UNIVERSITY OF TURKU

Environmentally friendly racemization process for the benzofuroquinolizines

A process for the racemization of T1143 for waste-stream recovery

Chemistry of Drug Development

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Master of Science in Chemistry

Author:

Müjgan Gamze Üstbas

Supervisors:

Prof. Pasi Virta (University of Turku)

Sr. Process Engineer Pasi Nieminen (Seqens)

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Abstract:

Control of racemization and chirality are of high importance in chemistry of active pharmaceutical ingredients (APIs), in terms of drug design, development and production. Even though it is desired to develop and produce *de novo* enantiopure compounds, certain syntheses cannot be achieved to yield enantiopure end-products. Thus, mastering the racemization techniques is of great importance to be able to recover the product from its stereoisomer in the process where enantiopure synthesis cannot be achieved. At the same time, financial burden and the environmental impact of the synthetic process should be managed. Herein, a novel and environmentally friendly method for racemizing a commercially available veterinary active pharmaceutical ingredient (VAPI) intermediate, named T1143, is introduced. T1143 is a small molecule containing a tertiary nitrogen, a ketone group, and a stereogenic center of interest at β-position to the ketone group. Racemization of this VAPI intermediate has been studied by various methods to recover the loss of half of the desired product, *(S)*-T1143, during production and purification. *(R)*-T1143, the undesired enantiomer, is collected from the waste stream, and converted into *(S)*-T1143 through formation of aza-retro-Michael/aza-Michael equilibrium by refluxing it at certain concentrations in aqueous solutions. This equilibrium mechanism leads to the racemization of benzofuroquinolizine-ones where the *(R)*-enantiomer can be converted into a racemic mixture, *(RS)*-T1143, in an efficient and environmentally friendly manner. The resolution of *(RS)*-T1143 is achieved by converting it to the (-)–*O, O'*- Di-p-toluoyl-L-tartaric acid (DTTA) salt, which can be crystallized in the presence of the corresponding *(S)*-T1143 salt.

Key Words: enantiomerization, chiral API, Michael reaction, aza-retro Michael, equilibrium, environmentally friendly, stereogenic center, racemization, conversion of enantiomers.

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Sincerely,

Müjgan Gamze Üstbas

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1 INTRODUCTION

1.1 Introduction to chirality.

Chirality is one of the key concepts in biological processes since natural systems have a proclivity for chirality. The building blocks of life, such as amino acids that have L-configuration or carbohydrates that have D-configuration, are chiral and thus biological systems that are created from these elements have a chiral distinction. As a result, physiological processes undergoing in biological systems have various response mechanisms dependent on the interactions of chiral compounds.[1] The compounds that have a stereogenic center and are non-superimposable by their mirror images are called enantiomers and they possess the same properties in non-chiral environments. Nonetheless, their properties differ when they encounter a chiral environment. Biological systems are the chiral environments that lead to changes in the interactions with the chiral drug molecules. Thus, two enantiomers of a molecule are likely to induce completely different responses in biological systems. Eutomer is the enantiomer that is active towards the preferred binding site and induces the desired effect, whereas the distomer is the inactive stereoisomer that might not bind at all or it binds to another target that leads to formation of an unwanted response.[2,3,4] Controlling the chirality of the drug substances is of utmost importance to provide specific interaction between the drug molecules and the target.[3] These selective interactions, stemming from the chiral nature of life, bestows varying pharmacological or pharmacokinetic properties to each drug compound. These diversified properties may affect the living systems over a range; from a completely undesired process taking place to efficacy of the drug molecule being diminished.[2,4] Thus, it is paramount for the active pharmaceutical ingredients (APIs) entering the biological systems to be under strict stereochemical control.

1.2 Drug substances containing stereogenic centers.

Synthesis of chiral pharmaceutical compounds as a single enantiomer may follow *de novo* enantiopure synthesis route or the desired enantiomer can be isolated from its racemic mixtures by different methods.[2] Synthesizing a drug substance as an enantiopure molecule requires an enantiopure starting material and / or integrating stereoselective synthesis steps (such as utilization of enzymes or chiral catalysts of high costs) to the synthesis. On the other hand,

chiral resolution provides an alternative that may usually be perceived as a lower-cost method. It also provides the benefit of evaluation and characterization of both enantiomers.

Despite the market consist of approximately 50% of chiral small molecules; less than a quarter of the chiral drug molecules are marketed in their pure enantiomeric form.[1] Enantiopure drugs consist of purely eutomers and are not marketed as a racemic mixture.[1,4] As mentioned in the previous section, chiral properties become prominent in chiral environments, i.e. biological systems. Therefore, chiral molecules being used as therapeutic agents may alter the interaction and / or selectivity of pharmaceuticals with the biological systems[4] and, chiral compounds encompass a high percentage presence in the market, i.e. about half of the small molecule drugs in the market are chiral. Yet, it is also reported that approximately a quarter of these chiral molecules are marketed as pure enantiomers and rest is still formulated as the racemate.[1] Even though the number of enantiopure to racemate drug molecules in the current market is relatively low, enantiopure drug molecules are becoming more prominent [1,4] because of the different pharmacokinetic properties, metabolic activities or toxicological profiles of enantiomers.[2,5] The presence of the undesired enantiomer (distomer) can cause dilution of the effect of the eutomer in the case where the two enantiomers bind to the same receptor but, e.g. one acts as the agonist while the other acts as the antagonist. It is also possible for the distomer to be the source of various adverse effects, if it induces a different biological activity at completely different sites.[5] The thalidomide drug molecule is one example where the eutomer, *(R)*-enantiomer, acting against the morning sickness experienced during pregnancy while the distomer leading to teratogenic effects by inducing a completely different mechanism in the body.[4,5]

Potency differences is another aspect of evaluation. When a chiral API is formulated as the racemic mixture, there also may be an issue of reduced potency. One prominent example is a very common API, ibuprofen. Ibuprofen is a molecule under the classification of non-steroidal anti-inflammatory drug (NSAID) and contains a stereogenic center. While it had been marketed as a racemate before it was found that the *(S)*-enantiomer is 100-fold more potent than the *(R)*-enantiomer. Therefore, it started to be marketed as the single enantiomer in 1994. Although the evidence suggested that *(R)*-enantiomer acts as a pro-drug for the *(S)*-enantiomer when entered the biological system as the racemate, enantiopure formulation became more appealing because of the increase in potency and thus allowing for lower dosage formulations.[1,3] Therefore, choosing enantiopure drug substances instead of the racemic mixtures in the formulation may provide a more reliable therapeutic response in each biological system by i.e. reducing the different response time by eliminating the pro-drug activation time which differ in each

individual. Thus, formulation of single enantiomer drugs may provide better pharmacokinetic and pharmacodynamic properties.[1,3,4]

1.3 Resolution of enantiomers.

As mentioned previously, there exists an option to synthesize the drug substance as single enantiomers, which is possible and feasible for some compounds, yet not for all. The alternative is the separation of enantiomers, which can be achieved by various methods.

The resolution of enantiomers can be applied either by covalent diastereomer formation, if an appropriate chiral reagent is available for derivatizing the racemic mixture, or by transient diastereomer formation when a chiral selector interacts with an enantiomer. Based on the type of the racemic drug, and therefore based on the crystal form of the racemate, various enantiomeric purification methods can be considered.[6] The racemic drug can be a racemic conglomerate (enantiomers are crystallized as enantiopure units and physically mixed in equal proportions)[7,8], a racemic mixture (each crystal unit contains both enantiomers in equal proportions)[7,8], a pseudo-racemate and a quasi-racemate.[7] These different forms of crystals present diverse characteristics. Due to the variance of behavior, achieving isolated enantiopure crystals from these crystals requires application of different methods.[6]

Isolation by column chromatography, such as high-performance liquid chromatography (HPLC) containing chiral column, is a simple and affordable option, however, it is not a feasibly applicable process for large amounts i.e. in production plants.[5,6] More commonly used option in pharmaceutical production is asymmetric synthesis which is performed through a reaction between a chiral compound (it can be a catalyst, reagent or a substrate) and an achiral compound. This method yields a compound with increased chiral purity. To apply this method, it is possible to choose a chiral auxiliary (for example, D-glucosamine or fluorous oxazolidinone), a chiral catalyst, a chiral reagent or a chiral substrate depending on the compound and the desired end-product.[6]

Additionally, racemic drug resolution can be performed through diastereomer formation with a chiral selector. The covalent or transient nature of the formed diastereomer depends on the interactions between the racemic compound and the chiral selector. This method provides the advantage where physically inseparable enantiomers become diastereomers where it is possible to distinguish them based on the differences in their physical properties.[6]

Resolution can be performed by direct (or chromatographic) resolution where chiral mobile phase additives (CMPAs), or chiral stationary phases (CSPs) are utilized during the process.

This method is developed based on the three-point interaction formed between the analyte and the CMPA or CSP. Formation of three-point interaction (rather than two-point or single point of interaction) prolongs the elution of the analyte and provides resolution based on their retention time.[6]

Additionally, utilization of a diastereomeric salts that form non-covalent interactions with the enantiomers provides a direct resolution method. One common example of this method is provided in the literature as formation of a diastereomeric salt between 1-phenylethylamine and *(R,R)*-tartaric acid, yielding 1-phenylethylammonium *(R,R)*-tartarate salt. There are several other methods of resolution yet differ in application. These methods are, namely, mechanical resolution, preferential, kinetic, diastereomeric, membrane-based resolution, inclusion complex formation, and molecular complex formation.[6]

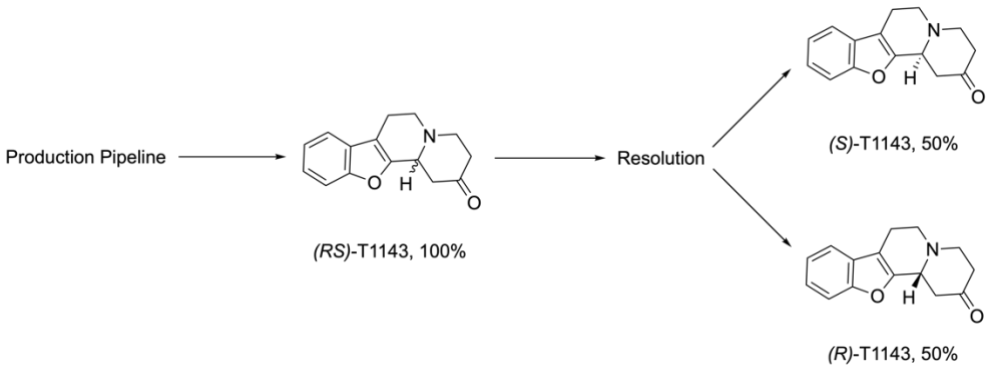

Figure 1. Representation of the theoretical loss of half of the product in distomer form during the resolution process of *(RS)*-T1143, in percentages.

The chromatographic methods provide a considerable time efficiency at analytical scale, whereas diastereoisomeric salt formation provides great advantages for larger scales, i.e., kilo-scale or larger. Therefore, during this work, both application of diastereomeric resolution by using (-)-*O,O'*- Di-p-toluoyl-L-tartaric acid (DTTA) salt (acidic resolving agent) and a direct resolution method by HPLC was applied.[3,5,6,9,10]

1.4 Defining the stereogenic center of T1143.

The stereogenic center of focus of the compound T1143 is located at the β-position of the ketone functionality, bonded to a tertiary nitrogen atom in a cyclic setting; a hexahydro-quinolizine-one. Process for production of *(S)*-T1143 also yields the undesired enantiomer, *(R)*-T1143. The

hydrogen bonded to the stereogenic center is between the hexahydro-quinolizine-one function and the benzofuran ring as you can see in Figure 1. To change the direction of this hydrogen, after several other modification attempts, it was found that breaking and re-forming the bond between the stereogenic carbon and nitrogen is required, so that a change in the chirality can be promoted.

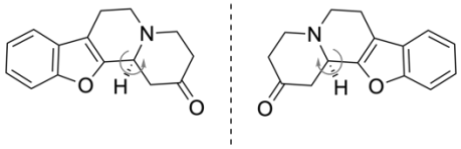

Figure 2. *(S)*-T1143 on the left and *(R)*-T1143 on the right as non-superimposable mirror images.

(S)-1,3,4,6,7,12b-hexahydro-2*H*-benzofuro[2,3-a]quinolizine-2-one, namely *(S)*-T1143, is a veterinary active pharmaceutical ingredient (VAPI) intermediate that cannot be synthesized in its enantiopure form, currently. Meaning that, the desired enantiomer must be separated from the racemic mixture of products by resolution using crystallization with *(-)-O,O'*-di-p-toluoyl-L-tartaric acid (DTTA). *(S)*-T1143·*(-)*-DTTA salt is obtained, and the half of the product gets lost as *(R)*-T1143·*(-)*-DTTA in the waste-stream.

This crystallization-based method is efficient in resolution of *(RS)*-T1143 and enantiomerically enriching the product to obtain *(S)*-T1143·*(-)*-DTTA salt with percentages reaching to the 98% ee in the solid state, while *(R)*-T1143·*(-)*-DTTA remains dissolved in the filtrate and removed. While the resolution is providing good yields of enantiomerically enriched salts, the production line still loses half of the product as the *(R)*-enantiomer since the maximum theoretical yield of resolution of racemates is 50%.[11]

Production of APIs or their intermediates are usually costly and environmentally challenging processes, thus recovery of the distomer that is corresponding to half of the yield of the API intermediate production batch, was of great importance. Therefore, the need of finding a feasible, integrable to the production pipeline easily, and environmentally friendly novel method to recover the other half of the material emerged. Hence, the focus of this work was to

find an industrially applicable method for racemization of *(R)*-T1143 to recover the product from the waste stream and increase the overall yield of each batch of production.

1.5 Racemization

The racemization is the process where a single enantiomeric form is transformed into the mixture of both enantiomers where the loss of optical activity takes place irreversibly. Conversion of an enantiomer into a racemic mixture usually requires conditions that either cause unwanted changes to the rest of the compound of interest, or modifications by addition of several synthesis steps to achieve a harmony between the temperature, pH, and concentration. Addition of steps, where increased temperature or altered pH are necessary, may make the whole process less favorable due to the increased economical and / or environmental burden.[11] Nonetheless, there are several methods for racemizing various compounds. From the table prepared by Ebbers *et al*.[11] it can be observed that a majority of methods successful for racemization of compounds such as amino acids or other carbonyl functionality containing molecules are based on either temperature or utilization of certain acid-base reactions. Despite the number of already existing racemization methods, a suitable method for obtaining *(RS)*-T1143 from *(R)*-T1143 could not be found in the literature.

1.6 Briefly: Tried and failed racemization methods.

The challenging task of finding a racemization method for a compound that does not fit in any of the previously defined class of successful racemization techniques was accomplished through experimentation of several methods. Figure 3 shows the structure of *(R)*-T1143·HCl, *(R)*-1,3,4,6,7,12b-hexahydro-2*H*-benzofuro[2,3-a]quinolizine-2-one hydrochloride that contains a benzofuran heterocycle fused with hexahydroquinolizine-2-one, in its hydrochloride salt form.

