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Eutectic Composition of a Chiral Mixture Containing a Racemic Compound

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Abstract:

The enantioseparation process is of great interest to the pharmaceutical industry since more than 50% of the pharmaceutically active ingredients are known to be chiral and one enantiomer is usually preferred over the racemic mixture. Crystallization is widely used as the final step to reject the enantiomeric impurity from a mixture. Yet, a fundamental guidance on developing a purification procedure for systems forming racemic compounds (which account for more than 90% of all chiral systems) is not available. In this work, it is shown that the enantiomeric excess (ee) of the eutectic point is the key information needed to assess the feasibility of a crystallization process and to predict the purity and the yield of the product. In a dilute solution, the eutectic ee is determined solely by a eutectic constant (K_{eu}), a new parameter introduced in this paper. K_{eu} is defined as the ratio of the activity of the major enantiomer to that of the minor enantiomer. A eutectic constant equation was derived from the basics of thermodynamics, and for the first time, it was shown that the K_{eu} is independent of solvent if no solvates are formed but varies with temperature. With an understanding of how the eutectic ee changes with solvent and temperature, the time and material required in developing a crystallization procedure for ee enhancement can be dramatically reduced. This theory was supported by experimental data, and its application was demonstrated on a system of pharmaceutical interest.

Introduction

More than 50% of pharmaceutically active ingredients are known to be chiral.[1,2] The two enantiomers of a drug candidate are generally different in potency and toxicity because the target receptor sites and the metabolic pathways are stereoselective.[3−5] Therefore, one enantiomer is usually preferred over the racemic mixture, and as such the enantioseparation process is of great interest to the pharmaceutical industry.

Roozeboom[6] identified three basic racemate types on the basis of their melting point phase diagrams: conglomerate, racemic compound, and pseudoracemate. Of the two main types of crystalline racemates, racemic compounds occur much more frequently than conglomerates.[7] The racemic compound forming system is also more complicated than the conglomerate forming system since one extra phase, the racemic compound, is involved. The separation of enantiomers which form a racemic compound requires the utilization of diastereomeric interactions, such as asymmetric reduction, diastereomeric compound formation, etc. If the process applied does not guarantee a high enough enantiomeric excess (ee) of the active pharmaceutical ingredient, API (99% or higher is desired), further chiral purification of API will be required.

Crystallization is widely used at small and large scales to reject impurities, including an enantiomeric impurity. Development of a crystallization method for an ee enhancement involves solvent screening and temperature selection and can be facilitated by construction of ternary solubility phase diagrams. This process is time-consuming, and also requires a large quantity of compound to develop the phase diagrams by varying solvent and temperature. Perhaps for this reason, phase diagrams are rarely used during process development.

Figure 1 shows a ternary phase diagram for a racemic system (racemic compound is thermodynamically more stable than conglomerate) at temperature T in an achiral solvent L when no solvates are formed. Points A and A′ represent the composition of the solution saturated with pure enantiomer S and R, respectively. Points B and B′ (eutectic points) correspond to the solution compositions when the three phases exist in equilibrium: racemic compound (r), one enantiomer (S or R), and the saturated solution. Based on the phase rule, once the temperature and the pressure are selected, points A, A′, B, and B′ are fixed. For this system, if the ee of the eutectic point (eutectic ee) is higher than the required ee (use ee_r for the rest of the paper) for the product, the desired enantiomer can be enriched in the liquid phase if the starting ee is lower than the eutectic ee (between E and r), and the ee of the filtrate will be the eutectic ee. If the eutectic ee is lower than ee_r and the starting material has ee higher than the eutectic ee (between S and E), pure enantiomer (theoretically) or mixtures of enantiomers with ee higher than the eutectic ee can be crystallized out. If the eutectic ee is lower than ee_r and the starting ee is even lower, neither the ee of the solid nor the ee of the solution will be higher than ee_r. With this system, no crystallization process controlled by thermodynamic equilibration will be able to generate a product with an ee meeting the requirement.

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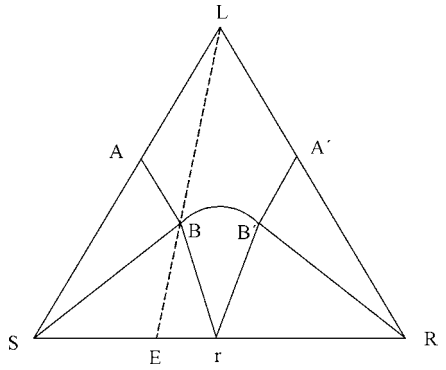

Figure 1. **Ternary phase diagram for a racemic compound forming system.**

Clearly, the eutectic ee is the key to assess the feasibility of a crystallization method and to predict the ee of the product. Therefore, it would be of great interest if a thermodynamic relationship was available to describe how the eutectic ee changes as a function of solvent properties and temperature.

