Molecular Recognition

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Highly Stereoselective Recognition and Deracemization of Amino Acids by Supramolecular Self-Assembly**

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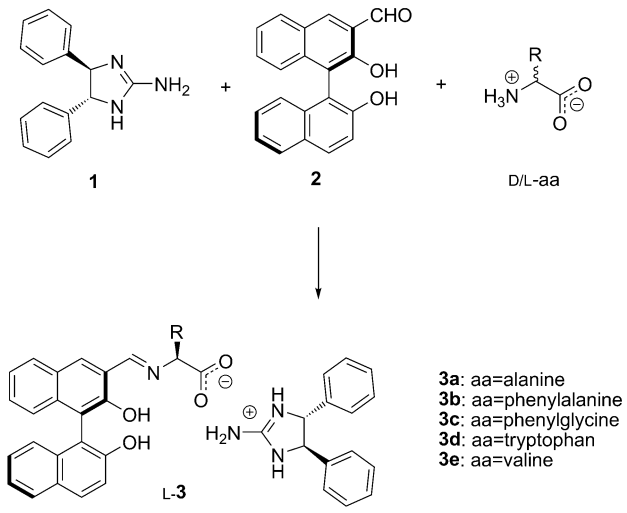
Abstract: *The highly stereoselective supramolecular self-assembly of α-amino acids with a chiral aldehyde derived from binol and a chiral guanidine derived from diphenylethylenediamine (dpen) to form the imino acid salt is reported. This system can be used to cleanly convert* D*-amino acids into* L*-amino acids or vice versa at ambient temperature. It can also be used to synthesize α-deuterated* D- *or* L*-amino acids. A crystal structure of the ternary complex together with DFT computation provided detailed insight into the origin of the stereoselective recognition of amino acids.*

There has been much interest in understanding the stereoselective recognition of small molecules, as it is central to the development of catalysts for the synthesis of chiral compounds.[1] Understanding the chiral recognition of amino acids is of particular interest, as they are fundamental building blocks of life. Cram[2] was one of the early pioneers in this field and developed highly stereoselective amino acid receptors based on chiral crown ethers. More recently, Soloshonok et al.[3] came up with modular designs of Ni^{II} complexes for the deracemization of amino acids on the basis of the earlier studies by Belokon et al.[4] Our own interest in this field led to rational designs of Co^{III} complexes[5] for the stereoselective recognition of amino acids. Inspired by the biomimetic studies of Breslow and co-workers,[6] we also developed chiral[7] and achiral[8] analogues of pyridoxal for the stereoselective recognition and deracemization of amino acids. There is much current interest in the synthesis of bioactive D-amino acids[9] by enantiomeric separation or deracemization.[10]

Supramolecular self-assembly has been a topic of considerable interest over the past two decades.[11] Whereas amino acid receptors have been based on single chiral molecules, supramolecular self-assembly could provide a new and interesting approach for the stereoselective recognition of amino acids. Indeed, supramolecular self-assembly has been shown to be useful for the development of stereoselective catalysts.[12] The appeal of supramolecular self-assembly is that complex structures can be built rapidly in excellent yield with minimal synthetic effort. In practice, it is more challenging to promote the stereoselective recognition of small molecules by supramolecular self-assembly than with single-molecule receptors. Although synthetic efforts are minimized with self-assembly, it is more challenging to control the precise positioning of functional groups for the stereoselective recognition of small molecules by supramolecular receptors. Herein we report how some of the difficulties may be overcome through the rational design of small chiral molecules that can assemble in a highly stereoselective way (Scheme 1). We investigated the use of this supramolecular system for the separation of racemic mixtures of amino acids as well as the mechanism for the deracemization of amino acids.



Scheme 1. Stereoselective self-assembly of **1**, **2**, and amino acids to form the ternary complex L-**3**.

