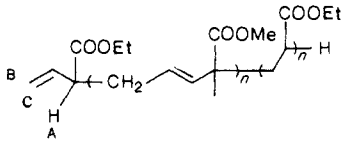
End Group Analysis of PMMA Initiated with 14. See Table II, entry 7. ^{1}H NMR (360 MHz, $CDCl_3$): δ 4.1 (m, 2 H, OCH_2), 5.01 (d, J = 12 Hz, 1 H, C=CH_2 cis H), 5.12 (d, J = 17 Hz, 1 H, C=CH_2 trans H), 5.38–5.6 (m, 1 H, C=CH), 5.9–6.3 (2 m, 2 H, C=CH), 3.0 (m, 1 H, methine CH).

End Group Analysis of Poly-2 Initiated with 10. To a solution of 0.22 mL (1 mmol) of **10** and 50 μL of tetrabutylammonium *m*-chlorobenzoate (0.1 M in THF) in 10 mL of THF was added 1.01 g (1.06 mL, 8 mmol) of **2**. When the exothermic reaction was finished, 0.86 mL (8 mmol) of ethyl acrylate was added. Then 1 mL of methanol was added, and the solution was evaporated to 1.9 g of oily poly(methyl 2-methylpentadienoate-*b*-ethyl acrylate). GPC: $\bar{M}_n$ 1980, $\bar{M}_w$ 4400, P = 2.23 (theory, $\bar{M}_n$ 1900). ^{1}H NMR (360 MHz, $CDCl_3$): δ 3.0 (m, 1 H, A), 5.05–5.16 (m, 2 H, B and C). The molar ratio of ethyl acrylate units and methyl 2-methylpentadienoate units is 1.0.



Acknowledgment. The authors are grateful to T. Fukunaga for critical review of the manuscript. Thanks are due B. M. Trost, O. W. Webster, and F. Davidson for valuable discussions of this work, to M. C. Han for molecular weight determinations by size-exclusion chromatography, and to Toni Anemone for help in preparation of the manuscript. The experimental assistance of C. E. Jackson, R. S. Peacock, and K. M. Stoltzfus is gratefully acknowledged.

Registry No. Poly-**1**, 26985-69-3; (**1**)(MMA) (block copolymer), 115078-18-7; **2**, 86952-00-3; poly-**2**, 115078-14-3; (**2**)(MMA) (block copolymer), 115078-19-8; (**2**)(MMA) (copolymer), 115078-20-1; poly-**3**, 115078-15-4; poly-**4**, 33774-25-3; (**4**)(MMA) (copolymer), 115078-21-2; (**4**)(MMA) (block copolymer), 115078-22-3; poly-**5**, 63747-18-2; **6**, 115078-12-1; poly-**6**, 115078-17-6; **7**, 31469-15-5; **9**, 51425-66-2; **10**, 73311-50-9; **11**, 73311-52-1; **12**, 87121-06-0; **13**, 115078-10-9; **14**, 115078-11-0; **15**, 80-62-6; **16**, 115078-13-2; MMA, 80-62-6; PMMA, 9011-14-7; TASF, 59218-87-0; $TASHF_2$, 85248-37-9; Bu_4NOAc, 10534-59-5; Bu_4N*m*CB, 60619-92-3; Bu_4NBB, 115116-46-6; 2-(diethylphosphono)-4-butyrolactone, 2907-85-9; sorbaldehyde, 142-83-6; poly(methyl sorbate), 30813-48-0.

Highly Enantioselective and Substrate-Selective Polymers Obtained by Molecular Imprinting Utilizing Noncovalent Interactions. NMR and Chromatographic Studies on the Nature of Recognition

Börje Sellergren,* Matti Lepistö,† and Klaus Mosbach*

Contribution from the Department of Pure and Applied Biochemistry, Lund Institute of Technology, University of Lund, P.O. Box 124, 221 00 Lund, Sweden.
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Abstract: By use of the molecular imprinting technique, highly enantioselective and substrate-selective polymers were prepared by utilizing noncovalent interactions between the print molecule (L-phenylalanine derivatives) and methacrylic acid monomers. In the chromatographic mode, such polymers resulted in almost base-line separation of the enantiomers of the respective print molecule (phenylalanine anilide) with a maximum separation factor (α) of 3.5. The polymers also showed a high capacity (1.5 mg of racemate/g of polymer with optically pure peak maxima). Substrates other than the respective print molecule were in most cases poorly resolved. The selectivity was shown to be governed by the number and nature of interactions between the substrate and the polymer stationary phase, as well as the shape and rigidity of the substituents of the print molecule. In order to investigate these interactions, a chromatographic and ^{1}H NMR study involving titration of the print molecule (phenylalanine anilide) with carboxylic acid was performed. The results were consistent with the existence of multimolecular complexes formed by electrostatic and hydrogen-bonding interactions and allowed an estimation of their formation constants and distribution. From this information, it was concluded that complexes between L-phenylalanine anilide and a maximum of three methacrylic acid monomers exist in solution prior to polymerization. On the basis of hydrogen bond theory, a model of the 1:2 complex is proposed. Finally, evidence is presented that is consistent with the existence of shape-specific cavities within the polymer.

The technique of molecular imprinting, that is, producing imprints of molecules in synthetic polymers, has received much attention in recent years. Apart from the general theoretical interest in this technique, potential practical applications are obvious, notably the use of such polymers for separations, facilitated synthesis, and possibly also as enzyme or receptor mimics. In most of the studies reported to date, the molecule forming the cavity (the print molecule) is allowed to interact with functionalized monomers prior to polymerization. After polymerization, the print molecule is removed and subsequent recognition occurs via a combination of reversible binding and shape complementarity. Essentially two different approaches have been followed in these studies: (a) Reversible covalent binding of the print molecule to the monomers has been used. Following this technique numerous reports have appeared describing highly selective polymers.[1] (b) Functionalized monomers are allowed to "prearrange" around the print molecule by noncovalent interactions (i.e., electrostatic, hydrophobic, hydrogen bonding).[2]

Whereas approach a has been used for the separation of the enantiomers of sugar derivatives,[1,3] approach b has in this and in other reports by us been applied in the separation of the enantiomers of amino acid derivatives.[4,5] Following both approaches, a variety of polymers have been used, encompassing styrene-

† Department of Organic Chemistry III, University of Lund, P.O. Box 124, 221 00 Lund, Sweden.