To the best of our knowledge, no theoretical discussion of the eutectic ee as a function of solvent properties or temperature has been reported for a racemic system. In this work, the relationship between the eutectic ee and solvent properties as well as temperature was derived from thermodynamic principles, and the results were supported by experimental data. A new term, eutectic constant (K_{eu}) is introduced, which connects eutectic ee, an experimentally obtainable perimeter, to solubility products of the enantiomer and corresponding racemic compound, two thermodynamic constants which dictate the phase diagram of the system.

Theoretical Derivation. (a) Introduction of Eutectic Constant. In the region SBr of the ternary phase diagram (Figure 1), solid S-1 and r-1 are in equilibrium with their saturated solution, which has the eutectic composition. The solubility equilibriums for this system (system X) are given by

$$S_{\text{solid}} \leftrightarrow S_{\text{solution}}$$

$$K_{sp(S)} = a_S$$

And

$$r_{solid} \leftrightarrow \frac{1}{2}S_{solution} + \frac{1}{2}R_{solution}$$

$$K_{sp(r)} = a_S^{1/2} * a_R^{1/2}$$

where, $K_{sp(S)}$ and a_S are the solubility product and activity of *S*, respectively; $K_{sp(r)}$ is the solubility product of the racemic compound; a_R is the activity of R. The factor $^1/_2$ is introduced since 1 mole of a racemic compound is defined on the basis of $^1/_2$ mole of S and $^1/_2$ mole of R enantiomer.

From the above equations, we obtain

$$\frac{a_S}{a_R} = \frac{(K_{sp})_S^2}{(K_{sp})_r^2}$$

The equation indicates that the activity ratio of the enantiomers at the eutectic point is determined by the solubility products of the enantiomer and racemic compound. For its importance, this ratio is named the eutectic constant (K_{eu})

$$K_{\text{eu}} = \frac{a_S}{a_R} = \frac{(K_{sp})_S^2}{(K_{sp})_r^2} \tag{1}$$

(b) Eutectic Constant Equation. (1) Expression of Eutectic Constant in Terms of Enthalpy of Solution. Applying classical thermodynamics on system X, we have

$$\frac{\mathrm{d}\ln K_{sp(S)}}{\mathrm{d}T} = \frac{\mathrm{d}\ln a_S}{\mathrm{d}T} = \frac{(\Delta H_S)_S}{RT^2} \tag{2}$$

$$\frac{\mathrm{d}\ln K_{sp(r)}}{\mathrm{d}T} = \frac{\frac{1}{2}\mathrm{d}\ln(a_S * a_R)}{\mathrm{d}T} = \frac{(\Delta H_S)_r}{RT^2} \tag{3}$$

where $(\Delta H_S)_S$ and $(\Delta H_S)_r$ are the molar enthalpy of solution for enantiomer S and racemic compound r, respectively.

Subtracting eq 3 from eq 2 results in

$$\frac{\mathrm{d}\ln\frac{a_S}{a_R}}{\mathrm{d}T} = \frac{2\,\mathrm{d}\ln\frac{K_{sp(S)}}{K_{sp(r)}}}{\mathrm{d}T} = \frac{2(\Delta H_S)_S - 2(\Delta H_S)_r}{RT^2}$$

or

$$\frac{\mathrm{d}\ln\frac{a_S}{a_R}}{\mathrm{d}\left(\frac{1}{T}\right)} = -\frac{2[(\Delta H_S)_S - (\Delta H_S)_r]}{R}$$

From eq 1

$$\frac{\mathrm{d}\ln\frac{a_S}{a_R}}{\mathrm{d}\frac{1}{T}} = \frac{\mathrm{d}\ln K_{\text{eu}}}{\mathrm{d}\frac{1}{T}} = -\frac{2[(\Delta H_S)_S - (\Delta H_S)_r]}{R} \tag{4}$$

The dissolution of a solid in a solvent is equivalent to the melting of the solid followed by mixing of the resulting liquid solute with the solvent. The enthalpies of solution of enantiomer S and racemic compound r are therefore given by

$$(\Delta H_S)_S = (\Delta H_f^T)_S + (\Delta H_{mix})_S \tag{5}$$

$$and\ (\Delta H_S)_r = (\Delta H_f^T)_r + (\Delta H_{mix})_r \tag{6}$$

where $(\Delta H_f^T)_S$ and $(\Delta H_f^T)_r$ are the enthalpies of fusion of S and r, respectively, at absolute temperature *T*, and $(\Delta H_{mix})_S$ and $(\Delta H_{mix})_r$ are the enthalpies of liquid S-solvent and liquid r (liquid S and R)-solvent mixing, respectively.

