J|A|C|S
COMMUNICATIONS

Published on Web 01/11/2007

Bioinspired Chemical Inversion of L-Amino Acids to D-Amino Acids

Hyunjung Park,† Kwan Mook Kim,*,† Areum Lee,‡ Sihyun Ham,‡ Wonwoo Nam,† and Jik Chin*,§

Department of Chemistry/Division of Nano Sciences, Ewha Womans University, Seoul 120-750, South Korea, Department of Chemistry, Sookmyung Women's University, Seoul 140-742, South Korea, and Department of Chemistry, 80 St. George Street, University of Toronto, Toronto, Ontario, Canada

Received October 29, 2006; E-mail: jchin@chem.utoronto.ca; kkmook@ewha.ac.kr

L-Amino acids dominate the natural world almost to the exclusion of D-amino acids. However, some important D-amino acids[1] have been found in biology, notably as neurotransmitters (D-serine)[2] and as building blocks (D-alanine) for bacterial cell wall synthesis.[3] There has been considerable interest in understanding the mechanism of the so-called "L to D conversion" of amino acids[4] since enzymes involved in these processes are often targeted for development of therapeutic agents (e.g., antibiotics and antidepressants). In nature, L-amino acids are converted to D-amino acids by pyridoxal phosphate dependent enzymes that racemize amino acids (Scheme 1a).[4] The first step of the racemization reaction involves the formation of a special type of imines with internal resonance-assisted H-bonds (RAHB).[5] We developed a chiral analogue (**1**) of pyridoxal that binds amino acids by formation of imines with RAHBs. The strong H-bonds can be used to activate the bound amino acids and convert L-amino acids (L-aa) to D-amino acids (D-aa) by epimerization of the imines (**1**-L-aa) formed between **1** and L-aa (Scheme 1b).

Receptor **1** was prepared as previously described.[6] Stereoselective recognition of amino acids by **1** can be studied in two different ways. In the first procedure, at least 2-fold excess of a racemic mixture of amino acid is added to **1** and allowed to equilibrate by imine exchange.[6] In the second procedure, 1 equiv of an amino acid (racemic or either enantiomer) is added to **1** and the mixture is allowed to equilibrate by triethylamine catalyzed epimerization (Scheme 1b). Without the base, epimerization is not significant even after weeks.

Epimerization of the imines (**1**-L-aa) formed between **1** and L-amino acids (Scheme 1b) were monitored by ^{1}H NMR. In a typical experiment, L-serine (10 mM) and **1** (10 mM) were mixed in a DMSO$_{d6}$ solution to give the corresponding imine (**1**-L-Ser) within minutes. Triethylamine (50 mM) was then added to the reaction mixture and the epimerization reaction was monitored for 2 days. Figure 1 shows the time dependent change in the ^{1}H NMR spectrum due to the epimerization of three imines ((a) **1**-L-Ser to **1**-D-Ser; (b) **1**-L-Ala to **1**-D-Ala, (c) **1**-L-His to **1**-D-His).

In all cases (Figure 1), the signals due to the two urea N−H and the imine C−H can be conveniently monitored free from other signals. A clear pattern in the ^{1}H NMR for the epimerization reaction emerges regardless of the amino acid used. The two urea N−H signals shift dramatically downfield and the imine C−H signal shifts upfield with the epimerization reaction. Figure 1 shows that the reactions are remarkably clean and complete for all amino acids. The epimerization reaction takes place by proton exchange at the α position of the amino acid as evidenced by deuteration at this position (solvent: 90/10 v/v DMSO-d_6/D_2O). (In principle, epimerization could also take place by rotation about the single bond connecting the two naphthyl groups of the receptor. This is unlikely since the energy barrier to such a rotation is expected to be high.[7] Epimerization by bond rotation or imine exchange would not be accompanied by deuteration).

