TCG Lifesciences | CHEMBIOTEK

TECHNICAL PACKAGE FOR BEDAQUILINE (BDQ) (MBR-M4ALL-BDQ-1)

Prepared By	Checked By	Reviewed By
Appana Ramakrishna	Sujoy Karmakar	Angshuman Ghosh
Designation: Research Scientist	Designation: Lead Scientist	Designation: Group Lead

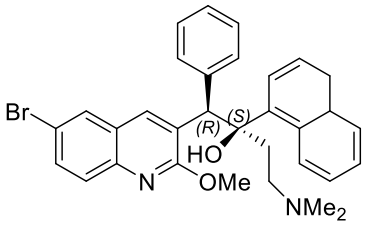
TCG Lifesciences | CHEMBIOTEK

Contents

INTRODUCTION

Bedaquiline, a diarylquinoline (DARQs), inhibits mycobacterial adenosine triphosphate (ATP) synthase and is licensed for use in the treatment of drug-resistant tuberculosis.

The description of Bedaquiline is shown below:

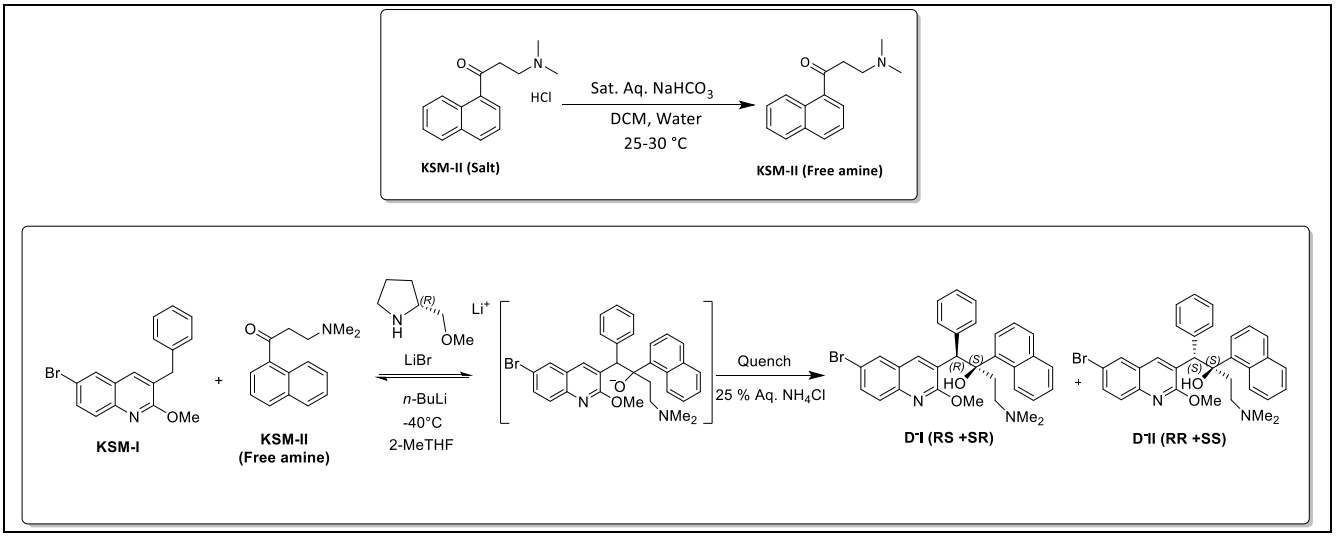
Project Code	CR592
MBR Number:	MBR-M4ALL-BDQ-1
Product Name:	Bedaquiline
IUPAC Name	(1*R*,2*S*)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-2-(naphthalen-1-yl)-1-phenylbutan-2-ol
CAS Number	843663-66-1
Chemical Formula	$C_{32}H_{31}BrN_2O_2$
Molecular Weight	555.52
Structure	
Physical Appearance	Crystalline white powder
Melting Point	118 °C
Solubility	Acetone, Tetrahydrofuran
Insoluble	Water
Stereoisomers	i) (1*S*,2*R*)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-2-(naphthalen-1-yl)-1-phenylbutan-2-ol ii) (1*R*,2*R*)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-2-(naphthalen-1-yl)-1-phenylbutan-2-ol iii) (1*S*,2*S*)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-2-(naphthalen-1-yl)-1-phenylbutan-2-ol
Polymorphic Details	Not available

OBJETIVE AND SCOPE

The objective of this development report is to create complete scientific information to support the process development of (1*R*,2*S*)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-2-(naphthalen-1-yl)-1-phenylbutan-2-ol (BDQ). This batch process record (BPR) document covers the first step in the process to make pure bedaquiline, namely the lithiation and 1,2-addition reaction, including the quench and isolation of the crude isomer mixture. The chiral resolution and subsequent purification steps will be handled in separate BPR documents.