(2) Heat of Fusion at Temperature *T*. Considering the following thermodynamic cycle of enantiomer S

$$\begin{array}{ccc} S, \text{solid at T and P} & \rightarrow & S, \text{solid at } T_m \text{ and P} \\ \uparrow & & \downarrow \\ S, \text{liquid at T and P} & \leftarrow & S, \text{liquid at } T_m \text{ and P} \end{array}$$

The enthalpy of fusion of enantiomer S at T can be expressed as

$$(\Delta H_f^T)_S = (\Delta H_f^{(T_m)S})_S + \int_T^{(T_m)S} (C^s)_S \, dT + \int_{(T_m)S}^T (C^l)_S \, dT \quad (7)$$

where $(\Delta H_f^{(T_m)S})_S$ is the enthalpy of fusion at the melting temperature $(T_m)_S$, and $(C^s)_S$ and $(C^l)_S$ are heat capacities of solid and liquid S, respectively.

Similarly, the expression for the enthalpy of fusion at T of racemic compound r is given by

$$(\Delta H_f^T)_r = (\Delta H_f^{(T_m)r})_r + \int_T^{(T_m)r} (C^s)_r \, dT + \int_{(T_m)r}^T (C^l)_r \, dT \quad (8)$$

where $(\Delta H_f^{(T_m)r})_r$ is the enthalpy of fusion at the melting temperature $(T_m)_r$, and $(C^s)_r$ and $(C^l)_r$ are heat capacities of solid and liquid r, respectively.

Since the heat capacities of the enantiomers in the liquid state and of their mixtures are indistinguishable,[8] eqs 7 and 8 can be rewritten as

$$(\Delta H_f^T)_S = (\Delta H_f^{(T_m)S})_S + \int_T^{(T_m)S} (C^s)_S \, dT + \int_{(T_m)S}^T (C^l) \, dT \quad (9)$$

$$(\Delta H_f^T)_r = (\Delta H_f^{(T_m)r})_r + \int_T^{(T_m)r} (C^s)_r \, dT + \int_{(T_m)r}^T (C^l) \, dT \quad (10)$$

(3) Heat of Mixing. Based on Hildebrand and Scott[9] as well as Scatchard,[10] the enthalpy change during liquid solute (A)−solvent (B) mixing is given by

$$\Delta H_{\text{mix}} = \frac{1}{2}(\mu_{AA} + \mu_{BB} - 2\mu_{AB}) \quad (11)$$

where μ is the pair potential energy between two molecules.

Applying eq 11 to the dissolution of S and r in solvent L results in

$$(\Delta H_{\text{mix}})_S = \frac{1}{2}(\mu_{SS} + \mu_{LL} - 2\mu_{SL}) \quad (12)$$

$$(\Delta H_{\text{mix}})_r = \frac{1}{2}(\mu_{RS} + \mu_{LL} - \mu_{SL} - \mu_{RL})$$

since $\mu_{SL} = \mu_{RL}$ for an achiral solvent

$$(\Delta H_{\text{mix}})_r = \frac{1}{2}(\mu_{RS} + \mu_{LL} - 2\mu_{SL}) \quad (13)$$

From eqs 12 and 13

$$(\Delta H_{\text{mix}})_S - (\Delta H_{\text{mix}})_r = \frac{1}{2}(\mu_{SS} - \mu_{RS}) \quad (14)$$

(4) Eutectic Constant. Combining eqs 4, 5, 6, 9, 10, and 14 results in

$$\frac{d \ln K_{\text{eu}}}{d \frac{1}{T}} = -\left(\frac{2}{R}\right) \times \left(\begin{array}{l} \{(\Delta H_f^{(T_m)S})_S - (\Delta H_f^{(T_m)r})_r\} + \\ \int_T^{(T_m)S} [(C^s)_S - (C^s)_r] dT + \int_{(T_m)_s}^{(T_m)_r} [(C^l) - (C^s)_r] dT + \left\{\frac{1}{2}(\mu_{SS} - \mu_{RS})\right\} \end{array} \right) \quad (15)$$

The upper temperature limit is the eutectic melting temperature, where the molar fraction of solvent becomes zero and K_{eu} is determined from the binary phase diagram. This equation (the eutectic constant equation) indicates that K_{eu} is independent of the solvent but changes as a function of temperature.

(c) Eutectic ee in Dilute Solution. For system X, if solute−solute interactions are negligible compared to the interactions between solute and solvent (i.e., dilute solution where Henry's law is obeyed, which is applicable to most crystallization solutions), the activity coefficients for the S enantiomer and the R enantiomer should approximately equal each other. Therefore, we have

$$\frac{[S]}{[R]} \approx \frac{a_S}{a_R} = K_{\text{eu}} \quad (16)$$

From the definition of ee, we obtain

$$\text{ee} = \frac{[S] - [R]}{[S] + [R]} * 100\% = \frac{\frac{[S]}{[R]} - 1}{\frac{[S]}{[R]} + 1} * 100\% = \frac{K_{\text{eu}} - 1}{K_{\text{eu}} + 1} * 100\% \quad (17)$$

Therefore the ee at the eutectic point in dilute solution is determined solely by K_{eu}. This leads to the conclusion that, in dilute solution, the eutectic ee is independent of solvent (achiral) but varies with temperature.