In a typical experiment, a diphenylethylenediamine-based guanidine[13] **1** (60 μmol) with the *R*,*R* configuration and a binol-based aldehyde[14] **2** (50 μmol) with the *R* configuration in $CDCl_3$ (1 mL) were mixed with a solution of a racemic mixture of an amino acid (100 μmol) in D_2O or H_2O (1 mL) for about 2 h at ambient temperature to give L-**3** as the ternary complex with excellent stereoselectivity (Scheme 1). ^1H NMR spectroscopy of the $CDCl_3$ layer revealed that L-**3** was formed cleanly with less than 2% of D-**3** for each of the five amino acids used (alanine, phenylalanine, phenylglycine, tryptophan, and valine). A thermodynamic equilibrium was reached between L-**3** and D-**3** under the experimental conditions by imine exchange in about 2 h. Recognition of the L-amino acid took place with the *R*,*R* form of **1** and the *R* form of **2**, whereas recognition of the D-amino acid took place with the *S*,*S* form of **1** and the *S* form of **2**.

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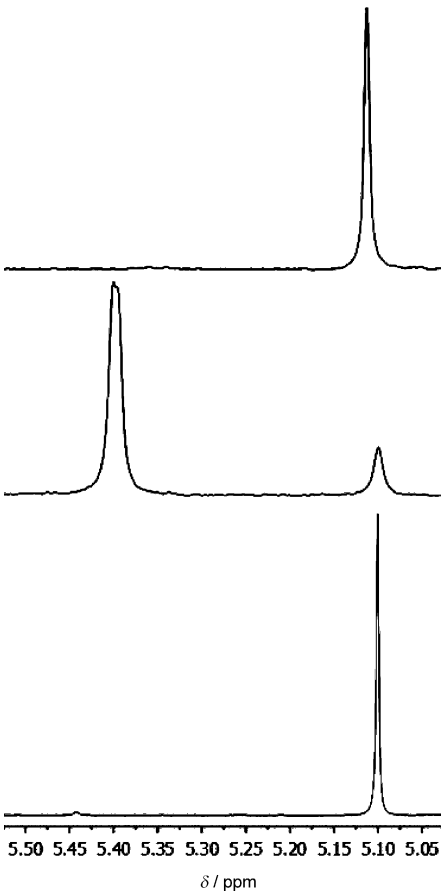


Figure 1. Partial ^{1}H NMR spectra showing the α-hydrogen-atom signals for ternary complexes formed with L-phenylglycine (after 20 min, top), D-phenylglycine (after 20 min, middle), and D/L-phenylglycine (after 2 h, bottom). When D-phenylglycine was used to make the ternary complex (middle), after 20 min, both D-**3c** and L-**3c** had formed as a result of partial epimerization.

Figure 1 shows ^{1}H NMR spectra of the ternary complexes **3c** formed with L-phenylglycine, D-phenylglycine, and D/L-phenylglycine. The NMR spectroscopic experiments were performed with $CDCl_3/H_2O$ as the solvent system. The ^{1}H NMR spectrum of the ternary complex L-**3c** formed with L-phenylglycine shows a sharp singlet for the α hydrogen atom at $\delta = 5.09$ ppm. For the ternary complex formed with D-phenylglycine, the signal for the α hydrogen atom was observed at $\delta = 5.40$ ppm for D-**3c** as the major product alongside that for L-**3c** as the minor product after 20 min. This conversion of D-**3c** into L-**3c** by α-proton exchange proceeded to near completion (98%) in about 24 h. Complexes D-**3a**, D-**3b**, and D-**3d** epimerized within 2 days, but D-**3e** did not epimerize under these conditions, apparently as a result of steric effects. The ternary complex formed with a more than twofold excess of D/L-phenylglycine showed signals for the α hydrogen atoms of L-**3c** and D-**3c** in a ratio greater than 50:1 after about 2 h. Thus, the equilibrium between L-**3c** and D-**3c** can be reached rapidly by imine exchange (2 h, Figure 1, bottom) or slowly by proton exchange (24 h, Figure 1, middle). Conversion of D-**3c** into L-**3c** took place in $CDCl_3$ even if only D-phenylglycine was used to form D-**3c** and the water layer was removed. Thus, equilibration by proton exchange can be separated from equilibration by imine exchange. The addition of D_2O (1M DCl) to this chloroform layer released L-phenylglycine into the water layer and left the aldehyde and guanidine in the chloroform layer. The catalytic turnover of the self-assembly system may be anticipated through continuous extraction. It is the reversibility of the self-assembly system, including imine and hydrogen-bond formation and α-proton-exchange reactions, under mild conditions at ambient temperature that makes it so effective for the deracemization of amino acids. Although self-assembly usually involves only weak bonds, reversible covalent-bond formation, such as imine formation, can provide greater rigidity, thus leading to higher stereoselectivity.

