at room temperature **C1** showed 86% conversion, while complete conversion was seen within a few hours when heating the samples to 60 °C.

Thus aqueous base or fluoride treatment is preferably used for cleavage of the phenylphosphonate linkage. As noted above and in the BOP-catalyzed synthesis of **A1** and **A2**, the phenolimidazole-containing templates require considerably milder conditions for cleavage.

Template Removal by Methanol Extraction (Procedures 1 and 2). Only templates from polymer series A and B were partly removed by methanol extraction (Table 1). A strongly UV- and ninhydrin-active spot with $R_f = 0.6$ is seen on TLC of the extracts of **PA2** and **PB2**. A weak ninhydrin-active spot with $R_f < 0.1$ was also seen in **PA2**. A UV-active spot was seen in **PA1**. These spots are most likely due to products resulting from the transesterification discussed above (see Schemes 2−4). This was confirmed by the ^{1}H NMR spectra of the extracts. As expected, all spectra showed that the splitting resulted in transesterifications giving the corresponding methyl esters. The absence of vinyl protons showed that all monomers including the templates have reacted. The higher splitting yield of **PA2** may be a result of its poor stability, implying that it has partly decomposed prior to polymerization. This is seen from the stability tests prior to polymerization (vide supra). The splitting yields of **PA3** and **PB3** based on the NMR data probably do not represent the true values. Loss of the volatile methyl benzoate upon evaporation in the sample preparation for the NMR analysis results in values that are too low. The values resulting from the HPLC analysis are therefore more reliable (values in parantheses in Table 1).

Template Removal by Fluoride (3) or Aqueous Base (4) Treatment. Series A and B. In both treatments 3 and 4 TLC showed only a weak ninhydrin-active spot corresponding to BOCPheOMe (**PB1**) and PheOMe (**PB2**). ^{1}H NMR analysis did not show any measurable amount of template. Instead in treatment 4 a substantial amount of MAA was released. Possibly this is due to hydrolysis of pendant unreacted methacrylate groups. Elemental analysis of residual phosphorus in the polymers indicated in contrast to the NMR data that almost quantitative splitting of the template had taken place. The reason for the disagreement between these data is unclear.

Series C. Aqueous base-catalyzed template removal was followed with HPLC (RP-18) and ^{1}H NMR analysis on the supernatants. For **PC31** and **PC32** HPLC data showed a template splitting of ca. 70%, while only ca. 20% was observed for the remaining polymers. The additional heat treatment did not result in any further increase in splitting. A considerable amount of MAA had also been released from these polymers. The NMR data after the aqueous base treatment showed a considerably higher splitting yield probably due to the extensive washing of the polymers. The splitting result obtained from the elemental analysis also shows that the yield of splitting is higher than that indicated by the HPLC analysis. In the CsF-catalyzed template removal the HPLC data showed that ca. 50% of methyl-*p*-chlorobenzoate (product from **PC31**−**2** splitting) had been released and that no MAA was detected. Moreover in ^{1}H NMR no vinylic protons were seen, indicating a high level of template incorporation. The low yield from **PC31**−**2** can, as in series A above, be explained by evaporation of the methylbenzoate formed. Transesterification was in all cases indicated by appearance of methyl ester signals at around 4 ppm.

Characterization of the Polymers. **PA1**−**3** and **PC31**−**2** showed a yellow color where the intensity depended on the pH of the surrounding medium. From being light yellow colored at low and neutral pH they adopt a clear yellow color at pH over 10−11, the color becoming darker with time. The color change correlates well with the pK_a of the phenol group,[24] indicating accessibility of the phenol function. The intensity decreased in the order **PA3**, **PA2**, **PA1**, probably reflecting the yield of splitting of the template. Indeed after the aqueous base and CsF treatment the difference in intensity between the polymers was less pronounced. When adding a small amount of $Cu(OAc)_2$ at pH 12, known to coordinate nitrogen bases,[39] a clear green color is formed in **PA1**−**3** again with intensity decreasing in

(38) Ogilvie, K. K.; Beaucage, S. L.; Theriault, N.; Entwistle, D. W. *J. Am. Chem. Soc.* **1977**, *99*, 1277.

the order **PA3**, **PA2**, **PA1**. No color was formed in the other polymers upon this treatment. The polymers not mentioned here remained white upon the above treatments. Depending on the nucleophilic treatment applied, the polymers showed different swelling factors in acetonitrile. The polymers subjected to aqueous base treament showed clearly a larger swelling than the polymers treated by either only methanol extraction or CsF treatment. This is probably due to hydrolytic cleavage of crosslinks in the polymer leading to an increased flexibility in the polymer backbone (vide infra).

