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ENTITLED Deracemization of a Keto Amide: A Study of Chiral Recognition

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Deracemization of a Keto Amide: 
A Study of Chiral Recognition

By

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Introduction

As an extension and in support of chiral recognition models developed from liquid chromatography, deracemization studies were conducted on thioesters of N-acylated amino acids. Deracemization was afforded through the use of a chiral solvating agent, (R)-1-undec-10-enyl N-(2-naphthyl)alaninate, in the presence of a base catalyst. Due to the success of this study, research was initiated to explore the applicability of this method to new classes of compounds.

Since the above chiral solvating agent was the best found to date, it was also employed in the following study. The compound targeted for deracemization was a keto amide, specifically 2-N-(3,5-dinitrobenzoyl)amino-1,2-diphenyl ethanone. Triethyl amine was used as the base catalyst. This paper discusses the results of the study of the deracemization of the above keto amide.

Historical

Early work in the area of chiral stationary phases (CSPs) in liquid chromatography by Pirkle and coworkers led to the development of a CSP made from either enantiomer of 1-undec-10-enyl N-(2-naphthyl)alaninate. Use of columns of this type led to the discovery that enantiomers of the thioesters of N-(3,5-dinitrobenzoyl)amino acids were easily separated by this CSP. Separation of these compounds on this phase is afforded by the formation of transient diastereomeric complexes between the analyte and the CSP. This mechanism is used to explain the separations done by all the
Pirkle columns, and is illustrated through the use of chiral recognition models.

As in all cases of chiral recognition, a minimum of three simultaneous interactions is required, with at least one of these interactions being stereochemically dependent. Because of the stereochemical dependence of one interaction, one of the diastereomeric complexes between the enantiomers of the analyte and the single enantiomer of the chiral stationary phase will have a lower ground state energy. The difference in ground state energies, $\Delta \Delta G$, can be calculated from the ratio of the retention times, $\alpha$, of the enantiomers of the analyte (most retained over least retained). This is given in the following equation:

$$\Delta \Delta G = -RT \ln \alpha$$

where $R$ is the universal gas constant and $T$ is the absolute temperature. This difference in ground state energy leads to preferred complexation with one of the enantiomers, which causes that enantiomer to more successfully compete with the solvent for adsorption. The end result is separation of the enantiomers.

Similar enantioselective complexation has, through spectroscopic studies, been shown to occur in solution. Taking advantage of this, Pirkle and Reno sought to use a chiral solvating agent in the presence of a base catalyst to deracemize the enantiomers of a compound with a stereogenic center containing an acidic hydrogen. Acting on the above chromatographic data, a single enantiomer of N-(2-naphthyl)alanine undecenyl ester, 3, was chosen as the chiral solvating agent, and thioesters of N-(3,5-dinitrobenzoyl) amino acids were chosen to be deracemized. The
enantioselective complexation utilized to afford chromatographic separation was expected to, in the presence of a base catalyst, shift the point of equilibrium between the thioester enantiomers. If the equilibrium could be shifted to a large enough extent, deracemization of the thioesters would occur. The shift in equilibrium would come about through the lowering of the ground state energy of a single enantiomer due to preferential complexation. This complexation can be described by the same chiral recognition model as that given for chromatographic separation. The chiral recognition model contains the following intermolecular interactions: (1) a π-π interaction between the electron rich naphthyl group and the electron deficient dinitrobenzoyl group, (2) a hydrogen bond between the dinitrobenzoyl amide hydrogen and the undecenyl ester carbonyl, (3) a hydrogen bond between the amino hydrogen and the thioester c-terminal carbonyl. As illustrated in Figure 1, with thioester 2, the third interaction is the one that is stereochemically dependent. In the presence of the (R)-enantiomer of N-(2-naphthyl) alanine undecenyl ester (3), the amino hydrogen of this compound can hydrogen bond with the c-terminal carbonyl of the thioester only if the thioester is also in the (R) configuration.
The enantioselectivity of complexation was found to be considerable. In one case, deracemization was carried out to a 91% enantiomeric excess. Pirkle and Reno established that, in order for thermodynamic deracemization to occur, the following conditions must be met: "(1) to eliminate the energetic degeneracy of the enantiomers, it is necessary but not necessarily sufficient, that the transformation solvent be either chiral nonracemic or contain a species which is chiral nonracemic; (2) the chiral nonracemic species must be stereochemically stable under the transformation conditions; (3) the species to be deracemized must be stereochemically labile." Due to the success of the deracemization of the thioesters of N-acylated amino acids, Pirkle looked for new classes of compounds with stereochemical lability. The compound next investigated was a keto amide. This paper offers results and discussion on the outcome of deracemization studies performed on 2-N-(3,5-dinitrobenzoyl) amino-1,2-diphenyl ethanone, 1.
Results and Discussion