The chiral guanidine **1** by itself extracts amino acids from water into chloroform, but with little or no stereoselectivity. The chiral aldehyde **2** by itself does not effectively extract amino acids from water into chloroform. Phenylalanine can be extracted with **2** in combination with an achiral quaternary ammonium hydroxide derived from *N*-methyl-*N*,*N*-dioctyl-octan-1-ammonium chloride (aliquat 336), but the stereoselectivity is low (<3:1). Thus, there is remarkable synergy between **1** and **2** for the stereoselective recognition of amino acids through supramolecular self-assembly.

To gain some insight into the origin of the stereoselectivity in amino acid recognition by supramolecular self-assembly, we obtained crystals of L-**3b** for structure determination. Equimolar amounts of the three small chiral molecules **1**, **2**, and L-Phe were mixed in dimethyl sulfoxide (DMSO) to form the iminocarboxylate–guanidinium salt L-**3b**. The ternary complex was crystallized from DMSO by slow diffusion of ether. The crystal-packing diagram (Figure 2; the phenyl group of the amino acid is omitted for clarity) reveals a hydrogen-bonded network of a supramolecular self-assembly consisting of the iminocarboxylate–guanidinium salt.

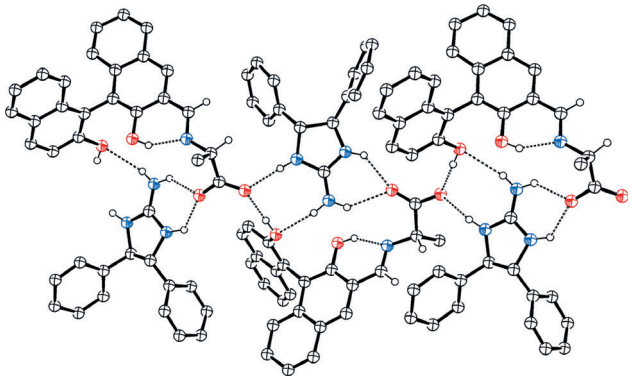


Figure 2. Crystal-packing diagram of three units of L-**3b** (the phenyl group of phenylalanine has been omitted for clarity). All hydrogen atoms attached to carbon atoms have been omitted, except for the imine C–H atom and the α hydrogen atom of the amino acid. Steric effects should increase in D-**3b**, as the positions of the α hydrogen atom and the amino acid side chain are interchanged. The two C–H groups shown are in van der Waals contact with one of the phenyl groups of the neighboring guanidine moiety.

