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Rational Design of New Polymerizable Oxyanion Receptors

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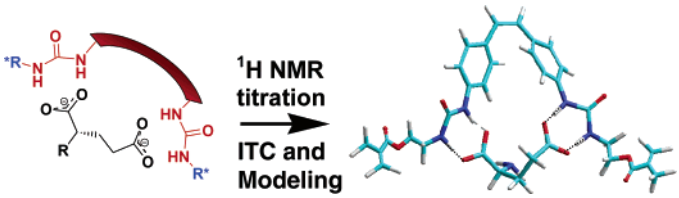

We report the synthesis of a library of new polymerizable functional monomers designed for complexing with the oxyanionic moiety of the chemotherapeutic drug methotrexate. The ^{1}H NMR and ITC binding studies allowed for the selection of receptors possessing the best association parameters. Subsequently, the design of a broad library of polymerizable moiety-specific binding monomers for the imprinting of dicarboxylate containing drugs was accomplished. Di(ureidoehylenemethacrylate)stilbene possesses the highest association properties and shows potential to act as a monomer in the molecularly imprinting technique to obtain photoswitchable cavities.

Introduction

Robust molecular recognition elements with the ability to bind and discriminate between chemicaly similar molecules can be synthesized by using molecular imprinting techniques.[1,2] In the past 20 years, molecular imprinting of polymer matrixes has become a widely used approach to generate macromolecular receptors for target molecules.[3] The noncovalent approach involves complexation of target molecules (templates) with functional monomers through supramolecular interactions in solution, followed by a polymerization reaction with an excess of cross-linkers.[4] Removal of the templates reveals specific recognition sites that are complementary to the template in terms of its shape, size, and functionality in the polymer network. The use of these molecularly imprinted polymers (MIPs) has seen application in several areas ranging from analytical chromatography to organic synthesis where they are exploited as catalysts.[5−7] MIPs are stable to a wide range of organic solvents, temperatures, and pH values, and to exposure to light and oxidative environments.[8] Their use in the field of Dynamic Combinatorial Chemistry (DCC)[9] as natural enzymes substitutes could surpass the limits encountered with biological receptors. To tailor MIPs with homogeneous cavities crafted for maximizing recognition toward a specific type of chemical structure, we initiated a procedure of systematic template-moiety/receptor complexation analysis. We report the synthesis of a library of new polymerizable functional monomers designed for imprinting the chemotherapeutic drug methotrexate (MTX) shown in Figure 1. Subsequent study and comparison of the strengths of the monomer−template interactions made possible the selection of "hit-monomers" possessing higher complexation affinity. Furthermore, we describe the measurement of their relative affinities

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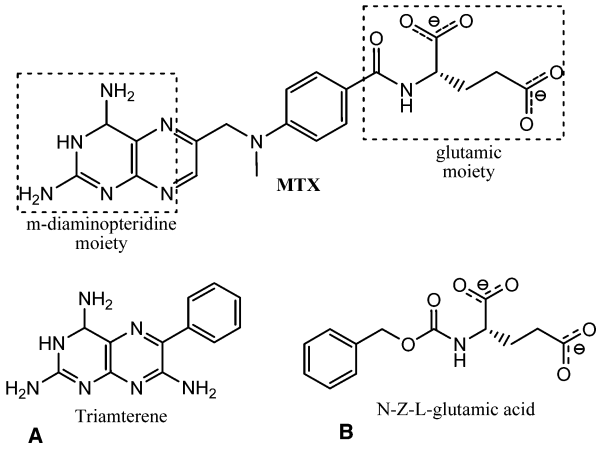

FIGURE 1. Structures of the methotrexate drug (MTX) and of the two substructural models (A: triampteren; B: bis(TBA)-*N*-Z-L-glutamate) used in the binding tests.

toward an MTX substructure template to identify specific properties that come to play in the creation of an MTX-imprinted polymer.