The polymers were finally characterized by IR and ^{13}C NMR spectroscopy. The FTIR spectra supported the correlation between the yield of template removal and the color intensity of the materials. Thus **PA3** showed the largest intensity of the band arising from the free −OH stretch at approximately 3550 cm^{-1} assigned to carboxylic acid groups and liberated phenol groups of the active sites. Unfortunately, the low concentration of aromatic residues in the polymer (0.5 mol %) prevented quantitative analysis of incorporated template by solid state ^{13}C NMR. These spectra revealed however a level of residual unsaturation in the polymers between 10 and 15%.

Monomer-Catalyzed Ester Hydrolysis. As a first step in the kinetic evaluation the homogeneous hydrolysis of BOCPheONP in the presence of the functionalized monomers was studied. The hereby obtained information about the catalytic role of the individual functional groups serves as a guideline in the following kinetic evaluation in the presence of the polymer catalysts. Only in the presence of the imidazole functionalized monomers (series A and B, monomers: **7** and **B4**) were appreciable rate enhancements observed (Figure 1). The observed rate, given as the pseudo-first-order rate constant k_{obs}, increased with increasing pH both in the background noncatalyzed reaction and in the presence of catalyst (Figure 1a). Also the catalytic rate k_{cat} (observed rate minus background rate) increased with increasing pH (Figure 1b), although this was more pronounced in the reactions catalyzed by **7**. In this case the increase in k_{cat} was accompanied by an increase in the UV absorbance of a shoulder at $\lambda = 350$ nm.

Kinetics of ester hydrolysis catalyzed by 4(5)-hydroxyphenylimidazole (4(5)HPI)[40] or 2-hydroxyphenylimidazole (2HPI)[31] (analogues of **7**) (Scheme 9) have been extensively studied. In the hydrolysis of the activated esters *p*-nitrophenylacetate (PNPA) or *p*-nitrophenyltoluate (PNPT) in the presence of 4(5)HPI the rate was of first order with respect to catalyst and increased with increasing pH, indicating involvement of the phenoxide group in the catalytic reaction mechanism.[40] This was further supported by a correlation between the rate and the pK_a of the phenol group.[31] In water the phenoxide of 2HPI was associated with a λ_{max} at 325 nm.[31] The correlation between the increase in k_{cat} and ϵ_{350} of **7** with pH (Figure 1b) is therefore evidence for involvement of phenoxide in the catalytic reaction mechanism at higher pH values. This probably takes place by either nucleophilic attack by phenoxide followed by intramolecular attack by imidazole and hydrolysis of the formed imidazolide or phenoxide-assisted nucleophilic catalysis by imidazole.[40] In the presence of **B4** the rate enhancement is ascribed to the imidazole group, which apparently is more efficient than the imidazole group in **7** in catalyzing the hydrolysis of BOCPheONP (compare the relative rates at neutral pH). For activated substrates nucleophilic displacement of ONP by imidazole is the exclusive pathway.[31]

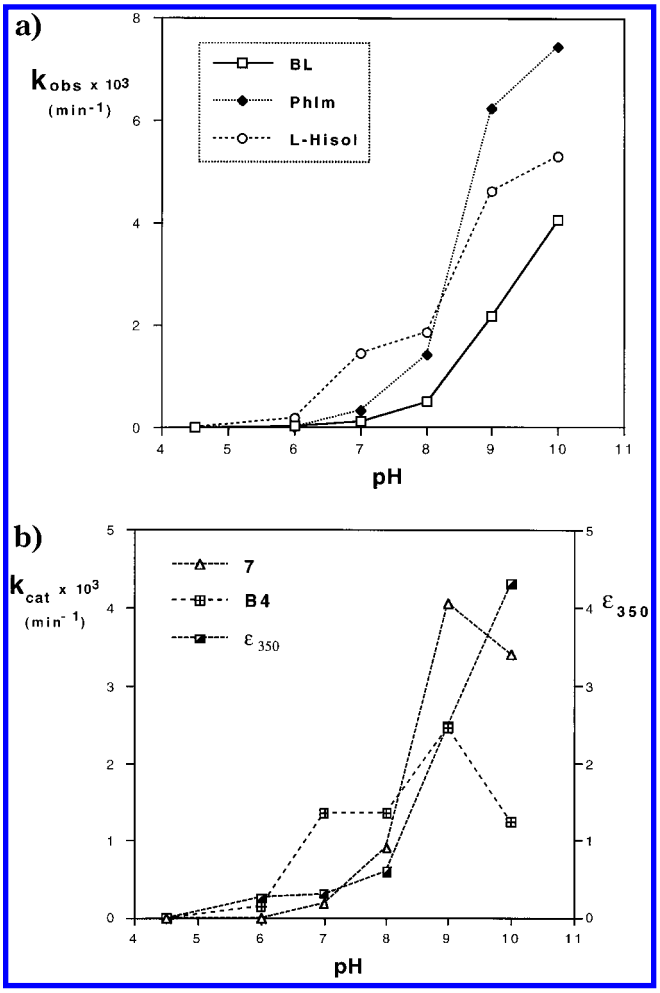
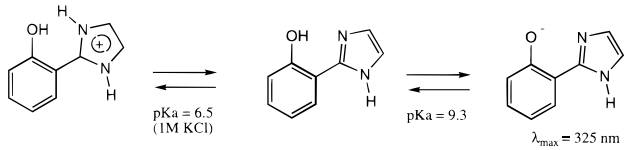


