Intermolecular $^1H-^1H$ Two-Dimensional Nuclear Overhauser Enhancements in the Characterization of a Rationally Designed Chiral Recognition System

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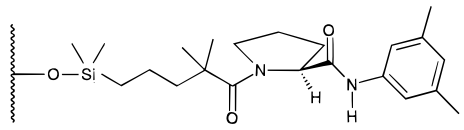
Chiral stationary phases (CSPs) for liquid chromatography derived from *N*-(acyl)proline-3,5-dimethylanilides separate the enantiomers of *N*-(3,5-dinitrobenzoyl)-α-amino esters and amides with high levels of selectivity. These CSPs have been used to assemble a large body of chromatographic data which indirectly supports the validity of the mechanistic rationale originally used in the design of these CSPs. We herein report ^1H and ^{13}C chemical shift data obtained when the (*S*)-enantiomer of chiral solvating agent (CSA) **3**, a soluble analogue of the selector used in CSP (*S*)-**1**, acts on each of the enantiomers of the dimethylamide of *N*-(3,5-dinitrobenzoyl)leucine, **2**. The changes in chemical shift in the mixture of (*S*)-**2** and (*S*)-**3** support the existence of those interactions thought to be essential to chiral recognition in this system. In addition, significant intermolecular NOESY enhancements are observed in this mixture. These NOE data are consistent with the structure expected for the more stable diastereomeric adsorbate formed between (*S*)-**2** and the (*S*)-proline-derived CSP **1**. No intermolecular NOEs are observed for corresponding mixtures of the chiral solvating agent (*S*)-**3** and (*R*)-**2**, the enantiomer least retained on (*S*)-CSP **1**.

We recently reported the preparation and evaluation of a new class of π-basic chiral stationary phases (CSPs) derived from anilides of *N*-(acyl)proline.[1,2] The *a priori* design of these CSPs was based on an understanding of the factors responsible for enantiodiscrimination in structurally different but mechanistically related systems.[3] In practice, these CSPs exhibit high levels of selectivity in the liquid chromatographic separation of the enantiomers of a variety of π-acidic racemates. For example, the separation factor (α) for the enantiomers of the dimethylamide of *N*-(3,5-dinitrobenzoyl)leucine, **2**, is 30, the capacity factor for the least retained enantiomer being 0.28.[4] Thus, (*S*)-CSP-**1** (Figure 1) displays a strong affinity for (*S*)-**2**, and very little affinity for (*R*)-**2**, an ideal situation from the standpoint of chiral recognition. In point of fact, separation factors exceeding 80 have been observed for the enantiomers of selected analytes with CSP **1**.[1]

The proline-based CSPs were designed to be mechanistically similar to the well-characterized *N*-(aryl)-α-amino ester-derived CSPs.[5,6] Choosing analyte **2** as a specific example, the interactions essential to the preferential retention of (*S*)-**2** on (*S*)-CSP **1** were expected to be (1) a face-to-face π−π interaction between the 3,5-dimethylanilido group of CSP **1** and the 3,5-dinitrobenzoyl group of (*S*)-**2**, (2) a hydrogen bond from the 3,5-dinitrobenzamide N-H of (*S*)-**2** to the *N*-(acyl)−carbonyl oxygen of CSP **1**, and (3) a hydrogen bond from the 3,5-dimethylanilide N-H of CSP **1** to the C-terminal carbonyl oxygen of (*S*)-**2** (Figure 2).



CSP 1

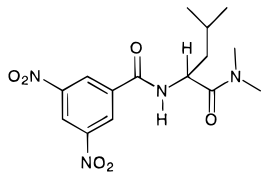


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Figure 1. Structures of the (*S*)-proline-derived CSP **1** and *N*-(3,5-dinitrobenzoyl)leucine dimethylamide (**2**).

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(4) α, the chromatographic separation factor, is the ratio of the two capacity factors (corrected retention times), k'_2/k'_1, and is related logarithmically to the difference in free energy between the two diastereomeric adsorbates formed as each enantiomer interacts with the CSP.

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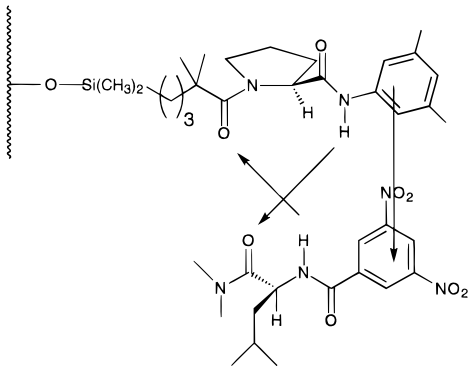

Figure 2. Interactions proposed to account for retention of the more retained enantiomer, (*S*)-**2**, on (*S*)-CSP **1**.