Results and Discussion

The design of the library of novel functional monomers **1** to **10** was based on a selection of three features that are relevant for the creation of selective MIPs. First is the choice of bis-urea binding functionalities, which exhibit strong affinity for dicarboxylate moieties.[10] The second feature is the selection of polymerizable end groups. We chose methacrylate functions, commonly used cross-linking chemical systems known for providing materials with good thermal stability.[7] In all the monomers we designed, the methacrylate group is placed two carbons away from the H-bond donating ureas to prevent destabilization of the template/monomer complex during the polymerization process. The final feature is to design species with enhanced affinity for the MTX template by synthesizing a variety of selected compounds, all differing by the chemical nature of their central spacer (Figure 2). The spacers differ in their length, rigidity, polarity, and space filling.

The monomers were synthesized in one-pot reactions by *N*-hydro-*C*-alkylamino additions of an amine on the appropriate isocyanate. ^{1}H NMR titrations were performed with the monomers **1** to **10**, using the substructural model bis(TBA)-*N*-Z-L-glutamate (**B**) as the guest compound. The complexation-induced chemical shift ($\Delta(\delta)$) of the urea protons of receptors **1** to **10** was monitored. Addition of increasing amounts of **B** (0−10 equiv) to DMSO-d_6 solutions of the functional monomers (Figure 3) allowed extraction of monomer/template interaction information (Table 1). All titration data fit well to 1:1 binding isotherms and association constants (K_a) were obtained by nonlinear least-squares fitting.[11] The competitive solvent DMSO-d_6 prevented self-association of monomer and/or guest to occur in these systems. The monomers that gave rise to the weakest $\Delta(\delta)$ and association constants are those containing the shortest spacer, namely receptors **1** and **2**. Although their outer pairs of

TABLE 1. Extrapolated ^{1}H NMR Chemical Induced Shifts ($\Delta(\delta)$)[a] and Association Constants[b]

	$\Delta(\delta)$ (ppm)		
receptor (−R−)	inner ^{1}H	outer ^{1}H	K_a (M^{-1})
di(ureidoethylenemethacrylate)			
1 (ethylene)	1.42	1.71	$(6.9 \pm 0.5) \times 10^2$
2 (propylene)	1.64	1.70	$(7.1 \pm 1.1) \times 10^2$
3 (butylene)	1.76	2.01	$(8.4 \pm 1) \times 10^2$
4 (pentylene)	1.74	1.91	$(7.4 \pm 0.5) \times 10^2$
5 (hexamethylene)	1.76	1.99	$(7.9 \pm 0.3) \times 10^2$
6 (heptylene)	1.74	1.98	$(7.7 \pm 0.8) \times 10^2$
7 (*p*-phenylene)	2.74	3.28	$(1.63 \pm 0.06) \times 10^3$
8 (*m*-xylylene)	1.74	1.88	$(8.5 \pm 0.4) \times 10^2$
9 (*p*-xylylene)	1.96	2.30	$(1.1 \pm 0.1) \times 10^3$
10 (*m*-phenylene)	2.00	2.35	$(1.6 \pm 0.2) \times 10^3$
16 (stilbene)	3.27	3.59	$(3.37 \pm 0.26) \times 10^3$
di(ureidostyrene)			
11 (butylene)	2.42	2.66	$(2.8 \pm 0.5) \times 10^3$
12 (hexamethylene)	2.76	2.89	$(2.7 \pm 0.8) \times 10^3$
13 (*m*-bis(methylethyl-benzene)	2.30	2.74	$(1.9 \pm 0.2) \times 10^3$
14 (*m*-xylylene)	3.04	2.95	$(2.9 \pm 0.5) \times 10^3$
15 (*m*-phenylene)	2.84	3.18	$(4.0 \pm 0.4) \times 10^3$

[a] The host concentration was 4 mM for entries **1**−**15** and 1 mM for **16**; the solvent was DMSO-d_6. [b] The association constants were determined from the experimental urea proton chemical shifts by the nonlinear refinement method.[11]

