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A Highly Enantioselective Receptor for *N*-Protected Glutamate and Anomalous Solvent-Dependent Binding Properties**

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The development of enantioselective receptors continues to be a challenging endeavor for supramolecular chemists, and enantioselective recognition of biologically relevant molecules in competitive solvents is particularly demanding.[1] Although numerous receptors have been developed for dicarboxylic acids and dicarboxylates,[2] only a few enantioselective receptors for chiral dicarboxylic acids (in the neutral diprotonated form) have been described,[3] and very few examples of enantioselective receptors for chiral dicarboxylates have been reported.[4]

We recently described an acyclic monothiourea receptor **1a**, which bound a range of *N*-protected amino acid carboxylate salts with modest enantioselectivity.[5] Building on this work, we have now prepared macrocyclic receptor **2**, which features two thiourea moieties flanked by carboxypyridines and separated by a chiral diamine. The receptor was designed

to produce a chiral pocket for dicarboxylates by forming up to eight hydrogen-bonding interactions with the carboxylate oxygen atoms and intramolecular hydrogen bonding with the pyridine unit to help preorganize[6] the receptor (Scheme 1). Binding measurements, by NMR titration and isothermal
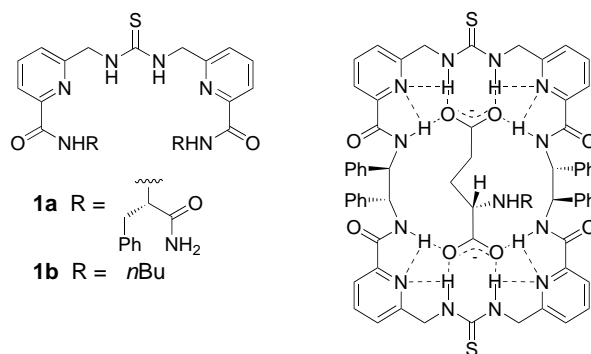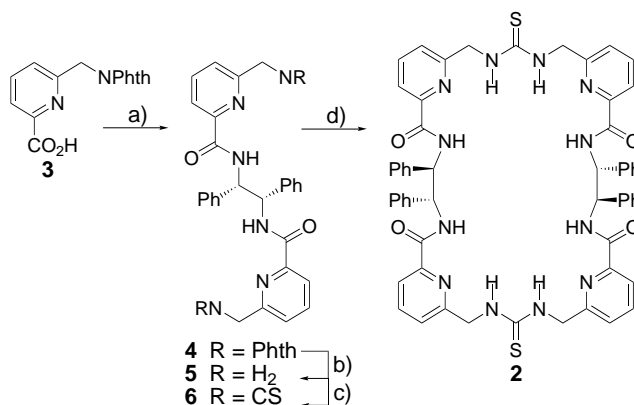

Scheme 1. Acyclic monothiourea **1** and the proposed complex of *N*-Boc-L-glutamate with macrocycle **2**. Boc = *tert*-butoxycarbonyl.

calorimetry, indicate that the macrocycle exhibits strong and highly enantioselective 1:1 binding of *N*-Boc-L-glutamate (as the dicarboxylate dianion) in relatively polar solvents (CH₃CN, DMSO) and that complexation involves a large, favorable entropic contribution. Remarkably, however, no binding of *N*-Boc-glutamate by the macrocycle is observed in a less polar solvent (CDCl₃).

The synthesis of receptor **2** was readily achieved by coupling acid **3**[5] with (*S,S*)-1,2-diphenylethylenediamine to give bisphthalimide **4** (Scheme 2). Removal of the phthalimide protecting groups and treatment of the resulting bisamine **5** with CS₂ and DCC produced the bis(isothiocyanate) **6**. A further equivalent of bisamine **5** was added to bisisothiocyanate **6**, in the presence of DMAP, by syringe pump over three hours to yield the macrocyclic bisthiourea **2** in 26% yield.

