JOC Article

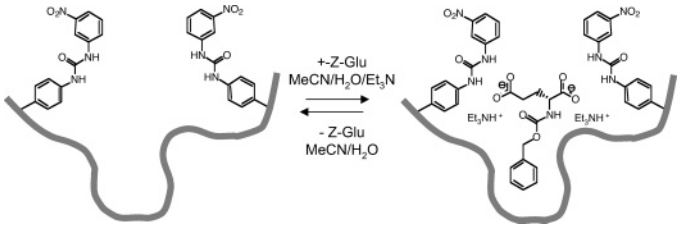
Urea Host Monomers for Stoichiometric Molecular Imprinting of Oxyanions[§]

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

INFU, Universität Dortmund, Otto Hahn Str. 6, 44221 Dortmund, Germany, and Department of Pharmaceutical Chemistry, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy

andy@infu.uni-dortmund.de; borje@infu.uni-dortmund.de

Received August 31, 2004



A series of urea-based vinyl monomers was synthesized and investigated for their ability to function as polymerizable hosts for the molecular imprinting of *N*-*Z*-D- or L-glutamic acid in polar media (DMSO or DMF). The monomers were synthesized in one step from a polymerizable isocyanate and a nonpolymerizable amine or vice versa, with yields typically over 70%. Prior to polymerization their solution binding properties vis-à-vis tetrabutylammonium benzoate in DMSO were investigated by ^{1}H NMR, UV−vis and fluorescence monitored titrations. The affinities of the urea monomers for benzoate depended upon the substitution pattern of the urea, with all diaryl ureas exhibiting high affinity. EDMA-based imprinted polymers prepared in DMF or DMSO against *Z*-D-(or L)-glutamic acid using 2 equiv of the urea monomer and 2 equiv of base were able to recognize the imprinted dianion as well as larger molecules containing the glutamic acid substructure. The affinity, reflected in liquid chromatography retention data, correlated with the solution binding properties of the corresponding monomers.

Introduction

Recent progress in the area of host−guest chemistry directed toward anion recognition has resulted in low molecular weight hosts capable of selective complexation of anions in water-rich media.[1−3] However, it is difficult to engineer them in useful formats for the recognition of guests of higher complexity or of larger size. Thus, general recognition strategies directed toward biomolecules based on artifical receptors remain an important challenge.[4] In this context the concept of molecular imprinting appears very appealing.[5−7] Here, monomers are chosen in order to complement functional groups of a template molecule. After incorporation of the monomer−template complexes in a cross-linked polymer matrix and removal of the template, binding sites remain which are capable of rebinding the template with high affinity and selectivity. The advantage of this "top down" approach in receptor design lies in its use of the self-assembly principle to guide the binding groups to their positions in the receptor site; thus, the structure of the final binding site is a priori unknown.

With few exceptions, imprinted polymers for anion recognition have been prepared from commercially available functional monomers (e.g., vinylpyridine, *N*,*N*-diethyl-2-aminoethyl methacrylate, methacrylamide), which are able to provide only weak interactions with the template molecule in solvents of low polarity.[8] Generally,

[§] Dedicated to Professor Günther Wulff on the occasion of his 70th birthday.

[†] Universität Dortmund.

[‡] University of Pavia.

(1) Best, M. D.; Tobey, S. L.; Anslyn, E. V. *Coord. Chem. Rev.* **2003**, *240*, 3−15.

(2) Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 191−221.

(3) Suksai, C.; Tuntulani, T. *Chem. Soc. Rev.* **2003**, *32*, 192−202.

(4) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479−2494.

(5) Molecularly imprinted polymers. Man made mimics of antibodies and their applications in analytical chemistry. In *Techniques and Instrumentation in Analytical Chemistry*; Sellergren, B., Ed.; Elsevier Science B.V.: Amsterdam, 2001; Vol. 23.

(6) Wulff, G.; Knorr, K. *Bioseparation* **2002**, *10*, 257.

(7) *Molecularly Imprinted Materials−Sensors and other Devices*; Shea, K. J., Roberts, M. J., Yan, M., Ed.; Materials Research Society Symposium Proceedings: San Francisco, 2002; Vol. 723, M1.3.