(d) Analysis of Temperature Effect. From eq 15, the rate at which the eutectic constant changes as a function of temperature depends on enthalpy of fusions, heat capacities, and intermolecular homochiral and heterochiral interactions. The first term equals the difference between the enthalpies of fusion of an enantiomer at its melting temperatures and that of the racemic compound. This term is a constant. Experimental data[11] suggest that the enthalpies of fusion range between 5 and 10 kcal/mol for most racemic compounds and their enantiomers. Moreover, Grant et al.[12] have shown that, among 25 racemic species they studied, all racemic compounds have a higher enthalpy of melting than their enantiomers if the racemic compounds are more stable. Therefore the first term of eq 15 can be taken as negative, from several kilocalories per mole to nearly 0 kcal/mol. The second term of eq 15 reflects the difference between the heat required to bring the enantiomer and the racemic compound from temperature T to the melting temperature of the enantiomer. There are minimal experimental data on the heat capacity of enantiomers and their racemic compound re-

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(12) Li, Z. J.; Zell, M. T.; Munson, E. J.; Grant, D. J. W. *J. Pharm. Sci.* **1999**, *88*, 337−346.

corded in the literature. The only data available (Leclercq et al.[11]) showed the specific heats of the pure enantiomers and racemic compounds of mandelic acid and erythro-phenylglyceric acid differ between 5 and 10 cal/mol K in the solid state and vary appreciably with temperature. Although the heat capacity difference between a enantiomer and the corresponding racemic compound is expected to be small in general, much lower than the difference between the enthalpy of melting in most cases, the second term can be significant. This is because the temperature of interest (T) is likely to be much lower than the melting temperature of the enantiomer, so the integration covers a large temperature range. The third term of eq 15 measures the difference between the heat needed to bring the racemic compound and the liquid enantiomer or racemic mixture from the melting temperature of the enantiomer to the melting temperature of the racemic compound. This term is independent of the temperature of interest. It should be positive if the enantiomer melts at a lower temperature than the racemic compound and negative otherwise, since the heat capacity of liquid phase is always higher than that of the corresponding solid phase. Finally the last term of eq 15 accounts for the difference between intermolecular homochiral and corresponding heterochiral interactions in the liquid state at temperature T. This term therefore corresponds to the heat associated with mixing two enantiomers in the liquid state in the absence of solvent. Various investigators estimated the enthalpies of mixing to be between several calories and tens of calories per mole.[13−17] This term's contribution is minimal in most cases. As it can be seen, the sum of the four terms (absolute number) can be quite large for one compound but very small for another. Therefore, K_{eu} can change significantly with temperature or can be insensitive to temperature, which is dependent on the compound of interest.

(e) Calculation of Eutectic ee in Dilute Solutions. Integration of eq 4 from temperatures T_1 to T_2 results in

$$(\ln K_{eu})_{T_2} = (\ln K_{eu})_{T_1} - \int_{T_1}^{T_2} \frac{2[(\Delta H_S)_S - (\Delta H_S)_r]}{RT} \quad (18)$$

From this equation, K_{eu} at T_2 can be calculated from K_{eu} at T_1 if the heat of solution for a pure enantiomer and the racemic compound are obtained. This indicates that the eutectic ee at T_2 can be calculated from the eutectic ee at T_1 (eqs 16 and 17).

Similarly, integration of eq 15 from temperatures T_1 to T_2 results in

$$(\ln K_{eu})_{T_2} = (\ln K_{eu})_{T_1} - \int_{T_1}^{T_2}\left(\frac{2}{RT}\right) \left(\begin{array}{l} \{(\Delta H_f^{(T_m)_S})_S - (\Delta H_f^{(T_m)_r})_r\} + \\ \int_T^{(T_m)_S}[(C^s)_S - (C^s)_r]\mathrm{d}T + \int_{(T_m)_s}^{(T_m)_r}[(C^l) - (C^s)_r]\mathrm{d}T + \left\{\frac{1}{2}(\mu_{SS} - \mu_{RS})\right\} \end{array}\right) \quad (19)$$

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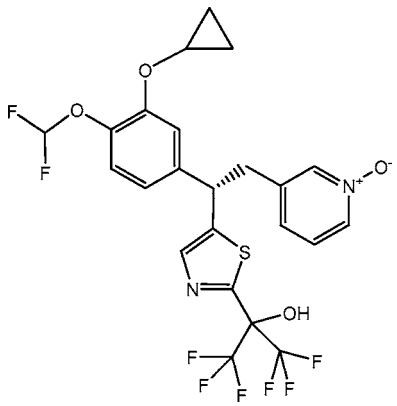