The ¹H NMR spectrum[7] of macrocycle **2** in CDCl₃ at room temperature is poorly resolved, with very broad signals for all protons, and resolution is not improved at higher temper-

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

Scheme 2. Reagents: a) SOCl₂; (1*S*,2*S*)-(−)-1,2-diphenylethylenediamine, DMAP, CH₂Cl₂ (68%); b) N₂H₄·H₂O, EtOH (84%); c) CS₂, DCC, CH₂Cl₂; d) simultaneous addition of 1 equiv of **5** and 1 equiv of **6** by syringe pumps over 3 h (26% over steps c and d). DMAP = 4-dimethylaminopyridine, DCC = *N,N'*-dicyclohexylcarbodiimide.

atures. At $-40\,°C$, however, a well-resolved spectrum is obtained, but it is not the simple spectrum expected, given the fourfold D_2 symmetry of the macrocycle. The spectrum instead indicates twofold C_2 symmetry. In particular, the thiourea NH protons give signals at $\delta = 8.96$ and 8.08 ppm and the amide NH protons give signals at $\delta = 10.10$ and 7.88 ppm.[7] A well-resolved and simple ^{1}H NMR spectrum is obtained in [D$_6$]DMSO or CD$_3$CN, however, which is consistent with the expected fourfold D_2 symmetry of the macrocycle.

Binding studies[7] with macrocycle **2** were carried out with the bis(tetrabutylammonium) salts of the enantiomers of N-Boc-glutamate in a range of solvents, by using standard NMR titration experiments[8] and isothermal calorimetry.[9, 10] The addition of the N-Boc-L-glutamate salt to a solution of **2** in CD$_3$CN led to significant downfield shifts of the thiourea NH ($\Delta\delta_{max} = 1.43$ ppm), the amide NH ($\Delta\delta_{max} = 1.06$ ppm), and the benzylic CH signals ($\Delta\delta_{max} = 0.59$), which is consistent with the formation of strong hydrogen bonds and the proposed mode of binding (Scheme 1). The binding data could be readily fitted to a 1:1 binding isotherm and gave $K_a^{1:1} > 10^4\,M^{-1}$.[11] Addition of the N-Boc-D-glutamate salt also led to significant downfield shifts of the NH and the benzylic CH signals. The binding data, however, could not be fitted to a 1:1 binding isotherm, but instead could be fitted to a 1:2 (host:guest) isotherm and yielded $K_a^{1:2} > 10^4\,M^{-1}$. The binding stoichiometry was confirmed by Job plots[7, 12] and is further evident from the ^{1}H NMR titrations, since saturation is rapidly reached after addition of approximately one equivalent of the N-Boc-L-glutamate salt, whereas saturation is reached only after addition of approximately two equivalents of the N-Boc-D-glutamate salt (Figure 1). The relatively smooth titration curve obtained with N-Boc-D-glutamate, and the lack of a clear transition after addition of only one equivalent of the guest, suggests that binding of the second guest molecule is stronger than the binding of the first (positive cooperativity), such that the receptor tends to saturate both binding sites simultaneously, that is, the 1:1 complex is only present in very small amounts relative to the uncomplexed receptor or the 1:2 complex.

The calorimetric binding data[13] obtained with the N-Boc-L-glutamate salt as the guest confirmed the strong 1:1 binding ($K_a^{1:1} = (2.83 \pm 0.48) \times 10^4\,M^{-1}$, $\Delta G_a^{1:1} = -25.5 \pm 0.5$ kJ mol^{-1}) which is dominated by the entropic contribution ($\Delta H_a^{1:1} = -4.5 \pm 0.2$ kJ mol^{-1}, $T\Delta S_a^{1:1} = 21.0$ kJ mol^{-1}). The calorimetric binding data obtained with the N-Boc-D-glutamate salt, using a two-site binding model, yielded a small 1:1 binding constant ($K_a^{1:1} = 38.4 \pm 1.66\,M^{-1}$, $\Delta G_a^{1:1} = -9.0 \pm 0.1$ kJ mol^{-1}) and a large 1:2 (host:guest) binding constant ($K_a^{1:2} = (4.92 \pm 0.07) \times 10^4\,M^{-1}$, $\Delta G_a^{1:2} = -26.8 \pm 0.1$ kJ mol^{-1}), which again is dominated by the entropic contribution ($\Delta H_a^{1:2} = -6.7 \pm 0.1$ kJ mol^{-1}, $T\Delta S_a^{1:2} = 20.1$ kJ mol^{-1}). This data confirms the stronger binding of the second N-Boc-D-glutamate guest relative to the first (positive cooperativity), as indicated by the NMR titration data, and suggests that the enantioselectivity exhibited by macrocycle **2** for the 1:1 binding of the N-Boc-glutamates is $> 700:1$.

