Final campaign report

Development of a safe and scalable process for the synthesis of pyrrolo[2,1-f][1,2,4]triazin-4-amine

Submitted to

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TCG GreenChem and Life sciences Pvt. Limited – Contract development and manufacturing organization (CDMO)

Report prepared by Dr. Sarabindu Roy & Dr Ajay K. Yadaw

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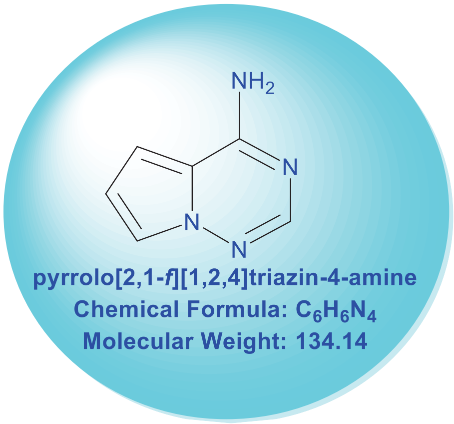
Major Scope of the work:

- Feasibility study of the proposed route
- Analytical method development for in-process control and release of the intermediates as well as final compound
- Optimization of the reaction in terms of yield, quality, time and safety
- Comprehensive safety studies of the optimized protocol
- Impurity profiling
- Synthesis of 1.0 Kg of the target pyrrolo[2,1-f][1,2,4]triazin-4-amine
- Preparation of lab development report and submit

Overview:

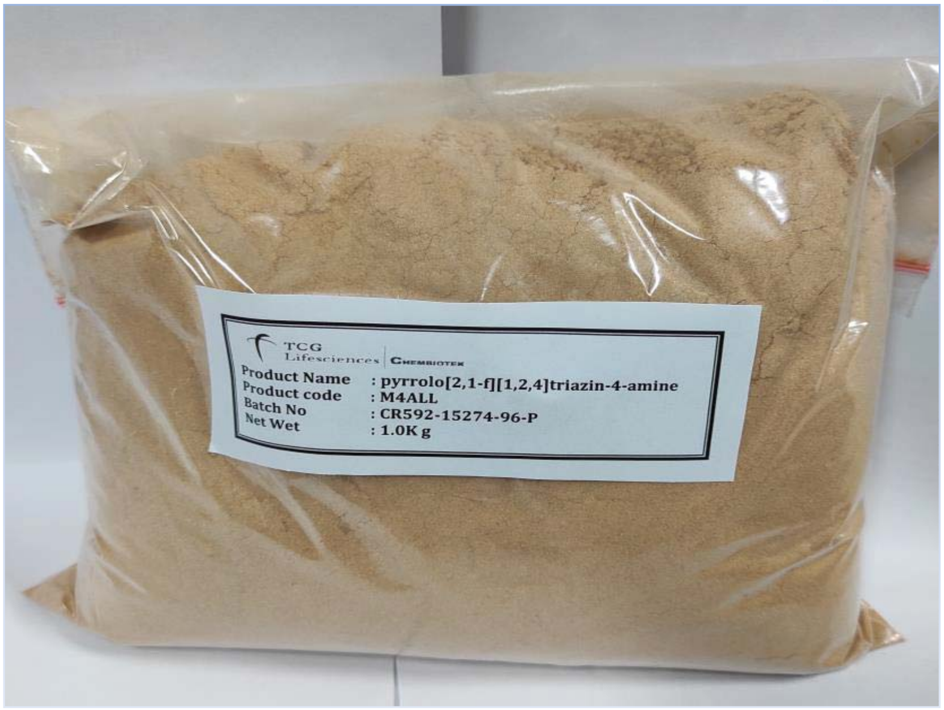
Pyrrolo[2,1-f][1,2,4]triazin-4-amine is a key starting material of Remdesivir which is one of the front line drug for the treatment of COVID-19. Structure of the Target compound is shown below.



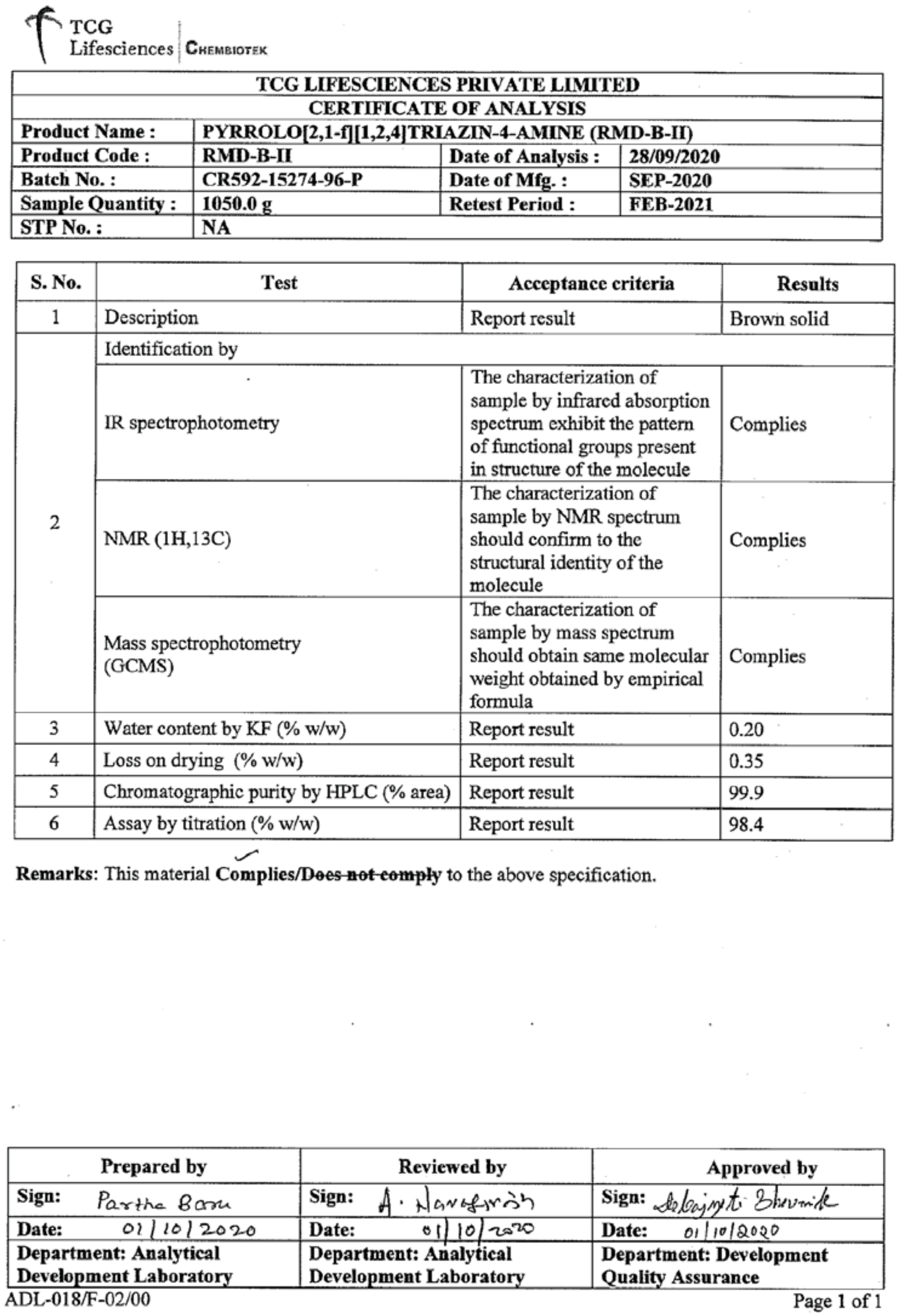
pyrrolo[2,1-*f*][1,2,4]triazin-4-amine
Chemical Formula: $C_6H_6N_4$
Molecular Weight: 134.14

Summary of work done and major accomplishments

- Various conditions were screened for all the steps and based on the study, a safe and scalable protocol has been established for the synthesis of target compound in Kg scale
- Extensive safety studies have been performed using RC1 and DSC to identify the safe protocol for scale up
- Many process impurities have been identified, isolated, synthesized and characterized
- Efficient purification protocol has been established to purge most of the process impurities
- Eventually final target compound has been synthesized on 1Kg scale with purity >99.9% with assay 98.4%


TCG Lifesciences | Chembiotek
Product Name : pyrrolo[2,1-f][1,2,4]triazin-4-amine
Product code : M4ALL
Batch No : CR592-15274-96-P
Net Wet : 1.0K g

Certificate of Analysis of Pyrrolo[2,1-f][1,2,4]triazin-4-amine



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TCG LIFESCIENCES PRIVATE LIMITED			
CERTIFICATE OF ANALYSIS			
Product Name :	**PYRROLO[2,1-f][1,2,4]TRIAZIN-4-AMINE (RMD-B-II)**		
Product Code :	**RMD-B-II**	**Date of Analysis :**	**28/09/2020**
Batch No. :	**CR592-15274-96-P**	**Date of Mfg. :**	**SEP-2020**
Sample Quantity :	**1050.0 g**	**Retest Period :**	**FEB-2021**
STP No. :	**NA**		

S. No.	Test	Acceptance criteria	Results
1	Description	Report result	Brown solid
2	Identification by		
	IR spectrophotometry	The characterization of sample by infrared absorption spectrum exhibit the pattern of functional groups present in structure of the molecule	Complies
	NMR (1H,13C)	The characterization of sample by NMR spectrum should confirm to the structural identity of the molecule	Complies
	Mass spectrophotometry (GCMS)	The characterization of sample by mass spectrum should obtain same molecular weight obtained by empirical formula	Complies
3	Water content by KF (% w/w)	Report result	0.20
4	Loss on drying (% w/w)	Report result	0.35
5	Chromatographic purity by HPLC (% area)	Report result	99.9
6	Assay by titration (% w/w)	Report result	98.4

Remarks: This material **Complies/~~Does not comply~~** to the above specification.

Prepared by	**Reviewed by**	**Approved by**
Sign: Partha Basu	**Sign:**	**Sign:** Debajyoti Bhowmik
Date: 01/10/2020	**Date:** 01/10/2020	**Date:** 01/10/2020
Department: Analytical Development Laboratory	**Department: Analytical Development Laboratory**	**Department: Development Quality Assurance**

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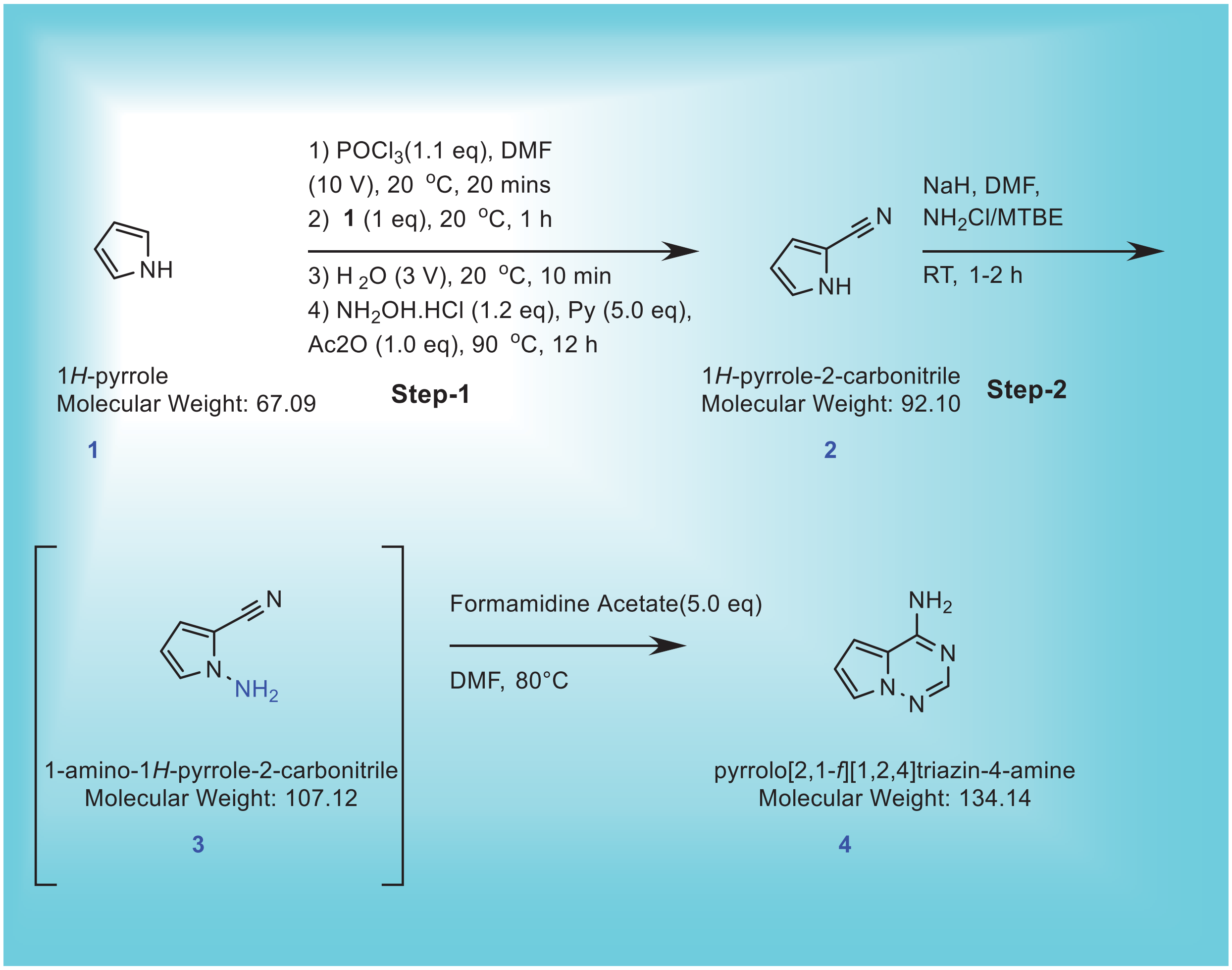
Final optimized synthetic scheme

Final optimized synthetic scheme is shown below.



Experimental:

Step-1

Actual batch size and quantity:

S. No.	Reagent		Unit	Qty	Mol Wt	Mole	Mole ratio/ wt times vol	Source
1	Pyrrole		g	1000	67.09	14.90	1	Commercial
2	Phosphorus oxychloride		mL	1544	153.33	16.56	1.11	Commercial
3	Hydroxylamine hydrochloride		g	1139	69.49	16.38	1.09	Commercial
4	Acetic anhydride		mL	1548	102.08	16.48	1.10	Commercial
5	Pyridine		mL	6000	79.10	74.18	4.97	Commercial
6	Process water	Lot-1	mL	3000	-		3 vol	
		Lot-2		10000			10 vol	
7	N,N-Dimethylformamide (anhydrous)		mL	10000	-		10 vol	Commercial
8	Methyl *tert*-butyl ether (MTBE)	Lot-1	mL	10000	-		10 vol	Commercial
		Lot-2		10000			10 vol	
		Lot-3		10000			10 vol	
9	2 N Hydrochloric acid		mL	10000	-		10 vol	Commercial
10	Aq. Sodium bicarbonate solution (10%)	Lot-1	mL	10000	-		10 vol	Commercial
		Lot-2		10000			10 vol	
11	Anhydrous Sodium sulfate		g	1000	-		1 w/w	Commercial

a) Process:

S.No.	Procedure	Qty.	Remarks
1.	Check the cleanliness of the reaction vessel (20 L 3-necked flask) fitted with an internal temperature probe, overhead stirrer, and addition flask and nitrogen line.		

S.No.	Procedure	Qty.	Remarks
2.	Charge **anhydrous DMF** (10000 mL) into the reaction flask.	10000 mL	
3.	Cool the reaction vessel under N_2 atmosphere so that internal temperature of the flask remains between 0-5 ºC		
4.	Stir the contents of the vessel at 0-5 ºC.		
5.	Charge **phosphorus oxychloride** (1544 mL) slowly into the reaction vessel maintaining the internal temperature of the vessel at 0-5ºC	1544 mL	Time : ~ 3.0 h
6.	Raise the reaction mixture to 15-20°C.		
7.	Stir the mixture at 15-20 ºC for another 30 min.		
8.	Cool the mixture again to 0-5 ºC.		
9.	Charge **pyrrole** (1000 g) slowly into the cooled reaction mixture maintaining the internal temperature of the vessel below 15 ºC.	1000 g	Time : 1.5 h
10.	Stir the mixture at 15-20 ºC for another 1 h.		
11.	Cool the light brown mixture to 0-5 ºC.		
12.	Charge **process water lot-1**(3000 mL) slowly (2-3 h) into the reaction mixture maintaining the internal temperature of the mixture below 15 ºC.	3000 mL	
13.	Stir the mixture at 15 ºC for 5 min.		
14.	Charge **solid hydroxylamine hydrochloride** (1139.2 g) into the mixture while maintaining the internal temperature below 15 ºC.	1139.2 g	
15.	Charge **acetic anhydride** (1548 mL) into the mixture while maintaining the internal temperature below 15 ºC.	1548 mL	
16.	Charge **pyridine** (6000 mL) into the mixture while maintaining the internal temperature below 15 ºC.	6000 mL	
17.	Heat the reaction mixture to 90 ºC and stir the mixture at this temperature for 16 h.		
18.	Send the sample of the reaction mixture for HPLC analysis to check the complete consumption of Aldehyde (IPC-1). Sample preparation: Take sample directly from reaction mass and submit as such		**IPC-1 (Aldehyde NMT 2%)** **Result: Below detection limit**
19.	Cool the reaction mass to 25-30°C.		
20.	Charge chilled (10-15°C) **process water lot-2** (10000 mL) into the reaction mass and stir the mass for 10 min.	10000 mL	
21.	Charge **MTBE Lot-1** (10000 mL) into the mixture and stir the mixture for 10 min.	10000 mL	
22.	Stop the stirring and allow the reaction mixture for layer separation.		

S.No.	Procedure	Qty.	Remarks
23.	Separate the organic and aqueous layer (with the help of separatory funnel) into two separate clean containers.		
24.	Charge back the aqueous layer into the reaction vessel again and charge **MTBE Lot-2** (10000 mL) into it.	10000 mL	
25.	Stir the mixture for 10 min. Stop the stirring and allow it for layer separation.		
26.	Separate the organic and aqueous layer (with the help of separatory funnel) into two separate containers.		
27.	Charge back the aqueous layer into the reaction vessel again and charge **MTBE Lot-3** (10000 mL) into it.	10000 mL	
28.	Stir the mixture for 10 min. Stop the stirring and allow it for layer separation.		
29.	Separate the organic and aqueous layer (with the help of separatory funnel) into two separate containers.		
30.	Charge the combined organic layers into the reaction vessel.		
31.	Charge **2N Hydrochloric acid** into the reaction vessel and stir the contents for 10 min.	10000 mL	
32.	Stop the stirring and allow it for layer separation.		
33.	Separate the aqueous layer.		
34.	Charge **10% aqueous sodium bicarbonate solution (**10000 mL) into the reaction vessel and stir the contents for 10 min.	10000 mL	
35.	Stop the stirring and allow it for layer separation.		
36.	Separate the aqueous and organic layer.		
37.	Charge **10% aqueous sodium bicarbonate solution (**10000 mL) into the reaction vessel and stir the contents for 10 min.	10000 mL	
38.	Stop the stirring and allow it for layer separation.		
39.	Separate the aqueous layer and organic layer into two separate containers.		
40.	Charge anhydrous sodium sulfate into the organic layer allows it for 15 min for drying.	1000 g	
41.	Charge the dried organic layer into the reaction vessel and distil out the organic layer under medium reduced pressure at 40-50 ºC.		The yield of crude dark brown liquid = 1380 g
42.	Purify the crude by fractional distillation. Desired product starts to distill off at 100-140 ºC under high vacuum pressure (2-4 mm Hg).		First fraction (~10-15%) contains mostly DMF and volatiles. Second fraction onwards desired compound starts to collect

S.No.	Procedure	Qty.	Remarks
43.	Send the distillate for checking purity by HPLC.		Purity by HPLC NLT 95% Assay by HPLC : Report result Assay of DMF by GC: Report result
44.	Yield range (1-1.2 w/w)		Yield is based on assay by HPLC

In process controls data:

S. No.	Batch Id	IPC-1 (Limit: NMT 2 %)	Crude Purity by HPLC
1	CR592-15288-25	IPC-01.pdf **Aldehyde (15.8 min) Below detection limit**	Crude-Purity by HPLC.pdf

Purification of Crude 2-cyanopyrrole to pure fraction:

Crude 2-cyanopyrrole was taken in a 2L single neck RBF equipped with distillation condenser and connected to other end with receiver and vacuum line. Set up the vacuum to maximum and slowly start to heat. First fraction collects at 50-70°C and second fraction starts to collect at 95-125°C.