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Table I. Results of Chromatography for the Different Substrates When Applied on the Corresponding L-Selective Polymers[a]

solute:	D,L-PheNHPh		PheNHEt		p-NH_2PheOEt		PheOEt	
polymer	k'_L	α	k'_L	α	k'_L	α	k'_L	α
L-PheNHPh	4.3	3.2	1.3	1.3	2.3	1.05	1.0	1.05
L-PheNHEt	2.4	2.1	2.0	2.0	1.2	1.1	1.1	1.3
L-p-NH_2PheOEt	1.6	1.1	1.4	1.2	2.7	1.8	1.0	1.3
L-PheOEt	2.6	1.1	0.9	1.3	2.5	1.2	1.4	1.3

[a] For L-PheNHPh, L-PheNHEt, L-p-NH_2PheOEt, and L-PheOEt the eluent HOAc was 0.8, 0.8, 0.6, and 0.3 M in acetonitrile, respectively; flow rate 0.3 mL/min; temperature, 85 °C; k'_L, capacity factor of the L form; α, separation factor (k'_L/k'_D).

based,[1,3,4a,6] silica,[1,7-9] and acrylic-based polymers.[1-5,10] A more recent variation of the topic of molecular imprinting represents the preparation of so-called distance-selective polymers containing functional groups at defined positions.[6,7,9]

We believe that the "prearrangement" approach b for the formation of molecular imprints may be advantageous for the following reasons: Since no covalent modification of the print molecule is required, and since a variety of different binding interactions may exist, such a procedure would be both simple and general. The kinetics of the noncovalent binding, analogous to enzyme–substrate binding, compare favorably to the reversible covalent binding in approach a. This argument is so far of less importance, since the binding kinetics of the imprinted polymers in general seem to be limited by other factors (see later discussion).

Efforts have been made in developing such an imprinting procedure for amino acid esters.[4,5] In this case, ion pairing between an amino acid ester and carboxylic acid groups of the monomer/polymer were utilized, resulting in macroporous polymers exhibiting substrate selectivity and enantioselectivity when applied in a chromatographic procedure.[5] The present paper reports on considerable improvements of this technique by utilizing hydrogen bonding between amino and amide groups of the print molecule (amino acid esters and amides) and carboxylic acid groups of the monomer/polymer, in addition to the ammonium–carboxylate ion pair. Chromatography on the formed polymers resulted in a base-line separation of the print molecule enantiomers. In addition, the polymers exhibited a pronounced selectivity for their complementary substrates.

Since selectivity was shown to be influenced by the number and nature of interactions between the substrate and the stationary phase, these interactions were examined in more detail. In particular, we decided to investigate the extent of complex formation and the nature of complexes formed between the print molecule and the acid monomers prior to polymerization. This was performed by a titration procedure employing a chromatographic technique and ^1H NMR spectroscopy.

Results and Discussion

Polymer Selectivity. After optimization of the polymerization conditions,[11] polymers were prepared and compared in a chromatographic procedure[5] where the column temperature and the eluent composition were optimized for each polymer. Under these conditions the substrate selectivity and enantioselectivity were investigated (Table I). Polymers prepared using L-phenylalanine anilide (L-PheNHPh), L-phenylalanine ethyl amide (L-PheNHEt), or L-p-aminophenylalanine ethyl ester (L-p-NH_2PheOEt) as print molecules showed a pronounced enantioselectivity as well as substrate selectivity for their complementary substrate (print molecule), as reflected in the obtained separation factor α. The substrate selectivity is additionally confirmed in the variation of the retention, k', of the different substrates. The results reveal that the complementary substrate is the most strongly bound, indicating that nonspecific interactions are unimportant.

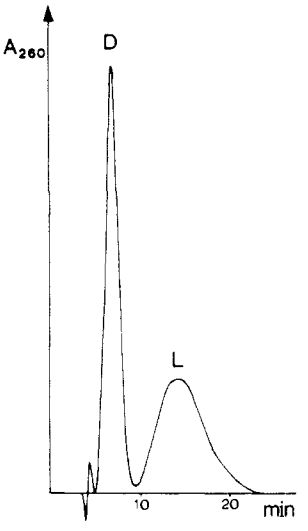

Figure 1. Elution profile of D,L-PheNHPh (10 nmol) applied on a L-PheNHPh-selective polymer. Eluent, HOAc, 1 M in acetonitrile; temperature, 90 °C; flow rate, 0.3 mL/min; separation factor (α), 3.5; resolution (R_s), 1.2.

Since these pronounced effects were obtained with print molecules only slightly different from the original print molecule, L-phenylalanine ethyl ester (L-PheOEt), additional noncovalent interactions are believed to participate within the sites.

In the case of PheNHPh almost base-line separation of its enantiomers on a L-PheNHPh-selective polymer was obtained (Figure 1). Relatively broad peaks were found,[12] but the high selectivity still resulted in acceptable resolution. This polymer also exhibited high capacity, giving a separation of 0.75 mg of D,L-PheNHPh (1.5 mg of racemate/g of polymer), with optically pure peak maxima. Broad peaks were also observed in chromatography using imprinted polymers based on reversible boronate ester formation[3] (approach a). These were concluded to be due to either (1) a slow binding reaction with a sterically hindered transition state of the covalent binding or (2) a slow orientation of the substrate in the cavity, when more than one binding interaction is possible. Since the peak broadening is of the same order in our case in spite of the more rapid and less sterically demanding noncovalent interactions, it is likely that (2) in both cases is the main reason for the peak broadening.

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(11) A polymerization procedure based on the one developed by Wulff et al. was employed. See, for example: Wulff, G.; Kemmerer, R.; Vietmeier, J.; Poll, H.-G. *Nouv. J. Chim.* **1982**, *6*, 681. Previously, we found that selectivity could be improved by increasing the ratio of the acid monomer to the print molecule.[5] With L-phenylalanine ethyl ester as print molecule, an optimal molar ratio of 4 was found, resulting in a degree of cross-linking of 83%. Since L-phenylalanine anilide forms multimolecular complexes prior to polymerization (see later discussion), the selectivity of polymers prepared with this as print molecule should exhibit a stronger dependence on the amount of acid monomer. As expected, the enantioselectivity was lower (α = 1.7) for polymers prepared with an above defined ratio of 2, compared to polymers with the original ratio of 4 (α = 3.5). The degree of cross-linking was kept the same by replacing methacrylic acid with methyl methacrylate. Further studies on the influence of the concentration of methacrylic acid on the uptake capacity and selectivity of the polymers are under way.[4c]

(12) $N_D \approx 100$ and $N_L \approx 30$ compared to an N of $\approx$1000 for nonretained substances.

