Chirality Sensors

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# Highly Stereospecific Generation of Helical Chirality by Imprinting with Amino Acids: A Universal Sensor for Amino Acid Enantiopurity**

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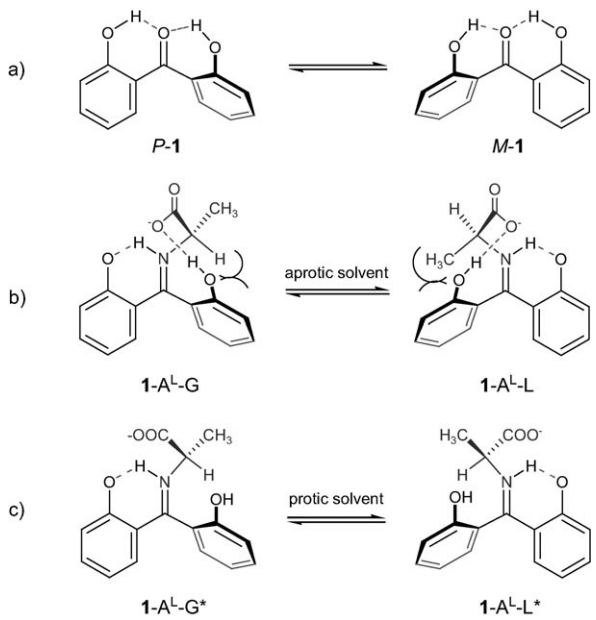
Generation of helical and axial chirality has been the topic of much interest in biology and chemistry. Controlling axial chirality with stereogenic-center-based chirality has shown to be useful for determining the absolute configurations or enantiopurities of chiral acids,[1] alcohols,[2] and natural[3] or unnatural amino acids,[4] and also for stereoselective synthesis of unnatural amino acids.[5] However, it has been a challenge to control axial chirality from stereogenic-center-based chirality with a high degree of stereospecificity. In biology, amino acid chirality (the L form) is used for the stereospecific generation of helical chirality (for example, the right-handed α helix of proteins). The energy difference between the right- and left-handed α-helical peptides[6] is small per amino acid residue,[7] and more than twenty L amino acids are required to favor the right-handed α helix. Finding the minimal structural requirement of a receptor for generating helical chirality from amino acid chirality may provide interesting insights into stereospecific folding[8] and stereoselective recognition[9] of molecules. Herein we report how helical chirality can be imprinted onto 2,2′-dihydroxybenzophenone (**1**) in a highly stereospecific manner with a single amino acid (Scheme 1). A signaling group can also be attached to the receptor **2** for general sensing of amino acid enantiopurity.

Compound **1** is readily available commercially. It has axial or helical chirality and exists as an equal mixture of rapidly interconverting *P* and *M* forms (*P*-**1** and *M*-**1** in Scheme 1 a). Receptor **1** resembles [4]helicene[10] in that they are both helical with four consecutive six-membered rings (including hydrogen bonds in **1**). Amines react with **1** to form imines within minutes at ambient temperatures (Scheme 1 b). Under the same conditions, it takes weeks to form imines with benzophenone. Thus the two hydroxy groups in **1** greatly activate the carbonyl group towards nucleophilic attack through double H bonding. If the tetramethylammonium salt of alanine (0.1M) is added to a solution of **1** (0.1M) in a protic solvent, such as $CD_3OD$ (Scheme 1 c), two sets of signals are detected in the $^1H$ NMR spectrum (Figure 1 a). The two compounds in Scheme 1 c are diastereomeric and are expected to give distinct NMR signals. However, if an aprotic solvent, such as $CD_3CN$ is used (Scheme 1 b), a remarkably clean $^1H$ NMR spectrum results with just one set of signals (Figure 1 b).

Computation can be used to help explain the high stereospecificity for imine formation in an aprotic solvent such as $CD_3CN$. Density functional theory computation (DFT; B3LYP at the 6-31G* level)[11] shows that the global minimum-energy structure of the imine **1**-$A^L$-G formed between anionic L-alanine and **1** has two internal H bonds (Figure 2 a). One internal H bond in **1**-$A^L$-G is a resonance-




***Scheme 1.*** Generating helical chirality in 2,2′-dihydroxybenzophenone **1** with a single amino acid. $A^L$ = L-alanine, G = global minimum, L = local minimum. See text for details.