Batch No. CR592-15288-25
Input: 1380.0 g
Yield = (144X 18.2% + 348 X 92.25% + 870 X 89.2%) = 26.0 g + 321.0 g + 776.0 g = 1123.0 g
W/w Yield = 1.13 Molar % yield = 81.78%

Fraction-1	Fraction-2	Fraction-3	Residue	Loss
144.0 g	348.0g	870.0g	10.0 g	8.0g
Assay 18.2%	Assay: 92.25%	Assay: 89.2%	Assay: 5.0%	
HPLC area% 94.15%	HPLC area% 99.54%	HPLC area% 99.38%	Not checked	

CR592-15288-25-F1.pdf CR592-15288-25-F1(Assay).pdf	CR592-15288-25-F2.pdf CR592-15288-25-F2(Assay).pdf	CR592-15288-25-F3.pdf CR592-15288-25-F3(Assay).pdf		

Step-2:

List of Raw materials

i) RMD-B-I (1H-pyrrole-2-carbonitrile)

ii) N,N-Dimethylformamide (DMF)

iii) 60% Sodium hydride (NaH)

iv) 12-15% aqueous sodium hypochlorite (NaOCl)

v) 25% Aqueous ammonia (NH_4OH)

vi) Ammonium chloride (NH_4Cl)

vii) Anhydrous calcium chloride (CaCl2)

viii) Methyl *tert*-butyl ether (MTBE)

ix) Formamidine acetate

x) Celite

xi) Process water

xii) Sodium chloride

Process Information

Actual batch size and quantity:

S. No.	Reagent	Unit	Qty	Mol Wt	Mole	Mole ratio/ wt times vol	Source
1	1H-pyrrole-2-carbonitrile (Pyrrole-2-carbonitrile)	Kg	0.60	92.04	6.52	1.0 eq	In-house
2	Sodium hydride (60 % dispersion in mineral oil)	Kg	0.29	23.99	7.17	1.1 eq	Commercial
3	Foramidine acetate	kg	2.04	104.11	19.56	3.0 eq	Commercial

S. No.	Reagent		Unit	Qty	Mol Wt	Mole	Mole ratio/ wt times vol	Source
4	Ammonium chloride (NH_4Cl)		Kg	2.7	53.49	-	4.5 T	Commercial
5	Ammonia aqueous (25%)		L	4.21	-	-	7.02 V	Commercial
6	Sodium hypochlorite solution (Available Chlorine 8-10%)		L	47.4	-	-	79 V	Commercial
7	Methyl *tert*-butyl ether (MTBE)	Lot-1	L	36	-	-	60 V	Commercial
		Lot-2		1.2			2V	
8	Anhydrous Calcium chloride ($CaCl_2$)		Kg	1.2	-	-	2T	Commercial
9	Anhydrous Sodium chloride (NaCl)		Kg	1.80	-	-	3T	Commercial
10	Anhydrous N,N-Dimethyl formamide (DMF)	Lot-1	L	6.0			10 V	Commercial
		Lot-2		0.6			1V	
11	Process water	Lot-1	L	12.0			25 V	
		Lot-2		2.4				
		Lot-3		0.6				

a) Process:

S.No.	Procedure	Qty.	Remarks
	Part-A: Preparation of 3-5% of Monochloroamine in MTBE		
1.	Check the cleanliness of the reactor-1 (fitted with an internal temperature probe, and addition flask and nitrogen line).		
2.	Charge MTBE into the reactor at 25-30 °C	36.0 L	
3.	Charge NH_4Cl into the reactor at 25-30 °C	2.7 kg	

4.	Cool the reactor to -8±2 °C (internal temperature)		
5.	Charge Ammonia aqueous (25%) into the reactor keeping the internal temperature -8±2 °C	4.21 L	
6.	Charge pre-cooled (below 5 °C) Sodium hypochlorite solution (chlorine 8-10%) via addition funnel over 50-80 min into the reactor keeping the internal temperature -8±2 °C	47.4 L	
7.	Stir the contents of the vessel at -8±2 °C for 30 min		
8.	Stop the stirring and settle the reaction mixture for 30-60 min at -8±2 °C		
9.	Separate the layers		
10.	Wash the organic layer with pre-cooled (0-5 °C) Saturated sodium chloride solution	12 L	1.8 kg Sodium chloride dissolved 10 lit process water lot-1
11.	Take back the organic layer into pre cooled reactor to 0-5 °C		
12.	Charge powdered anhydrous $CaCl_2$	1.2 kg	
13.	Stir the reaction mixture at 0-5 °C for 10-15 min		
14.	Submit the sample to QC for the strength (keep the sample at 2-8 °C) & Moisture content		Approximate concentration of monochloramine solution 3-5% (Chlorine content), Moisture content NMT 0.5%
15.	Keep the reaction mixture in the reactor at -5 to 0 °C until to charge into Part-B		
16.	**Part-B: Preparation of pyrrolo[2,1-f][1,2,4]triazin-4-amine (RMD-B-II)**		
17.	Check the cleanliness of the reactor-2		
18.	Charge DMF (Lot-1) into the reactor under nitrogen atmosphere at 25-30 °C	6.0 L	
19.	Cool the reactor to 0-5 ºC		
20.	Charge Sodium hydride (60 % dispersion in mineral oil) into the reactor keeping the internal temperature between 0-5 ºC	0.29 kg	
21.	Stir the reaction mixture at 0-5 ºC for 20-30 min		
22.	Charge Pyrrole-2-carbonitrile into the reaction mixture maintaining the internal temperature at 5-10 ºC under nitrogen atmosphere	0.60 kg	Gas evolution observe
23.	Stir the reaction mixture at 0-5 ºC for 30-40 min under N_2 atmosphere		
24.	Charge above prepared Monochloramine solution in MTBE into the reaction mixture while maintaining the internal temperature between 0-5 ºC	24.0 L	Reaction mass becomes yellowish to brown colour

25.	Send the sample for HPLC analysis to check the consumption of Pyrrole-2-carbonitrile (IPC-1). **Sample preparation: Take the sample directly from reaction mixture and submit**		**IPC-1: Pyrrole-2-carbonitrile NMT 3.0%** **Result: IPC-01: Comp-2: 1.31%**
26.	After HPLC complies, charge Formamidine acetate at 0-5 °C	2.04 kg	
27.	Heat the reaction mixture 85-90 °C and simultaneously distil the MTBE at atmospheric pressure until internal temperature reaches to 85-90 °C		
28.	Stir the reaction mixture for 20 h at 85-90 °C		
29.	Send the reaction mixture sample for HPLC analysis to check the consumption of RMD-B-IIa **Sample preparation: Take the sample directly from reaction mixture and submit**		**IPC-2:** Comp-3 content NMT 2.0% Result: 0.14%
30.	After complies of the HPLC results, cool the reaction mixture at 25-30 °C		
31.	Filter the reaction mixture		
32.	Wash the bed with DMF (Lot-2)	0.6 L	
33.	Concentrate the filtrate containing DMF up to ~4V under reduced pressure at below 70 °C		
34.	Cool the reaction mixture to 25-30 °C		
35.	Charge the water slowly (Lot-2)	2.4 L	
36.	Stir the reaction mixture for 1 h at 25-30 °C		
37.	Cool the reaction mixture and further stir for 2.0 h at 5-10 °C		
38.	Stop the stirring, filter the reaction mixture		
39.	Wash the wet solid with water (Lot-3)	0.6 L	
40.	Suck dry the wet solid for 30-45 min		
41.	Slurry wash the wet solid with MTBE (Lot-2)	1.2 L	
42.	Suck dry the wet solid for 1 h		
43.	Unload the wet solid and charge into dryer		
44.	Dry the material for 5-6 h under vacuum at 50-55 °C still constant weight		
45.	Pack the dry material under nitrogen atmosphere in LDPE transparent bag and followed by LDPE black bag and tie with pp strap and keep in HDPE drum.		
46.	Store the material in dark and dry place at ambient temperature		
47.	Yield	0.69 kg	67%

In process controls data:

S. No.	Batch Id	IPC-1 (Limit: NMT 3.0 %)	IPC-2 (Limit: NMT 2.0 %)	Purity by HPLC
1	CR592-15374-10	**1.51%**	**Below the detection level**	IPC-1 IPC-2
2	CR592-15288-28	**1.31%**	**0.14%**	IPC-1 IPC-2

Results of two batches:

S. No	Batch No.	Input (g)	Output (g)	Molar Yield	Analytical data
1	CR592-15374-10	500.0	590.0	68%	CR592-15374-10
2	CR592-15288-28	600.0	690.0	67%	CR592-15288-28

*** Both the batches are of grey coloured and assay 96-97.5%**

In an effort to improve the colour and assay , both the batches were mixed and once again purified to afford the desired compound in light brown colour with assay 98.4%. Details are shown below.

Purification detail:

Crude Aminotriazine (550 g, B. No. CR595-15374-10 & 650 g B. No CR592-15288-28), and DMF (4.8 L) were heated to 80°C to afford a clear solution. Reaction mass was cooled to 25-30°C and water (18L) was added to get a thick slurry which was aged at same temperature for 1.0 h and cooled to 0-5°C and stirred for 4 h and filtered and washed with 1V (1.2 L) of MTBE and dried the solid under vacuum at 50-55 °C for 6h till constant weight

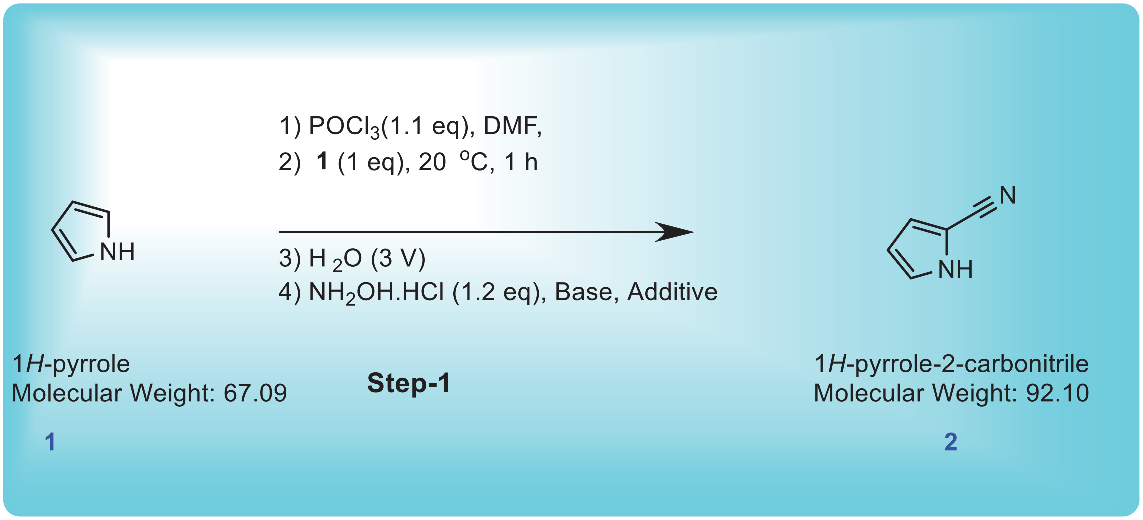
Y = 1050 g (87.5%)

Analytical data:

Triazine analytical data.pdf

Results and discussion

Optimization of Step-1:



General protocol: Without any base and additive

POCl3 (1.1 eq) was added to DMF (15V) slowly at 0°C for 30 min, Pyrrole (100 g, 1 eq) stirred for 1 h at 0 °C. RM was warmed to RT and H2O (3V) was added and stirred for 30 min and NH2OH.HCl (1.2 eq) was added and stirred at 70-80 °C.

Comparative data:

Batch No	Input	Output (Yield)	DMF (V)	Data
CR592-15288-5	100 g	126.3 g (92.0%)	15	Crude purity: 96% (by GC)-SMI-2.7%

CR592-15274-28	25.0 g	32.0 g (93.5%)	10	Crude purity: ~95 % (by GC)-SMI-3.5%
CR592-15274-29	25.0 g	30.3 g (88.3%)	5	Crude purity: 87 % (by GC)- SMI-9%

As the reaction performed well, it was further scaled up to 500.0 g scale using 10 V of DMF. Details are shown below.

Batch No	**Input** **(Pyrrole)**	**Out put- % yield**	**Remark/Analytical data**
CR592-15288-8	500.0g	853 g HPLC assay = 50 % Assay based yield = 62%	IPC-01: Pyrrole consumed completely IPC-02: after 12 h – Int-Aldehyde Consumed and could see 93.62% Product & SMI 2.57% (Amide impurity) pH of RM - 1-2 HPLC : 93.70%, SMI 1.57%. HPLC crude Assay: 49.4% **Observation:** RM was dark and found difficulty while layer separation. Aq. Layer contains product. Crude was very dark in colour

As the reaction showed difficulty in layer separation and formation of problematic black coloured solid impurities (probably polypyrrole and its derivatives), it was assumed that basic nature may help to prevent the formation of polymeric impurities, reaction was tried in presence of NaOAc and results are shown below.

General protocol: With NaOAc

POCl3 (1.1 eq) was added to DMF (10V) slowly at 0°C for 30 min, Pyrrole (1 eq) stirred for 1 h at 0 °C. RM was warmed to RT and H2O (3V) was added and stirred for 30 min and Sodium acetate (4 eq) and NH2OH.HCl (1.2 eq) was added and stirred at 70-80 °C.

Work up: RM was filtered added water (20V), aqueous layer extracted with EA (20V x 4). EA layer washed with saturated brine solution (20V) and concentrated the EA layer under reduced pressure.

Result:

Batch No	Input	Assay based Yield%	Solvent & Base	HPLC Purity	Remarks
CR592-15274-68	25.0 g	88	DMF (5 V), & Sodium acetate (4 eq)	94.14%, SMI 3.11%	Scale up from 25.0 g to 500.0 g produces consistent yield and purity
CR592-15274-75	500.0 g	92	DMF (5 V) & Sodium acetate (4 eq)	95.12%, SMI 2.25%	
Crude colour: Brown, layer separation was comparatively easy 					

Based on the initial result, reaction was scaled up to 1.0 Kg and details are shown below.

Batch No	Input (Pyrrole)	Out put- % yield	Remark/Analytical data

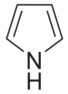
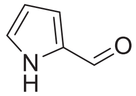
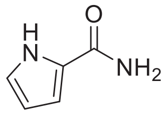
CR592-15288-22	1.0 Kg	1.3 Kg Assay based yield = 83%	IPC-01: No pyrrole was observed after 1h IPC-02: after 12 h HPLC 93.50%, SMI 2.57% **Observation:** Excessive black junk material formation was observed and did not observe clear layer separation using DCM. Modified work up protocol: Added chilled (5-10 °C) water (20V), and added MTBE (30V), stirred for 30 min and filtered the RM to remove the solid waste and separated the layers and further aqueous layer extracted with MTBE (10V) and MTBE layer was washed with 10% aqueous sodium bicarbonate solution (30V) and concentration of MTBE layer afforded the crude product Crude Purity by HPLC (area%): 93.66% Assay = 83% DMF assay = 0.1%

In an effort to further ease the isolation as well as scalable process, the reaction was tried in presence of Pyridine and acetic anhydride and comparative results are shown below.

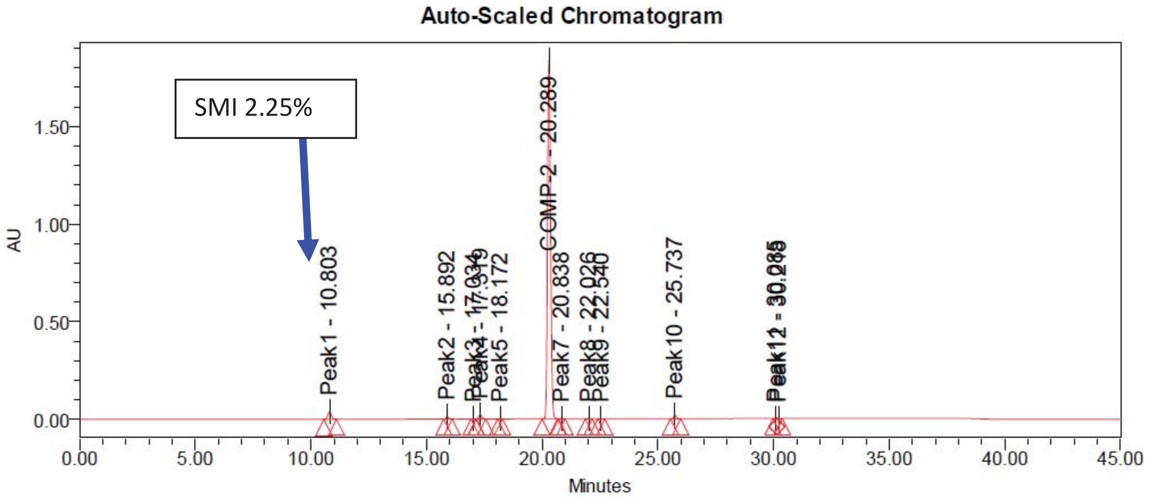
Batch Ref	Scale	IPC	Chemical purity (A%) by HPLC(after crude work up)	Yield/Assay	Remarks
CR592-15288-22 **(NaOAc batch)**	1 Kg	~94% product with SMI-2.5% (SM consumed)	94 %, SMI 2.36%	Assay based yield-83 % 1.288 Kg	More polymeric junk formation was observed
CR592-15288-25 **(Py/Ac2O)**	1 Kg	~98% product with SMI-0.8% (SM consumed)	98%, SMI 0.65%	Assay based yield-83 % 1.288 Kg	**Ease of work up** *

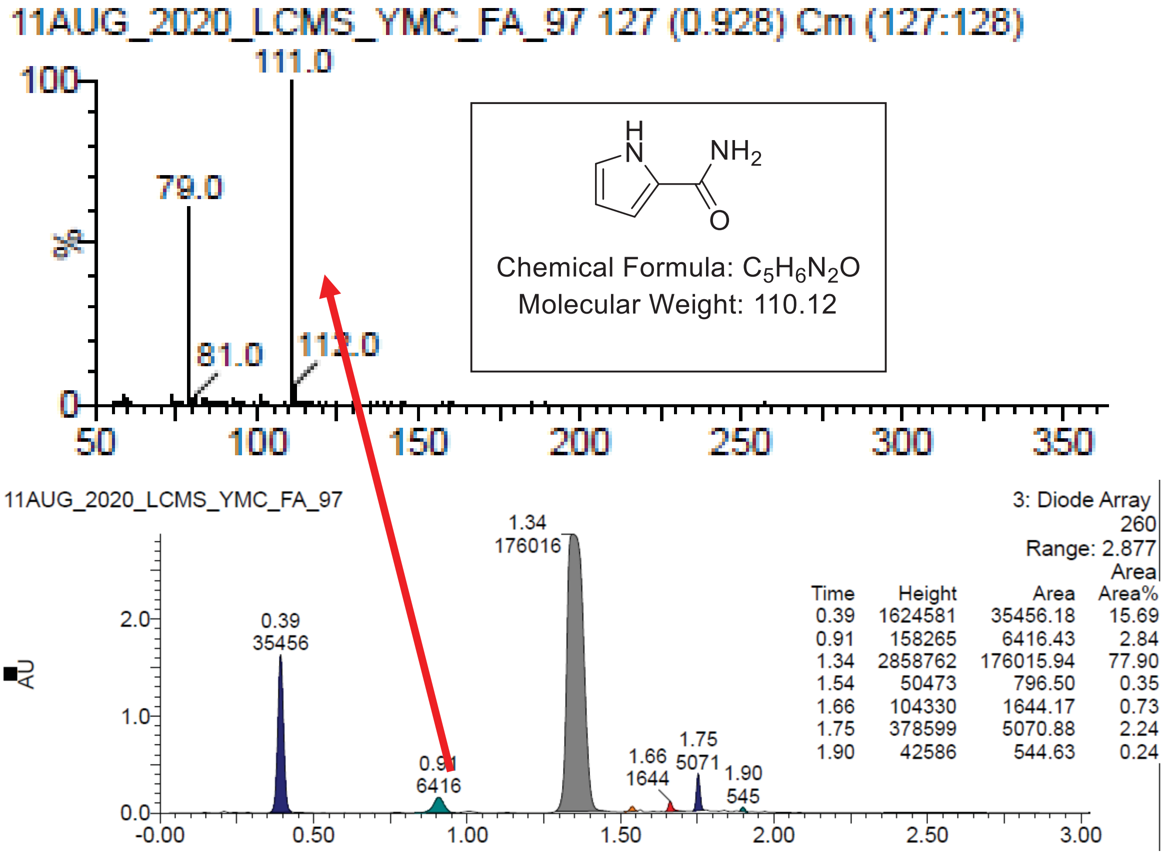
* No filtration of reaction mass which help to reduce the cycle time by minimizing one unit operation and eventually minimizes the handling loss of the product on scale up

Potential impurities and their origin

Name	Structure	Origin
Pyrrole		Process impurity/Un-reacted Starting material
1H-pyrrole-2-carbaldehyde		Process impurity/Un-reacted intermediate
1H-pyrrole-2-carboxamide		Process impurity/hydrolyzed product

1H-pyrrole-2-carboxamide has been proposed based on the LCMS data.