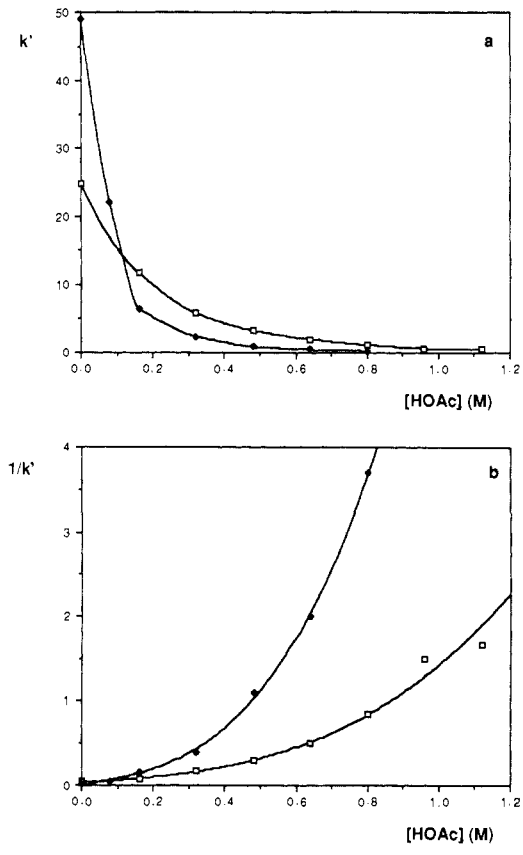

Figure 2. (a) Retention (k') of D-PheNHPh at 23 °C (◆) and at 60 °C (□) on a L-PheNHPh-selective polymer vs the concentration of HOAc in the eluent. (b) Plot of $1/k'$ vs concentration of HOAc in the eluent, calculated from the data in Figure 2a. The data points were fitted to theoretical curves defined by eq 6. At 23 °C (◆): $C_0 = 0.016$; $C_1 = 0.475\ M^{-1}$; $C_2 = 1.442\ M^{-2}$; $C_3 = 4.660\ M^{-3}$. At 60 °C (□): $C_0 = 0.039$; $C_1 = 0.213\ M^{-1}$; $C_2 = 0.259\ M^{-2}$; $C_3 = 0.820\ M^{-3}$.

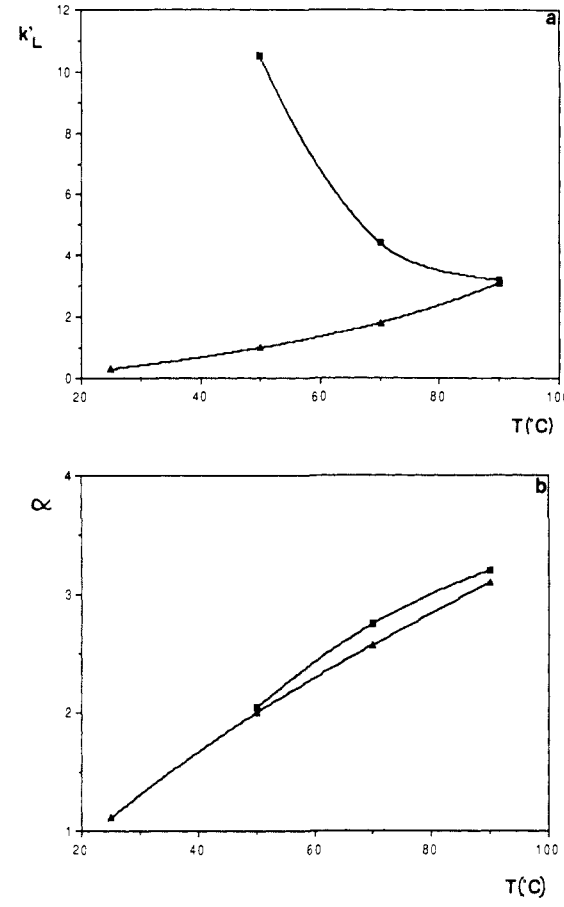

Figure 3. Retention of the L form, k'_L (a), and selectivity, α, (b) vs temperature, for D,L-PheNHPh on an L-PheNHPh-selective polymer at different eluent compositions: water (5.6 M) in acetonitrile (■), HOAc (1.1 M) in acetonitrile (▲).

In order to explain the pronounced selectivities observed (Table I), the following factors should be considered: (1) the number and nature of interactions between the substrate and the sites; (2) the rigidity of substituents around the chiral center; (3) the shape and size of these substituents.

Concerning (1), the importance of hydrogen bonding in compounds containing carboxylic acid and amide functional groups is well established,[13] as are the electrostatic and hydrogen-bonding interactions between carboxylic acids and amines.[14,15] With the presence of amide groups in PheNHPh and PheNHEt, hydrogen bonding between these and the methacrylic acid (MAA) prior to polymerization is likely to occur. In addition to the ammonium–carboxylate electrostatic interaction, this should result in a polymer offering multipoint interactions with the complementary substrate. This effect is probably the main reason for the difference in the α and k' values of D,L-PheNHEt and D,L-PheOEt obtained on their complementary L-selective polymers. A dramatic increase in selectivity is observed by introducing the amide function. The importance of (1) is also seen in the selectivity of the L-*p*-NH_2PheOEt-selective polymer. In this case two electrostatic interactions are anticipated, since the aromatic amino function of *p*-NH_2PheOEt is expected to be protonated under the conditions of elution as well as during polymerization.

The influence of (2) and (3) is less obvious. Amide bonds are more rigid than ester bonds and even more so when hydrogen bonded.[16,17] In addition to (1) (see above discussion), this could contribute to the higher selectivity of the L-PheNHEt polymer compared to that of the L-PheOEt polymer.

PheNHPh and PheNHEt are different in both shape and rigidity of their amide substituents, the anilide ring in PheNHPh being more rigid due to charge distribution and steric factors. Although their hydrogen-bonding interactions with carboxylic acids are probably different as well,[17b,18] the low α value of D,L-PheNHEt on the L-PheNHPh polymer compared to that obtained on its complementary polymer is most likely due to a difference in shape. This is supported by the high α value of D,L-PheNHPh obtained on its complementary polymer. Furthermore, the L-PheNHEt polymer recognizes L-PheNHPh as well as L-PheNHEt, which could be ascribed either to less-defined cavities (due to lower rigidity of the ethyl substituent) or to the above-mentioned difference in the interaction with the acid.

In summarizing the relative importance of (1)–(3), it can be concluded that probably all factors have an influence on selectivity but that the greatest effect is seen on the introduction of additional interactions.

Characterization of Complexes Formed between Print Molecules and Monomers Prior to Polymerization. Chromatographic Study of the Complex Formation. In order to elucidate the nature and importance of the interactions, the dependence of retention on the eluent composition for D-PheNHPh on a L-PheNHPh-selective polymer,[19] at room temperature and at 60 °C, was investigated (Figure 2).

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(19) Since the separation factor is apparently independent of the eluent composition (Figure 3b), the same dependence is seen for the L form. Because of the shorter time required for analysis, the D form was used in this investigation.

In acetonitrile (the solvent used in the polymerization) the retention was strongly dependent on the amount of acetic acid (HOAc) in the eluent, the effect being more pronounced at the lower temperature. Since interactions are to be expected between carboxyl groups, of either the stationary phase or the eluent, and amino and amide groups of the solute,[13-15] the results suggest the formation of complexes in the eluent. This was further supported by measuring the retention (Figure 3a) and selectivity (Figure 3b) as a function of temperature, at two different eluent compositions. At lower acid concentrations (<0.7 M), or in acetonitrile/water systems, the retention dropped by increasing the temperature. At higher acid concentrations, the behavior was reversed and the retention increased upon an increase in temperature. The selectivity, however, increased with the temperature[20] and seemed to be independent of the eluent composition. The above endothermic behavior at high acid concentrations is similar to that described for an adsorptive system,[21] which was also explained by complexation (solvation) in the eluent.