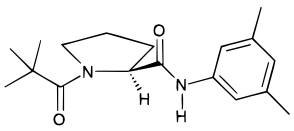

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Figure 3. Structure of the (*S*)-proline-derived chiral solvating agent **3**.

The investigation of molecular recognition phenomena is facilitated by high-field NMR instruments.[7] Of particular relevance, intermolecular nuclear Overhauser effect (NOE) experiments aid in the elucidation of chromatographically-derived chiral recognition mechanisms.[8] The nuclear Overhauser effect (or nuclear Overhauser enhancement) is manifest as a change in the intensity of one signal when another nucleus is saturated. The intensity change results from through space perturbation of the magnetic spin state distribution of nuclei near the nucleus which has been excited as it subsequently undergoes dipole−dipole cross-relaxation.[9] Thus, the utility of the NOE experiments in establishing conformations, making relative stereochemical assignments, and determining the spacial orientation of specific groups in a molecule or complex lies in the fact that an observed NOE indicates proximity of the irradiated and the observed nuclei in space.

In the present study, we have undertaken a detailed NMR investigation of each enantiomer of **2** with (*S*)-CSA **3**, a soluble analogue of (*S*)-CSP **1**, to improve our understanding of the structure of the diastereomeric complexes formed in solution (Figure 3).

Two-dimensional nuclear Overhauser spectroscopy (NOESY) is extremely useful in studying the conformations and higher order structures of proteins and other macromolecules. However, it has been used less frequently in studies of smaller molecules since tumbling of these molecules is generally sufficiently rapid so as to place dipole−dipole relaxation into the positive NOE regime.[10] For small molecules, the steady state one-dimensional experiments have advantages in sensitivity and quantitation of the observed enhancements. A disadvantage of one-dimensional experiments, however, is that they become increasingly more difficult to perform as spectra become more congested, since they require that individual resonances be irradiated selectively without affecting nearby resonances. Although the component molecules in this study are small, the mixtures have crowded NMR spectra with areas of significant overlap between multiplets. Because the need to selectively irradiate a particular signal is not of concern in the two-dimensional NOESY experiment, it is possible to observe NOEs of closely spaced signals in complex aromatic or aliphatic regions of the spectrum. Therefore, it seemed likely that NOESY experiments might be useful in studying chiral recognition systems involving small bimolecular complexes. Finally, *inter*molecular NOEs observed in the mixture are free from interfering COSY-type correlations since the two components in the mixture are not *J*-coupled to each other.

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Experimental Section

All reagents employed were of pharmaceutical or reagent grade. Elemental analyses were performed by T. McCarthy and associates of the University of Illinois microanalytical laboratory. Melting points are uncorrected.

(*S*)-*N*-Pivaloylproline. (*S*)-Proline (Aldrich) (10 g, 0.087 mol) was dissolved in 50 mL of 2 N NaOH, cooled to 0 °C in an ice−water bath, and stirred magnetically. Trimethyacetyl chloride (10.7 mL, 0.087 mol) and 40 mL of 2 N NaOH were added in several alternating portions over the course of 1 h so as to maintain the temperature at 5−10 °C. The pH was checked periodically to insure that the solution remained strongly alkaline. After addition of the acid chloride was complete, the reaction mixture was allowed to warm to room temperature and stirred vigorously for 30 min. The basic solution was extracted with two 75 mL portions of diethyl ether and then acidified to Congo red with 6 N HCl. The oil which separated was extracted into two 75 mL portions of diethyl ether which were combined, dried over anhydrous $MgSO_4$, and concentrated on a rotary evaporator. The white crystalline residue, 16.9 g (97.9%), was used without further purification: mp 130−132 °C; ^{1}H NMR (200 MHz) ($CDCl_3$) δ 1.30 (s, 9H), 1.95−2.20 (overlapping m, 4H), 3.70 (m, 2H), 4.55 (m, 1H), 11.10 (bs, 1H). Anal. Calcd for $C_{10}H_{17}NO_3$: C, 60.28; H, 8.60; N, 7.03. Found: C, 60.18; H, 8.64; N, 6.96.