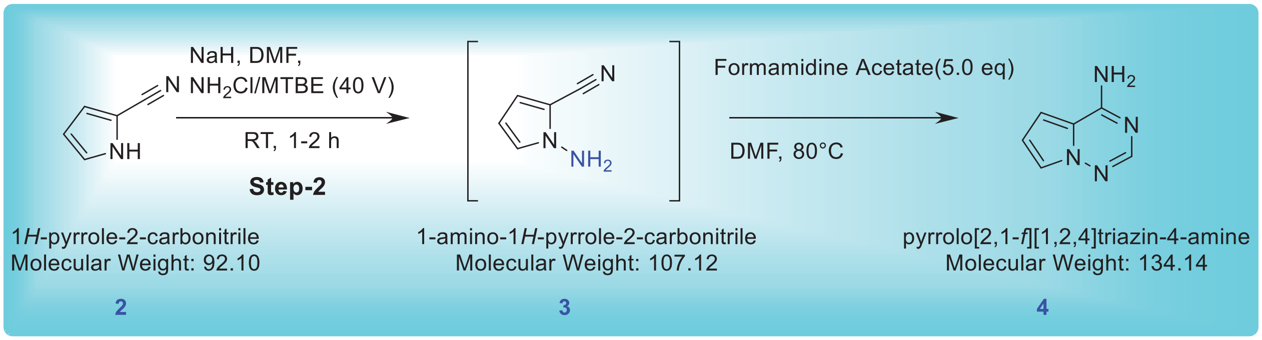


Chemical Formula: $C_5H_6N_2O$

Molecular Weight: 110.12

Time	Height	Area	Area Area%
0.39	1624581	35456.18	15.69
0.91	158265	6416.43	2.84
1.34	2858762	176015.94	77.90
1.54	50473	796.50	0.35
1.66	104330	1644.17	0.73
1.75	378599	5070.88	2.24
1.90	42586	544.63	0.24

Optimization of Step-2:



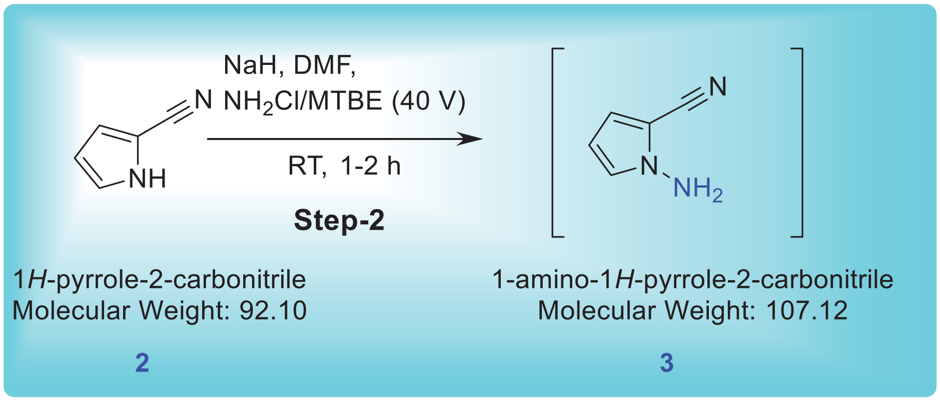
Screening of bases for the N-amination reaction:

Step-No/Batch No	Input Comp-2	Protocol	Remark/Analytical data
CR592-15274-73	25.0 g	To a stirred solution of 2-cyanopyrrole (1.0 eq) in DMF (10 V) was added **60%NaH (1.5 eq)** at 0-5 °C and the reaction was stirred for 30 min at 10-15 °C temperature. NH2Cl (50V of MTBE solution, assay 4.6%) was added via syringe at 10-15 °C.	IPC-01: **Comp-3 (92.85%), comp-2 (2.6%), SMI (3.37%)**
CR592-15274-64	2.0 g	**NaH (2.0eq).** Other parameters are same	IPC-01: **Comp-3 (85%), comp-2 (5%)**
CR592-15274-7	1.0 g	**K^{t}BuO (2.0 eq)**	IPC-01: **Comp-3 (26.5%), comp-2 (65.5%)**
CR592-15274-8	1.0 g	**Na^{t}OBu (2 eq)**	IPC-01: **Comp-3 (32%), comp-2 (57%)**
CR592-15274-9	1.0 g	**Na^{t}OAm (2 eq)**	IPC-01: **Comp-3 (38.8%), comp-2 (53%)**

CR592-15274-34	1.0 g	**KOH (1.5 eq)**	IPC-01: **Comp-3 (50%), comp-2 (46.6%)**
❑ **Above reactions showed that N-amination works well using NaH (1.5 eq) in DMF**			

Critical observation regarding excess addition of NH2Cl in the reaction mass:

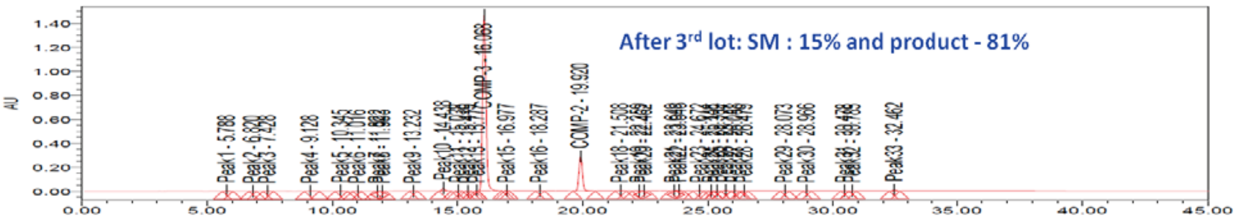
Following reaction was performed to assess the impact of addition of excess NH2Cl to push for the complete consumption of compound-2

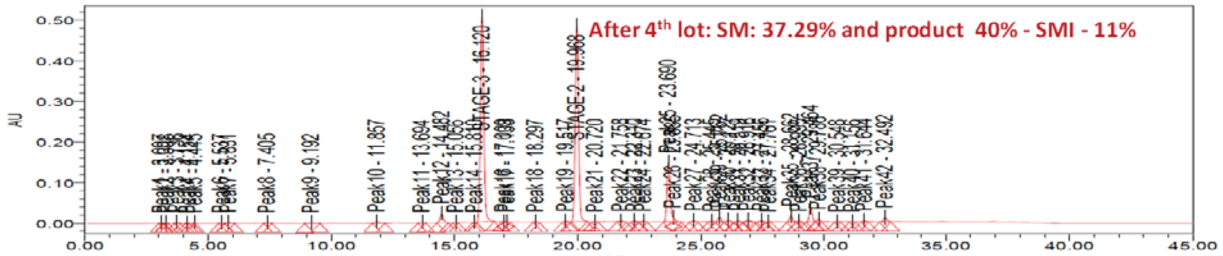


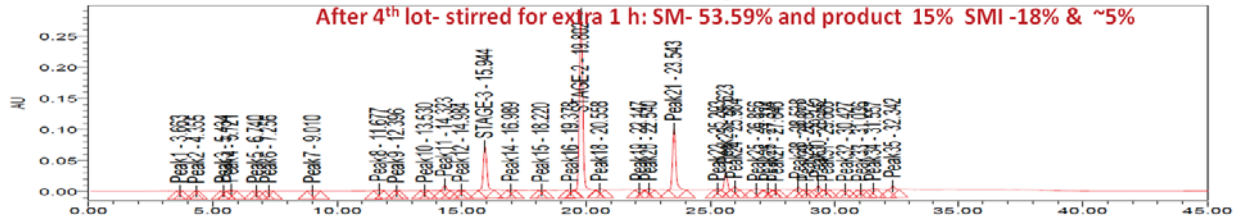
Batch No	**Input** **Comp-2**	**Protocol**	**Remark/Analytical data**
CR592-15274-59	1.0 g	To a stirred solution of 2-cyanopyrrole (1.0eq) in **DMF (10 V)** was added **60%NaH (1.5 eq)**and the reaction was stirred for 30 min at ambient temperature. NH2Cl (17V of MTBE solution, assay ~5.0%) was added via syringe. NH2Cl/MTBE was added lot wise (17V) after distilling MTBE	IPC data showed steady decease of SM up to 3 rd lot addition of NH2Cl. Afterwards degradation of product formed was observed. Needs to be investigated before scale up.

NH2Cl-MTBE solution	% of Un-reacted SM	% Product	Major Impurities
After Lot-1 addition	64%	30%	
After Lot-2 addition	17%	74%	
After Lot-3 addition	**15%**	**81%**	

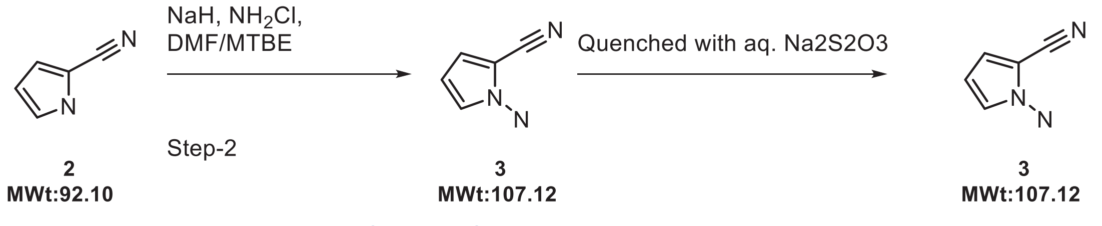
After Lot-4 addition	53%	15%	18% & 5%





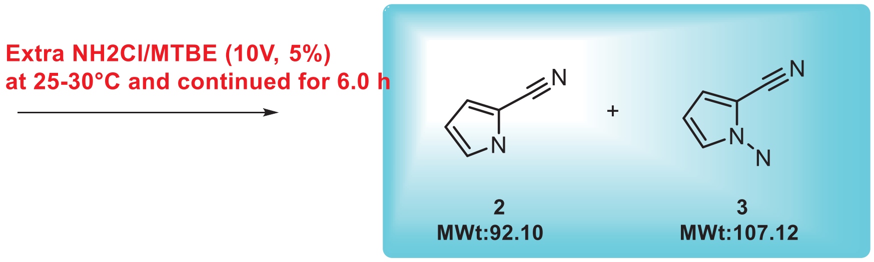


To prove the hypothesis that compound-3 reacts with excess NH2Cl in absence of base to generate impurities, compound-3 was isolated by quenching the reaction with aq. Na2S2O3 and reacted with NH2Cl. Observation of the reaction is shown below.

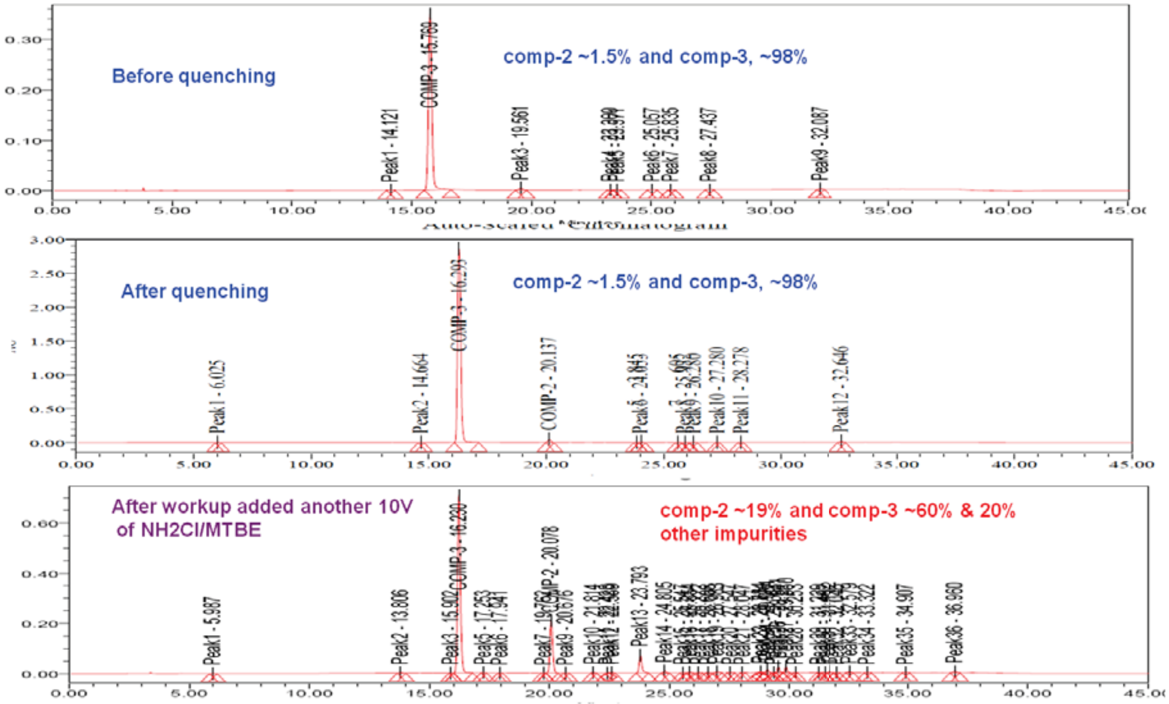


Comp-2: Comp-3: 1.23: 97.84

Comp-2: Comp-3: 1.47: 97.46



Comp-2: Comp-3: 20: 60 & 20% other multiple impurities



Excess NH2Cl generates back compound-2 along with other impurities.

So after 80-90% conversion of compound-2, excess NH2Cl should be added with extreme care. It needs further optimization.

Trail batches data of final Target compound synthesis

General reaction protocol:

To a stirred solution of 2-cyanopyrrole (1.0eq) in **DMF (10 V)** was added **60%NaH (1.5 eq)** at 0-5 °C and the reaction was stirred for 30 min at 10-15 °C temperature. **NH2Cl (50V of MTBE solution, assay 3.5%)** was added **at 10-15 °C.** Stirred 15 min at 10-15 °C, RM was monitored and Comp-2 ~1.51%, comp-3 ~97.4%. Added formamidineacetate (5 eq, Avra- Commercial Vendor) and distilled off MTBE (by warming the RM from 25 to 90°C under atmospheric condition) and continue stirring at the same temperature for 20 h.

Work up: RM cooled to 25-30°C and filtered to remove undissolved formamidine. Then distilled off DMF (~6V) and water (4 V) was added to afford slurry which was cooled to 5-10°C and aged for 3 h and filtered and waashed with water (2V) and MTBE (2V) and dried under vacuum to afford the target compound.

Summary of the lab batches data:

Batch No	**Input Comp-2**	**IPC-01**	**IPC-02**	**Yield (%)**	**Purity (Area%)**	**Remark**
CR592-15374-10	500.0 g	Comp-2: 1.5% Comp-3: 97%	Comp-3: BDL	68	99.95 Assay: 96%	**With Formamidine acetate from Avra (Assay 95%)**

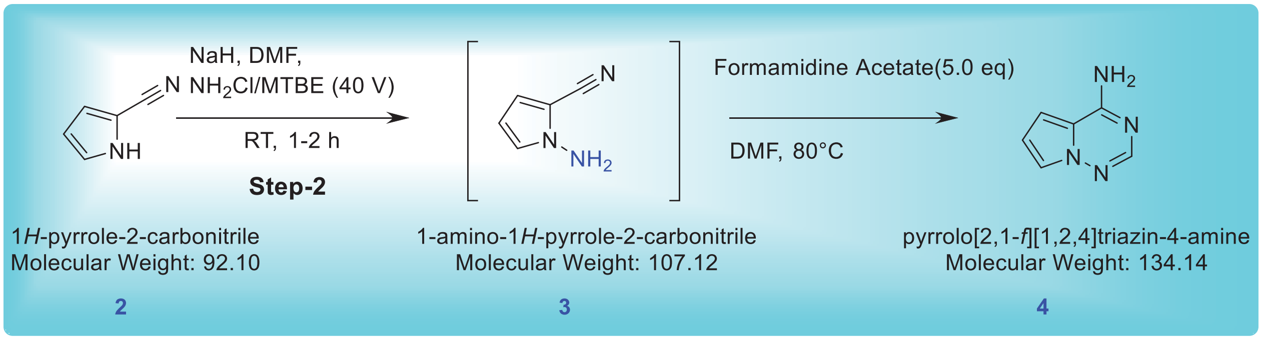
CR592-15274-85	100.0 g	Comp-2: 1.31%, Comp-3: 95.80%	Comp-3: 0.13%	50	99.44 Assay 97.8%	**With Formamidine acetate from Avra (Assay 78%)**
CR592-15274-83	25.0 g	Comp-2: 0.57%, Comp-3: 96.85%	Comp-3: 0.10%	66	99.89 Assay 100%	**With formamidine acetate from Aldrich(Assay 99%)**

In an effort to further reduce the reagent quantities, NaH (1.1 eq vs 1.5 eq) and Formamidine acetate (3.0 eq vs 5 eq) quantities were reduced and the following batches were performed.

Batch No	Input Comp-2	IPC-01	IPC-02	Yield (%)	Purity (Area %)	Remark
CR592-15365-8	5.0 g	Comp-2: 0.42% Comp-3: 96.5%	Comp-3: BDL Target Comp-92%	5.0 g (69)	99.80%	**Based on the result another 600.0 g batch was performed**
CR592-15288-28	600.0 g (100% Assay basis) **CR592-15306-14-F2** 260.0 g /Assay 98% **CR592-15288-25-F3**	Comp-2: 1.31% Comp-3: 98.22%	Comp-3 0.14%, Target 89.11%	670.0 g (77%),	HPLC (Area%) Purity: 99.87% Assay = 97.50%,	KF = 0.45% Colour grey

	165.0 g /Assay 95% **CR592-15306-15-F2** 189.0 g /Assay 101%					

Impurity profiling of the aminotriazine:



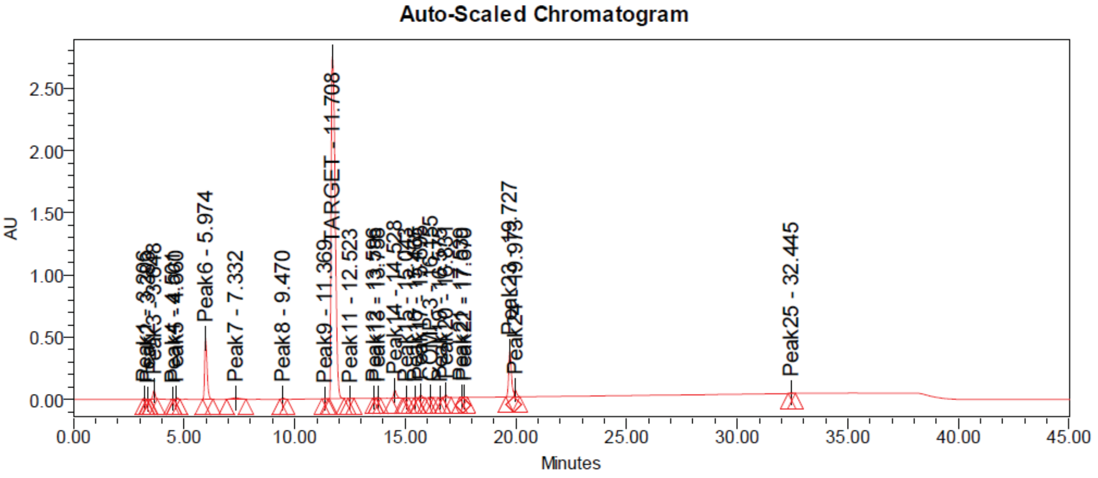
Step-No/Batch No	**Input Comp-2**	**Protocol**	**Remark/Analytical data**
CR592-15274-73	25.0 g	To a stirred solution of 2-cyanopyrrole (1.0eq) in **DMF (10 V)** was added **60%NaH (1.5 eq)** at 0-5 °C and the reaction was stirred for 30 min at 10-15 °C temperature. **NH2Cl (50V of MTBE solution, assay 4.6%)** was added via syringe **at 10-15 °C. After comp-2 below 5%,** distilled off the MTBE at 25-30 °C and added **formamidineacetate (5 eq)** and warmed to **80-85°C** and stirred for 20 h. **Work up:** Cooled the RM to below 30°C and added water (20V), solid precipitation was observed. Stirred 1 h at 20-25 °C.	IPC-01: **Comp-3 (92.85%), comp-2 (2.6%), SMI (3.37%)** Added Foramidineacetate IPC-02: after 20 h Target- 78.4%, Comp-3 (0.11%), SMI (8.5%) HPLC purity of Targeted compd: 99.79%, SMI 0.15%, **Yield : 25.0 g (70~%)** KF: 0.96%

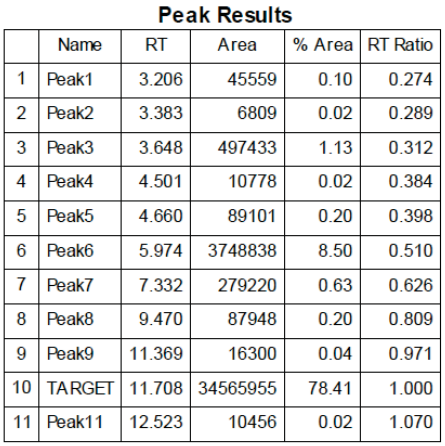
		Filtered the solid. Washed the wet solid with MTBE (1V) and dried under vacuum.	