N−H- - -O hydrogen bonds with the carboxylate group of **B** do give rise to significant $\Delta(\delta)$ (1.71 and 1.70 ppm, respectively), it is not unexpected to observe a weaker association of the inner proton considering that their respective spacers do not permit an ideal face-to-face positioning with **B**. The other linear aliphatic monomers **3** to **6** with butylene to heptylene spacers gave similar $\Delta(\delta)$ for both inner and outer urea protons. This observation gave topological information on the more suitable face-to-face relative positioning of **B**'s dicarboxylate moiety and the monomer's di(urea) part. This more favorable face-to-face positioning results in a slightly increased association constant for **3** to **6**. This aliphatic group of spacers are flexible and devoid of steric congestion and are adaptable for fitting to the substrate before rigidification into a polymerized structure. Monomers **7** to **10** carry more rigid, polar, and/or space filling properties not present in **1** to **6**. Surprisingly, the *p*-phenylene monomer **7** gave rise to the most intense binding and a very good fit to a 1:1 binding isotherm. Stronger $\Delta(\delta)$ and higher association constants were obtained for monomers **7** and **10**, in which urea protons are more acidic and positioned directly on the aryl moiety. Compared to monomer **7**, monomers **8** and **9**, in which urea protons are separated from the aryl moiety by one methylene group, showed weaker $\Delta(\delta)$ and association constants. However, monomer **9**, compared to monomer **8**, presented stronger $\Delta(\delta)$ and higher association constant. The association differences obtained for monomers **7** to **10** underline the fact that both tridimensional structure of the monomer and acidity of the urea protons are governing monomer/guest association. We did not anticipate the 1:1 stoichiometry for monomer **7** because of its linear geometry (Figure 2). However, data obtained from the titrations of **7** with the dicarboxylate **B** and the monocarboxylate analogue are also different (Figure 3). The values for the monoester do not display a 1:1 binding isotherm and the magnitude of the $\Delta(\delta)$ does not rise to the level observed for the dicarboxylate **B**. These results, as well as molecular modeling studies,[12] account for a face-to-face complexation between our receptors and dicarboxylate, rather than a monocarboxylate/receptor type of interaction. Molecular

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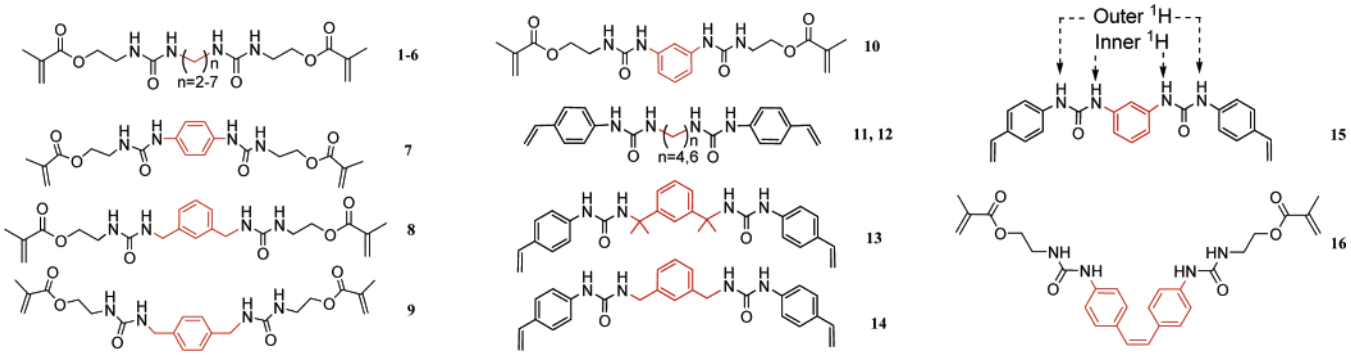

FIGURE 2. Library of novel polymerizable bis(ureidoethylenemethacrylate) monomers **1**−**10** designed for improved imprinting of methotrexate and second generation receptors **11**−**16**.