(*S*)-*N*-Pivaloylproline 3,5-Dimethylanilide (3). (*S*)-*N*-Pivaloylproline (2.50 g, 0.013 mol) was dissolved in 40 mL of dry dichloromethane. 2-Ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) (3.26 g, 0.132 mol) was added, and the solution was sonicated until homogeneous (about 5 min). When dissolution was complete, 1.77 g (0.013 mol) of freshly distilled 3,5-dimethylaniline (Aldrich) was added and the solution was stirred for 2 h and then poured into a 250 mL separatory funnel containing 50 mL of dichloromethane. The dichloromethane solution was washed sequentially with two 75 mL portions of 2 N HCl, two 75 mL portions of 5% $NaHCO_3$, and 100 mL of water, then dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. Upon addition of ethyl acetate and hexane, the remaining yellow syrup afforded a crystalline product determined to be greater than 99.9% enantiomerically pure by analytical HPLC using an (*S*)-*N*-(3,5-dinitrobenzoyl)leucine-derived CSP (Regis Technologies, Morton Grove, IL). **3:** ^{1}H NMR (500 MHz) (CD_2Cl_2) δ 1.27 (s, 9H), 1.87 (m, J_{cis} = 8.1, 10.0, J_{trans} = 3.8, J_{gem} = 12.0 Hz, 1H), 1.95 (m, J_{cis} = 7.1, 5.3, J_{trans} = 3.8, 3.6, J_{gem} = 12.5 Hz, 1H), 2.11 (m, J_{cis} = 10.0, 6.3, J_{trans} = 3.6, 6.2, J_{gem} = 12.5 Hz, 1H), 2.26 (s, 6H), 2.30 (m, J_{cis} = 7.1, J_{trans} = 3.2, 3.6, J_{gem} = 12.0 Hz, 1H), 3.67 (ddd, J_{cis} = 6.3, J_{trans} = 3.6, J_{gem} = 10.0 Hz, 1H), 3.74 (ddd, J_{cis} = 5.3, J_{trans} = 6.2, J_{gem} = 10.0 Hz, 1H), 4.72 (dd, J_{cis} = 8.1, J_{trans} = 3.2 Hz, 1H), 6.70 (s, 1H), 7.11 (s, 1H), 8.90 (bs, 1H); ^{13}C NMR (CD_2Cl_2) δ 178.7, 170.3, 138.9, 138.8, 125.8,

117.8, 63.0, 48.8, 39.6, 27.7, 26.6, 26.3, 21.4 ppm. Anal. Calcd for $C_{18}H_{26}N_2O_2$: C, 71.49; H, 8.67; N, 9.26. Found: C, 71.32; H, 8.60; N, 9.24.

Dimethylamide of (*R,S*)-*N*-(3,5-Dinitrobenzoyl)leucine (2). Racemic *N*-(3,5-dinitrobenzoyl)leucine, 2.95 g (0.009 mol), and 2.36 g (0.009 mol) of EEDQ were suspended in 50 mL of dry tetrahydrofuran, and the solution was stirred vigorously until dissolution was complete. After 20 min, anhydrous dimethylamine was bubbled through the homogeneous solution for 30 s, the reaction vessel was stoppered, and the mixture was allowed to stand for 4 h. The initially colorless solution turns purple upon addition of the gaseous amine, then orange, and finally remains yellow. After 2 h, the mixture was concentrated on a rotary evaporator, and the residue was taken up in 50 mL of dichloromethane, poured into a 100 mL separatory funnel, and washed sequentially with two 25 mL portions of 2 N HCl and two 25 mL portions of 5% $NaHCO_3$. The organic layer was removed from the separatory funnel, dried over anhydrous $MgSO_4$, and concentrated to dryness. Recrystallization of the remaining material from ethyl acetate−hexane yielded 2.46 g (77%) of the white crystalline powdery racemate, **2**: mp 205−207 °C; ^{1}H NMR (500 MHz) (CD_2Cl_2) δ 0.98 (d, J = 6.5 Hz, 3H), 1.00 (d, J = 6.5 Hz 3H), 1.49 (m, J = 10.5, 3.5, J_{gem} = 14.0 Hz, 1 H), 1.83 (m, J = 4.0, 11.5, J_{gem} = 14.0 Hz, 1H), 1.86 (m, J = 6.5, 10.5, 4.0 Hz, 1H), 3.07 (s, 3H), 3.20 (s, 3H), 5.16 (m, J = 3.5, 11.5, 8.0 Hz, 1H), 8.98 (d, J = 8.0 Hz, 1H), 8.82 (d, J = 2.0 Hz, 2H), 9.09 (t, J = 1.0 Hz, 1H); ^{13}C NMR (CD_2Cl_2) δ 173.5, 162.3, 148.8, 137.6, 127.7, 121.2, 49.6, 41.2, 37.4, 36.2, 25.4, 23.5, 21.5 ppm. Anal. Calcd for $C_{15}H_{20}N_4O_6$: C, 51.13; H, 5.72; N, 15.90. Found: C, 51.18; H, 5.73; N, 15.90.