Stereogenic center at the carbon 10 (C10 position) is the focus of interest that is bonded to two characteristic defining atoms and / or groups: 1) The tertiary nitrogen atom in the base form; and 2) the carbonyl group of the quinolizine-one ring. Other fused ring, benzofuran, has been omitted from the considerations of modifications due to the distance and stability due to aromaticity (although considered to be weak) of it. Despite the said omitting the benzofuran fraction, it has not been dismissed that the possibility of lability of the double bonds.

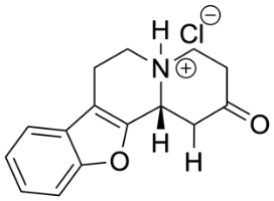

Figure 3. *(R)*-T1143·HCl.

1.6.1 Creating a change in planarity of T1143: Carbonyl group modifications.

Initially, protection of carbonyl-oxygen via acetal formation was thought to be necessary. This was due to the known lability of ketone group and decided to be protected prior to any other changes made to the structure. It was also evaluated whether it is possible to see a change in the absolute configuration of the compound, since the planarity of the molecule is assumed to change when the carbonyl group is removed. To achieve such results, the pH and temperature factors of racemization are also utilized, thus methods such as acetal formation through addition of alcohol in the presence of *p*-toluenesulfonic (*p*-TsOH) acid as the catalyst[12] and elevated temperatures accompanied by constant removal of water, i.e., by Dean-Stark apparatus; or by formation of acetals in the presence of methyl alcohol and trimethyl orthoformate as the scavenger of the water that is produced in the system[13], again in the presence of *p*-TsOH catalyst and accompanying heating procedure. Despite there had been minimal expectations, these methods were found to be easy to start this investigation and simple enough to try at the beginning. HPLC chromatograms as well as ^{1}H and ^{13}C NMR spectra did not show any indication of a change in C10 position; thus, these methods had been removed from the list of possible racemization techniques.

Similarly, thioacetal formation of the ketone, by applying the general method producing thioketals, through addition of Brönsted or Lewis acid catalyst, *p*-toluenesulfonic acid in this case, and 1,2-ethanedithiol in toluene at a reflux temperature and constant water removal through Dean-Stark apparatus integration to the set-up.[14] This method was not favorable due to two reasons: 1) the difficulties of recovery of the ketone functionality due to the stability of 1,3-ditholane form at the end of this step, and 2) the difficulty of handling 1,2-ethanedithiol due to its volatility and its foul smell.[15] Any indication of racemization was not observed in HPLC chromatograms.

1.6.2 Creating a change in planarity of T1143: Tertiary nitrogen modifications.

The approach of modifying carbonyl functionality was eliminated, and focus was shifted to the nitrogen atom on the quinolizine-one functionality. Electronegativity profile of this tertiary nitrogen atom was decided to be utilized as the next target of manipulation of stereochemistry of T1143. The initial attempt was to form an N-oxide to see if an increase of polarization between C10 and nitrogen bond would take place, then lead to cleavage of the bond.[16] To do so, it was considered that the removal of oxygen of N-oxide as the next step may lead to formation of C10 and nitrogen bond again. This way, however, the bond formation may take place from either side since the C10 would lose its chirality upon cleavage of C10 and nitrogen bond, and thus lead to formation of a racemic mixture.

The relative easiness of the oxygenation process was concealed when the deoxygenation process was included in this equation. Aligning with the initial concerns, the deoxygenation process needs to be cost efficient and environmentally friendly; nonetheless, most of the known methods were determined to be distant from lining up with this purpose. Methods of deoxygenation of N-oxide commonly found in literature consists of 1) use of metals that may cause exceeding the limitations of analytical margins for heavy metals of APIs, such as palladium (Pd) or copper (Cu),[17,18] 2) requirement of long reaction times with harsh conditions, which promotes undesired changes in the structure of T1143, which may be irreversible. It has been reported that it is possible to deoxygenize N-oxides of tertiary amines without the need of harsh conditions and the addition of heavy metals, if the phenyboronic acid was used as the deoxygenation reagent.[17] As a result of finding an appropriate deoxygenation method, *(R)*-T1143 and *(R)*-T1143·HCl was reacted in parallel to form the oxidized nitrogen atom by using *tert*-butyl hydroperoxide (*t*-BuOOH or TBHP) in dichloromethane (DCM) at room temperature.[19] Neither the structural analysis via ^{1}H and ^{13}C NMR indicated a change in the structure, nor the enantiomeric analysis by HPLC showed a variance in the stereochemical properties. Another method of oxygenation was experimented with the use of *meta*-Chloroperbenzoic acid (m-CPBA) in DCM.[20] As it was the case with TBHP oxidation, this method also failed to produce the desired N-oxide form, according to the initial analyses applied. Nonetheless, the phenylboronic acid reaction was set-up to observe if that would lead to any changes. This method was claimed to be so mild that it would not lead to the reduction of labile groups such as ketones, even if high temperatures were applied.[17] For the sake of scientific curiosity, this method applied to the material previously attempted to be oxidized to form N-oxide of *(R)*-T1143. Phenylboronic acid ($PhB(OH)_2$) was added to this material and

dissolved in DCM, then heated to 120 °C. When the reaction was finalized, samples for NMR and HPLC were collected. Analyses showed no desired results. Thus, other methods found in literature were continued to be studied.

1.6.3 Creating a change in planarity of T1143: Choosing a solvent system.

Based on the work done by Trauner *et al*. in 2017 on alkaloids such as sinoracutine alkaloid, a solution of *n*-heptane/*i*-PrOH/MeOH in 3/1/1 *(v/v/v)* ratio would result in racemization at prolonged reaction times when the sample was heated to 60 °C.[21] Based on these results, despite the reaction is defined to be extremely slow, the exclusion of additional materials that brings the risk of being toxic or expensive, or the moderate heat application leading to a racemization on a tetracyclic structure were the reasoning behind this method worth trying. This reaction was applied in parallel for both *(R)*-T1143 and *(R)*-T1143·HCl and at 100 °C, to decrease the time spent, with the solvent system used in the reference article.[21] The following HPLC analysis showed both enantiomers with additional peaks with much earlier retention times (see Appendix 2, S. Figure 16 for the related HPLC chromatogram). These additional peaks were interpreted as the result of some undesired changes took place on the compound, which was also verified by the NMR analysis, which showed the loss of signals of benzofuran ring on ^{13}C NMR (see Appendix 3, S. Figures 20 and 21 for ^{1}H and ^{13}C NMR spectra, respectively). In accordance with these results, a new search for another method was started.

1.7 How was this novel method found?

The previously mentioned methods in the tried and failed section taught plenty. It was proven that the application of heat at correct conditions would yield a racemic mixture. The initial clue was the evaluation of changes observed when the starting material was *(R)*-T1143·HCl or *(R)*-T1143. As it will be discussed in more detail in synthetic methods, these methods were applied to both base form and hydrochloride salt form of T1143 in parallel to compare the results of the reactions in accordance with the initial pH difference of T1143 or according to the state of the nitrogen atom, whether it is in tertiary nitrogen state or quaternary ammonium state. As in the case with the solvent system of heptane/isopropyl alcohol/methyl alcohol and heat application, the changes observed only when the starting material was *(R)*-T1143·HCl. With the consideration of both aspects, *(R)*-T1143 and *(R)*-T1143·HCl were continued to be evaluated in parallel to understand better what affects the course of reactions.

Better enantiomeric excess ratios, without the deformation of the compound, were observed during the consideration for an environmentally friendly removal of thioacetal protection on the ketone group. Thioacetals and thioketals, unlike acetals or ketals, are stable against acidic hydrolysis.[15,22] Thus, harsh reaction conditions and addition of halogens or metals to the reaction medium are known to be a part of removal of dithianes and dithiolanes. However, it was claimed that dissolving the thioacetal (or thioketal) in dimethyl sulfoxide (DMSO) and applying heat at high temperatures (140-160 °C), without addition of metals or halogens, the ketone functionality can be recovered.[22] DMSO is a solvent that may pose low risks of toxicity, otherwise accepted as a solvent that does not need strict regulations.[22] Based on the low environmental impact of DMSO[23] and simplicity of the method found in literature,[22] it was applied as a solvent for the racemization of *(R)*-1,3,4,6,7,12b-hexahydrospiro[benzofuro[2,3-a]quinolizine-2,2'-[1,3]dithiolane] (1,3-dithiolane protected *(R)*-T1143) obtained previously. Analysis of the resulting material in HPLC did not present a desirable chromatogram. Concomitantly, the ^{1}H and ^{13}C NMR spectra did not show the recovery of the ketone, however, it was visible that no other change occurred in the structure derived from T1143. Nonetheless, it was a great opportunity to experiment dissolving T1143 and T1143·HCl in DMSO and applying heat, since thermal applications were observed to lead to changes in the structure and their retention time in the chiral column; and DMSO would allow reaching to 160 °C, and even above. However, degradation of the compound would be observed if 160 °C or higher temperatures would be applied. *(R)*-T1143 and *(R)*-T1143·HCl was dissolved in DMSO in the same concentration and heated to 140 °C under reflux for 5 hours. Upon application of necessary work-up procedures, and the following HPLC analysis, it was found that racemization took place.

Despite DMSO as a solvent aligns well with the criteria set considering the environmental concerns, its price is relatively high and work-up procedure is laborious. Considering the properties of DMSO as a solvent, i.e. polarity and ability to accept hydrogen bonds; water, acetic acid, and ethyl acetate were decided to be used as the solvent while the same procedure was applied. These all four are good solvents and hydrogen bond acceptors. Depending on their boiling points, four reactions using *(R)*-T1143·HCl as a starting material, were set up. Enantiomerization was achieved in all cases, yet with a variation in the *(R)*- / *(S)*-enantiomeric ratios. While the best results were obtained when DMSO was chosen as the solvent at shorter

reaction times, similar ratios were obtained with water when the reaction time prolonged slightly. However, ethyl acetate did not yield an acceptable conversion even at prolonged reaction times. Thus, it was excluded from further evaluation. Acetic acid has a lower capacity of dissolving *(R)*-T1143·HCl, hence the handling and transferring of the material for the work-up was far from ideal, although the conversion ratio was better than that of ethyl acetate's even at shorter reaction times, it was decided not to be evaluated further. Nonetheless, water as the solvent was found to be the best choice among four solvents and since water is accepted as the universal solvent, the search for another solvent was halted. Instead, optimization of reaction was further investigated in terms of concentration, reaction time and temperature; and mechanism of the reaction was studied more closely.

1.8 Mechanism behind racemization of *(R)*-T1143·HCl: Formation of aza-Michael / aza-retro-Michael Equilibrium.

Michael reaction is a reaction that has been known since 1883 when Komnenos observed the reaction between anionic diethyl malonate and ethylene malonate and concluded that the nucleophilic carbon attacks to the carbon-carbon double bond.[24] The name of the reaction, however, is due to the systematic works by Arthur Michael on reactions of stabilized anions with α,β-unsaturated compounds in the presence of an alkoxide (RO^-). Michael reaction is a great tool in organic synthesis since it can also be applied to form carbon-carbon bonds.[24]

Michael reaction takes place either when stabilized anions (Michael donors) react with activated alkenes; or, when activated alkenes react with electron-deficient alkenes (Michael acceptors) in the presence of a base.

The mechanism of Michael reaction is initiated when the base in the media abstracts an acidic proton from the α-carbon, thus leading to formation of an enolate anion. The enolate anion is activated upon deprotonation, then reacts with a Michael acceptor, where a [1,4]-conjugate addition takes place. Formation of new carbon-carbon bond is concluded when the protonation occurs, and the base is regenerated to the media and finalized as the base-catalyst. [24] Until the final protonation step, Michael reaction is in the form of equilibrium, where the reverse reaction is called the retro-Michael reaction.

The racemization success is, however, due to a derivation of Michael reaction that is taking place, *aza*-Michael / retro-*aza*-Michael equilibrium formation. During this process, the

secondary amine of the azecine ring is attacking to the Michael acceptor. The *aza*- prefix indicates the involvement of nitrogen atom as the nucleophile in this version of Michael reaction. The function of this secondary nitrogen during the *aza*-Michael reaction is to act as a nucleophile due to its lone pair of electrons and lead to forming a carbon-nitrogen bond upon attack to the electrophilic β-carbon of the enolate formed (the Michael acceptor). During the reverse reaction, retro-*aza*-Michael, the nitrogen atom functions as a stabilizing factor for the electrons forming the carbon-nitrogen bond on the quinolizine-one structure. The ability of the nitrogen atom to bear lone pair electrons promotes the enolate formation, thus favors the retro-*aza*-Michael product during the equilibrium.

The formation of enolate is not direct in this case, since there is no base added to the media for the abstraction of the α-proton. However, the electron withdrawing capacity of quaternary ammonium ion might polarize the carbon-nitrogen bond to an extent where the bond is broken and the electrons forming this bond are thus transferred to the empty π-orbital of the nitrogen atom. While the dissociation of *aza*-Michael adducts taking place, formation of enolate must also be observed.

There are two α-carbons in T1143 molecule and each carry two α-hydrogens on them. For the racemization reaction to take place for *(RS)*-T1143, the more substituted alkene is formed upon promotion of *aza*-Michael / retro-*aza*-Michael equilibrium formation by heating T1143 in water. Unsymmetrical ketones are able to form two different enolates, as long as both α-carbons carry at least one α-hydrogen that can be removed from the carbon. According to the literature, thermodynamically and kinetically more stable enolates are formed, depending on following or not following the Zaitsev's rule, respectively. Zaitsev's rule dictates that if a more substituted carbon-carbon double bond has formed, it is the thermodynamically more stable product, whereas the formation of less substituted C=C is the resulting kinetically more stable product. The reason behind this is the latter carrying the more exposed, or less sterically hindered, hydrogen atom that is easier to remove from its position, while the former is more difficult i.e., in the case of base addition to the media to abstract an α-hydrogen, to reach to the more crowdedly surrounded hydrogen demands more energy. This means that the energy necessary for the thermodynamically stable product to form is more than that of kinetic product formation. Since the formation of retro-*aza*-Michael / *aza*-Michael equilibrium is strongly dependent on the energy supplied by heating the reaction media, there is a reason to assume that the

thermodynamically more stable enolate formation is achieved due to demand for higher temperature applications at prolonged times.[25]

2 RESULTS AND DISCUSSION

Racemization of a commercial veterinary active pharmaceutical ingredient (VAPI) intermediate is achieved through an environmentally friendly method. The already existing industrial production method of VAPI yields a racemic mixture of *(R)*-T1143 and *(S)*-T1143; however, only *S*-enantiomer is able to bind to the receptor to induce the desired effect on the patient. Due to the selectivity of the biological systems, a resolution technique has already been in use, so that enantiopure API can be obtained. This resolution is performed by crystallization with *(-)*-DTTA, and it can lead to a 50% yield success, that is the maximum theoretical yield of resolution of racemates. Even though this process gives the desired compound, there are financial and ecological concerns that give rise to the necessity of a recovery method that converts the undesired enantiomer into the desired one, *(S)*-T1143.