Figure 1. (a) Rate of hydrolysis of BOC-L-PheONP (0.025 mM) in MeCN/[potassium phosphate buffer 0.05 M], 7:3 (v/v) in the presence of soluble monomeric catalysts **7** and **B4** (0.7 mM) at varying pH. k_{obs} = observed pseudo-first-order rate constant. bg = background rate. (b) k_{cat} = catalytic rate constant = $k_{obs} - k_{bg}$, ϵ_{350} = extinction coefficient of **7** at 350 nm.

Scheme 9



Polymer-Catalyzed Ester Hydrolysis. pH Dependence. In Figure 2a the same experiment as in Figure 1 but in the presence of the polymer catalysts is shown. Only at pH's up to 8 is catalysis observed, while at higher pH the polymers all inhibit the reaction. At high pH the polymers are abundant in negatively charged carboxylic acid groups, suggesting electrostatic exclusion of nucleophilic OH^- from polymer-bound substrate as a possible cause of the inhibition. In a study of the effect of mobile phase pH on chiral separations using imprinted polymers

(39) For example see: Chin, J.; Jubian, V. *J. Chem. Soc., Chem. Commun.* **1989**, 839.
(40) Overberger, C. G.; Shen, C. M. *J. Am. Chem. Soc.* **1971**, *93*, 6992.

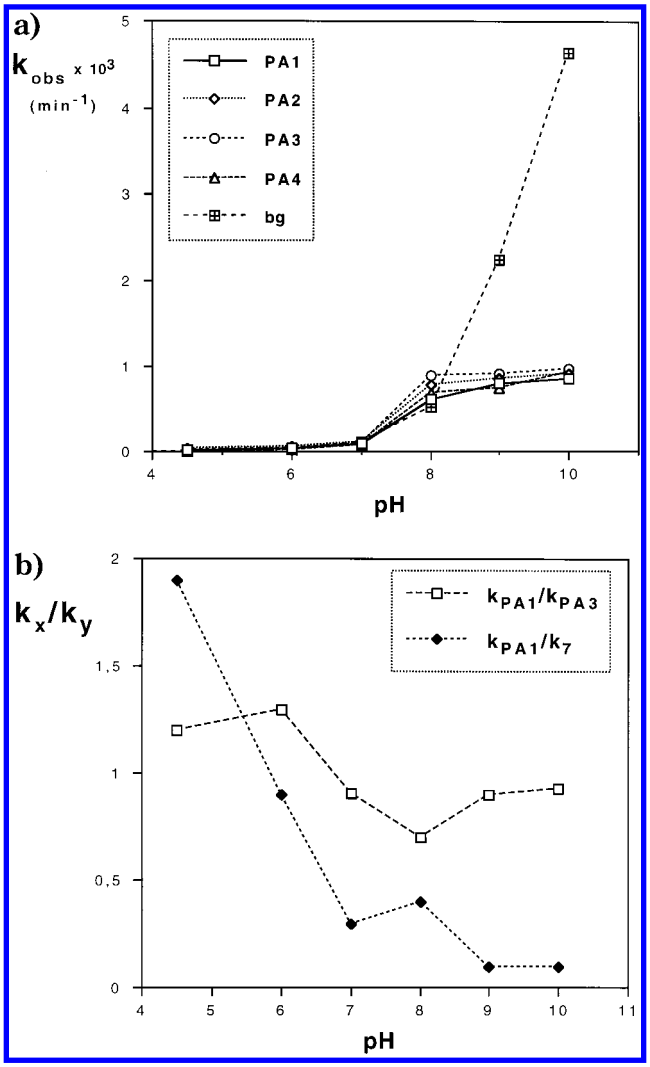


Figure 2. (a) Rate of hydrolysis of BOC-D-PheONP (0.025 mM) in MeCN/[potassium phosphate buffer 0.05 M], 7:3 (v/v) (2 mL) in the presence of series A polymer catalysts (50 mg) at varying pH. (b) Ratio of the observed pseudo-first-order rate constants (k_{obs}) for some of the catalysts in (a).