Racemic **2** (2.0 g) was dissolved in a minimal amount of 1,4-dioxane and preparatively resolved by MPLC employing a 75 × 2.5 cm column of (*S*)-*N*-(1-naphthyl)leucine-derived CSP bonded to 40 μm silica gel using a mobile phase consisting of 20% 2-propanol in hexane. The (*S*)-enantiomer of **2** is the more retained. The NMR spectrum of either enantiomer is essentially identical to that of the racemate.

Preparation of NMR Samples. Four NMR samples were prepared, containing respectively, (*S*)-*N*-pivaloylproline 3,5-dimethylanilide; (*S*)-*N*-(3,5-dinitrobenzoyl)leucine dimethylamide; (*S*)-*N*-pivaloylproline 3,5-dimethylanilide and (*S*)-*N*-(3,5-dinitrobenzoyl)leucine dimethylamide; and (*S*)-*N*-pivaloylproline 3,5-dimethylanilide and (*R*)-*N*-(3,5-dinitrobenzoyl)-leucine dimethylamide. Samples containing the individual components were initially 0.0505 M in **3** or **2**, respectively, and the mixtures were initially 0.0505 M in each component. Spectrophotometric grade CD_2Cl_2 was passed twice through Laroche basic alumina to remove any hydrogen chloride or water which might have been present. Each of the samples initially contained 1 mL of this CD_2Cl_2 before degassing under vacuum (10^{-3} Torr) and sealing after four freeze−thaw degassing cycles. Some concentration of the samples is inevitable during the degassing procedure, but if all samples are prepared consecutively under identical conditions, this should occur to the same extent for all samples.

NMR Experiments. NOESY experiments were performed on a Varian Unity 500 spectrometer using the standard software provided with VNMR version 4.3. The sample temperature was regulated at 30 or 0 °C. Spinning was not used. Spectra were acquired in the hypercomplex phase sensitive mode with 2048 data points in the directly acquired dimension and 256 real and 256 imaginary FIDs in the second dimension. The spectral width was 5085 Hz, centered near 5 ppm in both directions. The resulting acquisition time was 201 ms in the f_2 dimension and 50 ms in the f_1 dimension. A total of eight scans were acquired for each FID. The relaxation delay between scans was set to 20 s; this value was chosen on the basis of a T_1 experiment which showed that this was sufficient time to allow complete relaxation for all protons other than H13 (T_1 = 5.4 s) and H15 (T_1 > 20 s). Several experiments were performed using a range of mixing times from 0.25 to 4 s.

Data in the f_2 dimension were processed with no zero-filling and a matched Gaussian filter. Data in the f_1 dimension were extended from 512 to 2048 points using linear prediction and processed with the same Gaussian filter. The spectra were phased with diagonal peaks and chemical exchange cross-peaks positive and NOE cross-peaks negative. Volume integration was performed using standard VNMR "ll2d" software.

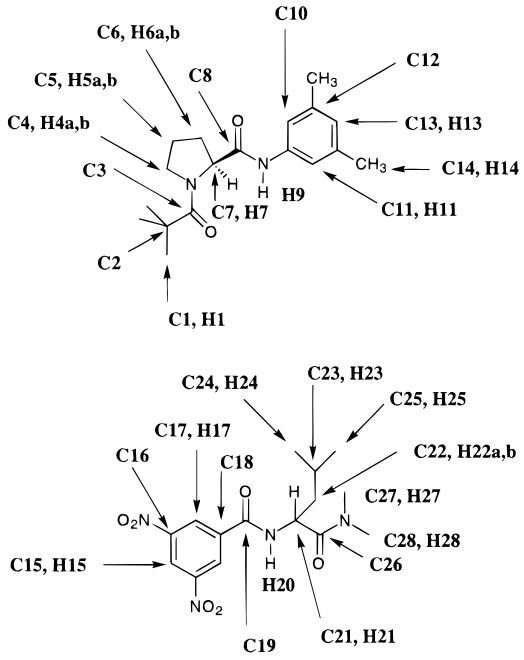

Figure 4. Numbering scheme for protons and carbon atoms in (*S*)-**3** and (*R*)- and (*S*)-**2**.

Results and Discussion

For convenience in discussion, the protons on the proline-derived chiral selector, (*S*)-**3**, and the *N*-(3,5-dinitrobenzoyl)leucine dimethylamide, **2**, are numbered as shown in Figure 4.