IPC-2:

S A M P L E   I N F O R M A T I O N

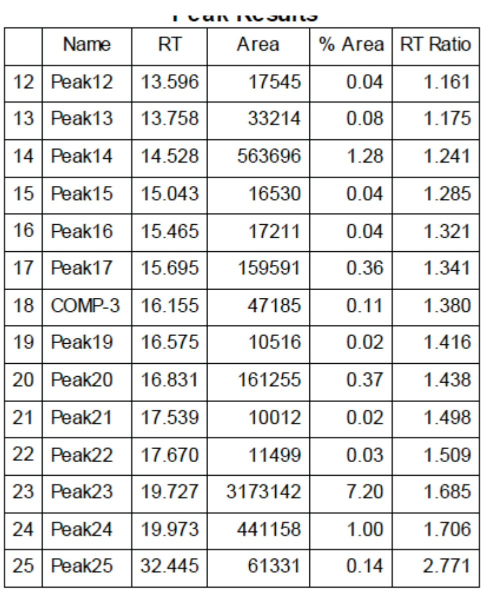
Sample Name:	CR592-15274-73-RM2-5	Acquired By:	AM0113345
Sample Type:	Unknown	Sample Set Name:	TCGLS_100820
Vial:	3	Acq. Method Set:	REM_KSM_DEV_2
Injection #:	1	Processing Method	REM KSM
Injection Volume:	10.00 ul	Channel Name:	230.0nm
Run Time:	45.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 230.0 nm (2998
Date Acquired:	10-08-2020 08:26:01 IST	Column Name:	SHIMPACK SOLAR C18 (250X4.6)5u
Date Processed:	12-08-2020 10:45:50 IST		





Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	3.206	45559	0.10	0.274
2	Peak2	3.383	6809	0.02	0.289
3	Peak3	3.648	497433	1.13	0.312
4	Peak4	4.501	10778	0.02	0.384
5	Peak5	4.660	89101	0.20	0.398
6	Peak6	5.974	3748838	8.50	0.510
7	Peak7	7.332	279220	0.63	0.626
8	Peak8	9.470	87948	0.20	0.809
9	Peak9	11.369	16300	0.04	0.971
10	TARGET	11.708	34565955	78.41	1.000
11	Peak11	12.523	10456	0.02	1.070



Peak Results

	Name	RT	Area	% Area	RT Ratio
12	Peak12	13.596	17545	0.04	1.161
13	Peak13	13.758	33214	0.08	1.175
14	Peak14	14.528	563696	1.28	1.241
15	Peak15	15.043	16530	0.04	1.285
16	Peak16	15.465	17211	0.04	1.321
17	Peak17	15.695	159591	0.36	1.341
18	COMP-3	16.155	47185	0.11	1.380
19	Peak19	16.575	10516	0.02	1.416
20	Peak20	16.831	161255	0.37	1.438
21	Peak21	17.539	10012	0.02	1.498
22	Peak22	17.670	11499	0.03	1.509
23	Peak23	19.727	3173142	7.20	1.685
24	Peak24	19.973	441158	1.00	1.706
25	Peak25	32.445	61331	0.14	2.771

Two major impurities were observed.

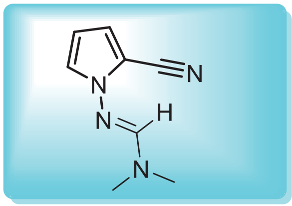
5.97 min RT ~9% which corresponds to DMF

19.727 min RT ~7%

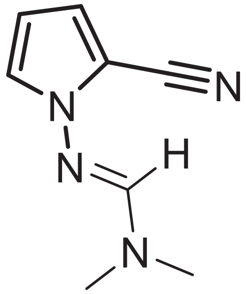
Impurity @ 19.727 min got enriched in the MTBE layer and isolated from the same and characterized by 1H & 13CNMR, LCMS.

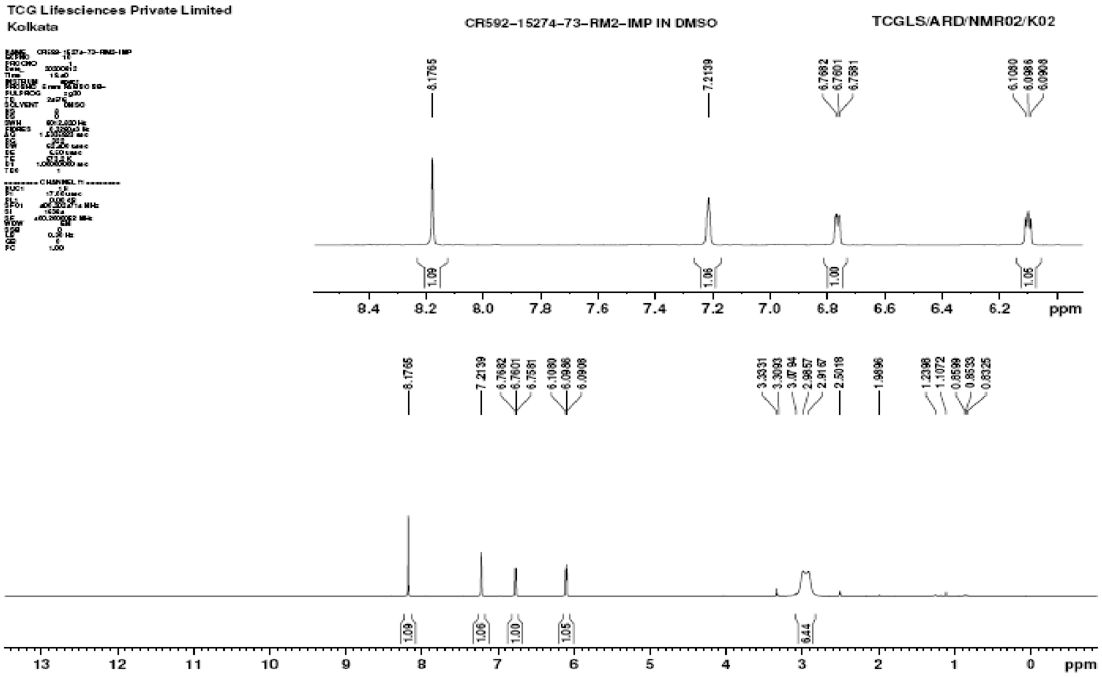
It was further synthesized and RT was matched.

Structure of the impurity is shown below

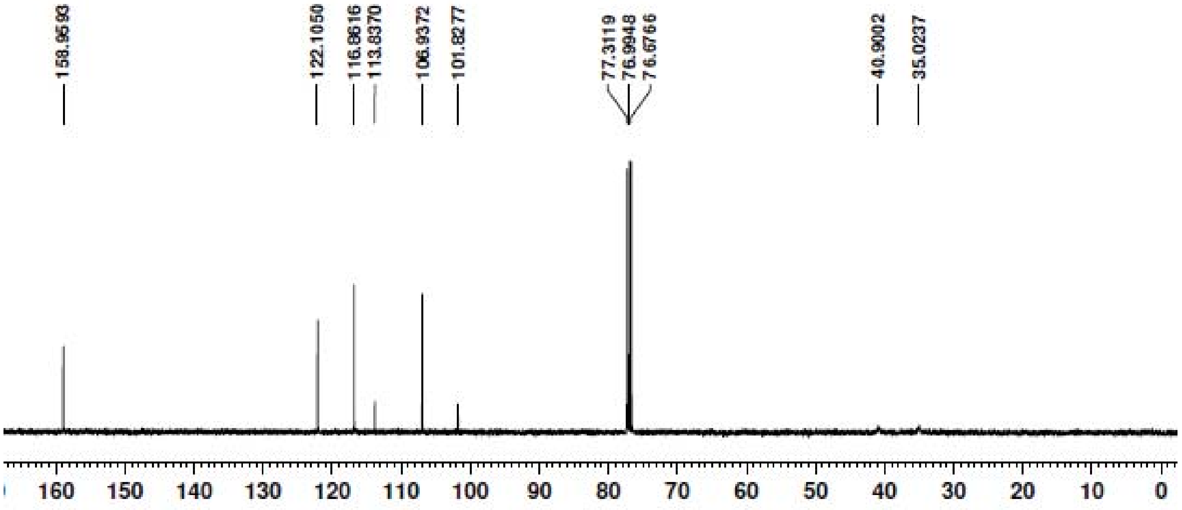


Molecular Weight: 162.20

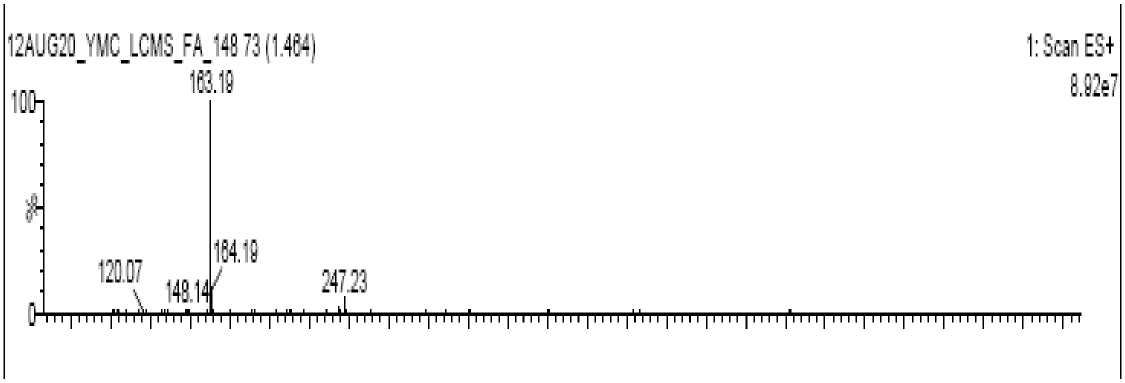



TCG Lifesciences Private Limited
Kolkata
CR592–15274–73–RM2–IMP IN DMSO
TCGLS/ARD/NMR02/K02
8.1765
7.2139
6.7682
6.7601
6.7581
6.1080
6.0986
6.0908
1.09
1.06
1.00
1.05
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
ppm
3.3331
3.3093
3.0794
2.9857
2.9167
2.5018
1.9896
1.2398
1.1072
0.8599
0.8533
0.8325
6.44
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

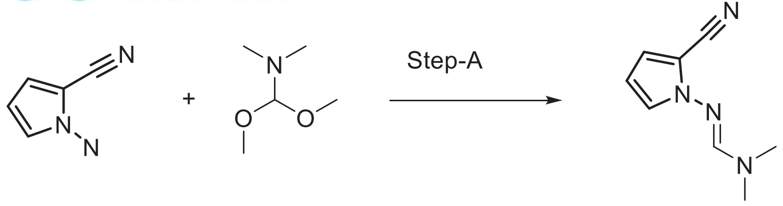
13C NMR



LCMS:



Synthesis of the impurity @ 19.727 min using literature protocol



3
MWt:107.12

DMFDMA
M. Wt: 119.16

DMF-Impurity
MWt: 162

Protocol:

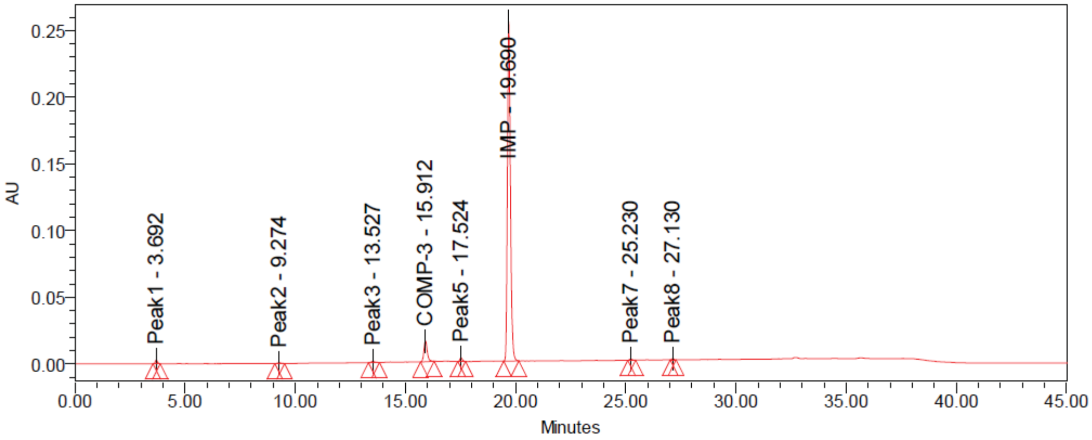
Comp-3 (1.0 eq, 5.0 g), $MgSO_4$ (5.0eq), AcOH (1.0 eq), DMF-DMA (1.3 eq), DCM (11.0V), N_2, 25-30°C, 12 h comp-3 converted to DMF-Impurity (As monitored by HPLC, 92% and ~6% comp-3)

Ref: Organic Letters, 22(3), 1217-1221; 2020

SAMPLE INFORMATION

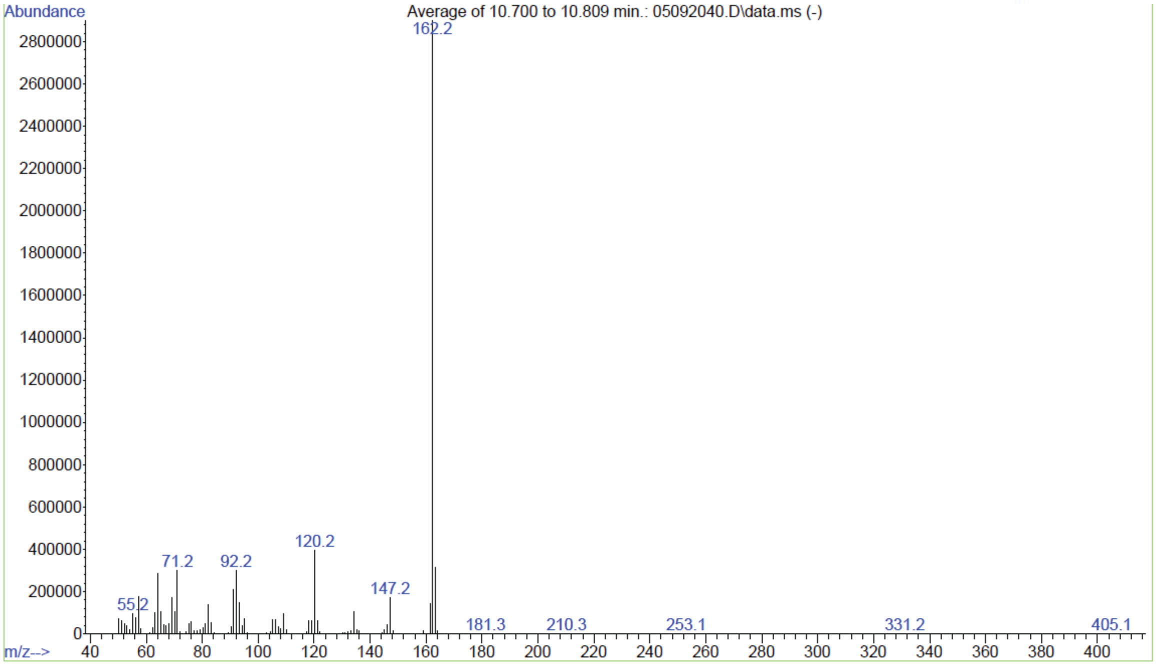
Sample Name:	CR592-15380-01-cr	Acquired By:	AM0113345
Sample Type:	Unknown	Sample Set Name:	TCGLS_050920
Vial:	37	Acq. Method Set:	REM_KSM_DEV_2
Injection #:	1	Processing Method	REM KSM
Injection Volume:	10.00 ul	Channel Name:	250.0nm
Run Time:	45.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 250.0 nm (2998
Date Acquired:	05-09-2020 18:17:43 IST	Column Name:	SHIMPACK SOLAR C18(250*4.6)5µ
Date Processed:	08-09-2020 07:03:05 IST		

Auto-Scaled Chromatogram

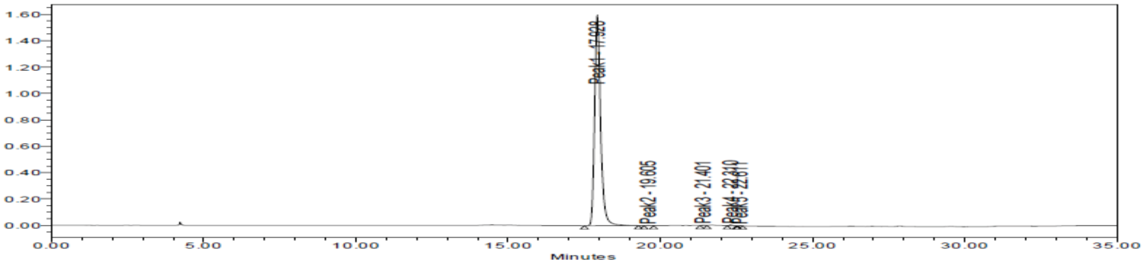


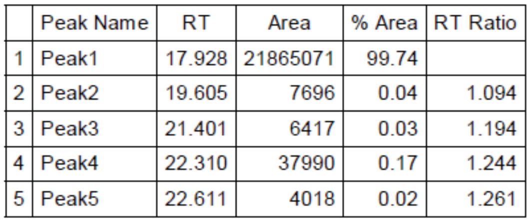
Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	3.692	18401	0.70	0.232
2	Peak2	9.274	6462	0.25	0.583
3	Peak3	13.527	9076	0.35	0.850
4	COMP-3	15.912	163559	6.24	1.000
5	Peak5	17.524	20891	0.80	1.101
6	IMP	19.690	2388408	91.14	1.237
7	Peak7	25.230	7743	0.30	1.586
8	Peak8	27.130	6063	0.23	1.705

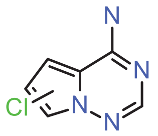


In few batches another impurity was observed at 1.3 RRT which could not be isolated but analyzed by LCMS and data is shown below.

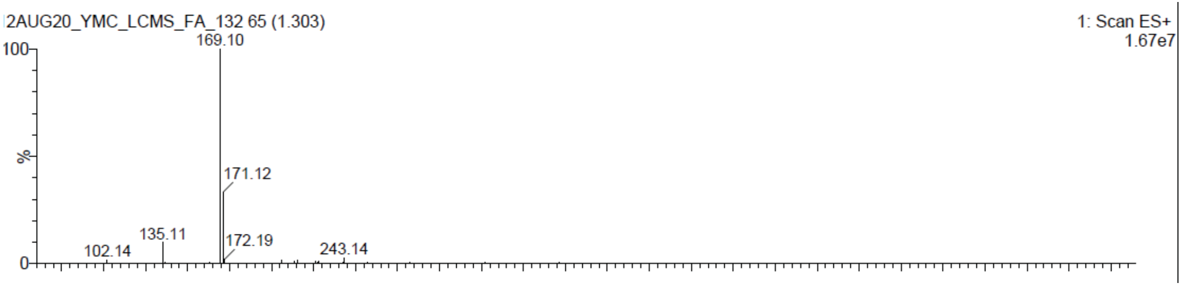




	Peak Name	RT	Area	% Area	RT Ratio
1	Peak1	17.928	21865071	99.74	
2	Peak2	19.605	7696	0.04	1.094
3	Peak3	21.401	6417	0.03	1.194
4	Peak4	22.310	37990	0.17	1.244
5	Peak5	22.611	4018	0.02	1.261



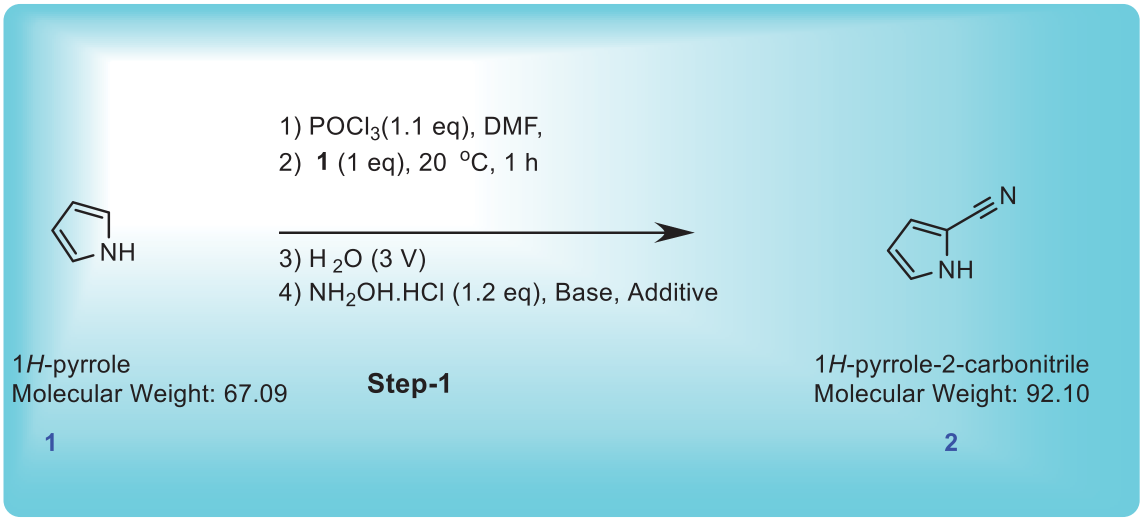
MW 168, 170



This impurity most probably formed due to presence of un-reacted NH2Cl which can act as chlorinating agent.