containing carboxylic acid groups, we observed that selective binding occurred preferentially at low pH, suggesting a difference in pK_a between the selective and nonselective sites.[41] At low pH the carboxylic acid groups of the sites should be mainly protonated and able to more efficiently form hydrogen bonds to the substrate. Interestingly, by comparing the observed rates in the presence of the template-imprinted polymers with the reference polymers or the soluble catalysts the relative rate increased with decreasing pH (Figure 2b). A relatively low pH is thus required for a selective polymer-catalyzed ester hydrolysis to occur in this system. As expected, carboxylic acid groups appear to be responsible for the substrate binding to the active sites. At pH 4.5 the imidazole group should be protonated (Scheme 9) and may therefore function as a general acid catalyst assisting the water attack on the substrate.[31]

Effect of Nucleophilic Treatments of the Polymers on the Observed Rate Enhancements. Series A. The catalytic activity of the polymers was sensitive to the nucleophilic treatments applied in order to remove the bound template. In series A the catalytic activity of **PA1** increased with the amount of template removed from the polymer (Table 4). After the methanol extraction (treatment 1 and 2) the control polymer **PA2** was more efficient than **PA1** in the catalysis, probably reflecting the difference in the amount of template removed from the polymers. As seen in Table 1, this treatment removed only 23% **A1** from **PA1**, while about 75% **A2** was removed from **PA2**. After the CsF treatment however the level of template removal should be similar for all polymers. The rate in the presence of **PA1** has now more than doubled compared to after treatment 1 and 2, while that in the presence of **PA3** remained approximately the same. This is evidence that the polymeric active sites generated by template removal are responsible for the rate enhancements observed here. Interestingly after treatment with aqueous base at room temperature **PA1** gave rise to an additional more than doubling of the rate, while the remaining polymers in the A series showed only modest increases. Note that the level of template removal is about the same as after the CsF treatment. A significant enantioselectivity is here also observed, with the enantiomer complementary to the configuration of **A1** being preferentially hydrolyzed. After 48 h treatment in aqueous sodium carbonate an even larger selectivity was observed. In presence of **PA1** BOC-D-PheONP was here hydrolyzed 1.9 times faster than the L enantiomer. Comparing **PA1** and **PA3**, a rate enhancement of 2.5 was observed, and compared to the soluble catalyst a 7-fold rate enhancement was observed. Using aqueous sodium hydroxide in methanol, a similar result is obtained. The selectivity of the binding site is here revealed by the control polymer **PA2** (prepared from template lacking the BOC group), which is clearly less efficient than **PA1** in catalyzing the hydrolysis. 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 polymer **PA3** was unaffected. Apparently the functional groups of the sites are still positioned for substrate binding, while the stereochemical complementarity has been lost. It should be noted that in the chromatographic enantiomer separations on polymers imprinted with L-phenylalanine anilide the selectivity decreased significantly (α 5.6 → 2.3) (swelling factor: 1.55 → 1.70) upon aqueous base treatment at room temperature.[42]

The larger rate enhancements exhibited by the polymers subjected to aqueous base treatment may be related to the flexibility of the polymer backbone reflected in the polymer swelling factor. The base-treated polymers swelled more in acetonitrile (series A: SF = 2.33 mL/mL (after heating for 24 h at 60 °C, SF = 2.47 mL/ml); series C: SF = 1.70 mL/mL) than those subjected to CsF treatment (series C: SF = 1.41 mL/mL) or those only subjected to methanol extraction (series A: SF = 2.00 mL/mL; series C: SF = 1.49 mL/mL). In the aqueous base treatment of cross-linked methacrylate polymers an increase in swelling has been explained by hydrolysis of ester cross-links, leading to a more flexible polymer structure.[43] Possibly the aqueous base treatment applied gives rise to a more flexible polymer network where the binding sites can more easily accommodate the transition state of the

(41) Sellergren, B.; Shea, K. *J. Chromatogr. A* **1993**, *654*, 17−28.

(42) Sellergren, B.; Shea, K. *J. Chromatogr. A* **1993**, *635*, 31.