SYNTHESIS OF BEDAQUILINE

Synthetic Approach BDQ



List of Raw materials used for the synthesis of crude BDQ

Stage-1:

i. **KSM-I**
ii. **KSM-II** ("HCl Salt")
iii. LiBr (Anhydrous)
iv. (*R*)-2-(Methoxymethyl)pyrrolidine ("*R*-Chiral amine", Anhydrous)
v. *n*-Butyllithium 2.5 M in hexane
vi. Ammonium chloride (NH_4Cl)
vii. Anhydrous Magnesium sulfate ($MgSO_4$)
viii. Sodium bicarbonate ($NaHCO_3$)
ix. 2-Methyltetrahydrofuran (2-MeTHF, Anhydrous)
x. Dichloromethane (DCM)
xi. Process water (Tap)
xii. Sodium chloride

List of Raw Material and Certificate of Analysis (COA)

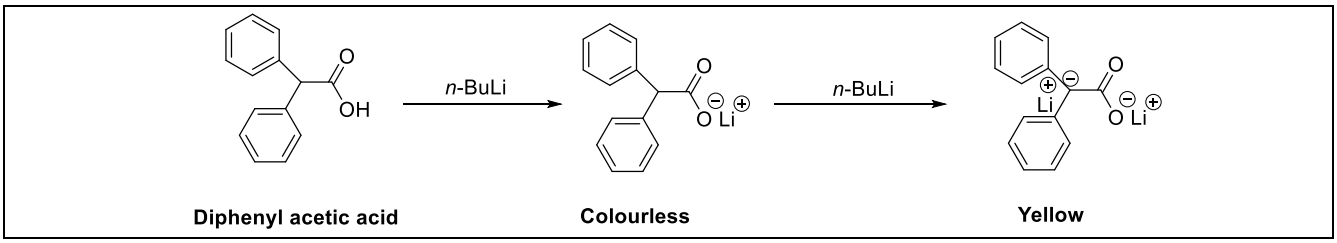
S. No.	Raw Material Name	Source	COA	Remarks
1.	**KSM-I**	Yibin Hongguang Pharma	KSM-I	

2.	**KSM-II** (Salt)	Yibin Hongguang Pharma	KSM-II	
3.	LiBr	Sigma-Aldrich	LiBr	
4.	*R*-Chiral amine	Combi-Block	COA-BD2450_CGP465_98.78_GC.pdf	*D*-Prolinol (Combi-Block/BLD Pharma) for making *R*-Chiral amine
5.	*n*-Butyllithium	Clear Synth	n-Butyl Lithium 2.5M in Hexane	Molarity determination is required before use
6.	NH_4Cl	Rankem	NH4Cl	
7.	Anhydrous $MgSO_4$	Sigma-Aldrich		
8.	Diphenyl acetic acid	Sigma-Aldrich		For tritration of *n*-Butyllithium
9.	$NaHCO_3$	Rankem	NAHCO3	
10.	DCM	Standard Reagent	DCM	
11.	2-MeTHF	Sigma-Aldrich	2-Me-THF	Batch No.: SHBN7180
12.	Sodium chloride (NaCl)	Rankem		
13.	Sodium sulfate (Na_2SO_4) (Anhydrous)	Finar Chemical		
14.	Raw Water			

Critical Material Attributes

S. No.	Reagents & Raw materials	Parameter Specification NMT KF (w/w %)	Purity Specification	Result
1.	**KSM-I**	<0.10	HPLC > 98 % (A %)	KF = 0.06 % HPLC = 99.81 (A %)
2.	**KSM-II**	<0.10	HPLC > 90 % (A %)	KF = 0.08 % HPLC = 95 % (A %)
3.	*(R)*-2-(Methoxymethyl)pyrrolidine	<0.10	GCHS > 98 % SOR ≤ −8.5° Concentration=1 g/100 cm^3 Solvent = Chloroform	GCHS = 99.03 % SOR = −9.637° Concentration = 1 g/100 cm^3 Solvent = Chloroform KF = 0.07 %
4.	LiBr	<0.10		KF = 0.05 %
5.	2-MeTHF	<0.05		KF = 0.05 %
6.	*n*-BuLi		Molarity > 2.0 M	Molarity determined = 2.5 M

General Procedure for *n*-BuLi Titration



To 1.00 mmol (212 mg) diphenylacetic acid (DPPA) in 8 mL dry THF at rt is added *n*-BuLi dropwise via syringe. Upon addition of each drop, a yellow cloud is formed which quickly dissipates. Toward the end of the titration, a white precipitate began to form. At the point when the yellow color persists, the titration is determined to be finished and the amount of molarity of active *n*-BuLi in the solution is calculated from the moles of DPPA that were required to reach the endpoint.

