Resolution of Racemic Mixtures by Phase Transition of PEGylated Resolving Agents

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Supporting Information

ABSTRACT: A novel, efficient, and simple method for the resolution of racemic mixtures is presented in which PEGylated resolving agents are subjected to diastereomeric complex formation in alcohols. The resulting complexes then undergo temperature-assisted phase transition, affording a precipitate that is enriched in one enantiomer and separable by filtration. In an aqueous solution, phase transition can be caused by the methods used in the precipitation of poly(ethylene glycol) (e.g., addition of ammonium sulfate). A number of racemic amines have been successfully resolved using this method. The first cycle of resolution affords the amines with an optical purity of 72–85% from their corresponding racemic mixture in good yields (78–90%). An additional cycle improved the optical purity to 87–95%. The PEGylated resolving agents can be recovered and reutilized without the loss of resolution efficiency.

1. INTRODUCTION

Chirality is a trademark of complexity and elegance of biological systems. Amino acids, proteins, carbohydrates, nucleosides, most alkaloids, and hormones are chiral compounds. The broad utility of chiral molecules in an optically pure form as biologically active compounds (pharmaceuticals and agrochemicals), additives for modification of polymer properties, and their application in electronic and optical devices is due to the importance of stereochemistry—the endeavors in which the recognition of amines with an optical purity of 72–85% from their corresponding racemic mixture in good yields (78–90%). An additional cycle improved the optical purity to 87–95%. The PEGylated resolving agents can be recovered and reutilized without the loss of resolution efficiency.

since the first separation of enantiomers by Louis Pasteur in 1848, a continuous search for new and efficient resolution procedures has been carried out, and many ingenious methods have been reported. To name a few, this includes the use of optically active resolving agents such as α-phenylethanesulfonic acid, d- or L-phenylglycine, R-mandelic acid, cinchona, and other alkaloids, polymers with chiral moieties such as poly(N-methacryloyl)-d-lysine, poly(N-isopropylacrylamide-co-ε-N or S-cis-butylacrylamide), polymer with chiral selector d-(-)-ditoluoyltartaric acid, and lysine-bearing vinyl monomer (S)-2-(tert-butoxycarbonylamo)-6-(4-vinylbenzamido)hexanoic acid. All of the reported methods are based on the formation of salt pair complexes between a resolving agent (itself a chiral molecule) and both enantiomers. Such complexes are endowed with two or more chiral centers and can therefore be thought of as diastereomers, which are known to have different physical properties and are therefore separable from one another by precipitation in a suitable solvent under appropriate conditions. The preference for crystallization of one salt pair over the other one is based on a lower free energy of formation. This is followed by separation from the solution and subsequent liberation of the enantiomer from the isolated complex.

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The two determining factors in a successful resolution of enantiomers are the choice of the proper resolving agent and a suitable solvent for crystallization. Chiral acids are valuable tools for the resolution of racemic amines and chiral amines are used for the resolution of enantiomeric acids. Salt formation per se does not result in resolution, which depends on the secondary parameters such as van der Waals and Coulombic interactions, H-bonding, and covalent derivative formation. However, most of the reported resolution methods are cumbersome and require multistage and complicated operations with limitations for industrial-scale resolution. Furthermore, the purified product usually requires further cycles of resolution to obtain the desirable optical purity. Consequently, besides the traditional well-known acid and amine resolving agents, the quest for new useful compounds with chiral moieties incorporated in their structures continues and it is desirable to have an efficient and simple process for the resolution of racemic mixtures that is easily scalable to industrial production.

PEGylation is the process of attaching strands of the poly(ethylene glycol) (PEG) to various molecules to endow them with new physicochemical properties. It has been applied successfully to peptides, proteins, and antibody fragments to improve the safety and efficiency of biopharmaceuticals by causing changes in conformation, electrostatic binding, hydrophobicity, etc. PEGylation of therapeutic agent can increase their systemic retention, influence their binding affinity to cell receptors, and alter their absorption and distribution patterns. PEG has been also used extensively for protein precipitation in the production of various biopharmaceuticals such as monoclonal antibodies. However, the use of PEG-assisted resolution of racemic mixtures has not been reported.