(43) Jelinkova, M.; Shataeva, L. K.; Tischenko, G. A.; Svec, F. *Reactive. Polym.* **1989**, *11*, 253.

Table 4. Pseudo-First-Order Rate Constants for the Hydrolysis of Boc-D(L)-PheONP in the Presence of Series A Polymer Catalysts Subjected to Nucleophilic Treatments[c]

treatment	catalyst	$k_D \times 10^3$ (min^{-1})	k_D(PA1)/k_D	k_D/k_L
MeOH Δ (1+2)	**PA1**	0.026	1.00	1.04
	PA2	0.038	0.68	
	PA3	0.024	1.08	1.00
	PA4	0.014	1.85	-
	7	0.014	1.9	-
CsF (3)	**PA1**	0.057	1.00	1.06
	PA2	0.046	1.24	
	PA3	0.035	1.63	1.00
	PA4	0.022	2.59	
	7	0.014	4	
Na_2CO_3(aq) 24 h	**PA1**	0.140 (±0.01)[a]	1.00	1.37 (±0.05)[a]
	PA3	0.080 (±0.004)[a]	1.75 (±0.05)[a]	1.00
	7	0.014	10 (±0.7)[a]	
Na_2CO_3(aq) 48 h	**PA1**	0.122 (0.410)[b]	1.00	1.85 (1.63)[b]
	PA3	0.048 (0.190)[b]	2.54 (2.10)[b]	1.00 (1.00)[b]
	7	0.014 (0.125)[b]	7 (3.3)[b]	
NaOH(aq)	**PA1**	0.102	1.00	1.60
	PA2	0.056	1.82	
	PA3	0.046	2.22	1.00
	PA4	0.022	4.64	
	7	0.014	6	
NaOH(aq) 60 °C	**PA1**	0.108	1.00	1.00
	PA3	0.046	2.35	1.00
	7	0.014	6	

[a] Average of two independent experiments with error in parantheses. [b] Reaction carried out at 40 °C. [c] The hydrolysis of BOC-D- or L-PheONP (0.025 mM) in MeCN/[potassium phosphate buffer 0.05 M, pH 4.5], 1:1 (v/v), in the presence of the various catalysts was followed by monitoring the formation of *p*-nitrophenol by HPLC (see Experimental Section). **7** was added to a concentration corresponding roughly to the site concentration determined from the amount of template removed from the polymers.

Table 5. Pseudo-First-Order Rate Constants for the Hydrolysis of Boc-L(D)-PheONP in the Presence of Series B Polymer Catalysts Subjected to Nucleophilic Treatments

treatment	catalyst	$k_L \times 10^3$ (min^{-1})	k_L(PB1)/k_L	k_L/k_D
MeOH Δ (1+2)	**PB1**	0.329	1.0	1.18
	PB2	0.258	1.3	
	PB3	0.243	1.4	1.08
	PB4	0.276	1.2	
	B4	0.039	8.2	1.07
CsF (3)	**PB1**	0.342	1.0	1.06
	PB2	0.155	2.2	
	PB3	0.199	1.7	1.11
	PB4	0.138	2.5	
	B4	0.039	8.8	1.07
Na_2CO_3(aq) 24 h	**PB1**	0.304	1.0	1.04
	PB3	0.236	1.3	1.01
	B4	0.039	7.8	1.07

[a] The hydrolysis of BOC-L- or D-PheONP (0.025 mM) in MeCN/[potassium phosphate buffer 0.05 M, pH 6], 1:1 (v/v), in the presence of the various catalysts was followed by monitoring the formation of *p*-nitrophenol by HPLC (see Experimental Section). **B4** was added to a concentration corresponding roughly to the site concentration determined from the amount of template removed from the polymers.