10.1021/jo048470p CCC: $30.25
Published on Web 01/29/2005

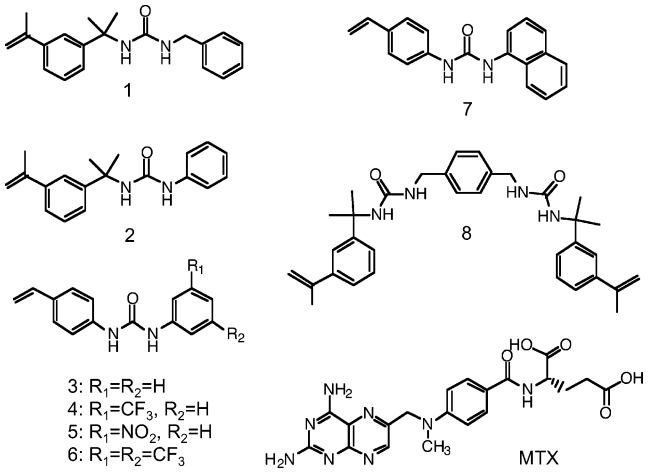
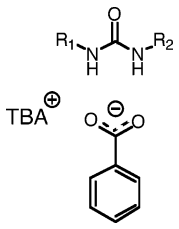


FIGURE 1. Urea monomers used to prepare imprinted polymers for oxyanion recognition. The monomers were prepared in one step from 1-isopropenyl-4-isopropyl-2-isocyanate and the corresponding amine (monomers **1**, **2** and **8**) or 4-vinylaniline and the corresponding isocyanate (**3**−**7**) as described in the experimental section.

this means that a large excess of functional monomer is used in order to ensure a high degree of complexation of the template. Such conditions are not compatible with the vast majority of biomolecules, which instead require polar or aqueous environments for imprinting.

One approach to improve this situation involves the preparation of functional monomers which provide strong and stoichiometric interactions with a given template.[6,9,10] If the monomer−template interactions are sufficiently strong, stoichiometric use of the monomer should lead to a high percentage of complex in the pre-polymerization solution, which would then be transformed into a high yield of imprinted sites in the polymer and lead to a drastic reduction in the degree of nonspecific binding in the obtained imprinted polymer. This has been demonstrated by Wulff et al. with the use of amidine-based

SCHEME 1



functional monomers for preparing polymeric receptors and catalytic sites.[9] However, the examples reported so far involve an elaborate multistep synthesis to prepare the host monomer. With synthetic accessibility as one design criterion, we were interested in extending the palette of functional monomers available for oxyanion imprinting. A further concern was to be able to achieve improved imprinting in more polar environments than those generally reported. There are numerous examples from the field of supramolecular chemistry showing that the 1,3-disubstituted urea moiety is a very capable binding element for this purpose.[2,11−14]

To exploit this binding element in molecular imprinting, we have prepared the monourea monomers **1**−**7** (Figure 1) and assessed their usefulness as binding and reporter monomers in the imprinting of *N*-*Z*-D-(or L)-glutamate (*Z*-Glu) (see Figure 2). For preliminary accounts on the behavior of monomers **8** and **5** in the imprinting of these templates, see refs 15 and 16, respectively.

Compounds **1**−**7** were prepared in a single step, from commercially available reagents, in moderate to good yields. We used benzoate (as its TBA-salt) as the model oxyanion for our ^{1}H NMR studies in the competitive solvent DMSO-d_6. Self-association of monomer and/or guest does not occur in these systems. A Job plot confirmed a 1:1 monomer−guest stoichiometry (Scheme 1) and fitting the raw titration data to a 1:1 binding isotherm afforded the respective association constants listed in Table 1.