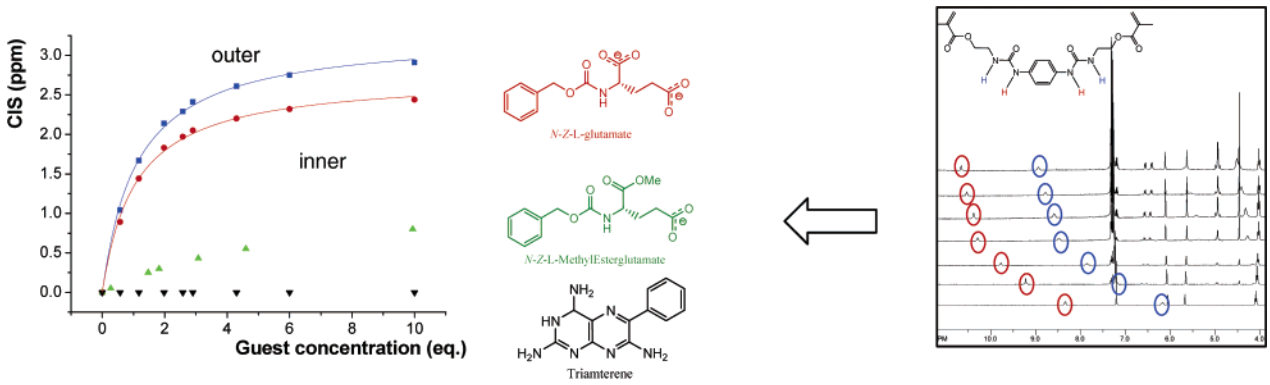

FIGURE 3. Left: Δ(δ) vs guest concentration; the nonlinear curve fitting process (nonlinear regression equation: CIS = $CIS_{max}\{K[S]/(1 + K[S])\}$, where $\Delta(\delta)_{max}$ is the extrapolated maximum complexation induced chemical shift, [S] is the added guest concentration, and *K* represents the curvature of the function) is represented by lines. In green is shown the titration against the monocarboxylate analogue. In blue, the chemical shifts are plotted as a function of triamterene concentration, showing the specificity of the bis-ureated receptors for the dicarboxylate moiety. Right: Chemical shifts of the inner and outer urea protons of the functionalized receptor **7** as a function of **B** concentration in DMSO-d_6.

modeling indicated energetic minima with the anticipated positioning of each **B**'s carboxylate interacting with one urea group.

We anticipated flexible linear monomers with the capacity to bend over the dicarboxylic glutamate moiety (like monomers **3** to **6**) or more rigid concave-shaped monomers (monomers **8** and **9**) to satisfy the criteria of face-to-face 1:1 complementarity. A reasonable interpretation of the result for monomer **7** is the formation of 2:2 or 6:6 binding suprastructures. The *p*-xylylene receptor **9** interacted strongly with the dicarboxy moiety of **B** in a 1:1 binding manner as already demonstrated.[13] The monomer/**B** association in such a case was due to the length and the shape of the spacer between the ureas on the monomers allowing suitable complementarity.[14]

Receptors **7** and **10**, the most rigid and inflexible compounds of the list, are an informative example to consider in this monomer assembling study for two reasons: (1) although the inner protons are located in the α-position of an electron-withdrawing aromatic ring making them much more acidic, their binding does not result in a Δ(δ) as high as the outer protons due to inappropriate spacer length and (2) the electron-withdrawing nature of the urea substituent (including the spacer itself) increases the acidity of the urea protons and, hence, compensates for the length by increasing the association constant. Monomers **3** to **6** showed effective association constants although the aliphatic nature of their spacers did not allow binding as intense as monomers **7** to **10** containing phenylene or xylylene spacers. It appears that hexamethylene and *m*- or *p*-xylylene confer the optimal distance between the urea moieties to bind the dicarboxylate anions as in molecule **B**. Moreover, when the topological criterion is satisfied, we can improve binding by increasing the acidity of the urea protons.

