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Receptor Binding Mimetics: A Novel Molecularly Imprinted Polymer

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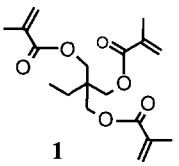
Abstract: *A novel molecularly imprinted polymer was prepared by copolymerization of trimethylolpropane trimethacrylate (1) and methacrylic acid (3) in the presence of a dipeptide acting as the template. The recognition capability of the synthetic receptor-like binding sites produced in the polymer network for the peptide was demonstrated by using the polymer as a chiral stationary phase in HPLC. The polymer was superior to previously reported molecularly imprinted polymers in that unusually high racemic resolution and load capacity were demonstrated.*

Molecular recognition plays a central role in interactions between biochemically important species. These interactions rely on selective binding between the receptor and its ligand. The aim in the design of artificial receptors has been to construct host systems possessing steric and electronic features complementary to those of the ligand to be bound by the receptor, which has resulted in *e.g.* crown ethers, cyclodextrins, cyclophanes and various molecular clefts and cavities.[1] Another approach to design synthetic receptor-like binding sites is molecular imprinting,[2] sometimes referred to as template polymerization, a technique that has been studied extensively by us[3] and others.[4]



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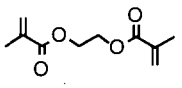

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The recent progress in the field of molecular imprinting presented here is believed to be of major importance for future practical applications of the technique. A novel type of imprinted polymeric binding sites, possessing high load capacity and excellent recognition with respect to the template molecule present during the imprinting procedure, was developed. The cross-linkers utilized for the preparation of molecularly imprinted polymers have, until now, been restricted to monomers with two vinyl groups. Previous studies to improve and develop novel polymer systems for non-covalent molecular imprinting have focused mainly on the functional monomers and their specific interactions with various functional groups of the print molecules.[3] In general, these polymers have been prepared with ethylene glycol dimethacrylate (**2**) as the cross-linking agent. However, by using cross-linking monomers containing more than two polymerizable groups, the network of the polymers seems to change profoundly,[5] resulting in polymers with better recognition properties. The specific model system described here was prepared by copolymerization of the branched cross-linker trimethylolpropane trimethacrylate (**1**) with methacrylic acid (**3**) in the presence of the template Z-L-Ala-L-Ala-OMe.[6] The templates were subsequently extracted from the resulting polymer, leaving enantio-

selective recognition sites. The recognition capability was demonstrated by using the ground polymer as a stationary phase in HPLC.[7] The main interactions responsible for the recognition are most likely hydrogen bonds between the positioned carboxyl groups of the polymer and the carbamate, amide and ester of the dipeptide (Figure 1).

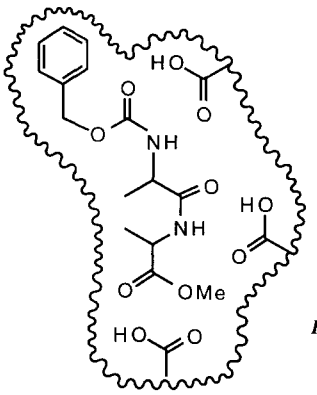

Figure 1. Schematic representation of the binding site.

Figure 2 shows the resolution of (a) 100 μg and (b) 1 mg of racemic mixtures containing Z-L-Ala-L-Ala-OMe and Z-D-Ala-D-Ala-OMe on a column containing 1.56 g polymer (column size: 250x4.6 mm) at a flow rate of 1 ml/min. The separation factors (α)[3f] were 3.19 and 1.92, respectively, and the resolution factors (R_S)[4b] 4.50 and 1.71, respectively. These figures are high compared to what can be achieved on a **2-3**-copolymer prepared with the same molar ratio Z-L-Ala-L-Ala-OMe : **3** as in the **1-3**-copolymer (Table 1). The separation and resolution factors were only 2.22 and 1.50, respectively, when 100 μg was injected on the **2-3**-copolymer, and no resolution was achieved at all when 1 mg was applied. Note also that eluents containing acetic acid (acting as a competing ligand) had to be used in the case of the **1-3**-copolymer in order to elute the species, but not in the case of the **2-3**-copolymer. This indicates that the interactions between the template molecules and the recognition sites of the polymer were stronger in the former case.

