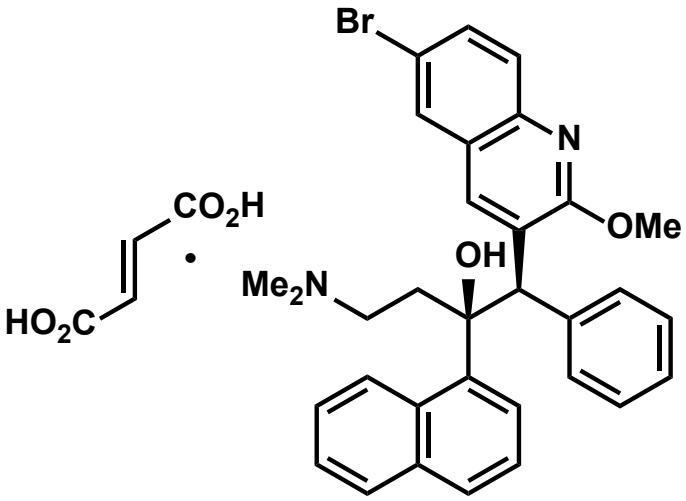
Medicines for All Institute

Summary of Process Development Work on the Synthesis of Anti-Tuberculosis Drug Bedaquiline Fumarate



Report Prepared by Medicines for All (M4ALL) Institute

Virginia Commonwealth University, Richmond, VA

In collaboration with TCG Life Sciences Pvt. Limited

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Contact: m4all@vcu.edu

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Scientists associated with the project from M4ALL

S.N.	Name of Scientist	Designation
1	Dr. Frank Gupton	CEO
2	Dr. Kai Donsbach	COO
2	Dr. Charles S. Shanahan	Director of Research
3	Dr. Ryan Nelson	Assoc. Director of Research
4	Dr. Juliana M. S. Robey	Research Scientist
5	Dr. Sanjay Maity	Postdoctoral Associate
7	Dr. Rodger Stringham	Director of Analytical
8	Dr. Justina Burns	Assoc. Director of Analytical
9	Dr. Sarah Aleshire	Senior Analytical Chemist

Scientists associated with the project from TCGLS

S.N.	Name of Scientist	Designation
1	Dr. Subho Roy	Vice President-Chemistry
2	Dr. Ajay K. Yadaw	Director – Chemistry
3	Dr. Angshuman Ghosh	Group Leader
4	Mr. Appana Ramakrishna	Research Scientist
5	Mr. Seelam Balaji Reddy	Research Scientist
6	Mr. Eerpina Ramudu	Senior Research Chemist
7	Mr. Gokavarapu Kishor	Senior Research Scientist

Scientists associated with TCG Green site

S.N.	Name of Scientist	Role / Title
1	Dr. Chris Senanayake	CEO & CSO
2	Dr. Joseph Armstrong	COO
3	Dr. Gopal Sirasani	AVP, Operations and Business Development

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EXECUTIVE SUMMARY

This Process Development Report (PDR) describes two cost-effective syntheses of Bedaquiline (BDQ) fumarate which offer significant cost-advantages compared to Janssen Pharmaceutical's current manufacturing process (Scheme 1).[1] Based on M4ALL's techno-economic (TE) analysis, the low yields for the 1,2-carbonyl addition reaction between **KSM-I** and **KSM-II** (Scheme 1) and the high cost associated with the chiral resolution used to obtain enantiopure BDQ (Scheme 2) were found to be the largest contributors to the raw material (RM) costs to make the API. To address these issues, we focused our development efforts on the optimization of the lithiation/1,2-addition sequence in order to improve reaction conversion and stereoselectivity as the primary tactic to improve the raw material cost of the final BDQ fumarate API.

The first synthetic approach we present in this report was a racemic synthesis of BDQ ("M4ALL-racemic") and focused on making small improvements to the original Janssen procedure, utilizing stronger lithium amide bases (i.e. pyrrolidine) and lithium salt additives (i.e. LiCl). These minor changes resulted in improved reaction conversion and enhanced diastereoselectivity towards the desired pair of diastereomers (Scheme 4). According to our TE calculations, this route has the potential to reduce RM costs by up to ~18% and improve PMI (Process Mass Intensity) by as much as ~8%, respectively, relative to the baseline route (Janssen).

The second approach we present in this report ("M4ALL-asymmetric") built off of our racemic approach by selecting a chiral pyrrolidine base to mediate and asymmetric coupling between **KSM-I** and **KSM-II**. The best chiral lithium amide base we screened was lithium (*R*)-2-(methoxymethyl)pyrrolidide (readily derived from D-proline) resulting in the induction of both diastereo- and enantioselectivity during the 1,2-addition reaction (Scheme 5). The increased stereoselectivity provided considerably higher yields of BDQ (**3**), and could offer a reduction of up to 52% in RM costs and improve PMI by ~48% relative to Janssen's baseline. A key cost-driver of this new asymmetric process is the chiral lithium amide base used to improve the yield of BDQ in the key step but, given the current low volume commercial supply, this chiral amine base would have to be made in-house starting from D-proline to realize the maximum cost-improvement. Fortunately, convenient processes to do so have been reported but would need verification at scale.[12] Outsourcing more advanced intermediates or the final chiral amine, (*R*)-2-(methoxymethyl)pyrrolidine, will decrease the savings potential of this approach. Also worth noting, is that in our asymmetric work we observed that switching the reaction solvent from tetrahydrofuran (THF) to 2-methyltetrahydrofuran (2-MeTHF) allowed for the reaction to be completed at increased temperature (-40 °C) compared to the baseline process (which uses −78 °C), and additionally improves solvent recycling opportunities. These two advantages of 2-MeTHF are likely to have a positive impact on cost reduction as well (though we have not modelled that in our TE analysis).

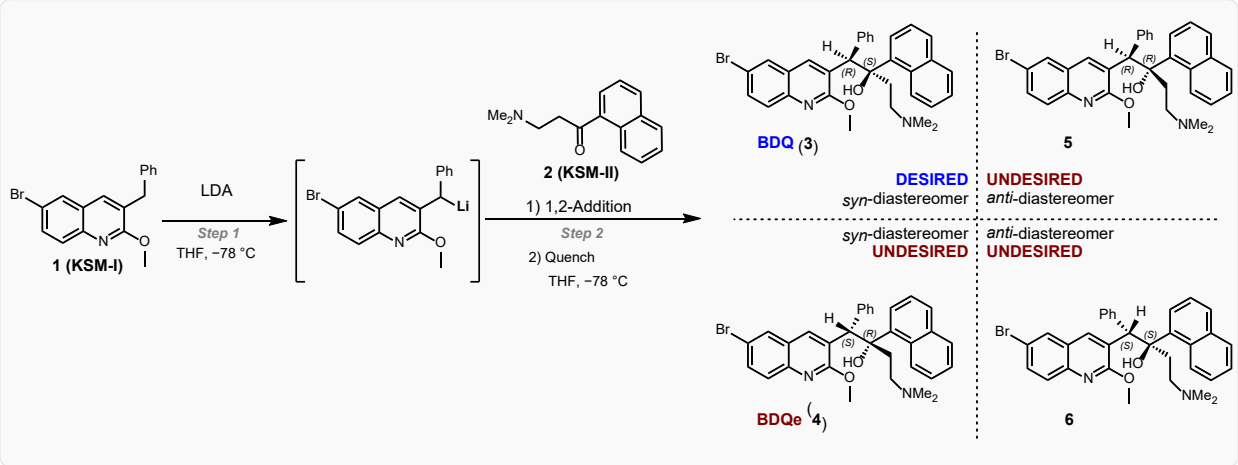
The work described in this PDR is a representative lab-scale process with batches starting with 75 to 100 g of **KSM-I**. Additional efforts will be needed for further optimization prior to scale-upand production in a cGMP (current Good Manufacturing Practices) environment.

INTRODUCTION

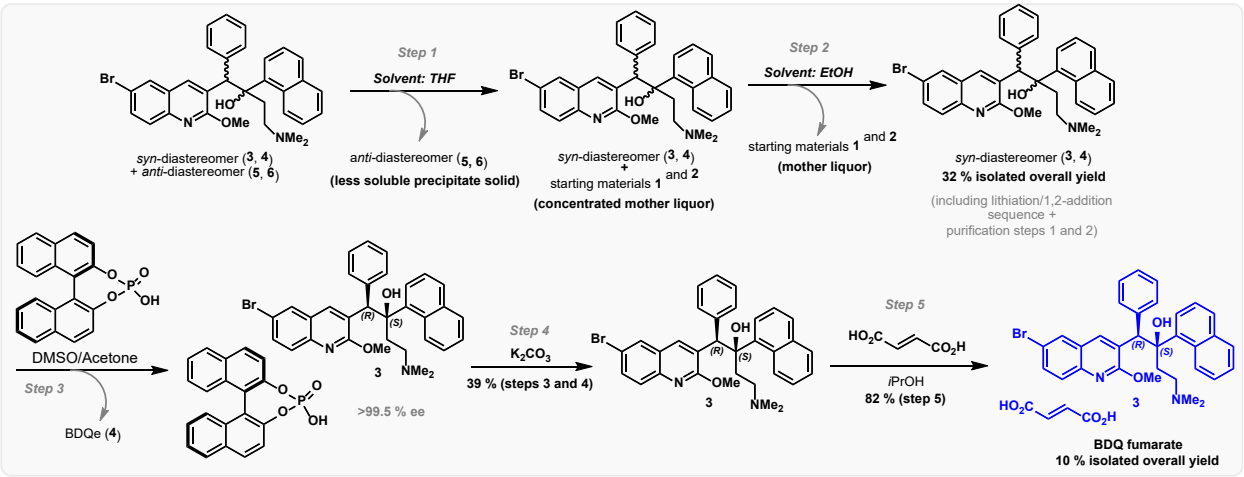
Bedaquiline fumarate (Sirturo™) was developed by Janssen in 2005 and later approved by the U.S. Food and Drug Administration (FDA) for treating patients with pulmonary multidrug-resistant tuberculosis (MDR TB) in cases where the most common treatments are no longer effective.**Error! Bookmark not defined.** BDQ is one of a family of diarylquinolines (DARQ) that show unique activity against drug-resistant TB strains, and it is used as a combination therapy. As such, bedaquiline is one of three drugs in the TB Alliance BPaL regimen (bedaquiline, pretomanid, and linezolid) used to treat extensively drug-resistant tuberculosis.[2] BDQ targets the *Mycobacterium tuberculosis* bacteria adenosine triphosphate (ATP) synthase enzyme, responsible for the generation of energy supply necessary for the bacteria's survival.[3]

The approach currently in use by manufacturers for the synthesis of BDQ fumarate starts with two key raw materials: the quinoline derivative **1** (**KSM-I**) and the substituted naphthalene ring **2** (**KSM-II**) (Scheme 1). The coupling of these two fragments occurs via an anionic 1,2-addition where **1** (**KSM-I**) is first deprotonated using lithium diisopropylamide (LDA). This coupling generates the overall structure of bedaquiline as a mixture of four stereoisomers. This mixture is distributed in two pairs of enantiomers, *syn*-(*RS*, *SR*) and *anti*-(*RR*, *SS*). BDQ is the (1*R*,2*S*) stereoisomer **3**, and it is the most active against TB.[4] Besides the lack of stereoselectivity, an additional drawback of this methodology is the low conversion of starting materials **1** (**KSM-I**) and **2** (**KSM-II**); 30 to 60% remain unreacted, also contributing to the low BDQ yields.

Isolation of the enantiopure BDQ (**3**) fumarate salt is achieved in 5 steps (Scheme 2). The first two steps consist of the isolation of the *syn*-diastereomer (**3**, **4**) from the undesired *anti*-pair and the remaining starting materials via precipitation in different solvents; this is possible due to the difference in solubility between the *syn* and *anti*-diastereomers. Step 2 product is a solid enriched with the *syn*-diastereomer pair (**3**, **4**), mostly free of the remaining starting materials **1** and **2**, and the *anti*-diastereomer pair (**5**, **6**) (Steps 1 and 2, Scheme 2). Resolution is achieved by selective recrystallization of the isolated *syn*-diastereomer pair with (*R*)-BINOL-phosphoric acid derivative (Step 3, Scheme 2). Neutralization with potassium carbonate liberates the BDQ (**3**) free base from the phosphoric acid salt (Step 4, Scheme 2), and the addition of fumaric acid generates the final active pharmaceutical ingredient (API) – BDQ (**3**) fumarate (Step 5, Scheme 2).

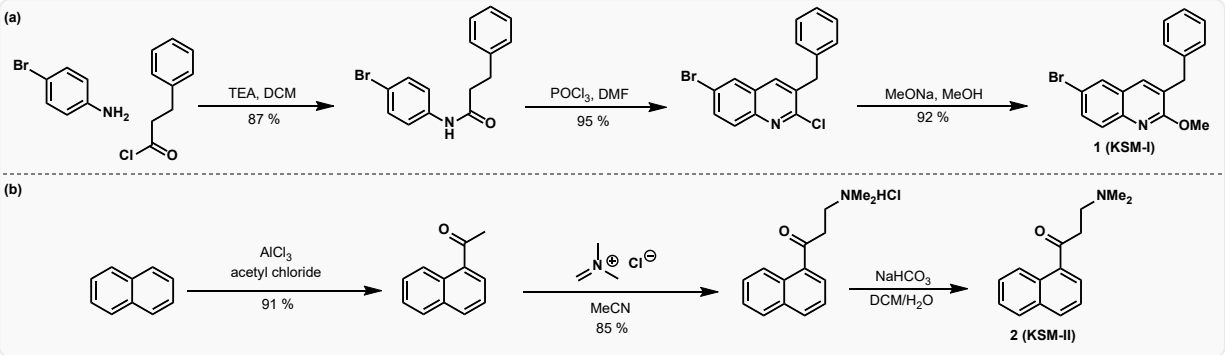


Scheme 1. *Registered steps to perform quinoline lithiation/1,2-addition sequence of reactions towards BDQ (**3**)*



Scheme 2. *Janssen's adopted steps for the isolation of the enantiopure BDQ (**3**) fumarate*

M4ALL reviewed materials provided by TB Alliance, current patents, and the open literature and identified the known routes to prepare the key starting materials necessary for the BDQ synthesis, **KSM-I** and **KSM-II**. It was concluded that the numerous synthetic routes documented to prepare these molecules and their relatively low raw materials costs would not leave much room for additional technological advancement for those molecules as a means to lower overall CoGs. A representative methodology for the preparation of quinolone **1** (**KSM-I**) is displayed in Scheme 3a.[5] Compound **2** (**KSM-II**) can be derived from naphthalene in two relatively high-yielding bond-forming steps (Scheme 3b).[6]



Scheme 3. *Typical synthetic routes to prepare the key starting materials* ***1 (KSM-I)*** *and* ***2 (KSM-II)***

The technical challenge for the BDQ project was to define a low-cost approach to establishing stereocontrol in the coupling reaction shown in Scheme 1, which would bypass the current unselective coupling and chiral resolution reactions. An obvious solution to this issue would be to design a stereoselective synthetic approach. A review of the total synthesis literature identified only two stereoselective syntheses of BDQ.[7] Even without the need for a full TE report, it was obvious that these approaches would not be feasible; they feature 11-12 total steps, utilize expensive asymmetric reagents, and exhibit low overall yields (5-12 %). Because the CoGs are quite low for the current BDQ synthesis, any new stereoselective synthetic approach is anticipated to have a very low probability of delivering cost improvements.

EDITORIAL NOTE

The Medicines for All Institute (M4ALL) contracted the TCG Lifesciences team to validate and scale up two different approaches developed by our team. These approaches can provide improved yields while applying similar conditions and using the same key starting materials as the ones currently used by manufactures in the production BDQ (**3**). Herein, we report the results of an initial optimization and scale-up of both racemic and asymmetric approaches for the bedaquiline assembly (BA) reaction, as well as the purification steps toward the final active pharmaceutical ingredient (API). Background data from the M4ALL development work can be found in the key references cited below. This report contains procedures and results from the development to support manufacturers interested in producing bedaquiline fumarate following this process.

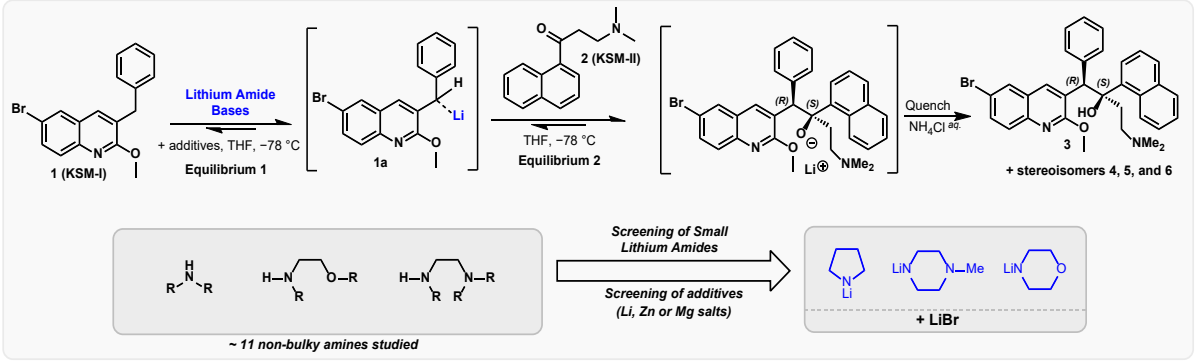
Timeline BDQ project – M4ALL

- **Part (1)/racemic approach:** Optimization of the lithiation/1,2-addition steps by screening different reaction parameters in order to find conditions that improve the conversion rate of starting materials **1** and **2**, while also exploring the use of additives to induce diastereoselectivity towards the desired *syn*-diastereomer (**3**+**4**). For this part of the project, M4ALL collaborated with Timothy Jamison (Massachusetts Institute of Technology, USA) and Till Opatz's (Johannes Gutenberg University, Germany) research groups. See the following reference: "Diastereoselectivity Is in the Details: Minor Changes Yield Major Improvements to the Synthesis of Bedaquiline" *Chemistry – A European Journal* **2022**, *28* (47), e202201311.[8]

- **Part (2)/asymmetric approach:** Development of an asymmetric approach for the same lithiation/1,2-addition sequence as described in Part (1), by performing the screening of a variety of chiral ligands derived from amino acids and analyzing their effect on the reaction outcome. The main goal was to find new conditions that provide enhanced diastereo- and enantioselective control towards BDQ (**3**) while maintaining good starting materials conversion rates. See the following reference: "Application of Chiral Transfer Reagents to Improve Stereoselectivity and Yields in the Synthesis of the Anti-Tuberculosis Drug Bedaquiline". ChemRxiv link: https://chemrxiv.org/engage/chemrxiv/article-details/64b563f2ae3d1a7b0dde20cc

- **Part (3)/partnership with TCG:** The main objective of the collaboration with TCG Life Sciences team was to explore further optimization of the reaction conditions for both approaches described in Part (1) and (2), and the best-encountered conditions were carried out at a larger scale (75 to 100 g of quinoline **1** (**KSM-I**), respectively).

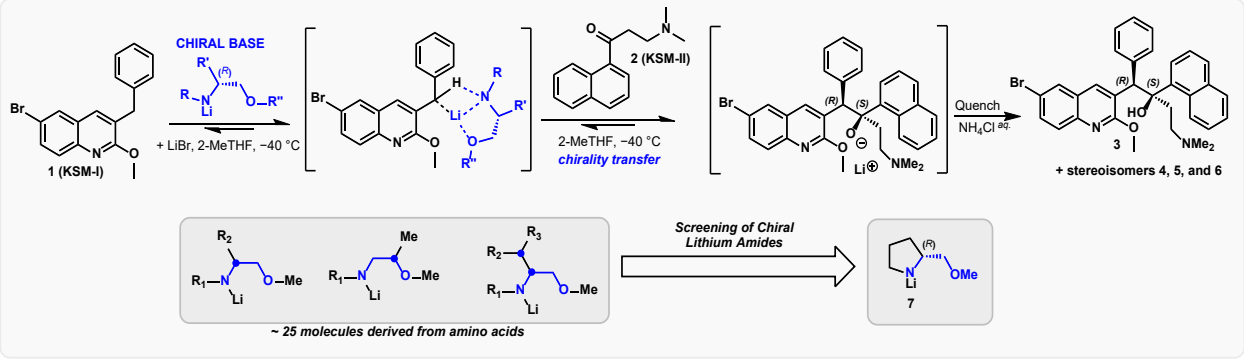
For Part (1), M4ALL reported a significant improvement in the conversion of starting materials for the lithiation/1,2-addition sequence. We demonstrated that the replacement of LDA with less hindered/stronger lithium amide bases obtained from pyrrolidine, morpholine, or *N*-methylpiperazine provided a substantial increase in the yield of the mixture of the *syn* and *anti*-diastereomers (78 to 97% assay yield). Furthermore, the use of lithium bromide (LiBr) as an additive improved the reaction's diastereomeric ratio (d.r.) from 1:1.2 to 2.1:1, favoring the *syn*-diastereomer pair, **3** and **4** (Scheme 4). This represents up to 33% yield of the desired BDQ (**3**) in the crude mixture (prior to purification). This is a significant improvement when compared to the racemic routes reported in the majority of patents available in the literature (BDQ (**3**) yield <15%).



Scheme 4. *Overview of the racemic approach adopted to synthesize BDQ (**3**)*

For Part (2), M4ALL's efforts were focused on the development of an asymmetric approach for the BA reaction. A variety of chiral ligands derived from amino acids containing an N-C-C-O bond

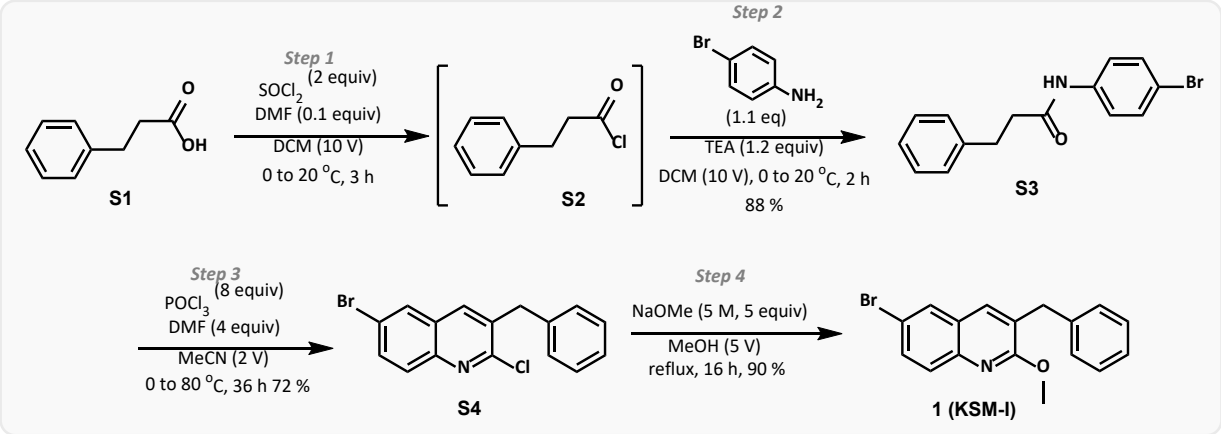
structure were employed in the methodology currently used by the manufacturers of BDQ (**3**). The D-proline derivative lithium (*R*)-2-(methoxymethyl)pyrrolidide (**7**) was employed for chirality transfer and found to be sufficiently basic to promote the deprotonation of quinoline **1** (**KSM-I**) while inducing both increased diastereoselectivity and enantioselectivity towards BDQ (**3)** and maintaining a high conversion rate of starting materials during the BA reaction (~70% HPLC, High Performance Liquid Chromatography, area of *syn*-diastereomer (**3**+**4**) in the crude mixture). When the reaction was performed in THF at higher temperatures, a considerable enhancement in enantioselectivity was observed (~55% ee at −40 °C versus 35% ee at −78 °C). The only drawback was that retro-addition is favored under these conditions, compromising the starting materials' efficient conversion. An initial optimization showed that switching solvent from THF to 2-MeTHF allowed the increase of the reaction temperature from −78 to −40 °C with no product deterioration due to the retro-addition. Moreover, the use of 2-MeTHF also allows easier solvent recovery by the end of the process. The reaction rates are slower in 2-MeTHF, which also enabled the reduction of 10-15 V (volumes, 1 g/1 mL = 1 V) of solvent compared to our racemic approach with THF, without affecting the reaction purity profile. In the same way as observed for THF, the higher temperatures were found to have a significant positive impact on the reaction enantioselectivity towards BDQ (**3**) (~55% ee/2-MeTHF at −40 ºC).



Scheme 5. *Overview of the asymmetric approach adopted to synthesize BDQ (3)*

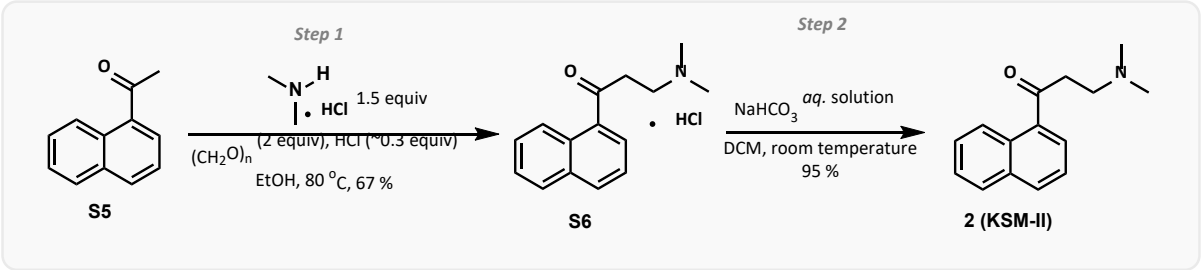
Part (3) covers an initial optimization and the validation of the M4ALL's racemic and asymmetric approaches (Parts 1 & 2) on a larger scale (100 g and 75 g, respectively) by TCG Lifesciences. Synthesis of key starting materials **1** (**KSM-I**) and **2** (**KSM-II**) was performed in-house following the aforementioned literature procedures.

Synthesis of quinoline fragment **1** (**KSM- I**):



1) Reactions were performed at different scales and reproducible results were obtained for all the steps. In general, the results and main observations are in accordance with the literature.
2) Largest scale for the in-house synthesis of **1** (**KSM-I**) was carried out starting from 500 g of 3-phenylpropanoic acid (**S1**) (a total of 4 steps are necessary).
3) All intermediates and final product **KSM-I** can be obtained in high purities (>98%, HPLC wt %). Washing of the crude solid material obtained in each step with adequate solvent (e.g., water (H_2O), methanol (MeOH), Methyl *tert*-Butyl Ether (MTBE)) is enough to efficiently purge out most of the undesired impurities.
4) When starting from 700 g of intermediate **S4** (step 4), 620 g of the desired quinoline **1** (**KSM-I**) was obtained (90% isolated yield, 98% purity HPLC wt %).

Synthesis of ketone fragment **2** (**KSM-II**):



1) Reactions were performed at different scales and reproducible results were obtained for all the steps. In general, the results and main observations are in accordance with the literature.
2) Compound **S6** can be obtained in 2 steps starting from 1-acetylnaphthalene (**S5**) (the largest scale this reaction was performed was 250 g). Formation of the **KSM-II** hydrochloride salt (compound **S6**) represents the lowest-yielding step among all the transformations used in the synthesis of the starting materials (67% isolated yield).

3) **KSM-II** hydrochloride salt (compound **S6**) can be obtained in high purity (99%, HPLC wt %). Washing the crude material with MeCN is enough to efficiently purge out most of the impurities.
4) Neutralization of **KSM-II** hydrochloride salt (compound **S6**) is the most sensitive step among all the transformations used for synthesizing the starting materials. The main reason is the reduced stability of **KSM-II** (free amine) – detailed information is provided in the "*Experimental*" and "*Good practices*" sections.
5) The largest scale the final step (step 2) was performed was 80.0 g of compound **S6**. Compound **2** (**KSM-II**) was obtained in 95% yield (66.0 g, 95% purity by HPLC wt %).
6) Taking **KSM-II** free amine decreased stability into account, it was decided that no further purification would be necessary for this key starting material. **KSM-II** possessing purity >95% was found to work well in the BA reaction.

<u>BA reaction (lithiation/1,2-addition sequence) – Good Practices</u>

Although the synthesis of starting materials **1** (**KSM-I**) and **2** (**KSM-II**) proceeded without issues, the same did not hold true for the BA reaction. The presence of moisture in the system represented the main challenge during attempts to achieve reproducible results. After adopting the following steps to ensure the lowest water content possible in the reaction medium, reproducible results were achieved:

1) Use inert atmosphere during the handling of reagents and during the entire reaction course.
2) Dry quinoline **1** (**KSM-I**) and LiBr via azeotropic distillation in THF or 2-MeTHF (see **MBR-M4ALL-BDQ-1** document for step-by-step procedure).
3) Use anhydrous solvents and check the water content percentage of each reaction component prior to their use in the BA reaction via Karl Fischer analysis (see **MBR-M4ALL-BDQ-1** to access the acceptable percentage values for each component).
4) Distillation of pyrrolidine and (*R*)-2-(methoxymethyl)pyrrolidine is recommended not only for drying purposes but also for the removal of any inorganic impurities present in the non-purified material.
5) Use *n*-BuLi from Sure/Seal™ bottles, and perform a titration before its use in the BA reaction (variation in *n*-BuLi concentration will alter the concentration of lithium salts in the reaction, which can have a direct impact on the diastereoselectivity).
6) Ketone **2** (**KSM-II**) can decompose at high temperatures; therefore, drying **2** (**KSM-II**) via azeotropic distillation is not an option. In this case, ketone **2** (**KSM-II**) should be dried under vacuum at room temperature.
7) When preparing a solution of **2** (**KSM-II**) in the desired solvent (THF or 2-MeTHF), the addition of activated molecular sieves (4 Å) is recommended to maximize the removal of remaining moisture from the solution prior to its use in the BA reaction.

<u>Other critical points that must be considered during the BA reaction:</u>

1) After neutralizing ketone **2** (**KSM-II**) hydrochloride salt, avoid storing ketone **2** (**KSM-II**) free amine for long periods of time since it can decompose. Decomposition rates is

considerably accelerated if the material is stored at room temperature. Purity check of **KSM-II** free amine is recommended prior to its use in the BA reaction.

2) 1,2-addition step is temperature and time sensitive; longer reaction times and higher temperatures favor the retro-addition (Equilibrium 2) towards lithiated quinoline **1a** and ketone **2** (**KSM-II**) (Scheme 4). Once **1a** is present in the solution, quinoline **1** (**KSM-I**) and the lithium amide base are formed in the reaction medium due to the reversible Equilibrium 1 (Scheme 4). The reaction of the lithium amide base with ketone **2** (**KSM-II**) leads to the enolate formation, a thermodynamic sink during BDQ (**3**) synthesis (see the detailed report to assess the differences between the racemic and asymmetric approaches).
3) Ensure the temperature is also well controlled during the reaction quench to avoid undesired retro-addition (quench must be performed at the same temperature the reaction was carried out, −78 ºC for the racemic approach, or −40 ºC for the asymmetric approach).
4) Slow addition of NH_4Cl (ammonium chloride) aqueous solution is not only important due to the temperature control but also to allow the formation of a slurry that can be easily stirred. Besides the undesired retro-addition, the faster rate of addition of the quenching solution can cause the formation of ice in the reaction flask and interfere with the homogeneous stirring.

When scaling up the racemic approach with 100 g of quinoline **1** (**KSM-I**), a brown color semi-solid material was obtained after reaction quench (177 g of crude material). The quenched crude mixture contained 55.6% of *syn*-diastereomer pair (**3**+**4**), 29.8% of *anti*-diastereomer (**5**+**6**) (~1.9:1 d.r., *syn*:*anti*), 3.62% of quinoline **1** (**KSM-I**), and 6.10% of ketone **2** (**KSM-II**) as determined by HPLC area %. Quantitative analysis showed that *syn*-diastereomer pair (**3**+**4**) was achieved in 74% assay yield (based on HPLC wt %), which corresponds to a 37% yield of BDQ (**3**) in the crude mixture. Considering the 6-step sequence of reactions necessary to obtain the enantiopure BDQ (**3**) fumarate (BA reaction = 1 step; precipitations, resolution, neutralization, reaction with fumaric acid = 5 steps), a 14% overall yield of BDQ (**3**) fumarate was achieved. Approximately 23% of BDQ (**3**) was lost during the purification steps. Resolution and fumarate salt formation steps were performed at a smaller scale, 20.0 g, and 1.00 g, respectively. We believe an increase in scale can minimize losses during the required precipitations.

