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An enantioselective imprinted receptor for *Z*-glutamate exhibiting a binding induced color change†

Panagiotis Manesiotis,[a] Andrew J. Hall,*[a] Marco Emgenbroich,[a] Milena Quaglia,[b] Ersilia De Lorenzi[b] and Börje Sellergren*[a]

[a] *INFU, Universität Dortmund, Otto Hahn Str. 6, 44221 Dortmund, Germany. E-mail: borje@infu.uni-dortmund.de; Fax: +49-231-7554084*

[b] *Department of Pharmaceutical Chemistry, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy*

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Using 1-(4-styryl)-3-(3-nitrophenyl)urea as host monomer for the imprinting of *Z*-(D or L)-Glu, a polymeric receptor exhibiting strong enantioselectivity and a change in color intensity upon binding of the guest was obtained.

Reagents capable of responding to specific target molecules by a visible color change are highly attractive for fast qualitative analysis.[1,2] This is reflected in the rapidly growing number of chromogenic anion sensors based on ingeniously constructed host molecules with appended chromophores. These typically respond to one or a group of target molecules depending on the host design. Thus in response to new target molecules, the host needs to be redesigned, often involving elaborate synthesis protocols. Molecular imprinting constitutes a more flexible approach in this context and robust polymeric receptors can now be readily prepared against a large variety of small molecules.[3–5] Although noncovalently imprinted polymers exhibiting a chromogenic or fluorogenic response have been previously reported,[6–9] to our knowledge there exist to date no examples where the molecular recognition event is associated with a color change visible to the naked eye. This is partly due to the common use of weakly associating monomers, precluding the exact placement of a chromophore in the imprinted binding sites.[10] Stoichiometric imprinting[4] may hold a solution to these problems. In our laboratory we have exploited disubstituted ureas, a functional group which is well established in anion recognition[11] and chromogenic sensing,[12–16] as easily synthetically accessible and potent host motif in monomers for oxyanions.[17] Here we wish to report that 1-(4-styryl)-3-(3-nitrophenyl)urea (**1**) (Fig. 1) can be used as functional monomer for the imprinting of *Z*-(D or L)-glutamate to form polymers exhibiting high enantioselective binding capacities and where the binding is visible to the naked eye.

In order to assess the solution binding ability of the monomer towards carboxylates we first performed a ^{1}H NMR titration in DMSO-d_6 using benzoate as its tetrabutylammonium (TBA) salt as guest. Self-association of monomer and/or guest does not occur in these systems and Job plot analysis confirmed the 1 : 1 stoichiometry of monomer–guest interactions as depicted in Fig. 1. Fitting of the raw titration data to a 1 : 1 binding isotherm gave an association constant of 6498 ($\pm$170) M^{-1}, in good agreement with previously published results.[11] This implies that carboxylates are quantitatively complexed by this host monomer at typical prepolymerization concentrations and, notably, in a competitive medium. As reported for similar low molecular weight host molecules,[12,13] the titration was accompanied by a bathochromic shift of 15 nm (Fig. 1) leading to a clearly visible increase in the yellow color intensity.

Imprinted (P1) and nonimprinted (P$_N$1) polymers were then prepared from **1**, with ethyleneglycol dimethacrylate (EDMA) as crosslinker, in the presence of *Z*-(D or L)-Glu and two equivalents of triethylamine (TEA) in DMF. Elemental analysis performed on the polymers after Soxhlet extraction indicated that the monomer conversion was high, that the monomers had been stoichiometrically incorporated into the polymers and that the template had been successfully removed from the polymer. The molecular recognition properties of the materials were then investigated *via* chromatography comparing the retention of the template, *Z*-Glu, with that of more complex biologically active molecules such as methotrexate (MTX), containing the glutamic acid substructure, and structurally related analogues *Z*-Asp and *Z*-Gly.

As mechanistically expected, the effect of imprinting depended strongly on the ionization state of the acid groups. Using an aqueous acetonitrile-based mobile phase, all solutes were weakly and similarly retained on both columns in the absence of added base, whereas in the presence of base the glutamate containing solutes were strongly and selectively retained (Table 1).

