An NMR Chiral Solvating Agent for the Chiral Recognition of the Two Enantiomers of *N*-(3,5-Dinitrobenzoyl)-α-amino Acids

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Received May 22, 2007

Key Words : Chiral recognition, NMR chiral solvating agent, *N*-(3,5-Dinitrobenzoyl)-α-amino acids, *trans*-1,2-Diaminocyclohexane

Two enantiomers of chiral drugs often show different pharmaceutical activities.[1] Consequently, the study on the stereochemical dependence of the pharmacokinetics and the pharmaceutical activities of chiral drugs is important. Determination of the enantiomeric composition of chiral drugs is, therefore, essential. Enantiomeric composition of chiral compounds has been determined most widely by high-performance liquid chromatographic chiral separations on chiral stationary phases (CSPs).[2] Another important method of determining the enantiomeric composition of chiral compounds is the NMR techniques utilizing chiral solvating agent (CSA).[3] The two transient diastereomeric adducts formed between a CSA and each of the two enantiomers of chiral analytes might induce anisochronous NMR resonances and consequently the enantiomeric composition of chiral analytes can be easily assessed from the NMR spectrum.

Most CSAs, which have been developed and successfully utilized in the determination of the enantiomeric composition of chiral compounds by NMR spectroscopy, contain aromatic functional group(s) to induce magnetic anisotropic influence and to invoke π-π interaction with analytes.[4] Derivatives of optically active *trans*-1,2-diaminocyclohexane has also been utilized as NMR CSAs and these CSAs have also been designed to contain aromatic functional group(s) in order to induce magnetic anisotropic influence and to invoke π-π interaction with analytes. Consequently, CSAs derived from optically active *trans*-1,2-diaminocyclohexane have been utilized for the determination of enantiomeric composition of chiral compounds containing aromatic functional group(s). For example, CSA **1** and CSA **2** derived from (1*R*,2*R*)-1,2-diaminocyclohexane shown in Figure 1 have been successfully applied to the determination of enantiomeric composition of chiral carboxylic acids by NMR spectroscopy.[5]

Recently, we found that a liquid chromatographic chiral stationary phase (CSP **3**, Figure 1) derived from optically active (1*S*,2*S*)-1,2-diaminocyclohexane is quite successful for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids even though the CSP does not contain any aromatic functional group, but contains only a simple primary amino group and a ureide tethering group.[6] From these results, we inferred that CSA **4**, which has the same structure as the chiral selector of CSP **3**, might show nonequivalences for the two enantiomers of *N*-(3,5-dinitrobenzoyl)-α-amino acids by NMR spectroscopy.

CSA **4** was prepared simply by treating (1*S*,2*S*)-1,2-diaminocyclohexane with ethyl isocyanate. CSA **4** thus prepared was used to see the ^{1}H NMR chemical shift nonequivalence of the two enantiomers of *N*-(3,5-dinitrobenzoyl)phenylglycine. As shown in Figure 2, the chemical shift nonequivalence of the two enantiomers of *N*-(3,5-dinitrobenzoyl)phenylglycine was observed at the NMR peaks corresponding to the protons on the 3,5-dinitrophenyl ring (denoted by a in Figure 1). In addition the chemical shift nonequivalence of the two enantiomers of *N*-(3,5-dinitrobenzoyl)phenylglycine was observed to be greatest when the ratio of CSA **4** and *N*-(3,5-dinitrobenzoyl)phenylglycine is 2:1 at ambient temperature (22 ± 0.5 ^{o}C).

The ^{1}H NMR experimental results for the chemical shift nonequivalences of the two enantiomers of five *N*-(3,5-dinitrobenzoyl)-α-amino acids in the presence of two equivalents of CSA **4** are summarized in Table 1. As shown

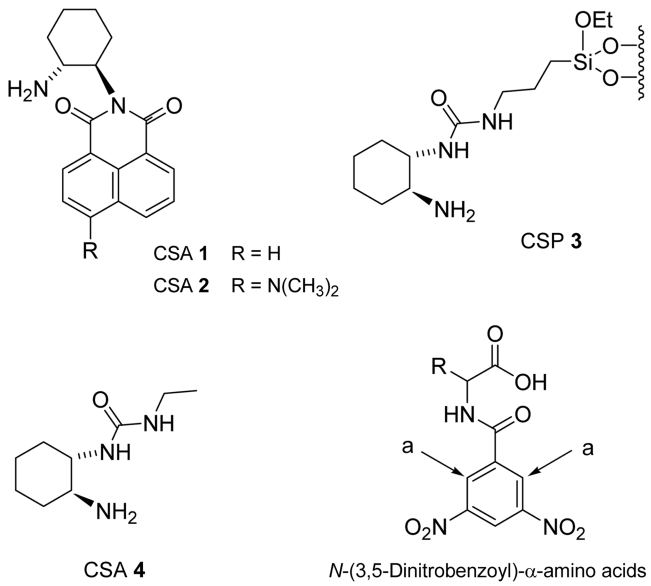

Figure 1. Structures of CSA **1**, CSA **2**, CSP **3**, CSA **4** and *N*-(3,5-dinitrobenzoyl)-α-amino acids. Labels a on the 3,5-dinitrophenyl ring of *N*-(3,5-dinitrobenzoyl)-α-amino acids correspond to the protons which show ^{1}H NMR chemical shift nonequvalences in the presence of CSA **4**.