2. RESULTS AND DISCUSSION

The most fundamental aspect of classical resolution is the salt formation between an amine and an acid. For example, when (1)-phenylalanine methyl ester was used as the resolving agent for the resolution of a racemic mixture of mandelic acid in diethyl ether, both diastereomeric salt pairs (R)-mandelic acid–(1)-phenylalanine methyl ester and (S)-mandelic acid–(1)-phenylalanine methyl ester precipitated immediately in equal quantities (Section 3.10). This is due to the fact that both precipitates are kinetic products with little or no difference in their free energies of formation. On the other hand, when the above experiment was carried out in ethanol, only (R)-mandelic acid–(1)-phenylalanine methyl ester was reported to precipitate after 24 h at 0 °C. This demonstrates the importance of the solvent in obtaining the thermodynamic product, the formation of which involves a number of factors other than simple salt formation. These include van der Waals and Coulombic interactions as well as H-bonding, ultimately resulting in more densely packed crystals with a lower lattice free energy and a higher thermodynamic stability. Therefore, ethanol is a “suitable” solvent in the above example because it allows for these interactions to come into play.
affording the lower energy complex as the desired precipitate.

It is clear from the above discussion that traditional resolution is achieved by free energy-dependent preferential crystallization of one diastereomeric salt pair over the other. This is time consuming and cumbersome because the suitable solvent as well as appropriate conditions for crystallization must be identified to afford the thermodynamic product. It would therefore be very desirable to expedite the process of precipitation of the desired enantiomer without affecting the enantiomeric recognition of the resolving agent. The present work is the result of endowment of known resolving agents with the physicochemical properties of poly(ethylene glycol) (PEG), which has afforded a new class of resolving agents with the retention of the original enantiomer recognition property as well as phase-transition properties unique to PEG. The properties of PEG imparted to the PEGylated resolving agents include phase transition of the complex from solution to solid by reducing the temperature in alcohols or by the addition of salt in water. The new resolving agents greatly facilitate the process of resolution of enantiomers. Their synthesis and utilization is shown in Figure 1.

Poly(ethylene glycol)-10 000 (I) (PEG-10 000) is activated with thionyl chloride, and the activated PEG (II) is condensed with an optically pure resolving agent such as (α)-hydroxy- or α-amino acid (III) through the hydroxy or the amine function of the resolving agent to afford optically pure PEGylated-(α)-hydroxy acid or PEGylated-(α)-amino acid (IV). In the general procedure for resolution, the optically pure PEGylated resolving agent (IV) is dissolved in methanol and racemic mixture of an amine such as DL-phenylalanine methyl ester (V) is added. The mixture is stirred for 4 h at room temperature and then cooled to 0−5 °C for 1 h, resulting in temperature-assisted phase transition of the desired diastereomeric salt pair in a few minutes (Section 3.5). The precipitate is isolated by filtration and the product is acidified with concentrated hydrochloric acid to liberate the desired phenylalanine methyl ester in good yields and good optical purity (Table 1, entries 1 and 2). The use of three different amines demonstrated the general applicability and scope of the method. Data on the resolution of the amines tested by this process are presented in the table below. The products can be subjected to another resolution cycle to increase their optical purity (vide infra).

The above table demonstrates that the racemic mixtures of phenylalanine methyl ester, 2-amino-1-butanol, and 1-phenylethylamine are resolved with PEGylated-(R)-mandelic acid and PEGylated-L-valine in good yields and with high enantiomeric excess in methanol. Also, improvement in % ee of resolved phenylalanine methyl ester, 2-amino-1-butanol and 1-phenylethylamine were achieved by an additional cycle of resolution with PEGylated-(R)-mandelic acid (Table 1). Furthermore, other investigators have reported improvement of % ee of amines from 96 to >99% by simple salt formation.48

It is interesting to note that the classical resolution of a racemic mixture of phenylalanine methyl ester with (R)-mandelic acid in methanol did not result in the precipitate formation even after standing at −15 °C for 24 h. However, the use of PEGylated-(R)-mandelic acid as the resolving agent in methanol afforded PEGylated-(R)-mandelic acid−(L)-phenylalanine methyl ester after cooling for a few minutes (Section 3.5). Water may also be used as a solvent. However, in contrast to methanol, the phase transition of the PEGylated-(R)-mandelic acid−(L)-phenylalanine methyl ester salt pair could not be achieved by cooling and required the addition of ammonium sulfate (Section 3.7), which is known to result in the precipitation of PEG.49,50 As indicated above, there are numerous interactions that come into play during the process of diastereomeric salt formation and precipitation. This is further complicated by the nature of the solvent used.51 Moreover, it has been reported that the functionalization of resolving agents increases their rigidity, which in turn affords a higher enantiomeric excess of the product.48 It should be noted that PEGylation, which can be considered as macromolecular functionalization of the resolving agent, does not affect the resolution characteristic of the resolving agent. It simply simplifies and expedites the process of precipitation. As indicated above, Wong52 has reported the formation of (R)-mandelic acid−(L)-phenylalanine methyl ester diastereomeric salt in ethanol after 24 h at 0 °C. The same diastereomic salt is also formed when PEGylated (R)-mandelic acid was used (Section 3.5). Furthermore, in the latter case, salt formation occurred in a few minutes at 0 °C.