Note: This titration must be repeated three times and the average used to calculate the molarity of the reagent used in the process.

See reference: A. F. Burchat, J. M. Chong, N. Nielsen, *J. Organomet. Chem.* **1997**, *542*, 281–283.

ABBREVIATION LIST

S. No.	Abbreviation	Full Form
1.	BDQ	Bedaquiline
2.	DCM	Dichloromethane
3.	HDPE	High Density Polyethylene
4.	*R*-Chiral amine	*(R)*-2-(Methoxymethyl)pyrrolidine
5.	MOC	Material of construction
6.	NMT	Not More Than
7.	2-MeTHF	2-Methyltetrahydrofuran
8.	MC	Moisture Content
9.	KF	Karl Fischer titration
10.	**KSM-I**	3-benzyl-6-bromo-2-methoxyquinoline
11.	**KSM-II**	3-(dimethylamino)-1-(naphthalen-1-yl) propan-1-one
12.	RM	Reaction mixture
13.	FLT	Filter
14.	RBF	Round-bottom flask
15.	T	Number of Times

EQUIPMENT LIST

S. No.	Equipment Name	Equipment ID.	MOC	Capacity	Remarks	Sign
1.	Reactor	RBF-1	All glass	1L 4-neck RBF		
2.	Reactor	RBF-2	All glass	1L 4-neck RBF		
3.	Reactor	RBF-3	All glass	1L 4-neck RBF		
4.	Reactor	RBF-4	All glass	1L 4-neck RBF		
5.	Reactor	RBF-5	All glass	1L 4-neck RBF		
6.	Reactor	RBF-6	All glass	5L 4-neck RBF		
7.	Reactor	RBF-7	All glass	5L 4-neck RBF		
8.	Reactor	RBF-8	All glass	5L 4-neck RBF		
9.	Buchner Funnel	FLT-1	Porcelain	1L 4-neck RBF		
10.	Buchner Funnel	FLT-2	Porcelain	2L 4-neck RBF		
11.	Separating funnel	Sep funnel-1	All glass	5L		
12.	Addition funnel	Addition funnel-1	All glass	2L		

PROCEDURE FOR DRYING REAGENTS AND PREPARING SOLUTIONS

i. Procedure-A (Drying of LiBr) - **RBF-1**
ii. Procedure-B (**KSM-II-Free Amine** Synthesis) - **RBF-8**
iii. Procedure-C (Drying of *R*-Chiral amine) - **RBF-2**
iv. Procedure-D (Drying of **KSM-I**) - **RBF-3**
v. Procedure-E (Drying of **KSM-II-Free amine**) - **RBF-4**
vi. Procedure-F (Solution of **KSM-II-Free amine**) - **RBF-5**

PROCEDURE FOR CLEANING AND DRYING GLASSWARE

Reactions and drying of the reaction components (LiBr, **KSM-I**, **KSM-II**, *R*-Amine) were performed in laboratory-scale using round-bottom flasks (RBF). RBF has been washed and dried as mentioned below:

- **Step 1:** Water was charged into the RBF, refluxed for 30 min, cooled at 25 °C, and water disposed.
- **Step 2:** MeOH was charged into the RBF, refluxed for 30 min, cooled at 25 °C and solvent disposed.
- **Step 3:** RBF was dried with N_2 flow.

i. Procedure-A (Drying of LiBr) [RBF-1]

Process Information:
Actual batch size and quantity (Calculation on the basis of 75 g KSM-I):

S. No.	Reagent		Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	LiBr		g	45.64	86.84	0.52	2.3 eq	Sigma-Aldrich
2	2-MeTHF	Lot-1	mL	300 mL	-	-	4.0 V	Sigma-Aldrich
		Lot-2		300 mL			4.0 V	
		Lot-3		300 mL			4.0 V	
		Lot-4		225 mL			3.0 V	

Table No. 1:

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
1.	Check the cleanliness of the reactor **RBF-1** (1 L 4-neck flask) and outfit with an internal temperature probe, overhead stirrer, distillation adapter, condenser and N_2 inlet.				
2.	Charge LiBr powder under N_2 atmosphere at 25-30 °C in **RBF-1**. **Note:** LiBr powder is extremely hygroscopic, operation was performed under N_2 atmosphere.	45.6 g	46.0 g		
3.	Charge anhydrous **2-MeTHF-Lot-1** via cannula into the **RBF-1**.	300 mL	300 mL		
4.	Stir (100-120 RPM) reaction mass for 15-20 min at 25-30 ° C. **Note:** Solution becomes homogeneous.	IPC-1: (MC by KF)		KF = 0.72 %	