Keto amides were targeted as the next class of compounds to be studied for deracemization because of their similarity to the thioesters studied by Pirkle and Reno. The similarity between these two classes of compounds results in almost identical interactions with chiral solvating agent 3, which can be demonstrated by comparing keto amide 1 and thioester 2. Both compounds contain a 3,5-dinitrobenzoyl group, which can undergo a π-π interaction with the naphthyl group on 3. The amide hydrogens on both 1 and 2 can hydrogen bond to the ester carbonyl on 3, and the amide hydrogen on 3 can hydrogen bond with the keto carbonyl on 1, as well as the thioester carbonyl on 2. Both compounds also possess a hydrogen-containing stereogenic center α to a carbonyl. This condition was found to make 2 stereochemically labile in the presence of base. This lability is due to the acidity of the α-hydrogen resulting from negative charge stabilization by the carbonyl. The α-hydrogen in 1 was also predicted to be sufficiently acidic to afford stereochemical lability in a basic medium. This was found to be the case, for enantiomerically pure 1
Figure 1. Chiral recognition model for 2 and 3. Most stable enantiomer shown.

was found to have racemized after 2 days in solution containing triethyl amine.

The similarity of the chiral recognition models, straightforward synthesis (see Scheme I), and stereochemical lability of 1 made the keto
amide an ideal compound for deracemization. Consequently, several mixtures of the following three components were prepared with different relative concentrations: (R,S)-2-N-(3,5-dinitrobenzoyl)amino-1, 2-diphenyl ethanone (1), (R)-1-undec-10-enyl N-(2-naphthyl)alaninate (3), and triethyl amine. Table 1 shows the exact concentrations, solvents, and end results of the deracemization studies. Reaction mixtures 1 and 2 show a very small shift in equilibrium towards the desired enantiomer. Due to the method used for calculating the enantiomeric ratios (cut and weigh), these mixtures can essentially be considered racemic. Mixture 3 shows a high enantiomeric excess of the desired enantiomer. This result, however, is misleading, because the concentration did not remain constant throughout the study due to solvent evaporation. The solvent evaporated to such an extent that the keto amide lost solubility, forming a precipitate in the sample tube. The high excess of the desired (R)-enantiomer is most likely due to preferred solubility rather than deracemization. This preferred solubility is due to the lower ground state energy of the diastereomeric complex formed by the (R)-enantiomer of the keto amide in the presence of (R)-3. This preferred solubility, then, is in itself supportive of the chiral recognition model purposed for this system.

One possible explanation for the lack of deracemization of mixtures 1 and 2 is solvent polarity. Pirkle and Reno reported that deracemization fails to occur in polar solvents.1 This is most likely due to competition between the polar solvent, in this case methylene chloride, and the enantiomers for interaction with the polar sites on the chiral solvating agent. That is, the two species, chiral solvating agent and racemic
Scheme I. Synthesis of 2-amino-\(N\)-(3,5-dinitrobenzoyl)-1,2-diphenyl ethanone.

The compound, will be solvated for a larger amount of time in a polar solvent than in a nonpolar solvent. The lack of complexation would cause a decrease in the shift of the equilibrium of the enantiomers.
Table 1. Mixtures of 1, 3, and Triethylamine.