Careful and thorough assignment of each signal in all four spectra is necessary if one is to accurately interpret the observed NOE enhancements. All resonances in each spectrum were unambiguously assigned using ^{1}H and ^{13}C NMR spectroscopy with various combinations of selective ^{1}H{^{1}H} decoupling, ^{1}H−^{1}H correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC), and heteronuclear multiple quantum coherence (HMQC) spectroscopy and intramolecular NOE data. No substantial change in chemical shifts occurs in either single component, **2** or (*S*)-**3**, as a function of temperature, save for the dinitrobenzamide N-H resonance, which moves from δ 8.98 at 30 °C to δ 10.24 at −60 °C. The signals arising from the diastereotopic protons of the chiral solvating agent (*S*)-**3** and the analyte **2** are complicated, particularly in the mixtures where changes in the chemical shifts occur and multiplets overlap.

The seriously interested reader is urged to assemble CPK space-filling molecular models (Havard Apparatus, South Natick, MA) of the more stable (*S*)−(*S*) complex to refer to in following the interpretation of the chemical shift and NOE data. For a cartoonlike representation of the more stable diastereomeric complex, the reader is directed to ref 1.

^{1}H and Chemical Shift Differences. The chemical shifts for all protons on **2** and **3** in each of the four samples at 26 °C are summarized in Table 1.

As the data in Table 1 show, significant changes in chemical shift occur for many of the protons of (*S*)-**3** and (*S*)-**2** in the (homochiral) mixture relative to those

Table 1. ^{1}H Chemical Shifts for (*S*)-3, (*S*)-2, and (*S*)-3 with (*S*)-2 and (*S*)-3 with (*R*)-2 at 30 °C in CD_2Cl_2 Reported in ppm Relative to TMS

proton	free	(*S*)−(*R*) mixture	(*S*)−(*S*) mixture
H1	1.27	1.28	1.36
H4a, H4b	3.68, 3.74	3.68, 3.74	3.78
H5a, H5b	1.95, 2.11	1.95, 2.11	1.92, 2.12
H6a, H6b	1.87, 2.30	1.87, 2.31	2.02
H7	4.72	4.72	4.71
H9	8.90	8.87	9.37
H11	7.11	7.11	6.82
H13	6.70	6.70	6.45
H14	2.26	2.26	2.14
H15	9.09	9.03	8.90
H17	8.82	8.83	8.86
H20	8.98	8.75	9.04
H21	5.16	5.16	5.16
H22a, H22b	1.49, 1.83	1.49, 1.80	1.44, 1.83
H23	1.86	1.90	1.91
H24	1.00	1.01	1.00
H25	0.99	0.98	0.98
H27	3.20	3.19	3.24
H28	3.07	3.05	3.06

observed for the noncomplexed species. H9 moves downfield as would be expected if this amide N-H participates in hydrogen bonding. Dinitrobenzamide H20 is also thought to be involved significantly in hydrogen bonding, but shows only a slight downfield chemical shift, most likely because it is simultaneously shielded (in the complex) by the 3,5-dimethylanilide ring.[11] Protons H11, H13, H14, and H15 show upfield chemical shifts, suggesting that the two aromatic rings mutually shield each other, consistent with a face-to-face approach of these groups during the π-donor/π-acceptor interaction. The signal of H1, the *tert*-butyl protons of the *N*-acyl group on (*S*)-**3**, shows a modest downfield shift in the stable complex, as these protons are placed in the deshielding zone of the 3,5-dinitrobenzoyl group during complexation.

The changes in the chemical shifts and coupling constants of the diastereotopic protons on the proline ring which attend formation of the stable complex are noteworthy. While H4b is downfield of H4a in uncomplexed (*S*)-**3**, both resonances are isochronous (δ 3.78 ppm) in the mixture of (*S*)-**3** and (*S*)-**2**. Both H6a and H6b experience a change in chemical shift, although in opposite directions, as a result of complexation. These induced shifts become greater as temperature is reduced owing to increased interaction of the two components. Many of the coupling constants of the proline ring protons change slightly in the mixture, suggesting that a small conformational change of the ring may accompany complexation.

There are no significant chemical shift differences between the free species and the heterochiral mixture of (*R*)-**2**−(*S*)-**3**, save for the two amide proton resonances, H9 and H20. Since the chemical shifts of these protons are sensitive to concentration and temperature even as the noncomplexed species, these shifts may reflect a change in the extent of self-association stemming from the slight extent of formation of the heterochiral complex(es).