Figure 2. **Structure of compound 1.**

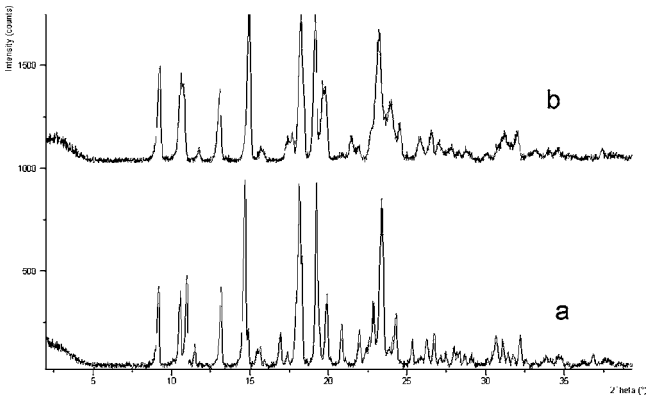

Figure 3. **X-ray powder diffraction pattern of the (a) S enantiomer of compound 1 and (b) racemate of compound 1.**

From this equation, K_{eu} at T_2 can be calculated from K_{eu} at T_1 if melting temperatures, enthalpy of fusions, and heat capacities for a pure enantiomer and the racemic compound are measured (ignoring the small differences between intermolecular homochiral and heterochiral interactions). Therefore, the eutectic ee at T_2 can be calculated from the eutectic ee at T_1.

Results and Discussion

Identification of Racemic Compound. Compound 1 (Figure 2), (+)-3-{2-[(3-cyclopropyloxy-4-difluromethoxy)-phenyl]-2-[5-(2-(1-hydroxy-1-trifluoromethyl-2,2,2-trifluoro)-ethyl)-thiazolyl]ethyl}pyridine *N*-oxide, was identified as a phosphodiesterase-4 (PDE4) inhibitor.[18] Its absolute configuration was assigned as S by X-ray crystallographic analysis.[18] Asymmetric reduction was used to generate a chiral intermediate, which was transformed into compound 1 through achiral reactions. The material generated was expected to have an ee close to 95%, so a crystallization process was required for further enantiomeric purification of the final compound (compound 1).

Figure 3 shows the X-ray powder diffraction patterns of compound 1 (S-1) and the corresponding racemate (an equimolar mixture of two enantiomers whose physical state is unspecified). The R enantiomer (R-1) obtained displayed the same pattern as S-1. However the patterns between S-1 (or R-1) and the racemate are different, indicating that the racemate is not a mechanical mixture of S-1 and R-1. This

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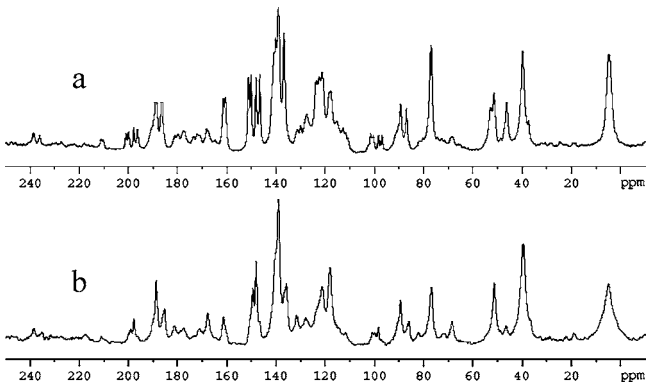

Figure 4. **SSNMR spectra of the (a) S enantiomer of compound 1 and (b) racemate of compound 1.**

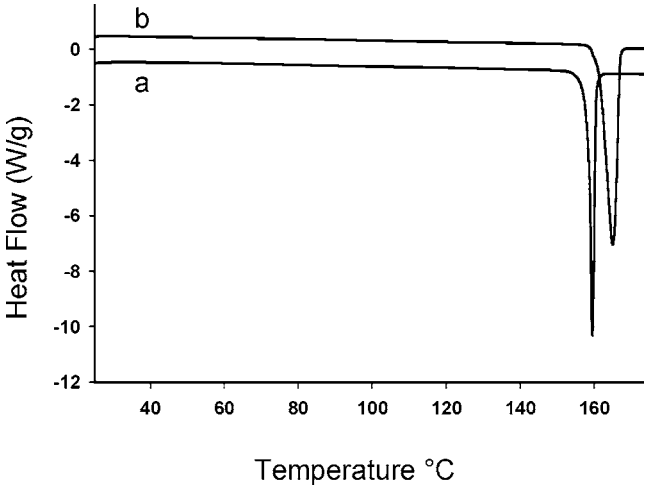

Figure 5. **DSC scans of the (a) S enantiomer of compound 1 and (b) racemate of compound 1.**