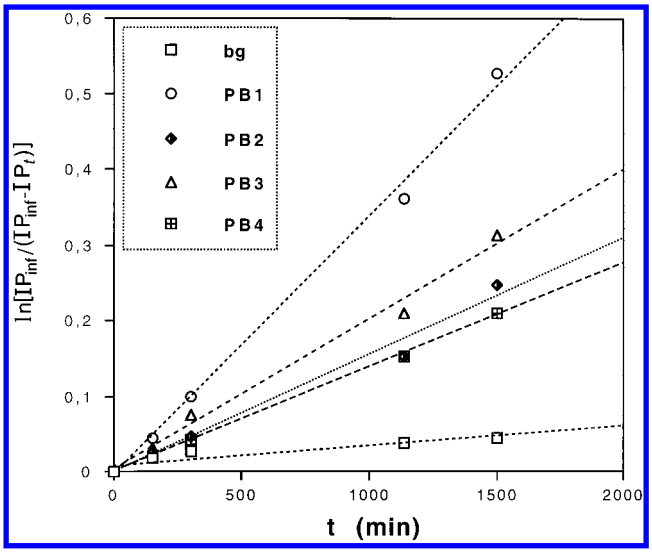


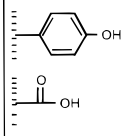
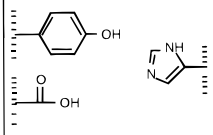
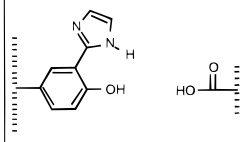
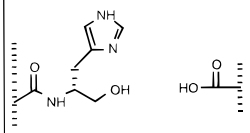
Figure 3. Evaluation of the observed pseudo-first-order rate constants for the hydrolysis of BOC-L-PheONP (0.025 mM) in MeCN/[potassium phosphate buffer 0.05 M, pH 6], 7:3 (v/v) (2 mL), in the presence of CsF-treated series B polymer catalysts (50 mg).

reaction. Note that conformational flexibility is a common requirement in enzyme catalysis.[13,14]

Series B. The rates of hydrolysis in the presence of the series B catalysts responded in a different way to the various nucleophilic treatments (Table 5). **PB1**, being the complement to BOC-L-PheONP, was in all cases most efficient in catalyzing the hydrolysis of the L enantiomer. A significant enantioselectivity in preference for the L enantiomer was observed. Comparing the rate enhancements after each treatment, no difference was noted in the presence of **PB1**, while upon CsF treatment the rates in the presence of the other polymers decreased slightly. This resulted in a slightly larger relative rate enhancement (Figure 3) of 2.5 for **PB1** relative to **PB4** and 8.8 for **PB1** relative to soluble catalyst after the CsF treatment. The other notable effect of the various treatments was that in contrast to the series A polymers the enantioselectivity decreased upon the more severe nucleophilic treatments. Note that the soluble chiral catalyst by itself exhibits some enantioselectivity in the catalysis.

The Role of the Active Site Functional Groups. In Table 6 CsF-treated catalytic polymers lacking the imidazole group (**PC11** and **PC31**), containing randomly distributed imidazole groups (**PC12** and **PC32**), or having an imidazole group in proximity to the binding site (**PA1**, **PA3** and **PB1**, **PB3**) have been compared. Due to the various concentrations of active sites in the polymers, second-order rate constants have been calculated (based on the amount of imidazole present in the monomer

Table 6. Second-Order Rate Constants for the Hydrolysis of Boc-D(L)-PheONP in the Presence of Various Polymer Catalysts[a]

POLYM.	FUNCTIONAL GROUPS	k_2	k_{2ref} ($M^{-1}min^{-1}$)	k_2/k_{2ref}
PC11, PC31		0.018	0.019	1.0
PC12, PC32		0.113	0.108	1.0
PA1, PA3		0.114	0.070	1.6
PB1, PB3		2.280[a]	1.330[a]	1.7[a]

[a] The hydrolysis of the complementary enantiomer of BOCPheONP (0.025 mM) in MeCN/[potassium phosphate 0.05 M, pH 4.5 ((a) pH 6)], 1:1 (v/v), in the presence of esterase-mimicking polymers **PC11**, **PC12**, **PA1**, and **PB1** and corresponding reference polymers **PC31**, **PC32**, **PA3**, and **PB3** was followed on HPLC as described in the Experimental Section. The second-order rate constants (k_2) were calculated from the theoretical concentration of accessible imidazole groups in the polymers or in the absence of imidazole groups, from the theoretical concentration of accessible sites.

mixture). The imidazole group is apparently required for catalysis since only small rate enhancements and no template-induced catalysis are seen using **PC11** and **PC31**. Introducing a randomly distributed imidazole group, a larger rate enhancement is seen but still no template-induced effect. Only when the imidazole group is implanted in conjunction with the site are template-induced rate enhancements observed (**PA** and **PB**). Clearly the positioning of the imidazole group in proximity to the binding site is necessary in the design of a template-imprinted polymeric esterase model.