Table 2. ^{13}C Chemical Shifts for (*S*)-3, (*S*)-2, and (*S*)-3 with (*S*)-2 and (*S*)-3 with (*R*)-2 at 26 °C in CD_2Cl_2 Reported in ppm Relative to TMS

carbon atom	free	(*S*)−(*R*) mixture	(*S*)−(*S*) mixture
C1	27.4	27.4	27.2
C2	39.6	39.6	39.3
C3	178.7	178.7	178.0
C4	48.8	48.8	49.1
C5	26.3	26.3	26.3
C6	26.6	26.6	28.0
C7	63.0	63.0	63.1
C8	170.3	170.3	171.1
C10	138.9	138.9	139.2
C11	117.8	117.7	116.6
C12	138.8	138.8	138.6
C13	125.8	125.8	125.1
C14	21.4	21.4	21.2
C15	121.2	121.2	120.6
C16	148.8	148.8	148.5
C17	127.7	127.7	127.9
C18	137.6	137.7	137.2
C19	162.3	162.3	162.9
C21	49.6	49.5	49.7
C22	41.2	41.4	40.5
C23	25.4	25.4	25.2
C24	23.5	23.5	23.7
C25	21.5	21.5	21.3
C26	173.5	173.3	173.9
C27	37.4	37.4	37.5
C28	36.2	36.1	36.3

^{13}C Chemical Shift Differences. The chemical shifts for the carbon atoms of **2** and **3** in each of the four samples are summarized in Table 2.

The resonances for the four carbonyl carbons are of particular interest. According to the proposed chiral recognition mechanism, two of the carbonyl carbons (C8 and C19) are in amides which are hydrogen bond donors (H9 and H20) and two are in amides which serve as hydrogen bond acceptors (carbonyl oxygens at C3 and C26). On formation of the more stable complex (the (*S*)−(*S*) mixture), the resonances for C8, C19, and C26 move downfield, while the resonance for C3 moves upfield. Intuitively, one expects participation in a hydrogen bond to cause downfield shifts for the carbons of carbonyls which serve as hydrogen bond acceptors. Likewise, the carbonyl carbon of an amide group in which the N-H is involved in hydrogen bonding should be deshielded and consequently should also show a downfield shift relative to the noncomplexed species. Thus, these experimental observations are consistent with expectation in three of the four cases. On the basis of the examination of space-filling molecular models of this complex, it appears as if C3 may be slightly shielded by the 3,5-dinitrobenzoyl system, thus accounting for the apparently anomalous direction of chemical shift.[12] Also noteworthy, the rela-

(11) Experience shows that the chemical shift of this amide hydrogen (H20) typically increases when it serves as a hydrogen bond donor. The close approach (2.05 Å) of this hydrogen (H20) to the pivaloyl carbonyl carbon (O3) in the crystalline 1:1 complex of (*S*)-**2** and (*S*)-**3** indicates that H20 is involved in a hydrogen bond in the solid state. Because the remaining induced shifts and intermolecular NOEs indicate that the dominant mode of interaction of (*S*)-**2** and (*S*)-**3** in solution is similar to that seen in the solid state, we believe this hydrogen bond to be present in solution. Study of a CPK space-filling model of this complex leads us to suspect that the absence of the expected downfield shift of H20 comes about owing to the anisotropic magnetic environment in the complex. One of the reviewers thought this explanation "too subjective" on the basis of his own modeling study and wanted a "more reasonable explanation" of the chemical shift of H20. We retain our original opinion but can offer no proof of this.

(12) The anilide N-H of uncomplexed **3** is believed to intramolecularly hydrogen bond to the pivaloyl carbonyl oxygen in view of the chemical shift of this N-H, the observation of but one pivaloyl rotamer, even at low temperature, and the lack of dependence of the N-H chemical shift on temperature. Complexation with (*S*)-**2** shifts the signal of the anilide N-H 0.5 ppm downfield and that of the anilide carbonyl carbon 0.7 ppm upfield. One of the reviewers points out that the anomalous upfield shift of C3 may be occasioned by the change from an intramolecular hydrogen to an intermolecular hydrogen bond. This seems quite plausible insofar as the complexation could entail some conformation alteration of the pivalamide group. Indeed, the changes in coupling constants which occur on complexation indicate that some conformation change occurs within the proline ring.