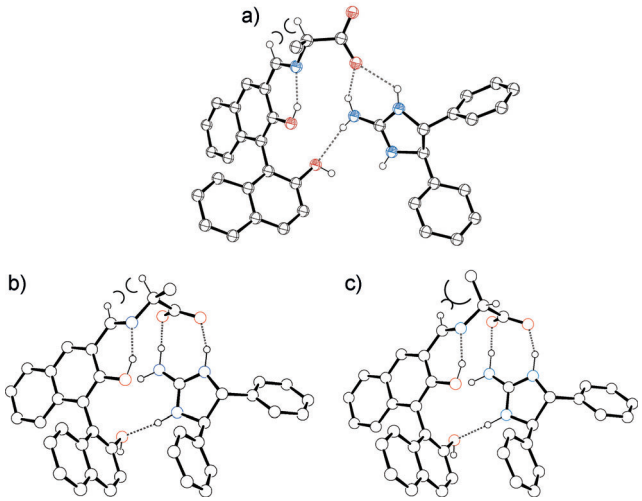
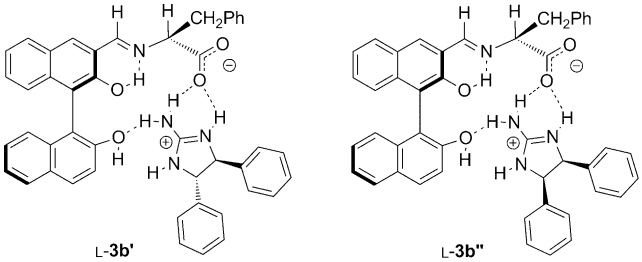


Figure 3. a) Crystal structure of one of the ternary complexes, L-**3b**. b) Computed structure of L-**3b**. c) Computed structure of D-**3b**. (The phenyl group of phenylalanine has been omitted for clarity.)

The choice of the three chiral molecules for self-assembly (Scheme 1) was initially based on computation. The global minimum structure of the ternary complex L-**3b** was obtained by molecular-mechanics calculations (Figure 3b; the phenyl group of the amino acid is omitted for clarity) and further refined by DFT computation (B3LYP at the 6-31G* level). The computed structure (Figure 3b) is in good agreement with the crystal structure (Figure 3a) and provides valuable insight into the origin of stereoselectivity in the binding of amino acids. Some discrepancies in the computed and crystal structures may be due to crystal packing. In the computed structure, both carboxylate oxygen atoms are hydrogen-bonded to one guanidinium ion (Figure 3b). In the crystal structure (Figure 3a), the carboxylate oxygen atoms bridge two guanidinium groups (Figure 1), possibly as a result of packing. In both the computed and the crystal structure, the imine formed between the amino acid and the aldehyde is stabilized by a resonance-assisted hydrogen bond (RAHB).[15] The guanidinium group forms a hydrogen-bonded bridge between the carboxylate group and one of the phenol groups (Figure 3a,b). All hydrogen atoms attached to carbon atoms have been omitted from Figures 1 and 3 for clarity, except for the imine C–H atom and the α C–H atom of the imino acid. These two hydrogen atoms are lined up in a parallel fashion, as shown in the crystal structure as well as the computed structure for L-**3b** (Figure 3a,b). In the case of D-**3b**, the imine C–H bond would be lined up with the side chain of the imino acid, thus resulting in greater steric interactions (Figure 3c). Computation showed that D-**3b** is less stable than L-**3b** by about 5 kcal mol^{-1}. This energy difference translates into a value for the equilibrium-constant ratio for the binding of L-Phe over D-Phe of about 3.7×10^3.

The crystal and computed structures show the synergistic interplay between the three chiral molecules. It is clear from these structures why the aldehyde alone or the guanidine alone cannot bind phenylalanine with a high degree of stereoselectivity. The lining up of the imine C–H and α C–H bonds is a direct result of the tight hydrogen-bond bridge that the guanidinium group provides between the carboxylate group of the amino acid and the phenol group of the aldehyde. Without this precise hydrogen-bond assembly in the ternary complex, the system would be too flexible to give much stereoselectivity. The tying together of the two ends of the iminocarboxylate by hydrogen bonding with the guanidinium moiety helps rigidify the system and dramatically increase the stereoselectivity.