In reversed-phase,[22] ion-exchange,[23] and adsorption[24] chromatography, complex equilibria in the eluent have been studied by using simple theoretical models. The assumptions made in these models are also justified in the present system as discussed below.

(a) According to the results shown in Figures 2 and 3, solute and solvent molecules are assumed to form complexes in the eluent.

(b) Since only noncovalent interactions are involved, the equilibration rate in the eluent is considered to be rapid on the chromatographic time scale.

(c) Solute–solute interactions are neglected because of the low solute concentration (<4 mM).

(d) In a limited interval, the retention at a fixed eluent composition is independent of the amount of sample injected.

Two additional assumptions can be made in the present system.

(e) Only the interactions between the noncomplexed solute molecules and the stationary phase are of importance for the solute retention. This is justified by the following observations: The selectivity at a given temperature was apparently independent of the eluent composition (Figure 3b). The extent of complex formation, therefore, does not seem to affect selectivity. In chromatography at room temperature and high HOAc concentrations, the retention was low and the number of theoretical plates relatively high.[25] This indicates that the interactions between the complexes and the stationary phase are unimportant.

(f) No retention was observed for HOAc, so we have neglected its interaction with the stationary phase. Since the latter is abundant in carboxyl groups, we have also neglected the dimerization of HOAc in the eluent. Although dimerization has been considered in studies of HOAc–amine interactions in the polar solvent nitrobenzene,[14] the dimerization constant is small compared to the ion-pair formation constant and that of homoconjugate complexes of the type $H(OAc)_2^-$. In addition, the good hydrogen bond acceptor acetonitrile should further reduce the extent of dimerization.

The above assumptions thus result in the equilibria 1 and 2, where S is the solute in the eluent, S_s the solute associated to the

$$S \overset{K_a}{\rightleftharpoons} S_s \tag{1}$$

$$S + nL \overset{K_n}{\rightleftharpoons} SL_n \qquad n = 1, 2, 3 \ldots, q \tag{2}$$

stationary phase, L the strong eluent (HOAc), SL_n the multimolecular complexes formed in the eluent, K_n their corresponding formation constants, and K_a the association constant of the solute to the stationary phase.

The retention described by the capacity factor can be expressed as[22]

$$k' = \phi[S_s]/[S]_{tot} \tag{3}$$

where ϕ is the phase ratio, $[S_s]$ the concentration of solute (noncomplexed) associated to the stationary phase, and $[S]_{tot}$ the total concentration of solute, including complexes, in the eluent. Based on (1) and (2), (3) can be written as

$$k' = \phi[S_s]/([S] + [SL] + [SL_2] + \ldots + [SL_q]) \tag{4}$$

which is equal to

$$k' = \phi K_a/(1 + K_1[L] + K_1K_2[L]^2 + \ldots K_1K_2 \ldots K_q[L]^q) \tag{5}$$

$$1/k' = (\phi K_a)^{-1}(1 + \beta_1[L] + \beta_2[L]^2 + \ldots + \beta_q[L]^q) = C_0 + C_1[L] + C_2[L]^2 + \ldots + C_q[L]^q \tag{6}$$

where $\beta_1, \beta_2, \ldots, \beta_q$ are overall formation constants, included in the constants $C_0, C_1, \ldots, C_q$ and $[L]$ is the concentration of noncomplexed HOAc in the eluent. Under the chromatographic conditions

$$[S]_{tot} \ll [L]_{tot} \tag{7}$$

which implies that

$$[L] \approx [L]_{tot} \tag{8}$$

According to (6)–(8), the inverse of k' thus depends on the addition of HOAc by a polynomial of degree q, where q should be informative about the maximum size of complexes formed in the eluent and the coefficients about their corresponding formation constants. In Figure 2b this relationship is seen for PheNHPh, together with the corresponding minimal degree polynomial showing the best fit to the experimental values. The resulting formation constants (Table II) reveal that multimolecular complexes are formed, of which the 1:1 complex is the strongest. This is to be expected since it should involve an electrostatic interaction. It should be mentioned that a small error in the curve fitting results in relatively large errors in K_2 and K_3. Characteristically, however, the 1:3 complex is favored over the 1:2 complex. A possible explanation for this is that the second acid molecule of the 1:2 complex offers a favorable binding site for the third acid.[14] Also, it could be due to a conformational change induced by the binding of the second acid.

Table II. Stepwise (K) and Overall (β) Association Constants[a]

temp, °C		SL_1	SL_2	SL_3
23	K, M^{-1}	30	3.0	3.2
	β, M^{-n}	30	90	291
60	K, M^{-1}	5.5	1.2	3.2
	β, M^{-n}	5.5	6.7	21

[a] Obtained from eq 6, using the coefficients in Figure 2b; SL_n, complexes formed in the eluent between PheNHPh (S) and HOAc (L).

The complex distribution is revealed by the size of the different terms of the polynomial (6). In Figure 4, this is seen under conditions similar to those prior to polymerization, at 60 °C and at room temperature.[26] Prior to polymerization at 60 °C ($[S] \approx 0.1$ M and $[L] \approx 0.4$ M), the 1:1 complex predominates (≈40% of $[S]_{tot}$) and the noncomplexed print molecule together with the higher complexes exist in roughly equal concentrations (each ≈20% of $[S]_{tot}$). It must be noted that self-association of the print molecule (see below), which in the model is neglected, could influence the complex formation prior to polymerization. The small difference in pK_a between HAc and MAA can, however, in this context be neglected. For D-PheOEt on its corresponding polymer, complex formation was much less pronounced. Mainly

(20) This has been ascribed to a higher number of accessible sites together with an increased rate of mass transfer at higher temperatures.[3]

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(25) At room temperature in the presence of 1.1 M HOAc, $k'_D \approx 0.3$ and $N_D \approx 60$, while in the absence of acid $k'_D > 10$ and $N_D < 10$.

(26) Under conditions prior to polymerization, $[S] \approx 0.1$ M, and therefore the assumption made in (7) is no longer valid. In order to obtain the complex distribution, the concentration of noncomplexed HOAc is first calculated.

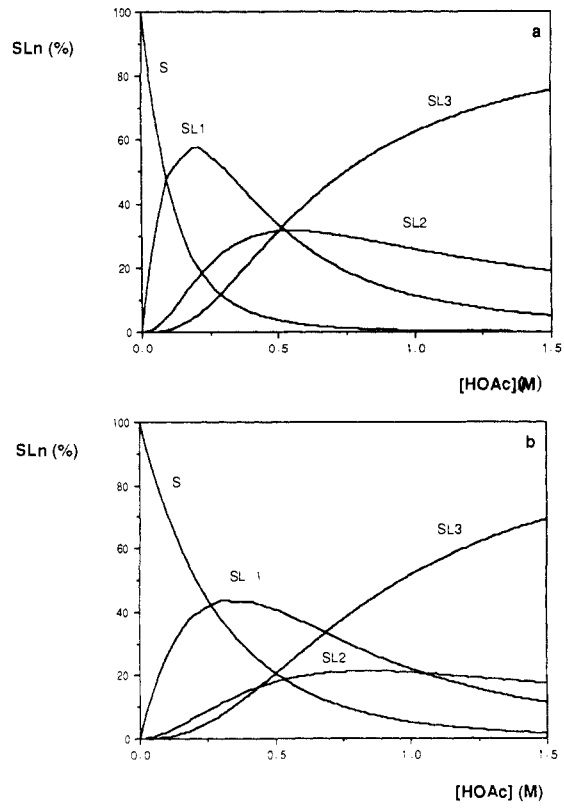


Figure 4. Distribution of complexes at 23 °C (a) and at 60 °C (b) as a result of the data in Figure 2, at the same concentration of PheNHPh (0.1 M) as prior to polymerization. [HOAc] represents the total concentration.