The results obtained from ^{1}H chemical shift studies of monomers **1**−**10** in binding **B** allowed the design of a second generation of di(ureidostyrene) monomers. We prepared monomers **11** and **12** (Figure 2), styrene analogues of the flexible aliphatic monomers **3** and **5**, in order to benefit from the spacer's nature while increasing the acidity of the urea protons. We also prepared monomers **13** and **14** which are modified versions of the *m*-xylylene monomer **8** and **15**, the analogue of **10**, as well as **16**. The stilbene moiety of **16** provides it with a controllable conformational change achievable by external stimulation via UV irradiation. This last feature makes it a very attractive compound in polymer matrixes with switchable-recognition cavities, an application that is notable since only a few photoswitchable receptors have been reported.[15] In all cases, the Δ(δ) values and association constants were increased relative

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TABLE 2. Association Data for Receptors via Isothermal Titration Calorimetry[a]

receptor	K_a (M^{-1})	ΔH (cal/mol)
1	1 200	−840
5	2 200	−1 350
8	2 800	−1 470
12	10 000	−3 700
15	27 000	−3 800
16	22 000	−3 100

[a] Measured by ITC at 25 °C. Performed in DMSO against a solution of **B**. Errors in K_a <15%.

to the original receptors, as a direct consequence of the increased acidity of the urea protons. Hence, although some receptors from the second set lack the two-carbon spacing between the polymerizable-extremity and the H-binding urea found in the initial set, the greater H-bonding magnitude could compensate for the potential thermodynamic complex-instability encountered during the polymerization process. Interestingly, the *m*-xylylene monomer **14** showed more $\Delta(\delta)$ magnitude than its tetramethyl-substituted analogue **13**, especially for the inner protons where the difference of magnitude is notable with a decrease of 24% for the inner ^{1}H shift against only 7% for the outer ^{1}H. Although they share the same skeletal architecture and length between the ureas, the presence of the methyl groups may twist the conformation of monomer **13**, not allowing it to adopt the well-matched conformation. This not well-matched conformation results in a significant difference in association constant for monomer **13** vs **14** (1900 M^{-1} vs 2900 M^{-1}). The highest association affinities were obtained for monomers **15** and **16**.

NMR titration analysis suggests that monomers **15** and **16**, demonstrating higher association affinity, are the most promising receptors among our library to bind dicarboxylate guest in a 1:1 complex. To assert the receptor's affinity properties, isothermal titration calorimetry (ITC) was employed. ITC is an acknowledged technique for measuring association constants between hosts and guests.[16] It measures the heat evolved or absorbed from the controlled addition of guest into host solution over a series of injections. Enthalpy values were negative for all receptor titrations indicative of exothermic, heat-releasing complexation behaviors. Titrations performed on six receptors are represented in Table 2. The association constants are higher for compounds **15** and **16** than for compound **12**, which is in turns much higher than those for compound **5** and **8**. These results are in good correlation with the association parameters measured by ^{1}H NMR titrations. Very strong binding constants of 27 000 and 22 000 M^{-1} were found for receptors **15** and **16** showing an obvious superior affinity for the dicarboxylate guest compared to the other receptors.

As receptors **1**−**16** were designed to complex the dicarboxylate part of MTX, we also evaluated the H-binding affinity of our monomers toward the diaminopteridine group as can be seen in Figure 3. The comparison of the chemical shifts of the receptor's urea protons vs triamterene clearly shows the specificity of the bis-ureated receptor for the dicarboxylate moiety over the diaminopteridine part, both of which are present on an MTX template.