Foremost concern is the maximum theoretical yield of this synthesis method. Production of APIs is costly and has considerable negative impacts to the environment. APIs are a group of fine chemicals which has a very expensive market due to multiple synthesis, analytical and purification steps to obtain APIs from raw materials. These steps include expensive and, in most cases, environmentally harmful solvents and catalysts used in large amounts of production batches, ranging from kilograms to tons, and has a high demand of energy, i.e. needed for sensitive temperature control. Materials collected from the reactors must undergo several other steps such as purification, quality control analyses, sterilization, and packaging in a downstream process. As a result of this stepwise production, a variety of waste production also takes place.[9] Considering these factors, loosing half of the product due to the lack of a racemization method adds a great deal of cost to the already expensive and impactful process. Thus, a method to transform the undesired, *(R)*- enantiomer into the desired one was essential.

2.1 Tried and failed methods: In detail

As briefly mentioned in the introduction part, enantiomerization of *(R)*-T1143·HCl, (12b*R*)-2-oxo-1,2,3,4,5,6,7,12b-octahydrobenzofuro[2,3-a]quinolizin-5-ium chloride was studied in detail by applying several methodologies.

2.1.1 Carbonyl group modification experiments.

Protection of carbonyl group by acetal formation was the initial choice since ketone group is labile and the procedure was simple, and it was evaluated if it is possible to achieve a change in absolute configuration when the planar structure of the carbonyl group is altered to a tetrahedral geometry by changing the hybridization of carbonyl carbon through this simple procedure.

Initially, acetal formation through addition of ethanol (EtOH) in the presence of *p*-toluenesulfonic (*p*-TsOH) acid as the catalyst[12] and elevated temperatures accompanied by constant removal of water by Dean-Stark equipment was studied.

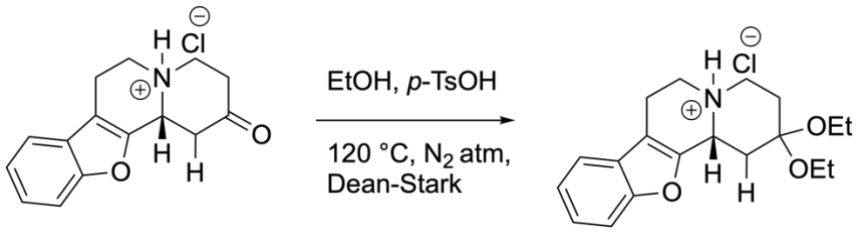

Figure 4. *(R)*-T1143·HCl is dissolved in ethyl alcohol upon heating in the presence of Brönsted acid catalyst, *p*-TsOH in 0.1 equivalency.

The resulting compound upon this reaction is the diethyl acetal protected (12b*R*)-2,2-diethoxy-1,2,3,4,5,6,7,12b-octahydrobenzofuro[2,3-*a*]quinolizin-5-ium chloride, which is confirmed by the loss of ketone carbon's signal in ^{13}C NMR spectrum. The transformation from *(R)*-enantiomer to *(S)*-enantiomer; on the other hand, was not observed in HPLC analysis.

Similarly, dimethyl acetal formation by dissolving *(R)*-T1143·HCl in methyl alcohol in the presence of *p*-TsOH as the organic acid catalyst at room temperature, and addition of trimethyl orthoformate (TMOF) as the water scavenger[13] was studied.

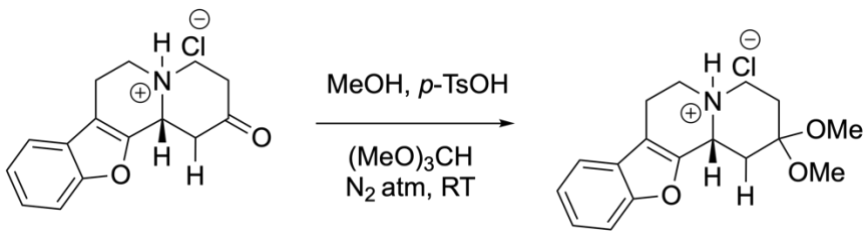

Figure 5. *(R)*-T1143·HCl is dissolved in methyl alcohol in the presence of 0.1 equivalent Brönsted acid catalyst, *p*-TsOH·H_2O, and 1.2 equivalent trimethyl orthoformate as the water scavenger.

(12b*R*)-2,2-dimethoxy-1,2,3,4,5,6,7,12b-octahydrobenzofuro [2,3-*a*]quinolizin-5-ium chloride was formed, however, any indication that enantiomerization is taking place was not observed.

The addition of ortho ester, TMOF, led to consumption of water molecules produced in the reaction medium. This reaction was confirmed by the new peak observed on ^{13}C NMR spectra near 160 ppm, while the protection of ketone functionality by dimethyl acetal was confirmed by the disappearance of ketone peak around δ = 206 and additional -O(CH_3) proton signals appeared around 3.5 ppm on ^{1}H NMR spectrum.

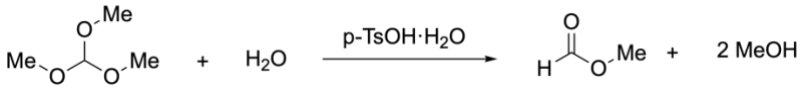

Figure 6. Reaction of trimethyl orthoformate in the presence of *p*-TsOH·H_2O and water lead to formation of methyl ester and methyl alcohol.

Additionally, thioacetal protection was achieved where *p*-toluenesulfonic acid was used as the catalyst and 1,2-ethanedithiol in toluene was refluxed. This method, similar to the diethyl acetal formation method, was integrated with Dean-Stark apparatus to remove water from the system. Despite the difficulty in handling of 1,2-ethanedithiol, the reaction was set up as mentioned and expected to proceed as shown in Figure 7.

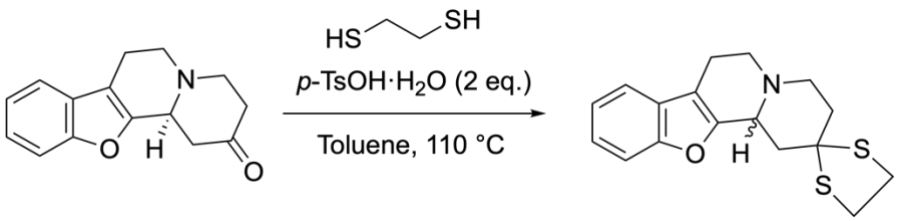

Figure 7. Expected reaction of *(S)*-T1143 dissolved in toluene in the presence of *p*-TsOH·H_2O and 1,2-ethanedithiol to be resulted with the formation of 1,3-dithiolane protection.

This reaction did not yield desired results. Two types of precipitates were formed after the work-up, which was then analyzed and concluded that the fused rings were cleaved and showed undesirable signals on ^{1}H, ^{13}C NMR and HPLC analyses indicating distorted compound. Two different NMR samples from two crystals of the same reaction showed higher number of peaks than expected. Nonetheless, there were signals that are matching the expected signals of protons of 1,3-dithiolane. Thus, it was decided to repeat the experiment and, after the work-up, divide

the product in half, then, to apply two different methods known for recovery of ketone from 1,3-dithiolane protection. The deprotection methods were found in literature as 1) use of *tert*-butyl hydroperoxide (TBHP) and methanol at reflux temperatures[26] and, 2) heating in DMSO up to 160 ºC [21] would lead to recovery of ketone functionality from dithioacetal protections. Both these methods were applied to the 1,3-ditholane protected T1143 derivative and the changes took place on the structure were followed by NMR analysis. Neither of these methods promoted the recovery of ketone functional group. Instead, the former method was observed to yield a very crowded ^{13}C NMR spectrum, and the latter was found to be ineffective for reinstalling the carbonyl group. Thus, the formation of 1,3-dithiolane protected T1143 was not decided to be beneficial at this stage.

2.1.2 Tertiary nitrogen modification experiments.

The nitrogen atom of the heterocyclic ring is bonded to the stereogenic center. Stands the reason that modifications on this position was expected to provide promising leads. Hence, the following oxidation methods were decided to be studied: 1) formation of N-oxide bond by *t*-BuOOH,[19] and 2) oxygenation by *m*-CPBA.[20] The method for removal of N-oxide bond was chosen to be via the addition of $PhB(OH)_2$ and application of heat up to 120 °C since it was claimed to deoxygenize tertiary amine oxides under milder conditions, such as lack of heavy metals in this method.[17]

(S)-T1143 was reacted with *tert*-butyl hydroperoxide (70% solution in H_2O) in dichloromethane at room temperature.[19] Prior to deoxygenation step, NMR sample was prepared and ^1H NMR spectrum was analyzed. Additional signals at $\delta \sim 1.2$ ppm and ~ 10.8 ppm were observed while the rest of the peaks were the same integration values and similar chemical shift values. These two observed signals might belong to *t*-butyl protons and hydroperoxyl- protons, respectively. However, the integration values do not correspond to the actual number of protons within the structure of TBHP. Nevertheless, to test if the use of phenylboronic acid would lead to desirable changes in the structure, this derivative of T1143 was dissolved in DCM and added $PhB(OH)_2$, then refluxed under N_2 atm. This process resulted in deformation of the T1143 structure, which was observed from both ^1H and ^{13}C NMR spectra where key signals were lost, i.e. ketone carbon signal at $\delta = 206$ ppm; signals of two carbon atoms at the fusion of benzene and furan rings at $\delta = 152$ and 154 or number of signals were almost doubled at $\delta = 110$ and 140 ppm on ^{13}C NMR. This was interpreted as the loss of aromatic double bonds upon this method's application.

After the consideration of the use of TBPH and $PhB(OH)_2$, same procedure was also worked with the use of *meta*-Chloroperbenzoic acid (*m*-CPBA) in DCM.[20] These two parallel reactions were tested and compared. The resulting ^1H NMR spectra showed additional signals between δ = 2 and 4.5 ppm and the signal splitting pattern was changed to a degree, which led to consideration of other changes taking place rather than formation of N-oxide alone. Following the same procedure as it was performed during the reaction of prepared with TBPH, $PhB(OH)_2$ was utilized to observe the changes in the structure. While the structure was preserved in terms of quinolizine and benzene rings, based on the ^{13}C NMR spectrum, disappearance of ketone functionality and distortion in the furan structure was apparent due to the disappearance of some peaks and increase in the number of peaks in certain ppm values, respectively. ^1H NMR spectrum showed 13 aromatic signals and an aldehyde signal while the integration between 2 and 4 ppm was reduced significantly, indicating that indeed the distortion on the quinolizine ring took place.

It is concluded that manipulations on tertiary nitrogen atom of the quinolizine-one ring through oxidation is not working without producing undesired by-products or cleaving the structure and formation of undesired functionalities on the structure of T1143. Other methods found in literature that contain harsh reaction conditions were omitted at that point to comply with the initial aim of this work. Hence, failure of this approach has led to experimenting other methods such as application of heat in various solvents and / or solvent systems, since thermal racemization has mentioned in the literature quite frequently.[11]

2.1.3 Solvent system experiments.

Literature contains good examples on heat application-induced racemization examples, provided an appropriate condition is found specific to the compound at hand. Finding that a solution of *n*-heptane / *i*-PrOH / MeOH in 3/1/1 (*v*/*v*/*v*) ratio leads to racemization of alkaloids when heat applied for long reaction times,[21] it was decided to be studied for T1143 as well. Two parallel reactions were set up, one with *(S)*-T1143, in the base form, and the other one with *(R)*-T1143·HCl, dissolved in a mixture of Heptane / Isopropyl alcohol / Methanol (3/1/1) and heated to reflux overnight. These reactions behaved considerably different from each other; while the former reaction gave the same ^{13}C NMR as the reference carbon NMR, and a very similar proton NMR; the latter was found out to be changed drastically. The main indication of such change is the loss of carbonyl carbon's signal, additionally, signal of fusion carbon at the

position of furan ring was lost and aromatic carbons' signals were shifted. An addition of a strong signal at δ = 96.2 ppm on the carbon NMR spectra was also found important. Due to the recognized changes observed in the latter, despite undesired, HPLC analysis was performed and found out certain amount of enantiomerization was measured. However, there was also additional peaks observed at RT = 7 - 9 minutes; showing there is more than one pair of enantiomers formed upon this application to *(R)*-T1143·HCl. The second experiment repeated, and similar results were reached (see Appendix 2, S. Figure 16).

Being convinced that the thermal applications might, in fact, be the solution for this problem, it was decided to follow this route. Considering the combination of heat and polar solvent changing the HCl form but not the base form, a parallel reaction was decided to be set to reach high temperatures in a polar solvent. Dimethyl sulfoxide was on the top of the list, due to its high capacity as solvent, high boiling point to provide thermal energy, if needed, and ability to tune in with the environmental expectations of this work, yet more importantly, showing no indication of undesired bond cleavage on previous works performed with DMSO and *p*-TsOH during the trial of removal of 1,3-dithiolane protection. Thus, parallel reactions, with the starting materials *(S)*-T1143 and *(R)*-T1143·HCl were designed by dissolving in 1 mol/L concentration and heating to 140 ºC in the duration of 5 hours. Reflux system was also integrated under nitrogen atmosphere. Although the boiling point of DMSO is well above the set temperature, 189 ºC, its flash point is 87 ºC if pressure is increased,[27] thus reactions were not left to continue overnight without control. The products were collected through aqueous phase chemistry[28] and extracted into dichloromethane (DCM), then evaporated to dryness. Both final products were collected in solid state in good mass yields, and samples were prepared for HPLC and NMR analysis.

Both ^{1}H and ^{13}C NMR spectra showed the same chemical shift values, however, signal splitting analysis was not performed. On the other hand, HPLC analysis showed these two products behave differently, to a degree that the product of *(S)*-T1143 showed no enantiomerization while the product of *(R)*-T1143·HCl yielded 35% *(S)*-enantiomer on the HPLC chromatogram. Since there was no loss or addition of signal patterns on NMR spectra, it was concluded that the observed enantiomerization is taking place without any unwanted cleavage on the T1143 structure.

2.1.4 Success in enantiomerization.

Supply of thermal energy to the solution of *(R)*-T1143·HCl in DMSO provided an applicable enantiomerization method. Hence, this experiment was repeated at different reaction times, concentrations and temperatures to observe the changes in the *(R)*- / *(S)*-enantiomer ratio depending on the reaction conditions, and Table 1 below is prepared.

Table 2. Dependence of formation of (*S)* - enantiomer to the reaction time and temperature in DMSO at the same concentration, 0.72 mol/L.

Entry	Reaction Time (h)	Temperature (ºC)	*S* - Enantiomer Area%
18	8	120	35.7
19	4	120	19.0
20	8	140	50.1
21	4	140	49.6
23/1	2	130	50.2
23/2	4	130	50.5
23/3	24	130	42.7
23/4	27	130	50.6
24/1	2	100	31.6
24/2	4	100	41.4
24/3	24	100	50.1
24/4	27	100	49.3

Table 1 indicates there is a fine balance between the reaction temperature and the extent of enantiomerization. The longer the reaction time, the higher the increase in the observed %area in HPLC for the *(S)*-enantiomer, i.e. 35.7% *versus* 19.0% for entries 18 and 19, respectively at 120 ºC. Nonetheless, same amount of increase in the reaction time leads to only 0.5 % change in the % area between the entries 20 and 21, when the reaction took place at 140 ºC. While the

entries 23/1, 23/2 and 23/3 seem like outliers when only these data are processed, it can also be explained by the formation of an equilibrium during this process which may end up reversing after exceeding certain heat supply limits.