Binding studies with the N-Boc-L-glutamate salt in [D$_6$]DMSO give a similar picture, with significant downfield
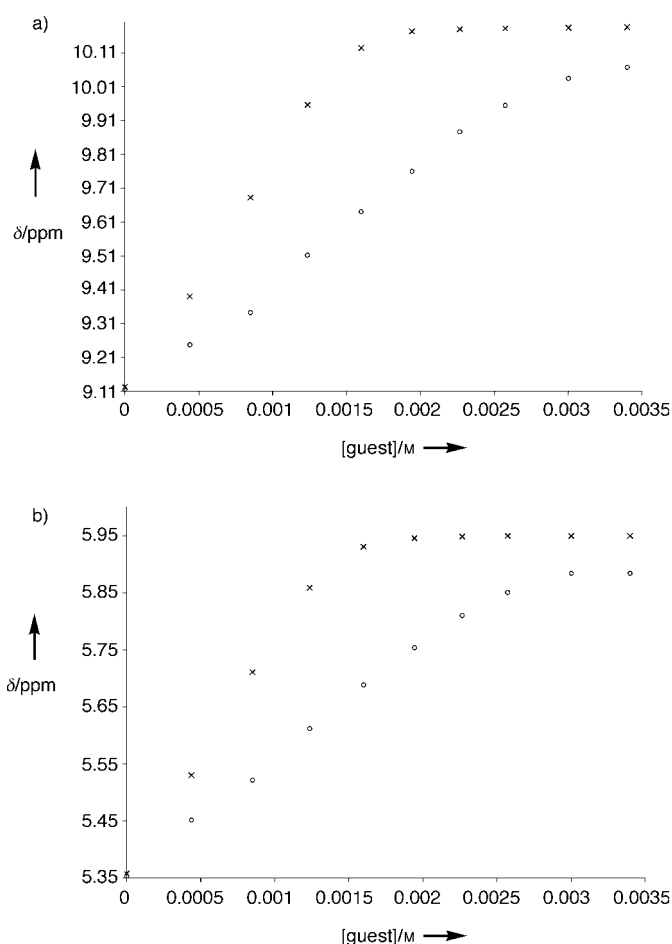

Figure 1. Binding titration curves for macrocycle **2** showing the shift of a) the amide and b) the benzylic CH protons on addition of the bis(tetrabutylammonium) salts of N-Boc-L-glutamate ($\times$) and N-Boc-D-glutamate ($\circ$) in CD$_3$CN. The graphs indicate the strong 1:1 binding with the former, and 1:2 binding with the latter (the 1:1 [host]:[guest] is reached when [guest] $= 1.5$ mM).

shifts of the NH signals observed on NMR titration and simple 1:1 binding, obtained by either NMR titration ($K_a^{1:1} = 3720\,M^{-1}$, $\Delta G_a^{1:1} = -20.4$ kJ mol^{-1}) or isothermal calorimetry ($K_a^{1:1} = 2280 \pm 258\,M^{-1}$, $\Delta G_a^{1:1} = -19.2 \pm 0.3$ kJ mol^{-1}), again with a large entropic contribution ($\Delta H_a^{1:1} = -10.7 \pm 0.3$ kJ mol^{-1}, $T\Delta S_a^{1:1} = -8.5$ kJ mol^{-1}). However, the binding data obtained with the N-Boc-D-glutamate salt in [D$_6$]DMSO deviates from the saturation curves expected for simple 1:1 or 1:2 binding, and reliable binding constants could not be obtained. Presumably several binding equilibria (1:2, 2:1, and possibly 2:2) are competing when the N-Boc-D-glutamate salt is the guest, although the NMR titration binding curve approaches saturation after addition of approximately two equivalents of guest, which indicates that a 1:2 (host:guest) binding stoichiometry is dominant.[7]

In the less competitive solvent, CDCl$_3$, addition of either enantiomer of the glutamate salt did not lead to any discernible change in the ^{1}H NMR spectrum of the macrocycle either at $-40\,°C$ or at room temperature. No change was observed even after warming the solution for several days. A 1:1 sample of macrocycle **2** and N-Boc-L-glutamate salt was also dissolved in CD$_3$CN (to allow formation of the 1:1

complex), the solvent removed, and redissolved in CDCl₃. The resulting ¹H NMR spectrum again showed unperturbed macrocycle. Thus, it is clear that macrocycle **2** does not bind the glutamate guests in the less polar solvent (CDCl₃), and it is improbable that this is because of slow binding kinetics.