The synthesis of similar compounds (PhCONHPhNHCOPh) had previously been reported[17] and the crystal structure of the benzoate complex of this receptor revealed that this compound is capable of binding carboxylates via four hydrogen bonds in an almost symmetrical arrangement.[18] We also obtained higher carboxylate affinities in solution by molecular modeling studies, suggesting that all four of the NH hydrogen bond donor groups are involved in dicarboxylate complexation in DMSO-d_6 solution.

An important factor to consider is that these new cross-linking monomers combine interactive monomer functionality with a cross-linking format.[4] These new cross-linking agents are readily copolymerizable under mild conditions[19] and may be used for noncovalent molecularly imprinted polymers (MIPs) with potential improved performance, under the premise that more functionality could be introduced without suffering performance losses due to reduced cross-linking.

Conclusions

The functionalized bis-ureated receptors **1** to **16**, whose assembly properties on a substructural model of the chemotherapeutic drug MTX were investigated, offer a variety of monomers for use in MIP applications. Among our library members we identified two monomers, monomers **15** and **16**, presenting highly enhanced binding affinities toward the dicarboxylate moiety. On the basis of those results and considering the stilbene features discussed above, we are looking forward to using **16** as an interesting compound in MIP preparations to come. In conventional polymer imprinting, removing the template remains a challenge. The retained template has been shown to be a serious obstacle to trace analysis applications and to be involved in apparent chiral recognition exhibited by imprinted polymers. In the case of monomer **16** described here, by switching the conformation of the cavity the affinity to the imprint may be altered and the imprint can be released. The presented methodology is under development and may lead to creation of new MIPs with high specific photoswitchable imprinted sites for several drugs.

Experimental Section

Synthesis of Bis(ureidoethylenemethacrylate) Receptors 1−10: General Procedure. To a stirred solution of the desired diamine (20 mmol) in anhydrous tetrahydrofuran (70 mL) under an inert atmosphere was added 2-isocyanatoethyl methacrylate (50 mmol) dropwise as a solution in dry THF (20 mL). The solution was allowed to stir at room temperature overnight under a stream of nitrogen and then the solvent was evaporated under reduced pressure. The resulting solid residue was recrystallized or washed with several volumes of solvent. The solid was dried under high vacuum.

1,2-Bis(ureidoethylenemethacrylate)ethylene (1): yield (washed six times in acetonitrile/*p*-dioxane) 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.85 (s, 6H), 2.97 (t, J = 2.5 Hz, 4H), 3.24 (m, 4H), 4.01 (t, J = 5.6 Hz, 4H), 5,66 (s, 2H), 5.99 (t, 2H), 6.03 (s, 2H), 6.06 (t, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.8, 125.0, 139.4, 160.5, 62.3, 40.1, 157.1, 40.0. HMRS (FAB$^+$) calcd for $C_{16}H_{26}N_4O_6$ $[M + H]^+$ 371.19, found 371.2029. Anal. calcd: C, 51.88; N, 15.13; H, 7.08. Found: C, 51.98; N, 15.10; H, 7.32.

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(19) For polymerization details and procedures see the Supporting Information.

Synthesis of Bis(ureiodostyrene) Receptors 11−15: General Procedure. To a stirred solution of the desired diisocyanate (20 mmol) in anhydrous tetrahydrofuran if not otherwise mentioned (50 mL) under an inert atmosphere was added 4-aminostyrene (50 mmol) dropwise as a solution in dry THF (20 mL). The solution was allowed to stir at room temperature overnight under a stream of nitrogen and then the solvent was evaporated under reduced pressure. The resulting solid residue was recrystallized or washed with several volumes of the mentioned solvent. The solid was dried under high vacuum.

Butylenedi(ureidostyrene) (11): yield (washed four times with toluene/acetonitrile/petroleum ether) 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (t, 4H), 3.06−3.08 (m, 4H), 5.06 (d, J = 10.8 Hz, 2H), 5.62 (dd, J = 16.0 Hz, J = 1.5 Hz, 2H), 6.14 (t, J = 5.5 Hz, 2H), 6.59(dd, J = 17.6 Hz, J = 10.9 Hz, 2H), 7.27−7.35 (m, 8H), 8.45 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 111.3, 138.5, 132.2, 126.6, 121.5, 138.5, 154.4, 42.1, 26.2. HMRS (FAB$^+$) calcd for $C_{22}H_{26}N_4O_2$ [M + H]$^+$ 379.21, found 379.2076. Anal. calcd: C, 69.82; N,14.80; H, 6.92. Found: C, 69.30; N, 14.73; H, 6.87.