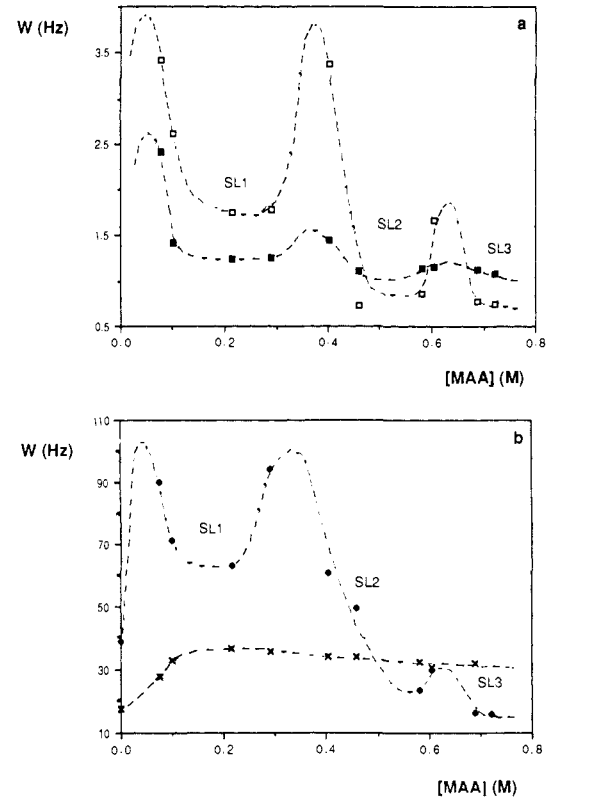


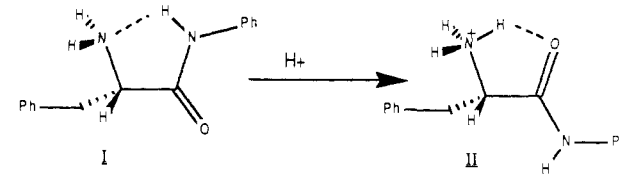
Figure 5. Line width (W) of the cis (□) and methyl (■) protons of MAA (a) and the exchangeable (◆) and amide (×) protons of PheNHPh (b) vs the concentration of MAA in the presence of 0.1 M PheNHPh in acetonitrile at 23 °C. The predominating complexes have been indicated (SL_n). The line width of the solvent reference was 0.29 ± 0.019 Hz (n = 13), and the digital resolution was 0.245 Hz.

1:1 complexes were observed, in agreement with the lower selectivities obtained with this polymer. This was further confirmed by temperature investigations where no endothermic behavior was observed.

^{1}H NMR Study of the Complex Formation—Line Widths. The use of ^{1}H NMR to study titration experiments is well-known and has been applied in determining protonation sites,[27] structure and hydrogen bonding,[28,29a] and association constants.[29]

We thus performed a titration experiment where methacrylic acid (MAA) was added to a fixed amount of PheNHPh in acetonitrile, mimicking the conditions prior to polymerization.[30] This resulted in changes in the line widths for the protons of MAA (Figure 5a) and for the exchangeable protons of the amino and carboxyl groups (Figure 5b). Common features of these are the maxima at 0–0.1, 0.3–0.4, and 0.55–0.65 M MAA. Because of a slow ligand exchange, an extensive broadening due to equivalent protons of the different complexes at equilibrium could be expected.[31] This should result in a maximum line broadening when two or more of the predominating complexes exist in equal amounts. We therefore ascribe the first maximum to the formation of the 1:1 complex ($[S] = [SL_1]$) and the second and third

Scheme I

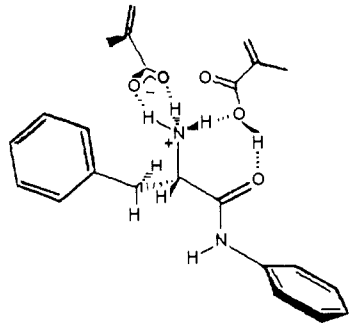


to the formation of the 1:2 ($[SL_1] = [SL_2]$) and 1:3 ($[SL_2] = [SL_3]$) complexes, respectively.

The first maximum agrees well with the complex distribution obtained in chromatography (Figure 4a). However, further comparison shows less agreement. According to the chromatographic results, only one additional maximum should be observed, due to an equilibrium between the 1:1, 1:2, and 1:3 complexes. A likely explanation for the disagreement between the chromatographic and NMR data is the existence of self-association of PheNHPh, which in the chromatographic model is not accounted for. Self-association was indicated by translational diffusion experiments[32] and has been observed for *N*-acetylphenylalanine methyl amide,[33] secondary amides,[17a,34] primary amines in ion pair with phenols,[35] and tertiary amines in ion pair with carboxylic acids.[14] In the case of the primary amines, it is interesting that the self-association is sometimes stronger to the already formed

(27) Sarneski, J. E.; Surprenant, H. L.; Reilley, C. N. *Spectrosc. Lett.* **1976**, *9*, 885–894.
(28) Yonezawa, T.; Morishima, I.; Takeuchi, K. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 1807–1813.
(29) (a) Rebek, J., Jr.; Askew, B.; Killoran, M.; Nemeth, D.; Lin, F.-T. *J. Am. Chem. Soc.* **1987**, *109*, 2426–2431. (b) Saunders, M.; Hyne, J. B. *J. Chem. Phys.* **1958**, *29*, 1319–1323.
(30) In order to fully mimic the situation prior to polymerization, a mixture of the same composition except for the initiator was initially used. In the ^{1}H NMR spectra, the signal of the α proton was not visible because of the strong methylene proton signal of the cross-linker ethylene glycol dimethacrylate. Since the titration curves were otherwise similar, the cross-linker was not added in the following investigation. The spectra were additionally recorded at 60 °C (polymerization temperature). These were similar to those recorded at room temperature, but the line-broadening maxima and shift inflections occurred at higher concentrations.
(31) Sandström, J. *Dynamic NMR Spectroscopy*; Academic: London, 1982.
(32) The samples for the diffusion study were prepared from a 120 mM solution of print molecule in acetonitrile-d_3 containing 2.5 equiv of MAA, diluted to the desired concentration with acetonitrile-d_3. The diffusion experiments were performed at 23 and 40 °C using a JEOL FX-60 NMR spectrometer equipped with a pulse gradient device and a PGSE program. For a review, see: Stilbs, P. *Prog. Nucl. Magn. Reson. Spectrosc.* **1987**, *19*, 1–45. The aromatic protons were monitored at concentrations of 5, 15, 30, 60, and 120 mM. At 23 °C, the diffusion coefficients decreased with concentration from ~2.5 × 10^{-9} m^2/s at 15 mM to ~1.4 × 10^{-9} m^2/s at 30 mM.
(33) Kobayashi, J.; Nagai, U. *Biopolymers* **1978**, *17*, 2265–2277.
(34) Pralat, K.; Jadzyn, J.; Balanicka, S. *J. Phys. Chem.* **1983**, *87*, 1385–1390.