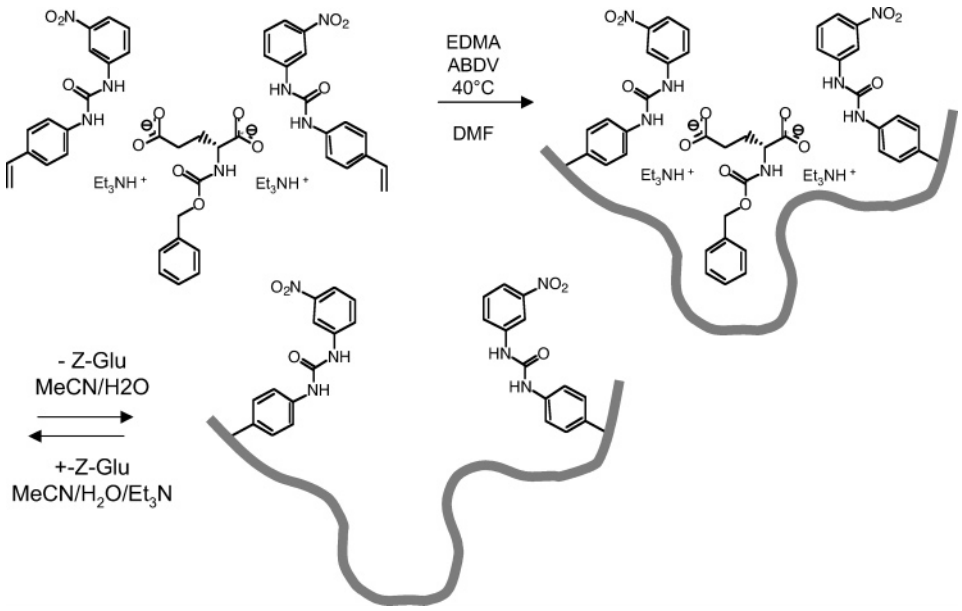


FIGURE 2. Imprinting of *Z*-D-Glu using urea host monomer (**5**), ethyleneglycol dimethacrylate (EDMA) as cross-linking monomer and DMF as solvent.

TABLE 1. Association Constants (K_a) and Complexation Induced Shifts (CIS) for the Interaction of 1,3-Disubstituted Monoureas with TBA-Benzoate in DMSO-d_6

monomer	K_a (M^{-1})[a]	CIS[a]
1	30 ± 4	1.21
2	121 ± 6	2.34
3	1322 ± 48	3.28
4	6520 ± 1099	3.48
5	6498 ± 170 (4600)[b]	3.54
6	8820 ± 1600	3.39
7	613 ± 61 (699)[c]	2.31
8	1500 ± 200[d]	1.80[d]

[a] CIS of both urea protons were monitored. K_a refers to the average of the individual values except for **6** where due to excessive broadening the ortho protons of the 3,5-bis(trifluoromethyl)phenyl group were monitored. [b] Value from Benesi−Hildebrand plot (see Supporting Information) of the UV−vis titration data. [c] Value from Stern−Vollmer plot (see Supporting Information) of fluorescence titration data. [d] Value calculated for the association of **8** with bis-TBA-glutarate.[15]

In agreement with previous reports,[17] dramatic increases in the binding strengths of the monomers are observed on varying the substitution of these simple monourea systems. These are attributed to the nature of the urea substituents, which leads to increases in the acidity of the urea protons[18] and, hence, increased magnitudes of association with the carboxylate guest.

We further assessed the ability of monomer **5** to bind benzoate in a water-containing environment. A ^{1}H NMR titration using DMSO-d_6/D_2O (9/1) as solvent led to K_a = 250 ± 9 M^{-1}. As reported for similar low molecular weight host molecules,[12,14] monomers **5** and **7**, with their extended π-systems, exhibited UV−vis and fluorescence spectral changes, respectively, upon association allowing corresponding association constants to be determined (Table 1 and Supporting Information).

Imprinted (**P1**, **P2**, **P5**) and nonimprinted (**P$_N$1**, **P$_N$2**, **P$_N$5**) polymers were then prepared from the monourea monomers **1**, **2** and **5**. We chose these monomers as representative examples of the three classes of monomer prepared. While monomer **6** exhibited a higher association with benzoate compared to monomer **5**, we were interested to extend our studies on the latter monomer because of its ability to signal, chromogenically, a specific binding event.[16] Polymerizations were performed in the presence of *Z*-D-(or L)-Glu and 2 equiv of triethylamine (TEA) in the polar solvents DMSO or DMF. Analysis of the extracts after polymer washing indicated that greater than 95% of the template was removed from the imprinted polymers and that the functional monomers were stoichiometrically incorporated in the polymer matrices.