The scale-up of the asymmetric approach with 75 g of quinoline **1** (**KSM-I**), using the lithium (*R*)-2-(methoxymethyl)pyrrolidide (**7**) base as chiral transfer agent, afforded the following composition of the quenched crude mixture: 77.7% of *syn*-diastereomer (**3**+**4**), 5.7% of *anti*-diastereomer (**5**+**6**) (13.6:1 d.r., *syn*:*anti*), 5.2% of quinoline **1** (**KSM-I**), 7.1% of ketone **2** (**KSM-II**) as determined by HPLC area %. Considering the 56% ee determined by SFC, and the 82% assay yield of the *syn*-diastereomer (**3**+**4**), 64% of BDQ (**3**) was present in the crude mixture prior to purification, the highest assay yield for BDQ (**3**) reported to date. In this case, since the BA reaction already provided a mixture enriched with the desired *syn*-diastereomer (**3**+**4**), a shorter sequence of steps could be used for purification and isolation of the final API. The first precipitation of the undesired *anti*-diastereomer (**5**+**6**) in THF was found no longer to be necessary. Considering the 5-step sequence of reactions necessary to obtain the enantiopure BDQ (**3**) fumarate (BA reaction = 1 step; precipitation, resolution, neutralization, reaction with fumaric acid

= 4 steps), BDQ (**3**) fumarate could be achieved in 43% overall yield. Approximately 21% of BDQ (**3**) was lost during the purification process.

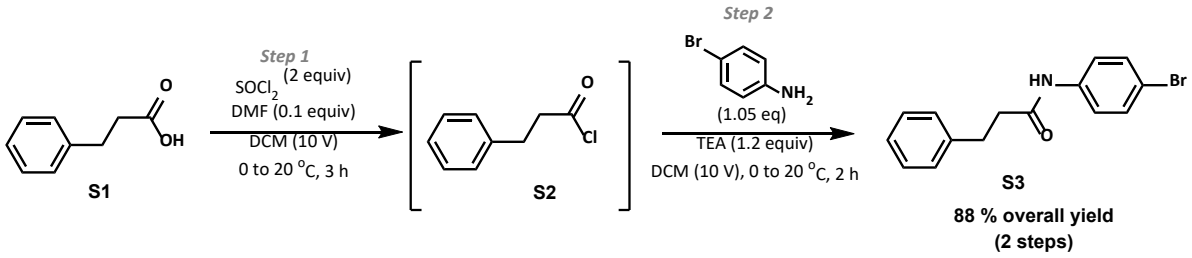
Master Batch Record (MBR) Documents

MBR-M4ALL-BDQ-1	MBR-M4ALL-BDQ-2
MBR-M4ALL-BDQ-1. docx	MBR-M4ALL-BDQ-2. docx

FINAL EXPERIMENTAL PROCEDURES USED FOR THE SYNTHESIS OF KEY STARTING MATERIALS 1 AND 2

Synthesis of quinoline 1 fragment (KSM-1)

Steps 1 and 2: *Synthesis of the acyl chloride of the 3-phenylpropanoic acid and its reaction with 4-bromoaniline*



N-(4-bromophenyl)-3-phenylpropanamide (S3). To a solution of 3-phenylpropanoic acid (**S1**) (500 g, 3.33 mol, 1 equiv) and DMF (24.3 g, 25.8 mL, 333 mmol, 0.1 equiv) in DCM (5 L, 10 V), $SOCl_2$ (792.1 g, 485.7 mL, 2 equiv) was added dropwise over 1 h at 0 °C. The reaction mixture was allowed to warm to 20 °C, stirred for 2 h, and concentrated under vacuum to give **S2** as a colorless oil (564 g, 100.5% yield crude **S2**). The obtained acyl chloride **S2** was solubilized in DCM (1.5 L, 3 V) and added dropwise to a solution of 4-bromoaniline (601.4 g, 3.50 mol, 1.05 equiv) and triethylamine (404.3 g, 553.8 mL, 4.00 mol, 1.2 equiv) in DCM (5 L, 10 V) over 1 h at 0 °C under N_2 atmosphere. The mixture was warmed to 20-25 °C and stirred for 1 h. After completion of the reaction, solvent was evaporated under reduced pressure at 35-40 °C. Water (1 L, 2 V) was added to the obtained solid and stirred for 6 h at 25-30 °C. The solid was filtered, washed with MTBE (500 mL, 1 V), and dried under vacuum to afford the product **S3** as a white solid in 88% yield (890 g, 99.9% purity by HPLC). NMR data is in accordance with the literature.**Error! Bookmark not defined.**

Notes:

1) The overall yield for the two transformations was satisfactory. Nevertheless, it is important to highlight that the crude material obtained in the first step was contaminated with traces

of thionyl chloride (not completely removed during solvent evaporation). Given the increased reactivity and instability of the acyl chloride **S2**, the immediate use of this intermediate is recommended, and storage should be avoided.

2) Reaction of the carboxylic acid **S1** with thionyl chloride is exothermic. Therefore, slow addition is recommended, while keeping the reaction temperature in a range between 0 and 5 °C.
3) Confirmation of the reaction completion was determined by TLC (step 1). An aliquot (0.5 mL) of the reaction mixture was quenched with MeOH (1 mL) to produce the methyl ester of **S1**. TLC eluent: 10% EtOAc/Hexanes.
4) Two unknown impurities (1 to 2%) were formed during Step 1 (see Table 1).

Table 1. *IPC profile (A %) of the reaction mixture obtained in Step 1*

HPLC/RRT* 500 g scale batch	RRT	IPC** Profile (A %)	Remarks
Compound **S1**	NA	ND	Completely consumed
Compound **S2**	1.00	89.19	Compound S1 DSC.pdf; Compound S1 BLD Pharma.pdf; DMF.pdf; 1H NMR.pdf; IPC.pdf; LCMS.pdf
Unknown-1	0.21	2.10	
Unknown-2	0.80	1.00	
Unknown-3	1.02	0.12	

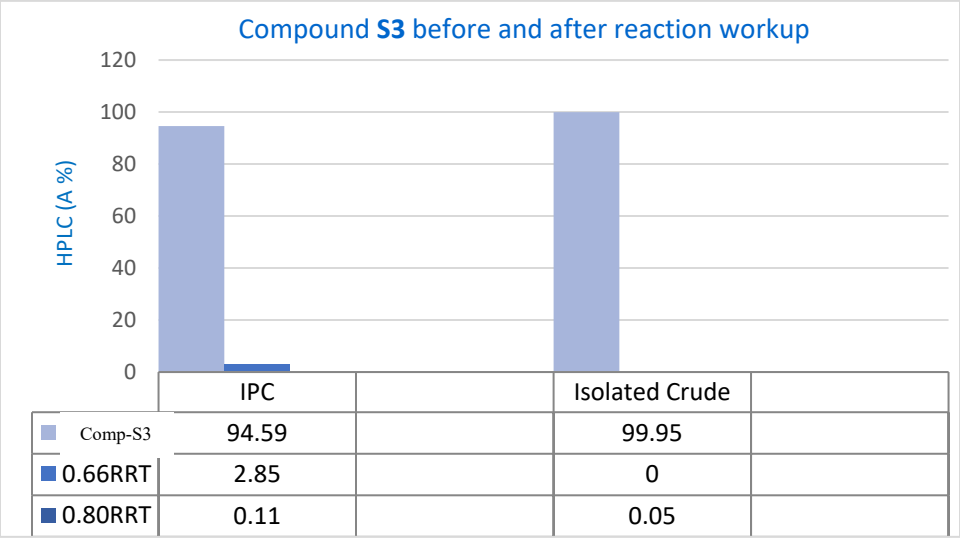
**RRT = Relative Retention Time; **IPC = Ion Pair Chromatography*

5) For step 2, two major unknown impurities (~1%) were detected in the crude mixture. Both impurities can be purged out during product **S3** isolation. Remaining impurities formed during the reaction (lower percentage, <1%) were also purged out below the level of 0.05% during reaction workup and isolation of the crude compound **S3** (see Table 2).
6) DSC (Differential Scanning Calorimetry) was recorded for compound **S3**. The analysis showed exothermicity above 270 °C. The drying temperature for compound **S3** was kept below 70 °C.

Table 2. *IPC profile (A %) of the reaction mixture obtained in Step 2*

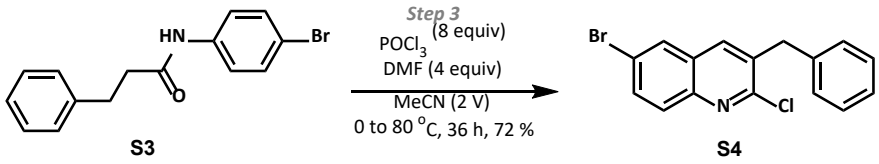
HPLC/RRT 500 g scale batch	RRT	IPC Profile (A %)	After reaction workup (A %)	Remarks
Compound **S2**	NA	ND	ND	Completely consumed
Compound **S3**	1.00	94.59	99.95	IPC-HPLC.pdf; HPLC after workup.pdf; Compound S3 - DSC.pdf; Compound S3 1H NMR after workup.pd
Unknown-1	0.81	0.11	0.05	
Unknown-2	0.66	2.85	ND	
Unknown-3	1.07	0.66	ND	

Unknown-4	1.09	0.18	ND	
Unknown-5	1.11	0.52	ND	
Unknown-6	1.15	0.12	ND	



Graph 1. *Simple reaction workup provided compound* ***S3*** *in high purity (99.9%, HPLC A %)*

Step 3: *Cyclization toward chloro-quinoline fragment*



3-benzyl-6-bromo-2-chloroquinoline (S4). DMF (854.8 g, 895.5 mL, 11.6 mol, 4 equiv) was added dropwise to a 5 L three-neck round-bottom flask containing $POCl_3$ (3.55 kg, 2.2 L, 23.1 mol, 8 equiv) at 0 °C and stirred for 1 h under N_2 atmosphere, followed by the addition of a solution of compound **S3** (880 g, 2.89 mol, 1 equiv) in MeCN (2.6 L, 2 V). The resulting mixture was stirred at 18-20 °C for 1 h. The temperature was increased to 80 °C, and reaction mixture was stirred for 36 h at the same temperature. After completion of the reaction, the mixture was cooled to 10-15 °C, and water was slowly added (12 L), and stirred for 30-45 min. After solid precipitation was observed, the reaction mixture was further stirred for another 1 h at 25-30 °C. The solid was filtered, and washed with water (2 x 880 mL, 2 V), followed by cold MeOH (2 x 880 mL, 2 V). After drying under vacuum, compound **S4** was obtained as an off-white solid in 72% yield (700.0 g, 99.9% purity by HPLC). NMR data is in accordance with the literature.[Error! Bookmark not defined.]

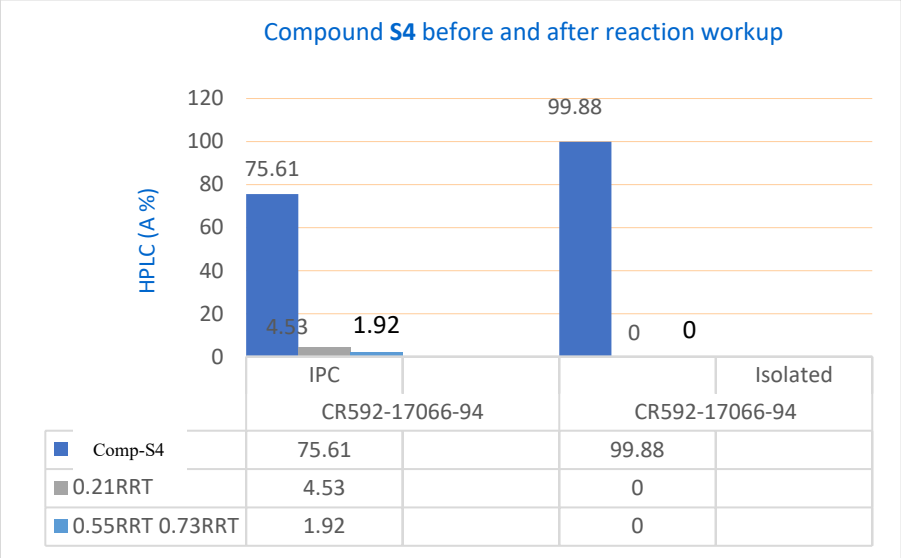
Notes:

1) The fast addition of DMF leads to exothermicity. For better temperature control, the slow addition of DMF was performed at 0-5 °C for ~1 h.

2) Two major unknown impurities (3-5% range) at 0.21 and 0.73 (RRT) were detected in the crude material prior to the reaction workup (see Table 3). After purification of compound **S4** via H_2O/MeOH wash, the obtained product still possessed the unknown impurities at 0.73 and 0.76 RRT (~ 0.1%). The remaining impurities formed during the reaction were detected in lower levels and purged out below the level of 0.01%.

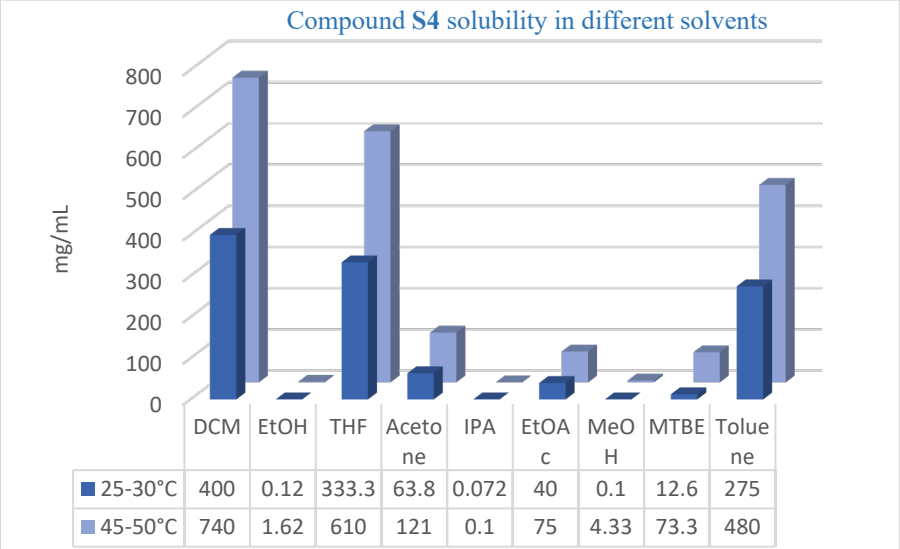
Table 3. *IPC profile (A %) of the reaction mixture obtained in Step 3*

HPLC/RRT	RRT	IPC Profile (A %)	Isolated (A %)	Remarks
Compound **S3**	0.83	ND	ND	Completely consumed
Compound **S4**	1.00	75.61	99.88	
Unknown-1	0.21	4.53	ND	IPC before workup.pdf; Compound S4 1H NMR after workup.pd; Compound S4 13C NMR after workup.pd; HPLC after workup.pdf; LCMS.pdf; Compound S4 DSC.pdf
Unknown-2	0.55	1.92	ND	
Unknown-3	0.73	3.34	0.02	
Unknown-4	0.76	0.51	0.10	



Graph 2. *Simple reaction workup provided compound **S4** in high purity (99.9%, HPLC A %)*

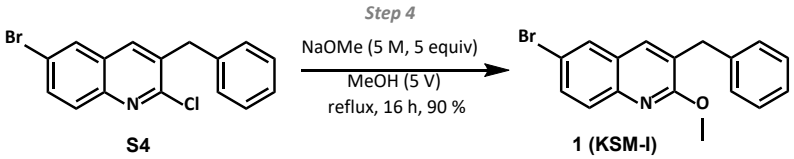
3) Although sequential washes with water and methanol purged the majority of impurities, other solvent mixtures can be employed in the purification of compound **S4** based on the solubility studies showed below (e.g., isopropanol (IPA), ethanol (EtOH), or toluene) (Graph 3).



Graph 3. *Compound **S4** solubility studies in different solvents (HPLC A %)*

4) DSC of compound **S4** showed two decomposition peaks with the liberation of heat at 240 °C and 318 °C, 78.04 J/g and 25.00 J/g, respectively. The reaction temperature was kept below 85 °C, while the drying temperature for compound **S4** was kept below 70 °C.

Step 4: *Chloride displacement with sodium methoxide*



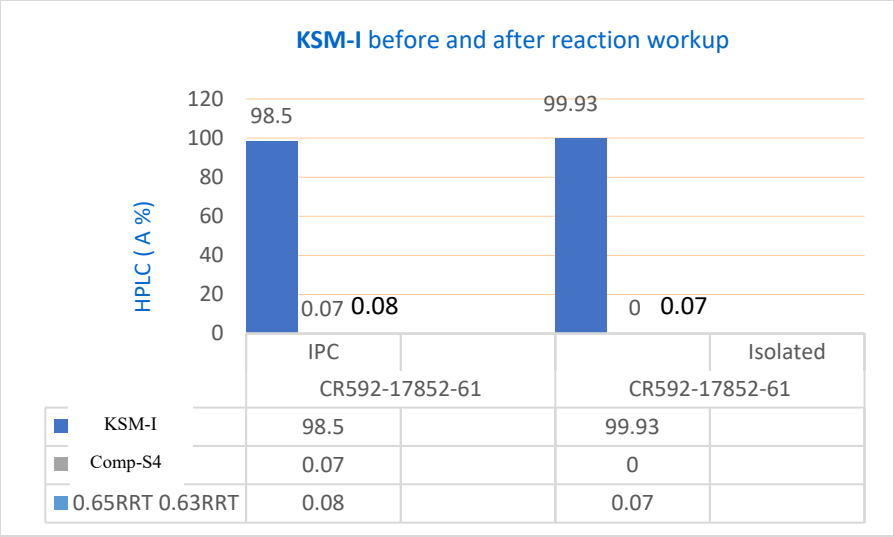
3-benzyl-6-bromo-2-methoxyquinoline (1). To a solution of compound **S4** (700.0 g, 2.10 mol, 1 equiv) in MeOH (3.5 L, 5 V), NaOMe (5 M in MeOH, 2.10 L, 10.52 mol, 5 equiv) was added and the reaction mixture was stirred for 8 h at 80 °C under N_2 atmosphere. After completion of the reaction, the resulting mixture was cooled to 25-30 ºC and concentrated under reduced pressure to remove the MeOH. Water was added (4 V) and stirred for another 6 h at 25-30 °C. The resulting solid was filtered, washed with water (1 V) and cold MeOH (1 V), and dried under vacuum at 40-45 °C for 6 h. Quinoline fragment **1** was obtained as an off-white solid in 90% yield (620.0 g, 98 % purity by HPLC wt %). NMR data is in accordance with the literature.**Error! Bookmark not defined.**

Notes:

1) To ensure low water content, quinoline **1** (**KSM-I**) was dried via azeotropic distillation (THF or 2-MeTHF) prior to its use in the BA reaction (see **MBR-M4ALL-BDQ-1** document for step-by-step procedure).
2) Two major unknown impurities (0.1 - 1.0% range) at 0.63 and 0.76 (RRT) were detected in the crude reaction mixture (IPC analysis).

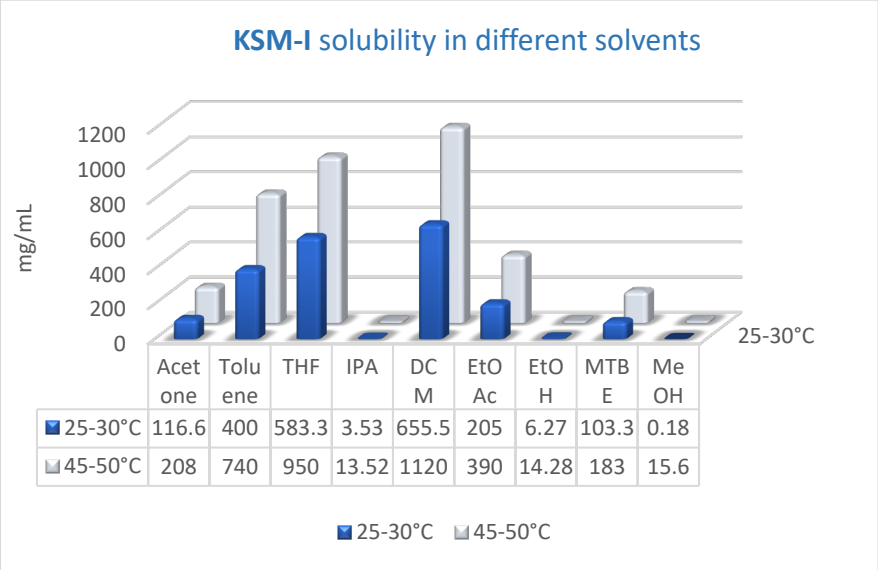
Table 4. *IPC profile (A %) of the reaction mixture obtained in Step 4*

HPLC/RRT	RRT	IPC Profile (A %)	Isolated (A %)	Remarks
Compound **S4**	0.93	0.07	ND	Almost entirely consumed
KSM-I	1.00	98.50	99.93	IPC-LCMS.pdf, IPC-HPLC.pdf, Crude reaction-HPLC.pdf, Crude reaction-LCMS.pdf, HPLC after workup.pdf, LCMS after workup.pdf, Aqueous MLs LCMS.pdf
Unknown-1	0.65	0.08	0.07	
Unknown-2	0.63	0.11	ND	
Unknown-3	0.73	0.03	ND	
Unknown-4	0.76	0.97	ND	



Graph 4. *Simple reaction workup provided compound **KSM-I** in high purity (99.9%, HPLC A %)*

3) Majority of impurities were purged out during the reaction workup (crude material wash with H_2O and MeOH). The isolated **KSM-I** presented a consistent unknown impurity at 0.65 RRT (<0.07%).
4) Although sequential washes with water and methanol purged the majority of impurities, other solvent mixtures can be employed in the purification of compound **KSM-I** based on the solubility studies showed below (e.g., IPA or EtOH) (Graph 5).

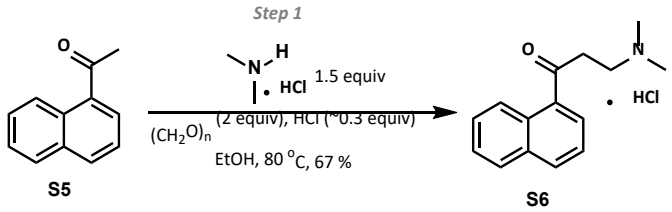


Graph 5. *Compound* ***KSM-I*** *solubility studies in different solvents (HPLC A %)*

5) DSC of **KSM-I** was recorded and showed decomposition at 370 °C. The drying temperature for **KSM-I** was kept below 50-55 °C.

Synthesis of ketone 2 fragment (KSM-II)

Step 1: *Reaction of 1-acetylnaphthalene with iminium ion*



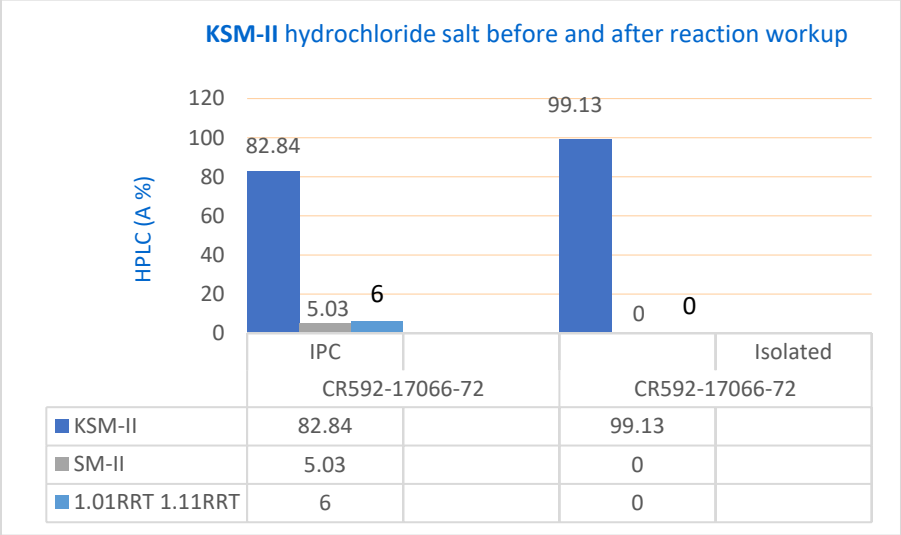
3-(dimethylamino)-1-(naphthalen-1-yl)propan-1-one hydrochloride salt (S6). To a suspension of *N*,*N*-dimethylamine hydrochloride (179.5 g, 2.2 mol, 1.5 equiv) and paraformaldehyde (88.2 g, 2.94 mol, 2 equiv) in EtOH (438 mL, 1.75 V) at room temperature, 1-acetylnaphthalene (**S5**) (250.0 g, 1.47 mol, 1 equiv) was added followed by the dropwise addition of concentrated HCl (12 M, 31.4 mL, ~0.3 equiv). The reaction mixture was warmed to 80 °C and stirred for 30 h at the same temperature. After completion, the resulting mixture was cooled down and concentrated under reduced pressure to remove the EtOH at 50-55 °C. MeCN (4 V) was added to the obtained crude mass (476.0 g, 123 % crude yield) and stirred for 5 h at 25-30 °C. The resulting solid was filtered, washed with MeCN (1 V), and dried under vacuum at 40-45 °C for 5 h to afford compound **S6** as an off-white solid (227.0 g, 67% yield, 99% purity by HPLC). NMR data is in accordance with the literature.[9]

Notes:

1) Two major unknown impurities (~3-6% range) at 1.36 and 1.01 (RRT) were detected in the crude reaction mixture (IPC analysis).
2) After the reaction workup (crude material wash with MeCN), compound **S6** still possessed ~1% of the unknown impurity at 1.11 (RRT). Most of the other impurities formed during the reaction and the remaining starting material **S5** was purged out during the isolation of the hydrochloride salt of **KSM-II**.

Table 5. *IPC profile (A %) of the reaction mixture obtained in Step 1*

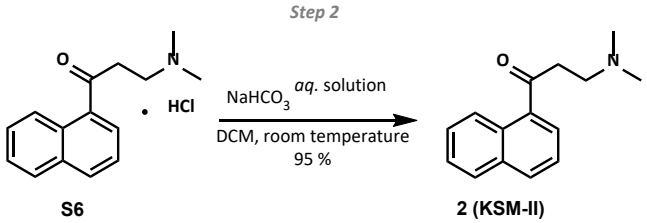
HPLC/RRT	RRT	IPC Profile (A %)	Isolated (A %)	Remarks
Compound **S5**	1.44	5.03	ND	Almost entirely consumed
Compound **S6**	1.00	82.84	99.13	IPC-LCMS.pdf, IPC before workup.pdf, LCMS.pdf, Compound S6 1H NMR.pdf, Compound S6 13C NMR.pdf, Compound S6 DSC.pdf
Unknown-1	1.01	6.0	0.07	
Unknown-2	1.11	1.15	0.78	
Unknown-3	1.36	2.8	ND	
Unknown-4	1.51	0.36	ND	



Graph 6. *Higher purity solid obtained after reaction workup (compound **S6**, 99.1%, HPLC A %)*

3) DSC analysis of compound **S6** showed onset temperature for the decomposition at 340 °C. The drying temperature for compound **S6** was kept below 50-55 °C.

Step 2: *Hydrochloride salt neutralization*

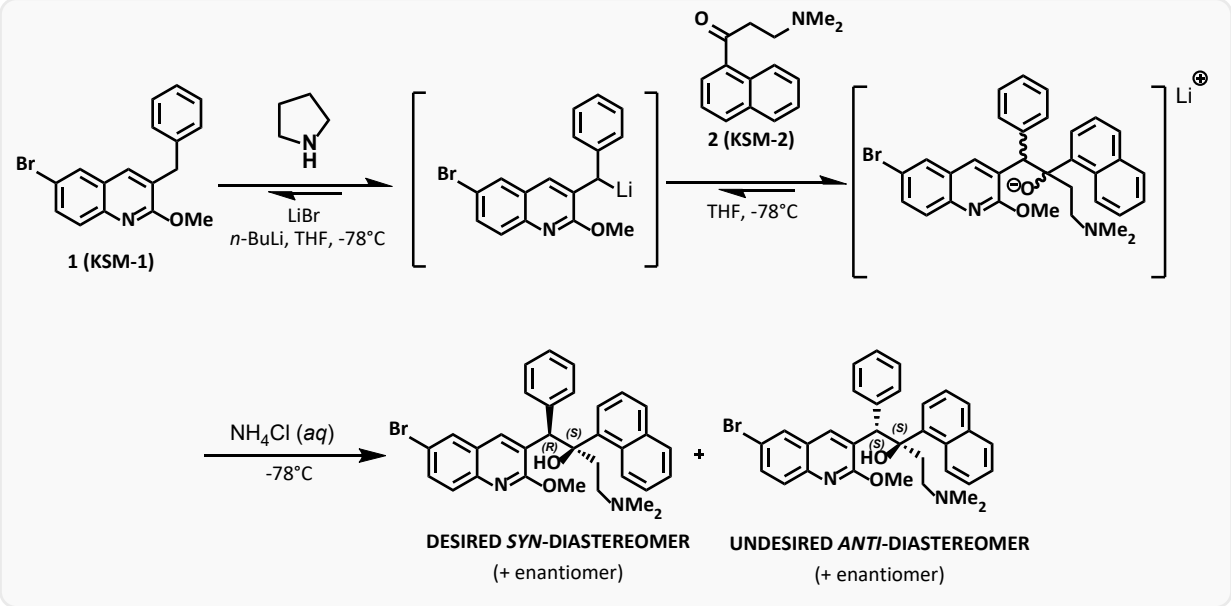


3-(dimethylamino)-1-(naphthalen-1-yl)propan-1-one (2, KSM-II). Hydrochloride salt (**S6**) (80.0 g, 303 mmol, 1 equiv, 98 % purity by HPLC) and water (800 mL, 10 V) were charged into a 5 L 3-neck round-bottom flask at 25-30 °C. The resulting mixture was stirred until full solubilization of the salt was achieved (10-15 min). DCM (800 mL, 10 V) was added, and the mixture was stirred for 15-20 min. A saturated aqueous solution of $NaHCO_3$ (800 mL, 10 V) was slowly added over 45 min via an addition funnel (rate of addition= 17.7 mL/min), and stirring was held for an additional 8-10 min. The biphasic mixture was transferred to a separatory funnel and the layers separated. The aqueous layer was extracted with DCM (2 x 400 mL, 10 V). Organic layers were combined and dried with anhydrous $MgSO_4$, filtered through a Buchner funnel, and the $MgSO_4$ bed was washed with DCM (80 mL, 1 V). Solvent was removed under vacuum at 30-35 °C for 1-2 h (740-750 mmHg) to afford compound **2** as a pale-yellow liquid (66.0 g, 95% yield, 95% purity by HPLC, 90% yield by purity). NMR data is in accordance with the literature.[9]

CAUTION: CO_2 release during acid neutralization.

Note: Prior to its use in the BA reaction, compound **2** (**KSM-II**) must be solubilized in anhydrous solvent (THF or 2-MeTHF) under inert atmosphere (N_2), followed by the addition of activated molecular sieves (4 Å) to ensure low water content in the solution. Drying ketone **2** (**KSM-II**) at high temperatures is not recommended (undesired side reactions). Avoid storing ketone **2** (**KSM-II**) for long periods since its decomposition can take place over time. Ideally, hydrochloride salt **S6** should be neutralized only prior to compound **2** (**KSM-II**) use in the BA reaction. If storage of **2** (**KSM-II**) cannot be avoided, opt for storing this material at low temperature and under inert atmosphere. See **MBR-M4ALL-BDQ-1** document for the step-by-step salt **S6** neutralization procedure.