Synthesis of Bis(ureidoethylenemethacrylate)stilbene Receptors 16. To a pyridine solution (30 mL) of 4,4′-diaminostilbene dihydrochloride (20 mmol) was added NaH/60% in mineral oil (20 mmol). The reaction mixture was gently stirred until no gas release was observed. The reaction mixture was filtrated to remove the NaCl deposit. To the crude pyridine mixture was then added a solution of 2-isocyanatoethyl methacrylate (50 mmol) in dry THF (50 mL) under an inert atmosphere. The solution was allowed to stir at room temperature over a period of 24 h and the solvent was then evaporated under reduced pressure. The resulting solid residue was washed six times in acetonitrile. The white solid was dried under high vacuum. Yield:65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.86 (s, 6H), 3.39 (dt, J = 6.0 Hz, J = 5.0 Hz, 4H), 4.11 (t, J = 5.2 Hz, 4H), 5.68 (s, 2H), 6.06 (s, 2H), 6.28 (t, J = 5.8 Hz, 2H), 6.97 (s, 2H), 7.34−7.41 (m, 8H), 8.62 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.7, 41.5, 63.0, 120.4, 128.6, 129.3, 131.0, 133.1, 135.0, 138.5, 157.0, 169.2. HMRS (FAB$^+$) calcd for $C_{28}H_{32}N_4O_6$ [M + H]$^+$ 521.24, found 521.2322.

Synthesis of Bistetrabutylammonium-*N*-*Z*-L-glutamate. *N*-*Z*-L-Glutamic acid (4 mmol) was dissolved in methanol (70 mL) and 1 M methanolic tetrabutylammonium hydroxide (8 mmol) was added in one portion. The solution was stirred at ambient temperature for 1 h, and then the solvent was removed in vacuo. The oily residue was dried under high vacuum pump at 75 °C for a period of 18 h and was not further purified.[13]

^{1}H NMR Titrations The receptor solutions were titrated by adding known quantities of a concentrated solution of the bis(TBA) *N*-*Z*-L-glutamate. Increasing the concentration of anion in the host−guest mixture solution induced a clear downfield shift in the NH peak of the receptor's ureas (as shown in the Supporting Information, Figure S3a). Fitting the observed data points to a 1:1 binding profile (see the Supporting Information, Figure S3b), using Microcal Origin 6.0, gave the extrapolated complex induced chemical shifts found in the Supporting Information, Figure S6.

ITC Titrations. Experiments were carried out with a Microcal VP-ITC MicroCalorimeter. The formation of the assembly **1·B**, **5·B**, **8·B**, **12·B**, **15·B**, and **16·B** was studied by introducing 28 × 10-μL injections of a 100 mM solution of **B** (bis(TBA) *N*-*Z*-L-glutamate) to 3.4 mM solutions of the receptors in the calorimetric cell and monitoring the heat change after each addition. The experimental temperature was 25 °C and the solvent DMSO. Calorimetric dilution experiments for the titrations were performed and they showed that the hosts do not undergo aggregation. The curves were corrected for the dilution heats and modeled by using a two-site nonlinear regression analysis. The ORIGIN software provided by Microcal Inc.[20] was used to calculate the binding constant (K_a) and the enthalpy change (ΔH).

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Supporting Information Available: Spectroscopic data for compounds **2**−**10** and **12**−**15**, NMR titration curves, polymerization procedure, ITC curves, and molecular modeling results. This material is available free of charge via the Internet at http://pubs.acs.org.

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