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 *N*-*Z*-Asp and *N*-*Z*-Gly. In our previous reports we concluded that addition of an organic base to a mobile phase consisting of pure MeCN led to enhanced imprinting factors and retentions, mainly for the template,[15] in agreement with the previously proposed mechanism of binding. Thus, our initial evaluations used a mobile phase consisting of MeCN containing 1% TEA. The column efficiency was in general very poor indicating slow mass transfer processes related to the strong carboxylate-urea interaction. As seen in Figure 3, the retention order seems to agree with the affinity toward TBA-benzoate displayed by the monomers in solution. Thus, the retention of the acid containing solutes increased in the order **P1**[19] < **P8** < **P2** < **P5**. Unfortunately, this is also the case for the nonimprinted reference polymer, leading to a total retention of all solutes on **P5** and **P$_N$5** in this mobile phase system.

To weaken the affinity displayed by this recognition element we added water to the above mobile phase system, keeping the base content fixed (Figure 4). Above 6% water, a dramatic drop in retention was observed. This was most pronounced for the nonimprinted polymer leading to high imprinting factors within a small interval of mobile phase water content. Thus, when passing from 6% to 7% (v/v) water in the mobile phase, the retention time dropped from more than 70 to 2.3 min on **P$_N$5**, whereas no elution was observed on **P5** within the run time of the measurement (120 min).

A further increase in the water content eluted the analyte from the imprinted polymer as well. A water content of 7% was thus chosen for further investigations of the selectivity of the materials. First we observed that **P5** exhibited no or very low enantioselectivity in the chromatographic mode in contrast to other imprinted polymers prepared using less strongly associating monomers where separation factors over 2 are common. This conflicts with the results under equilibrium conditions where high enantioselective uptake was observed,[16] with the amount of adsorbed *Z*-D-Glu exceeding that of *Z*-L-Glu by ca. 13 μmol/g.

We explain this dichotomy in the following manner. Three points of contact are required for enantioselective discrimination.[20] In our stoichiometrically imprinted polymers, two strong interactions to the analytes are provided by the polymeric urea units, while any further interaction must be provided by the polymer matrix. Such interactions, most probably van der Waals in nature, are necessarily weak and, under dynamic conditions, probably not strong enough to impart enantioselectivity to

(8) Sellergren, B. *Angew. Chem., Int. Ed.* **2000**, *39*, 1031−1037.
(9) Wulff, G.; Gross, T.; Schönfeld, R. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1962−9164.
(10) Lübke, C.; Lübke, M.; Whitcombe, M. J.; Vulfson, E. N. *Macromolecules* **2000**, *33*, 5098−5105.
(11) Linton, B. R.; Goodman, M. S.; Fan, E.; van Arman, S. A.; Hamilton, A. D. *J. Org. Chem.* **2001**, *66*, 7313−7319.
(12) Kato, R.; Nishizawa, S.; Hayashita, T.; Teramae, N. *Tetrahedron Lett.* **2001**, *42*, 5053−5056.
(13) Lee, D. H.; Lee, H. Y.; Lee, K. H.; Hong, J.-I. *Chem. Commun.* **2001**, 1188.
(14) Mei, M.; Wu, S. *New J. Chem.* **2001**, *25*, 471.
(15) Hall, A. J.; Achilli, L.; Manesiotis, P.; Quaglia, M.; De Lorenzi, E.; Sellergren, B. *J. Org. Chem.* **2003**, *68*, 9132−9135.
(16) Manesiotis, P.; Hall, A. J.; Emgenbroich, M.; Quaglia, M.; De Lorenzi, E.; Sellergren, B. *Chem. Commun.* **2004**, 2278−2279.
(17) Wilcox, C. S.; Kim, E.; Romano, D.; Kuo, L. H.; Burt, L. B.; Curran, D. P. *Tetrahedron* **1995**, *51*, 621.
(18) Meta substitution furthermore enhances the acidity of the ortho aromatic ring proton, which in turn enhances its ability to act as a hydrogen bond donor towards the carbonyl oxygen. This effect is important in the crystalline state leading it to cocrystallize with Lewis basic solvents but is likely to be of less importance in solution. See ref 17 and Etter, M. C.; Panunto, T. W. *J. Am. Chem. Soc.* **1988**, *110*, 5896−5897.
(19) Using the bis-TBA salt of *Z*-Glu as the oxyanion and acetonitrile as the mobile phase, **P1** exhibited a retention factor much lower ($k \ll 1$) than that of **P2** ($k > 1$) and **P8** ($k > 1$). **P1** was therefore not investigated any further.
(20) Dalgleish, C. E. *J. Chem. Soc.* **1952**, 3940.