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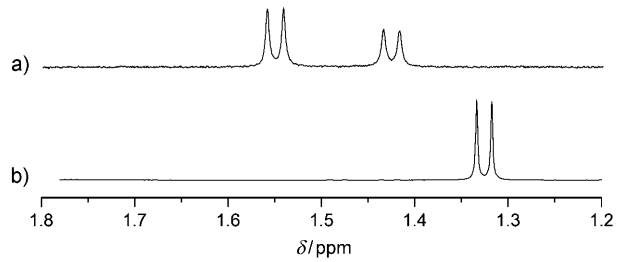


***Figure 1.*** The doublet signals in the $^1$H NMR spectrum of the alanine methyl group upon formation of imine(s) with **1** in a) $CD_3OD$ and b) $CD_3CN$.

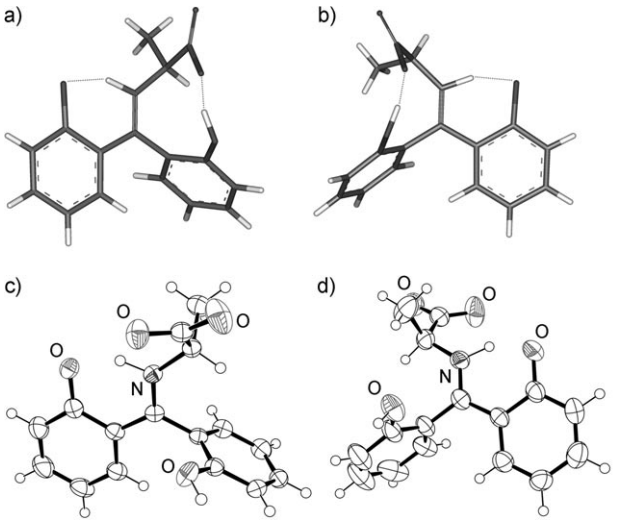


***Figure 2.*** a), b) Computed structures of **1**-A$^L$-G (a) and **1**-A$^L$-L (b). c), d) X-ray crystal structures of **1**-A$^L$-G* (c) and **1**-A$^L$-L* (d). The structures in (a), (c) have helical chirality in the *P* form; those in (b), (d) in the *M* form.

assisted hydrogen bond (RAHB)[12,13] between the protonated imine and the phenolate oxyanion. The other internal H bond in **1**-A$^L$-G is between the alanine carboxylate anion and the remaining phenolic hydrogen atom. Such charged H bonds are generally stronger than neutral H bonds.[13] The $^1$H NMR signals for the two H bonds determined in DMSO are shifted far downfield and are independent of concentration (Supporting information, Figure S1), as would be expected for strong intramolecular H bonds.[14] Comparing the structure of **1**-A$^L$-G and the structure of the receptor **1**, it is evident that L-alanine generates axial chirality in the *P* form upon formation of the imine.

The second most stable minimum energy structure of the imine formed between anionic L-alanine and **1** is **1**-A$^L$-L (Scheme 1b and Figure 2b). This local minimum energy structure also has the two strong internal H bonds and is closely related to the structure of **1**-A$^L$-G. However, a comparison of the structures of the imine complexes and the receptor (Scheme 1a,b), shows that **1**-A$^L$-L is in the *M* form whereas **1**-A$^L$-G is in the *P* form. It is likely that **1**-A$^L$-G and **1**-A$^L$-L are in equilibrium through rotation of the bond connecting the imine to one of the phenols. DFT computation shows that **1**-A$^L$-G is more stable than **1**-A$^L$-L by about 5.2 kcal mol$^{-1}$. It is interesting that the energy difference between **1**-A$^L$-G and **1**-A$^L$-L is so large for such closely related and simple diastereomers with just two H bonds. This energy difference translates to an equilibrium ratio of about $5.5 \times 10^3$ for [**1**-A$^L$-G]/[**1**-A$^L$-L] at 25 °C. Thus if both internal H bonds are maintained in aprotic solvents, only one imine should form to any observable extent, as confirmed by $^1$H NMR spectroscopy (Figure 1b).

It is apparent from the computed structures that **1**-A$^L$-G is more stable than **1**-A$^L$-L owing to the relative positioning of the alanine methyl groups (Scheme 1b). The methyl group in **1**-A$^L$-G is positioned in an unhindered area, whereas the methyl group in **1**-A$^L$-L is in a sterically crowded area close to one of the phenol groups. Thus L-alanine generates axial chirality in the *P* form stereospecifically upon formation of the imine complex in aprotic solvents (i.e., the equilibrium in Scheme 1b favors **1**-A$^L$-G over **1**-A$^L$-L).