It is a generally held view that binding interactions (for example, hydrogen bonds) between polar functionalities will lead to strong complexation in a nonpolar solvent (typically CHCl₃), but to weak (or negligible) complexation in more polar (competitive) solvents or solvent mixtures, and numerous examples of this phenomenon exist. For example, *N*-tolyl-*N′-n*-butylurea binds tetrabutylammonium benzoate with $K_a = 1300 \, M^{-1}$ in CDCl₃ and $K_a = 150 \, M^{-1}$ in [D₆]DMSO,[14] and thiourea **1a** binds the tetrabutylammonium salt of *N*-Ac-L-Phe with $K_a = 4800 \, M^{-1}$ in CDCl₃ and $K_a = 680 \, M^{-1}$ in 10% [D₆]DMSO/CDCl₃.[5]

The lack of binding exhibited by macrocycle **2** in CDCl₃, in contrast to the results observed in CH₃CN and DMSO, is thus remarkable. To probe the origin of this anomalous behavior the conformation of the macrocycle in CDCl₃ solution was established using torsion angle dynamics with NOE and scalar coupling constant constraints.[15] The NMR studies reveal that in CDCl₃ the macrocycle adopts a tightly wrapped conformation that is stabilized by a number of intramolecular hydrogen bonds, particularly from an amide carbonyl oxygen atom to the thiourea NH and adjacent amide NH protons (Figure 2). This hydrogen-bonding motif has been previously observed in the crystal structure of **1b** which forms a dimeric pair in the solid state.[5]
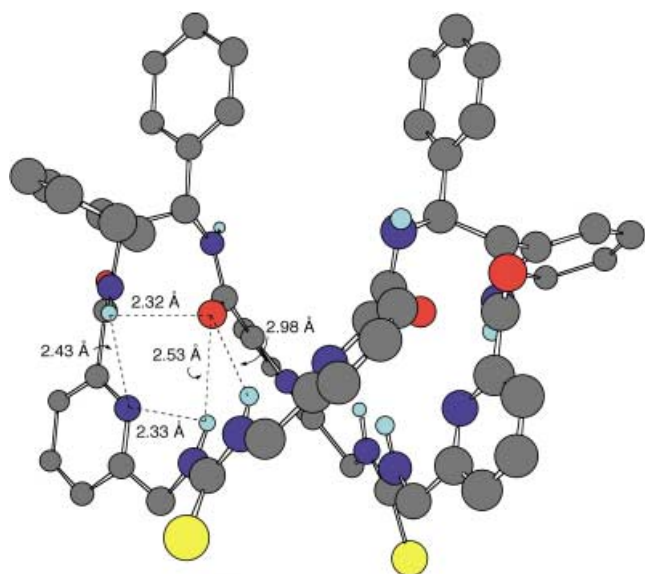

Figure 2. Conformation of macrocycle **2** in CDCl₃ as determined by ¹H NMR spectroscopy showing intramolecular H-bonding distances.

Clearly the energy required to reorganize the macrocycle into a suitable binding conformation (that is, to break the intramolecular hydrogen bonds) is not compensated for by binding interactions that would thereby be established. The more polar solvents, CH₃CN and DMSO, can solvate the hydrogen-bonding functionality of the macrocycle leading to a less rigidly constrained molecule and the ¹H NMR spectra

which reflect the fourfold D_2 symmetry of the molecule in these solvents. Solvation of the macrocycle by the more polar solvents allows tight binding of the *N*-Boc-L-glutamate salt—albeit with some conformational reorganization of the receptor (and associated energetic cost), as evidenced by the large shifts of various CH signals in the ¹H NMR spectrum on addition of the guest. Binding, in these solvents, is driven to a large extent by entropy, presumably produced by releasing bound solvent molecules from the cavity of the macrocycle. The same cavity is unable to accommodate the enantiomeric guest (*N*-Boc-D-glutamate) in a simple 1:1 binding mode, and instead binds two *N*-Boc-D-glutamate guests with a small binding constant for the first glutamate anion and a significantly larger binding constant for the second (positive cooperativity). At first sight this may seem surprising. However, the two carboxylate guests may bind on opposite faces and at opposite ends of the macrocycle, in which case the electrostatic repulsion between the two guests should be small. Furthermore, if binding of the first *N*-Boc-D-glutamate requires considerable reorganization of the receptor, and consequent energetic cost, then, once the first carboxylate is bound, the receptor may bind the second carboxylate without significant additional energetic penalty. Conformational changes in the receptor on binding *N*-Boc-D-glutamate in acetonitrile are, again, clearly evidenced by the large shifts in many of the CH signals in the ¹H NMR spectrum of the receptor (particularly the benzylic CH signals ($\Delta\delta = 0.53$ ppm)) on addition of *N*-Boc-D-glutamate.