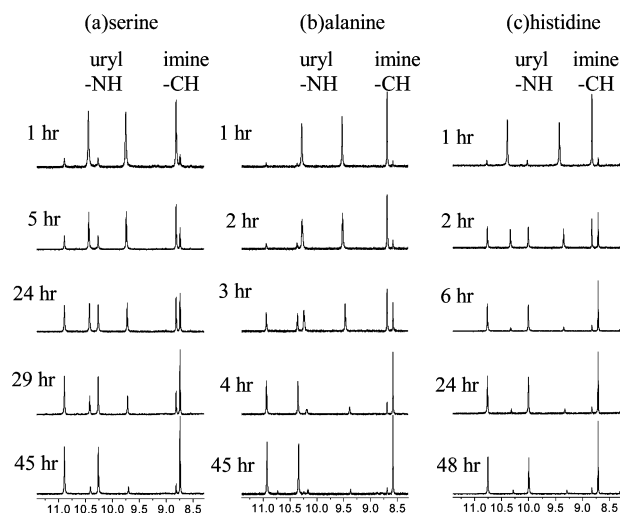
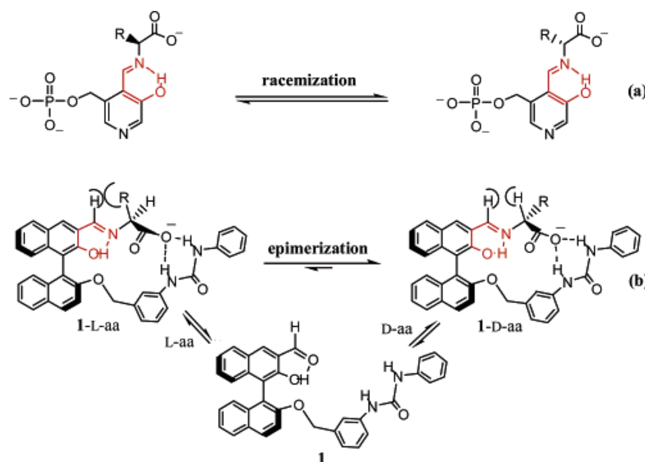


Figure 1. Partial ^{1}H NMR spectra showing the epimerization of (a) **1**-L-Ser to **1**-D-Ser, (b) **1**-L-Ala to **1**-D-Ala, and (c) **1**-L-His to **1**-D-His.

Scheme 1



The ratios of [**1**-D-aa] to [**1**-L-aa] at equilibrium (Scheme 1b) for thirteen different amino acids are listed in Table 1 (Figures S1−S13, Supporting Information). It is clear from the table that the receptor binds all amino acids with the same sense of stereoselectivity. Although the sense of the receptor stereoselectivity for binding α-amino acids is the same as that for it binding 1,2-amino alcohols,[6] the magnitude of the receptor stereoselectivity is considerably greater for binding α-amino acids (>10:1) than for binding 1,2-amino alcohols (∼4:1). The stronger H-bonding

† Ewha Womans University.
‡ Sookmyung Women's University.
§ University of Toronto.

10.1021/ja067724g CCC: $37.00 © 2007 American Chemical Society

Table 1. The Ratios of [**1**-D-aa] to [**1**-L-aa] at Equilibrium

amino acids	D/L ratio	amino acids	D/L ratio
threonine	20/1	methionine	11/1
glutamine	15/1	glutamic acid	11/1
histidine	14/1	serine	11/1
arginine	14/1	leucine	9/1
asparagine	13/1	tryptophan	8/1
tyrosine	12/1	alanine	7/1
phenylalanine	11/1		

between the urea group and the carboxylate group over the H-bonding between the urea group and the alcohol group may contribute to the greater receptor streoselectivity for binding α-amino acids than for binding 1,2-amino alcohols.

The stereoselectivity of **1** for binding a wide range of α-amino acids is remarkably high for such a simple organic receptor. Amino acids with basic (His) and acidic (Glu) side chains are tolerated as well as those with hydrophobic (Tyr, Trp, Phe, Leu, Ala) and hydrophilic (Ser, Thr, Gln, Arg, Asn, Met) side chains.[8] It is likely that the stereoselectivity is the highest for threonine because of the steric bulk next to the α-carbon (see **1**-L-aa of Scheme 1). Furthermore, the hydroxyl side chain of threonine is electron withdrawing and makes the α C−H more acidic for facile epimerization. Over the years, there has been much interest in developing organic,[9] biological,[10] polymeric,[11] and metal based[12] α-amino acid receptors. To our knowledge, **1** represents the most stereoselective small molecule organic receptor for binding a wide range of underivatized amino acids. In addition, it is the first receptor for deracemization of amino acids as shown below.