The highest stereoselectivities were observed when the *R* form of the binol aldehyde **2** was used together with the *R*,*R* form of the guanidine **1** to form L-**3**. For example, when the *R* form of **2** was used together with the *S*,*S* form of **1**, the stereoselectivity for the formation of L-**3b′** (Scheme 2) was reduced to about 2.4:1. Similarly, when the *R* form of **2** was used together with the *meso* form of the guanidine, the stereoselectivity for the formation of L-**3b″** (Scheme 2) was reduced to about 5.9:1.



Scheme 2. Ternary complexes formed with the *S*,*S* form of the guanidine derivative to give L-**3b′** and with the *meso* form of the guanidine derivative to give L-**3b″**.

^{1}H NMR spectroscopic and extraction experiments showed that the sense of stereoselectivity was the same for all three guanidine forms. However, the *R*,*R* form of **1** gave the highest stereoselectivity. The crystal-packing diagram (Figure 2) shows that the imine hydrogen atom and the α hydrogen atom are in van der Waals contact with one of the adjacent phenyl groups of the guanidine moiety. This interaction may be favorable and would be absent if the *meso* or *S*,*S* form of guanidine was used. In D-**3b**, the amino acid side chain would occupy the position of the α hydrogen atom in L-**3b**. Thus, there may be more steric crowding in D-**3b** between the amino acid side chain and the phenyl group of the neighboring guanidine moiety.

The less stable ternary complex D-**3** slowly epimerized to the more stable ternary complex L-**3** at ambient temperature over the course of a day. Interestingly, this epimerization initially took place without deuterium exchange at the α position when the reaction was carried out in $CDCl_3/D_2O$. In a ^{1}H NMR spectrum recorded part way through the epimerization of the ternary complex formed with D-alanine (conversion of D-**3a** into L-**3a**; see the Supporting information), the signal for the α hydrogen atom of not only D-**3a** ($\delta = 4.71$–4.81 ppm) but also L-**3a** ($\delta = 3.98$–4.06 ppm) was clearly visible even though the experiment was carried out in $CDCl_3/D_2O$. Imine exchange with L-alanine can be ruled out, since only D-alanine was added, and then most of the D_2O layer was removed. Thus, α-proton exchange for epimeriza-

tion does not initially take place with the solvent D_2O. We propose that the epimerization takes place by a "conducted tour"[16] mechanism (see the Supporting Information). Eventually, the α position of L-**3a** is deuterated through exchange with the solvent. The supramolecular system is thus useful for the synthesis of α-deuterated amino acids with excellent stereoselectivity.

In conclusion, we have developed the first stereoselective amino acid receptor based on supramolecular self-assembly. This supramolecular receptor not only binds amino acids with remarkable stereoselectivity through cooperative interactions between different components of the assembly but also allows detailed understanding of the origin of the stereoselectivity. Both crystal (Figure 3a) and computed structures (Figure 3b,c) indicate that the origin of the stereoselectivity is in the precise positioning of two key hydrogen atoms (the imine C–H atom and the α hydrogen atom of the amino acid) in the supramolecular structure. It has been a challenge to obtain such control and understanding even with a simple system let alone a supramolecular self-assembly. Extraction and ^{1}H NMR spectroscopic experiments showed that the supramolecular system is useful for separating racemic mixtures of amino acids (Scheme 1, Figure 2) as well as for deracemizing amino acids. It is the reversibility of the supramolecular system, including imine and hydrogen-bond formation and α-proton-exchange reactions, under mild conditions at ambient temperature that makes it so effective for amino acid deracemization. On the basis of deuterium-exchange reactions, we propose that deracemization takes place by the conducted tour mechanism and that the supramolecular system is useful for the synthesis of α-deuterated amino acids in their D or L form. Besides other advantages already mentioned, the supramolecular system is ideally set up for combinatorial studies.[17] The aldehyde and guanidine components can be varied readily to increase the stereoselectivity or to study the origin of stereoselectivity for amino acid recognition and deracemization.

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