Table 3. Intramolecular $^1H-^1H$ NOE Enhancements with Relative Volume Integrals Shown in Parentheses. Integrals Are Negative unless Otherwise Indicated (+)[a]

proton observed	intramolecular NOE enhancement (relative strength)
H1	H4a,b (s), H11 (w), H7 (vw)
H4a,b	H1 (s), H5a (m), H6a (w), H6b (w), H7 (vw)
H5a	H5b (vs), H4a,b (m)
H5b	H5a (vs), H4a,b (m), H7 (vw)
H6a	H6b (s), H7 (m), H4a,b (w)
H6b	H6a (s), H7 (m), H4a,b (w), H11 (vw)
H7	H6a (s), H9 (s), H6b (m), H1 (w), H11 (vw), H4a,b (vw), H5b (vw)
H9	H11 (s), H7 (s)
H11	H14 (vs), H9 (s), H13 (m), H1 (w)
H13	H14 (vs), H11 (w)
H14	H13 (vs), H11 (vs)
H15	
H17	H20 (vs), H23 (w), H24/25 (w), H27 (vw), H28 (vw)
H20	H17 (vs), H22b (m), H23 (m), H21 (w), H27 (vw), H28 (vw)
H21	H25a,b (s), H28 (s), H27 (m), H22a (m), H20 (w), H22b (w), H23 (w)
H22a	H22b (vs), H21 (m), H24/25 (m), H28 (m), H23 (w), H27 (w)
H22b	H22a (vs), H22 (m), H24/25 (m), H23 (w), H21 (vw), H27 (vw), H28 (vw)
H23	H24/25 (s), H20 (m), H22a (w), H17 (vw), H27 (vw), H28 (vw)
H24/25	H21 (s), H22a (s), H23 (s), H22b (m), H27 (w), H28 (w), H17 (vw)
H27	H28 (vvs, +), H21 (s), H17 (w), H22a (w), H24/25 (w)
H28	H27 (vvs, +), H21 (s), H22a (m), H24/25 (w), H17 (vw)

[a] Legend for relative volume integrals: vvs = very, very strong (>10); vs = very strong (~5); s = strong (~1); m = medium (~0.5); w = weak (~0.1); vw = very weak (~<0.1).

tively large upfield chemical shifts for C11, C13, and C15 in the homochiral mixture are consistent with the shielding caused by a parallel arrangement of the two aromatic rings. C10 is probably shielded by the dinitrobenzoyl ring but is simultaneously deshielded since it is attached to the hydrogen bond donating amide group, C8 and H9. These offsetting effects apparently result in a small net downfield shift.

By contrast, changes in the ^{13}C chemical shifts in the (*S*)-**3**−(*R*)-**2** mixture are small to nonexistent.

Intramolecular NOESY Experiments. Observed intramolecular two-dimensional NOE correlations for protons in **2** and (*S*)-**3** are presented in Table 3.

The intensities of the off-diagonal correlations were rated on a relative scale (from very, very strong to very weak) on the basis of the maximum peak volumes achieved. H24 and H25 are combined in Tables 3 and 4 because even though these methyl groups are distinguished as separate resonances in all spectra, they gave identical cross-correlations in every instance.

In the system under discussion, information obtained from the intramolecular NOE enhancements is useful in determining the average (most populated) conformations of the components, as free and complexed species. In the chiral solvating agent **3**, strong correlations between H9 and H7 and between H7 and H6a suggest that the *Z*-rotamer about the anilide bond is preferred and that H9 is synperiplanar to H7. This is supported by the fact that H4b is deshielded by the anilide carbonyl oxygen (C8) and appears downfield relative to H4a. *N*-(Acyl)-prolines are known to populate both conformations which result from rotation about the ring nitrogen to C3 bond. Both rotamers have been observed by HPLC and by NMR.[12,13] However, all NMR evidence related to the present system indicates that only the *trans*-rotamer of (*S*)-**3**, the one which places the C3 carbonyl group more or less eclipsed with the methine hydrogen H7, is populated in solution to any appreciable extent. Strong reciprocal NOEs between H1 and H4a and H4b support this assignment.

Table 4. Intermolecular $^1H-^1H$ NOE Enhancements with Relative Volume Integrals Shown in Parentheses[a]

proton observed	intermolecular NOE enhancement (relative strength)
H1	H22a (s), H22b (m), H24/H25 (m), H17 (m), H20 (w), H23 (w)
H4a, H4b	
H5a	
H5b	
H6a	H27 (vw)
H6b	H17 (vw), H27 (vw)
H7	H20 (w), H22b (w), H27 (vw)
H9	H20 (w), H17 (vw), H27 (vw)
H11	H17 (m), H15 (w), H21 (w), H27 (w)
H13	H17 (w)
H14	H15 (s), H17 (s), H27 (w), H28 (w)
H15	H14 (m), H11 (vw)
H17	H14 (s), H1 (m), H11 (m), H13 (vw)
H20	H7 (w), H9 (w), H11 (vw)
H21	
H22a	H1 (s)
H22b	H7 (vw)
H23	H1 (m)
H24/H25	H1 (m)
H27	H11 (vw), H14 (vw)
H28	H11 (m), H14 (m)

[a] Legend for relative volume integrals: vvs = very, very strong (>10); vs = very strong (~5); s = strong (~1); m = medium (~0.5); w = weak (~0.1); vw = very weak (~<0.1).