		(w/w %): Before azeotropic distillation			
5.	Raise the reaction's oil bath temperature to 90 ± 5 °C.			Internal Temp = 74 °C	
6.	Distill 2-MeTHF out so that ~75 mL is left in **RBF-1**. **Note**: Distill 2-MeTHF out by using simple distillation.		~ 250.0 mL collected		
7.	Cool the reaction mass up to 25-30 °C under N_2 atmosphere.				
8.	Charge **2-MeTHF-Lot-2** into **RBF-1** via cannula applying N_2 pressure.	300 mL	300 mL		
9.	Repeat Step-5 to 7.				
10.	Submit IPC-1. If not complies repeat operation from Step-3 to 7, and use **2-MeTHF-Lot-3**. **Sampling Procedure**: Take 5 mL of LiBr solution and submit for KF analysis.	IPC-1: (MC by KF) (w/w %): NMT 0.1		Complies KF = 0.05 %	
11.	Charge **2-MeTHF-Lot-4** via cannula applying N_2 pressure into the **RBF-1**.	225 mL	225 mL		
12.	Store LiBr solution under N_2 atmosphere in **RBF-1**.			LiBr in 300.0 mL of 2-MeTHF	

Results:

S. No.	Batch ID	LiBr KF before drying (w/w %)	LiBr KF after dry (w/w %)	Sign
1	CR592-20218-17-LiBr	0.72	0.05	

Sl. No.	Step No. 4	Step No. 10
	KF of Lithium Bromide before drying	**KF of Lithium Bromide after drying**
1	CR592-20218-17-LiBr_before_KF.pdf	CR592-20218-17-LiBr-after_KF.pdf

ii. Procedure-B (KSM-II Free Amine Synthesis) [RBF-8]

Actual batch size and quantity (Calculation on the basis of KSM-II salt - 80 g):

S. No.	Reagent		Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	**KSM-II** (Salt)		g	80.0	263.7	0.303	1.00 eq	Yibin Hongguang Pharma (Purity: 98.19 A %)
2	$NaHCO_3$		g	86.6	84.0	1.03	3.4 eq	Rankem $NaHCO_3$ (86.6 g) in Water (713 mL) 10 V
3	Water	Lot-1	mL	800 mL	-	-	10.0 V	Raw water
		Lot-2		400 mL			5.0 V	
4	DCM	Lot-1	mL	800 mL	-	-	10.0 V	Rankem
		Lot-2		400 mL			5.0 V	
		Lot-3		80 mL			1.0 V	
5	Mg_2SO_4		g	18.2	120.3	0.176	0.5 eq	Sigma-Aldrich

Table No. 2:

Procedure for Free amine of KSM-II

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
	KSM-II free base from KSM-II salt				
1.	Charge **KSM-II** salt into a well dried and clean 5 L 3-neck **RBF-8** at 25-30 °C.	80.0 g	80.0 g	Purity: 98.19 %, HPLC A %)	

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
2.	Charge **Water-Lot-1** through graduated measuring cylinder into **RBF-8** at 25-30 °C.	800.0 mL	800.0 mL		
3.	Stir the mixture (90-100 RPM) at 25-30 °C for ~10-15 min until the solid is completely dissolved.			Clear solution was observed	
4.	Charge **DCM-Lot-1** through graduated measuring cylinder into **RBF-8** at 25-30 °C.	800 mL	800.0 mL		
5.	Stir (90-100 RPM) the mixture at 25-30 °C for 15-20 min.				
6.	Slowly charge sat. solution of $NaHCO_3$ for 45 min into **RBF-8** via **addition funnel-1** at 25-30 °C. **Note:** Liberate CO_2 gas from mixture.	800 mL	800.0 mL	Rate of addition = 17.7 mL/min	
7.	Stir (90-100 RPM) the mixture at 25-30 °C for 8-10 min.			Temp = 28 °C	
8.	Transfer the reaction mass to 5 L separating funnel (**Sep funnel-1**).				
9.	Settle the mixture for layer separation (5-10 min).				
10.	Separate the DCM layer and store it in dedicated **HDPE-1** container. Aqueous layer goes to the **RBF-8**.		760.0 mL	Product is present in organic layer	
11.	Charge **DCM-Lot-2** through graduated cylinder into the aqueous layer.	400 mL	400.0 mL		
12.	Stir (90-100 RPM) the mixture at 25-30 °C for 5-10 min.				
13.	Transfer the reaction mass to the same separating funnel (**Sep funnel-1**).				
14.	Settle the mixture for layer separation (10-15 min).				
15.	Separate the DCM layer by separatory funnel and store in dedicated **HDPE-1**.		375.0 mL	Product is present in organic layer.	
16.	Discard the aqueous layer.				
17.	Charge DCM layer into 5L 3-neck RBF at 25-30 °C.			From dedicated **HDPE-1**	