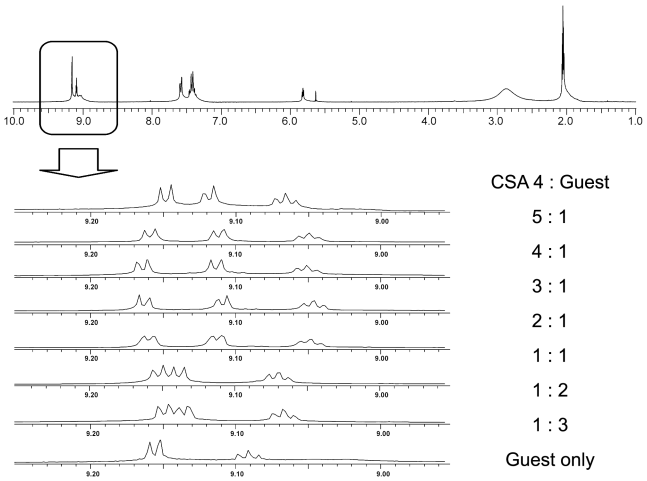

Figure 2. ^{1}H NMR spectra for the mixture of CSA **4** and racemic *N*-(3,5-dinitrobenzoyl)phenylgylcine in acetone-d_6 at ambient temperature (22 ± 0.5 °C). The two protons on the 3,5-dinitrophenyl ring of *N*-(3,5-dinitrobenzoyl)phenylgylcine (the two protons are indicated as a in Figure 1, R = Phenyl) show different chemical shifts for the two enantiomers and the spectral regions corresponding to these two protons are expanded. The chemical shift nonequivalence was observed with the variation of the ratio of CSP **4** and analyte from 1:3 to 5:1 [total mole numbers of CSP **4** and analyte were kept at $(21.0 \pm 1.0) \times 10^{-5}$ mole in 0.6 mL acetone-d_6].

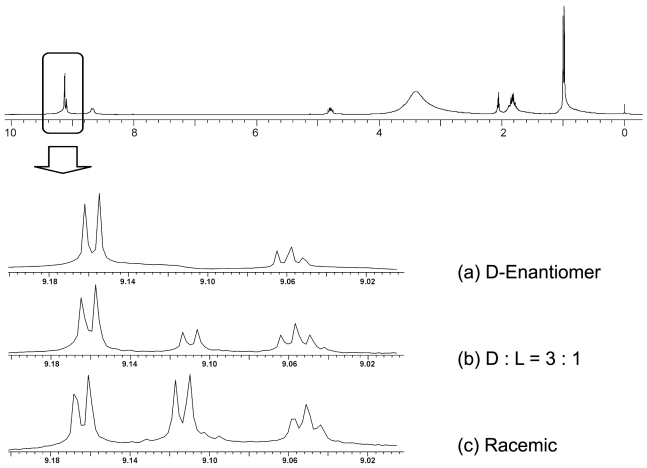

Figure 3. ^{1}H NMR spectral regions corresponding to the protons on 3,5-dinitrophenyl ring of *N*-(3,5-dinitrobenzoyl)leucine: (a) pure (D)-enantiomer (b) 3:1 mixture of (D)- and (L)-enantiomer and (c) racemic mixture in the presence of CSA **4**. CSA **4**: 14.6×10^{-5} mole, *N*-(3,5-Dinitrobenzoyl)leucine: 7.2×10^{-5} mole in 0.6 mL acetone-d_6.

in Table 1, the (D)-enantiomers always show larger chemical shift than the (L)-enantiomers, indicating that the chiral recognition mode is identical in every case. An ionic or ion-pairing interaction between the free primary amino group of the CSA and the carboxylic acid group of the analytes seems to play an important role for the chiral recognition. In addition, a hydrogen bonding interaction between the nitro group of the 3,5-dinitrophenyl ring of the analytes and the ureide N-H hydrogen of the CSA might play a role for the chiral recognition. However, the exact chiral recognition mode needs further study.