Protic solvents appear to disrupt the charged internal hydrogen bonds in **1**-A$^L$-G and **1**-A$^L$-L to give **1**-A$^L$-G* and **1**-A$^L$-L*, respectively (Scheme 1c). Indeed, crystal structures of **1**-A$^L$-G*[15] and **1**-A$^L$-L*[16] reveal that the internal charged H bonds can be broken while maintaining the resonance-assisted H bonds (Figure 2c and d). It appears that the weaker intramolecular H bond breaks to form intermolecular H bonds at the high concentrations required for crystallization. Computation shows that **1**-A$^L$-G* and **1**-A$^L$-L* are of comparable energy, which is in agreement with the integration ratio of the two doublet signals in the $^1$H NMR spectrum (Figure 1a). Apart from using protic solvents, a base can be used in aprotic solvents to eliminate the stereospecificity by breaking the charged H bond. Amino acid esters cannot form the charged H bond and do not control the helical chirality (see Supporting Information).

Helical contents of protein molecules are often measured with circular dichroism (CD) spectroscopy.[17] The CD signals are weak for amino acids bound to receptor **1**. We therefore covalently attached signal-amplifying diazo functional groups to the receptor **2** (Figure 3b). Figure 3a shows the CD and UV spectra of the imines **2**-A$^L$-G and **2**-A$^D$-G formed between L-alanine and **2** and between D-alanine and **2**, respectively.

It is evident from Scheme 1 and the computational studies (Figure 2a,b) that other natural or unnatural α-amino acids should behave similarly to alanine. Indeed, a wide variety of amino acids, including asparagine, phenylalanine, serine, and valine, all give one imine diastereomer each with **1** or **2** in aprotic solvents, as shown by $^1$H NMR spectroscopy (see Supporting Information). Computation shows that the stereospecificity increases with increase in the size of the amino acid side chain. Thus, alanine is expected to give the lowest stereospecificity (5500:1). Such a high degree of stereospecificity for generating helical chirality from stereogenic-center-based chirality has been observed with large biomolecules, such as proteins and nucleic acids, but not with small molecules. CD spectra of the imines formed with different L-amino acids not only have the same bisignate sign (*P* helicity) but they are also remarkably close in intensity (Table 1). If the CDs were identical for different amino acids,

  

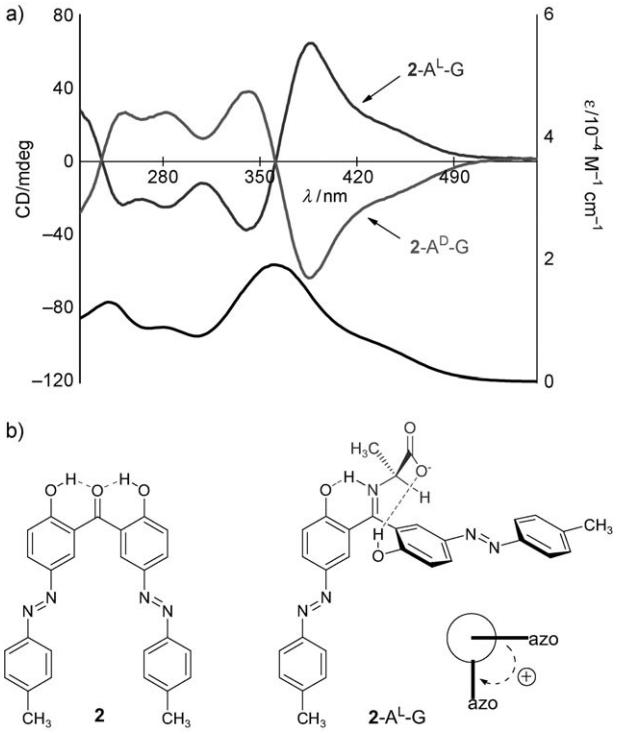


***Figure 3.*** a) Circular dichroism spectra of **2**-A$^{L}$-G and **2**-A$^{D}$-G (50 μM in acetonitrile, 1 cm cell, at 25 °C) and their UV/Vis spectra. b) Structure of **2** and **2**-A$^{L}$-G.