To gain insight into the binding energy and site density of the polymers we measured the equilibrium binding isotherm on a *Z*-D-Glu imprinted polymer in the optimum solvent system described above and in MeCN/TEA (99/1 v/v) where *Z*-Glu is expected to bind strongly to both the imprinted and nonimprinted polymers (Fig. 2). *Z*-Glu interacts strongly (K_a > 1000 M^{-1}) with both polymers in the latter system and the difference between the uptake of the solute by them is small. Interestingly, the curve levels off at a value close to the theoretical capacity of P1 based on the amount of template added to the monomer mixture. This shows that the matrix urea groups are functional and fully accessible.

The association to P$_N$1 could be selectively suppressed by addition of water, resulting in a large difference in adsorption properties between the materials. Thus, in addition to a preferential

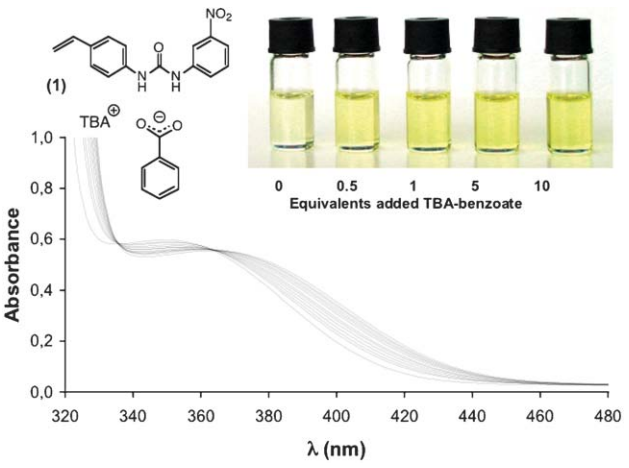

Fig. 1 UV–vis spectra of urea monomer **1** (0.33 mM in DMSO) in the presence of increasing concentrations of TBA benzoate. The position of λ_{max} shifts from 349 nm (0 equivalents TBA benzoate) to 364 nm (10 equivalents TBA benzoate). The inset shows solutions of urea monomer (1) (10 mM in DMSO) in the presence of, from left to right, 0, 0.5, 1, 5 and 10 equivalents of TBA benzoate, respectively.

† Electronic supplementary information (ESI) available: experimental procedure for the preparation of the imprinted polymers. Titration data containing ^{1}H NMR CIS curves and Job plot of monomer **1**. See http://www.rsc.org/suppdata/cc/b4/b407870e/

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Table 1 Chromatogaphic retention factors (k) and imprinting factors (IF ($= k_{P1}/k_{P_N1}$)) for *N*-substituted amino acids on P1 and P_N1

	−TEA[a]			+TEA[a]		
Solute	k_{P1}	k_{P_N1}	IF	k_{P1}	k_{P_N1}	IF
Z-Glu	4.4	2.6	1.7	>100	1.4	>70
Z-Asp	2.8	1.6	1.7	0.6	0.6	1.0
Z-Gly	5.2	3.0	1.8	0.5	0.5	1.0
MTX	4.0	2.7	1.5	2.5	0.6	4.3

[a] The mobile phase was MeCN/H_2O: 93/7 (v/v) (−TEA) or MeCN/H_2O/TEA: 92/7/1 (v/v/v) (+TEA).

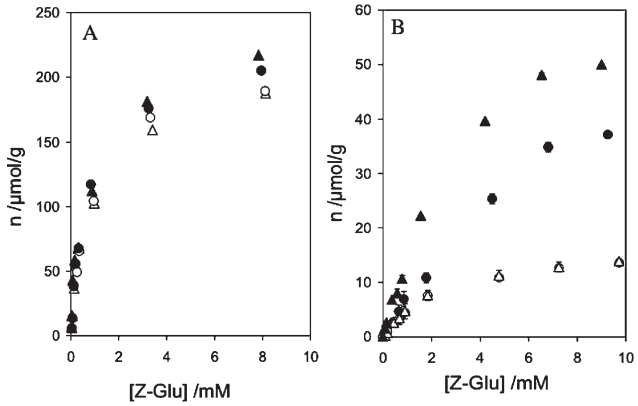

Fig. 2 Adsorption isotherms of *Z*-D-Glu (triangles) or *Z*-L-Glu (circles) on P1 (filled symbols) and P_N1 (open symbols) as solutions in MeCN/TEA: 99/1 (v/v) (A) or MeCN/H_2O/TEA: 92/7/1 (v/v/v) (B). The results are averages of two replicate experiments with error limits indicated.