More striking results are presented by the entries 23 to 24/4. Same reaction was sampled at the second, fourth, twenty fourth and twenty seventh hour of the reaction at 130 ºC and 100 ºC, respectively. Despite the lowest temperature applied in DMSO was 100 ºC, complete racemization is made possible by extending the reaction time to 24 hours. Additionally, a slight decrease is observed when the reaction continued up to 27 hours, which is an indication of reversibility of this mechanism; however, a more detailed study is necessary to present the factors affecting the reverse and forward reactions.

When the same reaction was observed from two hours to 27 hours when the temperature was set to 130 ºC, reaching to a complete racemization occurs much earlier than it happens when the temperature was kept at 100 ºC. This shows a clear dependence of this reaction to the thermal energy supplied. Although there is not a definite explanation about the decrease at 24 hour *(S)*-enantiomer area % measurements, it is probable that kinetics of this equilibrium prefers reverse reaction that reduces the presence of the *(S)*-enantiomer.

High prices and difficulty of the work-up process of collecting organic material from DMSO had led to search for other solvents that provides a success in promoting the same mechanism to achieve this racemization method. Glacial acetic acid was chosen as the solvent. Acetic acid is a hygroscopic liquid with a boiling point of 117.9 ºC. Due to its ability to absorb water molecules from the air, an assumption was made to contain water molecules to some extent, thus, the initial concentration was calculated to be 0.78 mol/L, theoretically. Reaction was set to continue for eight hours at 115 ºC refluxing under N_2 atmosphere. After the work-up, product showed *(S)*-enantiomer on HPLC chromatogram up to 36%; however, NMR spectra showed changes in the structure where the ketone carbon was disappeared in ^{13}C NMR while additional peaks were observed between 30-40 ppm; and ^{1}H NMR indicated changes occurring on the quinolizine protons and a considerable deshielding of the proton bonded to the stereogenic carbon, shifting from δ = 3.6 to 4.6 ppm.

Observation of changes in the structure of T1143 was the reason of pivoting from choosing the acetic acid to the ethyl acetate (EtOAc) as the solvent. One main advantage of EtOAc is that it is the general solvent of the production line of *(S)*-T1143, which means that the solvent-specific quality control analysis and analytical control methods were already worked extensively, and it

would be the best choice in terms of integrating the racemization method to the already existing production method. In a similar thought process, it is also possible to conclude to the point where the enantiomerization should already have been taking place during the reaction and work-up steps in the production. Nonetheless, the reaction conditions were set to be 0.18 mol/L concentration and refluxing overnight. This reaction, however, did not lead to any considerable change in the stereochemical properties of *(R)*-T1143·HCl. Measured area% in HPLC chromatogram was 5.7 for *(S)*-enantiomer. Upon obtaining this result, with the consideration of observation of no enantiomerization in the production line, ethyl acetate as the solvent of racemization reaction was not studied further.

Evaluation and comparison of these three solvents for thermal racemization was performed in terms of their polarity, hydrogen bond donor and acceptor capacity, and their boiling points. Finding the common properties; polar, being at least one hydrogen bond acceptor and better *(S)*-enantiomer formation at 100 ºC and above, water was decided to be used as the solvent.

Promising results were obtained when *(R)*-T1143·HCl was dissolved in water at 0.18 mol/L concentration and heated under reflux and N_2 atmosphere for twenty-four hours. Both ^{1}H and ^{13}C NMR analyses showed spectra consistent with the T1143 structure and HPLC analysis showed 49.6% *(S)*-T1143 in the chromatogram. Thus, this method was investigated further.

2.2 Optimization of reaction conditions

Table 2 below shows the resulting *(S)* - enantiomer formation, depending on the reaction time and concentration in water. Initially, the extent of the effect of the temperature change during the reaction was investigated. Entries starting from 29/1 to 30/3 display the lower the reaction temperature, the lower the observed *(S)* - enantiomer area %. Even when the reaction was provided thermal energy for four hours, stopping the heating leads to low percentages, even lower than the four-hour reaction, as it is the case with entry 30/3. Although the conversion is small, it is visible that the doubled reaction time results with almost doubled *(S)* – area % measurements from two-hours to four hours. After briefly overlooking the effects of the temperature changes, the initial concentration of the reaction and its effects were investigated as the following steps. From entries 31 to 35, reactions were compared in terms of their concentrations at 0.72, 0.36, 0.48, 0.12 and 0.18 mol/L, respectively. Reactions were at reflux temperature and continued for twenty hours. The results indicate that there is better conversion

success above certain concentration, i.e. 0.36 mol/L or higher. Besides obtaining better yield, practical conditions were also evaluated, and it was observed that transfer of material was easier below 0.48 mol/L concentration. Difficulties in the transfer was considered a reason behind loss of yield in mass balance. Thus, the best concentration to work in this system was decided to be 0.36 mol/L and the effects of the reaction time was investigated through entries #38 to #40/3.

Table 2. Dependence of formation of *S* – enantiomer to reaction time and temperature in water.

Entry	Reaction Time (h)	Temperature (ºC)	Concentration (mol/L)	S - Enantiomer Area %
27	24	100	0.18	49.6
29/1	2	100	0.18	9.2
29/2	4	100	0.18	16.1
29/3	24	100 → RT	0.18	18.7
30/1	2	80	0.18	5.2
30/2	4	80	0.18	9.2
30/3	24	80 → RT	0.18	4.8
31	20	100	0.72	50.3
32	20	100	0.36	50.6
33	20	100	0.48	50.7
34	20	100	0.12	46.1
35	20	100	0.18	38.9
38	25	100	0.36	51.2
39	24	100	0.36	50.9
40/1	9	100	0.36	49.7
40/2	15.5	100	0.36	49.7
40/3	24	100	0.36	49.8

It was found that the measured *(S)* - enantiomer area % is approximately 50 when the reaction carried out at 0.36 mol/L concentration and at 100 ºC in water. Therefore, dissolving *(R)*-

T1143·HCl in water and application of heat under reflux was determined as the most environmentally friendly choice of method for the racemization.

The effects of hydrochloride salt form were previously investigated when two parallel reactions were set with both *(R)*-T1143 and *(R)*-T1143·HCl in DMSO and concluded that this reaction does not undergo an enantiomerization in the base form. Therefore, the effects of other factors such as temperature, concentration and reaction time were investigated for the *(R)*-T1143·HCl form only. As can be seen in Table 2, this reaction follows certain trends, and they are further discussed in the following sections. Nonetheless, the data presented in Table 2 indicates an equilibrium is being formed during this change at the stereogenic center. Three elements are found important when this enantiomerization was achieved: 1) Tertiary nitrogen atom of the quinolizine-one cyclic structure must be in quaternary ammonium salt form, 2) a polar solvent must be used with the ability to accept hydrogen bonds, and 3) heat must be applied under certain conditions for racemization to take place. Hence, the mechanism was proposed to be an *aza*-Michael / retro-*aza*-Michael equilibrium formation as shown in Figure 8 below.

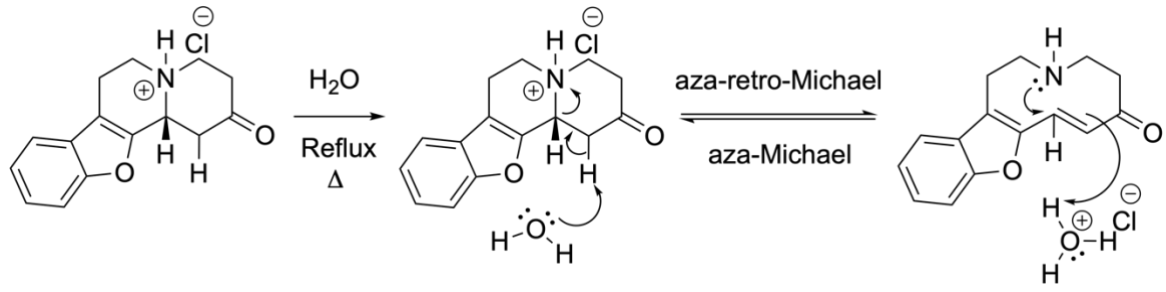

Figure 8. *(R)*-T1143·HCl is refluxed in H_2O and Retro-*aza*-Michael / *aza*-Michael equilibrium is formed through proton abstraction from the β-carbon and then attack of double bond to the proton on hydronium ion.

This mechanism is taking place through formation of an α,β-unsaturated carbonyl functionality, and the lone pair electrons of the nitrogen atom acting as the nucleophile in the presence of water and increased temperature. Since the nucleophile is a nitrogen atom, this equilibrium is called retro-*aza*-Michael / *aza*-Michael equilibrium.

The formation of racemic mixture is the result of generation of an intramolecular equilibrium between *aza*-Michael and retro-*aza*-Michael reactions. Michael reactions containing nitrogen atom functioning as the nucleophile have found a wide range of applications in chemistry, leading to formation of useful intermediates i.e. β-amino carbonyls. This conjugate addition reaction is considered to be simple due its nature that is allowing acid or base catalyzed

variations, as well as certain reactions requiring no catalyst at all. Therefore, it is one of the common reactions for the formation of carbon-nitrogen bond. Development of organocatalysis in organic chemistry has a huge impact on *aza*-Michael-type reactions as well. *Aza*-Michael reaction have been found quite useful for reactions taking place in the presence of organocatalysts to obtain enantiomerically enriched compounds.[29,30]

2.2.1 Determination of temperature dependency of the reaction.

Table 2 shows the overall data of how the area % of *(S)* - enantiomer changes when temperature, reaction time and initial concentration were varied in water. It is evident that reaction needs to be carried out for four hours or more. Comparison of entries 27, 29/2, and 35 shows supplying certain amount of energy for the enantiomerization to take place is necessary, either by longer reaction time or by higher degrees of heat application. Graph 1 shows how the measured *(S)*-T1143 % Area depends on the reaction time and the applied heat while the reaction is taking place. Shorter reaction times are leading to lower degrees of conversion. Experiments showed that an eight hour or longer times are leading to the formation of *(S)*-T1143 at a higher percentage. This is also valid for the other kinetic element, temperature. This reaction takes place after enough energy is put into the media so that formation of α, β – unsaturated ketone by the cleavage of carbon-nitrogen bond takes place to yield a higher ratio of intermediate leading to enantiomerization.

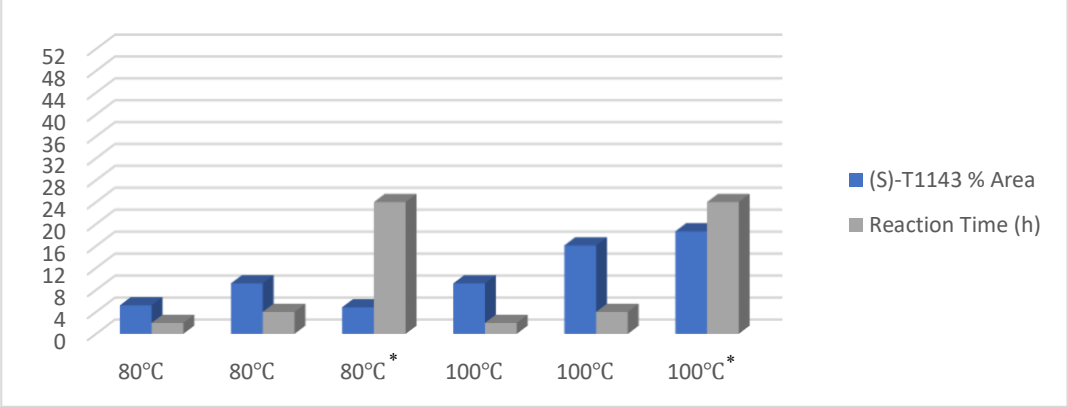

Figure 9. Bar graph presenting the effects of reaction temperature and time of *(R)*-T1143·HCl in H_2O. Asterisk (*) indicates reaction went to completion while cooling after four-hours.

The bar graph in Figure 9 shows how the retro-*aza*-Michael / *aza*-Michael equilibrium behaves at 80 °C *versus* 100 °C and, it is possible to compare how the reaction profile changes when

heating at set temperature continued for two hours, extended to four hours and finally heating mantle was turned off and the reaction continued to complete 24 hours (see green bars in Figure 9). When the supplied thermal energy is limited at 80 degrees Celsius, measured *(S)* - T1143 % Area is lower than the corresponding 100 degrees reactions. More importantly, the reverse reaction is more pronounced for the 80 °C reaction when it was cooled down to room temperature while the reaction was kept going (see the asterisks in Figure 9).

2.2.2 Determination of concentration dependency of the reaction.

The reaction showed concentration dependence. Investigation of the effects of concentration was performed at 100 °C for 20 hours. It was concluded that there is an optimal concentration for the retro-*aza*-Michael / *aza*-Michael equilibrium to yield an enantiomerization. At below and above certain concentrations, *(S)* - T1143 % area observed in HPLC was less than expected. The listed concentration and *(S)* - enantiomer Area% between entries 31 and 35 in the Table 2 shows the resulting Area % is varying with the changing concentration.

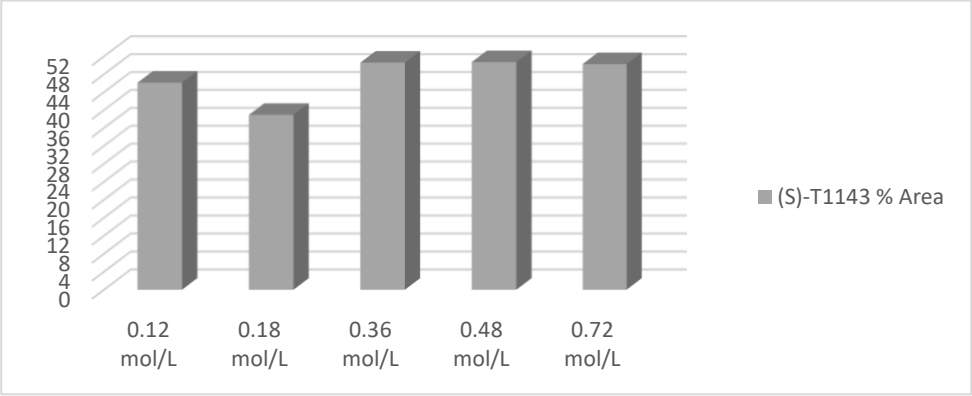

Figure 10. Bar graph indicating the variation of measured *(S)*-T1143 % Area in H_2O at different initial *(R)*-T1143·HCl concentration.