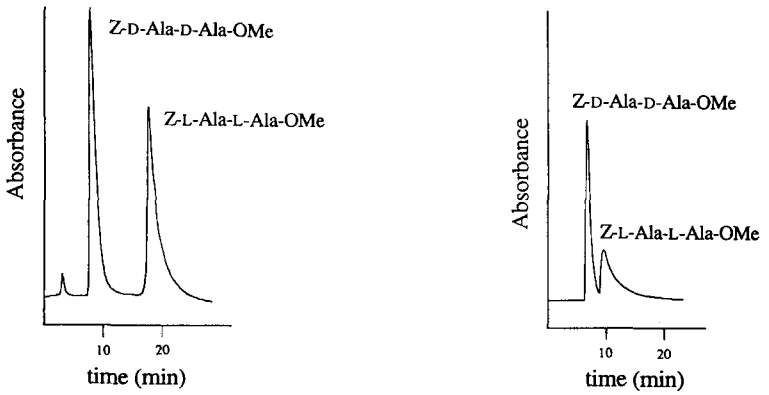

Figure 2. Chromatographic resolution of (a) 100 μg of a mixture of Z-L-Ala-L-Ala-OMe and Z-D-Ala-D-Ala-OMe on a Z-L-Ala-L-Ala-OMe-imprinted 1-3-copolymer using gradient elution with chloroform/acetic acid 99.75/0.25 (v/v) and chloroform/acetic acid 8/2 (v/v) (B) as the eluents at 1 ml/min (viz.: 0-10 min, 0% B; 10-18 min, 0-5% B; 18-22 min, 5% B; 22-24 min, 5-0% B); (b) 1 mg of a mixture of Z-L-Ala-L-Ala-OMe and Z-D-Ala-D-Ala-OMe on a Z-L-Ala-L-Ala-OMe-imprinted 1-3-copolymer at isocratic elution with chloroform/acetic acid 99.75/0.25 (v/v) as the eluent at 1 ml/min.

Table 1. *Chromatographic resolution of Z-L-Ala-L-Ala-OMe and Z-D-Ala-D-Ala-OMe on Z-L-Ala-L-Ala-OMe-imprinted chiral stationary phases prepared by co-polymerization of* ***3*** *and the indicated cross-linker.*

Cross-linker	Eluent	Separated amount (mg)	k'_{LL}	α	R_s
1	gradient[a]	0.1	4.28	3.19	4.50
1	chloroform/HOAc 99.75/0.25 (v/v)	1.0	1.79	1.92	1.71
2	chloroform	0.1	1.00	2.22	1.50
2	chloroform	1.0	0.22	1.00	--

[a] *The gradient is described in the legend of Figure 2a.*

We also addressed the question to what extent the two asymmetric centers of the dipeptide contribute to the recognition. As seen in Figure 3, which shows the elution-profiles of all stereoisomers of Z-Ala-Ala-OMe, Z-L-Ala-L-Ala-OMe was retarded most, as expected since this isomer was present as template during the polymerization. Z-D-Ala-D-Ala-OMe and Z-D-Ala-L-Ala-OMe coeluted and were less retarded than Z-L-Ala-D-Ala-OMe. From the elution order of these species, conclusions regarding the strength of the interactions between the recognition sites and the different parts of the peptide can be drawn. It appears that the N-terminal part of the molecule (Z-Ala-) is more important in the recognition than the C-terminal (-Ala-OMe).

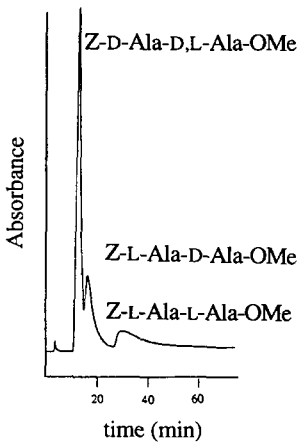

Figure 3. *Chromatographic resolution of 100 µg Z-Ala-Ala-OMe on a Z-L-Ala-L-Ala-OMe-imprinted* ***1-3****-copolymer at isocratic elution with chloroform as the eluent at 1 ml/min.*

The dissociation constants for the dipeptide-polymer complex, determined by frontal chromatography,[3d,8] were 2.5 mM for Z-L-Ala-L-Ala-OMe and 4.8 mM for Z-D-Ala-D-Ala-OMe. The number of binding sites in the polymer giving rise to these dissociation constants were 22 µmol/g dry polymer.