Safety studies of the finalized scheme: RC-1 studies

Step-1



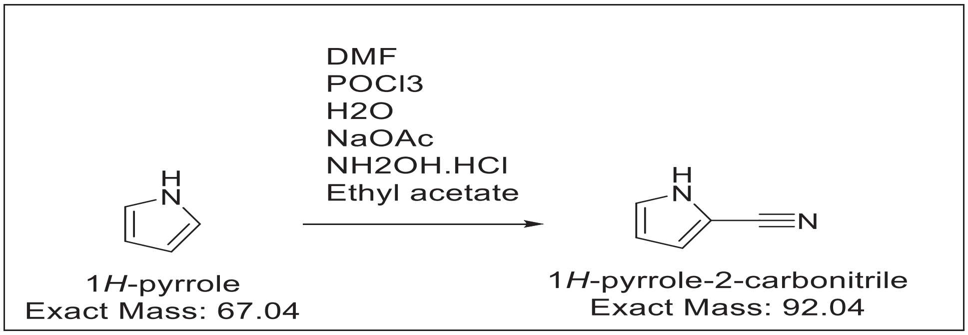
1

2

RC-1 report using Py/Ac2O and 10 of DMF

1.0 Subject/Aim: To evaluate the thermal risk factors i.e. heat of reaction, Adiabatic Temperature and MTSR (Maximum temperature of the synthesis reaction).

2.0 Route of synthesis (ROS)



3.0 Brief Process

1.0 Reaction: Charged DMF (as per B.Size : 50 grams) into the reactor at 25-30°C then Cool the solvent to 0°C. Slowly added POCL3 into the reaction mass at 0°C after addition maintained the reaction mass for 30 min.

Slowly added 1H-pyrrole into the Reaction mass at 0°C after addition maintained the reaction mass for 60 min. Drop wise added water into the reaction mass then maintained the mass for 30 min.

Added Acetic anhydride followed by pyridine at 0°C then maintained the reaction mass for 10 min after that added NH2OH.HCl to the reaction mass at 0°C and maintained for 30 min. Charged in 4 equal lots of Sodium acetate into the reaction at 0°C and maintained the mass for 30 min.

4.0 Raw materials used:

S.No	Raw materials Name	Molecular weight	Quantities	Equiv.
1	1H-pyrrole	67.09	50.0 g	1.0
2	POCl3	153.33	125.70 g	1.1
3	DMF	73.09	500 ml	10V
4	Water	18.00	150 ml	3V
5	NH2OH.HCl	69.49	62.15 g	1.2
6	Pyridine	79.10	295 g	5.0
7	Acetic anhydride	102.09	91.30 g	1.2

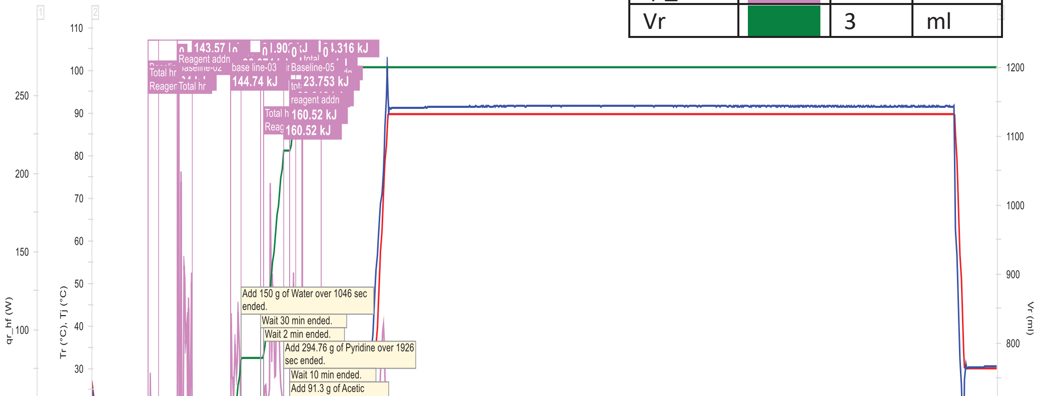
5.0 RC1 Study: Process Observations: B.Size 50 grams of 1H-pyrrole

Process	Observations
Charged DMF (500 ml)into the reactor at 25°C	Homogeneous clear solvent
Cool the reaction mass to 0°C	Homogeneous clear solvent
Calibrate it to find out UA & Cp values	Establish the base line and predict specific heat.
Slowly added POCl3 (125.70 g) into the reactor at 0°C over 15 min.	Homogeneous mass with light yellow color Observed high temperature raise during addition, Controlled addition is to be done. Gas liberation observed during addition
Maintain the reaction mass at 0°C over 30 min.	Homogeneous mass with light yellow color Reagent thermal accumulation at the end of dosing is 1.80%.
Drop wise added 1H-pyrrol (50 g) into the reactor at 0°C.	Homogeneous mass with dark orange color. Over all addition should be controlled even single drop addition shows high rise in reaction temperature.
Maintain the reaction mass at 0°C over 60 min.	Homogeneous mass with dark orange color. Reagent thermal accumulation at the end of dosing is 0.80%.

Drop wise added water (150 ml) into the reactor at 0°C.	Homogeneous mass with dark orange color. Over all addition should be controlled even single drop addition shows high rise in reaction temperature.
Maintain the reaction mass at 0°C over 60 min.	Homogeneous mass with dark orange color. Reagent thermal accumulation at the end of dosing is 1.86%.
Drop wise added pyridine (295 g) over 32 min into the reactor at 0°C and maintained for 10 min.	Homogeneous mass with dark orange color. Reagent thermal accumulation at the end of dosing is 0%.
Drop wise added Acetic Anhydride (91.30 g) over 10 min into the reactor at 0°C and maintained for 10 min.	Homogeneous mass with dark orange color. Reagent thermal accumulation at the end of dosing is 1.80%.
Slowly added NH2OH.HCl (62.15 g) into the reactor at 0°C.	Heterogeneous mass with dark orange color Initially heat of reaction is endothermic after addition shows exothermic reaction Addition should be in lot wise addition
Maintain the reaction mass at 0°C over 10 min.	Heterogeneous mass with dark orange color Reagent thermal accumulation at the end of dosing is 96.50%.
Calibrate it to find out UA & Cp values	Establish the base line and predict specific heat.
Heat the reaction mass to 90°C.	Heterogeneous mass with dark yellow color
Maintain the reaction mass at 90°C over 12 hr.	During heating maintenance dark yellow color turns to blackish color with heterogeneous mass nature There is no abnormal heat liberation was observed Heating maintenance was in smooth run and stable temperature.
Cool the reaction mass to 30°C.	Remains blackish color with heterogeneous mass nature
Unloaded the reaction mass	Remains blackish color with heterogeneous mass nature

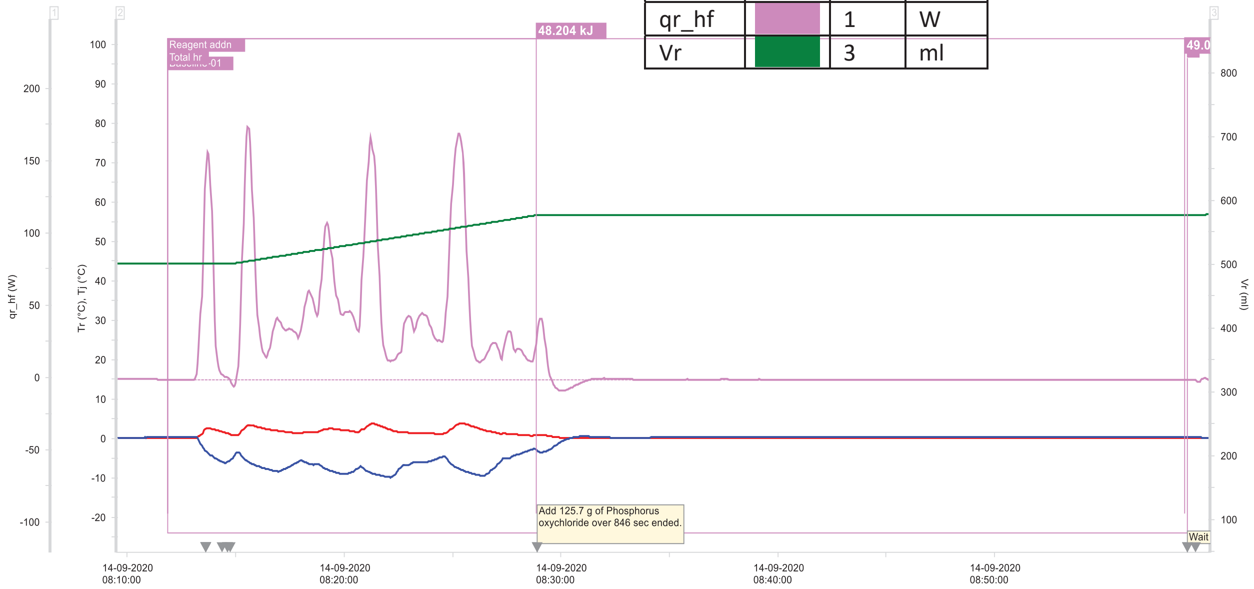
RC1 Graph: Overall Reaction

Trend	Color	Axis	Units
Tr	🟥	2	°C
Tj	🟦	2	°C
qr_hf	🟪	1	W
Vr	🟩	3	ml



RC1 Graph: POCl3 addition shows Exotherm

Trend	Color	Axis	Units
Tr	🟥	2	°C
Tj	🟦	2	°C
qr_hf	🟪	1	W
Vr	🟩	3	ml

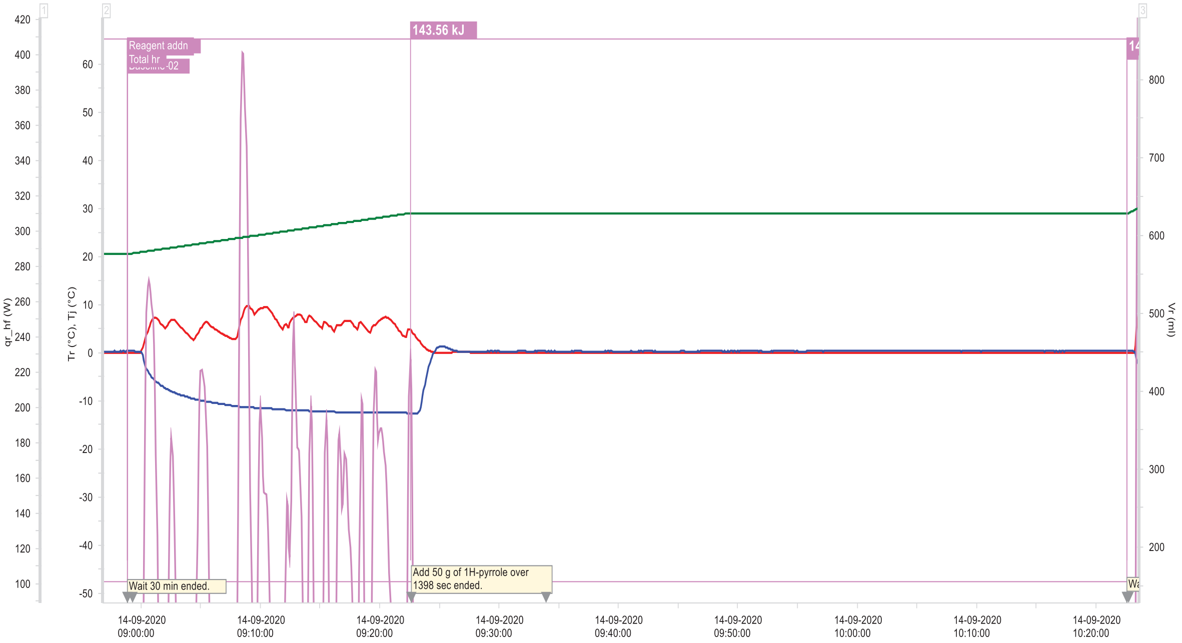


Add 125.7 g of Phosphorus oxychloride over 846 sec ended.

Rc1e Technical Data: During addition of POCL3 at 0°C.

RC parameters	Results	Remarks/Recommendations
Heat generated	49.08 KJ/ 50 grams of KSM 65.88 KJ/mole of KSM	Heat of reaction shows is mild exothermic considerable adiabatic temp rise is 30.40 °C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	30.40	Adiabatic temp rise shows medium hazard in reaction, controlled addition to be done
MTSR (°C) (As per 100% accumulation)	30.40°C (0 +30.40)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of reaction is less than MTT, so reaction safe for scale-up point of view
Thermal Accumulation at the end of Dosing	1.80 %	End of the dosing thermal accumulation is Considerable low
Thermal Conversion at the end of Dosing	98.20%	End of the dosing thermal Conversion is high
Specific Heat Before Reaction (kJ/Kg K)	2.83	Specific heat of reaction is high and it can accomplish medium heat transfer rate and slowly achieving temp due to high energy requirement.
Specific Heat After Reaction (kJ/Kg K)	1.95	

RC1 Graph: 1H-pyrrole addition shows Exotherm



Rc1e Technical Data: During addition of **1H-pyrrole** at 0°C.

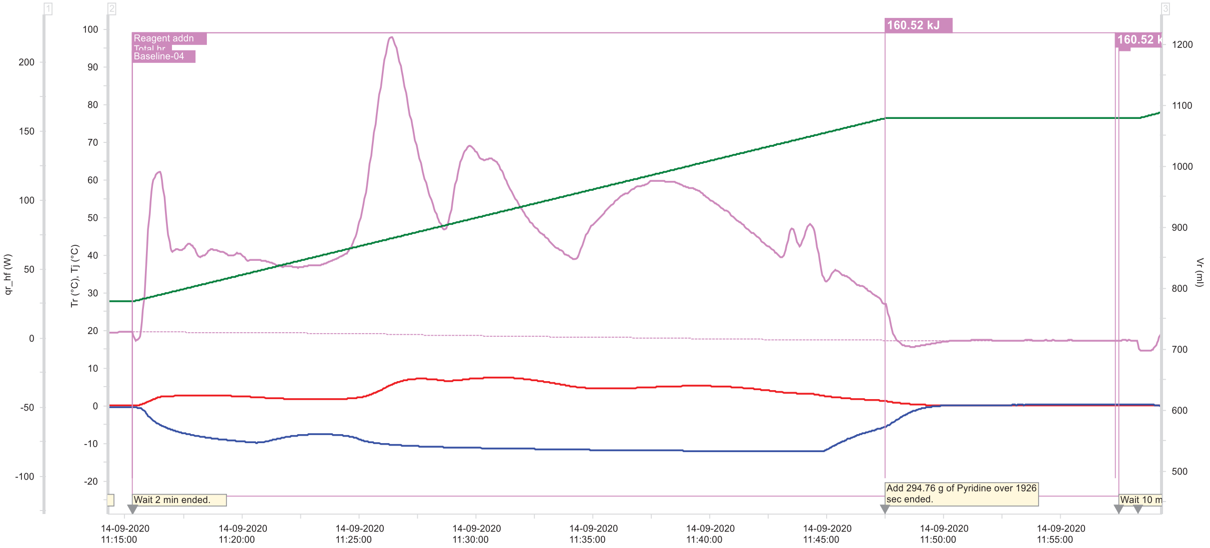
RC parameters	Results	Remarks/Recommendations
Heat generated	144.73 KJ/ 50 grams of KSM 194.27 KJ/mole of KSM	Heat of reaction shows is exothermic considerable adiabatic temp rise is 84.54 °C. The addition should be controlled due exothermic reaction.
Adiabatic temp rise (K)	84.54	Adiabatic temp rise shows medium hazard in reaction, controlled addition to be done
MTSR (°C)	84.54°C (0 + 84.54)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of the reaction is greater than MTT
Thermal Accumulation at the end of Dosing	0.80 %	End of the dosing thermal accumulation is Considerable low
Thermal Conversion at the end of Dosing	99.20 %	End of the dosing thermal Conversion is high
Specific Heat Before Reaction (kJ/Kg K)	2.83	Specific heat of reaction is high and it can accomplish medium heat transfer rate and achieving temp due to high energy requirement.
Specific Heat After Reaction (kJ/Kg K)	1.95	

RC1 Graph: Water addition shows Exotherm

Rc1e Technical Data: During addition of **water** at 0°C.

RC parameters	Results	Remarks/Recommendations
Heat generated	91.90 KJ/ 50 grams of KSM 123.35 KJ/mole of KSM	Heat of reaction shows is mild exothermic considerable adiabatic temp rise is 46.63°C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	46.63	Adiabatic temp rise shows medium hazard in reaction, controlled addition to be done
MTSR (°C)	46.63°C (0 +46.63)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of the reaction is less than MTT
Thermal Accumulation at the end of Dosing	1.86 %	End of the dosing thermal accumulation is Considerable low
Thermal Conversion at the end of Dosing	98.14 %	End of the dosing thermal Conversion is high
Specific Heat Before reaction (kJ/Kg K)	2.83	Specific heat of reaction is high and it can accomplish medium heat transfer rate and slow achieving temp due to high energy requirement.
Specific Heat After reaction(kJ/Kg K)	1.95	

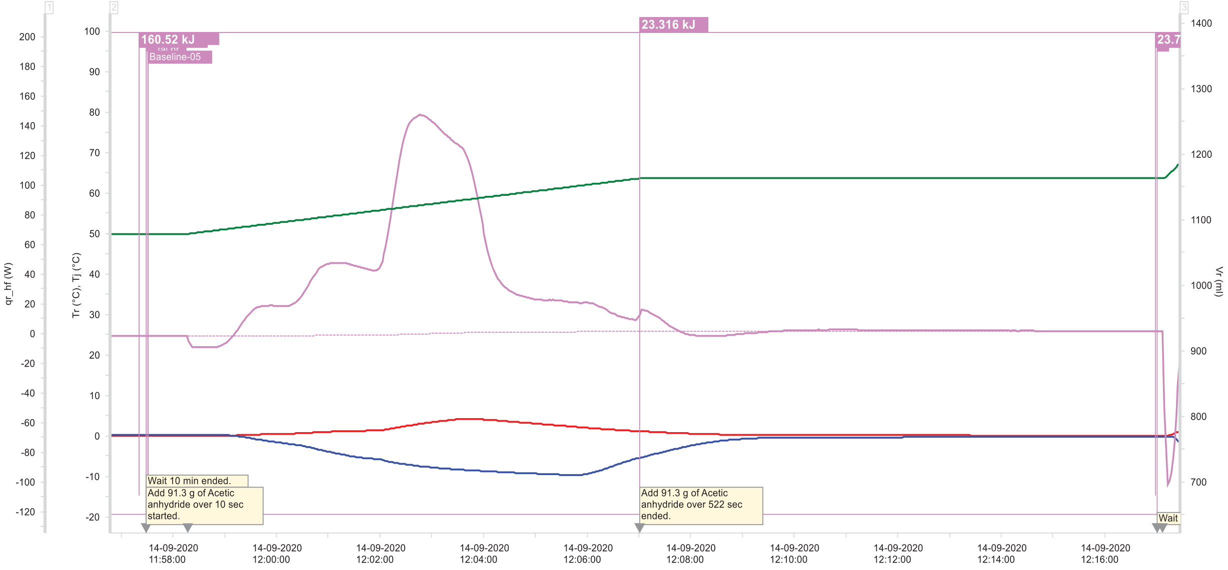
RC1 Graph: Pyridine addition shows Exotherm



Rc1e Technical Data: During addition of **Pyridine at** 0°C.