The five reactions shown in Figure 10 were refluxed for 20 hours. Formation of 46.1% *(S)* - enantiomer when the concentration was 0.12 mol/L was not found efficient, compared to 0.36, 0.48 and 0.72 mol/L reactions yielding 50.6, 50.7 and 50.3 % area, respectively. The plateau reached corresponding to 50% for concentrations 0.36, 0.48 and 0.72 mol/L. However, these three reactions were also evaluated in terms of the handling of the material in the reactor. *(R)* - T1143.HCl was difficult to transfer at concentrations higher than 0.36 mol/L upon heating, therefore causing the workup procedure to be more time consuming than acceptable. Considerably lower *(S)* - enantiomer area percentage was observed in HPLC chromatogram

when the concentration was adjusted to 0.18 mol/L. As a result, optimal concentration was determined to be 0.36 mol/L, considering both the area % measured in HPLC and the ease of application of workup.

The reason behind the concentration dependence is not known completely at this stage and there is a necessity to perform additional experiments to understand this notion better. Nonetheless, a kinetic explanation where intramolecular catalysis is taking place and it can be correlated with the acid dissociation constant, and additionally a comment where this mechanism can be both thermodynamic and concentration based can be mentioned.

2.2.3 Determination of time dependency of the reaction.

Formation of retro-*aza*-Michael / *aza*-Michael equilibrium is affected by kinetic factors and reaction time is considered to be one of the main factors. The temperature (100 °C) and concentration (0.36 mol/L) was set while the reaction time varied from 9-hours to 25-hours (see entries #38-40/3 in Table 2). The formed product is stable when the reaction is continued for long times. Product degradation does not exceed the amount of product formed; thus, this reaction is considered to be a robust and scalable process. The robustness of the process allows performing this reaction at large scales since the heating-cooling cycle takes several hours in the production scales. Fortunately, the observed *(S)* - T1143 area % does not differ largely from each other when the concentration was kept constant while the duration of the reaction varies from 9 to 25 hours.

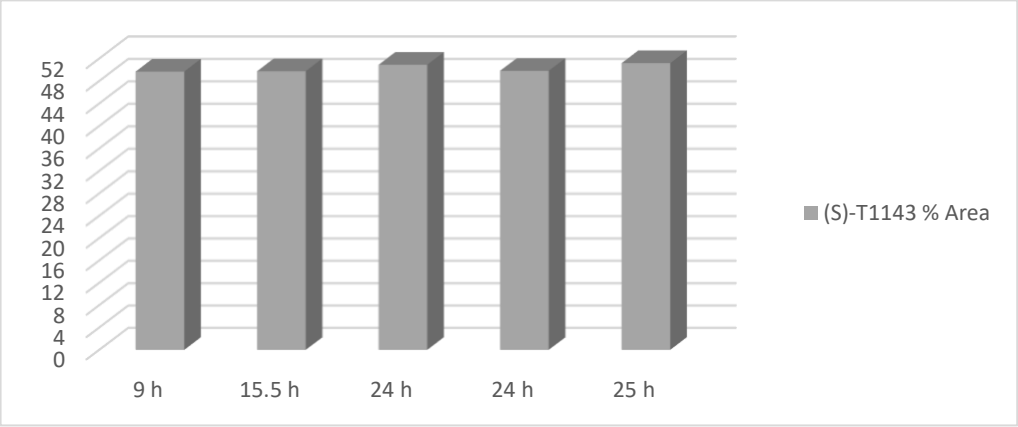

Figure 11. Bar graph representing the variation of measured *(S)* - T1143 % Area in H_2O at different reaction durations at the same *(R)*-T1143·HCl concentration. (Entry # 40/1, 40/2, 39, 40/3, and 38, from left to right).

The percentage of *(S)* - enantiomer detected in HPLC for the nine-hour reaction (see entry 40/1) is 49.7; while it was measured as 49.8 when the same reaction continued for twenty-four hours. When the same reaction is continued to be heated fifteen-hours longer at 100 °C, the improvement in the conversion from *(R)*- to *(S)*-enantiomer is only 0.1. Unlike other *aza*-Michael type reactions studied in the literature for enantiopure synthesis method improvements of compounds that need organocatalysts containing nitrogen to induce *aza*-Michael reaction by providing nucleophilic nitrogen, this reaction is not to be carried on for times exceeding nine hours.[31]

This means that the equilibrium can be formed in less than 24 hours at this specified concentration. The difference of the reaction time may not seem to provide a considerable energy efficiency in the lab-scale; however, for a production site designed to produce several kilograms, even tons of products, the smaller the amount of heat consumed, the more affordable and environmentally friendly the active pharmaceutical ingredient is. More detailed investigation of formation of *aza*-Michael / retro-*aza*-Michael equilibrium in terms of activation energy and other kinetic perspectives, along with energy management of the production, might be necessary for achieving a holistic approach for optimizing the production further.

2.3 Overall process and the yield.

The importance and the necessity of an enantiomerization method is previously explained. In theory, finding a method to succeed the racemization must recover the same amount of API product. However, in practice, the amount of re-gained material is dependent on the workup procedures and purification methods. Propitiously, the use water as the solvent is not conflicting with the already existing process and the absence of such contradiction is leading to a ground that a considerable amount of material can be recovered. The downstream process consists of 1) basification to release hydrochloride salt, 2) DTTA formation and resolution, 4) release of DTTA salt by basification, and 4) crystallization. Prior to these steps, the overall racemization can be shown as it is in Figure 12 below. The following procedures applied as shown in Figures 13, 14 and 15.

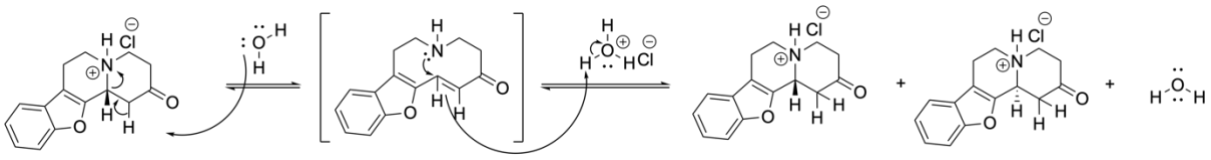

Figure 12. *(R)*-T1143·HCl ((12b*R*)-2-oxo-1,2,3,4,5,6,7,12b-octahydrobenzofuro[2,3-*a*] quinolizine-5-ium chloride) dissolved in water and refluxed. *(E)*-2,3,4,5-tetrahydro-benzofuro[3,2-*d*]azecin-6(1*H*)-one intermediate is formed, and an oxonium ion is produced. This intermediate is produced both *(R)*-T1143·HCl ((12b*R*)-2-oxo-1,2,3,4,5,6,7,12b-octahydro-benzofuro[2,3-*a*]quinolizin-5-ium chloride) and *(S)*-T1143·HCl ((12b*S*)-2-oxo-1,2,3,4,5,6,7,12b octahydro-benzofuro[2,3-*a*]quinolizin-5-ium chloride) in approximately 1:1 ratio.

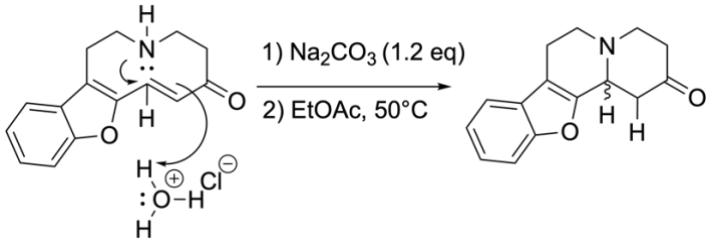

Figure 13. *(E)*-2,3,4,5-tetrahydro-benzofuro[3,2-*d*]azecin-6(1*H*)-one intermediate producing *(RS)*-T1143·HCl and the resulting racemic mixture is basified with sodium carbonate until the pH is above 10. Then extracted into ethyl acetate as *(RS)*-T1143.

The intermediate formed upon refluxing *(R)*-T1143·HCl in water is leading to the formation of hydrochloride salt of the racemic mixture of T1143. This salt needs to be removed to allow the resolution process to take place. The aforementioned resolution process is achieved by the use of DTTA, and it is performed to collect the desired enantiomer in an enantiomerically pure manner. To form the DTTA salt, HCl salt is necessary to be removed under basic conditions, then extraction must be performed. Basic conditions were met by addition of sodium carbonate (Na_2CO_3), where pH was adjusted to be at least 10. When it comes to selecting the organic solvent for to collect the organic material at this salt removal step, ethyl acetate was considered to be the best option since the overall production process already uses ethyl acetate in various steps as the organic solvent, thus, analytical studies related to ethyl acetate is completely done. Therefore, it was decided to be a solvent that is analytically easy to integrate for the additional steps. As a result, liquid-liquid extraction was performed where ethyl acetate was chosen to be

the organic phase. After performing phase separation in separatory funnel and washing the organic layer with water to remove the rest of the salt from the organic media, *(RS)*-T1143 was successfully collected into an evaporating flask. Ethyl acetate was evaporated by using rotary evaporator and a dry solid was obtained. The dried racemic mixture yielded 98% *(RS)*-T1143, and HPLC analysis showed 49.8 % *(S)* - enantiomer (see Table 2, entry 40/3). Upon obtaining the base form of the racemate, resolution step is applied by dissolving the racemate again in ethyl acetate, then addition of water and finally di-*p*-toluoyl-L-tartaric acid, in parts, and left for mixing for at least 10 hours at room temperature (21°C). This step is already a part of the production, thus additional analytical studies were not developed to control the reaction, instead, the previously defined procedures were applied. At the end of the mixing, the mixture is filtered through a No.3 sintered glass filter, and a beige-colored solid was obtained in 88 % yield, and HPLC analysis showed 90.4 % *(S)* - enantiomer. This step can be developed further to increase the percent yield and enantiomeric purity obtained by adjusting the reaction time and DTTA content, and / or temperature. Minor adjustments may promote the DTTA salt formation, thus may increase both the ee percent measured in HPLC and mass percent yield.

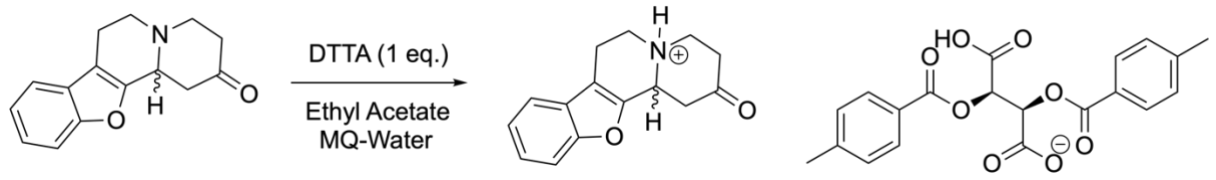

Figure 14. Reaction scheme of *(RS)*-T1143·DTTA formation from *(RS)*-T1143 for resolution of enantiomers between 10 to 72 hours with the presence of DTTA at room temperature. *(RS)*-T1143·DTTA can be separated into *(R)*-T1143·DTTA and *(S)*-T1143·DTTA by filtration.

The *(S)*-T1143·DTTA collected from the filter and reacted again in the presence of Na_2CO_3 to release the DTTA salt, and *(S)*-T1143 in the base form was obtained in the organic solvent (EtOAc).

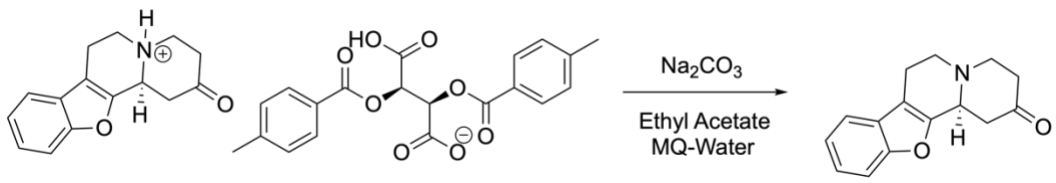

Figure 15. Reaction scheme of DTTA salt release from *(S)*-T1143·DTTA that is collected as a fine solid upon filtration.

The *(S)*-T1143 obtained as a result of the final basification step as shown in Figure 15 requires further purification. HPLC results varied between 90-98% *(S)*-enantiomer when DTTA resolution step was performed. After evaluating the parameters that affect the formation of enantiomeric excess percentages, it was found that there is a possibility of further improvement of the purity by adjusting the reaction time, i.e. it is acceptable to continue the DTTA salt formation step 10 to 72-hours, yet the longer the time spent, the more purity levels can be achieved. Additionally, the partial addition of DTTA is another parameter for obtaining better resolution. It was instructed to add the DTTA salt in four parts over at least an hour span. Evaluating this, it might be a better idea to divide the sum of DTTA into more than four fractions and addition to the reaction media at a longer time span might yield higher purity levels.

Nevertheless, the use of IPA crystallization was determined to be necessary to achieve higher levels of purity. Refluxing the crude *(S)*-T1143 in isopropyl alcohol was, again, an integral part of the API production. This method provides a lighter colored and more homogeneous solid, however, IPA dissolves the material to a degree that cannot be omitted; thus, an improvement study had been performed for this step. After refluxing in IPA from 15 to 45 minutes, mixing was continued at either 21 °C or 0 °C. Unfortunately, a major improvement of percent yield was not achieved after this work. The amount of isopropyl alcohol used as washing and transferring liquid was limited and the filtrate, or mother liquor, was collected to reduce the loss of *(S)*-T1143, which then can be purified and re-crystallized.

3 EXPERIMENTAL METHODS

3.1 Synthetic Methods

3.1.1 Synthesis of *(RS)* - T1143 from *(R)*-T1143·HCl

(R)-1,3,4,6,7,12b-hexahydro-2*H*-benzofuro[2,3-a]quinolizine-2-one hydrochloride is collected from the waste-stream of the current production of *(S)*-T1143, *(S)*-1,3,4,6,7,12b-hexahydro-2*H*-benzofuro[2,3-a]quinolizine-2-one, in 95% enantiomeric excess (ee) on average, where its color was ranging from intense brown to pale brown solid. *(R)*-T1143·HCl was then weighed 1 g (3.6 mmol) was added 10 mL MQ-water. The mixture obtained is of 0.36 mol/L concentration and it was mixed at reflux temperature of water (100 °C), under N_2 atmosphere, for 24-hours, samples were collected as presented in Table 2. Upon elongated reaction times, an orange-brown colored mixture was obtained, and a tar-like material was piling up on the peripheral of the round-bottom flask. After cooling down to approximately 60 °C, material inside the flask was added sodium carbonate (Na_2CO_3, 1.18 equivalent) and pH was checked to over 10, then ethyl acetate (EtOAc) was added and used as the organic phase at a 10 mL of volume. This mixture was mixed at 50 °C for approximately 15 minutes and cooled down to room temperature while mixing. Liquid-liquid extraction was performed by the use of a separatory funnel and then the organic layer was washed with one-fifth of initial water amount (~ 2 mL). Aqueous layer was removed, and the organic layer was transferred into a rotary evaporator flask, evaporated to dryness and the isolated *(RS)*-T1143 was analyzed by HPLC and NMR. The produced tar-like material was not transferred with the material of interest since this tar was stuck on the peripheral of the glassware. Later, this material was also analyzed and found to have no effect on the racemization reaction to be carried out. Depending on the reaction time and other variables, the results were also varied in terms of enantiomeric ratios. Upon confirmation of the racemization (50.3% / 49.7%, S- / R- %Area), the *(RS)*-T1143 was resolved by DTTA-crystallization technique as explained below.