is also confirmed by the facts that these two samples displayed distinct solid-state carbon-13 NMR spectra (Figure 4). The thermogravimetric analysis (TGA) determined no more than 0.2% residual solvents in any of the material used, indicating anhydrous materials. Figure 5 shows the DSC traces of S-1 and the racemate. S-1 melts at an extrapolated onset temperature of 158.4 °C with a heat of fusion of 9.09 kcal/mol, and the racemate melts at an extrapolated onset temperature of 161.0 °C with a heat of fusion of 9.88 kcal/mol. These data also indicate that the racemate is not a mechanical mixture of S-1 and R-1. Therefore the racemate is either a racemic compound or a mechanical mixture (conglomerate) of S and R enantiomers which have a different crystal form compared to S-1 and R-1. If the latter were true, the second crystal form of the enantiomers should melt at a temperature higher than the corresponding conglomerate (which would be the racemate) and the heat of fusion should be slightly higher than that of the conglomerate as well.[19] This means the second form of enantiomers compared to S-1 and R-1 would have a higher melting temperature and higher melting enthalpy. The second form would be thermodynamically more stable than S-1 and R-1 based on the theory from Burger and Ramberger.[20] To verify this, mixtures of S-1 and the racemate in various solvents were examined by XRPD after stirring several days to several weeks. No conversion of S-1 to the second form (which would have the same XRPD pattern as the racemate) was

Table 1. **Solubilities of S-1 and the conglomerate in various solvents at 24.2 °C**

solvent	equilibration time (days)	concn of S (mg/mL)	solubility of S-1 (mg/mL)	solubility of conglomerate (calculated) (mg/mL)
n-butyl acetate	10	26.0	25 (±1)	50
	10	24.8		
	14	24.7		
	14	24.3		
dichloroethane	10	20.7	20.8 (±0.5)	42
	10	21.3		
	14	21.0		
	14	20.1		
IPA/H_2O	3	34.8	35.4 (±0.3)	71
	3	35.2		
	5	35.1		
	5	35.6		
	7	35.0		
	7	35.5		
	7	35.6		

observed on this time scale. Therefore, it is concluded that the racemate is not an equimolar mixture of S and R enantiomers which are thermodynamically more stable than S-1 and R-1. Instead, the racemate is a racemic compound (r-1).

Stability of Racemic Compound versus Conglomerate. Comparing the solubility of the racemic compound to the solubility of the conglomerate in the same solvent is the most direct way to determine the relative stability of the two racemates. However, in many cases the limited supply of undesired enantiomer makes this direct comparison difficult. Instead, estimating the solubility of the conglomerate from the solubility of the desired enantiomer becomes a practical measure. It was shown from a theoretical basis in conjunction with experimental data that a conglomerate has approximately 2 times the solubility of the pure enantiomer in a dilute solution (provided that the compound does not dissociate in the solution).[21] The R enantiomer of compound 1 was not available for measuring the solubility of the conglomerate in this case, so the solubility of the S enantiomer was used to estimate the solubility of the conglomerate, which was then used to predict the relative stability of the racemic compound and the conglomerate in conjunction with the solubility data for the racemic compound.

The solubilities of S-1 and r-1 were determined in *n*-butyl acetate, dichloroethane, and IPA/H_2O (1/1 volume ratio). The solubilities of S-1 at 24.2 °C in the three solvent systems are listed in Table 1. An S-1 material with ee above 98% was used for the solubility measurements. The samples were equilibrated for different periods of time and the results indicate equilibria were reached in all three solvent systems. The solubilities of the conglomerate in these solvents were calculated and listed in Table 1 as well.

(19) (a) From Schröder−Van Laar equation, the melting temperature of a conglomerate and corresponding enantiomer has the following relationship: $1/T^f = 1/T^f_A - (R \ln 0.5)/\Delta H^f_A$, where T^f and T^f_A are the melting temperatures of the conglomerate and enantiomer, respectively, and ΔH^f_A is the heat of fusion of the enantiomer. It is clear that the enantiomer melts at a higher temperature than the conglomerate. (b) Jacques, J. and Collet, A. showed that $\Delta H^f_A - \Delta H^f_R$ (the heat of fusion of conglomerate) is positive and lies between 0.4 and 1.2 kcal/mol.

(20) Burger, A.; Ramberger, R. *Mikrochimica Acta* **1979**, **II**, 259−271.

(21) Jacques, J.; Collet, A.; Wilen, S. H. *Enantiomers, Racemates and Resolutions*; Wiley: New York, 1981; p 182.