weakening of the binding to P_N1, the difference in the adsorbed amount of *Z*-D-Glu to imprinted and nonimprinted polymers amounted to *ca.* 35 µmol g^{-1} and the imprinted polymer then exhibited a pronounced enantioselectivity with the adsorbed amount of *Z*-D-Glu exceeding that of *Z*-L-Glu by *ca.*13 µmol g^{-1}. This value exceeds the highest saturation capacities previously reported for weak imprinting systems.[18]

As seen in Fig. 3, the amount of guest bound under these conditions was sufficient to cause a color change in P1 in response to the added guest. Although the bathochromic shift is small, the yellowish polymers adopt a more intense yellow color in presence of added template (*Z*-L-Glu). Thus, prior to template removal, P1 exhibited a more intense yellow color than the corresponding nonimprinted polymer P_N1. Extensive washing of P1 resulted in polymer P1′ which showed a lighter color, indicating removal of the glutamate guest. The washed polymer was then incubated with the template *Z*-L-Glu in MeCN/H_2O/TEA: 92/7/1 (v/v/v) for 5 days giving P1″. As seen from the tone adjusted photographs, P1″ exhibits a significantly more intense yellow color than P1′. Interestingly, these binding induced changes were not observed for the corresponding nonimprinted polymer P_N1, indicating that the color changes originate from the template occupying imprinted sites in the polymer.

In conclusion, *Z*-glutamate imprinted polymers made using monomer **1** show strong binding to carboxylates in competitive aqueous-containing environments and the binding can be monitored with the naked eye. Given that a large range of biologically important molecules containing oxyanion functionality are compatible with imprinting in such solvent systems as those described above, this type of monomer will significantly expand the scope of imprinted polymer based applications.

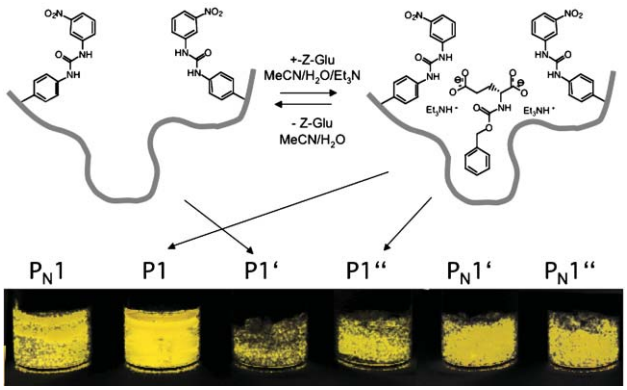

Fig. 3 Photographs after tone adjustment and with blackened background of polymer monoliths illuminated with a 60 W light bulb. The monoliths were prepared by copolymerization of **1** with EDMA in absence (P_N1) and presence (P1) of *Z*-L-Glu. P1′ is an imprinted polymer (P1) after template removal by extensive washing‡ and P1″ is a washed polymer (P1′) incubated§ with *Z*-L-Glu. P_N1' and P_N1'' are nonimprinted polymers subjected to the same treatments.

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Notes and references

‡ The polymers (P1 and P_N1) were washed as follows: MeOH (×7); 10% HCl (1 M, aqueous) in MeOH (×7); 10% HCl (1 M, aqueous) in DMF (×7), DMF (×6), THF (×6), DMF (×1).
§ The washed polymers (P1′ and P_N1)′ were dried *in vacuo* at room temperature and conditioned in MeCN. Thereafter they were incubated with a solution of *Z*-L-Glu (10 mM) in MeCN/H_2O/TEA: 92/7/1 (v/v/v) for 5 days.

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