3.1.2 Resolution of *(RS)*-T1143

Racemate *(RS)*-T1143 (0.82 g, 3.4 mmol) was resolved by one equivalent of (-) - *O, O'* - Di-*p*-toluoyl-L-tartaric acid (DTTA) (1.31 g, 3.4 mmol), added in four fractions, in the presence of 15 mass-fold of ethyl acetate and 0.3 mass fold of MQ-Water, over an hour-long span. Overnight mixing at room temperature (may vary from 10 to 72 hours for this step) yielded *(S)*-T1143·DTTA in solid form, which was collected by filtration via sintered glass, filter no: 3.

Collected residue had a lighter color, pale brown to beige, and fine solid texture. Compared to the *(R)*-T1143·HCl collected from the waste stream, the material was visibly pure and had a better homogeneity. Mass of T1143·DTTA (627.64 g/mol) was weighed as 1.02 g, which corresponds to 1.6 mmol of T1143·DTTA. The mass percent yield can be calculated 89%. This solid was analyzed and found to contain 90.4% *(S)*-enantiomer.

On the other hand, *(R)*-T1143·DTTA was mainly collected with ethyl acetate as the filtrate, which then was evaporated to dryness with rotary evaporator. The mass of T1143·DTTA solid obtained from the filtrate was 1.35 g corresponding to 2.15 mmol of T1143·DTTA. HPLC analysis showed that this solid obtained from the filtrate contained 55.3% *(S)*-enantiomer.

3.1.3 Isolation of *(S)*-T1143 from *(S)*-T1143·DTTA

Removal of DTTA salt was again performed by basification where addition of 10 mass-fold of ethyl acetate to 1.02 g *(S)*-T1143·DTTA (90.4% *(S)*-enantiomer), mixing at room temperature for 15 minutes, then addition of 0.7 mass fold of Na_2CO_3 (0.71 g, 7 mmol) and 6.4 mass fold MQ-Water (6.5 g). This mixture was then heated at 50 °C and mixed for another 15 minutes. After it was cooled down to room temperature, organic phase was separated, washed and evaporated. The isolated *(S)*-T1143 was collected and weighed as 0.31 g (1.29 mmol), where the yield was calculated to be 38% at a higher ee purity. According to HPLC peak area, this product contained 97.7 % S-enantiomer. Although the purity level was higher than initial resolution method, the 100% purity was desired. Thus, the following crystallization step was applied.

3.1.4 Crystallization of *(S)*-T1143

Isopropyl alcohol was used to prepare a slurry from the crude *(S)*- T1143 to increase ee further. For this purpose, 5-fold isopropyl alcohol (1.55 g, 2 mL) was added and mixed under reflux conditions for 15 minutes. Then it was cooled down to room temperature and filtered to obtain *(S)*-T1143 crystals. The mass of final dried product was 0.24 g (1 mmol) and contained 100 % *S*-enantiomer according to HPLC peak area.

3.2 Analytical Methods

3.2.1 Normal-phase high resolution liquid chromatography (HPLC)

Normal phase high performance liquid chromatography (HPLC) was performed with the following set-up: Agilent 1100 G1311A Quaternary pump, Agilent 1100 G1322A Degasser, Agilent 1100 G1313A Autosampler, Agilent 1100 G1316A thermostatted column compartment, Agilent 1100 G1315A Diode Array Detector (detection wavelength 254 nm), Chiralcel OD-H column 250 x 4.6 mm, 5 mm. Samples were filtered with 0.45 μm membrane filter during preparation. Reference solution (working standard, WS) and sample solutions were prepared in 0.6 g/mL concentration in hexane/ethyl alcohol (EtOH) diluent in 85/15 ratio. System solution was prepared by dissolving a 12 mg sample material in 2.0 mL of diluent in 10 mL volumetric flask, then adding 100 μL of reference solution, and following this step filling up the volume to the 10 mL with diluent.

The mobile phase was prepared by hexane/isopropyl alcohol (IPA)/diethylamine (DEA) in 90/10/0.1 ratio. All reagents used were HPLC-grade, except where DEA was Puriss grade (≥ 99.5%). Analysis was performed in the order of a blank, reference solution, system solution and sample vials where the flow rate was 0.5 mL/min, the injection volume was 2 μL and the run time was 30 minutes.

3.2.2 Nuclear magnetic resonance (NMR)

NMR spectra were recorded with Bruker-300 Ultrashield spectrometer [^{1}H: 300 MHz, ^{13}C: 75 MHz]. Measurements were performed at 300 K and, chloroform-d ($CDCl_3$) or dimethylsulfoxide-d_6 (DMSO-d_6) were used as solvent. The reference signals for T1143 WS (working standard) for ^{1}H and ^{13}C NMR was previously studied in DMSO-d_6. Chemical shifts are reported below with one decimal for both ^{1}H and ^{13}C. The reported signals are referenced to residual solvent signal (d_6-DMSO δ_H = 2.5 ppm, δ_C = 39.7 ppm).

^{1}H NMR (300 MHz, d_6-DMSO, 27 °C): δ = 2.3-2.8 (m, 2H, H-16 and H16'), 2.6-3.0 (m, 2H, H-14 and H-14'), 2.7-3.0 (m, 2H, H-13 and H-13'), 2.7-3.2 (m, 2H, H-17 and H-17'), 2.8-3.3 (m, 2H, H-12 and H-12'), 3.6 (m, 1H, J = 12.6 Hz, H-10), 7.3 (m, 2H, H-1 and H-2), 7.5 (m, 2H, H-3 and H-6) ppm.

^{13}C NMR (300 MHz, d_6-DMSO, 27 °C): δ = 20.7 (C-13), 40.9 (C-16), 43.5 (C-14), 50.2 (C-17), 52.9 (C-12), 57.3 (C-10), 111.1 (C-3, C-9), 119.1 (C-6), 122.7 (C-1), 123.8 (C-2), 127.4 (C-5), 152.3 (C-8), 154.0 (C-4), 206.6 (C-15) ppm.

4 CONCLUSION

Conversion of *(R)*-1,3,4,6,7,12b-hexahydro-2*H*-benzofuro[2,3-a]quinolizine-2-one, into its racemate can be performed by thermal enantiomerization in an appropriate choice of solvent when the correct concentration, reaction time, and temperature conditions are met. This specific compound had been studied to recover 50% of the undesired product, *(R)*-1,3,4,6,7,12b-hexahydro-2*H*-benzofuro[2,3-a]quinolizine-2-one, that is produced as a waste during the production of *(S)*-1,3,4,6,7,12b-hexahydro-2*H*-benzofuro[2,3-a]quinolizine-2-one active pharmaceutical ingredient. Despite various studies performed, based on the knowledge available in literature, no known method was successful to yield promising results. However, for this nearly planar, quinolizine-one, and benzofuro functionality carrying API small molecule, racemization is achievable when it is in hydrochloride salt form, upon dissolving it in a polar and a hydrogen bond acceptor solvent, such as DMSO or water; and heating for certain amount of time to supply enough thermal energy to the system, through formation of *aza*-Michael / retro-*aza* -Michael equilibrium. This novel method provides an environmentally friendly solution to the problem of losing half of the product due to unavailability of a *de novo* enantiopure synthesis method and a known racemization method that is applicable for this compound.

As a result of this finding, it is possible to convert an enantiomeric mixture of ≥ 90% *(R)*-enantiomer containing material into the desired form at 100% purity at 48 percent yield or more (up to 66%) in four steps. Improvements, such as adjusting temperature, reaction time or the amount of added DTTA salt may improve this yield further. Each step of racemization produces a 90% ee desired form in the resolution process, which is then recycled again. Additionally, a racemate is collected as the filtrate, which then can be subjected another cycle of resolution and purification easily.

This result shows the need of further understanding of stereogenic center containing molecules and, of enantiomerization methods based on thermal applications. The key point that might be missed easily is that any of the solvent-concentration-temperature combination worked for the racemization of *(R)*-T1143·HCl did not work for *(R)*-T1143. The pH value of the reactant and whether it's in quaternary ammonium salt or in tertiary nitrogen form affects the formation of an *aza*-Michael/retro-*aza*-Michael equilibrium. When the *(R)*-T1143 is in hydrochloride salt form, it is possible to cleave the bond between the quaternary ammonium nitrogen and the

stereogenic center carbon of the molecule, which then leads to the formation of an α,β-unsaturated carbonyl functionality and a lone-pair containing nitrogen atom in close proximity that can attack as the nucleophile. According to the general knowledge, the taking place of Michael reaction is closely related to the existence of a strong base in the reaction media. Nonetheless, the proximity of nitrogen atom-stereogenic carbon-ketone group allows the initiation of aza-retro-Michael reaction that later forms an equilibrium at elevated temperatures (100 °C) and at acceptable reaction times (2-25 hours). Similar compounds present in literature can be racemized by a similar method when correct conditions are met, which are assumed to be specific to the properties of the compound at hand and needs further studies.

The contribution of this work to the field is that it is possible to utilize environmentally friendly solvents and thermal racemization for compounds that may allow formation of any variation of Michael/retro-Michael equilibrium.

The importance of recovery of the waste produced at the end of this API production pipeline has been highlighted and it is a great opportunity, both in terms of ecological and economical aspects, for increasing the yields of each batch of production.

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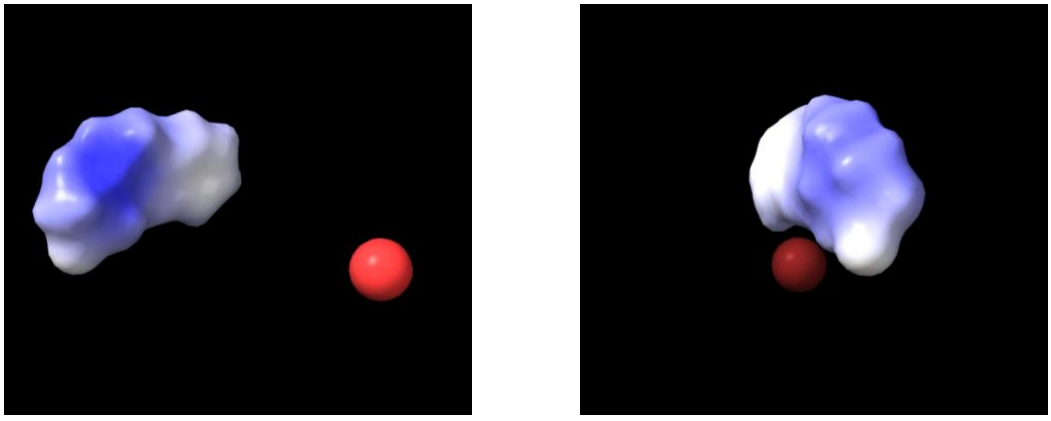
Appendices

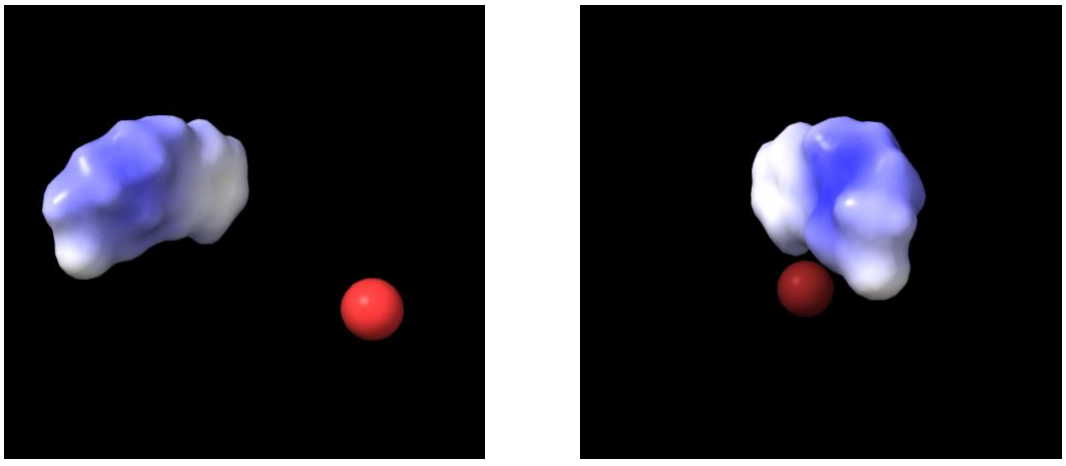
Appendix 1: Supplementary material of *(R)*- and *(S)*-T1143 and derivatives.

Molecular graphics and analyses performed with UCSF ChimeraX, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from National Institutes of Health R01-GM129325 and the Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases.

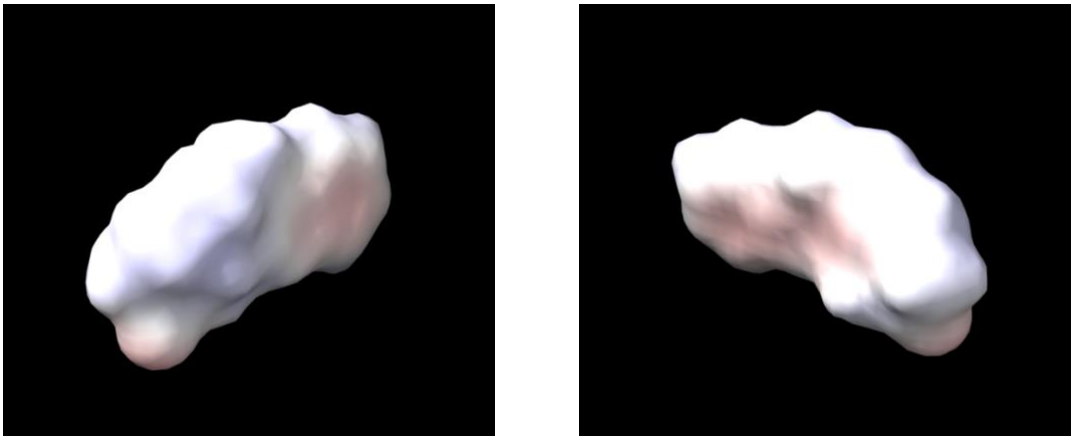


S. Figure 1. Both on the left and on the right are electrostatic properties of *(R)*-T1143·HCl. Coulombic values for iupac:(12bR)-2-oxo-1,2,3,4,5,6,7,12b-octahydrobenzofuro[2,3-a]quinolizin-5-ium chloride_ SES surface #3.1: minimum, -7.88, mean 2.94, maximum 7.98. (Ref. ChimeraX)

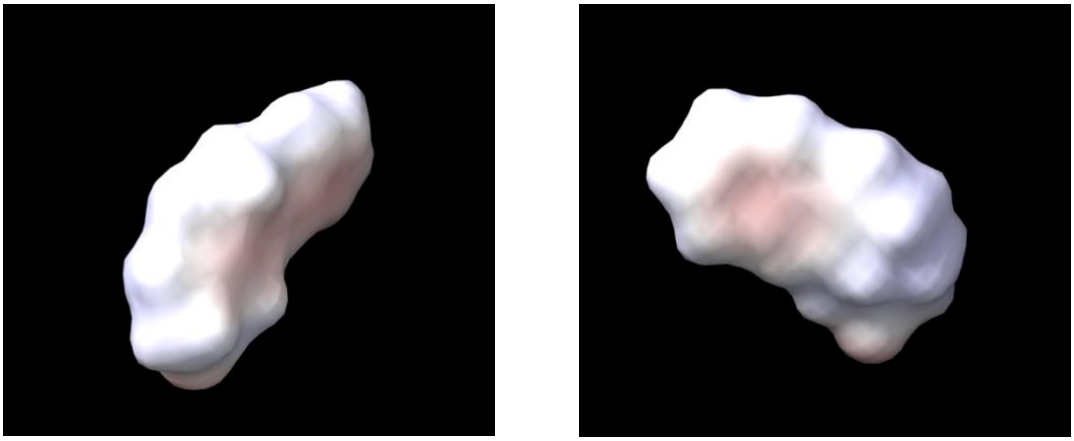
White to blue for positive potential. Red for negative potential. Palette red-white-blue range -10 to 10. Pettersen EF, Goddard TD, Huang CC, Meng EC, Couch GS, Croll TI, Morris JH, Ferrin TE. *Protein Sci.* 2021 Jan;30(1):70-82.