FINAL PROCEDURE FOR THE RACEMIC APPROACH



A 5 L four-neck round-bottom flask was equipped with an overhead stirrer and a thermometer for monitoring the reaction internal temperature, as well as a N_2 inlet/outlet to ensure inert atmosphere during the entire course of the reaction (see Figure 1). THF (100 mL, 1 V) was transferred to the 5 L flask, followed by a solution of LiBr (60.9 g, 0.701 mol, 2.3 equiv) in THF (400 mL, 4 V), which was dried via azeotropic distillation. The solvent and LiBr solution were transferred to the reaction flask via cannula through an addition funnel (see Figure 1). Freshly distilled pyrrolidine (0.457 mol, 32.5 mL, 1.5 equiv) was similarly transferred to the reaction flask. The reaction mixture was cooled to −20/−30 °C, and *n*-BuLi (1.8 M in hexanes, 0.396 mol, 220.0 mL, 1.3 equiv) was added dropwise (cannula/addition funnel). After 20 min, the flask was further cooled to −78 °C (acetone/dry ice bath), and a solution of quinoline **1** (**KSM-I**) (100.0 g, 0.305 mol, 1.0 equiv, 98% purity by HPLC wt %) in dry THF (400 mL, 4 V), also dried via azeotropic distillation, was transferred to the addition funnel, followed by additional THF (100 mL, 1 V). The quinoline **1** (**KSM-I**) solution in the addition funnel was added to the lithium amide base solution over 1 h (cannula/addition funnel). The resulting mixture was stirred for an additional 30 min. A solution of ketone **2** (**KSM-II**) (83.2 g, 0.366 mol, 1.2 equiv, 95% purity by HPLC wt %) in dry THF (500 mL, 5 V) was added to the reaction mixture over 1 h at the same temperature (cannula/addition funnel). The reaction was stirred for an additional 30-45 min, and quenched by the dropwise addition of a 25% NH_4Cl aqueous solution (500 mL, 5 V) at −78 °C (cannula/addition funnel). The reaction mass was directly poured into a separatory funnel. The phases were separated, and the aqueous layer was extracted with DCM (2 x 500 mL, 10 V). The combined organic layers were dried with anhydrous Na_2SO_4, filtered through a Büchner funnel, and the Na_2SO_4 bed washed with DCM (100 mL, 1 V). The solvent was removed under reduced pressure at 45-50 °C to afford a brown color semi-solid (177 g of crude material). The quenched crude mixture contained 55.6% of *syn*-diastereomer pair (**3**+**4**), 29.8% of *anti*-diastereomer (**5**+**6**) (1.87:1 d.r., *syn*:*anti*), 3.62% of

quinoline **1**, and 6.10% of ketone **2** (determined by HPLC area %). Quantitative analysis showed that *syn*-diastereomer pair (**3**+**4**) was achieved in 74% assay yield (based on HPLC wt %), which corresponds to a 37% yield of BDQ (**3**) in the crude mixture.


IPC-HPLC.pdf


IPC-LCMS.pdf


Crude mixture HPLC.pdf


Crude mixture LCMS.pdf


Crude mixture 1H NMR.pdf


HPLC wt % analysis.pdf

Notes:

1) Solvent volumes were calculated relative to the limiting reagent quinoline **1** (**KSM-I**) (e.g., 100 g of **1**/100 mL of solvent = 1 V).
2) After distillation, pyrrolidine was stored under inert atmosphere with activated molecular sieves (4 Å). In the case of ketone **2** (**KSM-II**), after neutralization of its hydrochloride salt **S6**, compound **2** (**KSM-II**) was dried under vacuum at room temperature, and after the addition of THF, activated molecular sieves (4 Å) were added to the solution prior to its use in the BA reaction.
3) Procedures for drying quinoline **1** (**KSM-I**) and LiBr via azeotropic distillation can be found in the **MBR-M4ALL-BDQ-1** document produced for the scale-up of the asymmetric approach.
4) Overall yield from BA reaction until BDQ (**3**) fumarate formation (6 steps) was lower than expected (14%). Circa of 23% of BDQ (**3**) was lost during the purification steps (maximum yield possible was 37% of BDQ (**3**)). Resolution and fumarate salt formation steps were performed at a smaller scale, 20.0 g and 1.00 g, respectively. We believe that increase in scale can minimized losses during the required precipitations.

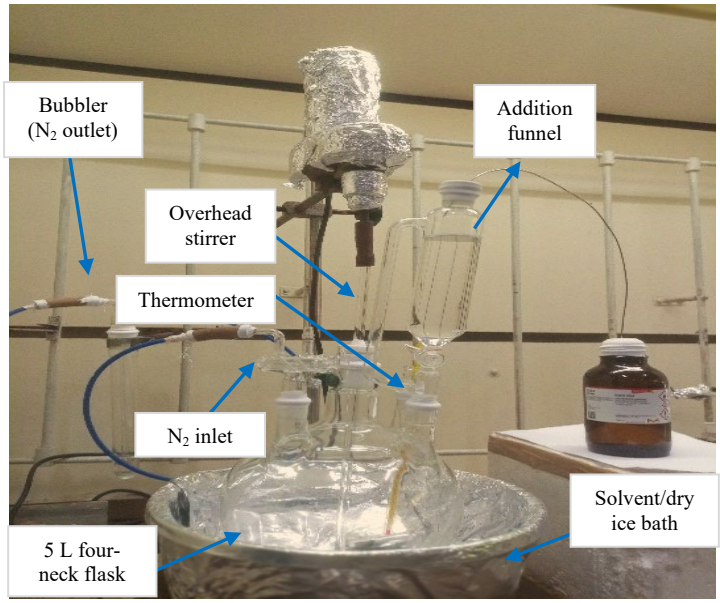


Figure 1. *Reaction setup utilized to synthesize BDQ (**3**) – racemic and asymmetric approaches, 100 g and 75 g of quinoline **1** (**KSM-I**), respectively*

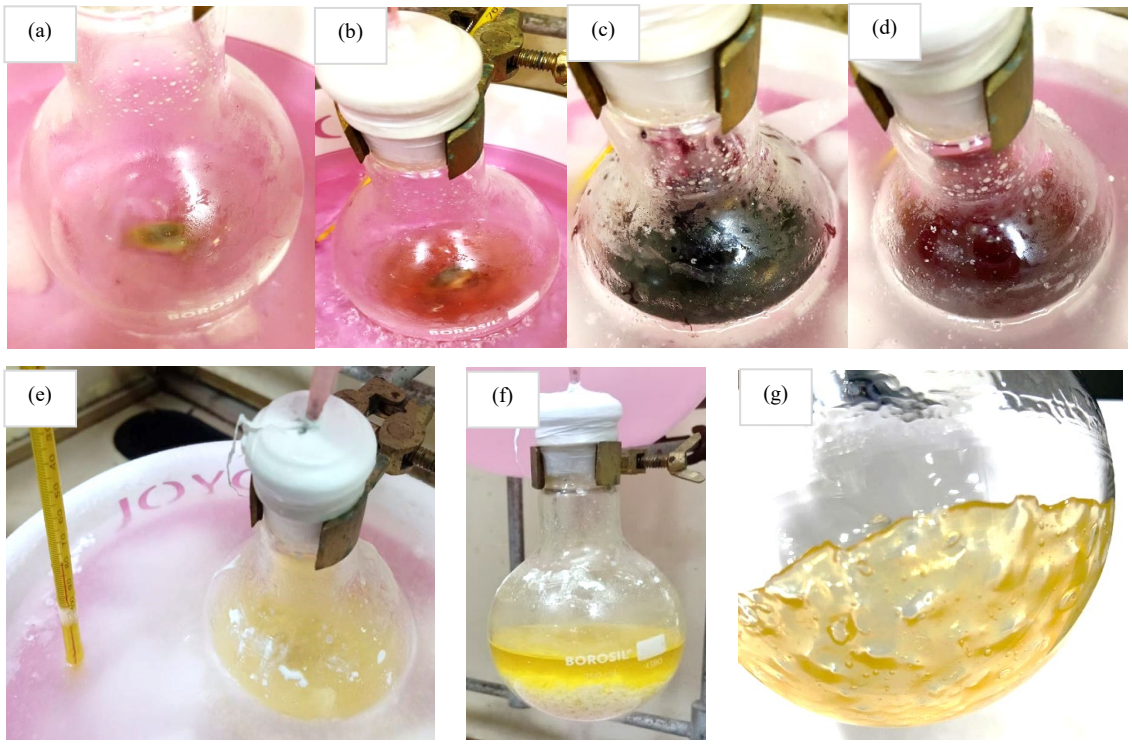
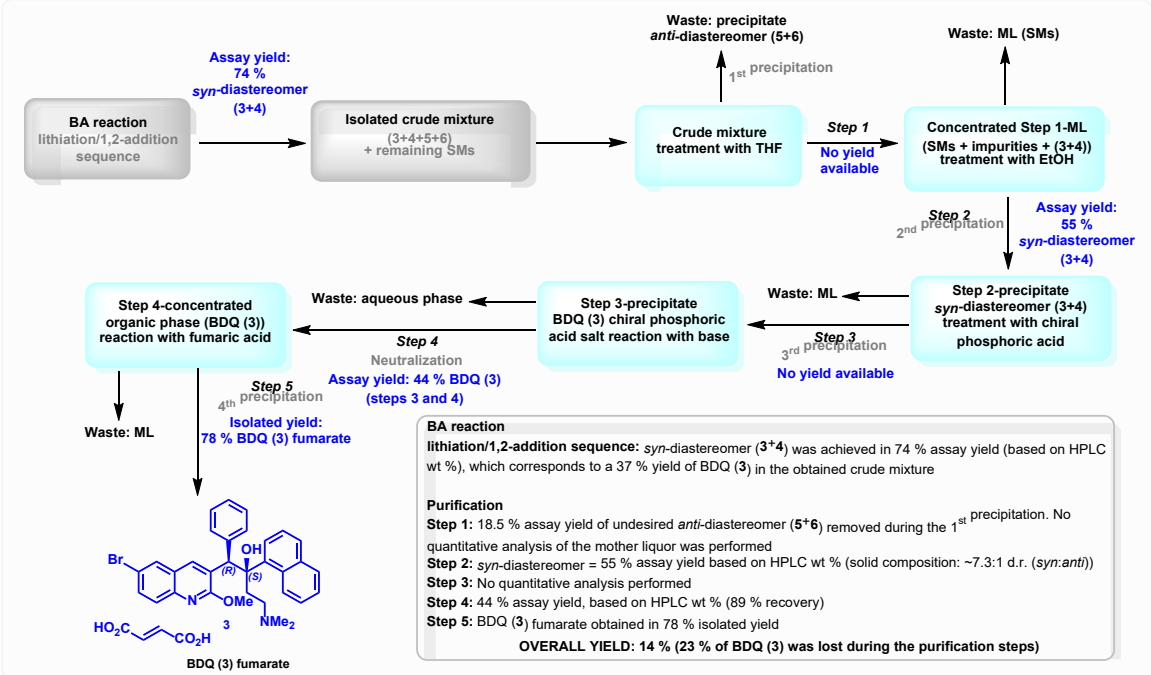


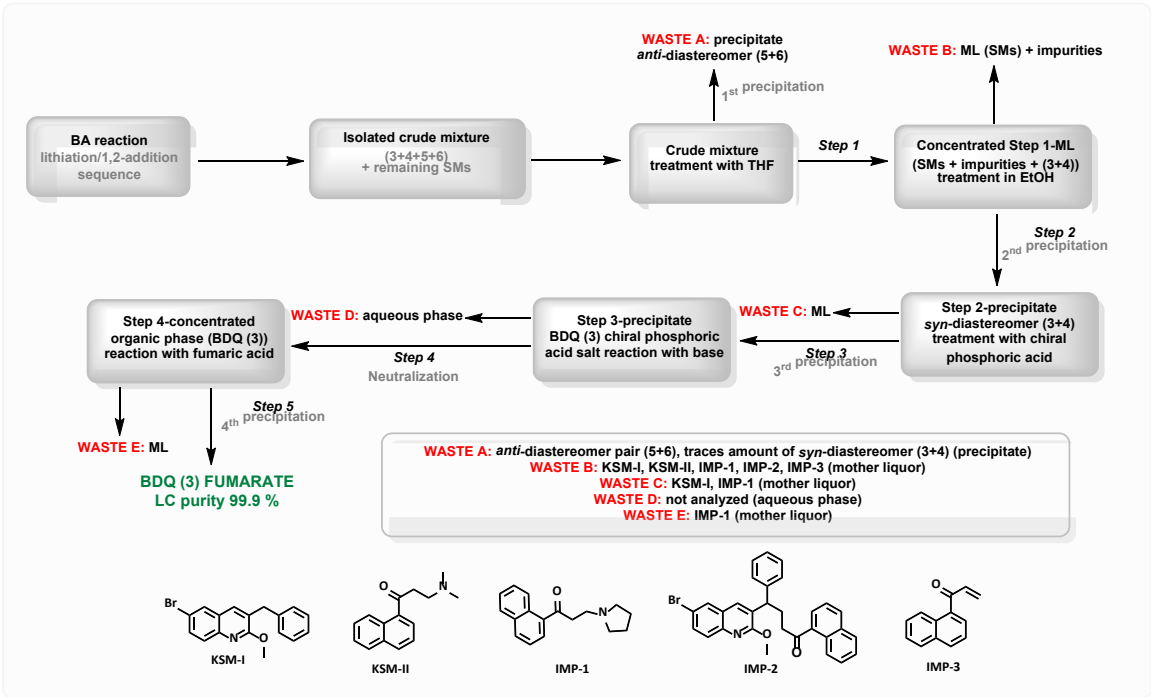
Figure 2. *Appearance of the reaction mixture at different points after (a) LiBr solubilization at 25-30 °C; (b) adding n-BuLi at −30 °C; (c) adding **KSM-I** solution at −78 °C; (d) adding **KSM-II** (free amine) at −78 °C; (e) adding 25% NH_4Cl aq. solution at −78 °C; (f) formation of ice precipitate during reaction quenching; and (g) solvent removal (crude material) – Images correspond to a 5.0 g batch experiment*

OBTAINING ENANTIOPURE BDQ (3) FUMARATE FROM THE CRUDE REACTION MIXTURE

Overview of the purification steps



Scheme 6. *Overview of the adopted purification steps to obtain enantiopure BDQ (**3**) fumarate salt*



Scheme 7. *Purgeability of impurities during the isolation of the BDQ (**3**) fumarate (API)*

Final Purification and Isolation of BDQ (3) fumarate

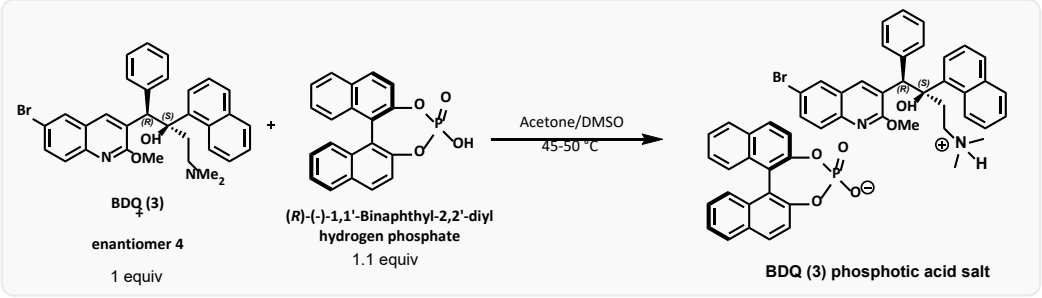
Purification – Steps 1 and 2

Removal of anti-diastereomer D-II (5+6). THF (600 mL, 6 V) was added to a round-bottom flask containing the concentrated crude material (175.0 g, ~2:1 d.r., *syn*:*anti*) obtained in the BA reaction, and stirred at 25-30 °C for 6 h. The resulting solid was filtered, washed with THF (100 mL, 1 V), and dried under vacuum at 40-45 °C to afford the *anti*-diastereomer D-II (5+6) as white solid (34 g, ~18.5% assay yield based on HPLC wt %). HPLC area % analysis showed 3.14% of D-I (3+4), and 95.7% of D-II (5+6). The mother liquor obtained in the previous step was taken into a round-bottom flask and the solvent was completely removed under vacuum at 40-45 °C. EtOH (500 mL, 5 V) was added to the concentrated crude material and stirred for 5 h at 25-30 °C. The obtained solid was filtered, washed with EtOH (1 V), and dried under vacuum at 40-45 °C to afford the syn-diastereomer **D-I** (**3**+**4**) as an off-white solid (98 g) in 55% assay yield based on HPLC wt %. HPLC area % analysis showed that the composition of the isolated solid was 87% of **D-I** (**3**+**4**) and 12% of **D-II** (**5**+**6**), representing a ~7.3:1 d.r. (*syn*:*anti*).

Note: Volumes of solvent (V) were calculated relative to the quinoline **1** (**KSM-I**) input mass (1 g/1 mL = 1 V).

Analytical data (HPLC A % analysis)		
Step 1: solid composition 34.0 g **Undesired:** 95.77% **Desired:** 3.14% **KSM-I:** 0.83% **KSM-II:** 0.20% S1-HPLC.pdf S1-LCMS.pdf S1-1H NMR.pdf S1-Assay.pdf	***Step 2: solid composition*** 98.0 g **Undesired:** 11.90% **Desired:** 86.98% **KSM-I:** 0.45% **KSM-II:** 0.06% S2-HPLC.pdf S2-LCMS.pdf S2-1H NMR.pdf S2-13C NMR.pdf S2-Assay.pdf	***Step 2: mother liquor composition*** 45.0 g **Undesired:** 0.69% **Desired:** 3.35% **KSM-I:** 24.32% **KSM-II:** 22.80% **Enone:** 5.00% Mother liquor HPLC.pdf Mother liquor LCMS.pdf

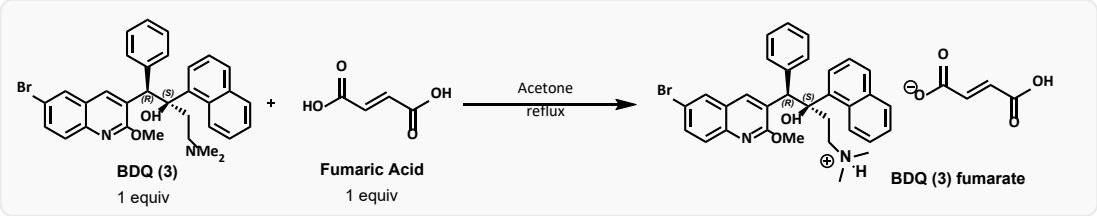
Resolution – Steps 3 and 4



Removal of undesired enantiomer 4 and remaining D-II (5+6). The solid obtained in the previous step (~7.3:1, 20.0 g ,1.0 equiv) was transferred to a round-bottom flask. Acetone (170 mL, 8.5 V) was added at 25-30 °C, and the resulting mixture heated to 50-55 °C. A heterogeneous slurry was observed. A solution of (*R*)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate (1.1 equiv) in DMSO (30 mL, 1.5 V) was added dropwise into the reaction flask at 50-55 °C. The reaction mixture became clear and stirring was continued for another 30 min at 50-55 °C. The reaction mass was gradually cooled to 25-30 °C and stirring maintained for 8 h. The resulting white solid was filtered, washed with acetone (60 mL, 3 V), and dried under vacuum at 45-50 °C to afford the BDQ (**3**) phosphoric acid salt. The obtained salt was transferred to a round-bottom flask followed by the addition of water (200 mL, 10 V), and a 10% aqueous solution of K_2CO_3 (200 mL, 10 V). After stirring for 10-20 min, the mixture was extracted with DCM (2 x 5 V, 200 mL). The combined DCM layer was dried over anhydrous Na_2SO_4, and the solvent removed under vacuum at 40-45 °C to afford the desired BDQ (**3**) (100% ee) as a white solid (7.8 g, 44% yield, based on HPLC wt % (89% recovery). HPLC area % analysis showed ~99.77% of BDQ (**3**) and 0.13% of undesired **D-II**.

Chiral phosphoric acid.pdf Chiral HPLC.pdf GLP HPLC.pdf Mother liquor SFC.pdf

Synthesis of BDQ (3) fumarate salt – Step 5



Enantiopure BDQ (**3**) obtained in the previous step (1.0 g, 1.0 equiv) was added into a round-bottom flask, followed by the addition of acetone (10 mL, 10 V), and fumaric acid (1.0 equiv). The reaction mixture temperature was slowly increased to 80-85 °C (clear solution observed), and stirred for 30 min. After this period, reaction flask was allowed to cool down to 25-30 °C, and stirring was maintained overnight (12-16 h) at the same temperature. The resulting white solid was filtered, washed with acetone (5 V), and dried under vacuum at 45-50 °C to give BDQ (**3**) fumarate (0.95 g, 78% isolated yield).

Notes:

1) The minor impurity at 1.5 RRT present in the Step 5's starting material was completely purged out in the mother liquor.

BDQ fumarate HPLC.pdf
BDQ fumarate LCMS.pdf
Mother liquor HPLC.pdf
Mother liquor LCMS.pdf

INITIAL BEDAQUILINE ASSEMBLY REACTION OPTIMIZATION

Reaction quench/Extraction

Reverse quenching with 25% NH_4Cl (aq) was attempted:

1) When the standard quench was used (addition of 25% NH_4Cl (aq) to the crude reaction mixture at −78 °C), large amount of solid was formed and stirring was not efficient (water freezes), which can provoke issues at a larger scale.
2) Transferring the reaction mixture to a NH_4Cl (aq) solution instead at 0-5 °C (reverse mode of addition), appears to result in similar outcome, but with slightly more retro-addition toward starting materials, and enone (**IMP-3**) formation (side product).
3) Both quenching modes were tested again, but slowing the rate of addition (over 1 h period). Slow addition of NH_4Cl (aq) provided a slurry which can be stirred well (procedure used for 100 g batch experiment).

Other quenching systems using 10% AcOH (acetic acid) (aq) at −78 °C were also tested:

4) Almost complete retro-addition was observed. SMs conversion decreased from 61% to 25%.
5) When quench was performed with 10% AcOH in THF, the outcome was better. However, it was not as effective as NH_4Cl (aq).

Extraction:

1) Extraction with greener EtOAc was attempted. However, the IPC analysis of the quenched mixture presented lower amount of product when compared to the one obtained when DCM was employed. This observation is due to the product solubility issues in EtOAc (see the **D-I** and **D-II** solubility studies described in this report).

Table 6: Reaction quench comparison data at −78 °C and 0-5 °C (HPLC area %)

S. No.	Batch No.	Input (g) of quinoline KSM-I	RM Quenched Temp (°C)	Isolated	Crude output (g)
1	CR592-17852-88	25.0 g **KSM-I** (CR592-17066-81-P) **KSM-I:** 97.22%	−78 Standard mode	**KSM-I:** 4.5% Racemic BDQ: **D-II:** 28.72% **D-I:** 55.76% **KSM-II:** 7.26%	47.0

2	CR592-17852-94	25.0 g **KSM-I** (CR592-17852-87-P) **KSM-I:** 99.5%	0-5 Reverse mode	**KSM-I:** 16.93% Racemic BDQ: **D-II:** 16.94% **D-I:** 46.17% **KSM-II:** 13.38% Enone at 20.6 min: 1.93%	49.0

Table 7: Reaction quench comparison data - Standard *vs.* Reverse quenching mode (slow addition over 1 h)

S. No.	Batch No.	Input (g) of quinoline KSM-I	Protocol	RM Quenched Temp (°C)	IPC	Isolated
1	Part-1 CR592-18567-4	25.0 g **KSM-I** (CR592-17066-81-P) **KSM-I:** 97.22%	**KSM-I** (1.0 equiv) **KSM-II** (1.2 equiv) Pyrrolidine (1.5 equiv) LiBr (2.3 equiv) *n*-BuLi (1.8 M, 1.3 equiv) Dry THF (15 V)	−78	Racemic BDQ: **D-II:** 29.77% **D-I:** 55.10% **KSM-I:** 5.20% **KSM-II:** 6.99% SMI@ 30.46 min:1.20%	Racemic BDQ: **D-II:** 31.31% **D-I:** 52.77% **KSM-I:** 5.44% **KSM-II:** 7.40% SMI at 15.5 min: 3.22%
2	Part-2 CR592-18567-4			0-5	Racemic BDQ: **D-II:** 27.82% **D-I:** 52.48% **KSM-I:** 7.85% **KSM-II:**8.99% Enone:1.56% SMI @ 30.6 min: 1.28%	Racemic BDQ: **D-II:** 26.81% **D-I:** 56% **KSM-I:** 7.69% **KSM-II:** 8.5% Enone: 1.76% SMI at 30.6 min: 1.26%

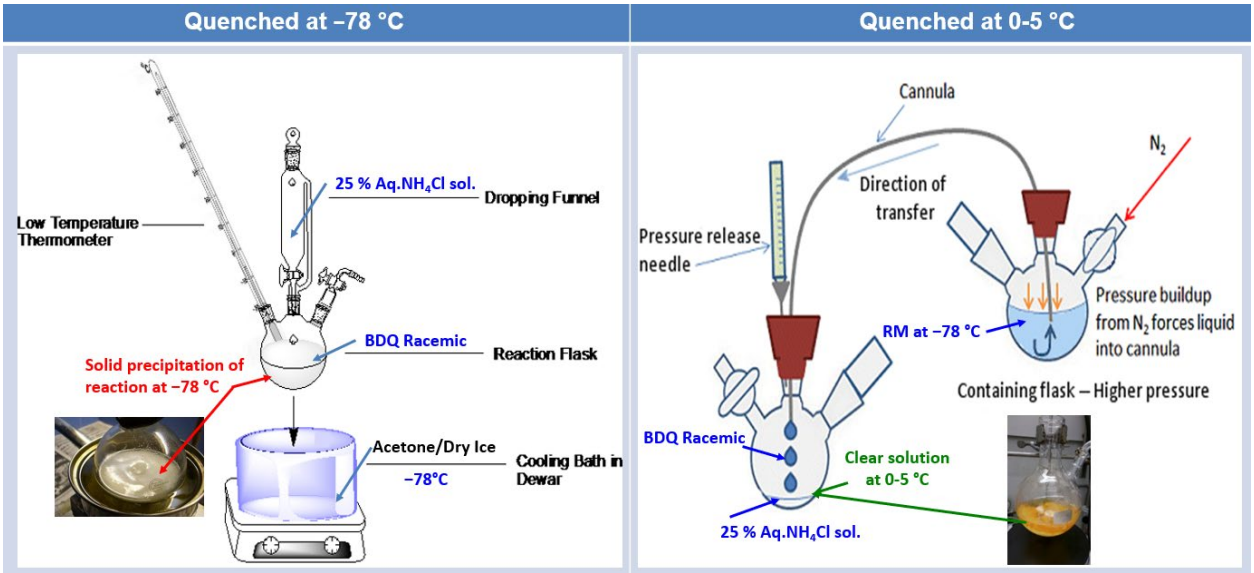
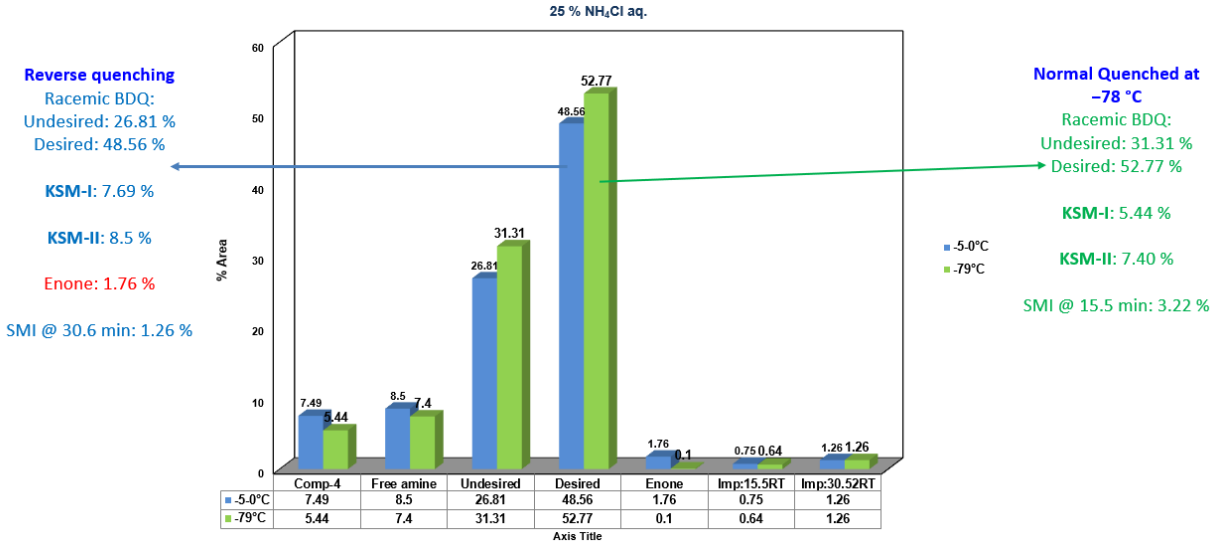


Figure 3. *Quenching setup for the standard mode of addition (−78 °C), and reverse (0-5 °C)*

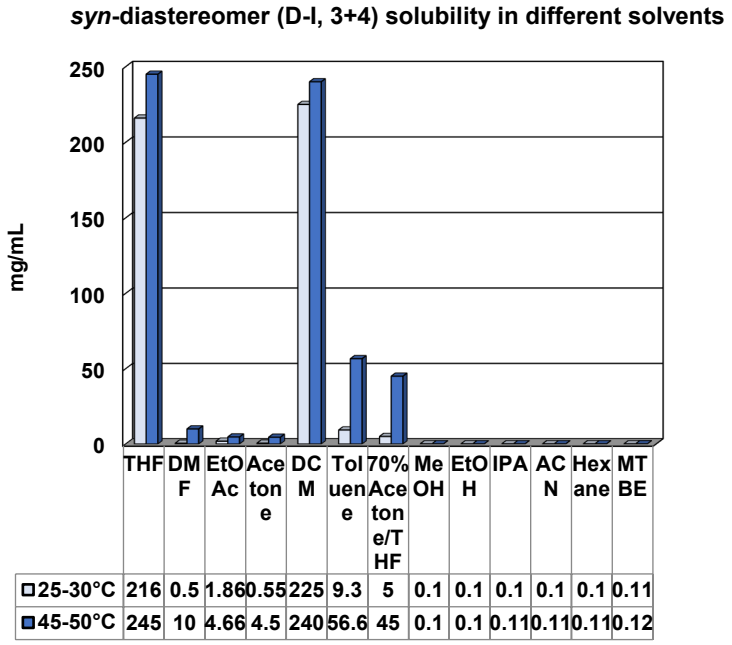


	Comp-4	Free amine	Undesired	Desired	Enone	Imp:15.5RT	Imp:30.52RT
-5-0°C	7.49	8.5	26.81	48.56	1.76	0.75	1.26
-79°C	5.44	7.4	31.31	52.77	0.1	0.64	1.26

Graph 7. *HPLC area % analysis of obtained crude mixture after quenching the reaction via the standard or reverse mode of addition, −78 °C or 0-5 °C, respectively*

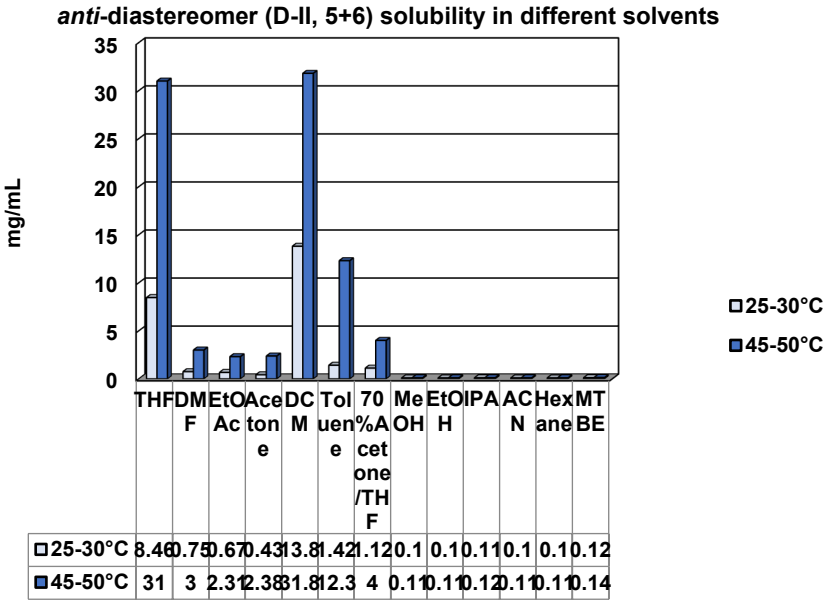
Exploring different solvent systems during BDQ (3) purification

Desired *syn*-diastereomer (D-I, 3+4):



Graph 8. *HPLC area % analysis of desired syn-diastereomer (**D-I**, 3+4) in different solvents at 25-30 °C and 45-50 °C*

Undesired *anti*-diastereomer (D-II, 5+6):



Graph 9. *HPLC area % analysis of desired syn-diastereomer (**D-II**, 5+6) in different solvents at 25-30 °C and 45-50 °C*

Experimental procedure used during the solvent screening (purification): same as previously described.