It should also be emphasized that the resolution efficiency of various resolving agents is conveyed to their PEGylated analogues. For example, (R)-mandelic acid is known to be a better resolving agent for the resolution of amino acid esters than valine52 and this was also observed in terms of a better resolution efficiency of PEGylated mandelic acid over PEGylated valine. The (L)-phenylalanine methyl ester liberated from the above PEGylated complexes by acid treatment had optical purities of 85% using PEGylated (R)-mandelic acid and 75% using PEGylated l-valine (Table 1 and Sections 3.5 and 3.6).

Excellent mass balance corresponding to optical rotation of the enantiomers was observed in all of the cases discussed above in that the filtrate in each case contained the other diastereomeric salt pair. For example, acid treatment of the filtrate in entries 1 and 2 of the table liberated (d)-phenylalanine methyl ester hydrochloride with an optical purity of 85 and 75%, demonstrating equal and opposite optical rotation to the optical purity of the product obtained from precipitation.

Our results demonstrate that imparting the unique characteristics of PEG to various resolving agents expedites the process of crystallization of the desired enantiomer. For example, when classic resolution of a racemic mixture of phenylalanine methyl ester

### Table 1. Resolution of Racemic Amines by PEGylated Resolving Agents

| Entry | Resolving Agent | Amines | Solvent | [α]θ,deg | Yield (%) | % ee first cycle | % ee second cycle | Ref |
|-------|----------------|--------|---------|----------|-----------|-----------------|------------------|-----|
| 1     | PEGylated-(R)-mandelic acid | (rac)-phenylalanine methyl ester | MeOH | +28 | 90 | 85 | 95 | 45 |
| 2     | PEGylated-(L)-valine | (rac)-phenylalanine methyl ester | MeOH | +24 | 81 | 75 | 45 |
| 3     | PEGylated-(R)-mandelic acid | (rac)-2-amino-1-butanol | MeOH | −8.6 | 86 | 81 | 46 |
| 4     | PEGylated-(L)-valine | (rac)-2-amino-1-butanol | MeOH | −7.1 | 78 | 72 | 46 |
| 5     | PEGylated-(R)-mandelic acid | (rac)-1-phenylethylamine | MeOH | −21.6 | 82 | 83 | 91 | 47 |
| 6     | PEGylated-(L)-valine | (rac)-1-phenylethylamine | MeOH | −19.1 | 80 | 76 | 47 |

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ester was carried out with (R)-mandelic acid in methanol, no precipitate was formed even after 1 week at −15 °C (Section 3.9). However, the use of PEGylated (R)-mandelic acid expedites the crystallization process as shown in Section 3.5 (methanol: 0–5 °C, 5 min). In the aqueous medium, immediate precipitation of the desired salt pair was observed upon the addition of ammonium sulfate (Section 3.7).

The use of acetonitrile, tetrahydrofuran, ethyl acetate, dioxane, halogenated solvents, or water did not result in the precipitation of the PEGylated diastereomeric complex by cooling of the mixture. However, methanol or ethanol afforded the complex as a precipitate at 0 °C overnight, a property imparted to the PEGylated resolving agent (vide supra). The underlying principle for salt-assisted phase transition of PEG from solution to solid in the aqueous solution has been proposed to be the interference of the added salt in the H-bond formation between the numerous oxygens of the polyether and the water molecules surrounding PEG. It has been suggested that such interference results in an increase in the local concentration of PEG and this in turn causes its precipitation in the aqueous medium.51

3. EXPERIMENTAL SECTION

3.1. Material and Methods. PEG was obtained from Kemiagaran Emrouz Ltd., Arak, Iran. All of the reagents were obtained from commercially available sources such as Sigma-Aldrich or Merck and were used without further purification. Thin-layer chromatography was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm). 1H NMR spectra were measured in CDCl3 with a Bruker DRX-500 Avance spectrometer at 500.13 MHz. Mass spectra were recorded on Avatar 380 (100), 55 (76), 43 (62).