In summary macrocycle **2** is highly enantioselective for the 1:1 binding of the *N*-protected glutamate dianion, but the binding shows anomalous solvent-dependent behavior. We are now developing analogous receptors incorporating recognition elements for the ammonium group to produce enantioselective receptors, and hence sensors, for zwitterionic glutamate.

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Catalytic Asymmetric 1,3-Dipolar Cycloaddition Reactions of Azomethine Ylides—A Simple Approach to Optically Active Highly Functionalized Proline Derivatives**

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The 1,3-dipolar cycloaddition reaction constitutes one of the most fundamental reactions for the stereoselective construction of five-membered heterocyclic compounds.[1] In recent years, the development of catalytic asymmetric reactions has been one of the challenging areas within the field of 1,3-dipolar cycloaddition reactions, and especially nitrones have been the focus of attention[2,3] as a result of the importance of the formed isoxazolidines as building blocks for more complex molecules.

Azomethine ylides can react in a 1,3-dipolar cycloaddition reaction with alkenes to form pyrrolidines, and several examples of the formation of optically active pyrrolidines based on diastereoselective reactions are known.[4,5] However, investigations on the metal-catalyzed enantioselective version of the 1,3-dipolar cycloaddition of azomethine ylides with alkenes are very limited. Grigg and co-workers[6] were the first to demonstrate that applying a stoichiometric amount of chiral cobalt or manganese complexes with ephedrine derivatives as the chiral ligand could give the cycloaddition product of azomethine ylides derived from imines of glycine alkyl esters with up to 96% *ee*. It has also been mentioned that silver(I) salts in combination with chiral phosphane ligands can catalyze the 1,3-dipolar cycloaddition reaction of azomethine ylides.[6b,7]

We present herein a new highly diastereo- and enantioselective 1,3-dipolar cycloaddition reaction of azomethine ylides with alkenes catalyzed by readily available chiral Lewis acids. Although there are few stable azomethine ylides,[8] most are unstable. Azomethine ylides **2** can be generated from, for example, imines of glycine alkyl esters **1** by reaction with a base in the presence of a Lewis acid complex. The metal-stabilized azomethine ylide **2** reacts with an alkene **3** to give highly functionalized pyrrolidines **4** (Scheme 1).
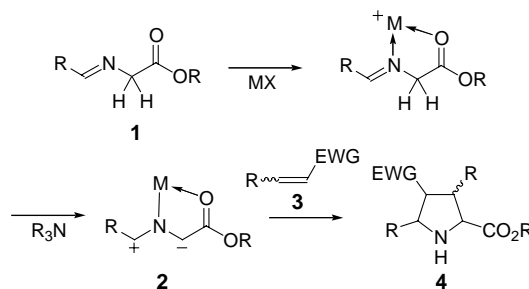
Scheme 1. Formation of highly functionalized pyrrolidines **4** from azomethine ylides **2** and alkenes **3**. EWG = electron-withdrawing group.

The reactions of *N*-benzylidene- and *N*-(2-naphthylmethylidene) glycinates **1a** and **1b**, respectively, with methyl acrylate (**3a**) and Et₃N as the base in the presence of chiral ligands such as the bisoxazolines (BOX) **5a,b**[9] and dibenzofuranyl-2,2′-bisoxazoline (DBFOX) **5c**[10] and Lewis acids were used in the screening process (Scheme 2). Some representative results are listed in Table 1.

The use of copper(II) salts in combination with the chiral bisoxazoline ligands **5a,b** as catalysts for the reaction of *N*-benzylidene glycinate **1a** with methyl acrylate (**3a**) gave high conversion only when using the (*S*)-*t*Bu-BOX (**5a**) ligand, but unfortunately product **4a** was formed as a racemate (Table 1, entries 1 and 2). When zinc(II)[9e] was used as the Lewis acid instead, the reaction proceeded smoothly and **4a** was formed with 76% *ee* using **5a** as the chiral ligand and THF as the solvent at room temperature (Table 1, entry 3). Furthermore, the reaction is also highly diastereoselective as only one

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