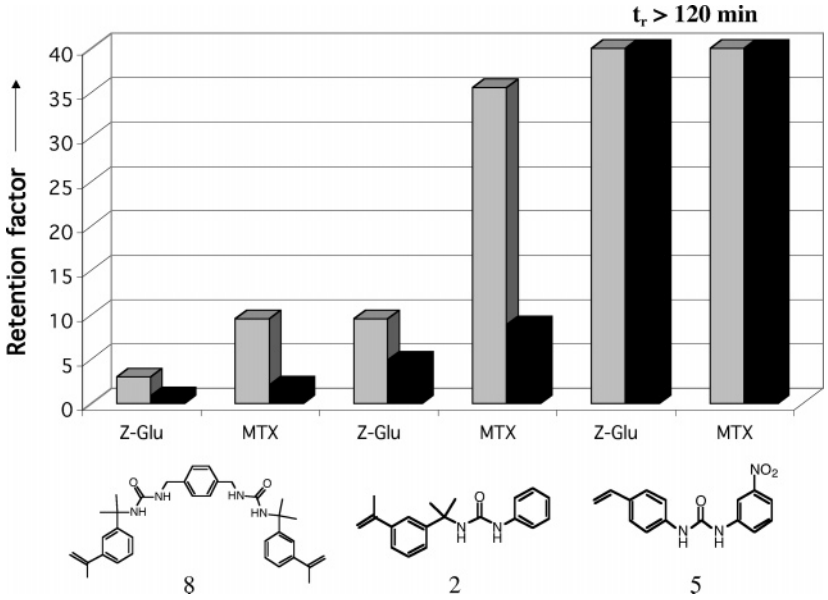


FIGURE 3. Retention factors for *Z*-Glu and MTX on imprinted (grey bars) and nonimprinted (black bars) polymers prepared using the depicted monomers. The mobile phase was MeCN/TEA: 99/1 (v/v), the injection volume was 20 μL, analyte concentration was 10 mM, flow rate was 1 mL/min and detection was performed at 262 nm for the *Z*-Glu and 260 nm for the MTX.

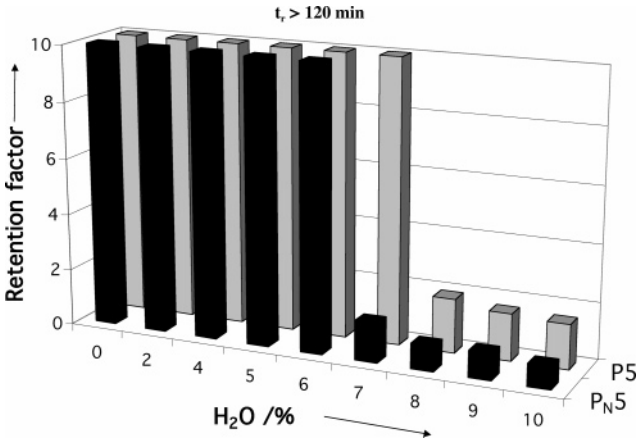


FIGURE 4. Retention factors for *Z*-Glu on imprinted (**P5**) and nonimprinted ($\mathbf{P_N5}$) polymers prepared using urea monomer (**5**). The mobile phase consisted of MeCN with increasing contents of water and a fixed concentration of TEA (1% (v/v)). Conditions were otherwise as described in Figure 3.