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
18.	Charge **Water-Lot-2** into the DCM layer.	400 mL	400 mL		
19.	Stir (100-120 RPM) the mixture at 25-30 °C for 5-10 min.				
20.	Settle the mixture for layer separation (10-15 min).				
21.	Separate the DCM layer by separatory funnel (**Sep funnel-1**) and store in dedicated **HDPE-1**.				
22.	Charged anhydrous $MgSO_4$ into **HDPE-1**.	18.2 g	20.0 g	DCM layer	
23.	Filter the organic layer through Buchner funnel (**FLT-1**).				
24.	Wash $MgSO_4$ bed with **DCM-Lot-3** and suck dry the solid.	80 mL	80 mL		
25.	Remove the collected solvent (DCM) under vacuum at 30-35 °C for 1-2 h.			Vacuum 740-750 mmHg	
26.	Unload pale yellow liquid (**KSM-II-free amine**) into RBF under N_2 and record the weight.		66.0 g		

Results:

Sl. No.	Batch ID	Batch size (g)	KSM-II salt purity (HPLC A %)	Output (g)	KSM-II Free amine purity after Workup (A %)	Molar yield (A %)	Sign
1	CR592-19938-59-Free amine	80	98.19 %	66.0	94.85 %	95.4 %	

Sl. No.	Step No. 1	Step No. 26
	KSM-II salt purity (HPLC A %)	**KSM-II free amine purity**
1	CR592-20218-7-KSM-II.pdf	CR592-19938-59-Free amine.pdf

iii. Procedure-C (Drying of *R*-Chiral amine with activated molecular sieves) [RBF-2]

Actual batch size and quantity:

S. No.	Reagent	Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	*R*-Chiral amine	L	45.0 mL	115.1	0.388	1.7 eq	CR592-20195-34-F2
2	Molecular sieves, 4 Å	g	9.0 g	-	-	0.2 T	Avra

Table No. 3:

Process Information:

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
1.	Check the cleanliness of the reaction **RBF-2** (1 L 2-neck flask) fitted with a vacuum line and N_2 inlet.				
2.	Charge molecular sieves 4Å at 25-30 °C.	9.0 g	9.0 g		
3.	Raise temperature of **RBF-2's** oil bath to 140-150 °C.			Digital Thermometer was used	
4.	Apply vacuum slowly and keep the same range of temperature for 4-5 h.			740-750 mmHg	
5.	Cool the **RBF-2** to 25-30 °C and keep N_2 atmosphere.				
6.	Charge *(R)*-2-(Methoxymethyl)pyrrolidine into **RBF-2**. N**ote:** Material has been transferred via cannula.	45.0 mL	45.0 mL	KF = 0.24 %	
7.	Store *(R)*-2-(Methoxymethyl)pyrrolidine for 3-4 h and submit sample for KF analysis. **Sampling Procedure:** Take 2 mL of *R*-Chiral amine and submit it for KF analysis.	IPC-2: (MC by KF) (w/w %: NMT 0.1)		Complies KF = 0.07 %	
8.	If complies, proceed to Step-9; If not, repeat Step-2 to7 using another clean RBF.				

9.	Store *(R)*-2-(Methoxymethyl)pyrrolidine under N_2 atmosphere in **RBF-2** over molecular sieves.	45.0 mL	~42.0 mL	40.2 mL was syringed out for reaction	

Results:

Sl. No.	Batch ID	Batch size (g)	*R*-Amine, KF before drying (w/w %)	Output (g)	*R*-Amine, KF after drying (w/w %)	Sign
1	CR592-20218-17-*R*-Amine	45.0	0.24	42.0	0.07	

Sl. No.	Step No. 6	Step No. 7
	KF of *R*-Amine before drying	**KF of *R*-Amine after drying**
1	CR592-20195-34-F-2 _KF.pdf	CR592-20218-17-R-base.pdf

iv. Procedure-D (Drying of KSM-I) [RBF-3]

Process Information:
Actual batch size and quantity:

S. No.	Reagent		Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	**KSM-I**		g	75.0	328.2	0.228	1.0 eq	Yibin Hongguang Pharma (Purity: 99.0 A %)
2	2-MeTHF	Lot-1	mL	225 mL	-	-	3.0 V	Sigma-Aldrich MC by KF (w/w %): NMT 0.06
		Lot-2		225 mL			3.0 V	
		Lot-3		150 mL			2.0 V	

Table No. 4:

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
1.	Check the cleanliness of the reaction **RBF-3** (1 L 4-neck flask) fitted with an internal temperature probe, overhead stirrer, and condenser with N_2 inlet.				
2.	Charge **KSM-I** via solid funnel at 25-30 °C.	75.0 g	75.0 g	(Purity: 99.0 A %, HPLC)	
3.	Charge **2-MeTHF-Lot-1** via cannula into **RBF-3**.	225.0 mL	225.0 mL		
4.	Stir reaction mass for 10-15 min at 25-30 °C under N_2 atmosphere. **Note:** Solution becomes homogeneous and pale-yellow in color. **Sampling Procedure:** Take 2 mL of **KSM-I** solution and submit it for KF analysis.	IPC-3: (MC by KF) (w/w %): Before azeotropic distillation.		KF = 0.18 %	
5.	Raise the oil bath temperature to 90 ± 5 °C.			RM internal temperature checked	
6.	Distill 2-MeTHF out so that ~75 mL is left in RBF.		125.0 mL	150.0 mL was collected	
7.	Cool the reaction mass to 25-30 °C under N_2 atmosphere. **Sampling Procedure:** Take 2 mL of **KSM-I** solution and submit it for KF analysis.	IPC-3: (MC by KF) (w/w %): After azeotropic distillation		KF = 0.06 %	
8.	If not complies repeat Step-2 to 7 using **2-MeTHF-Lot-2**.	225.0 mL			

9.	Charge **2-MeTHF-Lot-3** into **RBF-3**. **Note:** Handle the solvent under N_2 atmosphere.	150 mL	150 mL		
10.	Store **KSM-I** solution under N_2 atmosphere in **RBF-3**.				

Results:

Sl. No.	Batch ID	KSM-I KF before drying (w/w %)	KSM-I KF after drying (w/w %)	Sign
1	CR592-20218-17-KSM-I	0.18	0.06	

Sl. No.	Step No. 4	Step No. 7	Step No. 2
	KF of KSM-I before drying	**KF of KSM-I after drying**	**Purity of KSM-I (HPLC A %)**
1	CR592-20218-17-KSM-1-before-KF.pdf	CR592-20218-17-KSM-1-after-KF.pdf	CR592-20218-6-KSM-I (1).pdf

v. Procedure-E (Drying of KSM-II-Free amine with activated molecular sieves) [RBF-4]

Actual batch size and quantity:

S. No.	Reagent	Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	**KSM-II-Free amine**	mL	65.0	227.3	0.285	1.25 eq	CR592-19938-59
2	Molecular sieves, 4 Å	g	15.0	-	-		Avra

Table No. 5:

Process Information:

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
1.	Check the cleanliness of the reaction **RBF-4** (1 L 3-neck flask) fitted with a vacuum line and N_2 inlet.				

2.	Charge molecular sieves 4Å at 25-30 °C. **Note:** Handle the material under N_2.	11.0 g	11.0 g		
3.	Raise the oil bath temperature to 140-150 °C.				
4.	Apply vacuum slowly and keep the temperature at 140-150 °C for 4-5 h.				
5.	Cool the **RBF-4** to 25-30 °C under N_2 atmosphere.				
6.	Charge **KSM-II** into **RBF-4**. (Material taken from Table No. 2, Step-26). **Note:** Transfer material through graduated measuring cylinder under N_2 atmosphere.	65.0 g	65.0 g	KF = 1.13 %	
7.	Store **KSM-II** under molecular sieves for 3-4 h and submit sample for KF. **Sampling Procedure**: Take 2 mL of **KSM-II** solution and submit it for KF analysis.	IPC-4: (MC by KF) (w/w %): NMT 0.1		KF = 0.08 %	
8.	If it does not comply, repeat Step-2 to Step-7.				
9.	Store **KSM-II-Free amine** under N_2 atmosphere in **RBF-4**.	65.0 mL		62.30 mL was syringed out for reaction	

Results:

S. No.	Batch ID	Batch size (g)	KSM-II Free amine before drying (w/w %)	Output (g)	KSM-II Free amine KF after dry (w/w %)	Recovery	Sign
1	CR592-19938-59	65.0	1.13	62.5	0.08	96 %	

Sl. No.	Step No. 6	Step No. 7
	KF of KSM-II before drying	**KF of KSM-II after drying**
1	CR592-20218-17-KSM-2-before -KF.pdf	CR592-20218-17-KSM-2-after-KF.pdf

vi. Procedure-F (Solution of KSM-II Free amine) [RBF-5]

Process Information:
Actual batch size and quantity:

S. No.	Reagent		Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	**KSM-II-Free amine**		mL	62.30 mL	227.3	0.274	1.2 eq	**RBF-4**
2	2-MeTHF	Lot-1	mL	375 mL	-	-	5.0 V	Sigma-Aldrich MC by KF (w/w %): NMT 0.06