<table>
<thead>
<tr>
<th>Mixture</th>
<th>[1]</th>
<th>[3]</th>
<th>[Et3N]</th>
<th>Solvent</th>
<th>Area(S)²</th>
<th>Area(R)²</th>
<th>A(R)/A(S)</th>
<th>A(R)/A(S)³</th>
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</thead>
<tbody>
<tr>
<td>Racemic for 1-3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>MeCl₂</td>
<td>.062</td>
<td>.0587</td>
<td>.9468</td>
<td>1.000</td>
</tr>
<tr>
<td>1</td>
<td>.01</td>
<td>.01</td>
<td>.04</td>
<td>2:1 MeCl₂/Hexane</td>
<td>.0488</td>
<td>.0464</td>
<td>.9508</td>
<td>1.004</td>
</tr>
<tr>
<td>2</td>
<td>.01</td>
<td>.02</td>
<td>.04</td>
<td>2:1 MeCl₂/Hexane</td>
<td>.0589</td>
<td>.0586</td>
<td>.9949</td>
<td>1.051</td>
</tr>
<tr>
<td>3</td>
<td>.01</td>
<td>.03</td>
<td>.04</td>
<td>2:1 MeCl₂/Hexane</td>
<td>.0269</td>
<td>.0641</td>
<td>2.3829</td>
<td>2.517</td>
</tr>
<tr>
<td>Racemic for 4-8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>MeCl₂</td>
<td>.0547</td>
<td>.0845</td>
<td>1.5448</td>
<td>1.000(1.6316)</td>
</tr>
<tr>
<td>4</td>
<td>.01</td>
<td>.03</td>
<td>.02</td>
<td>3:1 Toluene/MeCl₂</td>
<td>.0613</td>
<td>.0676</td>
<td>1.1028</td>
<td>.7139(1.165)</td>
</tr>
<tr>
<td>5</td>
<td>.01</td>
<td>.03</td>
<td>.03</td>
<td>3:1 Toluene/MeCl₂</td>
<td>.0436</td>
<td>.0539</td>
<td>1.2362</td>
<td>.8002(1.306)</td>
</tr>
<tr>
<td>6</td>
<td>.01</td>
<td>.03</td>
<td>.04</td>
<td>3:1 Toluene/MeCl₂</td>
<td>.0570</td>
<td>.0688</td>
<td>1.2070</td>
<td>.7813(1.275)</td>
</tr>
<tr>
<td>7</td>
<td>.01</td>
<td>.03</td>
<td>.06</td>
<td>3:1 Toluene/MeCl₂</td>
<td>.0404</td>
<td>.0419</td>
<td>1.0371</td>
<td>.06713(1.095)</td>
</tr>
<tr>
<td>8</td>
<td>.01</td>
<td>.03</td>
<td>.07</td>
<td>3:1 Toluene/MeCl₂</td>
<td>.0517</td>
<td>.0628</td>
<td>1.2147</td>
<td>.07863(1.283)</td>
</tr>
</tbody>
</table>

1The values shown represent the status of the mixtures, after the following amounts of time: Mixtures 1-3, 84 d; Mixtures 5-8, 46 d; Mixture 4, 34 d. 2Areas determined by "cut and weigh" method, number is actually wt. in g of enlarged peak. There is +3% allowable error. (S)-enantiomer assumed to be that which eluted first, (R)-enantiomer that which eluted second. 3Normalization performed by dividing the area ratios of the mixtures by that of the appropriate racemic mixture. Numbers in parentheses for mixtures 4-8 are normalized by dividing [A(R)/A(S)] by racemic mixture for 1-3.
Mixtures 4 and 8 used 3:1 toluene/methylene chloride rather than the 2:1 methylene chloride/hexane solvent system used in mixtures 1 through 3. During analysis of the racemic mixture made from the same batch of keto amide used to prepare mixtures 4 and 8, an anomaly was noticed. The ratio of enantiomers for this mixture (entry 5, Table 1) is 1.5, with a larger amount of the (R)-enantiomer. Several attempts were made to resolve the problem. All analyses were done by HPLC using (R)-N-(2-naphthyl)alanine undecenyl ester column. The racemic mixture in question was analyzed again using the same method to ensure reproducibility. The results were the same. The mixture was then thought to contain some coeluting impurity, which would enhance the size of the peak corresponding to the (R)-enantiomer. To check this, the mixture was run through a racemic N-(2-naphthyl)alanine undecenyl ester column. If the impurity was achiral, the presence of the other (S)-enantiomer would not effect its retention time. The effect of the racemic column on the enantiomers of 1, however, is to cause coelution at the average of the retention times on the chiral column. When the sample was run through the racemic column, no impurity eluted at the retention time necessary to cause enhancement of the (R)-enantiomer peak.

The possibility of a chiral impurity has not been ruled out, but analysis of the data for mixtures 4 through 8 seems to indicate that an excess of the (R)-enantiomer was indeed present. At the end of this study, the ratio of peak areas actually decreased from the "racemic" value of 1.5. This may be interpreted as partial conversion of the excess (R)-enantiomer to the (S)-enantiomer. This would follow, since, as previously mentioned,
enantiomerically pure (R)-1 was racemized in the presence of base in the course of 2 days. The reason a totally racemic mixture was not obtained in samples 4 through 8 could be due to the presence of the chiral solvating agent. This indicates that deracemization would have occurred, had the starting mixture been racemic. The equilibrium point observed would remain the same regardless of the beginning ratio of enantiomers.

The way to interpret the enantiomeric ratios for mixtures 4 through 8 has become a problem, due to the apparent enantiomeric excess present in the starting material. The values in the last column of Table 1 show normalization by the "racemic" sample for these mixtures indicates an actual excess in the undesired enantiomer. This, however, supports the above argument that equilibrium was approached from an excess of the (R)-enantiomer. The values in parentheses in the last column are normalized values using the racemic sample for mixtures 1 through 3. If this value is accepted as truly racemic, the normalization is justifiable. This gives enantiomeric ratios indicating an excess of the desired (R)-enantiomer.