The imines (**1**-D-aa) formed between **1** and L-amino acids (Table 1) may be hydrolyzed under acidic conditions to yield the corresponding amino acids and the free receptor (**1**) once the epimerization reaction (Scheme 1b) has reached equilibrium.[13] The receptor (**1**) can be recycled repeatedly (alternating base-catalyzed imine epimerization and acid-catalyzed imine hydrolysis) without significant loss in yield or purity (Figure S14) to produce more D-amino acids from L-amino acids in excellent yields. The enantiopurity of the isolated amino acids were determined by ^{1}H NMR using the receptor (**1**) as the chiral shift reagent.[14] These experiments show that the enantiopurity of the isolated amino acids match the diasteriomeric ratios of the imines before hydrolysis (Table 1). Similarly, a one-pot chemical deracemization of amino acids can be accomplished by replacing L-amino acids in the above process with racemic ones. There has been much interest in deracemization of amino acids as a method for large-scale production of D or L amino acids. A chemoenzymatic dynamic kinetic resolution process has recently been developed for deracemization of amino acids.[15]

To gain some insight into the origin of stereoselectivity for the binding of α-amino acids to **1**, DFT computations were carried out for **1**-L-ala and **1**-D-ala (Figure S15).[16] Computation shows that **1**-D-Ala is more stable than **1**-L-Ala by about 1.1 kcal/mol in qualitative agreement with the experimental results (Table 1; 7/1 translates to 1.2 kcal/mol). In **1**-L-Ala, there appears to be considerable steric hindrance between the methyl group of the alanine group and the imine C−H of the receptor (Scheme 1b and Figure S15). In contrast, there is less steric hindrance between the α-proton of alanine group and the imine C−H of the receptor in **1**-D-Ala. The weaker steric interactions in **1**-D-Ala should result in stronger H-bonds to form between the two urea N−Hs and the carboxylate group (Scheme 1b). The ^{1}H NMR signals of the more strongly H-bonded protons should be more downfield shifted[17] as observed (see urea N−H signals in Figure 1).

Many interesting models of pyridoxamine including chiral ones have been developed to convert α-ketoacids to α-amino acids.[18] Receptor **1** may be considered a chiral model of pyridoxal. A rational design has led to the development of a general receptor (**1**) that binds a wide range of amino acids with remarkably good and even predictable stereoselectivity. Unlike other amino acid receptors, **1** can be used for deracemization of amino acids by epimerization of the imine intermediates that are activated by RAHBs.

Acknowledgment. This research was supported by the Ministry of Science and Technology of Korea (NRL M1060000269-06-J0000-26910), SRC programs of MOST/KOSEF (R11-2005-008-00000-0 and the Research Center for Women's Diseases), and by the Natural Sciences and Engineering Research Council of Canada.