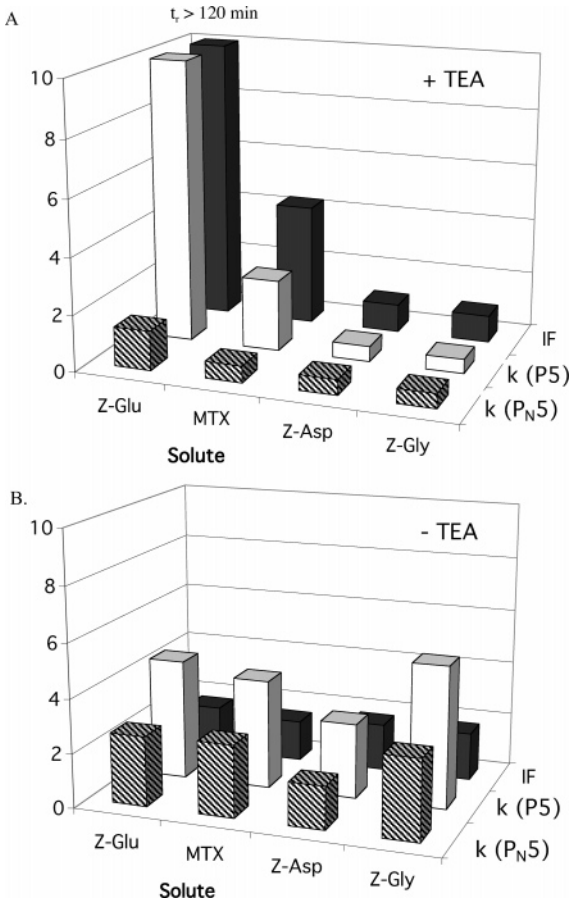


FIGURE 5. Retention factors (k) for *Z*-Glu and other carboxylic acid solutes on imprinted (**P5**) and nonimprinted ($\mathbf{P_N5}$) polymers and corresponding imprinting factors ($IF = k_{MIP}/k_{NIP}$). The mobile phase was (A) MeCN/H_2O/TEA 92/7/1 (v/v/v) and (B) MeCN/H_2O 93/7 (v/v).

our systems. Under equilibrium conditions, there is sufficient time for these low strength tertiary interactions to exert their influence.

Nevertheless, pronounced substrate selectivities were observed that were strongly dependent on the presence of base in the mobile phase (Figure 5). Thus, in the absence of TEA, the solutes exhibited similar retention factors and were, moreover, similarly retained on both **P5** and $\mathbf{P_N5}$. This resulted in low and similar imprinting factors and contrasts with the retention behavior in the presence of TEA where high imprinting factors were observed for *Z*-Glu and MTX. Interestingly, the other control substances, *Z*-Asp and *Z*-Gly, were still similarly retained on **P5** and $\mathbf{P_N5}$, indicating the presence of well-defined binding sites.

In summary, dramatic increases in the binding of oxyanions by simple, 1,3-disubstituted monourea monomers occurs on variation of the substitution pattern. Monomer (**5**) also shows moderate binding to benzoate in an even more competitive water-containing environ-

ment. Given that a large range of biologically important molecules containing oxyanion functionality are compatible with imprinting in such solvent systems as those described above, these monomers may lead to a new range of MIP-based applications.

Experimental Section

Benzylamine, aniline, phenyl isocyanate, 3-nitrophenyl isocyanate, 3-(trifluoromethyl)phenyl isocyanate and 1-naphthyl isocyanate, 4-aminostyrene, 3-isopropenyl-α,α-dimethylbenzyl isocyanate and 1,3-bis(trifluoromethyl)phenyl isocyanate were used as received. Ethylene glycol dimethacrylate (EDMA) was purified by the following procedure prior to use: the received material was washed consecutively with 10% aqueous NaOH, water, brine and finally water. After drying over $MgSO_4$, pure, dry EDMA was obtained by distillation under reduced pressure. All other reagents were used as received. *N*,*N*′-Azo-bis-(2,4-dimethyl)valeronitrile (ABDV) was purchased from Wako. DMSO-d_6 was purchased from Deuterio-GmbH (Kastellaun, Germany). Anhydrous solvents, dichloromethane and tetrahydrofuran, were stored over appropriate molecular sieves. Other solvents were of reagent grade or higher. ^{1}H NMR spectra were recorded at 400 MHz. UV−visible spectra were obtained using a Perkin-Elmer Lambda 20 instrument. Elemental microanalyses were performed using a CHN-rapid HERAEUS analyzer.

Synthesis of Monoureas 1−7. General Procedure. To a stirred solution of the desired amine (20 mmol) in THF (50 mL) under an inert atmosphere was added the required isocyanate (20 mmol) either neat (in the case of liquid isocyanates) or as a solution in THF (10 mL) (in the case of solid isocyanates). The solution was allowed to stir at room temperature overnight and then the solvent was evaporated under reduced pressure. The resulting solid residue was recrystallized from ethanol if not otherwise mentioned.