Solvent polarity (or the lack of it) may be suggested as the reason deracemization was allowed to occur in mixtures 4 through 8 and not in 1 through 3. Due to the way the two sets of mixtures were prepared, however, only mixtures 3 and 6 could be compared directly. Direct comparison is possible because the ratio of the components in these mixtures is the same, the only difference being the solvent systems. Unfortunately, mixture 3 was ruined due to solvent evaporation, yielding a deceptively high excess of the desired enantiomer. The 3:1
toluene/methylene chloride solvent system of mixtures 4 through 8 is, however, less polar than the 2:1 methylene chloride/hexane system of mixtures 1 through 3. Also, racemization occurred to some extent in all the mixtures using the less polar solvent, while virtually no deracemization occurred in the more polar solvent. This could be interpreted as a solvent polarity effect.

The only difference between mixtures 4 through 8 is the relative amount of base catalyst present in each mixture. It should be noted that mixture 4 was prepared about one week later than 5 through 8, allowing less time for the mixture to reach true equilibrium. As seen in Table 1, mixtures 5, 6, and 8 all have an enantiomeric ratio of about 1.3 [(R)/(S)]. This shows a slight excess of the desired enantiomer, indicating that deracemization did occur. It may be more accurate to assert that, since an excess of the (R)-enantiomer was present at the outset, the presence of the chiral solvative agent prevented the base from completely racemizing the keto amide enantiomers. Mixture 7 shows an enantiomeric ratio of about 1.1, indicating an excess of the desired enantiomer, but not to as large an extent as mixtures 5, 6, and 8. Mixture 4 has a ratio of approximately 1.2, although this mixture had less time to reach equilibrium. Due to the starting conditions (i.e. excess of the (R)-enantiomer), this value is, if anything, deceptively high. Analysis of this data, while noting that the amount of base increases with mixture number, indicates that the relative amount of base has little or no effect on the extent of deracemization. This finding is in agreement with the data presented by Pirkle and Reno.1

Deracemization of 1 did not occur to any great extent, the highest
enantiomeric ratio being 1.3. This indicates an approximate enantiomeric content of 56.5% (R) and 43.5% (S). One should note that, with this value, one assumes that a racemic sample gives a ratio of about 0.95, and that this can be incorporated as a normalization factor. Without normalization, the highest enantiomeric ratio is about 1.2, indicating the mixture contains 54.5% of the (R)-enantiomer and 45.5% (S). As noted earlier for mixture 4, the nature of the starting conditions indicates that these numbers are deceptively high if one assumes equilibrium has not been achieved. This is due to the indication that an excess of the desired enantiomer, (R)-1, was initially present. The lack of deracemization to a larger extent could be due to the solvent polarity. Mixtures 1 and 2, whose solvent system was more polar than that of 4 through 8, showed virtually no deracemization. Decreasing the solvent polarity afforded a small amount of deracemization, actually a shift of equilibrium towards the desired enantiomer, in mixtures 4 through 8. One may argue, then, that a further decrease in solvent polarity may afford a larger enantiomeric ratio. In comparing the least polar solvent system used in this study, 3:1 toluene/methylene chloride, with the solvents used by Pirkle and Reno, one sees support for this argument. In polar solvents, such as tetrahydrofuran and acetonitrile, no deracemization of thioester 2 was observed. In toluene, this thioester showed deracemization to the extent of 46% enantiomeric excess (ee) of the desired enantiomer. A 3:1 mixture of cyclohexane/toluene gave 65% ee, while 3:1 cyclohexane/CH2Cl2 gave a 67% ee (all values from Reference 1). If a nonpolar solvent capable of dissolving the keto amide could be found, one might expect a larger degree of deracemization to occur. These results
demonstrate one of the limiting factors of deracemization using 3: one must find a solvent system polar enough to dissolve the compound to be deracemized that is nonpolar enough not to cause detrimental solvation. The compounds that can be deracemized by 3 are intrinsically polar, since polar interactions are required in the chiral recognition model.

An attempt was made to monitor the rate of deracemization using an HPLC system containing an (R)-N-(2-naphthyl)alanine undecenyl ester column. Unfortunately, the system did not operate properly at the sensitivity used. Inconclusive data was obtained during this time. The data in Table 1 was obtained on another HPLC system with a more stable detector, using the same column. The data was collected within the linear range of operation.