Table No-6:

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
1.	Check the cleanliness of the reaction **RBF-5** (1 L 2-neck flask). **Note:** RBF should be under N_2 atmosphere.				
2.	Transfer **KSM-II-Free amine** to **RBF-5** from **RBF-4** via graduated measuring cylinder under N_2 pressure. **Note:** Density = 0.98 cm^{-3} at 20 °C.	62.3 mL	62.3 mL		
3.	Charge **2-MeTHF-(Lot-1)** into **RBF-5** via cannula at 25-30 ° C. **Note:** Homogeneous solution and pale-yellow in color.	375 mL	375 mL		
4.	Store **KSM-II-Free amine** solution under N_2 atmosphere in **RBF-5**. **Sampling Procedure:** Take 2 mL of **KSM-II-Free amine** solution and submit it for KF analysis.	IPC-5: (MC by KF) (w/w %): NMT 0.1		KF = 0.08 %	

Analytical method for IPC-1 to IPC-5 is attached herewith:

WATER CONTENT BY AUTO TITRATOR

List of raw materials after drying

S. No.	Reagents & Raw materials in 2-MeTHF	Parameter Specification NMT KF (w/w %)	Remarks
1.	LiBr solution (in 2-MeTHF solution)	<0.1	RBF-1
2.	*R*-Amine (neat)	<0.1	RBF-2
3.	**KSM-I** (in 2-MeTHF solution)	<0.1	RBF-3
4.	**KSM-II-Free amine** (in 2-MeTHF solution)	<0.1	RBF-5
5.	Dry 2-MeTHF	<0.05	Sure Seal™ bottle

Process Information

Actual batch size and quantity

S. No.	Reagent		Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	**KSM-I**		g	75.0	328.2	0.228	1.00 eq	RBF-3
2	**KSM-II-Free amine**		g/mL	62.3	227.3	0.273	1.20 eq	RBF-5
3	LiBr		g	45.64	86.84	0.524	2.30 eq	RBF-1
4	*R*-Chiral amine		mL	40.2	115.1	0.342	1.50 eq	RBF-2
5	*n*-BuLi (2.5 M in hexane)		mL	135.0	64.06	0.296	1.30 eq	Clear Synth
6	Aq. NH_4Cl (25 % w/v)		g	93.7	53.49	-	7.6 eq	NH_4Cl (93.7 g) in Water (281 mL)
7	2-MeTHF	Lot-1	mL	450.0	-	-	6.0 V	Sigma-Aldrich
		Lot-2		75.0			1.0 V	
8	DCM	Lot-1	mL	375.0	-	-	5.0 V	Standard Reagent
		Lot-2		75.0			1.0 V	
9	Nitrogen		-	As required			-	In-house

BEDAQUILINE ASSEMBLY REACTION

Table No. 7:

Synthesis of Bedaquiline from KSM-I & KSM-II-Free amine – [RBF-6]

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
1.	Check the cleanliness of the reaction **RBF-6** (5 L 4-neck flask) fitted with an internal temperature probe, and overhead stirrer vacuum line with N_2 inlet.				
2.	Charge **2-MeTHF-Lot-1** into **RBF-6** via cannula applying N_2 Pressure. **Note:** Continue under N_2 atmosphere.	450 mL	450 mL		
3.	Charge LiBr solution from **RBF-1** into **RBF-6** at 25-30 °C. **Note**: Transfer LiBr solution via cannula applying N_2 atmosphere.	45.64 g dissolved in 225 mL 2-MeTHF			
4.	Charge *R*-Chiral amine (neat) from **RBF-2** into **RBF-6** at 25-30 °C. **Note:** Transfer *R*-Amine via cannula applying N_2 atmosphere.	40.2 mL			
5.	Stir the mixture at 25-30 °C for ~10-15 min at 120-140 RPM. **Note:** Solution becomes homogeneous and colorless.				
6.	Cool the reaction mass to −20 °C / −22 °C. **Note:** Acetonitrile / dry ice bath.			Reaction mass internal temperature: −20° C	
7.	Charge *n*-BuLi into dropping funnel at 25-30 °C. **Note:** Transfer *n*-BuLi (2.5 M in hexane) into dropping funnel via cannula applying N_2 atmosphere.	135.0 mL	135.0 mL		
8.	Add *n*-BuLi (2.5 M in hexane) slowly into **RBF-6** at −20 ± 2 °C through dropping funnel for 25-30 min (under N_2 atmosphere). **Note:** Reaction mass color becomes pale-yellow.			Rate of addition = 5.3 mL/min	
9.	Stir the reaction mixture at −20 ± 3 °C for 25-30 min at 120-140 RPM.				