***Table 1:*** CD data for imines formed from amino acids and **2** in acetonitrile.

| Amino acid | UV/Vis[a] | | CD[a] | | Ratio [mdeg$_{max}$/$A_{max}$] |
|---|---|---|---|---|---|
| | $A_{max}$[b] | $\lambda_{max}$ [nm] | mdeg$_{max}$ | IP [nm][c] | |
| L-Ala | 1.84 | 360 | 64.5 | 361 | 35.0 |
| L-Asn | 1.84 | 355 | 67.8 | 355 | 36.8 |
| L-Gln | 2.06 | 363 | 77.4 | 357 | 37.6 |
| L-Ile | 2.02 | 363 | 66.2 | 363 | 32.8 |
| L-Leu | 2.11 | 363 | 69.6 | 362 | 33.0 |
| L-Met | 2.12 | 360 | 74.0 | 359 | 34.9 |
| L-Phe | 2.00 | 358 | 67.6 | 358 | 33.8 |
| L-Ser | 1.88 | 354 | 68.3 | 353 | 36.3 |
| L-Thr | 2.08 | 355 | 76.4 | 353 | 36.7 |
| L-Trp | 1.88 | 361 | 57.2 | 362 | 30.4 |
| L-Tyr | 1.96 | 360 | 66.0 | 359 | 33.7 |
| L-Val | 1.83 | 360 | 60.7 | 361 | 33.2 |

[a] 50 μM in acetonitrile, 1 cm cell, at 25 °C. [b] $\varepsilon/10^{-4}\,M^{-1}\,cm^{-1}$. [c] Isosbetic point.

a universal chirality sensor for all amino acids could be developed.

The uniform bisignate sign in the CD spectrum for different amino acids with the same configuration (Table 1) can be rationalized according to the exciton chirality method.[17,18a] A bisignate sign owing to exciton coupling of the two hydroxyphenyl rings in **2**-A$^{L}$-G is observed around its UV maximum at 360 nm (Figure 3a). The positive Cotton effect at 380 nm followed by the negative Cotton effect at 340 nm is in agreement with the computed structure (*P* helical chirality) of **2**-A$^{L}$-G (Figure 3b). Similarly, the negative Cotton effect at 380 nm followed by the positive Cotton effect at 340 nm is in agreement with the structure of **2**-A$^{D}$-G (*M* helical chirality) formed with D-alanine. This empirical approach has been used for determining the absolute configuration of diols,[18a] diamines,[18b] biphenyls,[18c] binols,[18d] and helicenes[18e] (Supporting Information).

Our CD and $^{1}$H NMR spectroscopy results indicate that different α-amino acids form imines with **2** and generate helical chirality not only with the same sense but also with a high degree of stereospecificity. Thus, **2** is an excellent chirality sensor for amino acids. There is excellent correlation between CD absorption at 360 nm and enantiomeric excess of valine in the imine complex formed with **2** (see Supporting Information). The crystal structure of the imine formed between L-valine and **2** is analogous to that of **1**-A$^{L}$-G* (Figure 2c). Although a variety of interesting chirality sensors have been developed for amino acids,[19,20] none to date have been shown to interact with a high degree of stereospecificity. Previously reported sensors give different CD signals for different amino acids. In contrast, CD spectra obtained from the reactions of **2** and different amino acids are remarkably close (Table 1).

Generation of helical chirality with unprecedented stereospecificity has been achieved by imprinting of amino acid chirality[21] onto a small molecule receptor (**1**). The control of the helical chirality is accomplished by an imine and two H bonds between underivatized amino acids and the receptor. The excellent agreement between DFT computational and experimental data, including CD and $^{1}$H NMR spectrscopy, and X-ray crystallography, provides valuable insight into the origin of the stereospecificity. Furthermore, a universal chirality sensor **2** for amino acids has been developed by attaching a CD signal-amplifying group to **1**.

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[15] Crystal was grown in $CH_3NO_2$. Crystal data of **1**-$A^L$-G*: $C_{20.5}H_{27.5}N_{2.5}O_5$, $T = 150(2)$ K, $C2/c$, $Z = 8$, $a = 37.757(3)$, $b = 7.4155(3)$, $c = 16.4442(12)$ Å, $\beta = 115.008(3)°$, $V = 4172.5(5)$ Å$^3$, $R_1 = 0.0580$, $wR_2 = 0.1234$ for $I > 2\sigma(I)$, GOF on $F^2 = 1.015$. At high concentrations required for crystallization, intermolecular charged H bonds appear to be favored over the intramolecular H bonds.
[16] Crystal was grown in $CH_3OH$. Crystal data of **1**-$A^L$-L*: $C_{20}H_{26}N_2O_4$, $T = 150(2)$ K, $P2_1/n$, $Z = 4$, $a = 11.7534(5)$, $b = 8.2201(5)$, $c = 23.1214(10)$ Å, $\beta = 98.134(3)°$, $V = 2211.38(19)$ Å$^3$, $R_1 = 0.0829$, $wR_2 = 0.2389$ for $I > 2\sigma(I)$, GOF on $F^2 = 1.122$. Crystal structures of imines formed with serine and with phenylalanine have also been determined (see Supporting Information).
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