3.2. Preparation of Activated PEG (Chlorination of PEG). PEG (10 000) (50 g, 5 mmol) was dissolved in 500 mL of toluene, followed by the distillation of 100 mL of the solvent to remove traces of moisture. After cooling to 35 °C, freshly distilled anhydrous triethylamine (3.75 mL, 27 mmol) was added. Freshly distilled thionyl chloride (1.5 mL, 21 mmol), distilled anhydrous triethylamine (3.75 mL, 27 mmol) was then dissolved in 50 mL of dry toluene, was then added dropwise over 1 h at 35 °C under a dry nitrogen atmosphere with continuous stirring. The mixture was refluxed for 1 h and triethylammonium hydrochloride was removed by passing the hot solution through a bed of Celite. The solution was treated with 5 g of decolorizing carbon at 50 °C and filtered over Celite. The filtrate was stored at 4 °C overnight, affording activated PEG, which was filtered at 4 °C. The solid product was further purified by dissolving in 2.5 L of absolute ethanol at 60 °C and treating with 30 g of decolorizing carbon, followed by filtration over Celite. The ethanolic filtrate was stored overnight at 4 °C. The solid material was separated by filtration and washed with cold ethanol and then ether. After drying in a vacuum desiccator, 49 g of a pale yellow product was obtained (97%).

3.3. Preparation of PEGylated-(R)-Mandelic Acid. (R)-Mandelic acid (0.6 g, 4 mmol) was dissolved in 200 mL of acetonitrile, followed by the addition of potassium carbonate (1.1 g, 8 mmol). Activated PEG (10 000) (20 g, 2 mmol), prepared in Section 3.2 was dissolved in 50 mL of acetonitrile and added dropwise over 30 min with continuous stirring at 25 °C. Thereafter, the resulting pale yellow solution was stirred at reflux for 24 h. The reaction mixture was then cooled to ambient temperature and adjusted to pH 2–3 using concentrated hydrochloric acid. This solution was treated with decolorizing charcoal and filtered over a layer of Celite. The filtrate was evaporated under reduced pressure to obtain a solid that dissolved in methanol and stored at 4 °C overnight. The solid material was separated by filtration and washed with cold methanol and then ether. After drying in a vacuum desiccator, 18 g of a pale yellow product was obtained (88%). The product was characterized by NMR and mass spectra.1H NMR (500 MHz, CDCl3): δ 3.57 (s, CH2), 7.21–7.40 (m, 10H of Ar), 9.22 (bs, OH), 5.12 (s, CH). MS (EI) m/z: 152 (15), 107 (80), 79 (100).

3.4. Preparation of PEGylated-(L)-Valine. L-Valine (0.469 g, 4 mmol) was dissolved in 200 mL of acetonitrile. Triethylamine (1.11 mL, 8 mmol) was added to the reaction mixture. Activated PEG (10 000) (20 g, 2 mmol), prepared in Section 3.2, was dissolved in 50 mL of acetonitrile and added dropwise to the reaction mixture with continuous stirring at 30 °C. The resulting yellow solution was stirred at room temperature for 24 h and then treated with decolorizing charcoal and filtered over a layer of Celite. The filtrate was evaporated under reduced pressure to obtain a solid, which was dissolved in methanol and the solution was stored at 4 °C. The solid material was separated by filtration and washed with cold methanol and then ether. After drying in a vacuum desiccator, 18.5 g of a pale yellow product was obtained (90%). The product was characterized by NMR and mass spectra.1H NMR (500 MHz, CDCl3): δ 0.98 (m, 2CH3), 2.63 (m, CH(CH3)n), 3.59 (s, nCH3), 3.59 (m, CH(NH)). MS (EI) m/z: 116 (15), 72 (100), 55 (76), 43 (62).

3.5. General Procedure for the Resolution of Racemic Amines with PEGylated-(R)-Mandelic Acid. PEGylated-(R)-mandelic acid (10 g, 1 mmol) was dissolved in 50 mL methanol followed by the addition of racemic mixture of the amine (2 mmol) and the mixture was stirred at room temperature for 12 h. It was then cooled to 0–5 °C and stirred for 1 h. A voluminous precipitate of white solids was formed, followed by the addition of 20 mL cold methanol. The slurry was filtered and the cake was washed with 10 mL cold methanol to afford a white solid consisting of optically impure PEGylated-(R)-mandelic acid—(R or S)-amine. The cake was then dissolved in 50 mL methanol and acidified with concentrated hydrochloric acid and cooled to 0–5 °C. The resulting white solid was filtered and washed with 10 mL cold methanol. The filtrate was evaporated under reduced pressure to dryness at 50 °C to afford (R or S)-amine hydrochloride. Yields and optical purities are shown in Table 1.