Table 2. **Solubilities of r-1 in various solvents at 24.2 °C**

solvent	equilibration time (days)	tube no.	concn of S (mg/mL)	concn of R (mg/mL)	solubility of r-1 (mg/mL)
n-butyl acetate	7	1	5.53	5.52	11.07 (±0.04)
	7	2	7.54	4.09	
dichloroethane	7	1	5.57	5.56	11.08 (±0.08)
	7	2	7.38	4.12	
IPA/H_2O	7	1	10.6	10.6	21.1 (±0.3)
	7	2	13.6	8.23	
	7	3	13.2	8.28	

The solubilities of r-1 at 24.2 °C are listed in Table 2. Due to the limited amount of r-1 material available, pure r-1 (0% ee) was used in only one tube for each solvent system. Materials with 17% ee or lower were used in the second and third tubes. The equation used for calculating the solubility of r-1 based on the second and third samples is

$$S_{r-1} = 2\sqrt{[S] * [R]} \tag{20}$$

where [S] and [R] are the concentrations of S and R in the supernatant, respectively. This approach is valid only when the system composition is in the region BrB′ of the ternary phase diagram (Figure 1). In addition, the lower the ee, the more accurate this approach is. In this study, the solubility results generated using material with low ee agree well with the data generated using r-1.

The estimated solubilities of the conglomerate in all three systems are much higher than the solubilities of r-1, respectively. This indicates that the racemic compound r-1 is more stable than the conglomerate. This hypothesis was confirmed by the fact that no conversion of r-1 to S-1 and R-1 was observed during the mixing experiments described earlier.

Eutectic Constant of Compound 1. It was shown in eq 16 that K_{eu} is approximately equal to the ratio of the concentration of major enantiomer to the concentration of minor enantiomer in a dilute solution saturated with one enantiomer and racemic compound. K_{eu} of compound 1 was examined in the three solvent systems listed earlier, using the same S-1 and r-1 material as the solubility measurements (Table 3). The results from the three very different solvent systems (protic and aprotic solvents) are reasonably close to each other. The slight deviation can be attributed to the fact that the activity coefficients of S and R are slightly different since solute−solute interactions do not equal zero although they are much smaller than solute−liquid interactions in dilute solution. Taking this into account, the results agree with the conclusion from the mathematical derivation that the eutectic constant is independent of solvent.

K_{eu} of compound 1 was also measured at 5.2 °C in *n*-butyl acetate and dichloroethane, using the same S-1 and r-1 material as above (Table 4). The precision of the results are not as good as for the data collected at 24.2 °C, possibly due to the slow process of dissolution−crystallization of enantiomer and racemic compound at low temperature. At 5.2 °C, K_{eu} of compound 1 acquired from the *n*-butyl acetate system is in good agreement with that from the dichloroethane system. In addition, K_{eu} at 5.2 °C is significantly different from that at 24.2 °C, which is also in agreement with the conclusion from mathematical derivation that K_{eu} is dependent on temperature.

Jacques et al.[22] demonstrated the application of the Schröder−Van Laare equation and Prigogine−Defray equation in the binary phase diagram of the racemic compound system. With this approach, the eutectic temperature and eutectic composition of the binary mixture of compound 1 can be calculated using melting temperatures and heats of fusion of S-1 and r-1. The results obtained are 423.94 K and 0.8258/0.1742 (S/R). Therefore the K_{eu} at 150.8 °C is 4.74, and the eutectic ee is 65.2%, which is significantly different from those at 24.2 °C and 5.2 °C. This again proves the conclusion that the eutectic constant and thus the eutectic ee are dependent on temperature. The results show that it is misleading to use a calculated or measured eutectic composition at the binary eutectic temperature in place of the eutectic ee at the temperature of interest.

Strategy of Developing a Crystallization Method for Compound 1. The eutectic ee's for this compound ranged from 88.8% at 5.2 °C to 65.2% at 150.8 °C. Since the starting material is expected to have an ee above the eutectic ee at a given temperature, the desired enantiomer (S) can be isolated in the solid phase. Therefore, the lower the eutectic ee is at the separation condition, the lower the loss of the desired S in the filtrate will be. As discussed earlier and supported by the experimental data, the eutectic ee is independent of achiral solvent(s) in dilute solution if no solvates are formed, so no time should be wasted in screening various solvents hoping for a significant lower ee. Instead, a screening for solvents that can form solvates with S may identify a system that has significantly lower eutectic ee. Moreover, increasing crystallization temperature will decrease the eutectic ee and therefore reduce the loss of the desired enantiomer. By employing the guidance of the eutectic constant equation, a clearer picture with regards to developing an efficient and robust purification procedure may be obtained.