In **2**, stronger NOEs observed between H21 and one of the *N*-methyl groups permit assignment of H27 as the downfield methyl singlet, since this group is *syn* to the methine hydrogen H21 and closer in space than H28. H20 is correlated to H21 which is eclipsed by the carbonyl oxygen of C19. Interestingly, reciprocal NOEs between H27 and H28 are positive, since these methyl groups are exchanging positions, even though slowly relative to the NMR time scale.

Intermolecular NOESY Experiments. Observed NOE correlations in the more stable (*S*)-**2**−(*S*)-**3** complex are summarized in Table 4.

Many protons in the complex show significant intermolecular NOESY enhancements which are consistent with the originally proposed chiral recognition mechanism. Reciprocal NOEs are abundant between protons on both aromatic rings, including the methyl protons H14, indicating the close alignment of these groups required for effective face-to-face $\pi-\pi$ overlap. Both H9 and H11 show correlations to H27 and H28, the two methyl groups on (*S*)-**2**, suggesting that these groups are in close proximity to the 3,5-dimethylanilide owing to the hydrogen bond from H9 to the carbonyl oxygen of C26. This arrangement also places H27 in the vicinity of H6a and H6b. H9 shows further correlations to H17 and H20, suggesting that the two amide groups are close neighbors. H20, in turn, shows correlations to H11 and to H9. There are reciprocal NOEs between many of the protons on the isobutyl group of (*S*)-**2** and the *tert*-butyl group of (*S*)-**3** (H1). This is significant because it verifies approach of (*S*)-**2** to that face of the selector which presents the C3

(13) (a) Jocobsen, J.; Melander, W.; Vaisnys, G.; Horvath, C. *J. Phys. Chem.* **1984**, *88*, 4536−4542. (b) Gustafsson, S.; Eriksson, B.; Nilsson, I. *J. Chromatogr.* **1990**, *506*, 75−83.

(14) (a) Madison, V.; Schellman, J. *Biopolymers* **1970**, *9*, 511−567. (b) Garner, R.; Watkins, W. B. *Chem. Commun.* **1969**, 386. (c) Maia, H. L.; Orrell, K. G.; Rydon, H. N. *Chem Commun.* **1971**, 1209−1210.

carbonyl oxygen for hydrogen bonding to H20. All of the observed intermolecular NOEs increase in intensity (become more negative) as the temperature is lowered because of more extensive complexation.

Intermolecular NOEs are lacking where, according to the mechanistic hypothesis, there should be little interaction between the components (*S*)-**3** and (*S*)-**2**. For example, no intermolecular correlations are observed to or from protons H4a, H4b, H5a, or H5b.

Significantly, no intermolecular NOEs are observed in the (*R*)-**2**−**3** mixture. While the absence of an observed NOE should not be taken as evidence that no interactions occur, this does suggests that, if present, such complexation makes a negligible contribution to the time-weighted average of all processes affecting these components.[15]

(15) A reviewer suggested that intermolecular interatomic distances from the accompanying crystallographic study be cited to further support the conclusions drawn from the NOE study. A complete listing of these distance is available from author S.W.R. (see following paper in this issue). The observed NOEs are time-averaged values and are influenced by the extent of association. For protons in symmetrical rotors, the major portion of the NOE is presumably generated during the periods in which the proton(s) in the rapidly spinning group must closely approach the NOE partner. This is a consequence of the $1/r^6$ dependence of NOE magnitude upon the distance between the protons involved. From the lengthy list of intermolecular H−H distances obtained from the crystallographic study, one notes that strong NOEs are observed only between those protons which are within about 4 Å of each other in the solid state complex.

Conclusion

Two-dimensional nuclear Overhauser effect spectroscopy appears to be a worthwhile probe in investigating the nature of diastereomeric complexes formed in the course of chiral recognition, particularly if high levels of enantioselectivity are present in the system under study. In this instance, these experiments appear to be entirely consistent with the mechanism initially proposed on the basis of the examination of space-filling molecular models and supported by extensive chromatographic data. The chromatographic separation of enantiomers will continue to function as an effective screen in the search for new systems to study by NMR. Likewise, direct NMR evidence for complexation, particularly NOESY-derived information about the more stable diastereomeric complexes, will become increasingly important in the design of improved chiral selectors for the chromatographic separation of enantiomers.

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