Summarizing, here we report the advantages of branched cross-linkers with three polymerizable groups, such as **1**, for the preparation of molecularly imprinted polymers. The model system studied here was

superior to previously reported preparations, in that considerably higher load capacities and better resolutions were achieved. **1** was subsequently used as cross-linker in polymers imprinted with an array of other amino acid derivatives and peptides. The polymers showed excellent recognition for the template molecules in all cases. From preliminary studies on related cross-linkers, such as pentaerythritol triacrylate, we have indications for the formation of polymers with similar ideal properties. Besides the possibilities of applying this kind of synthetic binding sites in the studies of molecular recognition phenomena, we feel this to be an important step towards the application of molecularly imprinted polymers as synthetic receptors in diagnostic analyses and as large-scale separation media.

REFERENCES AND NOTES

1. (a) Cram, D.J. *Angew. Chem. Int. Ed. Engl.* **1986**, 25, 1039-1057. (b) Lehn, J.-M. *Angew. Chem. Int. Ed. Engl.* **1988**, 27, 89-112.
2. (a) Ekberg, B; Mosbach, K. *Trends Biotechnol.* **1989**, 7, 92-96. (b) Wulff, G. *Trends Biotechnol.* **1993**, 11, 85-87. (c) Mosbach, K. *Trends Biochem. Sci.* **1994**, 19, 9-14.
3. (a) Arshady, R.; Mosbach, K. *Makromol. Chem.* **1981**, 182, 687-692. (b) Sellergren, B.; Lepistö, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, 110, 5853-5860. (c) Fischer, L.; Müller, R.; Ekberg, B.; Mosbach, K. *J. Am. Chem. Soc.* **1991**, 113, 9358-9360. (d) Kempe, M.; Mosbach, K. *Anal. Lett.* **1991**, 24, 1137-1145. (e) Vlatakis, G.; Andersson, L.I.; Müller, R.; Mosbach, K. *Nature* **1993**, 361, 645-647. (f) Kempe, M.; Fischer, L.; Mosbach, K. *J. Mol. Recogn.* **1993**, 6, 25-29. (g) Kempe, M.; Mosbach, K. *J. Chromatogr.* **1994**, 664, 276-279. (h) Kempe, M.; Mosbach, K. *Int. J. Pept. Prot. Res.* **1994**, 44, 603-606.
4. (a) Wulff, G.; Heide, B.; Helfmeier, G. *J. Am. Chem. Soc.* **1986**, 108, 1089-1091. (b) Wulff, G.; Poll, H.-G.; Minarik, M. *J. Liq. Chromatogr.* **1986**, 9, 385-405. (c) Shea, K.J.; Dougherty, T.K. *J. Am. Chem. Soc.* **1986**, 108, 1091-1093. (d) Shea, K.J.; Sasaki, D.Y. *J. Am. Chem. Soc.* **1989**, 111, 3442-3444.
5. The pore volume of pores in the interval 17-3000 Å and the pore size distribution of the **1-3**-copolymer were measured by nitrogen adsorption and desorption after outgassing at 150 °C for 3 h using a Micromeritics ASAP 2400. The adsorption and desorption isotherms were recorded using a 72-point pressure table and 10 s equilibration time. The average pore diameter was 282 Å and the pore volume was 0.022 ml/g. The specific surface area of the **1-3**-copolymer was 4.4 m^2/g polymer.
6. The **1-3**-copolymer was prepared by dissolving 30 mmol **1**, 30 mmol **3** and 5 mmol Z-L-Ala-L-Ala-OMe in 20 ml chloroform. The **2-3**-copolymer was prepared by dissolving 50 mmol **2**, 10 mmol **3** and 1.67 mmol Z-L-Ala-L-Ala-OMe in 15 ml chloroform. The polymerizations were initiated by photolytic homolysis of 2,2'-azobis(2-methylpropionitrile) at 366 nm. The bulk polymers were ground in an end runner mill (Model RM O, Retsch, Germany) and the resulting particles were wet-sieved by hand with water and ethanol through a 25 μm sieve. The particles that passed the sieve were collected and dried on a sintered glass funnel. The particles were sedimented in acetone and the fines were removed by decantation.
7. The sieved and sedimented particles were slurried in chloroform/acetone (17/3, v/v) and packed with acetone as solvent into stainless-steel HPLC-columns (250x4.6 mm) at 300 bar using an air driven fluid pump (Haskel Engineering Supply Co., USA). The print molecules were extracted from the polymers by eluting with methanol/acetic acid (7/3, v/v) at 1 ml/min until a stable baseline was achieved. The elutions were performed at ambient temperature and monitored spectrophotometrically at 260-265 nm. The flow rate was 1.0 ml/min.
8. The frontal chromatography was performed at 0.5 ml/min using chloroform as the eluent. The polymer was packed into a 50x4.6 mm column.

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