Supporting Information Available: Experimental details and ^{1}H NMR spectra for the conversion of L-amino acids to D-amino acids. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kreil, G. *Science* **1994**, *266*, 996−997.(b) Corrigan, J. J. *Science* **1969**, *164*, 142−149.
(2) (a) Wolosker, H.; Blackshaw, S.; Snyder, S. H. *Proc. Nat. Acad. Sci. U.S.A.* **1999**, *96*, 13409−13414. (b) Wickelgren, I. *Science* **1999**, *286*, 1265−1266. (c) Fujii, K.; Maedal, K.; Hikida, T.; Mustafa, A. K.; Balkissoon, R.; Xia, J.; Yamada, T.; Ozekil, Y.; Kawahara, R.; Okawa, M.; Huganir, R. L.; Ujike, H.; Snyder, S. H.; Sawa, A. *Mol. Psychiatry* **2006**, *11*, 150−157.
(3) Stryer, L. *Biochemistry*, 4th ed.; W. H. Freeman and Co.: New York, 2000; pp 201−202.
(4) (a) Shaw, J. P.; Petsko, G. A. Ringe, D. *Biochemistry* **1997**, *36*, 1329−1342. (b) Walsh, C. T. *J. Biol. Chem.* **1989**, *264*, 2393−2396.
(5) (a) Gilli, P.; Bertolasi, V.; Ferretti, V.; Gilli, G. *J. Am. Chem. Soc.* **2000**, *122*, 10405−10417. (b) Kim, H.-J.; Kim, W.; Lough, A. J.; Kim, B. M.; Chin, J. *J. Am. Chem. Soc.* **2005**, *127*, 16776−16777. (c) Kim, H.-J.; Kim, H.; Alhakimi, G.; Jeong, E. J.; Thavarajah, N.; Studnicki, L.; Koprianiuk, A.; Lough, A. J.; Suh, J.; Chin, J. *J. Am. Chem. Soc.* **2005**, *127*, 16370−16371.
(6) Kim, K. M.; Park, H.; Kim, H.-J.; Chin, J.; Nam, W. *Org. Lett.* **2005**, *7*, 3525−3527.
(7) Meca, L.; Reha, D.; Havlas, Z. *J. Org. Chem.* **2003**, *68*, 5677−5680.
(8) Some limitations for the other six amino acids are given in the Supporting Information.
(9) (a) Newcomb, M.; Toner, J. L.; Helgeson, R. C.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 4941−4947. (b) Zhang, X. X.; Bradshaw, J. S.; Izatt, R. M. *Chem. Rev.* **1997**, *97*, 3313−3361. (c) Breccia, P.; Van Gool, M.; Perez-Fernandez, R.; Martin-Santamaria, S.; Gago, F.; Prados, P.; Mendoza, J. *J. Am. Chem. Soc.* **2003**, *125*, 8270−8284. (d) Oliva, A. I.; Simon, L.; Hernandez, J. V.; Muniz, F. M.; Lithgow, A.; Jimenez, A.; Moran, J. R. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1050−1052. (e) Pazicky, M.; Gaspar, B.; Solladie-Cavallo, A.; Salisova, M.; Bohac, A.; Hutta, M.; Addova, G. *Synthesis* **2006**, *12*, 2013−2018.
(10) (a) Famulok, M. *Science* **1996**, *272*, 1343−1346. (b) Hofstetter, O.; Hofstetter, H.; Schurig, V.; Wilchek, M.; Green, B. S. *J. Am. Chem. Soc.* **1998**, *120*, 3251−3252.
(11) (a) Osawa, T.; Shirasaka, K.; Matsui, T.; Yoshihara, S.; Akiyama, T.; Hishiya, T.; Asanuma, H.; Komiyama, M. *Macromolecules* **2006**, *39*, 2460−2466. (b) Okuno, H.; Kitano, T.; Yakabe, H.; Kishimoto, M.; Deore, B. A.; Siigi, H.; Nagaoka, T. *Anal. Chem.* **2002**, *74*, 4184−4190.
(12) (a) Job, R. C.; Bruice, T. *J. Am. Chem. Soc.* **1974**, *96*, 809−819. (b) Chin, J.; Lee, S. S.; Lee, K. J.; Park, S.; Kim, D. H. *Nature* **1999**, *401*, 254−257. (c) Reeve, T. B.; Cros, J.-P.; Gennari, C.; Piarulli, U.; Vries, J. G. *Angew. Chem., Int. Ed.* **2006**, *118*, 2509−2513.
(13) See Supporting Information.
(14) Chin, J.; Kim, D. C.; Kim, H.-J.; Panosyan, F. B.; Kim, K. M. *Org. Lett.* **2004**, *6*, 2591−2593.
(15) Turner, N. J. *Curr. Opin. Chem. Biol.* **2004**, *8*, 114−119.
(16) Gaussian 03 was used for the geometry optimizations (DFT at the B3LYP/6-31* level). (a) Kohn, W.; Sham, L. J. *Phys. Rev.* **1965**, *140*, A1133−1138. (b) Gill, P. M. W.; Johnson, B. G.; Pople, J. A. *Chem. Phys. Lett.* **1993**, *209*, 506−512. (c) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652. (d) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B: Condens. Matter Mater. Phys.* **1988**, *37*, 785−789.
(17) Kumar, G. A.; McAllister, M. A. *J. Org. Chem.* **1998**, *63*, 6968−8972.
(18) (a) Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146−153. (b) Liu, L.; Zhou, W.; Chruma, J.; Breslow, R. *J. Am. Chem. Soc*. **2004**, *126*, 8136−8137.

JA067724G