Purification Step 1 – Removal of undesired *anti*-diastereomer (5+6) by using different solvents

S. No.	Batch No.	Input (g)	Solvent	Volume /Wash	Temperature (°C)	Output (g)	Undesired/Desired LCAP	Recovery (%)
1	CR592-17087-44	2.0	Toluene	5+2	25-30	0.92	93.54/2.41	26.6
2	CR592-17087-52	**2.0**	Toluene	5+2	25-30	**1.1**	**74.99/23.98**	**32.5**
3	CR592-17852-70	25	Toluene	5+2	0-5	25	41.56/58.10	59.1
4	CR592-17852-70(Repeat)	25	Toluene	5+2	85-90/ 25-30	12.5	41.56/58.10	50.0
5	CR592-17087-54	50	Toluene	5+2	85-90/ 25-30	42.5	41.53/57.52	50.23
6	CR592-17087-59	**10.0**	**Acetone:THF(70: 30)**	**10+5**	**25-30**	3.2	57.39/41.70	32
7	CR592-17087-60	5.0	**Acetone:THF (60:40)**	10+5	25-30	1.3	96.75/2.46	26
8	CR592-17087-61	5.0	**Acetone:THF (30:70)**	10+5	25-30	1.2	96.01/3.22	24
9	***CR592-17066-93***	***2.0***	***THF***	***8+1***	***25-30***	***0.25***	***99.08/0.52***	***12.5***
10	CR592-17852-83	2.0	**THF**	5+1	25-30	0.35	95.0/0.98	17.5

Purification Step 2 – Precipitation of desired *syn*-diastereomer (3+4) by using different solvents

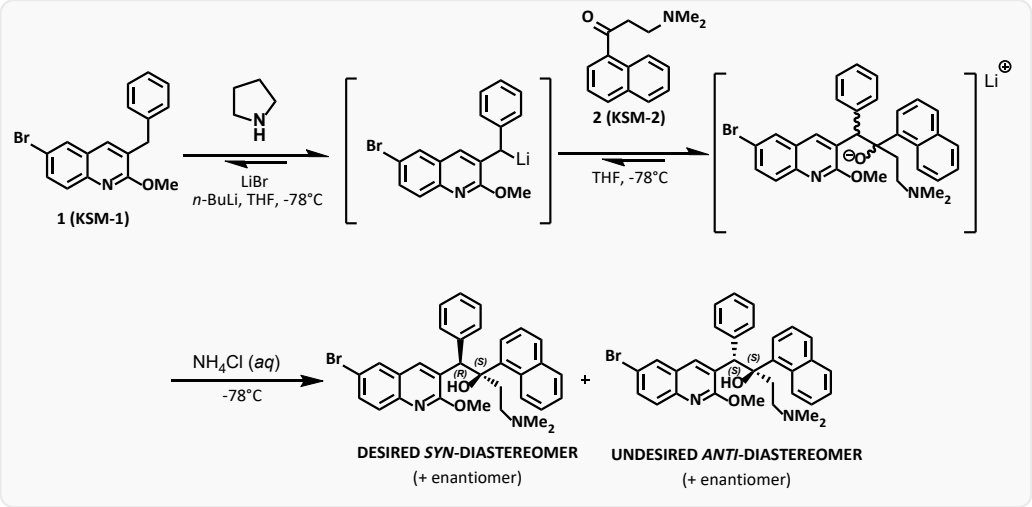
S. No.	Batch No.	Input (g)	Solvent	Volume/Wash	Temperature (°C)	Output (g)	Undesired/Desired LCAP	Recovery (%)
1	CR592-17087-44	2.0	Ethanol	5+2	25-30	1.3	8.05/82.2	38.4
2	CR592-17087-45	**2.0**	Ethanol	**5+2**	25-30	**1.2**	**9.2/88.17**	**35.44**
3	CR592-17852-70	25	Ethanol	5+2	25-30	11.0	7.39/89.61	25.99
4	CR592-17852-70(Repeat)	25	Ethanol	5+2	25-30	12	41.56/58.10	43
5	CR592-17087-54	50	Ethanol	5+2	25-30	25.83	7.06/90.39	30.47
6	***CR592-17087-59***	***10.0***	***Ethanol***	***5+5***	***25-30***	***5.82***	***6.76/97.27***	***58***
7	CR592-17087-60	5.0	Ethanol	4+1	25-30	2.5	10.30/88.24	50
8	CR592-17087-61	5.0	Ethanol	4+1	25-30	3.2	17.69/80.60	64
9	CR592-17066-93	2.0	Ethanol	5+1	25-30	1.2	12.73/86.42	60
10	CR592-17852-83	2.0	Ethanol	5+1	25-30	1.1	12.24/87.07	55

Notes:

1) Diastereomers **D-I** and **D-II** were insoluble in the majority of tested solvents.

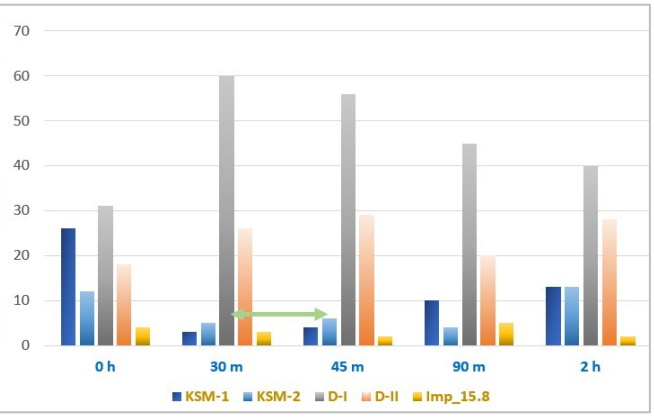
2) THF and DCM were the best solvents encountered to solubilize **D-I** and **D-II**.
3) Desired diastereomer **D-I** is considerably more soluble than **D-II**.
4) Solubility in EtOAc is low, which may explain inferior results observed during extraction using this solvent.
5) Purification (step 1) using toluene or different ratios of acetone:THF was attempted. However, results were not as good as the original Janssen procedure using THF (step 1).
6) Step 1 was carried out using different volumes of THF. Higher selectivity toward **D-II** precipitation was obtained when applying 8 V + 1 V (wash).
7) Solvent was kept the same for step 2, and different volumes were analyzed. When using 5 V + 5 V (wash), higher selectivity toward **D-I** precipitation was achieved.

Parameters optimization



1) In general, optimization performed by TCG was in accordance with M4ALL small scale optimization.
2) **Time after KSM-II addition (1,2-addition step):** Increasing reaction time while keeping the temperature at −78 °C had a negative effect in the reaction outcome. The longer the reaction time, greater was the amount of SMs in the crude mixture due to the retro-addition. Optimum reaction time was 30-45 min (after ketone **KSM-II** addition was completed).

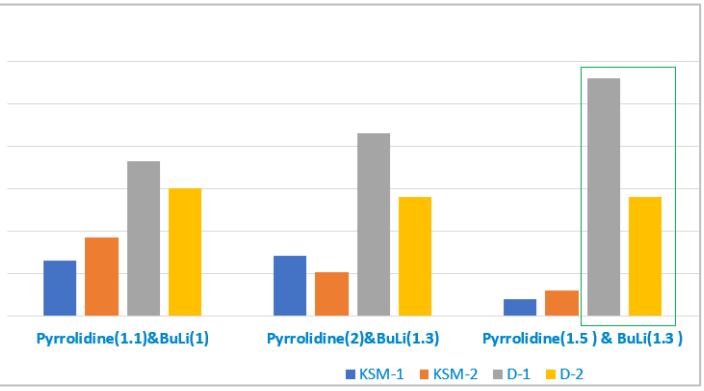
HPLC A % - Crude mixture after reaction quench at different times					
	KSM-1	KSM-2	D-I	D-II	Imp_15.8
0 min	26	12	31	18	4
30 min	3	5	60	26	3
45 min	4	6	56	29	2
90 min	10	4	45	20	5
2 h	13	13	40	28	2



3) **Stoichiometry *n*-BuLi/pyrrolidine:** three different combinations were tested (1.1 equiv pyrrolidine/1.0 equiv *n*-BuLi; 2.0 equiv pyrrolidine/1.3 equiv *n*-BuLi; 1.5 equiv pyrrolidine/1.3 equiv *n*-BuLi).

4) 1.5 equiv pyrrolidine/1.3 equiv *n*-BuLi provided the best outcome.

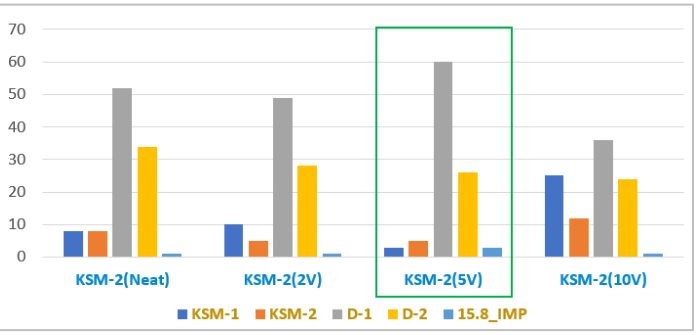
HPLC A % crude mixture after reaction quench				
	KSM-I	KSM-II	D-I	D-II
Pyrrolidine (1.1 equiv) & *n*-BuLi (1 equiv)	13	19	37	30
Pyrrolidine (2.0 equiv) & *n*-BuLi (1.3 equiv)	14	10	43	28
Pyrrolidine (1.5 equiv) & *n*-BuLi (1.3 equiv)	4	6	56	28



5) **Ketone KSM-II concentration:** four different concentrations were tested (neat, 2 V, 5 V, and 10 V).

6) Higher concentration of ketone **KSM-II** has shown the best results, with 5 V representing the optimum point. Less concentrated solution such as 10 V resulted in the lowest BDQ yield. Retro-addition was observed at a larger extent for this case.

HPLC A % crude mixture after reaction quench					
	KSM-I	KSM-II	D-I	D-II	15.8_IMP
KSM-II (Neat)	8	8	52	34	1
KSM-II (2 V)	10	5	49	28	1
KSM-II (5 V)	3	5	60	26	3
KSM-II (10 V)	25	12	36	24	1



7) **Experiments at −40 °C -** Although lower conversion of SMs was observed, formation of desired diastereomer **D-I** seems to be favored over undesired **D-II** at higher temperatures:

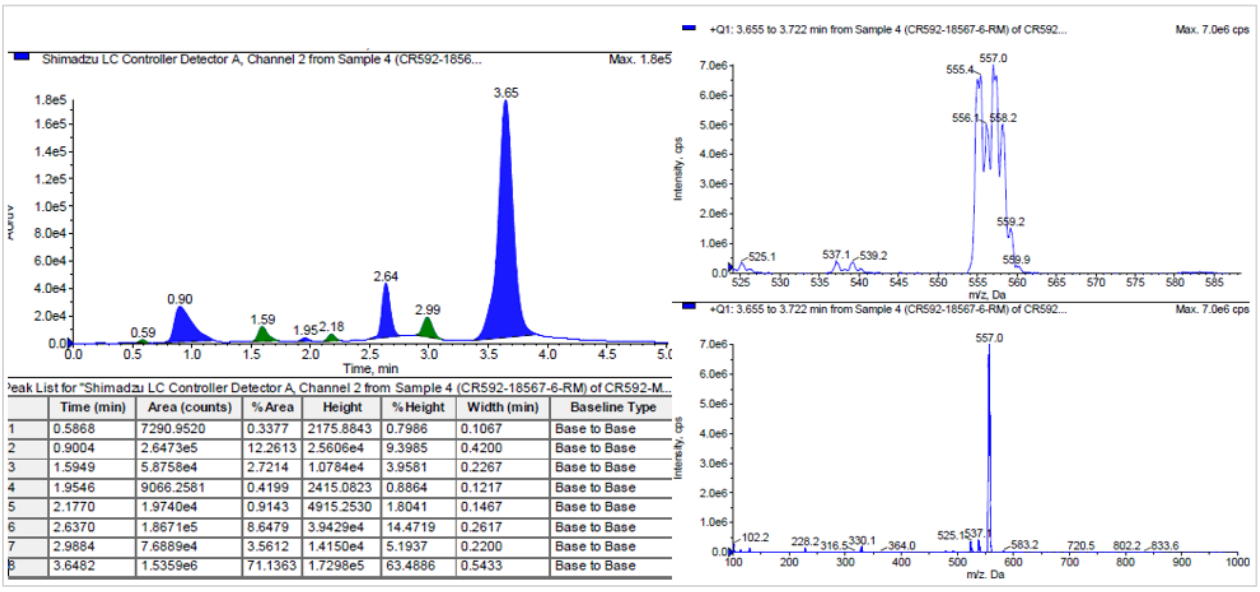
a) When 1,2-addition was carried out at −40 °C during 45 min, 41% of quinoline **KSM-I** was detected along with ~29% of desired diastereomer **D-I**, and 0.5% of undesired **D-II** (HPLC area %). Increase of reaction time from 45 min to 8 h had a negative effect in the reaction outcome – retro-addition was favored (HPLC area %: 62% quinoline **KSM-I**, 1.6% desired diastereomer **D-I**, and 0.07% undesired **D-II**). Increased amount of enone (**IMP-III**) side product was also observed.

b) It was hypothesized that earlier quench of the reaction (right after ketone **KSM-II** addition is finished) could have helped to minimize retro-addition. However, an unknown new impurity was detected with retention time very similar to the BDQ stereoisomers (HPLC A %: 30% new impurity, 20% quinoline **KSM-I**, 16% desired diastereomer **D-I**, and 9% undesired **D-II**).

c) When the BA reaction temperature was decreased to −60 °C, impurity amount dropped from 30% to 14%, but BDQ yield was still not as good as the one obtained when reaction was carried out at −78 °C.

LCMS AND NMR ANALYSIS (RACEMIC APPROACH)

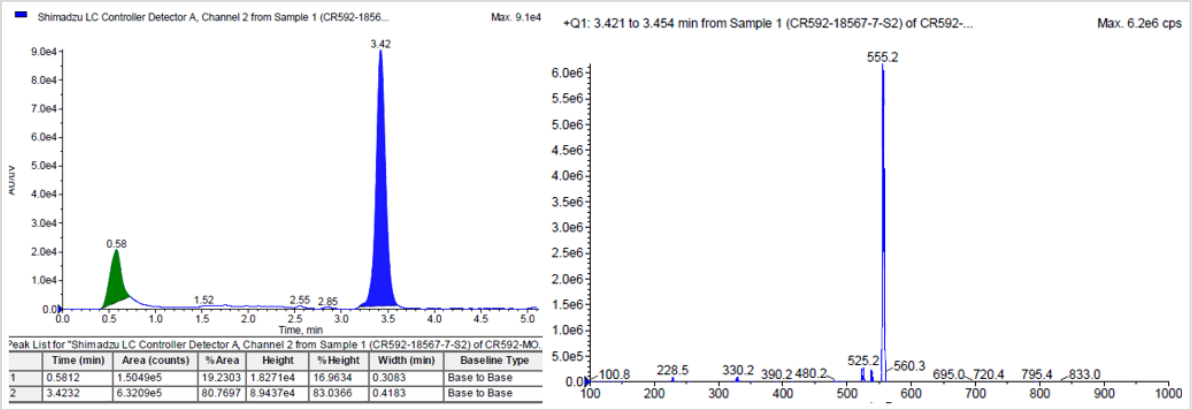
LCMS of crude reaction mixture (after quench):



Peak List for "Shimadzu LC Controller Detector A, Channel 2 from Sample 4 (CR592-18567-6-RM) of CR592-M...

	Time (min)	Area (counts)	% Area	Height	% Height	Width (min)	Baseline Type
1	0.5868	7290.9520	0.3377	2175.8843	0.7986	0.1067	Base to Base
2	0.9004	2.6473e5	12.2613	2.5606e4	9.3985	0.4200	Base to Base
3	1.5949	5.8758e4	2.7214	1.0784e4	3.9581	0.2267	Base to Base
4	1.9546	9066.2581	0.4199	2415.0823	0.8864	0.1217	Base to Base
5	2.1770	1.9740e4	0.9143	4915.2530	1.8041	0.1467	Base to Base
6	2.6370	1.8671e5	8.6479	3.9429e4	14.4719	0.2617	Base to Base
7	2.9884	7.6889e4	3.5612	1.4150e4	5.1937	0.2200	Base to Base
8	3.6482	1.5359e6	71.1363	1.7298e5	63.4886	0.5433	Base to Base

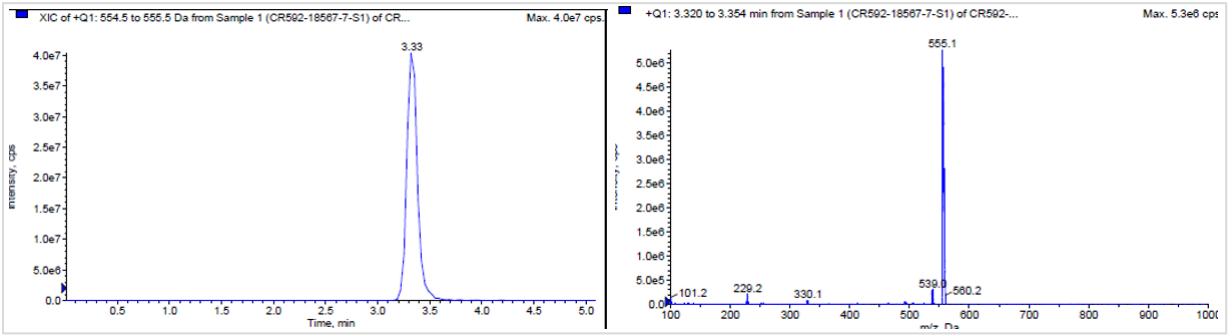
LCMS of desired diastereomer **D-I**:



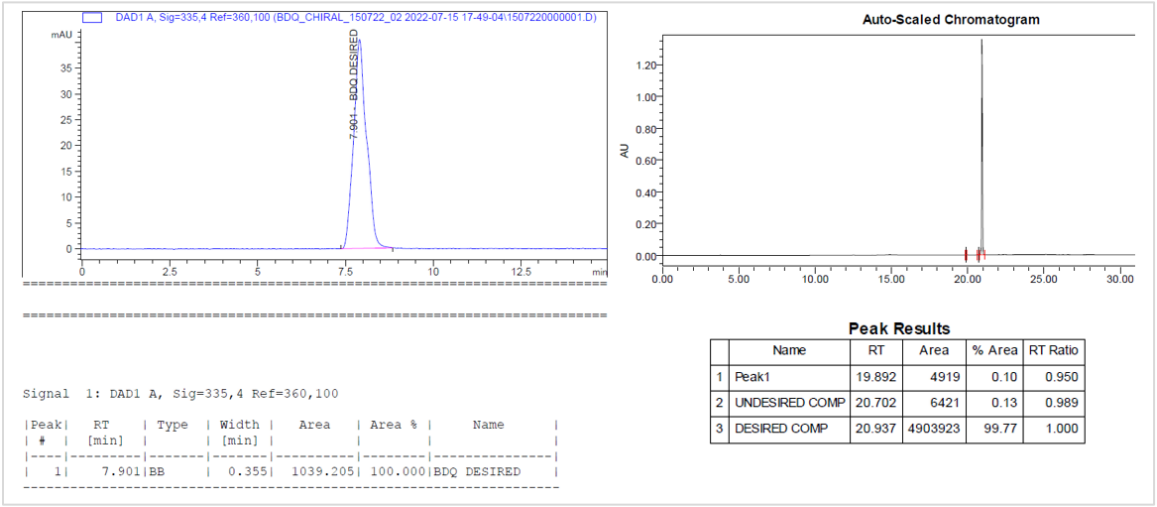
Peak List for "Shimadzu LC Controller Detector A, Channel 2 from Sample 1 (CR592-18567-7-S2) of CR592-MO...

	Time (min)	Area (counts)	%Area	Height	%Height	Width (min)	Baseline Type
1	0.5812	1.5049e5	19.2303	1.8271e4	16.9634	0.3083	Base to Base
2	3.4232	6.3209e5	80.7697	8.9437e4	83.0366	0.4183	Base to Base

LCMS of desired diastereomer **D-II**:



Chiral purity and HPLC of BDQ (**3**):



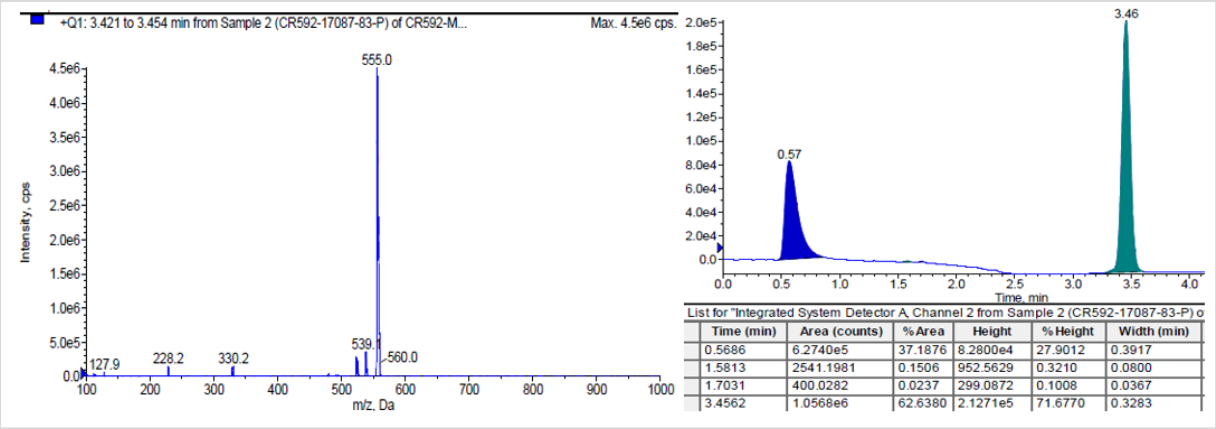
Signal 1: DAD1 A, Sig=335,4 Ref=360,100

Peak #	RT [min]	Type	Width [min]	Area	Area %	Name
1	7.901	BB	0.355	1039.205	100.000	BDQ DESIRED

Peak Results

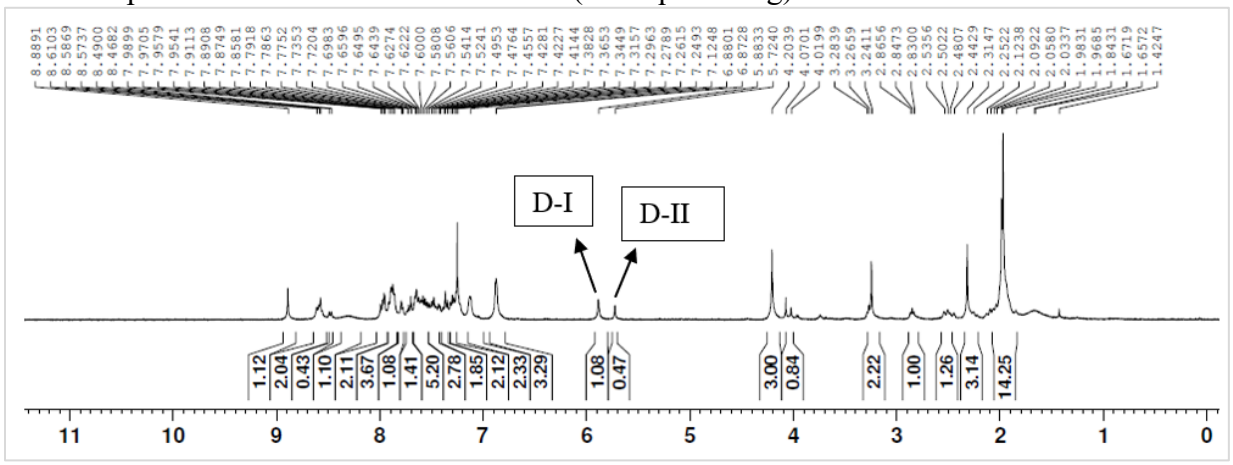
	Name	RT	Area	% Area	RT Ratio
1	Peak1	19.892	4919	0.10	0.950
2	UNDESIRED COMP	20.702	6421	0.13	0.989
3	DESIRED COMP	20.937	4903923	99.77	1.000

LCMS of BDQ (**3**):

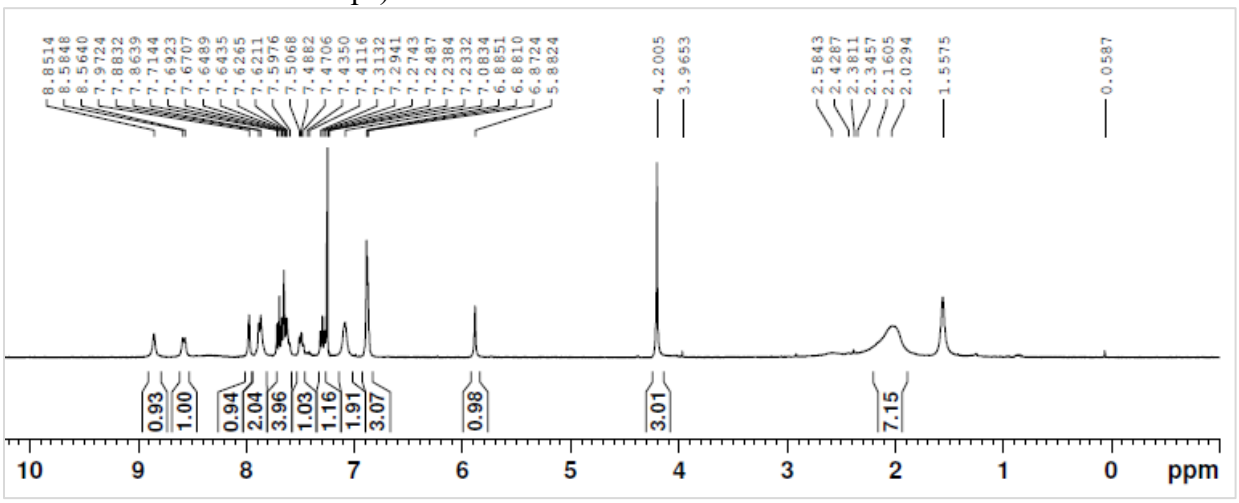


Time (min)	Area (counts)	% Area	Height	% Height	Width (min)
0.5686	6.2740e5	37.1876	8.2800e4	27.9012	0.3917
1.5813	2541.1981	0.1506	952.5629	0.3210	0.0800
1.7031	400.0282	0.0237	299.0872	0.1008	0.0367
3.4562	1.0568e6	62.6380	2.1271e5	71.6770	0.3283

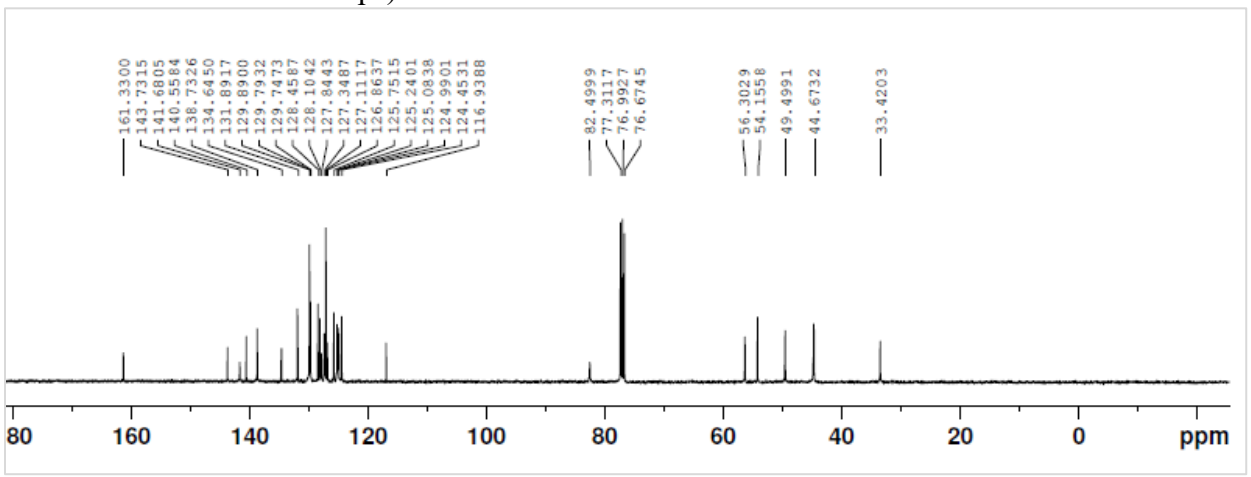
^{1}H NMR spectrum of reaction crude mixture (after quenching) in $CDCl_3$:



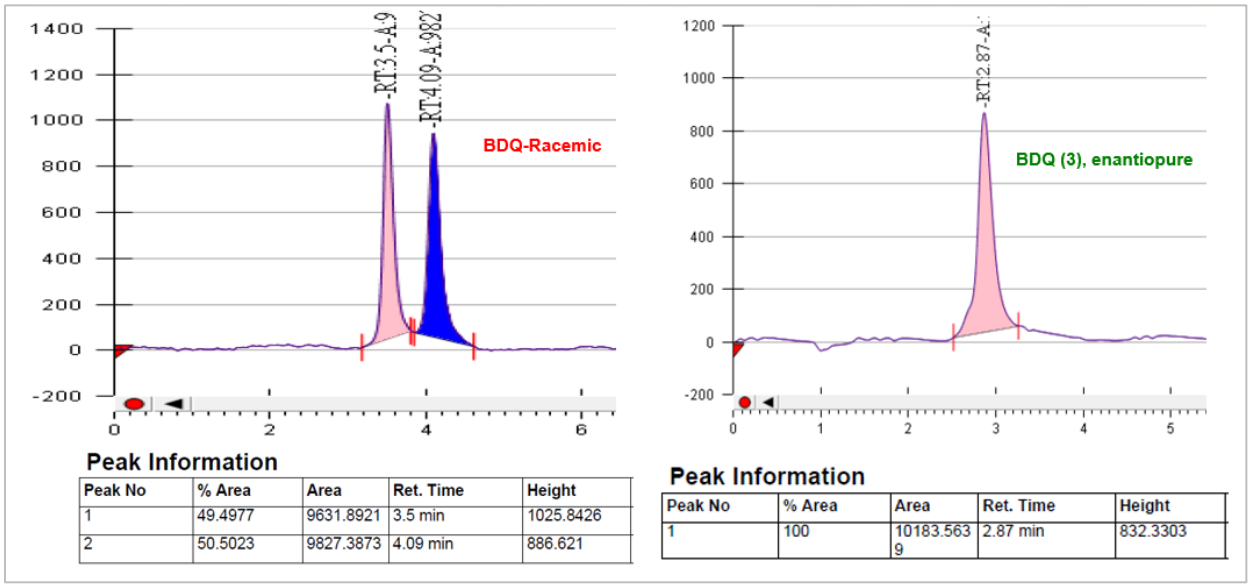
^{1}H NMR spectrum of BDQ (**3**) in $CDCl_3$ (SFC analysis = 100% ee - Material obtained after resolution/neutralization steps):



^{13}C NMR spectrum of BDQ (**3**) in $CDCl_3$ (SFC analysis = 100% ee - Material obtained after resolution/neutralization steps):



SFC analysis of racemic and enantiopure BDQ:



Peak Information

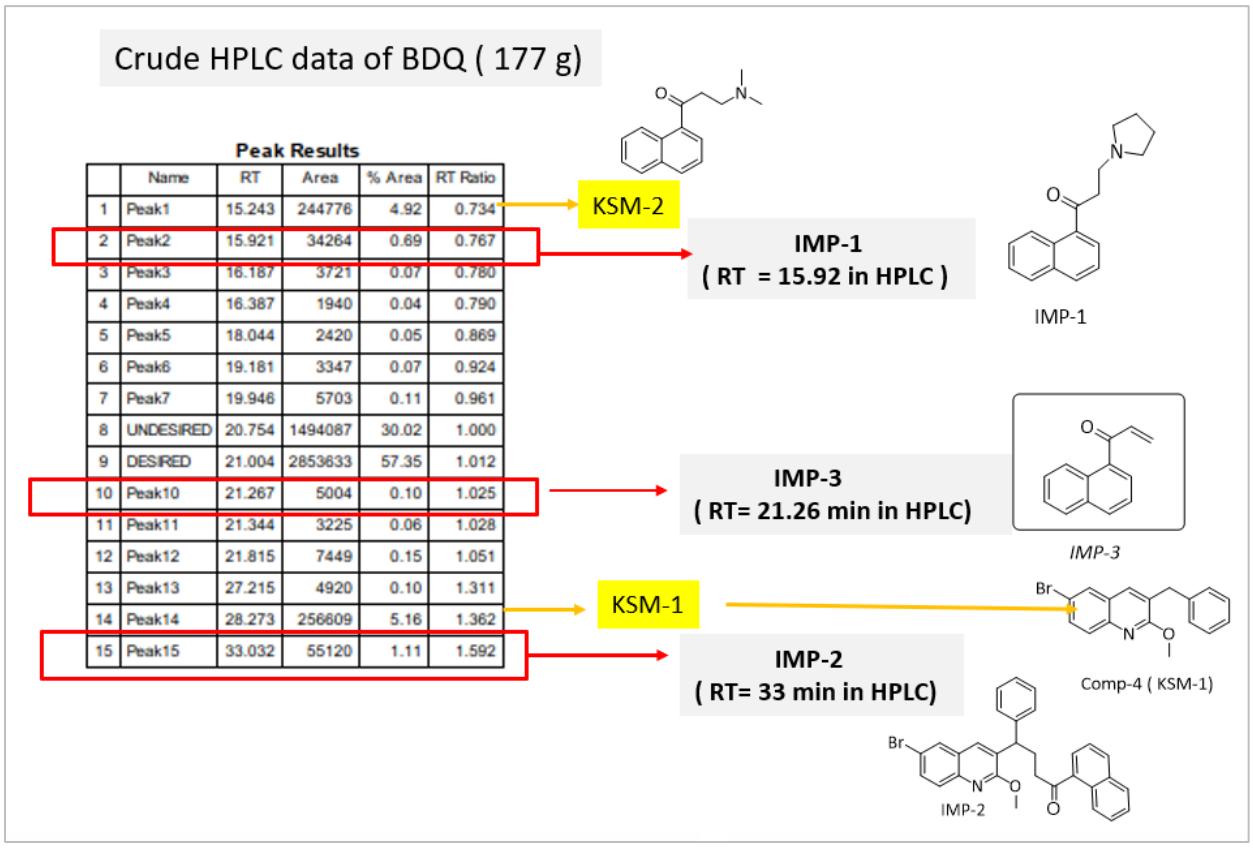
Peak No	% Area	Area	Ret. Time	Height
1	49.4977	9631.8921	3.5 min	1025.8426
2	50.5023	9827.3873	4.09 min	886.621