Rate of Hydrolysis versus Substrate Concentration. The rate of hydrolysis of BOC-D-PheONP in the presence of aqueous base-treated **PA1** and **PA3** was studied up to substrate concentrations of 10 mM (Figure 4). The rate enhancement of **PA1** relative to **PA3** and soluble catalyst persists at higher substrate concentrations. Only a weak saturation tendency is seen, implying that the substrate binding is quite weak. An Eadie-Hofstee plot of the kinetic data gave for **PA1** $K_m = 5.4$ (± 0.4) mM, $k_{cat} = 1.40 \times 10^{-3}$ min^{-1} and for **PA3** $K_m = 10.8$ (± 0.1) mM, $k_{cat} = 1.14$ (± 0.04) $\times 10^{-3}$ min^{-1}. The K_m values agreed well with those that could be estimated from the substrate peak integrals at the start of the reaction. Apparently the rate enhancements are mainly the result of a stronger substrate binding to the complementary polymers. At a substrate concentration of 10 mM turnover kinetics was observed. Over the time course followed the soluble catalyst performed 0.7 turnovers.

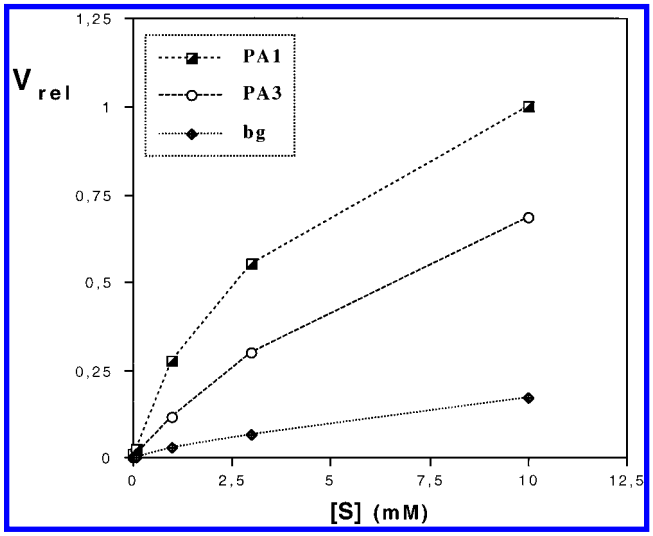


Figure 4. Rate of hydrolysis of BOC-D-PheONP in MeCN/[potassium phosphate buffer 0.05 M, pH 4.5], 7:3 (v/v) (2 mL), in the presence of series A polymer catalysts (50 mg) as a function of substrate concentration. The reactions were followed over 3 days and the rates calculated as the average of the rate of substrate consumption and the rate of product formation.

Meanwhile **PA1** and **PA3** had performed 5 and 3 turnovers, respectively.

Polymer-Catalyzed Hydrolysis of L(D)-Phenyl Alanine Ethyl Ester (L(D)-PheOEt). The hydrolysis of

Table 7. Pseudo-First-Order Rate Constants for the Hydrolysis of D(L)-PheOEt[a]

catalyst	$k_{Dobs} \times 10^5$ (min^{-1})	$k_{Lobs} \times 10^5$ (min^{-1})	k_{Dobs}/k_{BLD}	k_{Lobs}/k_{BLL}	k_D/k_L[b]
PA1	2.50	1.97	3.05	2.43	1.44
PA2	2.23	1.92	2.68	2.37	1.26
PA3	1.97	1.86	2.37	2.29	1.08

[a] The hydrolysis of D(L)-PheOEt (0.025 mM) was monitored in MeCN/[potassium phosphate 0.05 M, pH 7.4], 1:1 (v/v), in the presence of esterase-mimicking polymers as described in the Experimental Section. [b] Ratio of first-order rate constants after subtraction of the rate constants for the background reaction.

(PheOEt), a nonactivated substrate, was studied at pH 7.4 using the polymer catalysts **PA1, PA2**, and **PA3** (see Table 7). Polymer **PA2** has the exact size complementarity to D-PheOEt. A rate enhancement up to 3 was observed when compared to the blank rate. **PA2** hydrolyzed D-PheOEt 1.3 times and polymer **PA1** 1.4 times faster than the L ester. The Michaelis−Menten kinetic studies for the hydrolysis of D- and L-ethyl esters was carried out for polymers **PA1** and **PA2.** The concentration was varied from 0.5 to 5 mM.(Figure 5). The K_m and k_{cat} values for polymer **PA1** for L-PheOEt are 1.96 (±0.05) mM and 1.91×10^{-5} min^{-1}, respectively. The corresponding values for polymer **PA2** are $K_m = 2.32$ (±0.06) mM and 1.82×10^{-5} min^{-1} . The values for the hydrolysis of D-PheOEt are for **PA1** $K_m = 1.92$ (±0.05) mM, $k_{cat} = 2.32 \times 10^{-5}$ min^{-1} and for polymer **PA2** $K_m = 2.04$ (±0.04) mM and $k_{cat} = 2.07 \times 10^{-5}$ min^{-1}. The ratio of the resulting apparent second-order rate constants k_{cat}/K_m for D-PheOEt over L-PheOEt is informative about the D-enantioselectivity of the polymers within a larger concentration interval. These figures are 1.24 for polymer **PA1** and 1.30 for **PA2**, which is in agreement with the expected structural complementarity.