10.	Cool the reaction mass to −42 ± 3 °C.		−40 °C		
11.	Charge **KSM-I** solution from **RBF-3** into **RBF-6** through dropping funnel at 25-30 °C. **Note:** Transfer **KSM-I** solution into dropping funnel via cannula applying N_2 atmosphere.	75.0 g	75.0 g	75.0 g in 300.0 mL 2-MeTHF	
12.	Charge **2-MeTHF-Lot-2** into dropping funnel at 25-30 °C. **Note:** Transfer 2-MeTHF via cannula applying N_2 pressure.	75.0 mL			
13.	Add **KSM-I** slowly into **RBF-6** at −42 ± 3 °C through dropping funnel for 1 h (under N_2 atmosphere). **Note:** Reaction mass color becomes red wine.		−40 °C	Rate of addition = 10 mL/min	
14.	Stir the reaction mixture at 120-140 RPM for 25-30 min at −42 ± 3 °C.				
15.	Charge **KSM-II** solution from **RBF-5** into dropping funnel at 25-30 °C. **Note**: Transfer **KSM-II** solution into dropping funnel via cannula applying N_2 pressure.	375 mL	375 mL		
16.	Add **KSM-II** solution slowly into **RBF-6** at −42 ± 3 °C via dropping funnel for 1 h (under N_2 atmosphere). **Note:** Reaction mass color continues red wine.			Rate of addition = 10 mL/min	
17.	Stir the reaction mixture at 120-140 RPM for 40-45 min at −42 ± 3 °C.				
18.	Charge Aq. NH_4Cl solution (25 % w/v in water) into dropping funnel at 25-30 °C. **Note**: Transfer Aq. NH_4Cl solution into dropping funnel using cannula applying N_2 pressure.	375 mL	375 mL	Aq. NH_4Cl is prepared in a separate **Container-1**	
19.	Add Aq. NH_4Cl solution into **RBF-6** at −42 ± 3 °C slowly for 35-40 min through dropping funnel. **Note**: Reaction mass color becomes yellow/brownish.			Rate of addition = 12.5 mL/min	
20.	Allow reaction mixture to warm to 25-30 °C.				
21.	Stir (120-140 RPM) the reaction mixture at 25-30 °C for 10-15 min.				

22.	Settle the reaction mixture for layer separation (5-10 min).				
23.	Separate the aqueous layer using **Sep funnel-1** and keep the organic layer in a clean **Container-2**.			Product is present in organic layer	
24.	Transfer aqueous layer to **RBF-6** and charge **DCM-Lot-1** via graduated cylinder.	375 mL	375 mL		
25.	Stir the mixture at 25-30 °C for 5-10 min.				
26.	Settle the mixture for layer separation (10-15 min).				
27.	Separate the organic layer via **Sep funnel-1** and collect organic layer in **Container-2** and discard aqueous layer.			Product is present in organic layer	
28.	Charge Na_2SO_4 into dedicated **Container-2**.	20 g			
29.	Filter the organic layer through Buchner funnel (**FLT-2**) followed by washing successively with **DCM-Lot-2** and suck dry the solid.	75 mL	75 mL		
30.	Charge organic layer (DCM) into a well dried and clean 5 L **RBF-7** at 25-30 °C.				
31.	Remove the solvent under vacuum at 45-50 °C for 1-2 h. **Note**: Until no distillate comes out. A pale-yellow semi-solid (**Crude-Bedaquiline**) residue was obtained.			Vacuum 740-750 mmHg	
32.	Submit for purity analysis (Related Substance) of crude BDQ and weight is taken.	~25 mg	50 mg	IPC: Related Substance by HPLC for Information only	
33.	Take **Crude-Bedaquiline** as such for further purification in **RBF-7**.				

IPC METHOD FOR PURITY FOR PURITY ANALYSIS OF CRUDE BDQ

CHROMATOGRAPHIC PURITY OF BDQ.pc

IN-PROCESS CONTROL DATA

S. No.	Batch ID	Input (g)	Output (g)	Purity by HPLC (A %)					Sign
				D-I	D-II	KSM-I	KSM-II	Imp-1	
1	CR592-20218-17	75.0	136	77.74	5.70	5.18	7.11	2.87	

Sl. No.	Step No. 32	Step No. 32	Step No. 32	Step No. 32	Step No. 32
	NMR	HPLC	Assay	LC	SFC
1	CR592-20218-17-CR_NMR.pdf	CR592-20218-17-CR-R.pdf	CR592-20218-17-CR-assay.pdf	CR592-20218-17-CR-R1_LC.pdf	CR592-20218-17-CR-SFC-CK571_Chiral P