Peak Information

Peak No	% Area	Area	Ret. Time	Height
1	100	10183.5639	2.87 min	832.3303

HPLC profile of detected impurities

HPLC profile of crude mixture after reaction quench (~2:1 d.r., *syn*:*anti*):



	Name	RT	Area	% Area	RT Ratio
1	Peak1	15.243	244776	4.92	0.734
2	Peak2	15.921	34264	0.69	0.767
3	Peak3	16.187	3721	0.07	0.780
4	Peak4	16.387	1940	0.04	0.790
5	Peak5	18.044	2420	0.05	0.869
6	Peak6	19.181	3347	0.07	0.924
7	Peak7	19.946	5703	0.11	0.961
8	UNDESIRED	20.754	1494087	30.02	1.000
9	DESIRED	21.004	2853633	57.35	1.012
10	Peak10	21.267	5004	0.10	1.025
11	Peak11	21.344	3225	0.06	1.028
12	Peak12	21.815	7449	0.15	1.051
13	Peak13	27.215	4920	0.10	1.311
14	Peak14	28.273	256609	5.16	1.362
15	Peak15	33.032	55120	1.11	1.592

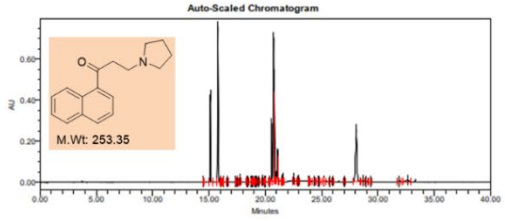
Impurity Characterization - Impurity Structure **IMP-1** (15.8 RT in HPLC):

Spike data of impurity at RT 15.8 min HPLC(0.92 min in LC)

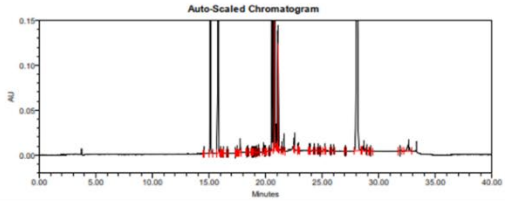
SAMPLE INFORMATION

Sample Name:		Acquired By:	SD0113647
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_270722_01
Vial:	26	Acq. Method Set:	BDQ_CP_LC42_01
Injection #:	1	Processing Method:	BDQ_CP_270722_02
Injection Volume:	10.00 ul	Channel Name:	225.0nm
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 225.0 nm (2998
Date Acquired:	27-07-2022 19:20:21 IST	Column Name:	SHIMPACK SOLAR C18(250*4.6)mm,5
Date Processed:	27-07-2022 20:37:59 IST		

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram

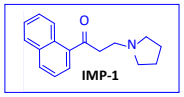


RT = 15.8

Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	14.515	5504	0.04	0.919
2	Peak2	15.131	1440373	10.77	0.958
3	IMP	15.788	2648526	19.80	1.000
4	Peak4	16.045	4456	0.03	1.016
5	Peak5	16.263	3881	0.03	1.030
6	Peak6	16.624	7131	0.05	1.053
7	Peak7	17.351	893	0.01	1.099
8	Peak8	17.518	7823	0.06	1.110
9	Peak9	17.767	42044	0.31	1.125
10	Peak10	18.331	1374	0.01	1.161
11	Peak11	18.446	5317	0.04	1.168
12	Peak12	18.740	2201	0.02	1.187
13	Peak13	18.829	1550	0.01	1.193
14	Peak14	18.920	1343	0.01	1.198
15	Peak15	19.024	2832	0.02	1.205
16	Peak16	19.161	766	0.01	1.214
17	Peak17	19.204	8055	0.06	1.216
18	Peak18	19.403	10447	0.08	1.229
19	Peak19	19.777	17387	0.13	1.253
20	Peak20	19.929	3937	0.03	1.262
21	Peak21	19.991	2717	0.02	1.266
22	Peak22	20.343	3504	0.03	1.288
23	Peak23	20.564	1012599	7.57	1.302
24	Peak24	20.738	2664865	19.92	1.314
25	Peak25	20.807	1914838	14.32	1.318
26	Peak26	20.956	59599	0.45	1.327
27	Peak27	21.076	529198	3.96	1.335
28	Peak28	21.126	392720	2.94	1.338

Spike Data



Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	15.243	244776	4.92	0.734
2	Peak2	15.921	34264	0.69	0.767
3	Peak3	16.167	3721	0.07	0.780
4	Peak4	16.387	1940	0.04	0.790
5	Peak5	18.044	2420	0.05	0.869
6	Peak6	19.181	3347	0.07	0.924
7	Peak7	19.946	5703	0.11	0.961
8	UNDESIRED	20.754	1494087	30.02	1.000
9	DESIRED	21.004	2853633	57.35	1.012
10	Peak10	21.267	5004	0.10	1.025
11	Peak11	21.344	3225	0.06	1.028
12	Peak12	21.815	7449	0.15	1.051
13	Peak13	27.215	4920	0.10	1.311
14	Peak14	28.273	256609	5.16	1.362
15	Peak15	33.032	55120	1.11	1.592

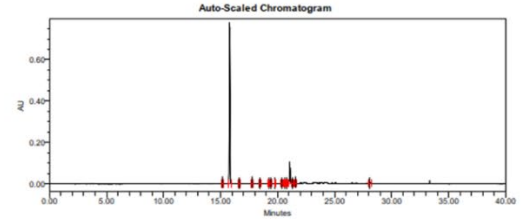
Crude BDQ

Imp-I: RT 15.8 min in HPLC (0.92 min in LCMS)

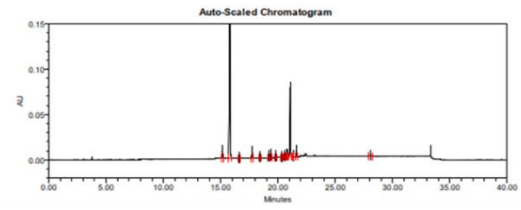
SAMPLE INFORMATION

Sample Name:		Acquired By:	SD0113647
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_270722_01
Vial:	25	Acq. Method Set:	BDQ_CP_LC42_01
Injection #:	1	Processing Method:	BDQ_CP_270722_02
Injection Volume:	10.00 ul	Channel Name:	225.0nm
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 225.0 nm (2998
Date Acquired:	27-07-2022 17:09:18 IST	Column Name:	SHIMPACK SOLAR C18(250*4.6)mm,5
Date Processed:	27-07-2022 20:29:50 IST		

Auto-Scaled Chromatogram

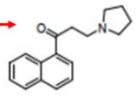


Auto-Scaled Chromatogram



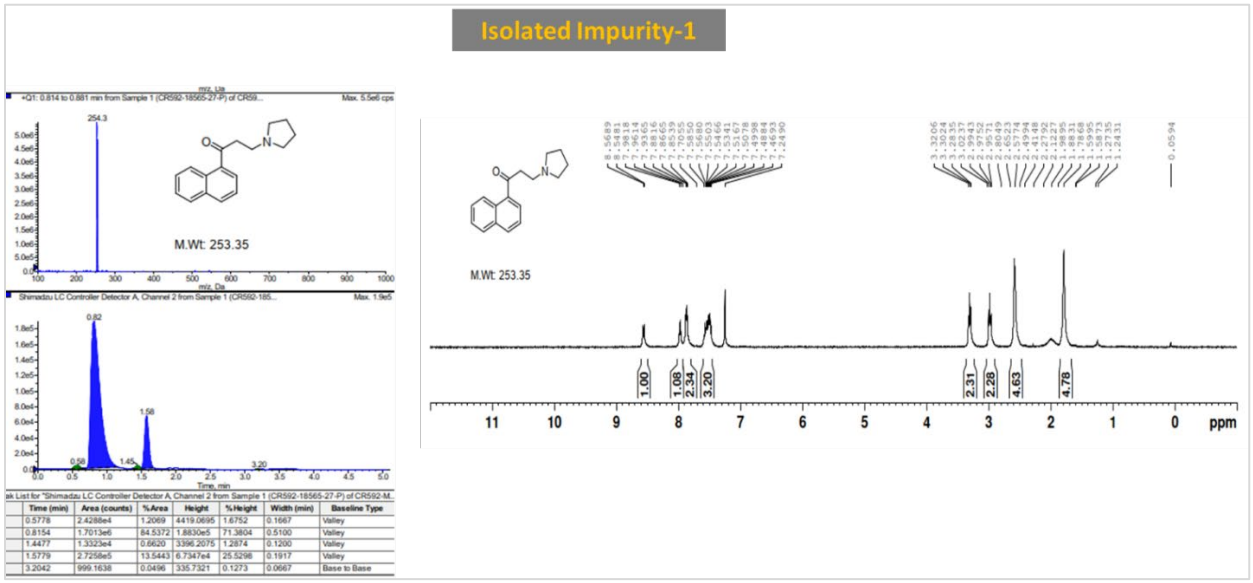
Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	15.144	26355	0.84	0.959
2	IMP	15.793	2659512	84.41	1.000
3	Peak3	16.610	2626	0.08	1.052
4	Peak4	17.770	27300	0.87	1.125
5	Peak5	18.452	5969	0.19	1.168
6	Peak6	19.204	6445	0.20	1.216
7	Peak7	19.408	11328	0.36	1.229
8	Peak8	19.784	8384	0.27	1.253
9	Peak9	20.344	4930	0.16	1.288
10	Peak10	20.577	3686	0.12	1.303
11	Peak11	20.756	8853	0.28	1.314
12	Peak12	20.819	7334	0.23	1.318
13	Peak13	21.080	341928	10.85	1.335
14	Peak14	21.319	2532	0.08	1.350
15	Peak15	21.615	23461	0.74	1.369
16	Peak16	28.087	10027	0.32	1.778

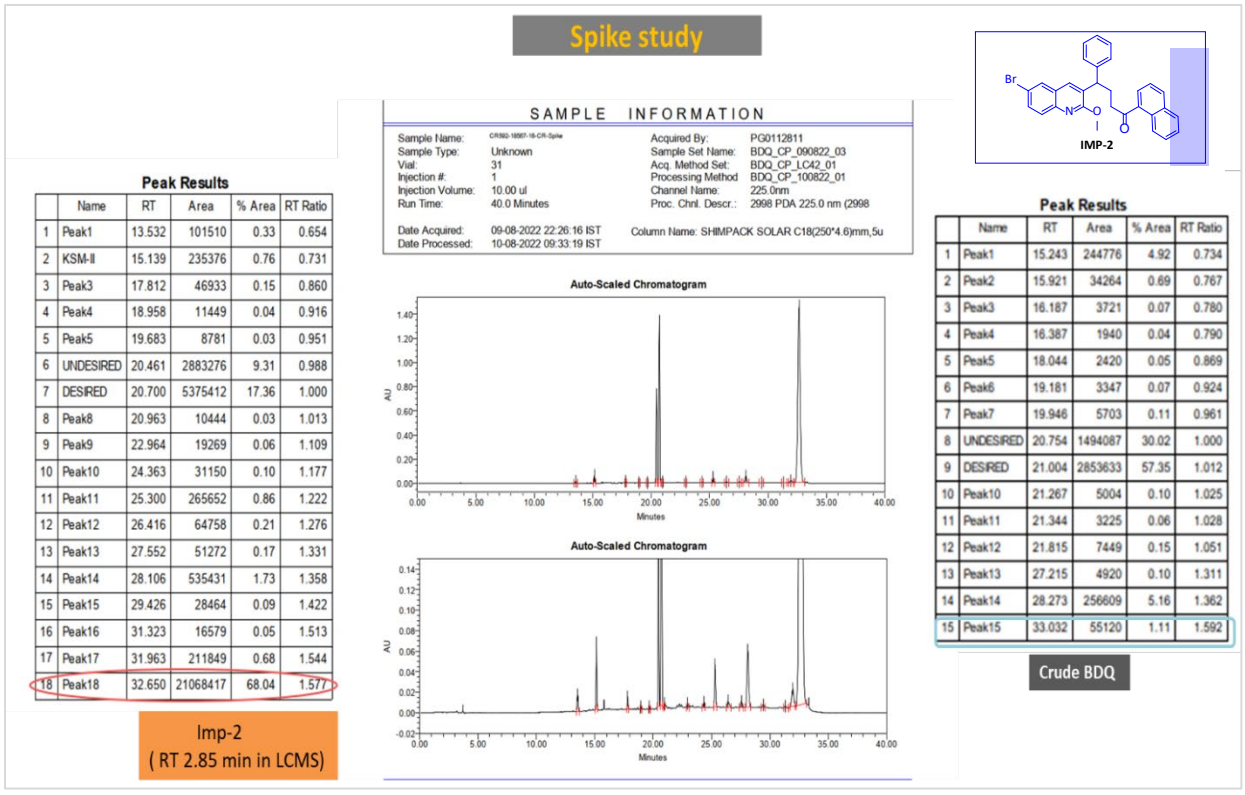


M.Wt: 253.35

RT = 15.8



Impurity Characterization - Impurity Structure **IMP-2** (33.0 RT in HPLC):



	Name	RT	Area	% Area	RT Ratio
1	Peak1	13.532	101510	0.33	0.654
2	KSM-II	15.139	235376	0.76	0.731
3	Peak3	17.812	46933	0.15	0.860
4	Peak4	18.958	11449	0.04	0.916
5	Peak5	19.683	8781	0.03	0.951
6	UNDESIRED	20.461	2883276	9.31	0.988
7	DESIRED	20.700	5375412	17.36	1.000
8	Peak8	20.963	10444	0.03	1.013
9	Peak9	22.964	19269	0.06	1.109
10	Peak10	24.363	31150	0.10	1.177
11	Peak11	25.300	265652	0.86	1.222
12	Peak12	26.416	64758	0.21	1.276
13	Peak13	27.552	51272	0.17	1.331
14	Peak14	28.106	535431	1.73	1.358
15	Peak15	29.426	28464	0.09	1.422
16	Peak16	31.323	16579	0.05	1.513
17	Peak17	31.963	211849	0.68	1.544
18	Peak18	32.650	21068417	68.04	1.577

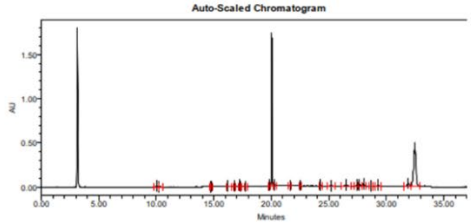
	Name	RT	Area	% Area	RT Ratio
1	Peak1	15.243	244776	4.92	0.734
2	Peak2	15.921	34264	0.69	0.767
3	Peak3	16.187	3721	0.07	0.780
4	Peak4	16.387	1940	0.04	0.790
5	Peak5	18.044	2420	0.05	0.869
6	Peak6	19.181	3347	0.07	0.924
7	Peak7	19.946	5703	0.11	0.961
8	UNDESIRED	20.754	1494087	30.02	1.000
9	DESIRED	21.004	2853633	57.35	1.012
10	Peak10	21.267	5004	0.10	1.025
11	Peak11	21.344	3225	0.06	1.028
12	Peak12	21.815	7449	0.15	1.051
13	Peak13	27.215	4920	0.10	1.311
14	Peak14	28.273	256609	5.16	1.362
15	Peak15	33.032	55120	1.11	1.592

Spike study

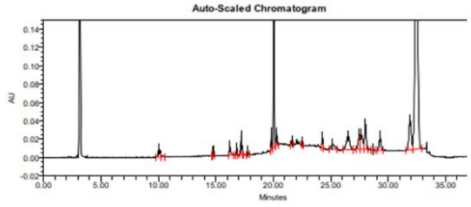
SAMPLE INFORMATION

Sample Name:		Acquired By:	PG0112811
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_2220822_01
Vial:	25	Acq. Method Set:	BDQ_CP_LC42_01
Injection #:	1	Processing Method	BDQ_CP_220822_01
Injection Volume:	5.00 ul	Channel Name:	225.0nm
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 225.0 nm (2
Date Acquired:	22-08-2022 18:01:45 IST	Column Name:	SHIMPACK SOLAR C18(250*4.6
Date Processed:	22-08-2022 19:09:09 IST		

Auto-Scaled Chromatogram

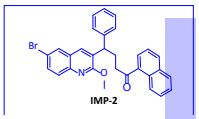


Auto-Scaled Chromatogram



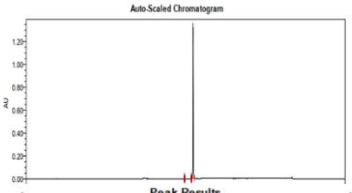
Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	10.060	98406	0.65	0.502
2	Peak2	10.245	14101	0.09	0.511
3	Peak3	14.722	12173	0.08	0.734
4	Peak4	14.805	15859	0.11	0.738
5	Peak5	16.212	83787	0.56	0.809
6	Peak6	16.831	35066	0.23	0.840
7	Peak7	17.216	91814	0.61	0.859
8	Peak8	17.328	18956	0.13	0.864
9	Peak9	17.783	18753	0.12	0.887
10	UNDESIRED COMP	19.821	65239	0.43	0.989
11	DESIRED COMP	20.049	6434856	42.67	1.000
12	Peak12	20.293	65996	0.44	1.012
13	Peak13	21.621	18546	0.12	1.078
14	Peak14	22.507	15366	0.10	1.123
15	Peak15	24.277	63203	0.42	1.211
16	Peak16	25.159	118573	0.79	1.255
17	Peak17	26.506	311423	2.06	1.322
18	Peak18	27.495	171337	1.14	1.371
19	Peak19	27.643	231045	1.53	1.379
20	Peak20	28.036	261443	1.73	1.398
21	Peak21	28.686	7184	0.05	1.431
22	Peak22	29.296	162714	1.08	1.461
23	Peak23	31.917	441077	2.92	1.592
24	IMP	32.471	6324521	41.94	1.620



Sample Name:		Acquired By:	PG0112811
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_260722_02
Vial:	12	Acq. Method Set:	BDQ_CP_LC42_01
Injection #:	1	Processing Method	BDQ_CP_260722_02
Injection Volume:	10.00 ul	Channel Name:	225.0nm
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 225.0 nm (2998
Date Acquired:	26-07-2022 17:33:41 IST	Column Name:	SHIMPACK SOLAR C18(250*4.6)mm,5
Date Processed:	26-07-2022 20:28:03 IST		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	19.892	4919	0.10	0.950
2	UNDESIRED COMP	20.702	6421	0.13	0.989
3	DESIRED COMP	20.937	4903923	99.77	1.000

Purified BDQ

Impurity Characterization - Impurity Structure **IMP-3** (21.0 RT in HPLC):

IMP-3 (enone) synthesized in-house

Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	10.191	733278	5.78	0.484
2	Peak2	16.574	51798	0.41	0.787
3	Peak3	16.841	82835	0.65	0.800
4	Peak4	17.054	7634	0.06	0.810
5	Peak5	17.497	206676	1.63	0.831
6	Peak6	17.770	8478	0.07	0.844
7	Peak7	18.366	15065	0.12	0.872
8	Peak8	18.947	37540	0.30	0.900
9	Peak9	19.186	745107	5.88	0.911
10	Peak10	20.138	112272	0.89	0.956
11	Peak11	20.373	338803	2.67	0.968
12	Peak12	20.682	208480	1.64	0.982
13	ENONE	21.055	8740363	68.94	1.000
14	Peak14	21.435	25864	0.20	1.018
15	Peak15	21.620	18216	0.14	1.027

IMP-3 (enone) spike data with Crude BDQ:

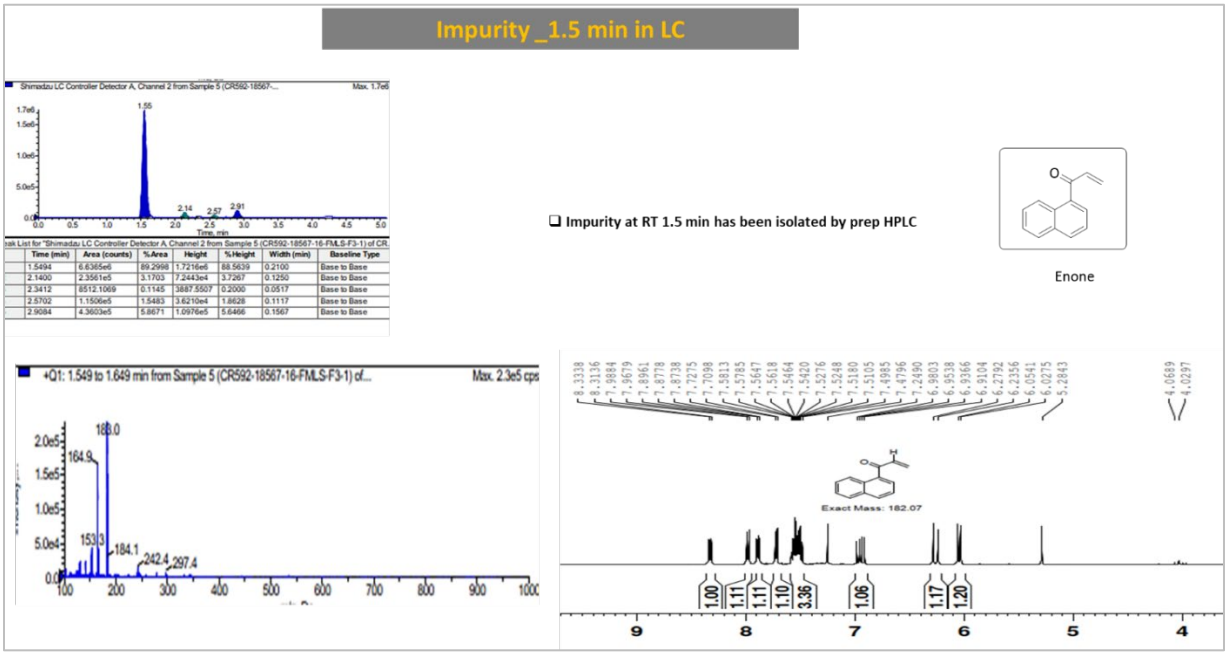
Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	9.863	204496	1.63	0.477
2	Peak2	10.102	140293	1.12	0.489
3	Peak3	14.782	9208	0.07	0.715
4	KSM-II	15.088	129311	1.03	0.730
5	Peak5	15.748	37471	0.30	0.762
6	Peak6	15.980	42897	0.34	0.773
7	Peak7	16.560	34205	0.27	0.801
8	Peak8	16.823	42332	0.34	0.814
9	Peak9	17.481	131094	1.04	0.846
10	Peak10	17.757	37805	0.30	0.859
11	Peak11	18.357	25552	0.20	0.888
12	Peak12	18.643	10618	0.08	0.902
13	Peak13	18.932	41394	0.33	0.916
14	Peak14	19.173	495294	3.94	0.928
15	Peak15	20.125	49246	0.39	0.974
16	UNDESIRED COMP	20.423	2634574	20.98	0.988
17	DESIRED COMP	20.663	4771233	37.99	1.000
18	ENONE	21.044	2540410	20.23	1.018

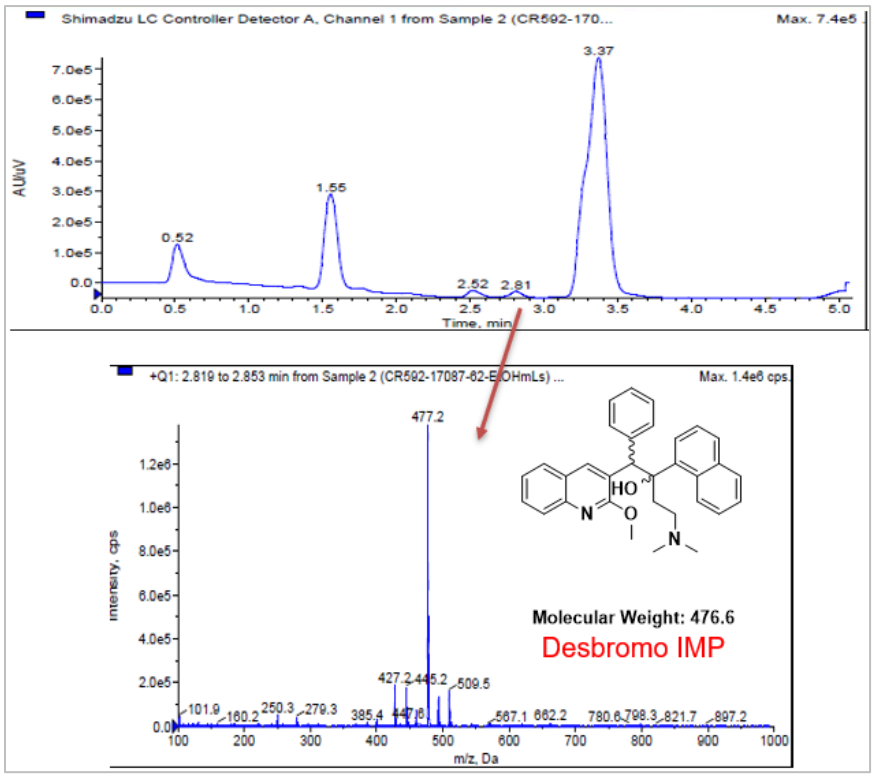
Crude mixture (BDQ)

Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	14.789	9538	0.11	0.715
2	KSM-II	15.097	132692	1.60	0.730
3	Peak3	15.757	36285	0.44	0.762
4	Peak4	15.989	43129	0.52	0.774
5	Peak5	17.762	31908	0.38	0.859
6	Peak6	18.378	13061	0.16	0.889
7	Peak7	18.651	6197	0.07	0.902
8	Peak8	19.652	5388	0.06	0.951
9	UNDESIRED COMP	20.432	2600164	31.29	0.988
10	DESIRED COMP	20.670	4840927	58.26	1.000
11	Peak11	20.891	9277	0.11	1.011
12	Peak12	20.925	8419	0.10	1.012
13	Peak13	21.044	5441	0.07	1.018

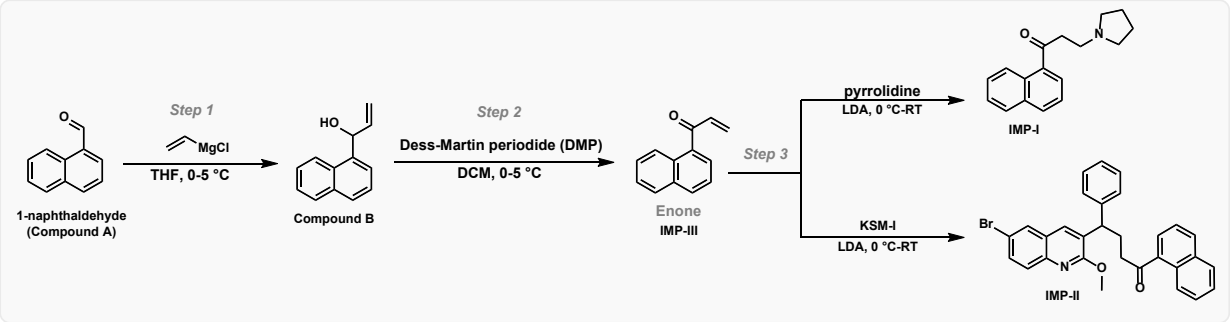


LCMS – Minor desbromo-BDQ impurity:



SYNTHESIS AND CHARACTERIZATION OF IMPURITIES

Preparation of **IMP-I** and **IMP-II**:



Step 1:

Synthesis of 2-(tert-butyl (R)-2-(hydroxymethyl) pyrrolidine-1-carboxylate (Compound B). To a stirred solution of 1-napthaldehyde (15 g, 96.04 mmol, 1 equiv) in THF (5 V), vinyl magnesium bromide (1 M in THF, 115.25 mL, 1.2 equiv) was added dropwise at 0-5 °C under nitrogen atmosphere. The reaction mass was stirred at this temperature for 4 h. After completion of the reaction (determined by TLC), a saturated solution of NH_4Cl (420 mL) was slowly added to the solution at 0 °C and the aqueous phase was extracted with EtOAc (3 x 420 mL). The combined organic phases were washed with water (500 mL) followed by brine (400 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to give a residue which was purified by silica gel flash chromatography (eluent: 10% EtOAc/hexanes). **Compound B** was obtained as light-yellow oil in 46% yield (8.2 g).

CR592-18828-55-P_NMR.pdf

Step 2:

Synthesis of 1-(naphthalen-1-yl)prop-2-en-1-one (IMP-III): A solution of **Compound B** (8 g, 43.42 mmol, 1 equiv) in DCM (80 mL, 10 V) was cooled to 0-5 °C under nitrogen atmosphere. Dess-Martin periodide (22.1 g, 52.10 mmol, 1.2 equiv) was added portion wise to the reaction mass at the same temperature. The reaction was allowed to warm to 25-30 °C, and was stirred for an additional 8 h. After completion, the reaction was quenched with sodium thiosulphate (hypo solution, 250 mL) at 0 °C, and stirring was maintained for 0.5 h. The reaction mass was filtered through a Celite® bed, which was washed with DCM (24 mL, 3 V). The filtrate was transferred to a separatory funnel, and was extracted with DCM (3 x 80 mL). The combined organic phase was washed with water (80 mL), followed by brine (80 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to give **IMP-III** as reddish gum and in 91% yield (7.2 g). The obtained material was used in the next step without further purification.
^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 6.04 - 6.01 (m, 1H), 6.28 - 6.24 (m, 1H), 7.58 -7.42 (m, 3H), 7.72 - 7.70 (m, 1H), 7.89-7.86 (m, 1H), 7.98 - 7.95 (m, 1H), 8.36 - 8.33 (m,1H).


CR592-18828-58-CR-R_LC.pdf


CR592-18828-58-CR_NMR.pdf


CR592-18828-58-CR_HPLC_Enone.pdf


CR592-18828-58-CR-R-PG1.pdf

Step 3 for IMP-I:

Synthesis 1-(naphthalen-1-yl)-3-(pyrrolidin-1-yl)propan-1-one (IMP-I). A solution of pyrrolidine (0.948 g, 1.5 equiv) in THF (5 mL, 5 V) was cooled to −20 °C, and LDA (1 M in THF/hexanes, 7.13 mL, 1.3 equiv) was slowly added to the reaction flask under nitrogen atmosphere. The resulting mixture was stirred for 30 min at the same temperature. A solution of **IMP-III** (enone) (1.0 g, 5.48 mmol, 1.0 equiv) in THF (5 V) was added to the reaction flask at −20 °C, and stirred for 2 h at 25-30 °C. After completion of the reaction (determined by TLC), a saturated solution of NH_4Cl (10 mL) was added slowly at 0 °C. The layers were separated, and the aqueous phase was extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with water (100 mL), followed by brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to give a residue which was purified by silica gel flash chromatography (eluent: 10% EtOAc/hexanes). **IMP-I** was obtained as a light-yellow oil in 30% yield (0.5 g).


CR592-18565-27-P_LC.pdf


CR592-18565-27-P_HPLC.pdf


CR592-18565-27-P_NMR.pdf

Step 3 for IMP-II:

Synthesis of 4-(6-bromo-2-methoxyquinolin-3-yl)-1-(naphthalen-1-yl)-4-phenylbutan-1-one (IMP-II): A solution of **KSM-I** (2.7 g, 1.5 eq) in THF (5 ml, 5 V) was cooled to −20 °C, and LDA (1 M in THF/hexanes, 7.13 mL, 1.3 equiv) was slowly added to the reaction mass under nitrogen atmosphere. The resulting mixture was stirred for 30 min at the same temperature. A solution of **IMP-III** (enone) (1.0 g, 5.48 mmol, 1 equiv) in THF (5 V) was added to the reaction flask at −20 °C, and stirred for 2 h at 25-30 °C. After completion of the reaction (determined by TLC), a saturated solution of NH_4Cl (10 mL) was added slowly at 0 °C. The layers were separated, and the aqueous phase was extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with water (100 mL), followed by brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to give a residue which was purified by silica gel flash chromatography (eluent: 50% EtOAc/hexanes). IMP-II was obtained as off-white solid in 40% yield (1.12 g).