Chart I



ion pair than to the free amine. This could explain the good agreement of the first complex transition with the chromatographic results.

The influence of the self-association should then be an increasing competition for the available binding sites and should thus result in a displacement of the transition between the different complexes to higher concentrations.

Shifts. In the absence of acid, inter- and intramolecular interactions can be thought to occur. The intermolecular interactions should result in self-associates as discussed above, while the intramolecular interaction is possibly a five-membered ring, I, formed by an intramolecular hydrogen bond (Scheme I). Such interactions have been observed in other amino anilides,[18] although the solvation by acetonitrile may in this case reduce its importance. The side-chain conformation is, according to our molecular mechanics calculations (MM2) and other studies,[33] dominated by the rotamer having the phenyl and the carbonyl groups in a trans orientation, along the C_α–C_β bond (Chart I). This agrees well with our observed $^3J_{\alpha\beta}$ couplings (in pure acetonitrile). In this context, it must be noted that important conformational changes have been observed upon an increase in solvent polarity.[33] This was explained by the change in dipole–dipole interactions, but increased steric hindrance due to solvation was not excluded. Also, in our studies, large changes in the couplings were observed when MAA was added. In view of the above observations it is therefore not possible to ascribe these to steric factors only, i.e. to the bulkiness of an associated MAA molecule.

At 0–0.1 M MAA, protonation of the amino group occurs and the ion pair is formed. This is accompanied by shift displacements and clear inflections at 0.1 M MAA for the exchangeable (+3.5 ppm) (Figure 6a), α (+0.2 ppm), β (+0.08 ppm), and amide (–0.1 ppm) protons and a shift displacement for the ortho protons of the anilide ring (–0.03 ppm). It is interesting to note that the shift displacements of the β' and para (anilide) protons are much smaller (<0.005 ppm). However, clear inflections can be seen for these as well as for the MAA protons (Figure 6b). The large downfield shift of the exchangeable protons and the downfield shift of the α and β protons are in the same order as observed in the case of primary amine protonation.[36]

The intramolecular hydrogen bond should be disrupted upon amine protonation, possibly in favor of another intramolecular hydrogen bond, II (Scheme I).[16] This is supported by the upfield shift of the amide proton accompanied by a clear change in its line width (Figure 5).

When further MAA is added (0.1–1 M) the exchangeable, α, and β protons continue to move downfield (4, 0.4, and 0.2 ppm, respectively) while the amide and ortho move upfield (0.07 and 0.15 ppm, respectively). All curves level off at ~1 M MAA, which may indicate predominance of the 1:3 complex (see Figures 4 and 5). According to the complex transitions (Figure 5), further inflections should occur at 0.3–0.4 M as well as at 0.55–0.65 M MAA. Although the shift changes observed were small, the transition at 0.3–0.4 M can be seen as clear inflections for the MAA cis and trans protons (Figure 6b) as well as for the β' and

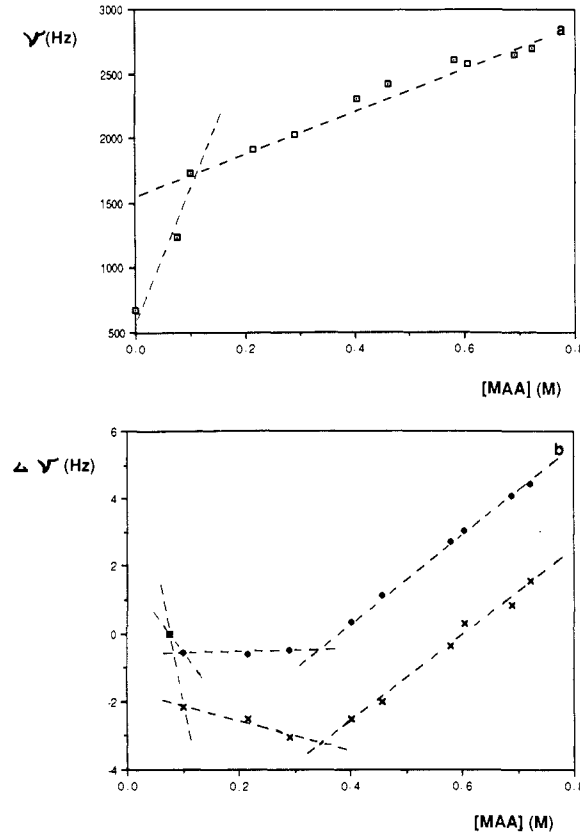


Figure 6. Shift (ν) of the exchangeable protons (a) and shift displacement ($\Delta\nu$) of the cis (◆) and trans (×) protons of MAA (b) vs the MAA concentration at conditions as in Figure 5.

para protons (not shown). Inflections could also be seen at 0.55–0.65 M for the ortho and para protons.

In conclusion, the shift and line width dependences are in agreement, indicating the existence of higher complexes.

Proposed Structure of Formed Complexes. In Chart I we have proposed a model of the 1:2 complex based on the following arguments. As expected, the above results support the 1:1 complex as being represented by the ion pair. Since the best hydrogen bond acceptor in this complex is probably the amide oxygen,[13] we suggest that the second acid forms a hydrogen bond to it, which would allow stabilization by an additional hydrogen bond to an ammonium proton [see earlier discussion on the stability of homoconjugate complexes $H(OAc)_2^-$]. Another possibility would be hydrogen bonding to the amide proton. However, because of its continuous upfield shift we consider this as less likely. The third acid could associate either to the carboxylate of the ion pair,[14] to the amide oxygen, or to the amide proton, although the latter possibility again seems less likely. More definite structural conclusions cannot be drawn at this stage.