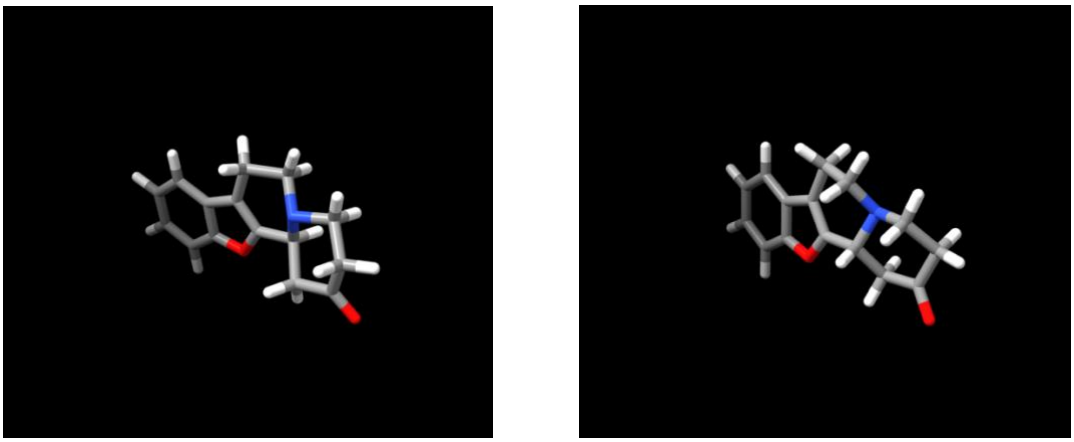
S. Figure 2. Both on the left and on the right are electrostatic properties of *(S)*-T1143·HCl. Coulombic values for iupac:(12bS)-2-oxo-1,2,3,4,5,6,7,12b-octahydrobenzofuro[2,3-a]quinolizin-5-ium chloride_ SES surface #2.1: minimum, -7.88, mean 2.95, maximum 8.04. (Ref. ChimeraX)



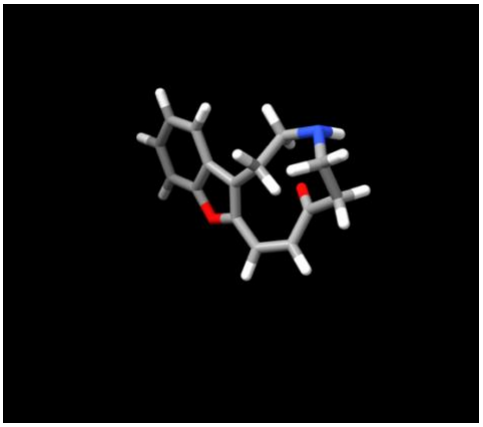
S. Figure 3. Both on the left and on the right are electrostatic properties of *(S)*-T1143. Coulombic values for iupac:(S)-1,3,4,6,7,12b-hexahydro-2H-benzofuro[2,3-a]quinolizine-2-one_ SES surface #5.1: minimum, -2.51, mean 0.10, maximum 1.75. (Ref. ChimeraX)



S. Figure 4. Both on the left and on the right are electrostatic properties of *(R)*-T1143. Coulombic values for iupac:(R)-1,3,4,6,7,12b-hexahydro-2H-benzofuro[2,3-a]quinolizine-2-one_ SES surface #7.1: minimum, -2.52, mean 0.14, maximum 1.76. (Ref. ChimeraX)

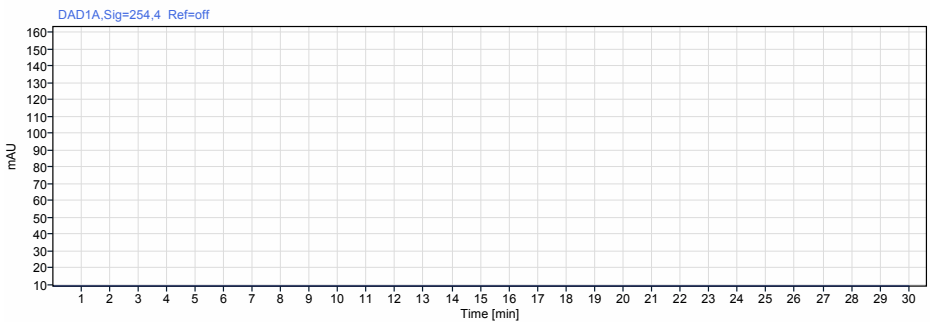


S. Figure 5. On the left side 3D model of (S)-enantiomer and on the right side (R)-enantiomer of T1143. (Ref. ChimeraX)

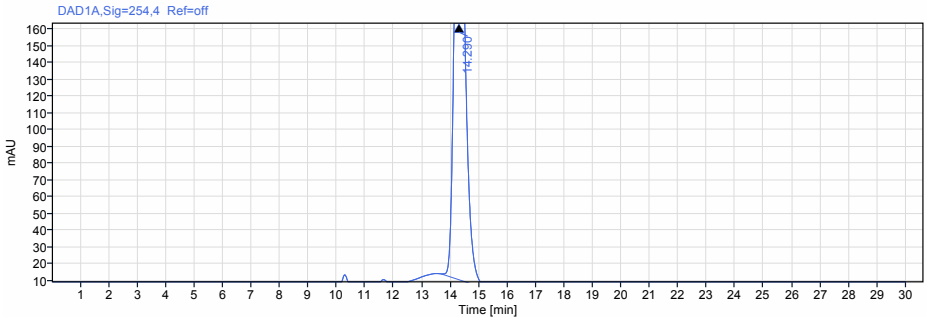


S. Figure 6. 3D stick model of (Z)-2,3,4,5-tetrahydrobenzofuro[3,2-d]azecin-6(1H)-one. (Ref. ChimeraX).

Appendix 2: Supplementary material of *(R)*-, *(RS)*- and *(S)*-T1143 HPLC chromatograms.

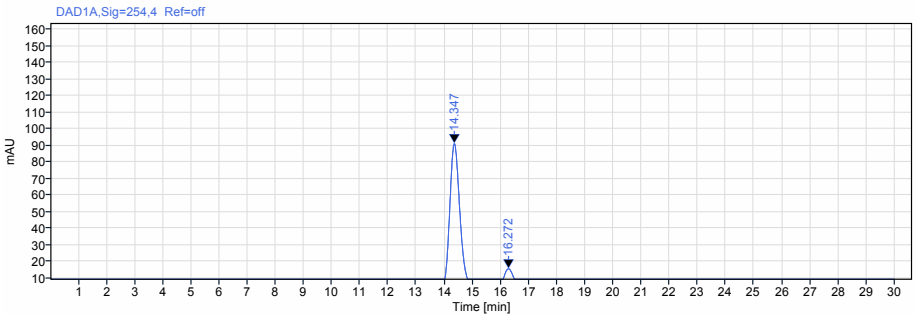


S. Figure 7. Blank analysis HPLC chromatogram.



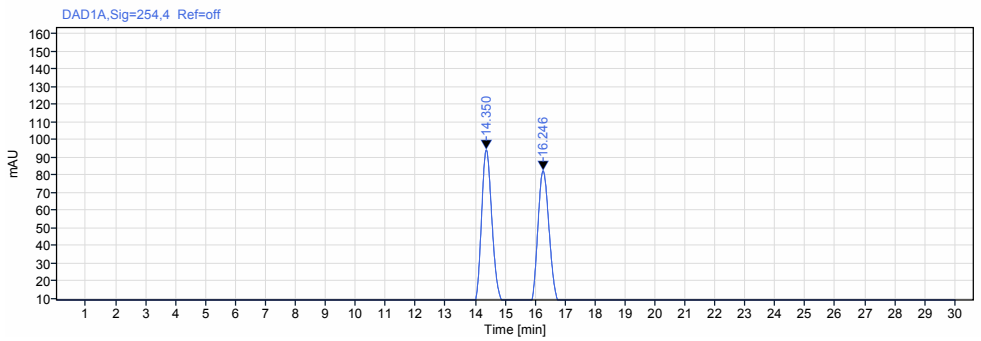
Signal:	DAD1A,Sig=254,4 Ref=off					
RT [min]	**Type**	**Width [min]**	**Area**	**Height**	**Area%**	**Name**
14.290	BB	1.81	7662.6309	305.3929	100.0000	T1143
		Sum	7662.63			

S. Figure 8. T1143 wrong enantiomer *(R)*-T1143 reference analysis HPLC chromatogram.



Signal:	DAD1A,Sig=254,4 Ref=off					
RT [min]	**Type**	**Width [min]**	**Area**	**Height**	**Area%**	**Name**
14.347	BB	1.89	2223.7873	87.7608	84.7206	T1143
16.272	BB	1.78	401.0612	14.2705	15.2794	T1143 R-enantiomer
		Sum	2624.85			

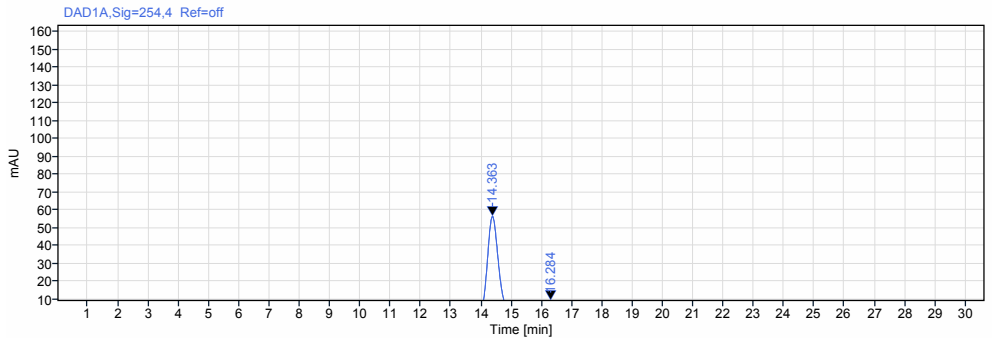
S. Figure 9. System solution analysis HPLC chromatogram.



Signal: DAD1A,Sig=254,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name
14.350	BB	1.95	2268.6477	89.4580	50.2748	T1143
16.246	BB	2.09	2243.8454	79.6517	49.7252	T1143 R-enantiomer
		Sum	4512.49			

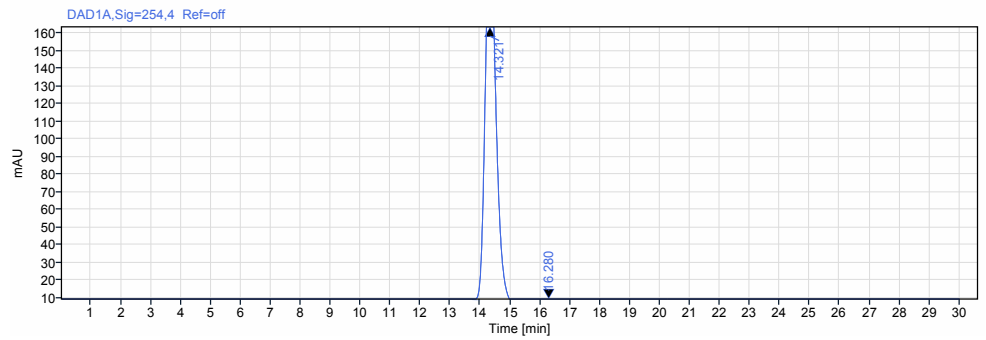
S. Figure 10. Racemate-base, *(RS)*-T1143 formation upon 24-hour reaction in refluxing in water, enantiomeric ratio confirmation by HPLC.



Signal: DAD1A,Sig=254,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name
14.363	BB	1.87	1383.9958	54.4803	90.4002	T1143
16.284	BB	1.50	146.9701	5.2397	9.5998	T1143 R-enantiomer
		Sum	1530.97			

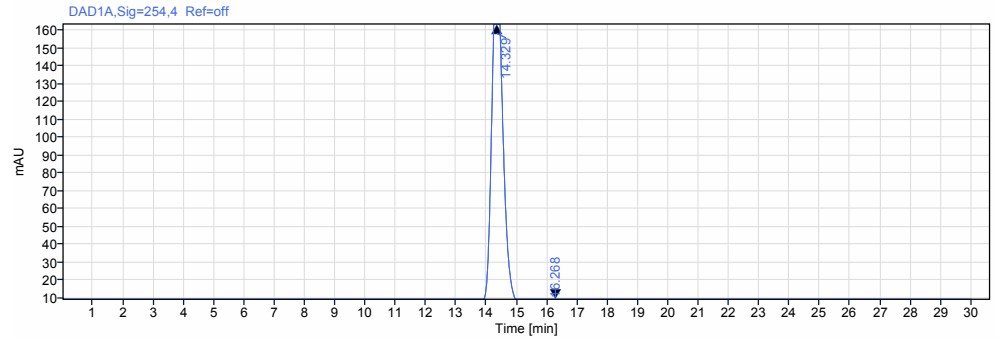
S. Figure 11. *(S)*-T1143·DTTA salt formation and resolution, enantiomeric ratio confirmation by HPLC for the compound obtained upon filtration.



Signal: DAD1A,Sig=254,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name
14.321	BB	1.83	5341.4178	211.3807	97.6887	T1143
16.280	BB	1.24	126.3790	4.6957	2.3113	T1143 R-enantiomer
		Sum	5467.80			

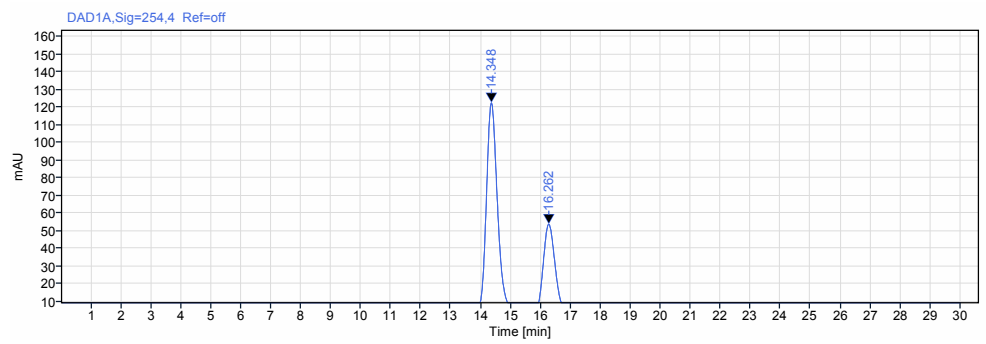
S. Figure 12. *(S)*-T1143-base form enantiomeric ratio confirmation by HPLC after DTTA salt is removed by basification process.