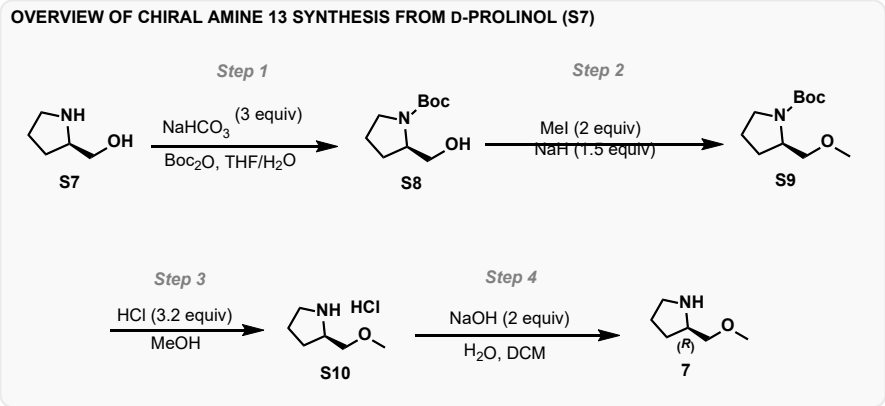

CR592-18567-36-F2-2-LC.pdf


CR592-18567-36-F2-2_NMR.pdf


CR592-18567-36-F2-2_HPLC.pdf

FINAL PROCEDURES USED FOR THE ASYMMETRIC APPROACH

Synthesis of (*R*)-2-(methoxymethyl)pyrrolidine (7)



Tert-butyl (R)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (S8). A suspension of D-prolinol (**S7**) (290.0 g, 2.87 mol, 1.0 equiv) and $NaHCO_3$ (602.1 g, 7.17 mol, 2.5 equiv) in THF (2.9 L, 10 V) and H_2O (2.9 L, 10 V) was cooled to 5-10 °C, and Boc_2O (938.6 g, 4.3 mol, 1.5 equiv) was slowly added to this mixture. The reaction was stirred for 12 h at 25 °C, and water (1.2 L, 4 V) was added to the reaction flask. After phases separation, the organic layer was extracted with EtOAc (2 x 2.9 L, 20 V). The combined organic phase was washed with brine (2.9 L, 10 V), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure in the rotavap to give crude **S8** (599.0 g, 104% yield). *n*-Heptane (600 mL, 2 V) was charged into the flask and stirred for 12 h at 25-30 °C. The resulting white solid was filtered and washed with cold *n*-heptane (150 mL, 0.5 V), and dried under vacuum for 4-5 h at 45-50 °C resulting in **S8** (485 g, 84% yield, 78% purity by qNMR, 66% yield by purity). NMR data is in accordance with the literature.[10]

Note: The moderate yield is due to compound **S8** losses in the mother liquor (this step needs to be further optimized).

1H NMR Step-1.pdf

Tert-butyl (R)-2-(methoxymethyl)pyrrolidine-1-carboxylate (S9). To a solution of compound **S8** (480.0 g, 2.38 mol, 78% purity, 1.0 equiv) and MeI (677 g, 4.77 mol, 2.0 equiv) in THF (2.9 L, 6 V), NaH (60% dispersion in mineral oil, 143 g, 3.58 mol, 1.5 equiv) was added portion-wise at 0-5 °C. The suspension was warmed to 25 °C and stirred at 25 °C for 12 h, and a saturated NH_4Cl solution (2.9 L, 6 V) was slowly added to the reaction mixture. After phase separation, the aqueous layer was extracted with EtOAc (3 x 2.9 L, 18 V). The combined organic phase was washed with brine (2 x 2.9 L, 12 V), dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to give crude **S9** (514.0 g, 100% yield, 95% purity by qNMR, 82% yield by purity). Compound **S9** was used without further purification in the next step. NMR data is in accordance with the literature.[11]

1H NMR Step-2.pdf

(R)-2-(methoxymethyl)pyrrolidine hydrochloride (S10). To a stirred solution of HCl (4 M in methanol, 1.8 L, 7.51 mol, 3.2 equiv) at 0 °C, compound **S9** (505.0 g, 2.35 mol, 95% purity, 1.0 equiv) was added and the resulting solution was stirred for 2 h at 25 °C. After completion of the reaction, the solvent was removed under reduced pressure to give hydrochloride salt **S10** as a light-yellow solid (383.0 g, 108% yield, 87% purity by qNMR, 94% yield by purity). Compound **S10** was used without further purification in the next step.

1H NMR Step-3.pdf

(R)-2-(methoxymethyl)pyrrolidine (7): The hydrochloride salt **S10** (380.0 g, 2.51 mol, 87% purity, 1.0 equiv) was dissolved in water (1.5 L, 4 V), and a solution of NaOH (210.5 g, 5.26 mol, 2.1 equiv) in water (3.8 L, 10 V) was added dropwise into the reaction mixture at 0 °C. This reaction mass was stirred for 15 min at room temperature. DCM (15 V) was poured into the reaction flask and vigorously stirred for an additional 15 min. The biphasic reaction mixture was transferred to a separatory funnel, and the organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to provide crude **7** (130.0 g, 45% yield, 94% purity by qNMR, 42% yield by purity). NMR data is in accordance with the literature.[12]

Note: The low yield is due to amine **7** losses in the aqueous phase (this step needs to be further optimized).

1H NMR Step-4.pdf

Step-4_free base_HPLC.pdf

Free Base SOR.pdf

Distillation of (R)-2-(methoxymethyl)pyrrolidine (7). Crude **7** (130 g, 1.13 mol, 94% purity) was distilled under vacuum (oil bath temperature was ~110 °C). The first fraction was collected at vapor temperature of 30-35 °C at 0.4 mmHg. This fraction comprises the desired compound **7** (68 g, 52% yield, >99% purity by HPLC area %). A second fraction (dark brown colored) was collected at vapor temperatures of 37-42 °C at 0.4 mmHg. According to the ^{1}H NMR analysis, this fraction contains a small amount of product along with other impurities. The first fraction was stored under argon atmosphere with activated molecular sieves (4 Å). The mass of the residue left in the flask after distillation was 30.0 g (~23%), while the mass of chiral amine captured in the cold trap was ~25.0 g (~19%) which can be purified in the next distillation batch.

Note: It is important to check the purity of the isolated chiral amine by GCHS (Head Space Gas Chromatography), to ensure no other volatile impurities are present in the collected sample prior to its use in the BDQ (**3**) synthesis. Further optimization for the distillation purification will be developed.

R-Chiral base_Distilled.pdf

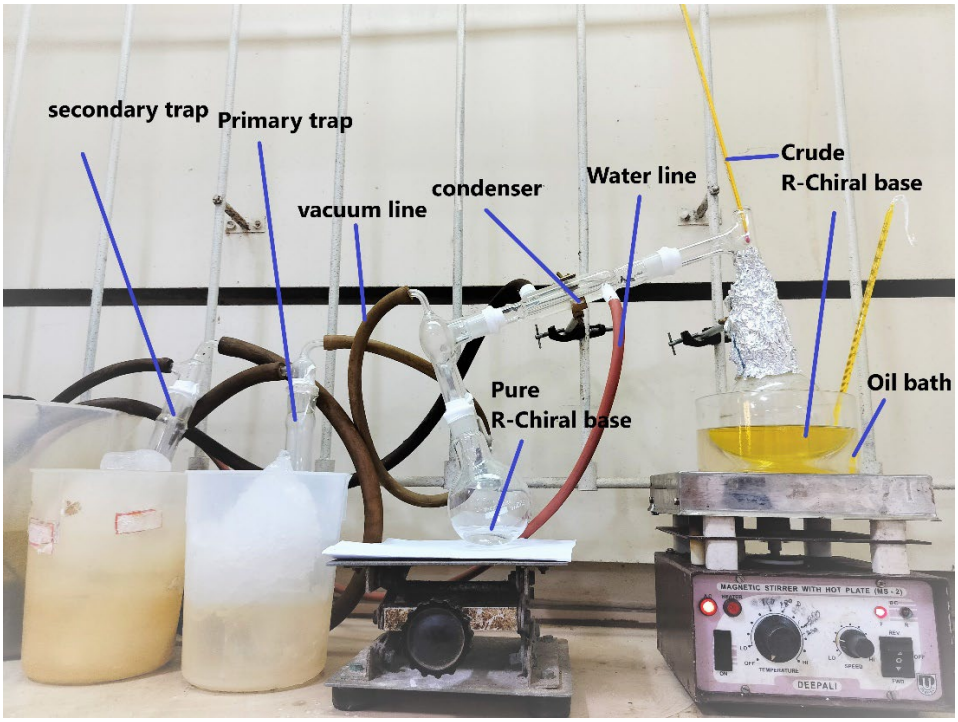


Figure 4. *Setup used for vacuum distillation of chiral amine 7*

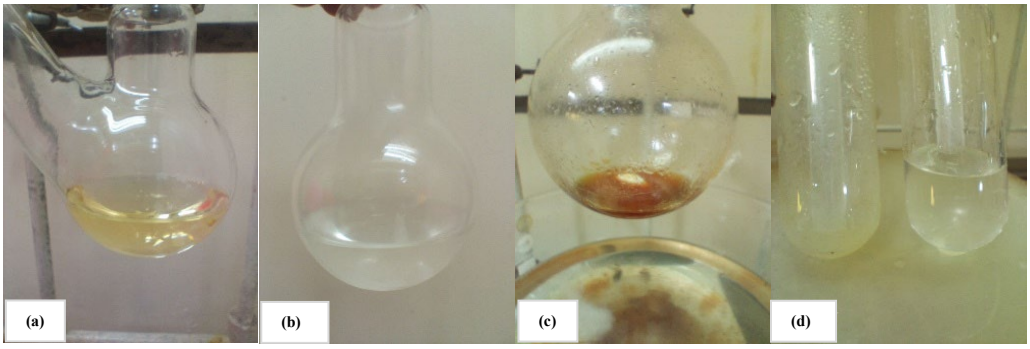
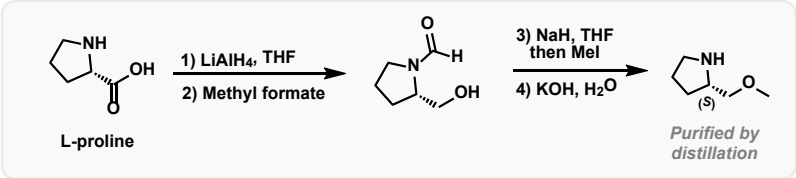


Figure 5. *Vacuum distillation of (R)-2-(methoxymethyl)pyrrolidine (7) - (a) crude amine prior to distillation; (b) First fraction of 7 collected (>99% by HPLC); (c) Brown residue obtained by the end of distillation; (d) Chiral amine 7 vapor captured in the traps (containing other volatile impurities):*

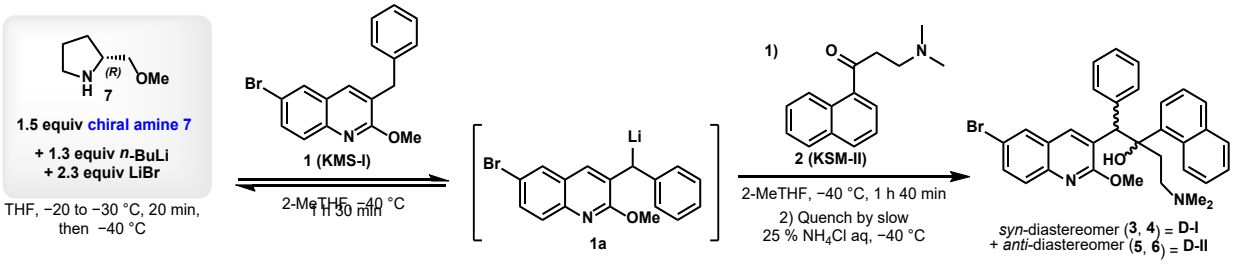
Note: Although the procedures described above were adopted to produce (*R*)-2-(methoxymethyl)pyrrolidine (**7**) for the BA reaction scale-up displayed in this report, TE analysis had shown the use of D-proline as starting material is essential to make the asymmetric approach more cost-effective than the racemic one. The in-house production of **7** from D-proline is recommended due to the high values some vendors can charge for more advanced intermediates or the final amine **7** itself, which have a significant impact on the total RMs cost. After analyzing different methodologies in the opened literature, we identified a more straightforward and lower-

cost 4-step telescoped process covering the transformation of L-proline to (*S*)-2-(methoxymethyl)pyrrolidine in 70% overall yield (Scheme 6).[12, 13] We believe that switching L-proline with D-proline should not cause considerable variation in the reaction outcome. Therefore, this 4-step sequence becomes a valuable option to obtain **7** as cheaply as possible.



Scheme 6. *Four-step process for the synthesis of (S)-2-(methoxymethyl)pyrrolidine from L-proline*

BA Reaction – Asymmetric Approach



A 5 L four-neck round-bottom flask was equipped with an overhead stirrer and a thermometer for monitoring the reaction internal temperature, as well as a N_2 inlet/outlet to ensure inert atmosphere during the entire course of the reaction (see Figure 1). Anhydrous 2-MeTHF (450 mL, 6 V) was transferred to the 5 L flask, followed by a solution of LiBr (0.526 mol, 45.64 g, 2.3 equiv) in anhydrous 2-MeTHF (225 mL, 4 V), which was dried via azeotropic distillation (see **MBR-M4ALL-BDQ-1** for detailed drying procedure). The solvent and LiBr solution were transferred to the reaction flask via cannula through an addition funnel (see Figure 1). Anhydrous (*R*)-2-(methoxymethyl)pyrrolidine (**7**) (39.5 g, 0.343 mol, 1.5 equiv) was similarly transferred to the reaction flask. The reaction mixture was cooled to −20/−30 °C range, and *n*-BuLi (1.8 M in hexanes, 0.297 mol, 165.0 mL, 1.3 equiv) was added dropwise (cannula/addition funnel). After 20 min, the flask was further cooled to −40 °C, and a solution of quinoline **1** (**KSM-I**) (0.228 mol, 75.0 g, 1.0 equiv, 98% purity by HPLC) in dry 2-MeTHF (300 mL, 4 V), also dried via azeotropic distillation, was transferred to the addition funnel, followed by additional 2-MeTHF (75 mL, 1 V). The quinoline **1** (**KSM-I**) solution contained in the addition funnel was added to the lithium amide base solution over 1 h (cannula/addition funnel). The resulting mixture was stirred for an additional 30 min. A solution of ketone **2** (**KSM-II**) (0.274 mol, 62.3 g, 1.2 equiv, 95% purity by HPLC) in dry 2-MeTHF (375 mL, 5 V) was added to the reaction mixture over 1 h at the same temperature (cannula/addition funnel). The reaction was stirred for an additional 45 min, and quenched by the dropwise addition of a 25% NH_4Cl aqueous solution (375 mL, 5 V) at −40 °C (cannula/addition funnel). The reaction mass was directly poured into a separatory funnel. The phases were separated, and the aqueous layer was extracted with DCM (2 x 375 mL, 10 V). The combined organic layers were dried with anhydrous Na_2SO_4 (20.0 g), filtered through a Büchner funnel, and

the Na_2SO_4 bed washed with DCM (75 mL, 1 V). The solvent was removed under reduced pressure at 45-50 °C to afford the crude product (136 g, 107% yield). Analysis of the crude material showed that the *syn*-diastereomer pair (**3**+**4, D-I**) was obtained in 82% assay yield based on HPLC wt % purity. Further analysis by chiral SFC showed there to be 64% of BDQ (**3**) in the crude mixture prior to purification. See (see **MBR-M4ALL-BDQ-1** document for step-by-step procedure).


Crude mixture assay.pdf


SFC crude mixture.pdf


HPLC crude mixture.pdf

Crude: 136.0 g	
Analytical data	
D-I	77.74
D-II	5.70
KSM-I	5.18
KSM-II	7.11
IMP-I	2.87
Assay_D-I	76.4 % w/w
SFC_BDQ	74.20 %

Assay D-I = Amount of **D-I** in the crude mixture (determined by HPLC A %)

Note: After distillation, (*R*)-2-(methoxymethyl)pyrrolidine (**7**) was stored under inert atmosphere and with activated molecular sieves (4 Å). See (see **MBR-M4ALL-BDQ-1** document for step-by-step procedure).

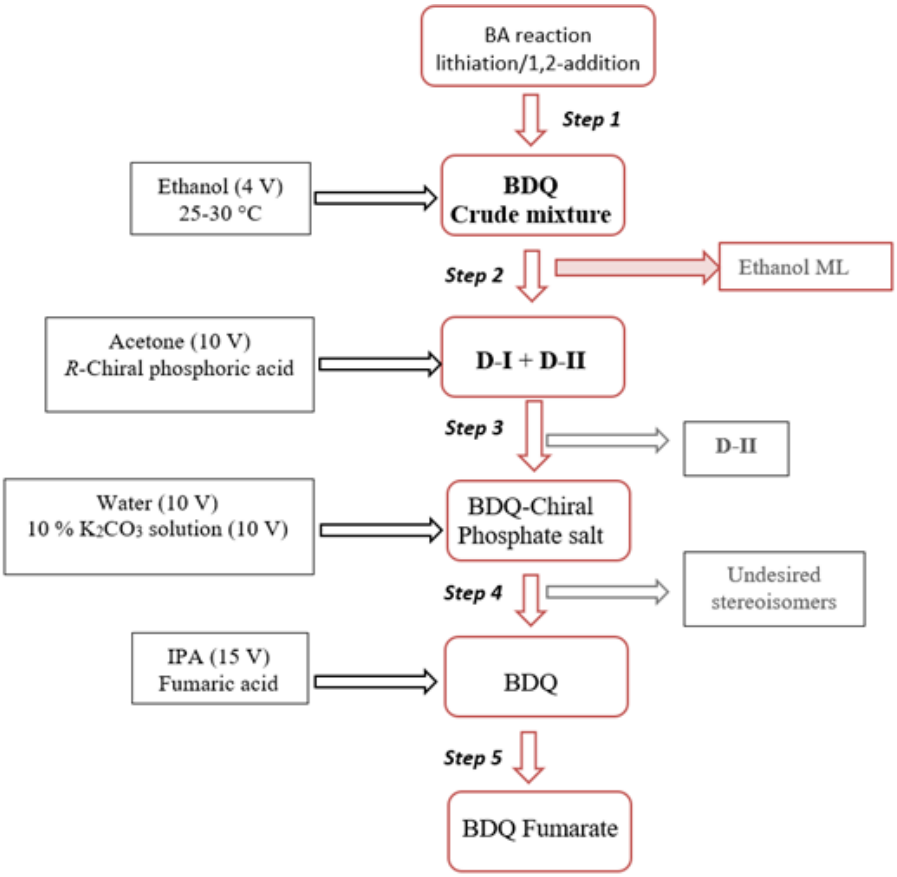
Note: After neutralization of its hydrochloride salt, compound **2** (**KMS-II**) was dried under vacuum at room temperature, and after the addition of 2-MeTHF, activated molecular sieves (4 Å) were added to the solution prior to its use in the BA reaction. See (see **MBR-M4ALL-BDQ-1** document for step-by-step procedure).

Reaction optimization

- Reaction initial optimization and information on purity profile can be found at "Application of Chiral Transfer Reagents to Improve Stereoselectivity and Yields in the Synthesis of the Anti-Tuberculosis Drug Bedaquiline". ChemRxiv link: https://chemrxiv.org/engage/chemrxiv/article-details/64b563f2ae3d1a7b0dde20cc

PURIFICATION AND ISOLATION OF BDQ (3) FUMARATE – ASYMMETRIC APPROACH

Overview of the purification process adopted for the asymmetric approach

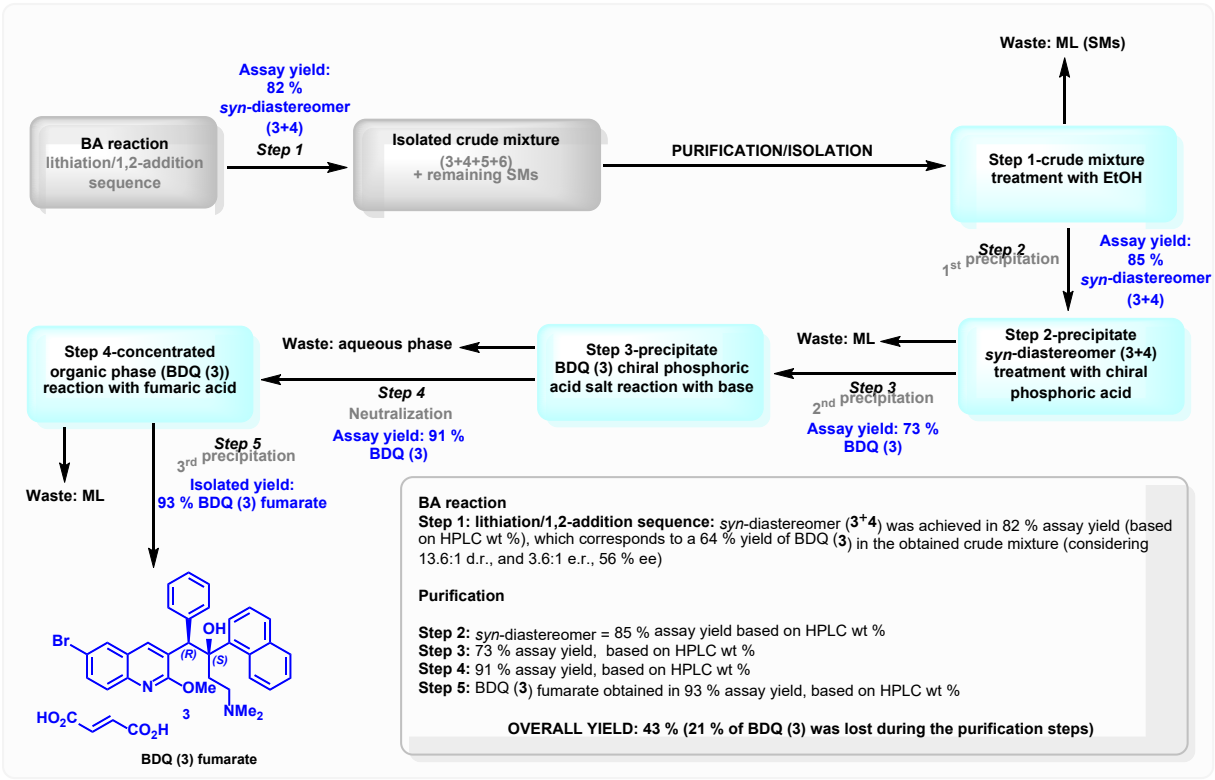


Main modifications and relevant remarks

- This purification procedure possesses one less step when compared to the racemic approach (4 steps *vs* 5 steps).
- Initial precipitation of the undesired *anti*-diastereomer (**5**+**6**) (treatment of concentrated crude reaction mixture with THF) was skipped. Since the asymmetric approach already provided a mixture with a high d.r. value favoring the desired *syn*-diastereomer (**3**+**4**), precipitation of undesired diastereomer in THF is not necessary.
- Although this possibility was not further explored by M4ALL/TCG, we believe that the chiral resolution step can be avoided, which will potentially decrease the overall cost of BDQ production. A Chinese patent filed by Fujian Institute of Microbiology claimed to be able to achieve enantiopure BDQ (**3**) without the use of the resolution step with the expensive chiral phosphoric acid (resolving agent).[14] In this case, the BA reaction was performed using LDA/chiral lithium alkoxide system, which afforded 84% ee, 5:1 d.r.

syn:*anti* (crude mixture) favoring the desired BDQ stereoisomer **3**. The authors claimed that after carrying out three crystallizations of the partially resolved material using BDQ (**3**) seed, they were able to achieve enantiopure BDQ (**3**) (100% ee). Similar results were observed during the development of this work at TCG (not further explored due to project time constrains). There are a few references available in the literature that show how, in some cases, each enantiomer possesses a different crystalline structure, which allow its selective separation during crystallizations.[15]

Results overview of asymmetric approach purification

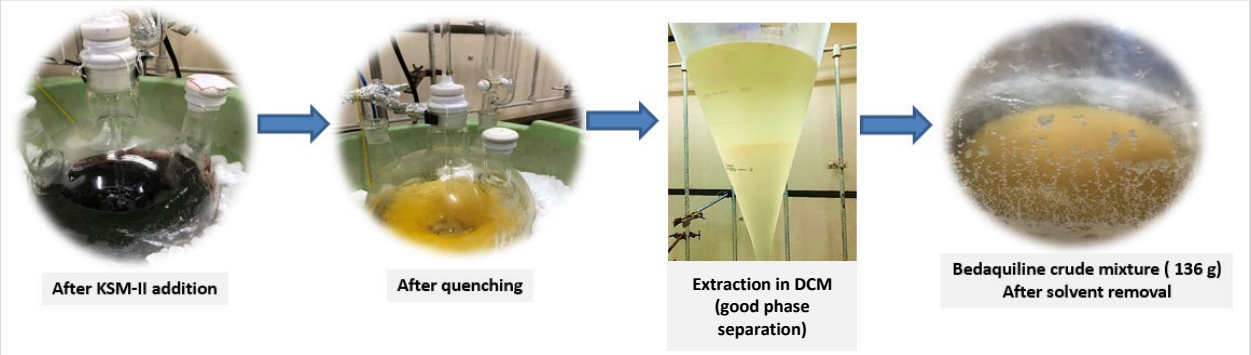


Purification steps (asymmetric approach)

See **MBR-M4ALL-BDQ-2** for complete analytical data and step-by-step procedures.

Stepwise purification (Output and yield of BDQ): 130 g of crude BDQ has been used for purification out of 136 g of crude BDQ obtained from 1,2-addition step.

Step 1: BA reaction (lithiation/1,2-addition sequence), then quench with NH_4Cl aqueous solution



Step 2: Precipitation of desired *syn*-diastereomer (**D-I**, **3**+**4**) via precipitation in EtOH.

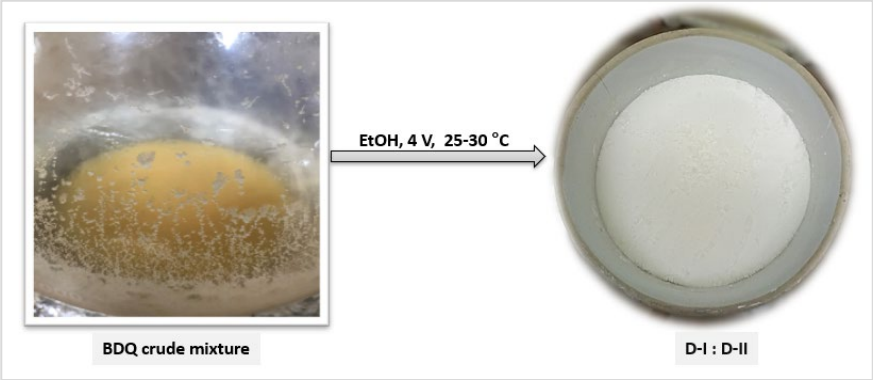
The concentrated crude mixture of BDQ obtained after BA reaction quench (130.0 g crude, 99.32 g (178.8 mmol) of **D-I** considering 76.4% purity) was taken into a round-bottom flask at 25-30 °C, EtOH (300 mL, 4 V, with respect to the 75.0 g input batch of quinoline **KSM-I**) was added, and the resulting mixture stirred for 12 h at 25-30 °C. The precipitate was filtered, washed with EtOH (1 V), and dried under vacuum at 40-45 °C to afford the desired *syn*-diastereomer (**D-I**) along with other impurities as an off-white solid (91.0 g crude, 84.4 g (151.9 mmol) of **D-I** considering 92.7% purity, 85% assay yield of **D-I**).

Mother liquor: Mass balance, **D-I**: 99.32 g (input) – 84.4 g (output) = 15 g of **D-I** remained in the mother liquor or during the operations (filtration, transferring of product to different containers, etc).

1H NMR.pdf Assay D-I.pdf HPLC D-I.pdf LCMS.pdf SFC D-I.pdf

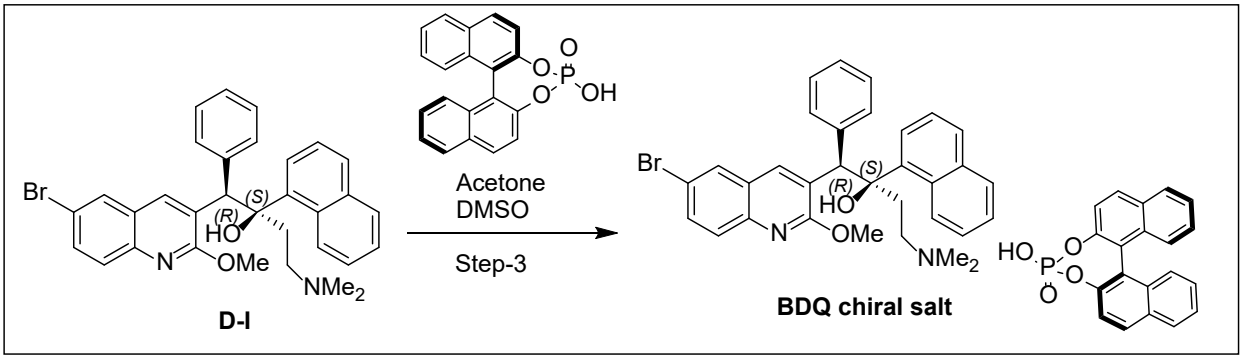
HPLC FML.pdf

SFC FML.pdf



Sl. No.	Batch ID	Input	D-I	Total Output (crude)	Step 2 – assay corrected yield
1	CR592-20218-18	130.0 g	84.4 g	91.0 g	85%

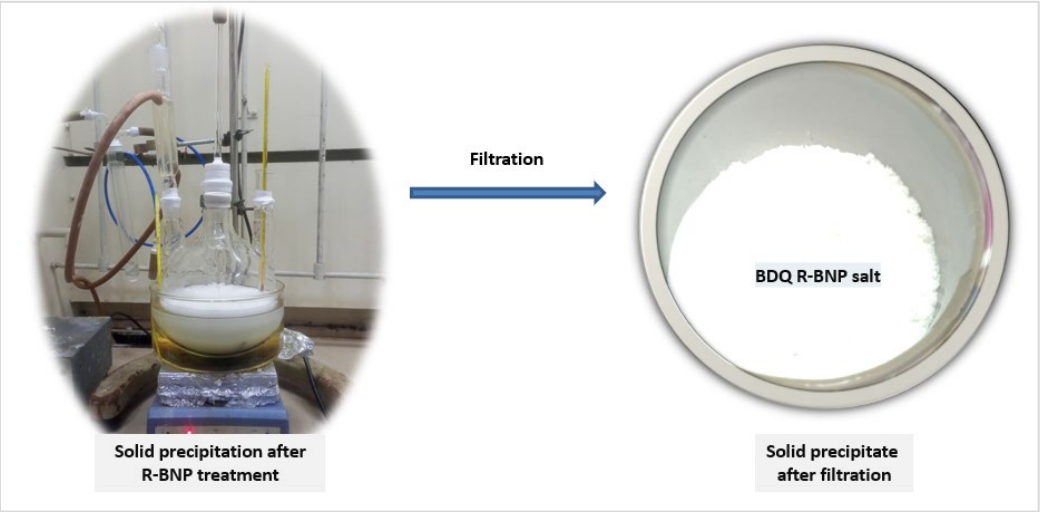
Step 3: Resolution using chiral phosphoric acid (resolving agent)



The solid obtained in the previous step (91.0 g crude (1 equiv), 84.4 g (151.9 mmol) of **D-I** based on 92.7% purity assay) was taken into a round-bottom flask, and acetone (8.5 V) was added at 25-30 °C. The resulting mixture was heated to 50-55 °C (not clear solution). A solution of (*R*)-(-)-1,1'-binaphthyl-2,2'-diyl hydrogen phosphate (63.47 g, 182.2 mmol, 1.2 equiv relative to crude mass of **D-I**) in DMSO (1.5 V) was added dropwise to the reaction flask at 50-55 °C (solution became clear at this point). Stirring was kept for 30 min at 50-55 °C, and the reaction was allowed to cool down to 25-30 °C, and maintained under these conditions for 8 h. Solid formation was observed. The obtained solid was filtered, washed with acetone (3 V), and dried under vacuum at 45-50 °C. BDQ (**3**) chiral salt was obtained as a white solid in 73% yield (95.0 g crude, 61.7 g of BDQ chiral salt (111.1 mmol) considering that 64.9% of the solid composition corresponds to BDQ (**3**)).