Conclusion

From the data in Table 1, one can conclude that the equilibrium between the enantiomers of 1 was shifted slightly towards the desired (R) enantiomer. This lends support to the proposed chiral recognition model describing the interaction between racemic 1 and (R)-3. This model, illustrated in Figure 2, consists of: (1) a π-π interaction between the electron rich naphthyl group on 3 and the electron deficient 3,5-dinitrobenzoyl group on 1, (2) hydrogen bonding between the amide hydrogen on 1 and the ester carbonyl on 3, and (3) a stereochemically dependent hydrogen bond between the amide hydrogen on 3 and the keto carbonyl on 1. The extent of the equilibrium shift was decreased, however,
by the polarity of the solvent system used in these mixtures. The polarity of the solvent system for mixtures 1 through 3 was such that, under the set conditions, solvation of the racemic compound and chiral solvating agent

Figure 2. Chiral recognition model for 1 and 3. Most stable enantiomer shown.
was great enough to prevent deracemization. Future studies of this system, as well as other keto amides, should include less polar solvents, temperature studies, and deracemization rate determination.

Experimental

Elemental analyses were performed by J. Nemeth and associates of the University of Illinois microanalytical service. 1H NMR were obtained on a Varian XL-200 200 MHz FT NMR. All 1H resonances are recorded in parts per million using trimethyl silane as a zero reference. Chromatographic analyses were performed using a Rainin Rabbit HP pump, a Milton Roy uvMonitorTM D fixed wavelength detector, and a Kipp and Zonen BD 41 recorder.

(R,S)-2-N-(3,5-dinitrobenzoyl)amino-1,2-diphenyl-1-ethanol (4).
Procedure using aqueous base. To 0.50 g 2-amino-1,2-diphenyl-1-ethanol partially dissolved in 40 ml methylene chloride was added 0.55 g 3,5-dinitrobenzoyl chloride, with stirring. A white precipitate formed immediately, but the mixture was stirred for 2 hours to ensure completion of the reaction. The mixture was then shaken with 20 ml 1N NaOH. The precipitate was filtered and recrystallized from methanol and water to give 4 as a white solid: 0.43 g (60.5%); m.p. 246-247 oC; NMR (DMSO, 200 MHz) \( \delta \) 5.18 (d, 1H), \( \delta \) 5.39 (t, 1H), \( \delta \) 7.22 (m, 5H), \( \delta \) 7.38 (m, 5H), \( \delta \) 9.04 (s, 1H), \( \delta \) 9.08 (s, 2H), \( \delta \) 9.15 (d, 1H); Anal. Calcd. for C21H17N3O6: C, 61.91; H, 4.21; N, 10.31. Found: C, 61.95; H, 4.45; N, 10.26.
(R,S)-2-N-(3,5-dinitrobenzoyl)amino-1,2-diphenyl-1-ethanol (4). Procedure using propylene oxide. To a solution of 0.50 g 2-amino-1,2-diphenyl-1-ethanol and 0.21 ml (0.17 g) propylene oxide in 100 ml methylene chloride was added, with stirring, 0.70 g 3,5-dinitrobenzoyl chloride. A precipitate formed immediately, but the mixture was stirred for 16 h to ensure complete reaction. The precipitate was isolated by filtration. No further purification was performed. This procedure gave 0.91 g (95.4%) of 4 as a white solid. Identification was done by TLC (solvent: 1:1 Ether/CH2Cl2), which showed a single spot under a UV lamp.

(R,S)-2-N-(3,5-dinitrobenzoyl)amino-1,2-diphenyl ethanone (1). To a solution of 0.09 g 2-N-(3,5-dinitrobenzoyl) amino-1,2-diphenyl-1-ethanol dissolved in 3 ml dimethyl sulfoxide was added 2ml acetic anhydride. The resulting solution was stirred overnight. The volatile components of the mixture were removed via rotary evaporation. Residual DMSO was removed through the use of a Kugelrohr, leaving a yellow oil. The oil was crystallized from hot methanol to give 1 as a white solid: 0.02 g (18%); m.p. 202-203 oC; NMR (DMSO, 200 MHz) δ 6.86 (d, 1H), δ 7.33 (m, 5H), δ 7.52 (m, 5H), δ 8.10 (d, 1H), δ 9.10 (m, 3H); Anal. Calcd. for C21H15N3O6: C, 62.22; H, 3.73; N, 10.37. Found: C, 61.90; H, 3.70; N, 10.23.
References


(5) Deracemize in this paper is used in the way defined by Pirkle and Reno in J. Am. Chem. Soc. 1987, 109, 7189.