Signal: DAD1A,Sig=254,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name
14.329	BB	1.76	4879.3003	193.6588	99.4509	T1143
16.268	BB	0.98	26.9381	1.0744	0.5491	T1143 R-enantiomer
		Sum	4906.24			

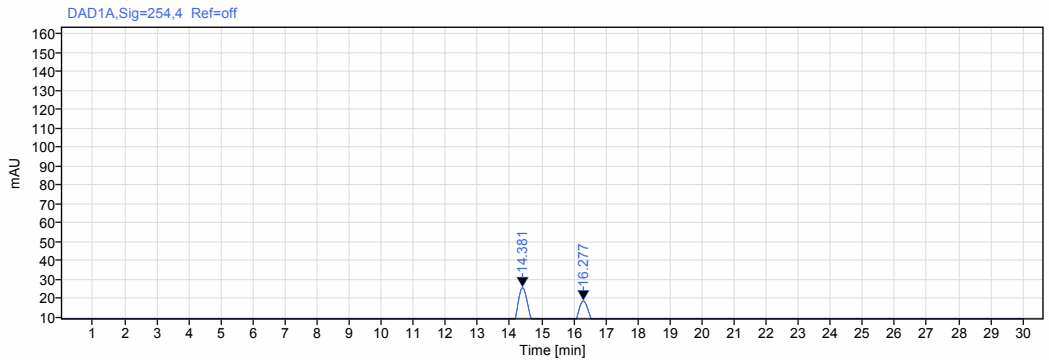
S. Figure 13. *(S)*-T1143 crystallization by IPA treatment and the resulting HPLC chromatogram.



Signal: DAD1A,Sig=254,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name
14.348	BB	1.96	2979.9468	117.6154	67.2802	T1143
16.262	BB	1.80	1449.2104	51.4234	32.7198	T1143 R-enantiomer
		Sum	4429.16			

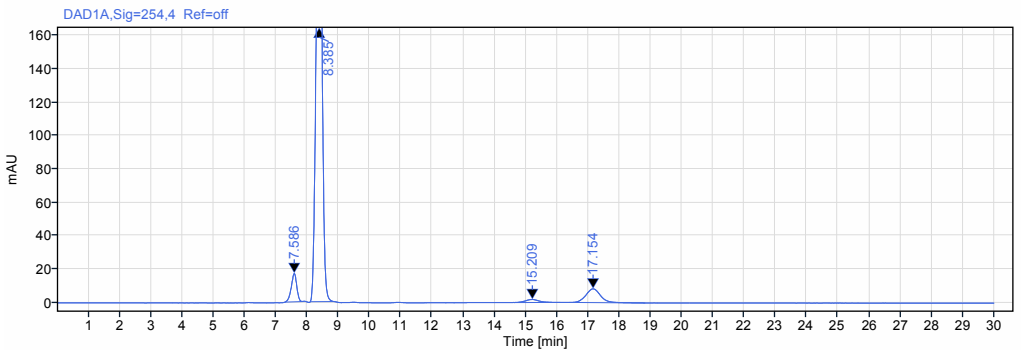
S. Figure 14. *(S)*-T1143 IPA treatment mother liquor, analysis of loss of the desired form into the isopropyl alcohol filtrate.



Signal: DAD1A,Sig=254,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name
14.381	BB	1.87	628.4454	24.4390	55.2974	T1143
16.277	BB	1.74	508.0378	17.8948	44.7026	T1143 R-enantiomer
		Sum	1136.48			

S. Figure 15. *(RS)*-T1143 DTTA resolution step, analysis of loss of the desired form into the DTTA-ethyl acetate filtrate.

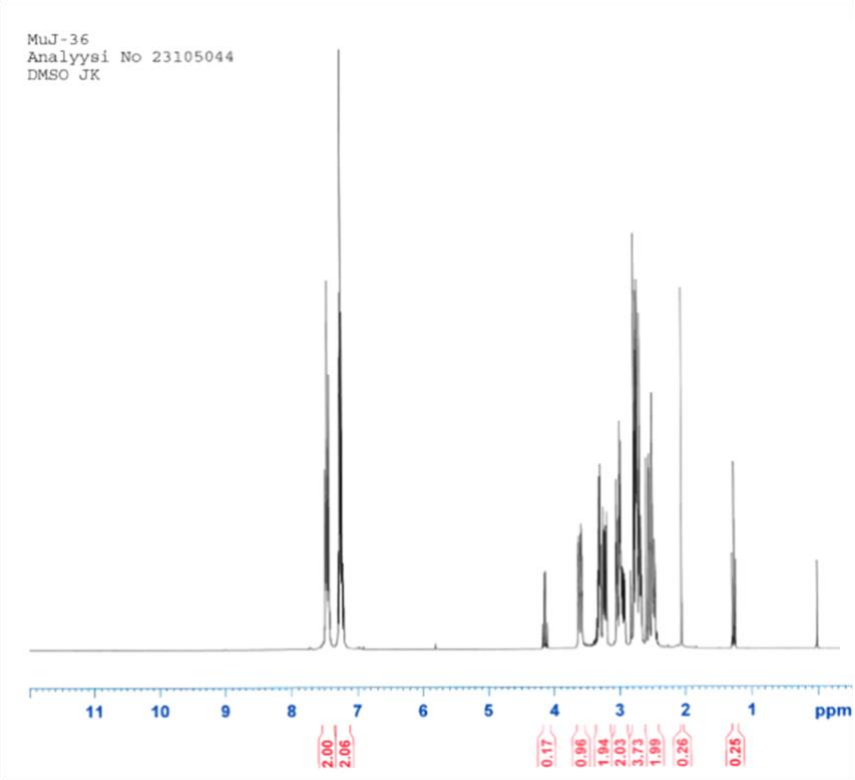


Signal: DAD1A,Sig=254,4 Ref=off

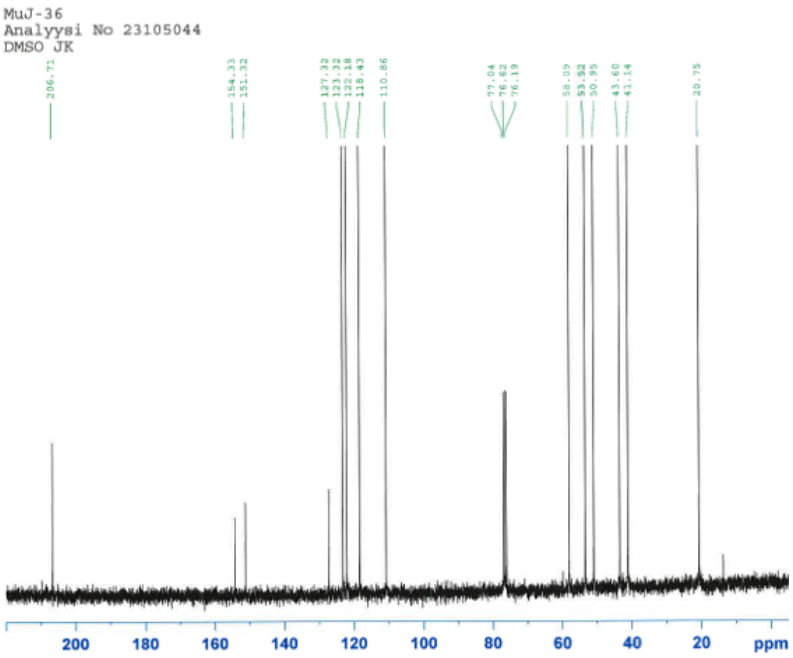
RT [min]	Type	Width [min]	Area	Height	Area%	Name
7.586	MM m	0.55	205.19	16.97	4.56	
8.385	MM m	0.74	3974.76	329.26	88.26	
15.209	BB	1.47	54.09	1.79	1.20	
17.154	BB	2.43	269.66	8.26	5.99	
		Sum	**4503.71**			

S. Figure 16. Additional peaks that were observed in HPLC analysis when Heptane/*i*-PrOH/MeOH solution at 3/1/1 ratio was used as the solvent and heated to 100 °C.

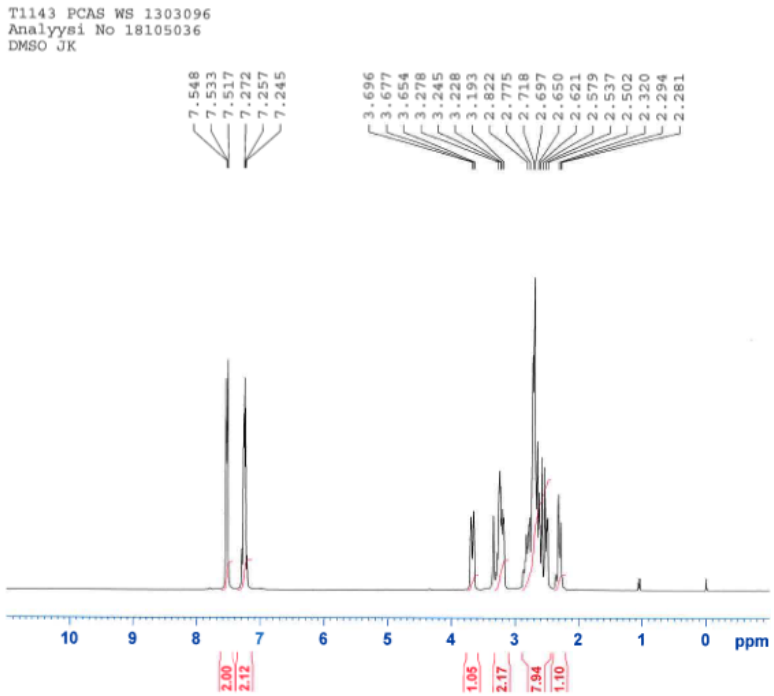
Appendix 3: Supplementary material of *(R)*-, *(RS)*- and *(S)*-T1143 NMR spectra.



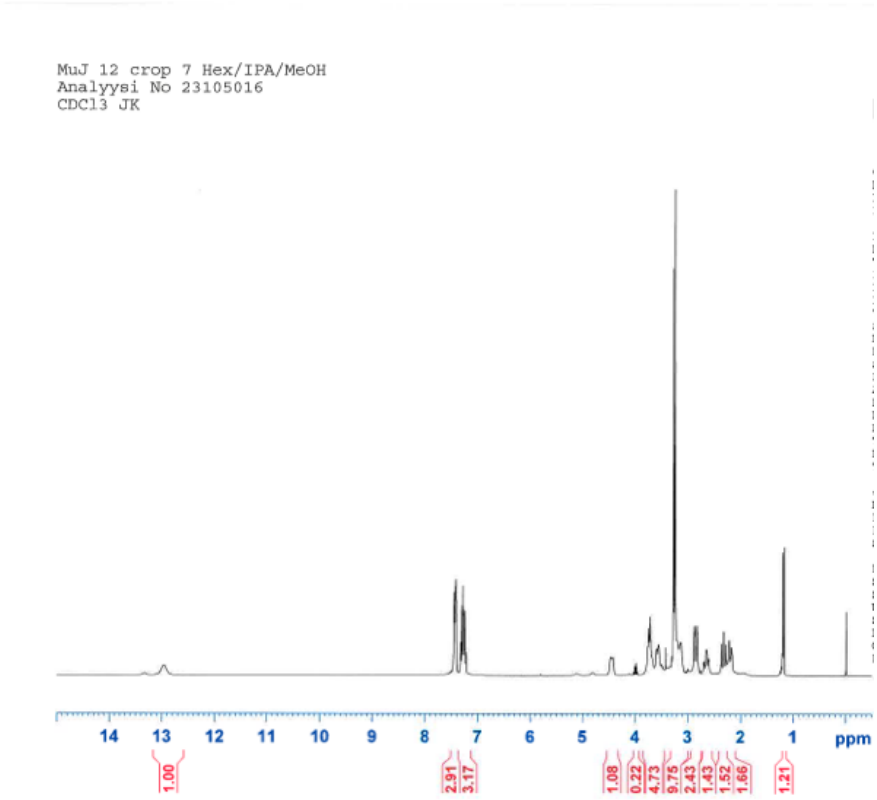
S. Figure 17. ^{1}H NMR spectra of the *(RS)*-T1143 obtained after 20-hours of refluxing in water. Analysis performed in DMSO at 300.0 K

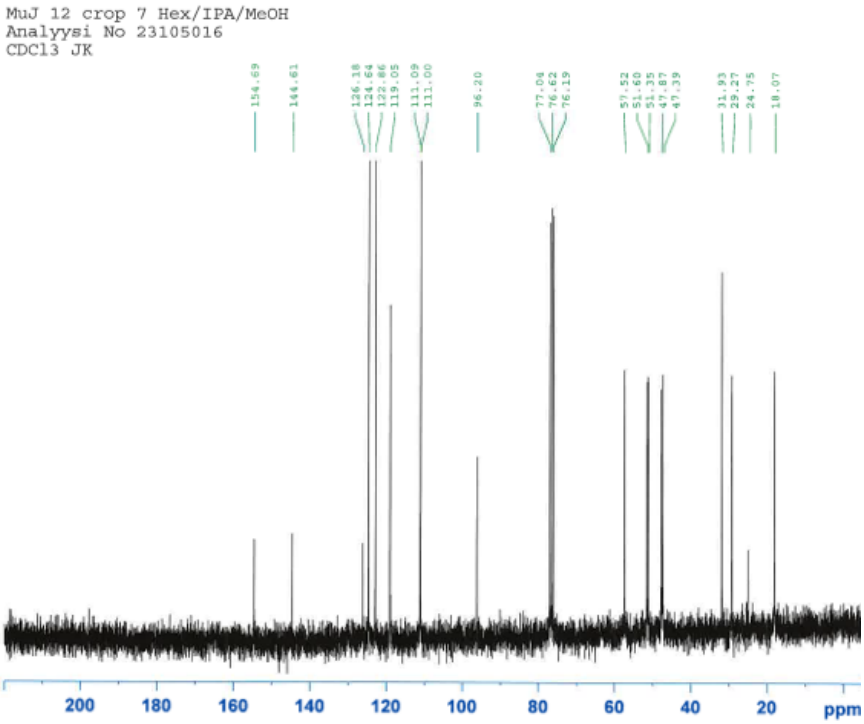


S. Figure 18. ^{13}C NMR spectra of the *(RS)*-T1143 obtained after 20-hours of refluxing in water. Analysis performed in DMSO at 300.0 K.



S. Figure 19. ^{1}H NMR spectra of the *(R)*-T1143 working standard analysis performed in DMSO at 300.0 K.

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60
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20
ppm