1H NMR salt.pdf Assay salt.pdf HPLC salt.pdf SFC salt.pdf

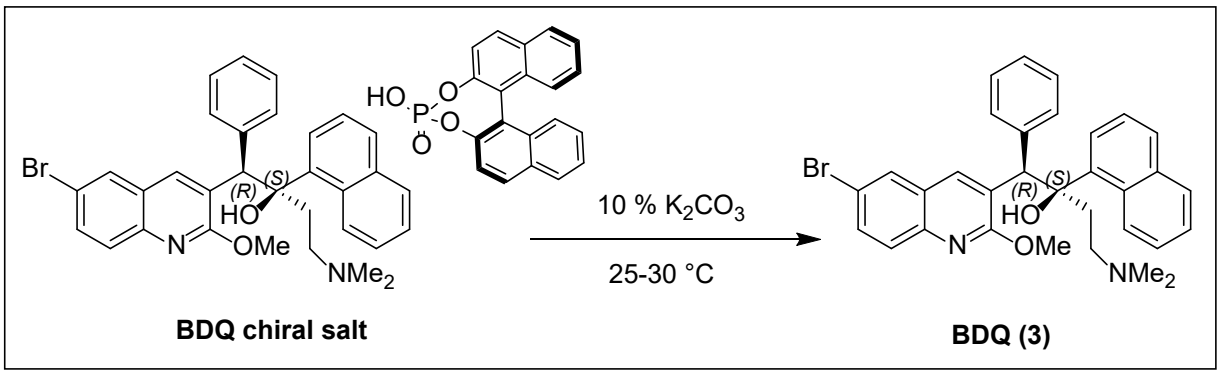
Assay ML.pdf HPLC ML.pdf SFC ML.pdf



Bedaquiline chiral phosphoric salt (BDQ-phosphoric acid salt)

Sl. No.	Batch ID	Input	Output (Including sample for analysis)	Step 3 – assay corrected yield
1	CR592-20218-19	91.0 g HPLC: **D-I** = 91.55, **D-II** = 7.06 Assay: **D-I** = 92.7, **D-II** = 6.1 SFC: **BDQ** = 71.45% **BDQ enan** = 23.15% **D-II** = 2.76 & 2.63%	95.08 g HPLC: **D-I** = 98.92, **D-II** = 0.69 Assay: **D-I** = 64.9% SFC: **BDQ** = 98.94% **BDQ enan** = 0.72% **D-II** = 0.33% & ND	73%

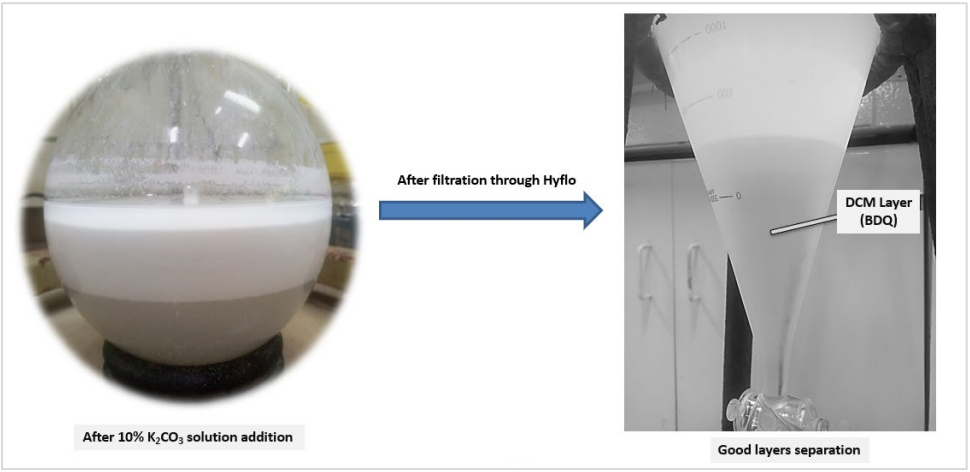
Step 4: Bedaquiline free base (BDQ-free base)



The BDQ (**3**) phosphate salt obtained in the previous step (95.0 g crude, 61.7 g (111.1 mmol) considering 64.9% of the solid composition corresponds to BDQ (**3**), 1 equiv relative to the crude mass) was taken into a round-bottom flask, and water (950 mL, 10 V) was added at 25-30 °C (heterogeneous mixture). A 10% aqueous solution of K_2CO_3 (950 mL, 10 V) was added to the reaction flask at 25-30 °C, followed by DCM (950 mL, 10 V). The resulting heterogenous mixture was stirred for 30 min at the same temperature. After this period, mixture was filtered through Celite® pad, which was washed with DCM (95 mL, 1 V), and the layers were separated. Aqueous phase was extracted with DCM (1 x 475 mL, 5 V), and the solvent removed under vacuum at 45-50 °C. BDQ (**3**) was obtained in 91% (57.0 g crude, 56.1 g (101 mmol) of BDQ (**3**) considering 98.4% purity assay).

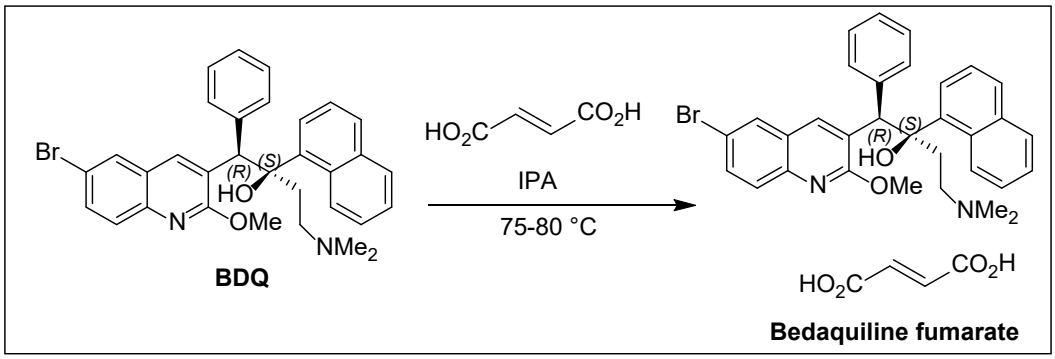
1H NMR BDQ.pdf BDQ assay.pdf BDQ HPLC.pdf BDQ SFC.pdf

Aq phase HPLC.pdf Aq phase assay.pdf



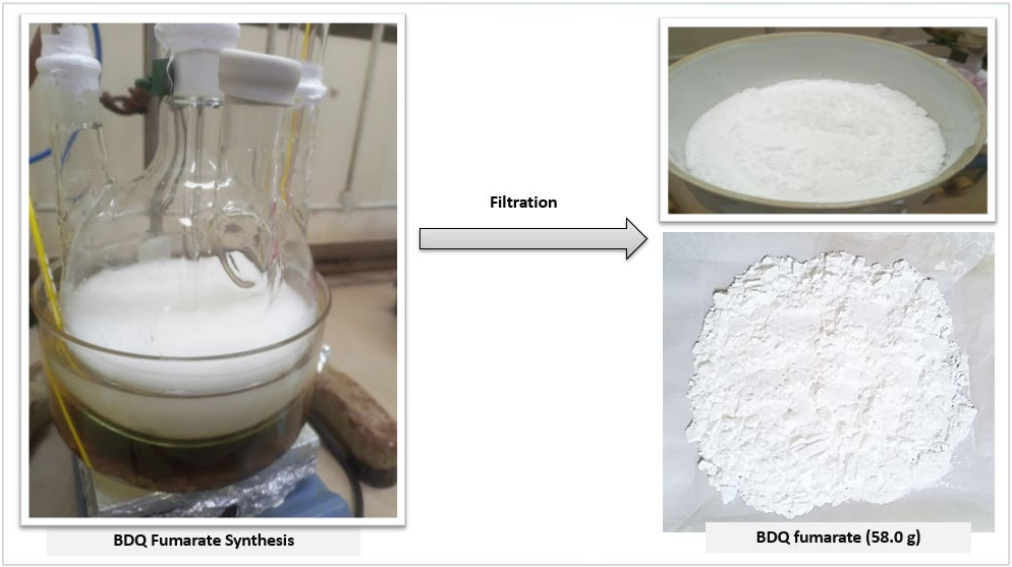
Sl. No.	Batch ID	Input (BNP Salt)	Output, Free Base (Including sample for analysis)	Step 4 – assay corrected yield
1	CR592-20218-23	95.0 g HPLC: **D-I** = 98.92, **D-II** = 0.69 Assay: **D-I** = 64.9 SFC: **BDQ** = 98.94 **BDQ enan** = 0.72 **D-II** = 0.33 & ND	57.09 g HPLC: **D-I** = 99.16, **D-II** = 0.77 Assay: **D-I** = 98.4, **D-II** = 0.8 SFC: **BDQ** = 99.48 **BDQ enan** = 0.42 **D-II** = 0.08 & ND	91%

Step 5: BDQ (3) fumarate salt formation



The solid obtained in the previous step (55.0 g crude, 54.1 g (97.4 mmol) of BDQ (**3**) considering 98.4% purity assay), 1.0 equiv relative to the crude mass) was taken into a round-bottom flask, and IPA (825 mL, 15 V) was added at 25-30 °C. Resulting mixture was heated to 50-55 °C (not clear solution). Fumaric acid (1.1 equiv) was added to the reaction flask at 50-55 °C. The reaction mixture became a clear solution, which was stirred for an additional 30 min at 75-80 °C. After this period, the solution was allowed to cool down to 25-30 °C, and maintained under these conditions for 12 h. The obtained solid was filtered, washed with IPA (2 V), and dried under vacuum at 45-50° C. BDQ (**3**) fumarate was obtained as a white solid in 93% yield (59.0 g, 50.2 g (90.3 mmol) considering that 85% of the solid composition corresponds to BDQ (**3**)).

Fumarate Assay.pdf
Fumarate 1H NMR.pdf
Fumarate HPLC.pdf
Fumarate LCMS.pdf
Fumarate SFC.pdf
ML 1H NMR.pdf
ML assay.pdf
ML HPLC.pdf
ML LCMS.pdf
ML SFC.pdf



Sl. No.	Batch Id	Input	Output (Including sample for analysis)	Step 5 – assay corrected yield
1	CR592-20218-24	57.0 g HPLC: **D-I** = **99.16, D-II = 0.77** Assay: **D-I** = 98.4, **D-II** = 0.8 SFC: **BDQ** = 99.48 **BDQ enan** = 0.42 **D-II** = 0.08 & ND	59.12 g HPLC: **BDQ** = 99.74, **D-II** = ND Assay: **BDQ** = 84.8, **D-II** = ND SFC: **BDQ** = 100.0 **BDQ enan** = ND **D-II** = ND & ND	93%

ANALYTICAL PROCEDURES

Chromatographic purity of KSM-I by HPLC (w/w %):

Chemicals / Reagent references:
Perchloric acid (70%): HPLC Grade/Equivalent
Acetonitrile: HPLC Grade/Equivalent
Purified Water: HPLC Grade/Equivalent

Chromatographic conditions:

Column	Shim-pack solar C-18 (250 x 4.6 mm, 5.0 µm) (Cat No: 227-30600-02)
Injection Volume	10.0 µL
Flow rate	1.0 mL/min
Column temperature	35 °C
Sample temperature	10 °C
Wavelength	225 nm
Run time	40 min
Diluent	Buffer:Acetonitrile (50:50)
Needle wash	Acetonitrile (100%)
Seal wash	Water: Acetonitrile (90:10 v/v)

Gradient:

Time (min)	M.P-A (%)	M.P-B (%)
0	90	10
5	90	10
20	10	90
30	10	90
31	90	10
40	90	10

Buffer preparation:
Pipette out 1ml of Perchloric acid (70%) and transfer into 1000 mL of water, sonicate to dissolve and filter through 0.45 µm filter paper.

Mobile phase preparation:

Mobile phase-A: Buffer.

Mobile Phase-B: Acetonitrile (100%).

Preparation of Blank: Buffer:Acetonitrile (50:50)

Preparation of sample solution:
Weigh accurately about 20 mg of test sample into 100 mL volumetric flask, add 50 mL of diluent, sonicate to dissolved, and dilute to volume with diluent and mix (concentration: 200 ppm).

Procedure:
Establish chromatograph by running mobile phase through the column and inject blank (diluent). After blank inject the sample solution.

Injection sequence:

S. No.	Solution details	No. of injections
1.	Blank (diluent) solution	1 (at least)
2.	Sample solution	1

Processing Procedure:
No interfering peak should be observed at the retention times of main peak in the blank.

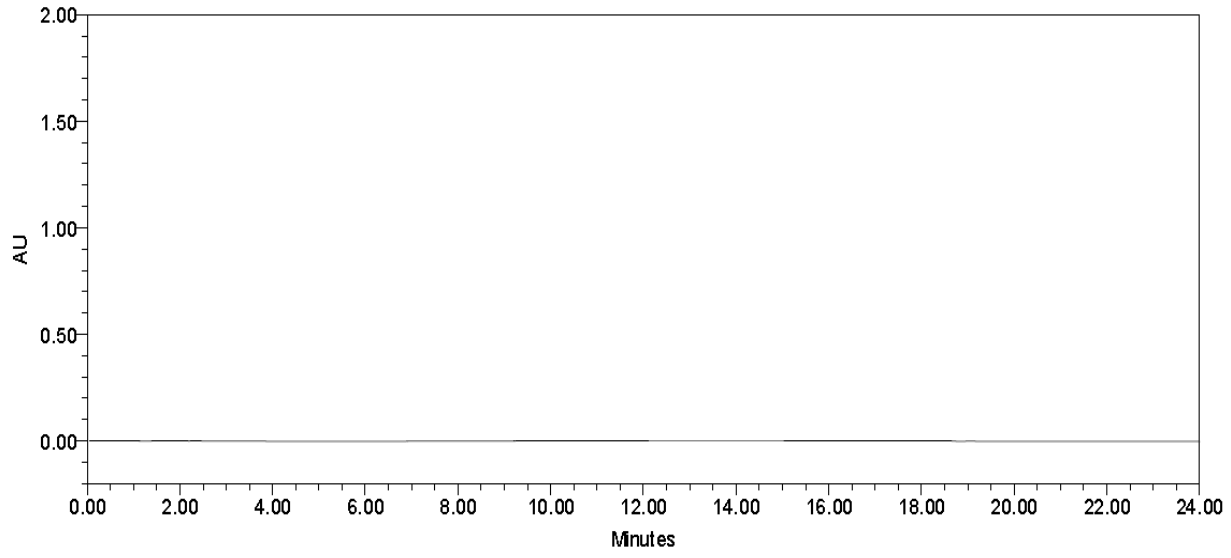
The retention time of the compound:

S. No.	Compound	Retention time (min)	RRT
1.	**KSM-I**	~27.47	1.00

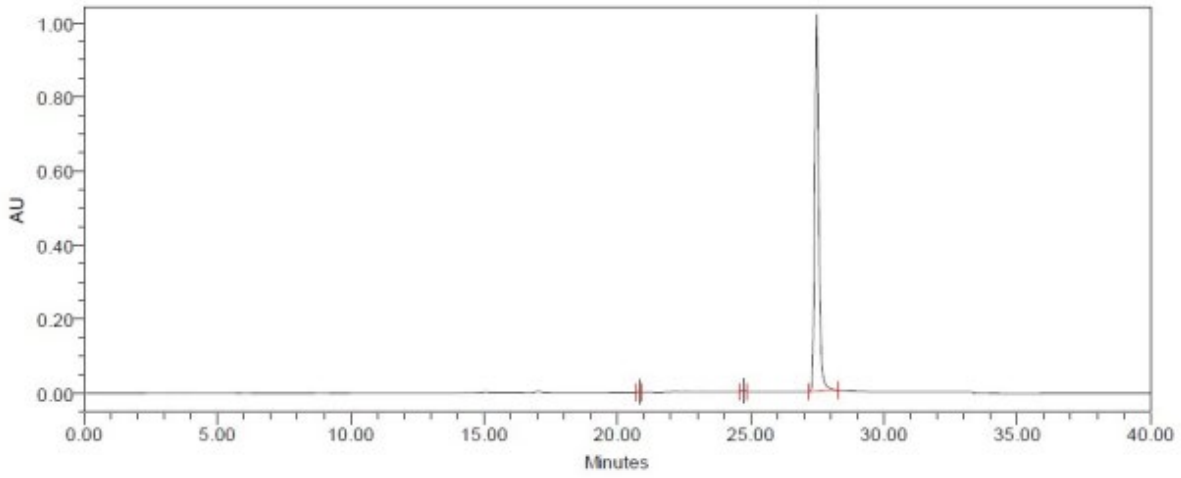
Calculation: All impurities and compound purity should be calculated as per area normalization procedure.

$$\% \text{ Purity: } \frac{\text{Area of desired peak}}{\text{Area of total peak}} \times 100$$

Blank Chromatogram:



Sample Chromatogram:



Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	20.813	5468	0.05	0.757
2	Peak2	24.733	15761	0.14	0.900
3	BDQ-KSM-I	27.477	10921141	99.81	1.000

Chromatographic purity of KSM-II by HPLC

Chemicals/Reagent references:
Perchloric acid (70%): HPLC Grade/Equivalent
Acetonitrile: HPLC Grade/Equivalent
Purified Water: HPLC Grade/Equivalent

Chromatographic conditions:

Column	Shim-pack solar C-18 (250 x 4.6 mm, 5.0 µm) (Cat No: 227-30600-02)
Injection Volume	10.0 µL
Flow rate	1.0 mL/min
Column temperature	35 °C
Sample temperature	10 °C
Wavelength	225 nm
Run time	40 min
Diluent	Buffer:Acetonitrile (50:50)
Needle wash	Acetonitrile (100%)
Seal wash	Water:Acetonitrile (90:10 v/v)

Gradient:

Time (min)	M.P-A (%)	M.P-B (%)
0	90	10
5	90	10
20	10	90
30	10	90
31	90	10
40	90	10

Buffer preparation: Pipette 1 mL of perchloric acid (70%) and transfer into 1000 mL of water, sonicate to dissolve and filter through 0.45 µm filter paper.

Mobile phase preparation:
Mobile phase-A: Buffer
Mobile phase-B: Acetonitrile (100%)
Preparation of blank: Buffer:Acetonitrile (50:50)

Preparation of sample solution: Accurately weigh about 20 mg of test sample into 100 mL volumetric flask, add 50 mL of diluent, sonicate to dissolve, and dilute to volume with diluent and mix (concentration: 200 ppm).

Procedure: Establish equilibrium by running mobile phase through the column. Inject at least 1 blank (diluent). After blank injection(s), inject the sample solution.

Injection sequence:

S. No.	Solution details	No. of injections
1.	Blank (diluent) solution	1 (at least)
2.	Sample solution	1

Processing Procedure: No interfering peak should be observed at the retention times of main peak in the blank.

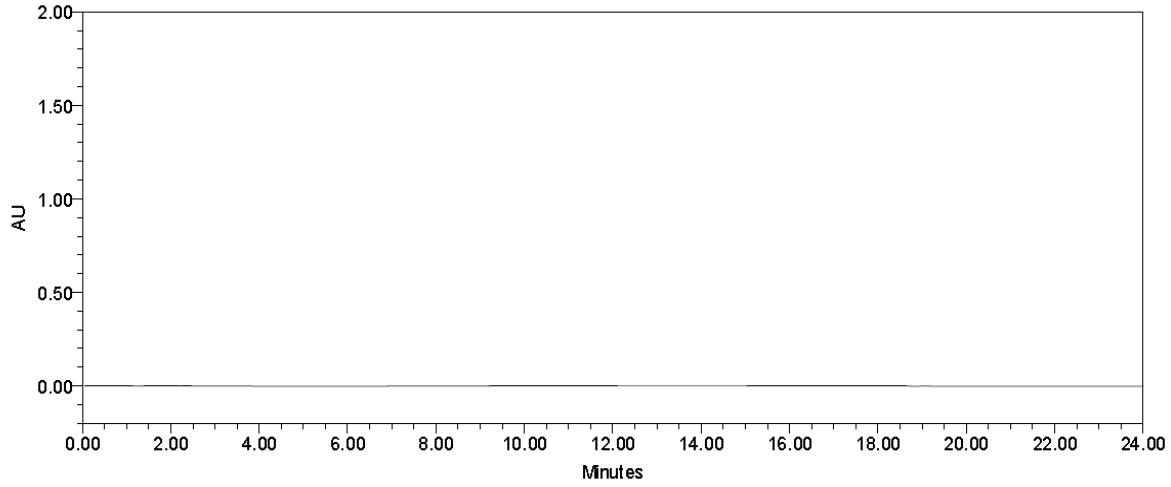
The retention time of the compound:

S. No.	Compound	Retention time (min)	RRT
1.	**KSM-II**	~14.99	1.00

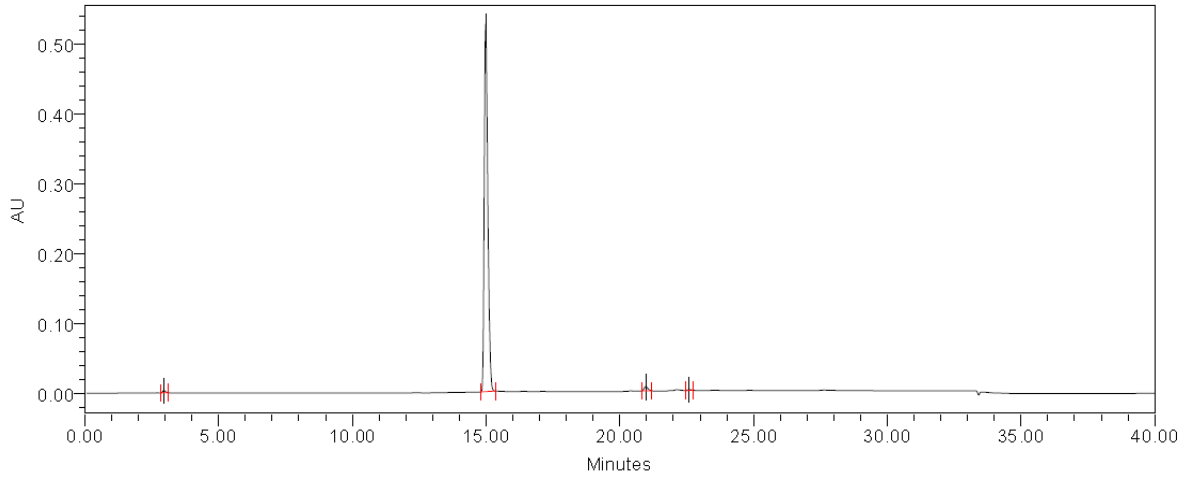
Calculation: All impurities and compound purity should be calculated as per area normalization procedure.

$$\% \text{ Purity: } \frac{\text{Area of desired peak}}{\text{Area of total peak}} \times 100$$

Blank chromatogram:



Sample chromatogram:



Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	2.960	21447	0.43	0.197
2	BDQ-KSM-II	14.988	4898385	98.19	1.000
3	Peak3	20.969	58476	1.17	1.399
4	Peak4	22.593	10242	0.21	1.507

Chiral purity by HPLC

Chemicals/Reagent references:
n-Hexane: HPLC Grade/Equivalent
Ethanol: HPLC Grade/Equivalent
Dichloromethane: HPLC Grade/Equivalent

Chromatographic conditions:

Column	Chiral Art Cellulose SC (250 x 4.6 mm, 5.0 µm) (Cat No: KSC99S05-2546 WT)
Injection Volume	5.0 µL
Flow rate	0.6 mL/min
Column temperature	40 °C
Sample temperature	25 °C
Wavelength	230 nm
Run time	45 min
Diluent	Dichloromethane (DCM)
Needle wash	Dichloromethane (DCM)
Seal wash	Dichloromethane (DCM)

Mobile phase preparation: Accurately measure individually 900 mL *n*-hexane, 50 mL ethanol, and 50 mL dichloromethane into a 1000 mL glass bottle, add 1 mL of isopropyl amine, and shake well. Briefly sonicate to mix and degas the solution.

Preparation of Blank: Dichloromethane

Preparation of racemate solution: Accurately weigh about 100 mg of racemate standard into 100 mL round bottom flask, add 10 mL of dichloromethane, 5 mL of triethylamine, and 150 mg of tosyl chloride. Stir for 2 hours at room temperature. After this period, add 5 mL of water and stir for 15 min. Transfer the lower layer (DCM layer) into a 20 mL volumetric flask and add anhydrous Na_2SO_4 (100 mg). Shake the flask well so the salt absorbs any water. Filter the DCM layer into another 20 mL volumetric flask. This is the sample solution. Dilute the sample solution up to 20 mL with DCM and inject. [Concentration: Sample solution:DCM (1:1).

Preparation of sample solution: Accurately weigh about 100 mg of test sample into 100 mL round-bottom flask, add 10 mL of dichloromethane, 5 mL of triethyamine, and 150 mg of tosyl chloride. Stir for 2 hours at room temperature. After this period, add 5 mL of water and stir for 15 min. Transfer the lower layer (DCM layer) into a 20 mL volumetric flask and add anhydrous Na_2SO_4 (100 mg). Shake the flask well so the salt absorbs any water. Filter the DCM layer into

another 20 mL volumetric flask. This is the sample solution. Dilute the sample solution up to 20 mL with DCM and inject (concentration: Sample solution:DCM (1:1)).

SYSTEM SUITABILITY:

The tailing factor of racemate solution should not be more than 2.0
The theoretical plates should not be less than 5000
The resolution between two peaks should not be less than 1.5

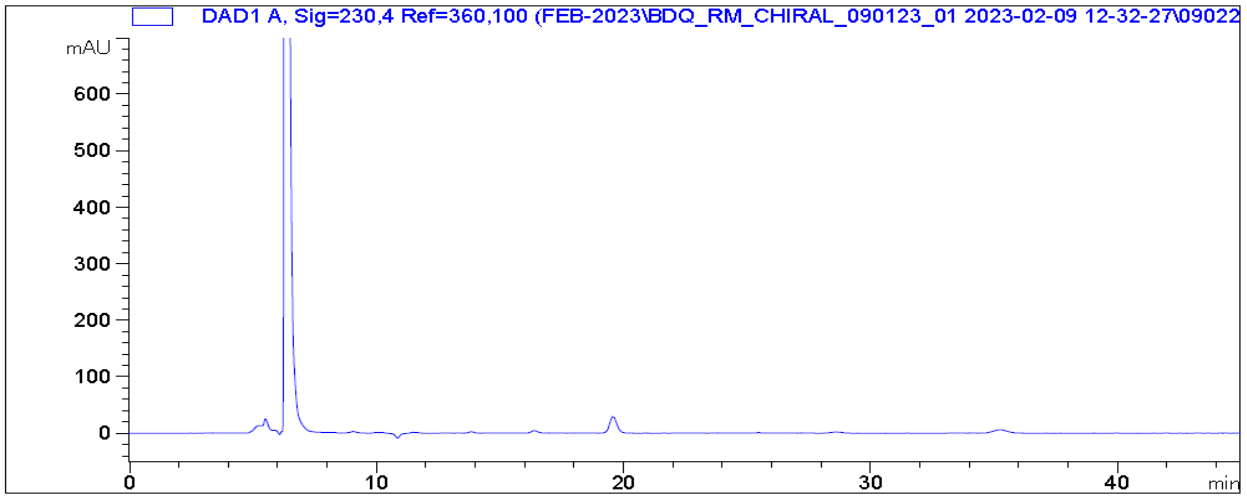
Procedure: Establish equilibration by running mobile phase through the column Inject at least 1 blank (diluent). After blank injection(s), inject the racemate and sample solution.

Injection sequence:

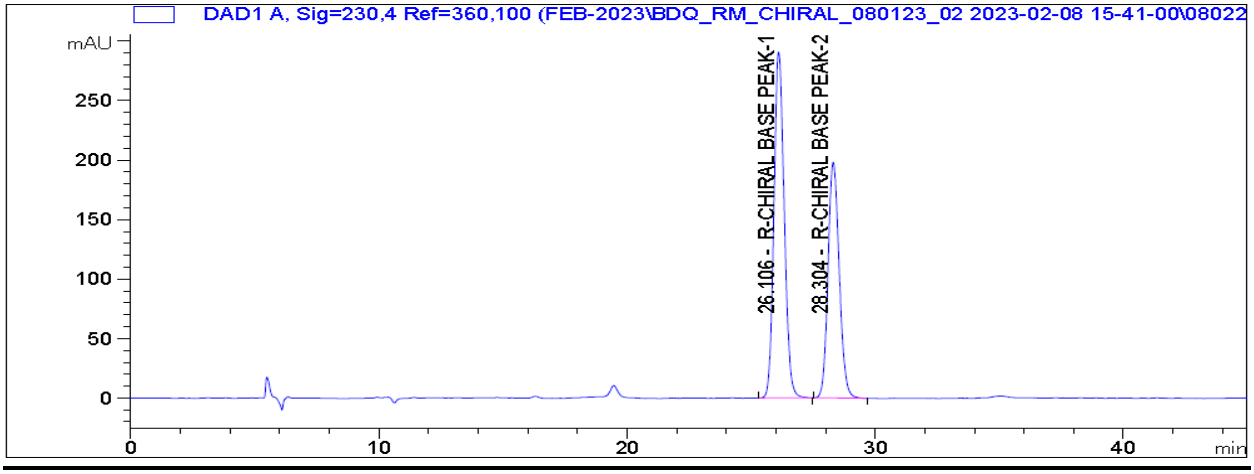
S. No.	Solution details	No. of injections
1.	Blank (diluent) solution	1 (at least)
2.	Racemate solution	1
3.	Sample solution	1

Processing Procedure: No interfering peak should be observed at the retention times of main peak in the blank.

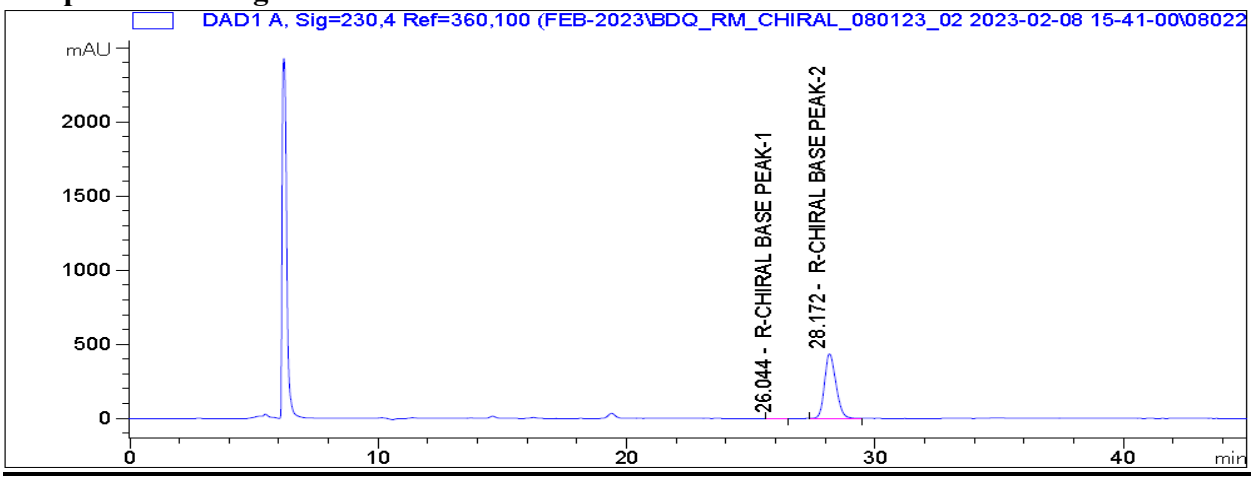
Blank chromatogram:



Racemate chromatogram:



Sample Chromatogram:



Water content by Karl Fischer (KF) (w/v %)

Take about 40 mL methanol in titration vessel of Karl Fischer Titrator. Pre-titrate with Karl Fischer titrant. Weigh about 1.0 gm of the sample and transfer immediately into the titration vessel and stir it for about 1 min and then initiate titration with Karl Fischer titrant. Record the volume of Karl Fischer reagent consumed and calculate the water content of sample as given below. Report the value to one decimal point.

$$\text{Water content of sample (\% w/v)} = \frac{\text{KFT Factor (mg/mL) X Titer Value (mL) X 100\%}}{\text{Weight of sample (gm) X 1000}}$$

Assay purity of BDQ crude by HPLC (% w/w)

Chemicals / Reagent references:
Perchloric acid (70%): HPLC Grade/Equivalent
Acetonitrile: HPLC Grade/Equivalent
Purified Water: HPLC Grade/Equivalent

Chromatographic conditions:

Column	Shim-pack solar C-18 (250 x 4.6 mm, 5.0 µm) (Cat No: 227-30600-02)
Injection Volume	10.0 µL
Flow rate	1.0 mL/min
Column temperature	35 °C
Sample temperature	10 °C
Wavelength	225 nm
Run time	40 min
Diluent	Buffer:Acetonitrile (50:50)
Needle wash	Acetonitrile (100%)
Seal wash	Water: Acetonitrile (90:10 v/v)

Gradient:

Time (min)	M.P-A (%)	M.P-B (%)
0	30	70
3	30	70
10	5	95
15	5	95
16	30	70
24	30	70

Buffer preparation: Pipette 1 mL of perchloric acid (70%) and transfer into 1000 mL of water, sonicate to dissolve and filter through 0.45 µm filter paper.