The Nature of the Sites. It is often argued that the observed selectivities of polymers prepared by molecular imprinting are not due to cavities but rather to print molecules left in the polymer or to chiral helicity in the main chain of the polymer.[1] It is therefore of interest to present all the evidence consistent with the cavity interpretation, in a more condensed form. The arguments are the following:

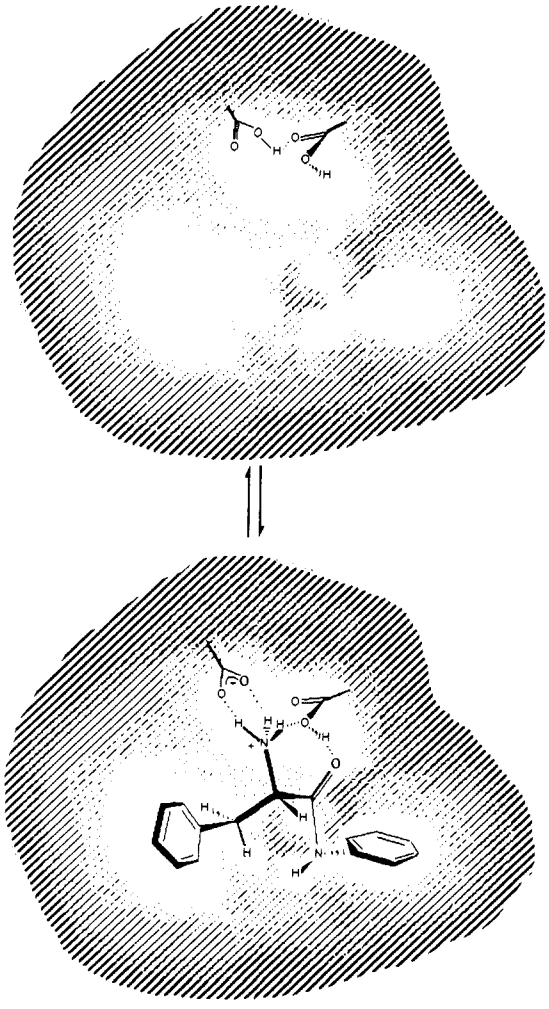
(1) The high substrate selectivity as such is not observed in systems used for chiral separations with covalently bound amino acid derivatives.[37]

(2) Neither enantioselectivity nor substrate selectivity was observed when the print molecule was left covalently bound to the polymer.[4,5]

(3) Leaving out the functional monomers resulted in nearly quantitative removal of the print molecules.[5] Although the

(35) Huyskens, P. L. *J. Am. Chem. Soc.* **1977**, *99*, 2578–2582.
(36) Sudmeier, J. L.; Reilley, C. N. *Anal. Chem.* **1964**, *36*, 1698.
(37) Blaschke, G. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 13.

Scheme II



polymers had low capacity, enantioselectivity was still observed.

(4) Translational diffusion experiments[32] showed that the diffusion constant in the PheNHPh–MAA system exhibited a lower limit at higher concentrations, indicating small aggregates (dimers) as being the largest formed under these conditions. Thus, polymerization along chiral chain segments, according to the so-called template effect,[38] seems unlikely in this system.

In addition to the above evidence, Wulff and co-workers have shown[1] that swelling experiments in some cases irreversibly destroyed selectivity and that asymmetry in the main chain in such systems was unlikely. In view of the above arguments, we therefore ascribe the enhanced selectivities observed in the present investigation to polymer cavities equipped with carboxyl groups at defined positions. A schematic picture of how such cavities may interact with the substrate is seen in Scheme II. It is interesting to note here that carboxyl groups coupled to a synthetic molecular cleft have proved to be efficient in similar molecular recognitions.[29a,39]

A question arises as to whether or not the carboxyl groups of the polymer in our case have the same geometry as in the complex prior to polymerization. Indirect evidence for this is provided by the polymer selectivity investigations, but information regarding the polymer structure would also be of great interest. For this purpose FTIR and solid-state NMR techniques should prove useful.

Assuming the same geometry in the polymer as in solution, cavities containing a various number of carboxyl groups, determined by the actual complex distribution, are formed. It is reasonable to assume that higher complexes gain in importance upon polymer formation, since carboxyl groups of the same polymer chain, by a chelate mechanism, should preferentially bind to the same print molecule. In this context, our aim is now to further stabilize the higher complexes, which we believe will lead to polymers exhibiting even higher selectivities. Principally, this should be feasible by lowering the polymerization temperature, by increasing the MAA content, or by changing the inert solvent prior to polymerization.[4c,11]

(38) Chapiro, A. *Pure Appl. Chem.* **1981**, *53*, 643–655.
(39) Rebek, J., Jr. *Science (Washington, D.C.)* **1987**, *235*, 1478–1484.

Conclusions

Polymers prepared by molecular imprinting utilizing noncovalent interactions exhibit enantioselectivity and substrate selectivity for their original print molecules. The main factors influencing selectivity were shown to be the number and nature of noncovalent interactions between the polymer and the print molecule and the rigidity and shape of the substituents of the latter.

Titration of the print molecule (phenylalanine anilide) with acid, studied by chromatography and by ^{1}H NMR, revealed that multimolecular complexes between the print molecule and the acid monomers are formed prior to polymerization, and a model of the 1:2 complex between phenylalanine anilide and methacrylic acid was proposed. The extent of complex formation was shown to have an influence on the observed polymer selectivity. In addition, results were presented which strongly indicate that recognition takes place in cavities and not by interaction with print molecules left in the polymer or by chiral recognition of the polymer as such.

These studies should facilitate further utilization of the concept of molecular imprinting, allowing tailor-made chiral stationary phases for a particular chromatographic application to be designed. Moreover, it may assist in developing receptor or enzyme mimics.[40]

Experimental Section

Preparation of Amino Acid Derivatives and Polymers. Amino acids were purchased from Sigma Chemical Co. (St. Louis, MO), and the amino acid derivatives D- and L-phenylalanine anilide, *p*-aminophenylalanine ethyl ester, phenylalanine ethyl amide, and phenylalanine ethyl ester were synthesized by standard procedures.[5,41] The polymers were prepared as previously described.[5] In a typical preparation, 5640 mg of ethylene glycol dimethacrylate (Merck, Darmstadt, W. Germany), 520 mg of methacrylic acid (83 and 17 mol %, respectively, of vinyl monomers), 1.5 mmol of print molecule (L-PheOEt, L-*p*-NH_2PheOEt, L-PheNHPh, or L-PheNHEt), and 60 mg of azobis(isobutyronitrile) in 8.2 mL of acetonitrile were mixed in a glass tube. After being degassed, the tube was sealed under nitrogen and consecutively heated for 24 h at 60, 90, and 120 °C. Subsequently, the polymer was ground and subjected to continuous extraction in acetonitrile for 24 h. To determine the recovery of print molecules, a TNBS assay[42] on the extract and an elemental analysis on the polymers were carried out in addition to the earlier used method. In the TNBS assay the amino group of the extracted print molecule was determined and in the elemental analysis the residual nitrogen in the polymers was determined before and after extraction and compared with a blank polymer with no print molecules. According to these methods, about 90% of the print molecules had been removed from the polymers, in agreement with our previous study.[5]

Chromatographic Experiments. The polymers were milled and sieved in a wind sieving machine (Alpine multiplex 100 MZR). A fraction (10–30 μm) was collected and used for packing of stainless steel chromatographic columns (250-mm length, 3-mm i.d.).[5] In the case of the L-PheOEt- and L-PheNHEt-selective polymers, a fraction of 45–65 μm was collected by mechanical sieving. The chromatographic investigations were performed at elevated temps. using acetonitrile, with addition of various amounts of acetic acid (in some cases water), as eluent. The amount of substrate applied varied between 5 and 40 nmol, injected in a volume of 10 μL; the flow rate was 0.3 mL/min and the elution was monitored at 260 nm, unless otherwise stated. Both racemic mixtures and pure enantiomers were injected, and the retention times were determined by measurement of the peak maxima elution times. In the case of poorly resolved peaks, separation factors were calculated from the separately applied enantiomers. The void elution time was determined by injecting a small amount of an inert substance. Thus, acetone,