1-(3-Isopropenyl-α,α-dimethylbenzyl)-3-(benzyl)urea (1). Yield: 85%. ^{1}H NMR (DMSO-d_6) δ: 1.53 (s, 6H), 2.07 (s, 3H), 4.13 (d, 2H), 5.05 (s, 1H), 5.33 (s, 1H), 6.26 (t, 1H), 6.40 (s, 1H), 7.15−7.27 (m, 8H), 7.47 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 21.85, 30.44, 42.78, 54.45, 112.48, 122.02, 123.07, 124.51, 126.75, 127.08, 128.10, 128.43, 140.28, 141.28, 143.34, 149.36, 157.35. Calculated for $C_{20}H_{24}N_2O$: C 77.89, H 7.84, N 9.08. Found: C 77.47, H 8.07, N 8.95

1-(3-Isopropenyl-α,α-dimethylbenzyl)-3-(phenyl)urea (2). Yield: 80%. ^{1}H NMR (DMSO-d_6) δ: 1.56 (s, 6H), 2.06 (s, 3H), 5.03 (s, 1H), 5.33 (s, 1H), 6.55 (s, 1H), 6.81 (t, 1H), 7.11−7.15 (m, 2H), 7.21−7.29 (m, 5H), 7.46 (s, 1H), 8.35 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 21.84, 29.99, 54.60, 112.64, 117.65, 121.16, 121.99, 123.26, 124.48, 128.24, 128.87, 140.36, 140.75, 143.26, 148.73, 154.34. Calculated for $C_{19}H_{22}N_2O$: C 77.52, H 7.53, N 9.52. Found: C 77.32, H 7.66, N 9.35

1-(4-Vinylphenyl)-3-(phenyl)urea (3). Yield: 50%. ^{1}H NMR (DMSO-d_6) δ: 5.11 (d, 1H), 5.66 (d, 1H), 6.63 (dd, 1H), 6.94 (t, 1H), 7.25 (m, 2H), 7.33−7.45 (m, 5H), 8.63 (broad s, 1H), 8.71 (broad s, 1H). Calculated for $C_{15}H_{14}N_2O$: C 75.60, H 5.92, N 11.76. Found: C 75.45, H 5.69, N 11.63

1-(4-Vinylphenyl)-3-(3-trifluromethylphenyl)urea (4). Yield: 65%. ^{1}H NMR (DMSO-d_6) δ: 5.12 (d, 1H), 5.68 (d, 1H), 6.64 (dd, 1H), 7.27−7.56 (m, 7H), 7.99 (s, 1H), 8.83 (broad s, 1H), 9.01 (broad s, 1H). Calculated for $C_{16}H_{13}F_3N_2O$ (%): C 62.74, H 4.28, N 9.14. Found: C 62.41, H 4.40, N 9.01

1-(4-Vinylphenyl)-3-(3-nitrophenyl)urea (5). Yield: 70%. ^{1}H NMR (DMSO-d_6) δ: 5.06 (d, 1H), 5.68 (d, 1H), 6.64 (dd, 1H), 7.36−7.80 (m, 7H), 8.54 (s, 1H), 8.87 (broad s, 1H), 9.18 (broad s, 1H). Calculated for $C_{15}H_{13}N_3O_3$: C 63.59, H 4.63, N 14.84. Found: C 63.98, H 4.53, N 14.69

1-(4-Vinylphenyl)-3-(3,5-bis(trifluromethyl)phenyl)-urea (6). Yield: 64%, mp 192.4 °C. ^{1}H NMR (DMSO-d_6) δ: 5.11 (d, 1H), 5.68 (d, 1H), 6.63 (dd, 1H), 7.37 (d, 2H), 7.43 (d, 2H), 7.59 (s, 1H), 9.02 (s, 1H), 9.35 (s, 1H). ^{13}C NMR (DMSO-d_6) 112,7, 114.7, 122.3, 118.3, 119.1, 125.0, 127.0, 130.9, 131.2, 131.8, 136.5, 139.1, 142.2, 152.6. MS (FAB) m/z (M^+) 374.0, ($[M + H]^+$) 375.0. Calculated for $C_{17}H_{12}F_6N_2O$: C 54.55, H 3.23, N 7.48. Found: C 54.10, H 3.15, N 7.40