RC1 Graph: Acetic Anhydride addition shows Exotherm

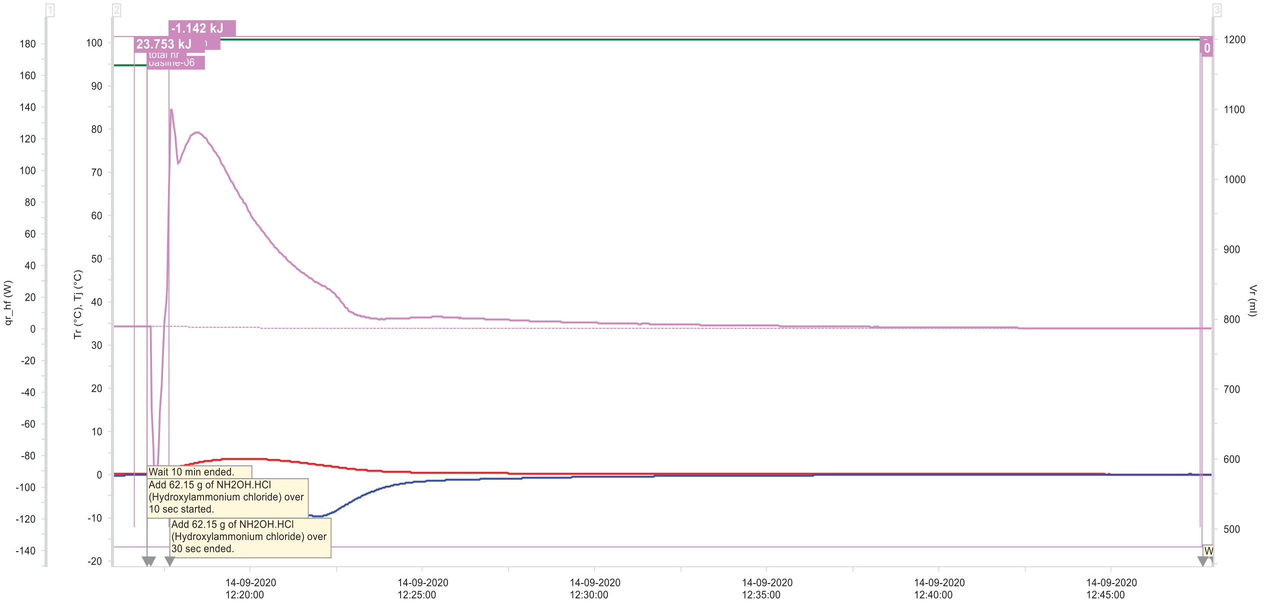
RC parameters	Results	Remarks/Recommendations
Heat generated	160.52 KJ/ 50 grams of KSM 215.46 KJ/mole of KSM	Heat of reaction shows is exothermic considerable adiabatic temp rise is 68.85°C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	68.85	Adiabatic temp rise shows medium hazard in reaction, controlled addition to be done
MTSR (°C)	68.85°C (0 +68.85)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of the reaction is greater than MTT
Thermal Accumulation at the end of Dosing	0 %	End of the dosing thermal accumulation is Considerable low
Thermal Conversion at the end of Dosing	100 %	End of the dosing thermal Conversion is high
Specific Heat Before reaction (kJ/Kg K)	2.83	Specific heat of reaction is high and it can accomplish medium heat transfer rate and achieving temp due to high energy requirement.
Specific Heat After reaction(kJ/Kg K)	1.95	



Rc1e Technical Data: During addition of **Acetic Anhydride** at 0°C.

RC1 Graph: NH2OH.HCl addition shows Exotherm

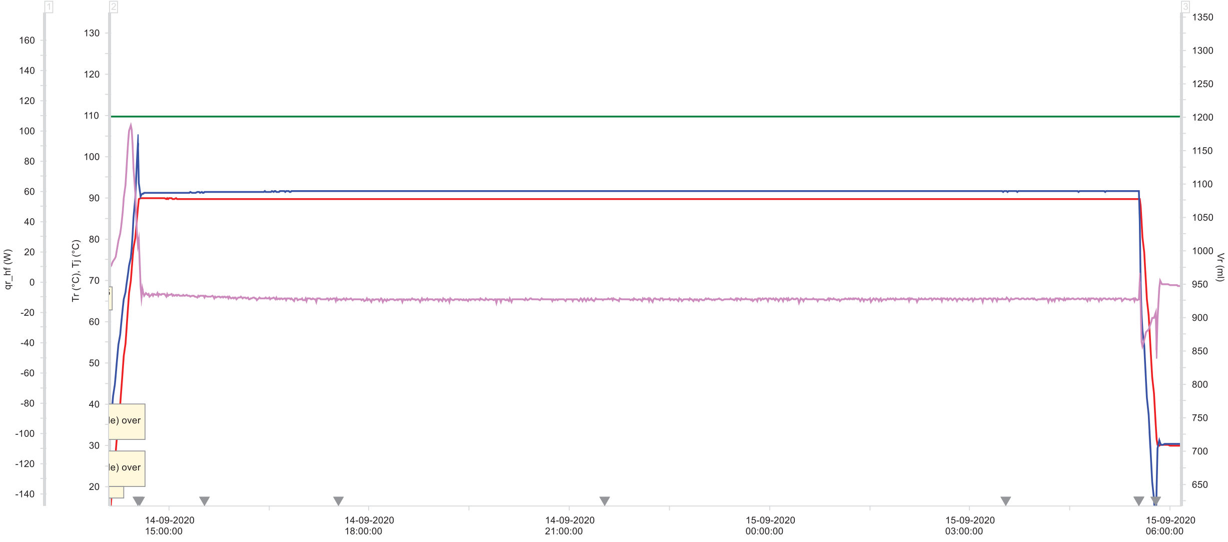
RC parameters	Results	Remarks/Recommendations
Heat generated	23.75 KJ/ 50 grams of KSM 31.88 KJ/mole of KSM	Heat of reaction shows is mild exothermic nature considerable adiabatic temp rise is 9.88°C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	9.88	Adiabatic temp rise shows low hazard in reaction, controlled addition to be done
MTSR (°C)	9.88°C (0+9.88)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of the reaction is less than MTT
Thermal Accumulation at the end of Dosing	1.85 %	End of the dosing thermal accumulation is Considerable medium To be add sodium acetate for longer hours
Thermal Conversion at the end of Dosing	98.15 %	End of the dosing thermal Conversion is medium
Specific Heat Before reaction (kJ/Kg K)	2.83	Specific heat of reaction is high and it can accomplish medium heat transfer rate and slow achieving temp due to high energy requirement.
Specific Heat After reaction(kJ/Kg K)	1.95	



Rc1e Technical Data: During addition of NH2OH.HCl at 0°C.

RC1 Graph: graph for reaction maintenance for 14 hrs

RC parameters	Results	Remarks/Recommendations
Heat generated	23.32 KJ/ 50 grams of KSM 31.30 KJ/mole of KSM	Initially heat of reaction is endothermic after addition shows exothermic reaction
Adiabatic temp rise (K)	9.96	Adiabatic temp rise shows low hazard in reaction, controlled addition to be done
MTSR (°C)	9.96°C (0+9.96)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of the reaction is less than MTT
Thermal Accumulation at the end of Dosing	96.50 %	End of the dosing accumulation of reagent is high , so add reagent in equal lot wise
Thermal Conversion at the end of Dosing	3.50 %	End of the dosing thermal Conversion is medium
Specific Heat Before reaction (kJ/Kg K)	2.83	Specific heat of reaction is high and it can accomplish medium heat transfer rate and slow achieving temp due to high energy requirement.
Specific Heat After reaction(kJ/Kg K)	1.95	



Observation : There is no abnormal heat liberation and internal temperature raise observed

6.0 Conclusion and Recommendation:

1. Addition of POCl3 at 0°C shows mild exothermic and indicates Low severity reaction with adiabatic temperature rise of 30.40°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 30.40°C.
2. Recommended for POCl3 charging should be through double controlled valve under nitrogen atmosphere.
3. Recommended for during addition of POCl3 into The reactor condenser vent should be connected to the Scrubber.
4. Ensure Condenser efficient cooling before startup the addition of POCL3.
5. Addition of 1H-pyrrol at 0°C shows Exothermic and indicates Medium severity reaction with adiabatic temperature rise of 84.54°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 84.54°C.
6. Recommended for 1H-pyrrol charging should be through double controlled valve under nitrogen atmosphere.
7. Ensure Condenser efficient cooling before startup the addition of 1H-pyrrol.
8. Addition of Water at 0°C shows Exothermic and indicates Medium severity reaction with adiabatic temperature rise of 46.63°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 46.63°C.
9. Recommended for Water charging should be through double controlled valve.
10. Ensure Condenser efficient cooling before startup the addition of water.
11. Addition of Pyridine at 0°C shows Exothermic and indicates Medium severity reaction with adiabatic temperature rise of 68.85°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 68.85°C.
12. Addition of Acetic anhydride at 0°C shows mild Exothermic and indicates low severity reaction with adiabatic temperature rise of 9.88°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 9.88°

13. Addition of NH2OH.HCl at 0°C shows mild Exothermic and indicates low severity reaction with adiabatic temperature rise of 9.96°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 9.96°C.
14. Recommended for NH2OH.HCl charging should be through double valve Hopper and in equal lot wise addition to avoid thermal accumulation of reagent as per RC shows 96.50% thermal accumulation at the end of dosing.

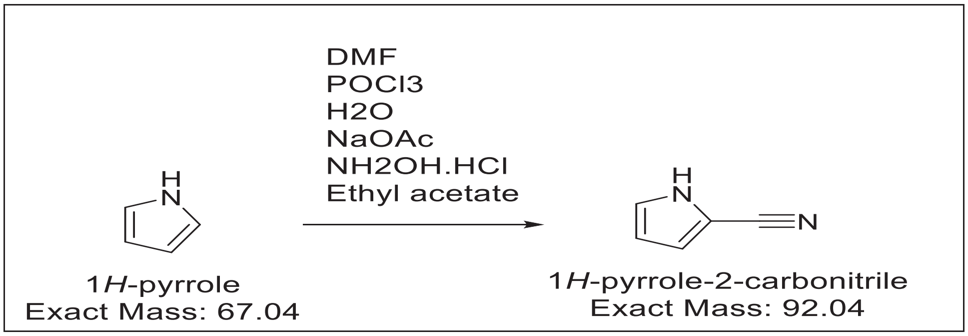
7.0 Reference

1. Thermal safety of Chemical Processes (Risk assessment and Process Design By Francis Stoessels

RC-1 study of Step-1 using 5V DMF and NaOAc base

4.0 Subject/Aim: To evaluate the thermal risk factors i.e. heat of reaction, Adiabatic Temperature and MTSR (Maximum temperature of the synthesis reaction).

5.0 Route of synthesis (ROS)



6.0 Brief Process

2.0 Reaction: Charged DMF (as per B.Size : 100 grams) into the reactor at 25-30°C then Cool the solvent to 0°C. Slowly added POCL3 into the reaction mass at 0°C after addition maintained the reaction mass for 30 min. slowly added 1H-pyrrole into the Reaction mass at 0°C after addition maintained the reaction mass at for 60 min. Drop wise added water into the reaction mass then maintained the mass at for 60 min. after that added in equal lots of NH2OH.HCl to the reaction mass at 0°C and maintained for 30 min. Charged in 4 equal lots of Sodium acetate into the reaction at 0°C and maintained the mass for 30 min.

4.0 Raw materials used:

S.No	Raw materials Name	Molecular weight	Quantities	Equiv.
1	1H-pyrrole	67.09	100.0 g	1.0

2	POCl3	153.33	251.36 g	1.1
3	DMF	73.09	500 ml	5V
4	Water	18.00	300 ml	3V
5	NH2OH.HCl	69.49	124.28 g	1.2
6	CH3COONa	77.08	459.56 g	4.0

5.0 RC1 Study: Process Observations: B.Size 100 grams of 1H-pyrrole

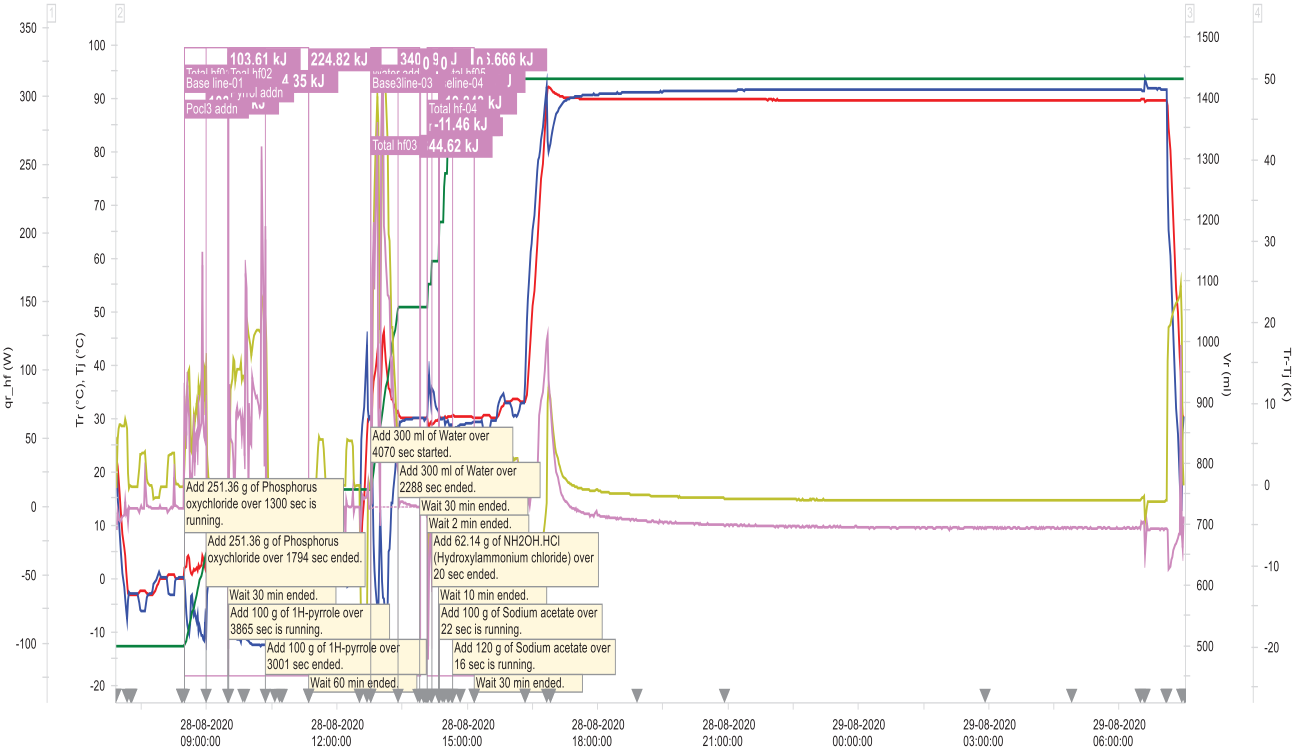
Process	Observations
Charged DMF (500 ml)into the reactor at 25°C	Homogeneous clear solvent
Cool the reaction mass to 0°C	Homogeneous clear solvent
Calibrate it to find out UA & Cp values	Establish the base line and predict specific heat.
Slowly added POCl3 (251.36 g) into the reactor at 0°C.	Homogeneous mass with light yellow color Observed high temperature raise during addition, Controlled addition is to be done. Gas liberation observed during addition
Maintain the reaction mass at 0°C over 30 min.	Homogeneous mass with light yellow color Reagent thermal accumulation at the end of dosing is 0.53%.
Drop wise added 1H-pyrrol (100 g) into the reactor at 0°C.	Homogeneous mass with dark orange color. Over all addition should be controlled even single drop addition shows high rise in reaction temperature.
Maintain the reaction mass at 0°C over 60 min.	Homogeneous mass with dark orange color. Reagent thermal accumulation at the end of dosing is 0.2%.
Calibrate it to find out UA & Cp values	Establish the base line and predict specific heat.
Raise the reaction mass to 30°C	No impact on raising temperature of the reaction mass
Drop wise added water (300 ml) into the reactor at 30°C.	Homogeneous mass with dark orange color. Over all addition should be controlled even single drop addition shows high rise in reaction temperature.
Maintain the reaction mass at 30°C over 60 min.	Homogeneous mass with dark orange color. Reagent thermal accumulation at the end of dosing is 1.10%.

Slowly added NH2OH.HCl (124.28 g) in 2 lots equal quantity into the reactor at 30°C.	Heterogeneous mass with dark orange color Addition shows the reaction is endothermic Nature. Addition should be longer hours to reduce thermal accumulation
Maintain the reaction mass at 30°C over 10 min.	Heterogeneous mass with dark orange color Reagent thermal accumulation at the end of dosing is 42%.
Slowly added CH3COONa (459.56 g) in 4 lots equal quantity into the reactor at 30°C.	Heterogeneous mass with dark yellow color Addition should be longer hours to reduce thermal accumulation
Maintain the reaction mass at 30°C over 30 min.	Heterogeneous mass with dark yellow color Reagent thermal accumulation at the end of dosing is 45%.
Calibrate it to find out UA & Cp values	Establish the base line and predict specific heat.
Heat the reaction mass to 90°C.	Heterogeneous mass with dark yellow color
Maintain the reaction mass at 90°C over 12 hr.	During heating maintenance dark yellow color turns to blackish color with heterogeneous mass nature There is no abnormal heat liberation was observed Heating maintenance was in smooth run and stable temperature.
Cool the reaction mass to 30°C.	Remains blackish color with heterogeneous mass nature
Unloaded the reaction mass	Remains blackish color with heterogeneous mass nature

RC1 Graph: Overall Reaction

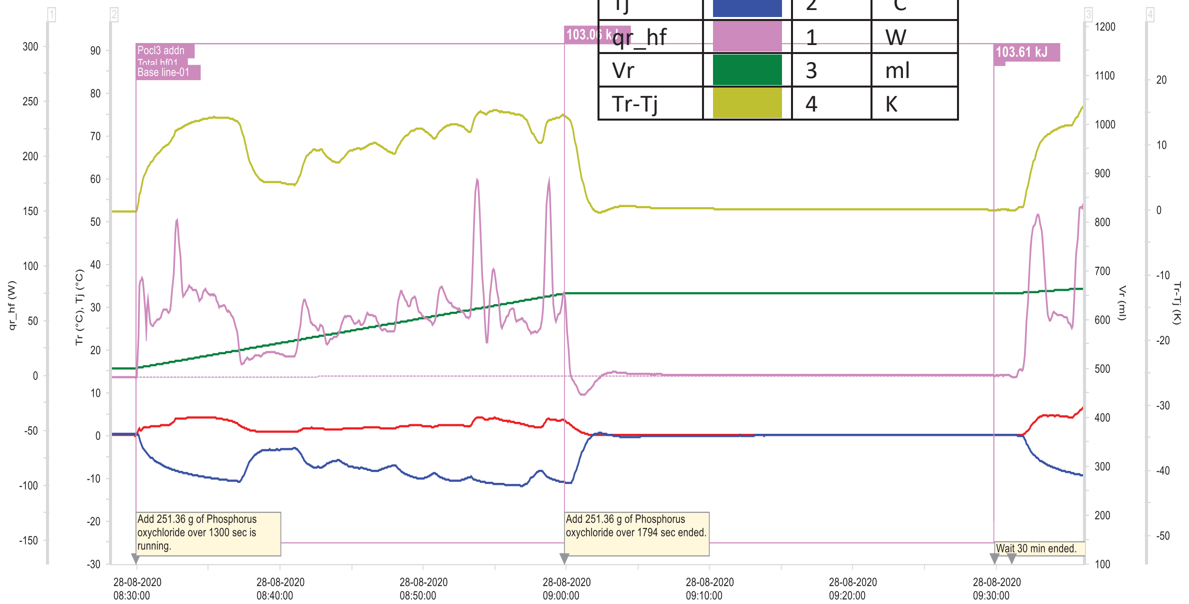
Trend	Color	Axis	Units
Tr	🟥	2	°C
Tj	🟦	2	°C
qr_hf	🟪	1	W
Vr	🟩	3	ml
Tr-Tj	🟨	4	K

TCG Life sciences Private limited, Kolkata



RC1 Graph: POCl3 addition shows Exotherm

Trend	Color	Axis	Units
Tr	🟥	2	°C
Tj	🟦	2	°C
qr_hf	🟪	1	W
Vr	🟩	3	ml
Tr-Tj	🟨	4	K



Rc1e Technical Data: During addition of POCL3 at 0°C.