Conclusions

For chiral systems where racemic compounds are more stable than the conglomerates, and where no solid solution competes, a new parameter, the eutectic constant, was introduced. A mathematical relationship between the eutectic constant and the thermodynamic parameters of an enantiomer and the racemic compound was obtained. The equation indicates that the eutectic constant is independent of solvent (achiral) properties if no solvates are formed. In most cases, crystallization solutions can be treated as dilute solution, so the eutectic ee is solely determined by the eutectic constant. Therefore, the eutectic ee is independent of solvent in dilute solution. The equation also indicates that the eutectic constant, thus eutectic ee in dilute solution, varies with temperature. Applying the eutectic ee determined from a binary phase diagram in the place of eutectic ee for a ternary system (which would be at a different temperature) is incorrect. With the guidance of the eutectic constant equation, the time and material required to develop a crystallization

(22) Jacques, J.; Collet, A.; Wilen, S. H. *Enantiomers, Racemates and Resolutions*; Wiley: New York, 1981; p 90.

Table 3. **Eutectic constant of compound 1 at 24.2 °C**

solvent	equilibration time (days)	concn of S (mg/mL)	concn of R (mg/mL)	eutectic constant	average	eutectic ee
n-butyl acetate	8	23.8	1.84	13.1 (±0.2)	12.7	85.4%
	8	23.7	1.78			
	8	23.6	1.83			
dichloroethane	8	20.0	1.59	12.4 (±0.3)		
	8	19.2	1.54			
	8	21.7	1.80			
IPA/H_2O	8	33.2	2.66	12.6 (±0.1)		
	8	33.4	2.66			
	8	33.4	2.63			

Table 4. **Eutectic constant of compound 1 at 5.2 °C**

solvent	equilibration time (days)	concn of S (mg/mL)	concn of R (mg/mL)	eutectic constant	average	eutectic ee
n-butyl acetate	7	16.3	0.87	17.2 (±2)	16.9	88.8%
	7	17.2	1.09			
	10	16.6	1.09			
	14	17.4	0.88			
	14	16.9	0.95			
	14	16.3	1.02			
dichloroethane	14	12.0	0.85	16.5 (±2)		
	14	10.7	0.58			
	14	11.9	0.71			

procedure for ee enhancement in dilute solution can be significantly reduced.

Experimental Section

Materials. Compound 1 was prepared at Merck Research Laboratories, Rahway, New Jersey. *n*-Butyl acetate (99+%) and 1,2-dichloroethane (99%) were purchased from Aldrich. 2-Propanol (99.9%), hexanes (98.5+%), and ethanol (200 proof) were purchased from Fisher.

XRPD. The data were generated on a Philips Analytical X′Pert PRO X-ray Diffraction System with a PW3040/60 console. A PW3373/00 ceramic Cu LEF X-ray tube K-Alpha radiation was used as the source. The experiments were run at ambient condition.

SSNMR. The solid-state carbon-13 NMR spectrum was obtained on a Bruker DSX 400WB NMR system using a Bruker 7 mm double resonance CPMAS probe. The carbon-13 NMR spectrum utilized proton/carbon-13 cross-polarization magic-angle spinning with variable-amplitude cross polarization. The sample was spun at 5.0 kHz, and a total of 1024 scans were collected with a recycle delay of 5 s. A line broadening of 40 Hz was applied to the spectrum before FT was performed. Chemical shifts are reported on the TMS scale using the carbonyl carbon of glycine (176.03 ppm) as a secondary reference.

TGA. Thermogravimetric analyses were conducted using a Perkin-Elmer TGA 7 or Pyris 1 thermogravimetric analyzer. A heating rate of 10 °C/min was employed, and a nitrogen purge was used. The balance was calibrated using a standard weight, and the sample temperature was calibrated using Curie-point standards.

DSC. Thermograms were acquired using a TA Instrument Q1000 differential scanning calorimeter. The experiments were run in a crimped pan with nitrogen flow at a heating rate of 10 °C/min. Calibration of the temperature and cell constants were performed with indium under the same condition.

Solubility Measurement. A solid sample was suspended in solvent in a sealed glass tube and agitated in a temperature controlled water bath for a period of time. After the equilibration, the solid was allowed to settle by rapid centrifugation, and the glass tube was then opened and the supernatant was filtered and then diluted. The concentration of the solution was determined by HPLC, and the solubility was calculated. The remaining solids from each tube were analyzed by XRPD.

HPLC Analysis. The chromatographic system used was an Agilent 1100 HPLC system equipped with a diode array detector. Chromatograms were processed using a PE Nelson version 3.1 data acquisition system (Cupertino, CA). The column employed was Chiralcel OD-H, 250 mm × 4.6 mm i.d., particle size 5 μm. The chromatographic experiments were carried out in isocratic mode with a premixed mobile phase of 80 v/v% hexanes and 20% ethanol (dried under sieves, water less than 0.1 w/w%). The flow rate used was 0.6 mL/min, and the wavelength for detection was 215 nm. The target concentration was 0.6 mg/mL, diluent was ethanol, and the injection volume was 10 μl. The total run time was 35 min, and the pressure of the system was 28 bar.

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Supporting Information Available

Derivation of eq 20. This material is available free of charge via the Internet at http://pubs.acs.org.

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