3.6. General Procedure for the Resolution of Racemic Amines with PEGylated-(L)-Valine. Using the method of Section 3.5, PEGylated-(L)-valine was used for resolving of racemic amines. Yields and optical purities are shown in Table 1.

3.7. Ammonium Sulfate-Assisted Resolution of Racemic Phenylalanine Methyl Ester with PEGylated-(L)-Mandelic Acid in Water. PEGylated-(R)-mandelic acid (10 g, 1 mmol) and racemic phenylalanine methyl ester (0.36 g, 2 mmol) were added to 50 mL water and the mixture was stirred at room temperature for 12 h. The resulting mixture was cooled to 0–5 °C and stirred for 1 h. Unlike Section 3.5, no
precipitation occurred. Solid ammonium sulfate (34.85 g, 0.26 mol) was added to the mixture. After 1 h, a voluminous precipitate of a white solid occurred. The slurry was filtered and the cake was washed with 10 mL saturated ammonium sulfate. The cake, which consisted of optically impure PEGylated-(R)-mandelic acid–(L)-phenylalanine methyl ester, was dissolved in 50 mL water and acidified with concentrated hydrochloric acid and stirred for 1 h to liberate (L)-phenylalanine methyl ester hydrochloride. Dry ammonium sulfate was then added to the resulting solution to precipitate PEGylated (R)-mandelic acid, which was filtered and the cake washed with 10 mL saturated ammonium sulfate. The filtrate was adjusted to pH 7 using sodium bicarbonate (1 M) to neutralize (L)-phenylalanine methyl ester. The resulting white precipitate was filtered, washed with 5 mL water, and dried to afford 0.20 g of optically impure (D)-phenylalanine methyl ester (yield: 93%). Specific optical rotation of +24 (EtOH, c = 2) was obtained, corresponding to 75% of theory.

3.8. Improvement of Optical Purity by an Additional Cycle of Resolution. A mixture of PEGylated-(R)-mandelic acid (5 g, 0.5 mmol), optically impure (R or S)-amine (Table 1, 1 mmol), and 25 mL methanol was stirred at room temperature for 12 h and then cooled to 0–5 °C with stirring for 1 h. A voluminous white precipitate was formed, followed by the addition of 20 mL cold methanol. The slurry was filtered and washed with 10 mL cold methanol. The resulting white solid was dissolved in 50 mL methanol and acidified with concentrated hydrochloric acid to pH 2–3 and cooled to 0–5 °C. The white solid was filtered and washed with 10 mL cold methanol. The filtrate was evaporated to dryness at 50 °C under vacuum to afford (R or S)-amine hydrochloride with improved % ee (87, 91, and 95% for 2-amino-1-butanol, 1-alanine methyl ester, and phenylalanine methyl ester, respectively).

3.9. Indiscriminate Precipitation of L- and D-Phenylalanine Methyl Ester–(R)-Mandelic Acid Salt Pairs in Diethyl Ether. To a solution of racemic mixture of phenylalanine methyl ester (0.72 g, 4 mmol) in diethyl ether (10 mL) was added (R)-mandelic acid (0.6 g, 4 mmol). A white precipitate formed immediately and the mixture was stirred at room temperature for an hour. The mixture was then adjusted to pH 8 using 1 M solution of sodium bicarbonate to afford a biphasic mixture. The organic phase was washed with 5 mL water and dried over MgSO₄. The solvent was removed under reduced pressure to afford 0.72 g racemic phenylalanine methyl. This is equal to the quantity of racemic mixture of phenylalanine methyl ester used, indicating indiscriminate precipitation of L- and D-phenylalanine methyl ester–(R)-mandelic acid complexes in diethyl ether.

3.10. Indiscriminate Precipitation of R- and S-Mandelic Acid–(L)-Phenylalanine Methyl Ester Complexes in Diethyl Ether. The procedure of Section 3.9 was followed using (0.6 g, 4 mmol) a racemic mixture of mandelic acid, which afforded 0.6 g racemic mandelic acid. Again, this is equal to the quantity of racemic mixture of mandelic acid used, indicating indiscriminate precipitation of L- and D-phenylalanine methyl ester–(R)-mandelic acid complexes in diethyl ether.

**ASSOCIATED CONTENT**

- Supporting Information
  The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01070.

- **H** NMR and mass spectra of PEGylated resolving agents (PDF)

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**Notes**
The authors declare no competing financial interest.

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