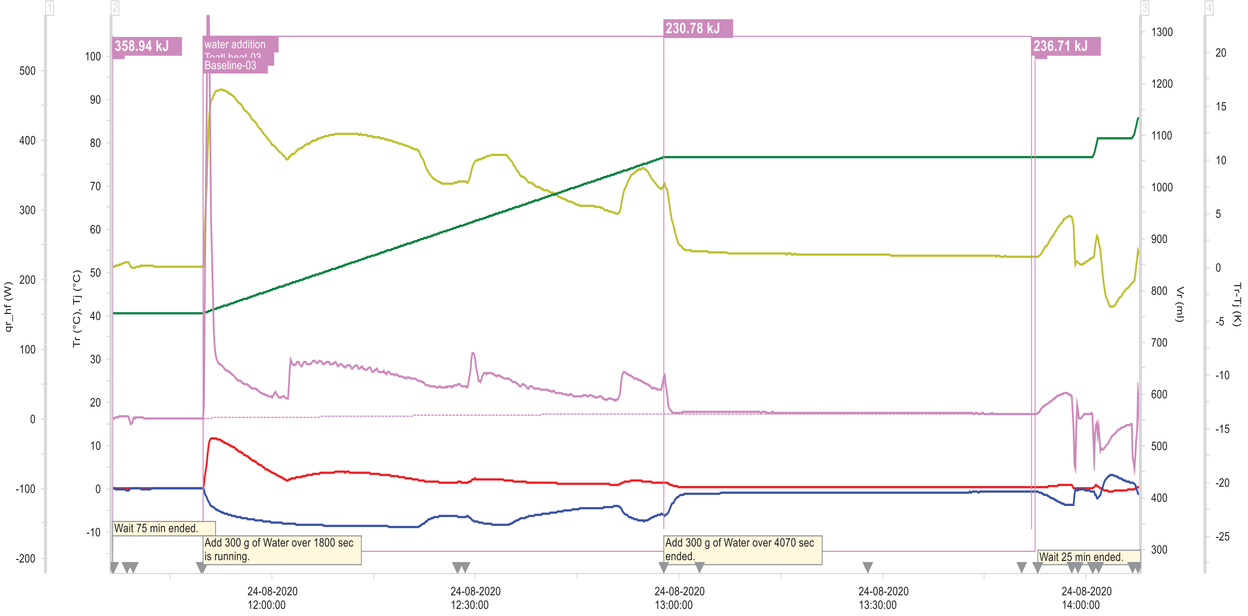
RC parameters	Results	Remarks/Recommendations
Heat generated	103.60 KJ/ 100 grams of KSM 69.53 KJ/mole of KSM	Heat of reaction shows is exothermic considerable adiabatic temp rise is 68.70 °C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	68.70	Adiabatic temp rise shows medium hazard in reaction, controlled addition to be done
MTSR (°C)	68.70°C (0 + 68.70)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of reaction is less than MTT, so reaction safe for scale-up point of view
Thermal Accumulation at the end of Dosing	0.53 %	End of the dosing thermal accumulation is Considerable low
Thermal Conversion at the end of Dosing	99.47%	End of the dosing thermal Conversion is high
Specific Heat Before Reaction (kJ/Kg K)	2.75	Specific heat of reaction is high and it can accomplish medium heat transfer rate and slowly achieving temp due to high energy requirement.
Specific Heat After Reaction (kJ/Kg K)	1.81	

RC1 Graph: 1H-pyrrole addition shows Exotherm

Rc1e Technical Data: During addition of **1H-pyrrole** at 0°C.

RC parameters	Results	Remarks/Recommendations
Heat generated	224.82 KJ/ 100 grams of KSM 150.88 KJ/mole of KSM	Heat of reaction shows is exothermic considerable adiabatic temp rise is 150.40°C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	150.40	Adiabatic temp rise shows medium hazard in reaction, controlled addition to be done
MTSR (°C)	150.40°C (0 + 150.40)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of the reaction is greater than MTT
Thermal Accumulation at the end of Dosing	0.2 %	End of the dosing thermal accumulation is Considerable low
Thermal Conversion at the end of Dosing	99.80 %	End of the dosing thermal Conversion is high
Specific Heat Before Reaction (kJ/Kg K)	2.75	Specific heat of reaction is high and it can accomplish medium heat transfer rate and achieving temp due to high energy requirement.
Specific Heat After Reaction (kJ/Kg K)	1.81	

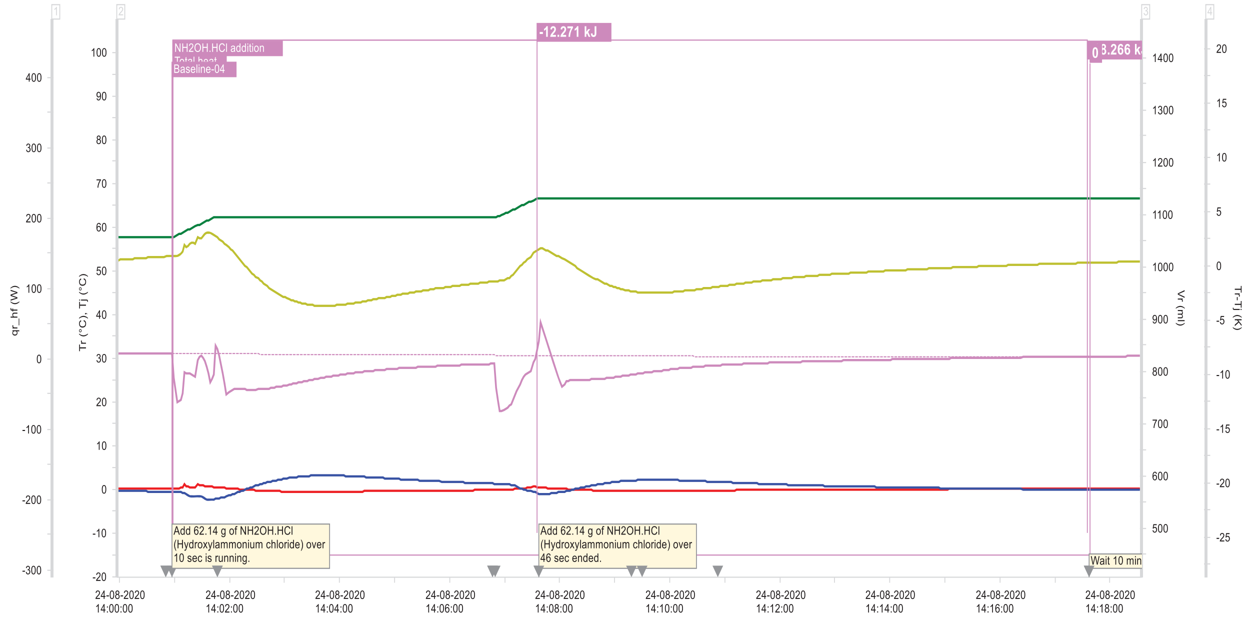
RC1 Graph: Water addition shows Exotherm



Rc1e Technical Data: During addition of **water** at 30°C.

RC parameters	Results	Remarks/Recommendations
Heat generated	344.62 KJ/ 100 grams of KSM 231.28 KJ/mole of KSM	Heat of reaction shows is exothermic considerable adiabatic temp rise is 187.66 °C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	157.66	Adiabatic temp rise shows medium hazard in reaction, controlled addition to be done
MTSR (°C)	187.66°C (30 + 157.66)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of the reaction is less than MTT
Thermal Accumulation at the end of Dosing	1.10 %	End of the dosing thermal accumulation is Considerable low
Thermal Conversion at the end of Dosing	98.90 %	End of the dosing thermal Conversion is high
Specific Heat Before reaction (kJ/Kg K)	1.81	Specific heat of reaction is high and it can accomplish medium heat transfer rate and slow achieving temp due to high energy requirement.
Specific Heat After reaction(kJ/Kg K)	2.20	

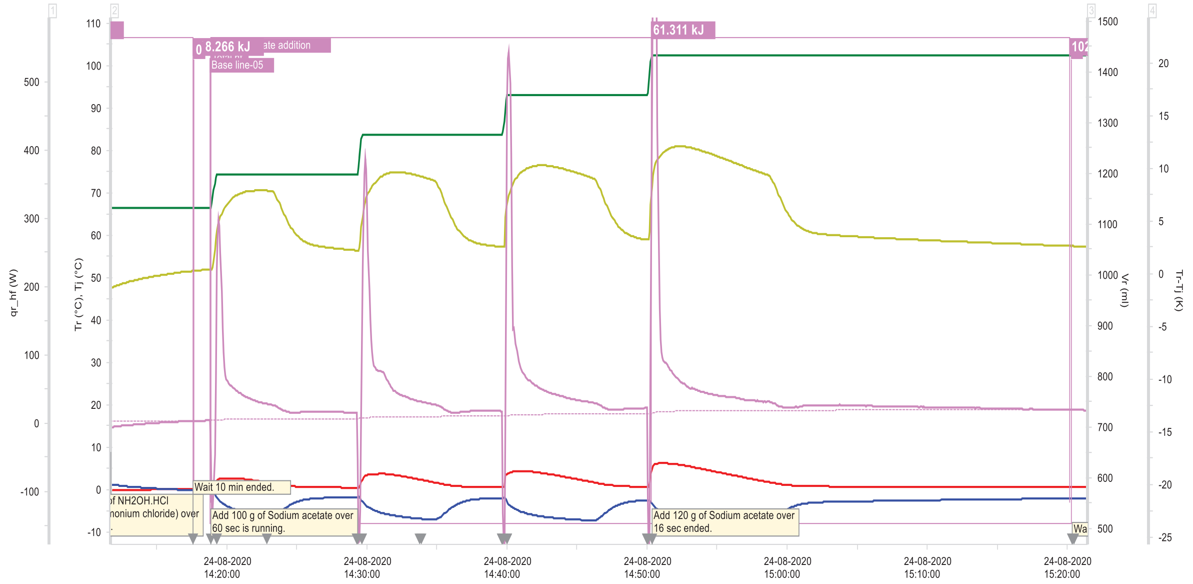
RC1 Graph: NH2OH.HCl addition shows Exotherm



Rc1e Technical Data: During addition of **NH2OH.HCl** at 30°C.

RC1 Graph: NaOAc addition shows Exotherm

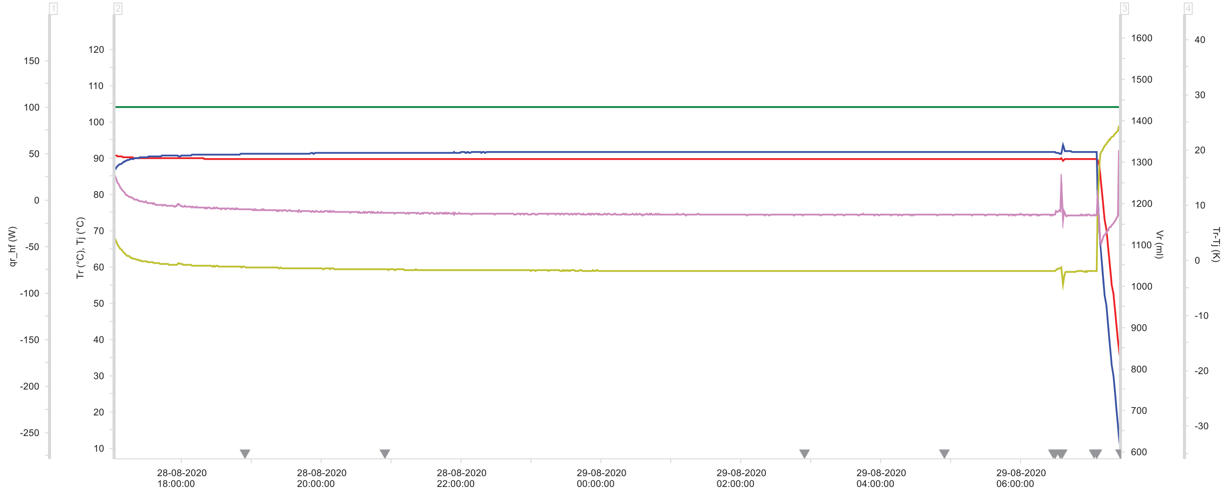
RC parameters	Results	Remarks/Recommendations
Heat generated	19.84 KJ/ 100 grams of KSM 13.31 KJ/mole of KSM	Heat of reaction shows is endothermic nature considerable adiabatic temp rise is -7.95°C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	-7.95	Adiabatic temp rise shows medium hazard in reaction, controlled addition to be done
MTSR (°C)	22.05°C (30 – 7.95)	Maintain temperature during addition due to endothermic reaction
MTT (°C)	152°C	The MTSR of the reaction is less than MTT
Thermal Accumulation at the end of Dosing	42.22 %	End of the dosing thermal accumulation is Considerable low
Thermal Conversion at the end of Dosing	57.78 %	End of the dosing thermal Conversion is high
Specific Heat Before reaction (kJ/Kg K)	1.81	Specific heat of reaction is high and it can accomplish medium heat transfer rate and slow achieving temp due to high energy requirement.
Specific Heat After reaction(kJ/Kg K)	2.20	



Rc1e Technical Data: During addition of **NaOAc** at 30°C.

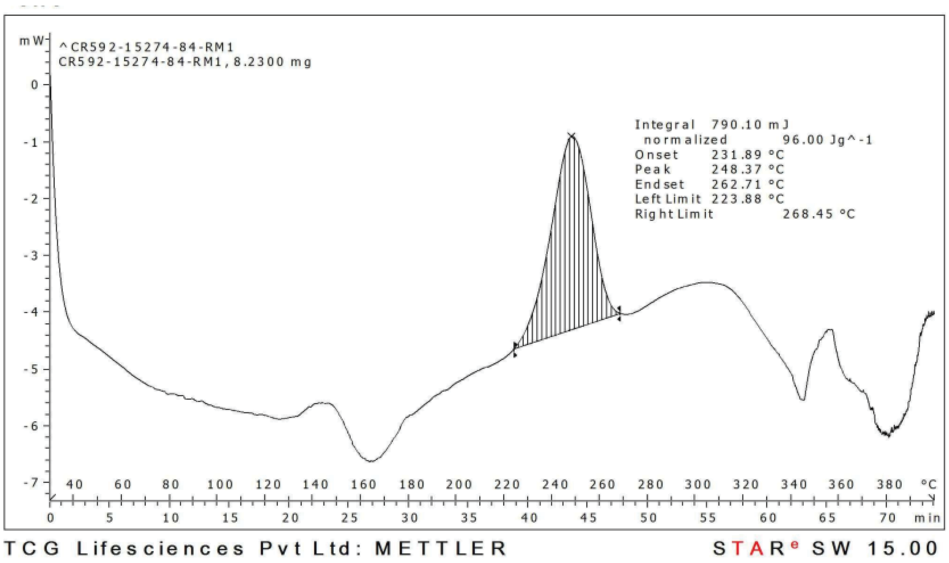
RC1 Graph: graph for reaction maintenance for 12 hrs

RC parameters	Results	Remarks/Recommendations
Heat generated	26.67 KJ/ 100 grams of KSM 17.90 KJ/mole of KSM	Heat of reaction shows is mild exothermic nature considerable adiabatic temp rise is 7.08°C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	7.08	Adiabatic temp rise shows low hazard in reaction, controlled addition to be done
MTSR (°C)	37.08°C (30 + 7.08)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of the reaction is less than MTT
Thermal Accumulation at the end of Dosing	45 %	End of the dosing thermal accumulation is Considerable medium To be add sodium acetate for longer hours
Thermal Conversion at the end of Dosing	55 %	End of the dosing thermal Conversion is medium
Specific Heat Before reaction (kJ/Kg K)	1.81	Specific heat of reaction is high and it can accomplish medium heat transfer rate and slow achieving temp due to high energy requirement.
Specific Heat After reaction(kJ/Kg K)	2.20	



DSC Report : Identify the Probability of Severity

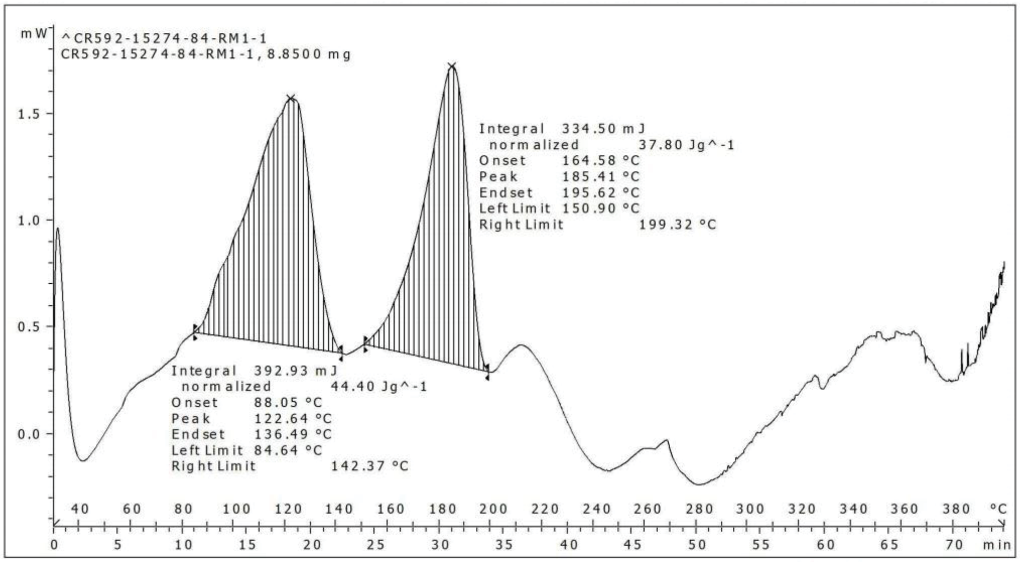
DSC Report 01 : Reaction mass after 1H-pyrrole addition



DSC CONCLUSION:

1. As per the DSC Report of the reaction mass after addition of 1H-Pyrrole observed heat liberation observed due to scientifically reason the heat of liberation is for formation of aldehyde and there is no thermal decomposition.

2. **DSC Report 02 : Reaction mass after Water addition**



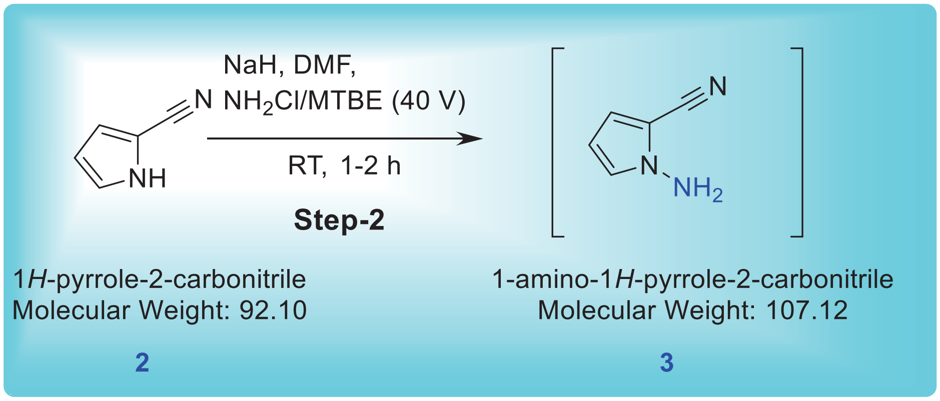
DSC CONCLUSION:

1. As per the DSC Report of the reaction mass after addition of water observed heat liberation observed due to scientifically reason the heat of liberation is for Pocl3 and water incompatibility under heating, And there is no thermal decomposition in the reaction mass.

Observation : There is no abnormal heat liberation was observed

6.0 Conclusion and Recommendation:

1. Addition of $POCl_3$ at 0°C shows exothermic and indicates Medium severity reaction with adiabatic temperature rise of 68.70°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 68.70°C.
2. Recommended for $POCl_3$ charging should be through double controlled valve under nitrogen atmosphere.
3. Recommended for during addition of $POCl_3$ into The reactor condenser vent should be connected to the Scrubber.
4. Ensure Condenser efficient cooling before startup the addition of $POCL_3$.
5. Addition of 1H-pyrrol at 0°C shows Exothermic and indicates Medium severity reaction with adiabatic temperature rise of 150.40°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 150.40°C.
6. Recommended for 1H-pyrrol charging should be through double controlled valve under nitrogen atmosphere.
7. Ensure Condenser efficient cooling before startup the addition of 1H-pyrrol.
8. Addition of Water at 30°C shows Exothermic and indicates Medium severity reaction with adiabatic temperature rise of 157.76°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 187.76°C.
9. Recommended for Water charging should be through double controlled valve.
10. Ensure Condenser efficient cooling before startup the addition of water.



1*H*-pyrrole-2-carbonitrile
Molecular Weight: 92.10

2

1-amino-1*H*-pyrrole-2-carbonitrile
Molecular Weight: 107.12

3

11. Addition of NH2OH.HCl_at 30°C shows endothermic and indicates low severity reaction with adiabatic temperature rise of -7.95°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 22.05°C.
12. Recommended for NH2OH.HCl_charging should be through double valve Hopper and longer hours addition to avoid thermal accumulation of reagent as per RC shows 42% thermal accumulation at the end of dosing.
13. Addition of NaOAc at 30°C shows mild Exothermic and indicates low severity reaction with adiabatic temperature rise of 7.08°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 37.08°C.
14. Recommended for NH2OH.HCl_charging should be through double double valve Hopper and longer hours addition to avoid thermal accumulation of reagent as per RC shows 45% thermal accumulation at the end of dosing.