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(41) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; p 925.
(42) Satake, K.; Okuyama, T.; Ohashi, M.; Shinoda, T. *J. Biochem. (Tokyo)* **1960**, *47*, 654.

methanol, toluene, heptane, and acetic acid all eluted at the same time (t_0). The capacity factors (k'), separation factors (α), and the number of theoretical plates (N) were calculated according to standard chromatographic procedures[43] as $k'_D = (t_D - t_0)/t_0$, $\alpha = k'_L/k'_D$, and $N_D = 5.54\,(t_D/t_{1/2})^2$, where t_D is the retention time of the D enantiomer, t_0 the void elution time, and $t_{1/2}$ the width of peak at half-height. Resolution (R_s) was calculated according to Wulff et al.[3]

NMR Investigation. The samples for the titration study were prepared in sample tubes (5-mm o.d.) by adding 0.50 mL of a 100 mM solution of the print molecule dissolved in acetonitrile-d_3 or a mixture of acetonitrile-d_3 and EDMA of the same composition as in the preparation of the polymers. To this was added MAA with a gas-tight 50-μL syringe to obtain samples with desired acid concentrations.

The samples were degassed by sonication. All spectra were recorded at room temperature and at 60 °C, with a Varian XL-300 NMR spectrometer operating at 300 MHz. To obtain the spectra, 32 FIDs were accumulated by using 30° pulses with 10-s pulse intervals. All shifts are relative to the central peak of the CHD_2CN heptet at 1.980 ppm (relative to TMS).

Acknowledgment. We are grateful to Dr. Torbjörn Drakenberg, Dr. Torbjörn Frejd, Dr. Ingmar Persson, Prof. Bertil Törnell, Rolf Servin, Dr. Roland Isaksson (all at the Chemical Center, University of Lund), and Prof. Margaret C. Etter (University of Minnesota) for valuable discussions and assistance. This work was supported in part by the National Swedish Board for Technical Development and by the Swedish National Science Foundation to K.M.

Registry No. (EDMA)(MAA)(L-PheOEt) (copolymer), 114395-75-4; (EDMA)(MAA)(L-*p*-NH_2PheOEt) (copolymer), 114422-53-6; (EDMA)(MAA)(L-PheNHPh) (copolymer), 114395-77-6; (EDMA)(MAA)(L-PheNHEt) (copolymer), 114395-79-8.

(43) Poole, C. F.; Schuette, S. A. *Contemporary Practice of Chromatography*; Elsevier: Amsterdam, 1984; pp 1-8.

Radiation Chemical Production, Lifetimes, and Structure–Activity Relations for α-Dialkoxyalkyl Carbocations in Aqueous Solutions: Importance of Solvation for Cation Reactivity

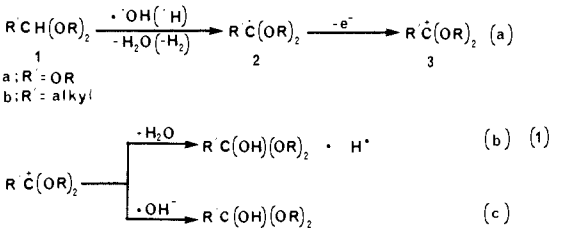
Robert A. McClelland*,† and Steen Steenken*,‡

Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1, and Max-Planck-Institut für Strahlenchemie, D-4330 Mülheim, West Germany. *Received January 4, 1988*

Abstract: α-Dialkoxyalkyl carbocations $R'C(OR)_2^+$ were produced in aqueous solution by H abstraction from acetals by OH and H radicals followed by one-electron oxidation of the so-formed α-dialkoxyalkyl radicals. Rate constants for the reactions of the cations with H_2O (and in some cases OH^-) have been determined by using time-resolved conductance techniques. For example, $MeC(OMe)_2^+$ reacts with water with a rate constant of 1.3×10^5 s^{-1} and with OH^- with a rate constant of 3.7×10^8 M^{-1} s^{-1}. Rate constants for the hydration of 30 open-chain and cyclic aliphatic cations have been measured, enabling structure–activity relations to be seen. For substitution in the OR group a reactivity order OMe > OEt > O-*i*-Pr is observed with a good correlation versus σ^* encompassing a number of ions of different structural type with a ρ^* of +4.4. However, substitution of R′ (directly on the carbocation center) does not follow the expected steric/electronic order, *t*-$BuC(OEt)_2^+$, for example, reacting with water 2.3-fold *more* rapidly than $MeC(OEt)_2^+$. The reaction with water of these cations is a very close model of the rate-limiting step of H^+-catalyzed ester hydrolysis, the defining reaction for the E_s steric substituent constant. The observation that the effects of alkyl substituents at C_α are described neither by their σ^* nor by their E_s constants is explained in terms of solvation effects. A consequence of this is that solvational effects, in particular on the equilibrium protonation step of ester hydrolysis, must also play an important role in determining the E_s parameter. This parameter is therefore not a pure measure of steric effects.

In a previous paper, a radiation chemical technique was described for the production of trialkoxymethyl carbocations **3a** in aqueous solution, with subsequent direct measurements of lifetime.[1] The ions were formed by one-electron transfer to an oxidant from trialkoxymethyl radicals **2a**, which were produced by hydrogen abstraction from orthoformates **1a**. The overall reaction consists of removing a hydride ion from the precursor in a step-wise fashion, i.e. by consecutive removal of a hydrogen atom and an electron (eq 1a). Time-resolved conductance measurements provided rate constants for the reactions of **3a** with water and hydroxide ion (eq 1b,c).

Hydrogen abstraction from acetals **1b** to produce radicals **2b** is also well established,[2] and thus dialkoxy carbocations **3b** should be accessible through the same procedure. These cations are probably of greater interest and relevance to physical organic chemistry, since they are intermediates in the mechanistically



well-studied reaction of ortho ester hydrolysis.[3] They are also models for hydroxyalkoxyalkyl cations, the intermediates of acid-catalyzed ester hydrolysis.

† University of Toronto.
‡ Max-Planck-Institut für Strahlenchemie.

(1) Steenken, S.; Buschek, J.; McClelland, R. A. *J. Am. Chem. Soc.* **1986**, *108*, 2808.
(2) Eibenberger, J.; Schulte-Frohlinde, D.; Steenken, S. *J. Phys. Chem.* **1980**, *84*, 704.
(3) Cordes, E. H. *Prog. Phys. Org. Chem.* **1967**, *4*, 1. Fife, T. H. *Acc. Chem. Res.* **1972**, *5*, 264. Cordes, E. H.; Bull, H. G. *Chem. Rev.* **1974**, *74*, 581.

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