Mobile phase preparation:
Mobile phase-A: Buffer
Mobile phase-B: Acetonitrile (100%)
Preparation of blank: Buffer:Acetonitrile (50:50)

Preparation of standard solution: Accurately weigh about 10 mg of **D-I** and **D-II** standard individually into a 100 mL volumetric flask, add about 50 mL of diluent, sonicate to dissolve, dilute to volume with diluent and mix well (concentration:100 ppm).

Preparation of sample solution: Accurately weigh about 10 mg of test sample into 100 mL volumetric flask, add 50 mL of diluent, sonicate to dissolve, and dilute to volume with diluent and mix (concentration:100 ppm).

Procedure: Establish equilibrium by running mobile phase through the column and inject blank (diluent) followed by standard solution into the chromatograph to evaluate the system suitability criteria. If the system suitability criteria passes, then inject sample solution.

Injection sequence:

S. No.	Solution details	No. of injections
1.	Blank (diluent) solution	1 (at least)
2.	Standard solution	5
3.	Sample solution	1
4.	System suitability solution-BKT	1

System suitability test:

1. No interfering peak should be observed at the retention times of main peak in the blank.
2. From the standard solution, the USP tailing factor for peak should not be more than 2.0 and USP theoretical plates should not be less than 8000% RSD of area of five injection of standard chromatogram should not be more than 2.0%.
3. For standard bracketing also need to meet the same system suitability criteria.

The retention time of the compound:

S. No.	Compound	Retention time (min)	RRT
1.	BDQ Desired	~5.67	1.00
2.	BDQ Undesired	~5.26	0.93

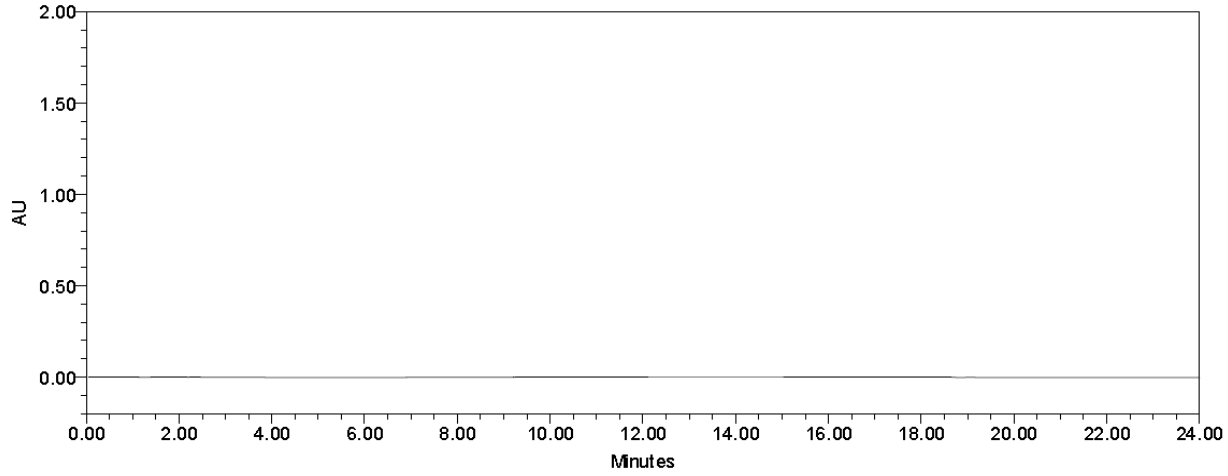
CALCULATION:

$$\text{Assay purity (\% w/w)} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{100}{WT} \times P$$

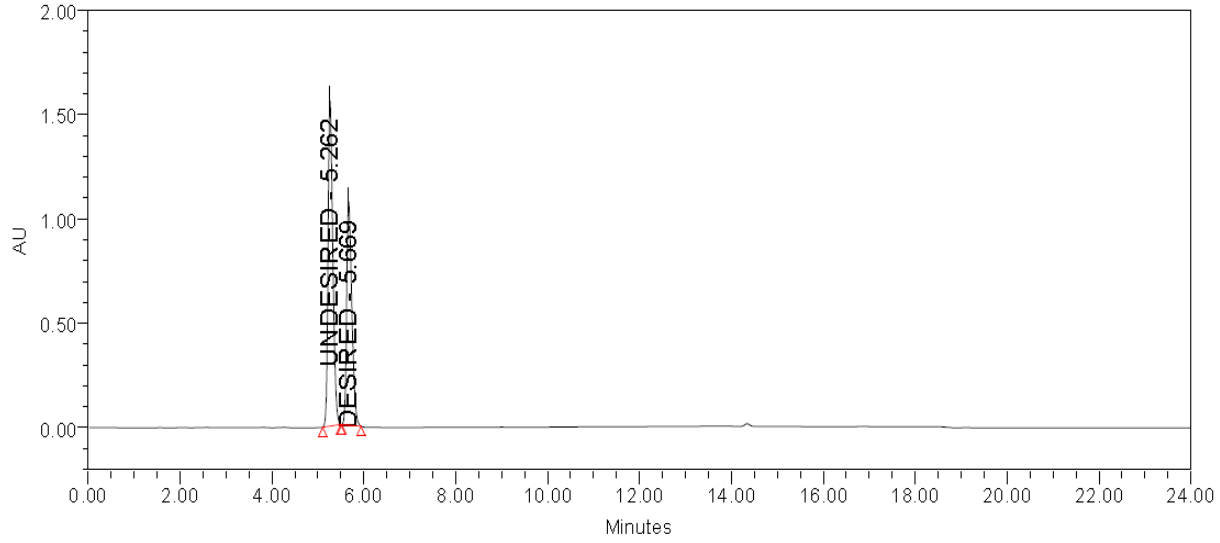
Where:

AT = Area of peak in the chromatogram obtained with test preparation
AS = Average area of peak due to five injections of standard preparation
WS = Weight of standard taken for standard preparation in mg
WT = Weight of test sample taken in mg
P = purity of standard

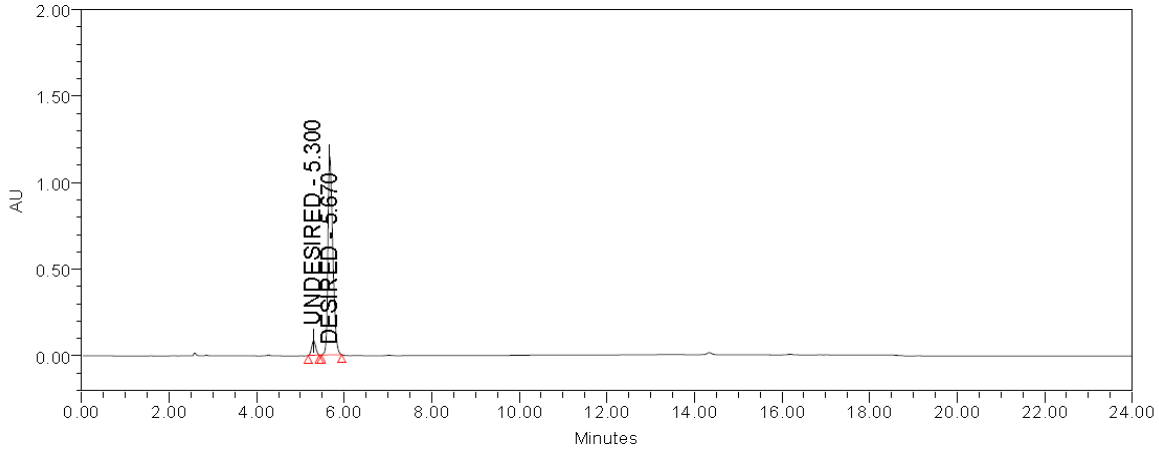
Blank chromatogram:



Standard chromatogram:



Sample Chromatogram:



Chromatographic purity of BDQ crude by HPLC (LCAP)

Chemicals / Reagent references:
Perchloric acid (70%): HPLC Grade/Equivalent
Acetonitrile: HPLC Grade/Equivalent
Purified Water: HPLC Grade/Equivalent

Chromatographic conditions:

Column	Shim-pack solar C-18 (250 x 4.6 mm, 5.0 µm) (Cat No: 227-30600-02)
Injection Volume	10.0 µL
Flow rate	1.0 mL/min
Column temperature	35 °C
Sample temperature	10 °C
Wavelength	225 nm
Run time	40 min
Diluent	Buffer:Acetonitrile (50:50)
Needle wash	Acetonitrile (100%)
Seal wash	Water:Acetonitrile (90:10 v/v)

Gradient:

Time (min)	M.P-A (%)	M.P-B (%)
0	90	10
5	90	10
20	10	90
30	10	90
31	90	10
40	90	10

Buffer preparation: Pipette 1 mL of Perchloric acid (70%) and transfer into 1000 mL of water, sonicate to dissolve and filter through 0.45 µm filter paper.

Mobile phase preparation:
Mobile phase-A: Buffer
Mobile phase-B: Acetonitrile (100%)
Preparation of blank: Buffer:Acetonitrile (50:50)

Preparation of sample solution: Accurately weigh about 25 mg of test sample into 100 mL volumetric flask, add 50 mL of diluent, sonicate to dissolve, and dilute to volume with diluent and mix (concentration: 250 ppm).

Procedure: Establish equilibrium by running mobile phase through the column. Inject at least 1 blank (diluent). After blank injection(s), inject the sample solution.

Injection sequence:

S. No.	Solution details	No. of injections
1.	Blank (diluent) solution	1 (at least)
2.	Sample solution	1

Processing Procedure: No interfering peak should be observed at the retention times of main peak in the blank.

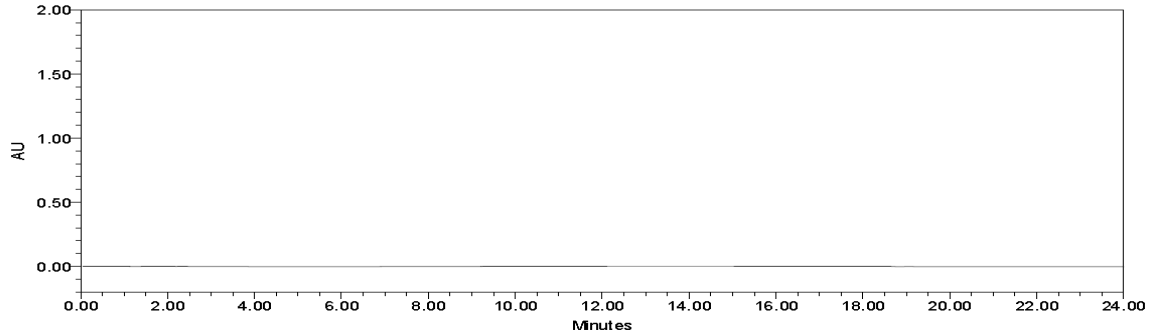
The retention time of the compound:

S. No.	Compound	Retention time (min)	RRT
1.	BDQ Desired	~20.87	1.00
2.	BDQ Undesired	~20.65	0.99
3.	**KSM-I**	~27.73	1.33
4.	**KSM-II**	~15.32	0.73

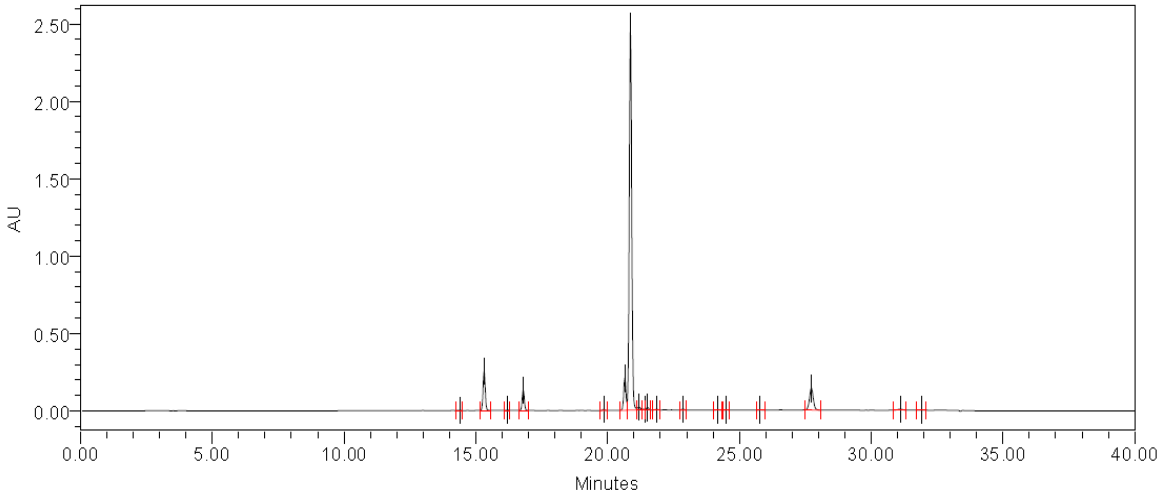
Calculation: All impurities and compound purity should be calculated as per area normalization procedure.

$$\% \text{ Purity: } \frac{\text{Area of desired peak}}{\text{Area of total peak}} \text{ X } 100$$

Blank chromatogram:



Sample chromatogram:



Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	14.396	11570	0.05	0.690
2	KSM-II	15.315	1471347	6.84	0.734
3	Peak3	16.203	13673	0.06	0.776
4	Peak4	16.800	764167	3.55	0.805
5	Peak5	19.851	36474	0.17	0.951
6	UNDESIRED COMP	20.653	1228708	5.71	0.990
7	DESIRED COMP	20.868	16030794	74.52	1.000
8	Peak8	21.192	165722	0.77	1.016
9	Peak9	21.433	30848	0.14	1.027
10	Peak10	21.516	104119	0.48	1.031
11	Peak11	21.863	30745	0.14	1.048
12	Peak12	22.857	40549	0.19	1.095
13	Peak13	24.178	26251	0.12	1.159
14	Peak14	24.501	18408	0.09	1.174
15	Peak15	25.793	18769	0.09	1.236
16	KSM-I	27.731	1412241	6.57	1.329
17	Peak17	31.096	86298	0.40	1.490
18	Peak18	31.901	20420	0.09	1.529

Optical rotation of (*R*)-2-(methoxymethyl)pyrrolidine (7) (*R*-Chiral amine) by Specific Optical Rotation (SOR)

Specific optical rotation at 25 °C: Accurately weigh and transfer about 1.0 g of sample in a 100 mL volumetric flask. Add about 90 mL of chloroform and shake to dissolve completely and then dilute to volume with chloroform and mix well. Measure the optical rotation at 25 ºC and 589 nm wavelength. Perform the blank rotation of chloroform at the same temperature and wavelength. Calculate the specific optical rotation using the following formula.

Specific optical rotation $[\alpha]^{20}_{D} = (\alpha \times V \times 100)/d \times W$

Where:

α = angle of rotation of the sample in (º)

d = Length of the polarimeter cell in decimeters

V= Volume of the volumetric flask used for sample solution

W = Weight of the sample in grams taken for sample solution

Chromatographic purity by Gas Chromatography Flame Ionization Detection (GC-FID)

Chemicals/Reagent references:
Acetonitrile: GC Grade/Equivalent

Chromatographic conditions:

Column	DB-1301 (30 m x 0.32 mm, 1.0 µm) (Part No: 123-1333)
Run time	20 min
Injection temperature	260 °C
Detector temperature	280 °C
Volume of injection	1.0 µL
Carrier gas	Helium
Mode	Split
Split ratio	10:1
Control mode	Constant flow
Column flow	5.0 mL/min
Hydrogen flow	40 mL/min
Air flow	400 mL/min
Make up gas	Helium
Make up flow	25 mL/min
Liner	Ultra inert inlet liners (5190-2295, Agilent)

Oven program:

Rate (°C/min)	Value (°C)	Hold time (min)
	50 °C	2.0
20 °C/min	260 °C	7.5

Diluent: Acetonitrile

Preparation of sample solution: Accurately weigh about 50 mg of sample and transfer in a 10 mL volumetric flask. Add about 5 mL of diluent. Sonicate for about 2 min. Dilute to volume with acetonitrile and mix well (concentration: *R*-Chiral amine - 5000 ppm).

Procedure: After equilibrating the column, separately inject diluent as blank and sample solutions as per the sequence given below.

Injection sequence:

S. No.	Solution details	No. of injections
1.	Blank (diluent) solution	1 (at least)
2.	Sample solution	1

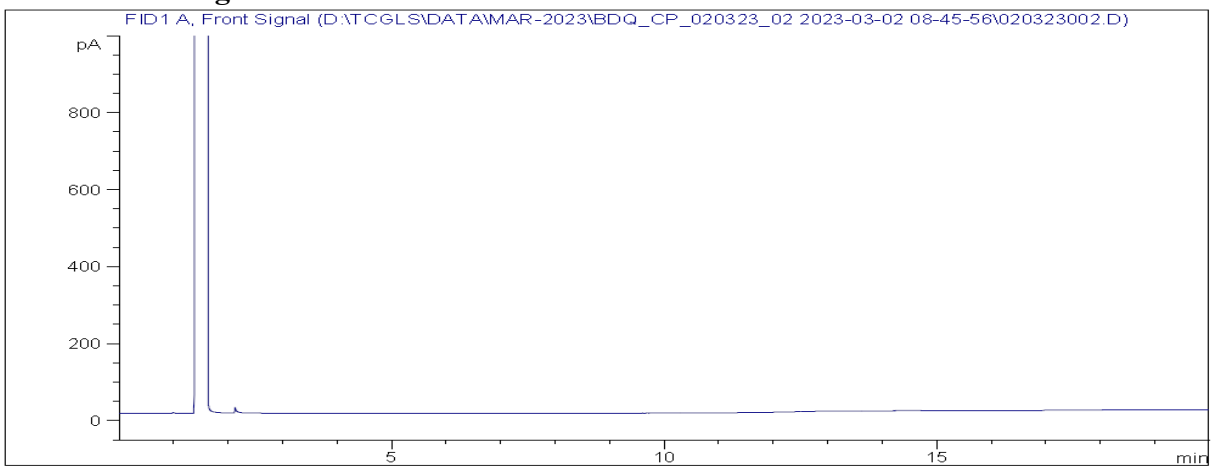
System suitability test: No interfering peak should be observed at the retention times of *R*-Chiral amine peak in the blank.

The retention times, relative retention times (RRT) of the compounds:

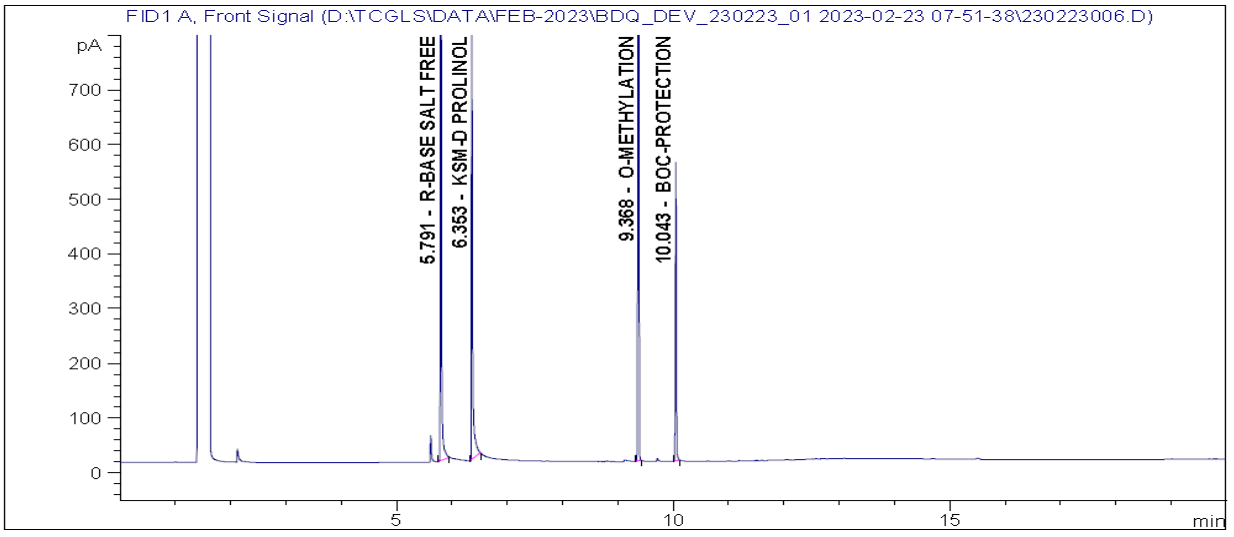
S. No.	Compound	Retention time (min)	RRT
1	*R*-Chiral amine	~5.79	1.00
2	D-Prolinol	~6.35	1.10
3	*O*-Methylation	~9.37	1.62
4	Boc-Protection	~10.04	1.73

Reporting: Disregard the peaks due to blank, and below 0.05% peak area. Report the single maximum unknown impurity and total impurities by % area normalization to two decimal points if less than 1.0% and one decimal point if equal to or greater than 1.0% from the sample solution.

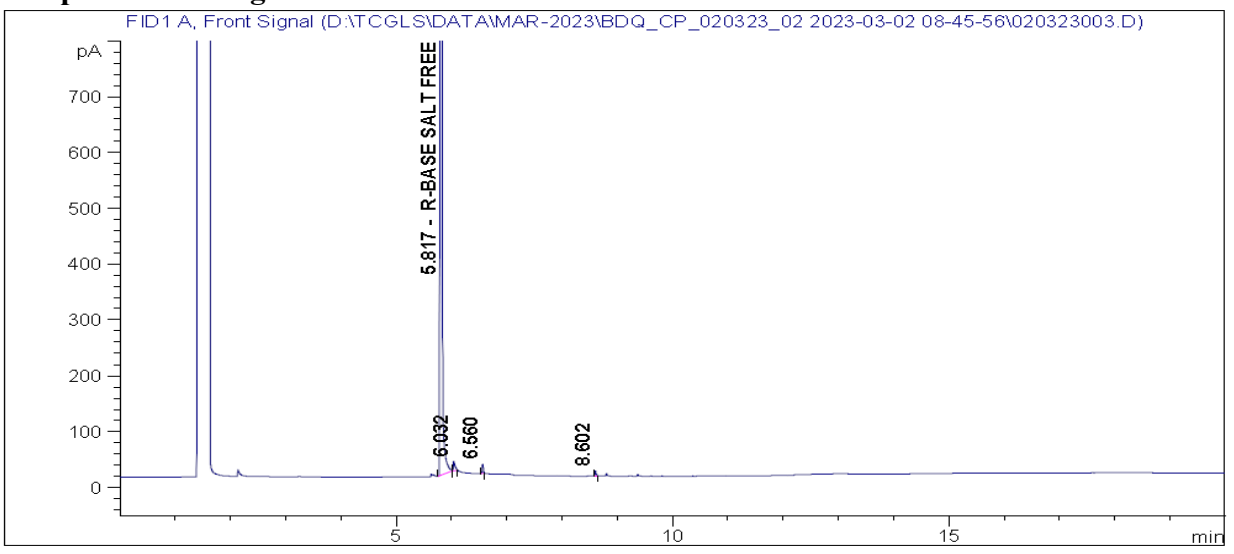
Blank Chromatogram:



Spiked Chromatogram:



Sample Chromatogram:



Assay purity by GC-FID

Chemicals / Reagent references:
Dichloromethane: GC Grade /Equivalent
Toluene: GC Grade /Equivalent

Chromatographic conditions:

Column	RTX-5 Amine (30 m x 0.53 mm, 3.0 µm) (Part No: 12385)
Runtime	20 min
Injection temperature	240 °C
Detector temperature	280 °C
Volume of injection	0.2 µL
Carrier gas	Helium
Mode	Split
Split ratio	10:1
Control mode	Constant flow
Column flow	5.0 mL/min
Hydrogen flow	40 mL/min
Air flow	400 mL/min
Make up gas	Helium
Make up flow	25 mL/min
Liner	Ultra inert inlet liners (5190-2295, Agilent)

Oven program:

Rate (°C/min)	Value (°C)	Hold time (min)
	60 °C	1.0
20 °C/min	260 °C	5.0

Diluent: Dichloromethane (DCM)

Preparation of toluene solution: Accurately weigh about 500 mg of toluene and transfer in a 100 mL volumetric flask. Add about 50 mL of diluent. Sonicate for about 2 min. Dilute to volume with DCM and mix well (concentration: Toluene - 5000 ppm).

Preparation of standard solution: Accurately weigh about 40 mg of standard and transfer in a 10 mL volumetric flask. Add about 1 mL of toluene solution and 5 mL of diluent. Sonicate for about 2 min. Dilute to volume with diluent and mix well (concentration: *R*-Chiral amine - 4000 ppm & toluene - 500 ppm).

Preparation of sample solution: Accurately weigh about 40 mg of sample and transfer in a 10 mL volumetric flask. Add about 1 mL of toluene solution and 5 mL of diluent. Sonicate for about

2 min. Dilute to volume with diluent and mix well (concentration: *R*-Chiral amine - 4000 ppm & Toluene - 500 ppm).

Injection sequence:

S. No.	Solution details	No. of injections
1.	Blank (diluent) solution	1 (at least)
2.	Standard solution	5
3.	Sample solution	1
4.	System suitability solution-BKT	1

System suitability test:

1. No interfering peak should be observed at the retention times of the main peak in the blank.
2. The % RSD of the area of five injections of standard chromatogram should not be less than 15.0%.
3. For standard bracketing also need to meet the exact system suitability criteria.

The retention time of the compound:

S. No.	Compound	Retention time (min)	RRT
1.	Toluene	~6.24	0.65
2.	*R*-Chiral amine	~9.61	1.00

CALCULATION:

$$\text{Assay Purity (\% w/w)} = \frac{AT}{AS} \times \frac{WS}{10} \times \frac{10}{WT} \times P$$

Where:

AT = Area ratio of toluene and *R*-Chiral amine peaks in the chromatogram obtained with test preparation

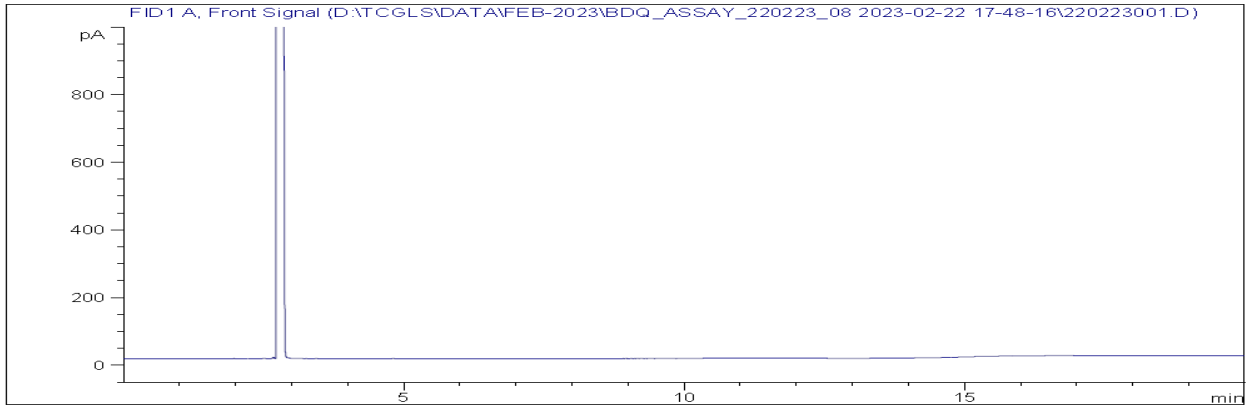
AS = Average area ratio of toluene and *R*-Chiral amine peaks from five injections of standard preparation

WS = Weight of standard taken for standard preparation in mg

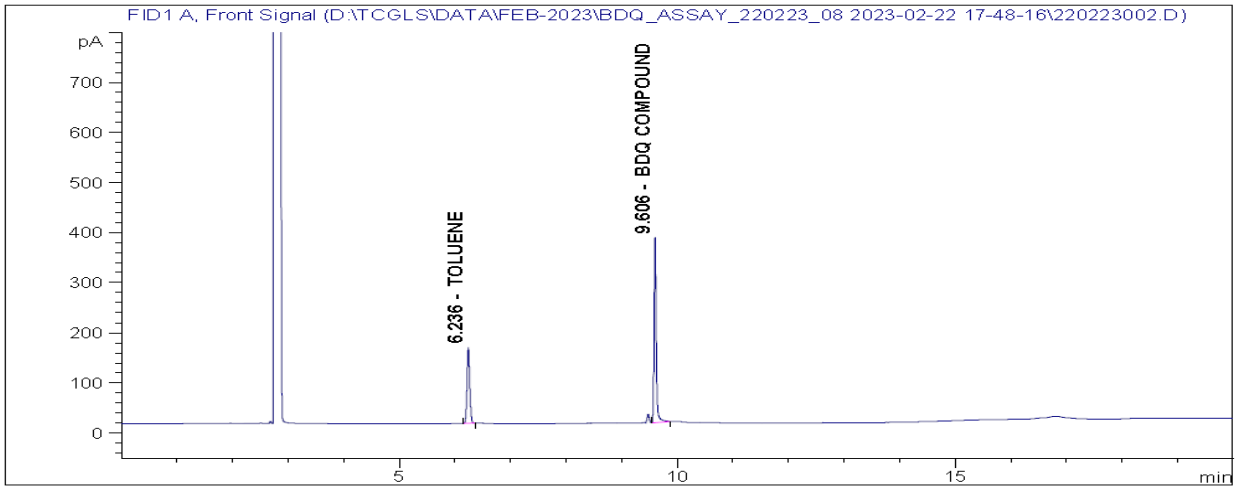
WT = Weight of test sample in mg

P = purity of standard

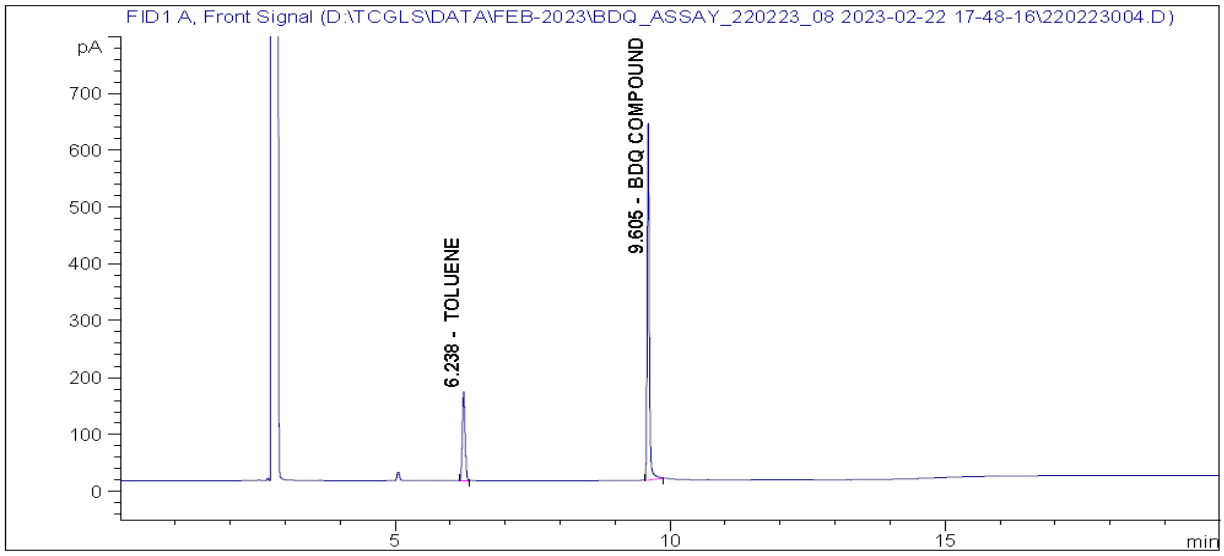
Blank Chromatogram:



Standard Chromatogram:



Sample Chromatogram:



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