7.0 Reference

1. Thermal safety of Chemical Processes (Risk assessment and Process Design By Francis Stoessels

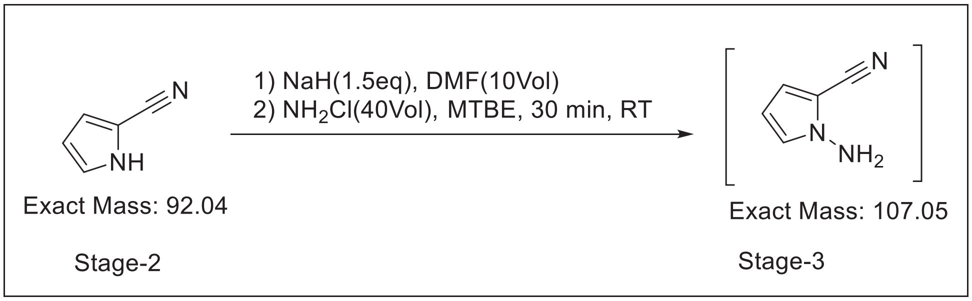
Software reference document:

Remdesivir M4ALL
STG-01- 02_Report.d

RC-1 study of N-amination reaction:

1.0 Subject/Aim: To evaluate the thermal risk factors i.e. heat of reaction, Adiabatic Temperature and MTSR (Maximum temperature of the synthesis reaction).

2.0 Route of synthesis (ROS)



3.0 Brief Process

Reaction: Charged NaH and DMF into the reactor at 25°C after that cool the mass to 0°C. Slowly added Pyrrole-2-carbonitrile into the reactor at 0°C after addition maintained the reaction mass for 30 min. Charged NH2Cl in MTBE solution into the reaction mass at 0°C, after addition maintained the reaction mass for 30 min. Reaction was monitored by TLC then charged Formamidine acetate into the mass at 0°C.

4.0 Raw materials used:

Raw materials	Quantity	Molecular weight	Equivalent
Pyrrole-2-carbonitrile	35.0 gm	92.04	1.0eq
60% NaH	22.81 gm	23.99	1.5eq
NH2Cl in MTBE	1.4 Lit	-	40Vol
DMF	350 mL	73.09	10Vol
Formamidine acetate	158 g	104.11	4.0eq

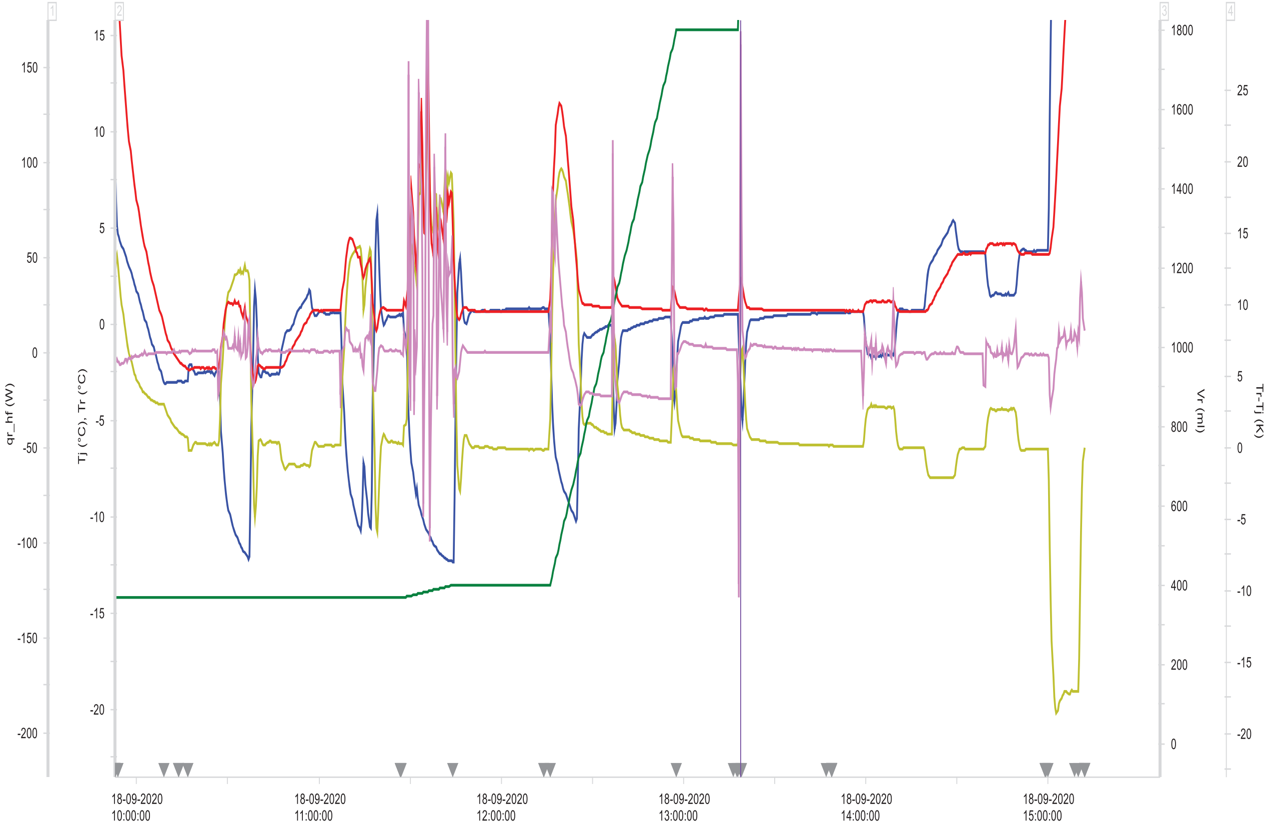
5.0 RC1 Study: Process Observations:

Reaction: B.Size: 35 grams of Pyrrole-2-carbonitrile (STG-02)

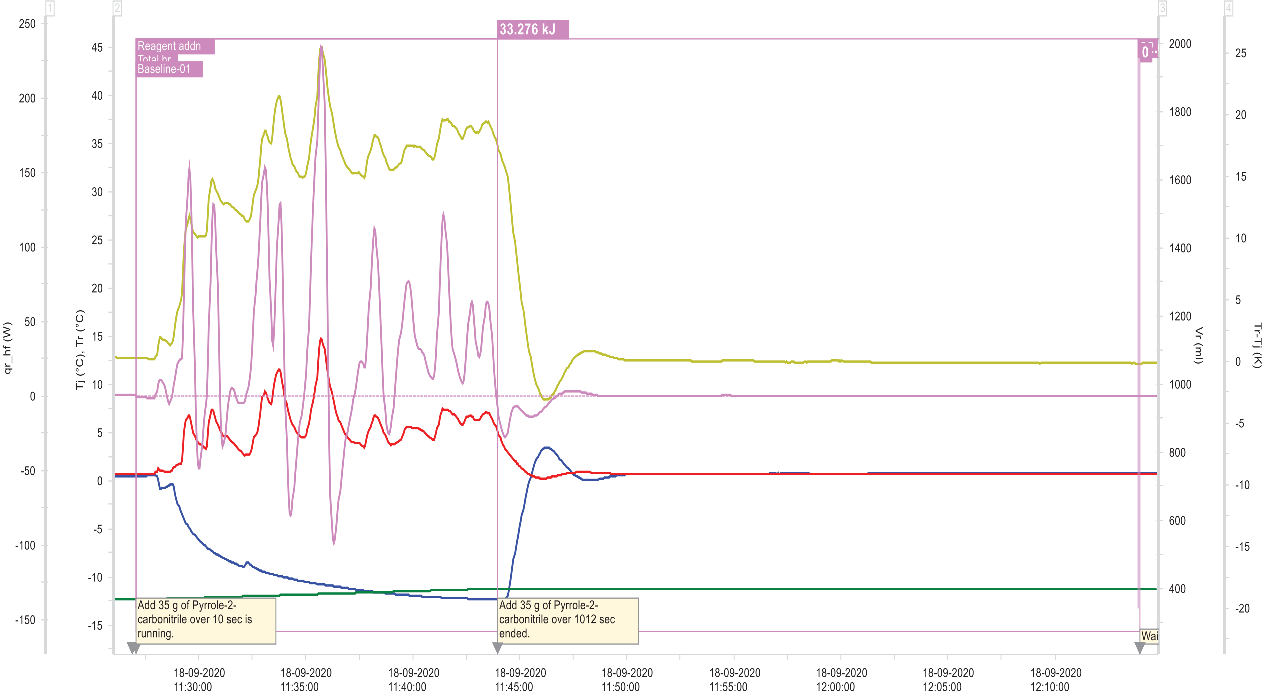
Process	Observations
Charged NaH and DMF into the reactor at 25°C	Heterogeneous mass with light ash color
Cool the reaction mass to 0°C	Heterogeneous mass with light ash color
Calibrate it to find out UA & Cp values	Establish the base line and predict specific heat.
Slowly added Pyrrole-2-carbonitrile into the reactor at 0°C.	Heterogeneous mass with Dark ask color Addition Shows exothermic during addition and temperature Max rise up to 55.00 °C. Reagent sudden dump (under Cooling failure) the maximum attainable temperature is 55.00°C
Maintain the reaction mass at 0°C over 30 min.	End of the dosing thermal accumulation is 0% Due to end of addition shows immediate endothermic nature
Charged 1400 ml of NH2Cl in MTBE Solution at 0°C.	Addition Shows low exothermic during addition and temperature Max rise up to 26.40 °C. Reagent sudden dump (under Cooling failure) the maximum attainable temperature is 26.40°C During addition reaction mass changes Heterogeneous Dark ash color to light pink color then changed to off white color. No Gas liberation observed during addition.
Maintain the reaction mass for 30 min at 0°C	Heterogeneous mass with off white Color End of the dosing thermal accumulation is 27.6%
Charged Formamidine acetate into the reactor at 0°C	Addition Shows initially endothermic reaction after end of dosing shows exothermic addition and temperature Max rise up to 1.67 °C. Reagent sudden dump (under Cooling failure) the maximum attainable temperature is 1.67°C
Maintain the reaction mass for 30 min at 0°C	Heterogeneous mass with off white Color End of the dosing thermal accumulation is 19.40%
Calibrate it to find out UA & Cp values	Establish the base line and predict specific heat.
Raised the reaction mass to 30°C.	Remains Heterogeneous mass with off white Color
Unloaded the reaction mass	Remains Heterogeneous mass with off white Color

RC1 Graph 1: Overall Reaction

Trend	Color	Axis	Units
Tr	🟥	2	°C
Tj	🟦	2	°C
qr_hf	🟪	1	W
Vr	🟩	3	ml
Tr-Tj	🟨	4	K



RC1 Graph 1.1: Pyrrole-2-carbonitrile shows Exothermic

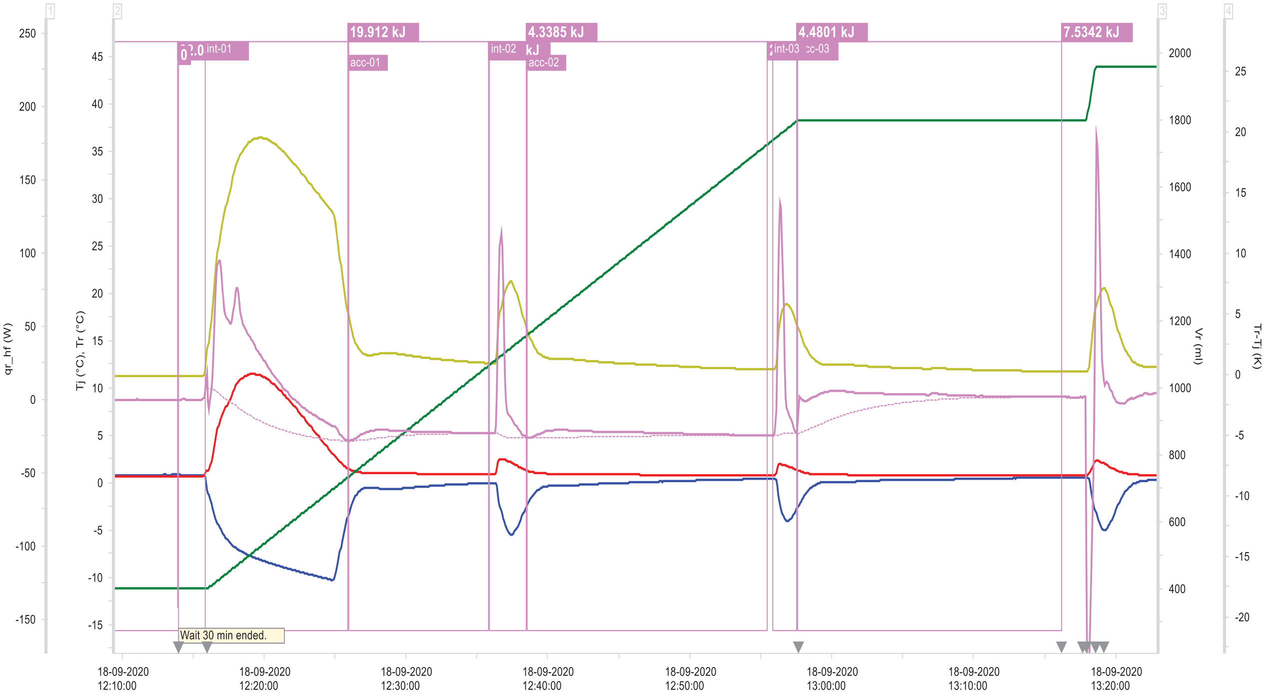


Rc1e Technical Data: During addition of **Pyrrole-2-carbonitrile** at 0°C.

RC parameters	Results	Remarks/Recommendations
Heat generated	33.27 KJ/ 35 grams of KSM 87.55 KJ/mole of KSM	Heat of reaction shows exothermic considerable adiabatic temp rise is 55.00 °C. The addition should be controlled due to exothermic reaction.
Adiabatic temp rise (K)	55.00	Adiabatic temp rise shows medium hazard in reaction, controlled addition to be done
MTSR (°C) (As per 100% Accumulation)	55.00°C (0 +55.00)	Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of reaction is less than MTT, so reaction safe for scale-up point of view
Thermal Accumulation at the end of Dosing	0 %	End of the dosing thermal accumulation is Considerable low After addition shows immediate endothermic nature
Thermal Conversion at the end of Dosing	100 %	End of the dosing thermal Conversion is high
Specific Heat Before Reaction (kJ/Kg K)	1.55	

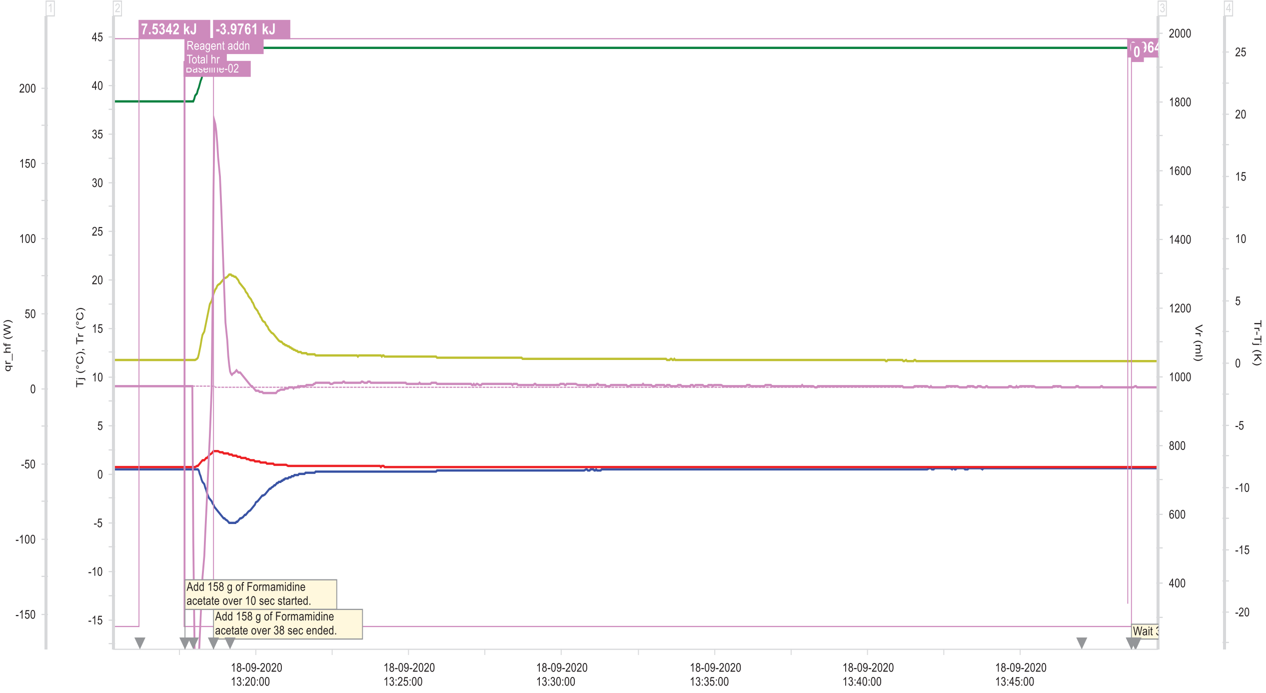
Specific Heat After Reaction (kJ/Kg K)	1.52	Specific heat of reaction is low and it can accomplish high heat transfer rate and fast achieving temp due to low energy requirement.

RC1 Graph 1.2: NH2Cl in MTBE Solution shows Exothermic



Rc1e Technical Data: During addition of **NH2Cl in MTBE Solution** at 0°C.

RC1 Graph 1.3: Formamidine acetate shows Exothermic



Rc1e Technical Data: During addition of Formamidine acetate at 0°C.

RC parameters	Results	Remarks/Recommendations
Heat generated	3.97 KJ/ 35 grams of KSM 13.06 KJ/mole of KSM 49.74 KJ/ 35 grams of KSM 104.05 KJ/mole of KSM	Heat of reaction shows mild exothermic considerable adiabatic temp rise is 26.40 °C. The addition should be controlled due to exothermic reaction. Heat of reaction shows mild exothermic considerable adiabatic temp rise is 1.67 °C. The addition should be in lot wise.
Adiabatic temp rise (K)	1.67 26.40	Adiabatic temp rise shows low hazard in reaction, controlled addition to be done
MTSR (°C) (As per 100% Accumulation)	1.67°C (0 + 1.67) 26.40°C (0 + 26.40)	Slightly temperature raise due to sudden charge of reagent Addition should be under double valve control.
MTT (°C)	152°C	The MTSR of reaction is less than MTT, so reaction safe for scale-up point of view
Thermal Accumulation at the end of Dosing	19.45 % 27.63 %	End of the dosing thermal accumulation is Considerable medium
Thermal Conversion at the end of Dosing	80.55 % 72.37 %	End of the dosing thermal Conversion is medium
Specific Heat Before Reaction (kJ/Kg K)	1.55	Specific heat of reaction is low and it can accomplish high heat transfer rate and fast achieving temp due to low energy requirement.
Specific Heat After Reaction (kJ/Kg K)	1.52	

6.0 Conclusion and Recommendation:

1. Addition of Pyrrole-2-carbonitrile at 0°C is medium severity reaction with adiabatic temperature rise of 55.00°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 55.00°C.
2. Recommended for Pyrrole-2-carbonitrile charging should be through double controlled valve under nitrogen atmosphere.
3. Addition of NH2Cl in MTBE at 0°C is Low severity reaction with adiabatic temperature rise of 26.40°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 26.40°C.
4. Observed there is no noticeable gas liberation during addition of NH2Cl in MTBE Solution.
5. Addition of Formamidine acetate at 0°C is Low severity reaction with adiabatic temperature rise of 1.67°C. In case of cooling failure or dumping, the maximum temperature attainable by the synthesis reaction mixture is 1.67°C.
6. During addition observed initially endothermic reaction then completion slightly exothermic reaction with accumulation of 19.43% so recommended for lot wise addition.
7. The reaction is scalable for bigger batch size with RC recommendations.

7.0 Reference

1. Thermal safety of Chemical Processes (Risk assessment and Process Design By Francis Stoessels

Reference Document:

Remedisvir Stg-03
Revision_Report.doc

RC software Report:

Further areas of developments / improvements

- ✓ **Although the process has been scaled up to kg scale, there is still scope for further improvements**
- ✓ **Step-1 isolation needs to be further optimized in terms of waste generation, reduction of PMI**
- ✓ **Step-2 is still run under high dilution which is major road block for further scale up**

Acknowledgement: TCG would like to thank VCU to provide an opportunity to work on such a challenging development project. We would also like to thank Dr. David Snead and Dr. Frank Gupton for the valuable comments, continuous guidance, encouragement to the team and unprecedented support throughout the journey**.** It was indeed a joy working with M4ALL, VCU

Look forward to continue our collaboration further and strengthen our relationship