Conclusions

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. The catalysts described here are truly tailor-made since the enantioselectivity in all cases could be predicted. They furthermore provide yet an example of an imprinting strategy combining noncovalent and covalent binding forces between the template and the functionalized monomers. This allows simultaneous introduction of different functional groups into the sites. Hydrogen bonding, involving carboxylic acid groups at the sites, is the main driving force in the stereoselective binding step and an imidazole group associated with the binding site responsible for the catalytic action. For the first time the enantioselective hydrolysis of a nonactivated ester is reported. The polymer catalysts were found to be catalytically active and retained the enantioselectivity over a period of about 10 years. This clearly highlights the advantage of polymer mimics over the other less stable soluble models. The influence of transition state complementarity is still unknown, but imprinted polymers are apparentely capable of discriminating between a planar and tetrahedral structure similar to the substrate and template **A1**, respectively. It may be of interest that the optimum pH for the selective catalysis of the activated ester is at the acidic side. This is quite unusual in esterolytic reactions and may be of general synthetic interest when the substrate contains for instance base labile protecting groups.

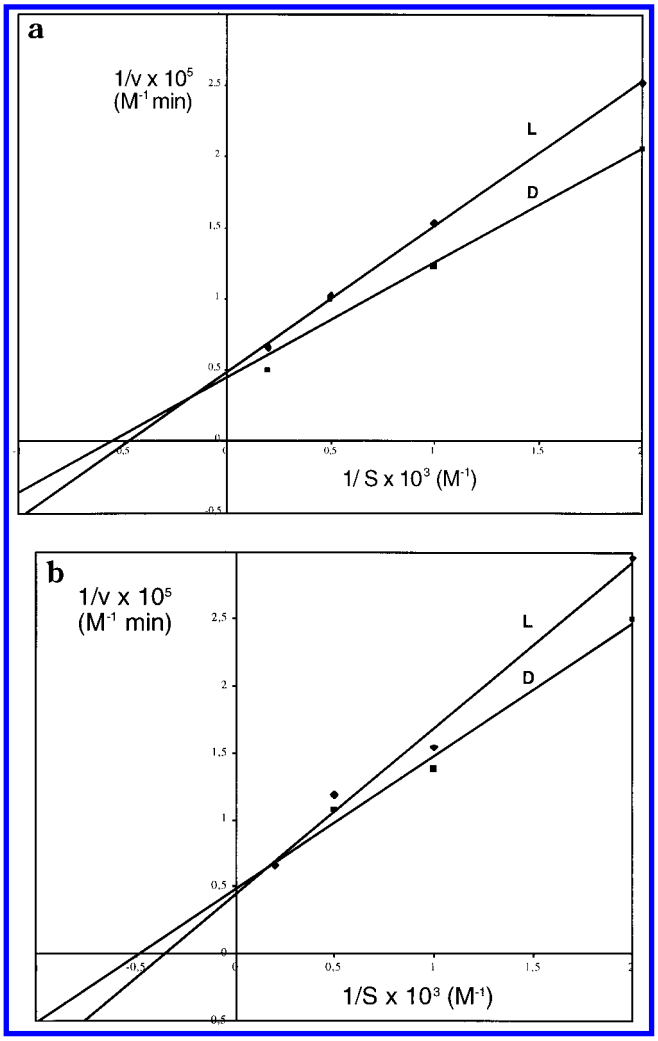


Figure 5. Lineweaver−Burk plots of the initial reaction rates versus the substrate concentration for the hydrolysis of L-phenyalanine ethyl ester in the presence of (a) **PA1** and (b) **PA2**. The hydrolysis reactions were followed for over 30% conversion, and the rates were calculated as the average of the rate of product formation for duplicate samples after subtracting the blank rates.

Although modest, the rate enhancements reported here should be considered as a first step in the development of imprinted polymers for enantioselective catalysis. The major problem with the present system is the rather weak substrate binding. Introduction of additional stronger binding interactions directed both toward the acyl part and the leaving group of the substrate are guidelines for our future efforts to achieve an efficient mimic of chymotrypsin.

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Supporting Information Available: Detailed synthetic procedures and supporting structural characterization not included in the Experimental Section are available for the compounds described in this work . This material is available free of charge via the Internet at http://pubs.acs.org.

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