The chemical shift non-equivalences ($\Delta\Delta\delta$) for the two enantiomers of *N*-(3,5-dinitrobenzoyl)-α-amino acids are always greater than 0.037 ppm as shown in Table 1. Consequently, determination of the enantiomeric composition of *N*-(3,5-dinitrobenzoyl)-α-amino acids is expected to be possible by comparing integration values of the peaks corresponding to the two enantiomers. Figure 3 shows the comparison of the ^{1}H NMR spectra for (a) pure (D)-enantiomer, (b) 3:1 mixture of (D)- and (L)-enantiomer and (c) racemic mixture of *N*-(3,5-dinitrobenzoyl)leucine in the presence of two equivalents of CSA **4**. The relative peak intensities corresponding to the two enantiomers are exactly consistent with the ratio of the two enantiomers present. From these results, it is concluded that CSP **4** can be successfully utilized as an NMR chiral solvating agent for the determination of the enantiomeric composition of *N*-(3,5-dinitrobenzoyl)-α-amino acids even though the CSA does not contain any aromatic functional group.

Table 1. ^{1}H NMR chemical shift nonequivalences of the two enantiomers of *N*-(3,5-dinitrobenzoyl)-α-amino acids induced by CSA **4**[a]

R	$\Delta\delta$(D) ppm[b]	$\Delta\delta$(L) ppm[c]	$\Delta\Delta\delta$[$\Delta\delta$(D) - $\Delta\delta$(L)] ppm
CH_3 (alanine)	9.182	9.130	0.052
$(CH_3)_2CH$ (valine)	9.163	9.122	0.041
$(CH_3)_2CHCH_2$ (leucine)	9.182	9.141	0.041
C_6H_5 (phenylglycine)	9.160	9.112	0.048
$CH_3(OH)CH$ (threonine)	9.159	9.122	0.037

[a]Chemical shift nonequivalences for the protons indicated as a in Figure 1 on the 3,5-dinitrophenyl ring of *N*-(3,5-dinitrobenzoyl)-α-amino acids. CSA **4**: 18.4×10^{-5} mole. *N*-(3,5-Dinitrobenzoyl)-α-amino acid: 9.2×10^{-5} mole in 0.6 mL acetone-d_6. [b]Chemical shifts of protons a of (D)-enantiomers. [c]Chemical shifts of protons a of (L)-enantiomers.

In summary, CSA **4** was quite efficient as an NMR chiral solvating agent for the determination of the enantiomeric composition of *N*-(3,5-dinitrobenzoyl)-α-amino acids even though the CSA does contain only simple a free primary amino group and a ureide group. The ^{1}H NMR chemical shift non-equivalences for the protons on the 3,5-dinitrophenyl ring of *N*-(3,5-dinitrobenzoyl)-α-amino acids were always greater than 0.037 ppm and these were large enough to be utilized for the assessment of the enantiomeric composition. Consequently, it is concluded that CSP **4** can be successfully utilized as an NMR chiral solvating agent for the determination of the enantiomeric composition of *N*-(3,5-dinitrobenzoyl)-α-amino acids.

Experimental

General. All ^{1}H NMR spectra were taken on a Varian Mercury 300 spectrometer (300 MHz) at ambient temperature (22 ± 0.5 °C). For the measurement of chemical shift non-equivalences of the two enantiomers of *N*-(3,5-dinitrobenzoyl)-α-amino acids, the sample solutions were prepared by dissolving CSA **4** (18.4×10^{-5} mole) and analyte ($9.2 \times$

10^{-5} mole) in 0.6 mL of acetone-d_6. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. IR spectrum was measured with a Jasco FT/IR-300E. Melting point was taken on an Electrothermal Capillary Melting Point Apparatus and reported without correction. Optical rotation was measured on a Rudolph Research Analytical AUTOPOL IV Polarimeter (Flanders, NJ, USA).

Preparation of CSA 4 [*N*-ethylaminocarbonyl-(1*S*,2*S*)-diaminocyclohexane]. (*S*,*S*)-1,2-Diaminocyclohexane (1.14 g, 10 mmol) and triethylamine (1.4 mL, 10 mmol) were dissolved in 100 mL of dry CH_2Cl_2. The solution was stirred at room temperature for 30 min. And then, ethyl isocyanate (0.8 mL, 10 mmol) was added to the solution. After stirring the whole reaction mixture at room temperature for 12 h, the solvent and triethylamine were removed by rotary evaporation. The residue was purified by silica gel chromatography (MeOH:$CHCl_3$ = 1:1) to afford CSP **4** (0.33 g, 18%) as a white solid. mp 122-124 °C. $[\alpha]_D^{21.8}$ −18.5 (0.1, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, 3H), 1.20-1.40 (m, 4H), 1.60-1.75 (m, 2H), 1.80-2.10 (m, 4H), 2.35-2.45 (m, 1H), 3.05-3.20 (m, 3H), 4.50 (d, 1H), 5.20 (br s, 1H). IR (KBr) 3343, 2930, 2857, 1630, 1577, 1379, 1308 cm^{-1}.

Acknowledgement. This work has been supported by the grant from KOSEF (NRL program: M1-0318-00-0005) and by grants-in-aid for the National Core Research Center Program from MOST/KOSEF (No. R15-2006-022-03001-0).

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