1-(4-Vinylphenyl)-3-(1-naphthyl)urea (7). Yield (ethanol/toluene) 81%, mp 240.8 °C. ^{1}H NMR (DMSO-d_6) δ: 5.10 (d, 1H), 5.67 (d, 1H), 6.64 (dd, 1H), 7.37−7,62 (m, 8H), 7.89−8.10 (m, 3H), 8.75 (s, 1H), 9.10 (s, 1H). ^{13}C NMR (DMSO-d_6) 112.4, 117.8, 118.2, 121.7, 123.3, 126.1, 126.2, 126.3, 127.1, 128.8, 131.2, 134.1, 134.6, 136,6, 129.9, 153.1. MS (EI) m/z (M^+) 288. Calculated for $C_{19}H_{16}N_2O$: C 79.14, H 5.59, N 9.72. Found: C 78.80, H 5.55, N 9.55

^{1}H NMR Titrations and Estimation of Association Constants. All ^{1}H NMR titrations were performed in DMSO-d_6. Association constants (K_{SL}) for the interactions between hosts and guests were determined by titrating an increasing amount of guest (tetrabutylammonium benzoate, TBABz) into a constant amount of functional monomer. The concentration of functional monomer was 1 mM and the amounts of added guest were 0, 0.5, 1, 2, 3, 4, 5, 7.5 and 10 equiv, respectively. The complexation induced shifts ($\Delta\delta$) of the host urea protons were followed and titration curves were then constructed of $\Delta\delta$ versus guest concentration. The raw titration data were fitted to a 1:1 binding isotherm by nonlinear regression using Microcal Origin 5.0 from which the association constants could be calculated.

Polymer Preparation. An imprinted polymer was prepared in the following manner. The template molecule, *Z*-D-Glu-OH (1 mmol), if not otherwise stated, functional monomer (2 mmol) and EDMA (20 mmol) were dissolved in DMF (**P5**) or DMSO (**P1**, **P2**, **P8**) (5.6 mL). To the solution were added TEA (2 mmol) and the initiator ABDV (1% w/w of total monomers). The solution was transferred to a glass ampule, cooled to 0°C and purged with a flow of dry nitrogen for 10 min. The tubes were then flame-sealed while still under cooling and the polymerization initiated by placing the tubes in a thermostated water bath preset at 40°C. After 24 h (**P5**) or 48 h (**P1**, **P2**, **P8**) the tubes were broken and the polymers lightly crushed. Removal of the template molecule from the polymers was achieved by extraction with methanol in a Soxhlet apparatus for 24 h. Thereafter, the polymers were crushed and sieved to obtain particles in the size range 25−50 μm. A nonimprinted polymer (**P_N#**) was prepared in the same way as described above, but with the omission of the template molecule and TEA from the pre-polymerization solution. Elemental analyses of extracted polymers: **P5/P_N5**, calculated C 60.97; H 6.81; N 1.86; found **P5** C 60.0; H 7.0; N 1.7. P_N5: C 59.9; H 7.1; N 1.6

HPLC Evaluation. The 25−36 μm particle size fraction was repeatedly sedimented (80/20 methanol/water) to remove fine particles and then slurry-packed into HPLC columns (100 mm × 4.6 mm, i.d.) using the same solvent mixture as pushing solvent. Subsequent analyses of the polymers were performed using an Agilent HP1100 system equipped with a diode array-UV detector and a workstation. Analyte detection was performed at 262 nm (*Z*-Glu), 260 nm (MTX), 282 nm (*Z*-Asp), and 284 nm (*Z*-Gly).

Acknowledgment. Work supported in part by the European Community's program for Training and Mobility of Researchers (TMR) under contract FMRX-CT-98-0173, [MICA] and the European Community's Improving Human Potential Program under contract HPRN-CT-2002-00189, [AquaMIP]. Ms. Scilla Pelletti and Ms. Ilaria Cei are gratefully acknowledged for their technical support with the chromatographic experiments.

Supporting Information Available: Titration data containing ^{1}H NMR CIS curves of monomers **1**−**7**, UV−vis spectra and Benesi-Hildebrand plots of **5**, fluorescence emission spectra and Stern−Volmer plot of **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO048470P