Final Report for Pyronaridine_INV-054926-2 Project

Project	Pyronaridine_INV-054926-2
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Starting Date	2023-05-19
Achieving Date	2024-06-07
Report ID	INV_054926_PDR version 2.0

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Distribution

Irvin Zhou, Claude Mercier, Pyronaridine (INV-054926-2) project team, BMGF

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1. Introduction and Acknowledgements

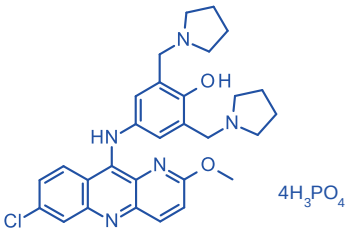
Pyronaridine tetraphosphate and Artesunate form the active ingredients of the anti-malaria treatment Pyramax.

The Bill & Melinda Gates Foundation (BMGF) asked for a partner to develop a cost-efficient and robust process to produce Pyronaridine tetraphosphate at a commercial scale. The strategy of BMGF is to sponsor a development and initial scale up program and to transfer the obtained process to an established API producer.

PHT tech Started the Phase Ⅰ work from May 2023 based on the initial study by Professor Lipshutz team from UC Santa Barbara, which was completed in December 2023. During Phase Ⅰ, three different routes **(Convergent route named as route 1, Linear route named as route 2 and modified convergent route named as route 3, see page 36)** were successfully developed. The study showed that impurity profile of API and costs were different for these 3 routes. And route 3 was defined as the best one among these three routes after the comparison on the raw material cost, operation cost and E factor.

Consequently, Phase Ⅱ work was proposed based on the study in Phase Ⅰ to do the further improvement on the process and to address the unsolved issues in Phase Ⅰ study. Phase Ⅱ work started from Feb 2024 and was completed at the end of May 2024.

This report combines the Phase Ⅰ and Phase Ⅱ work.



TGF-001
(PND-$4H_3PO_4$)
CAS 76748-86-2

With the completion of this project, we would like to express our heartfelt thanks to Prof. Bruce H. Lipshutz, professor at the University of California, Santa Barbara, Dr. Claude Mercier, the CTO of PHT International and BMGF expert team for their supporting throughout this project. Their extensive knowledge and expertise are fundamental in ensuring the success of this project.
We also would like to give our sincerely thanks to our colleagues, especially to Mr. Rack Dong and Mr. Harry Shi who are responsible for Process Development, Mr. Alain Cai who is for Analytical Development, for their innovative contributions and hard work in this project.

2. Executive summary

PHT have designed and completed a comparative study of three synthetic routes for pyronaridine phosphate. These three synthetic routes can meet the quality requirements of the Chinese Pharmacopoeia. But route 3 has the highest purity and lowest cost.

PHT have provided 3 choices for purification of API to meet the requirements of BGMF.

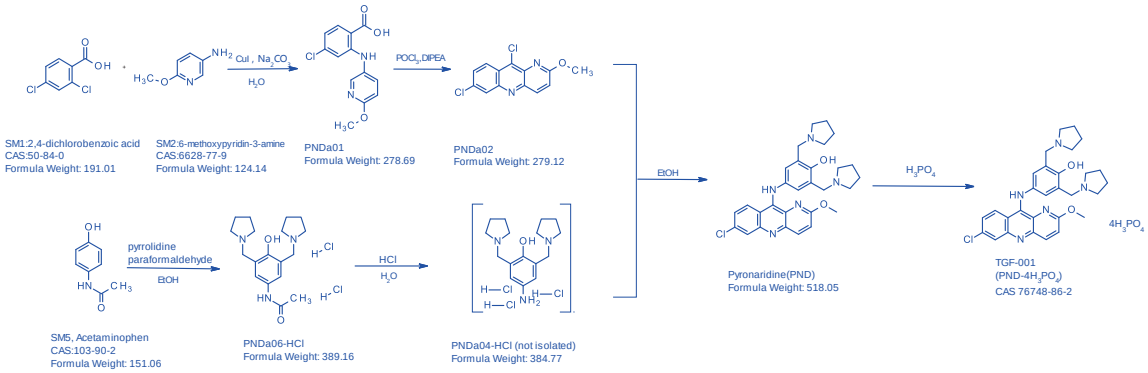
PHT have developed a cost-effective, environmentally friendly, and reliable process for scaling up the production of pyronaridine phosphate. This process can meet the continuously changing requirement.

PHT also developed a process for to make 5A2MP (key raw material). The overall yield of 3 steps was 56%.

PHT also completed a use test of 5A2MP from 3 different suppliers.

Impurity control in the API (TGF-001) is successful, however, the application of purification procedure of water/EtOH (additional 1 eq. H_3PO_4) to water/acetone purification directly is not suitable (OOS of HPLC assay). The reason maybe acetone cannot wash the additional H3PO4 as EtOH does.

Due to the late and rush request of the latest specification (HPLC purity and crystal form), the modified purification process gave the OOS material. It should be further investigated.



Scheme 1. Synthesis of TGF-001 by route 3

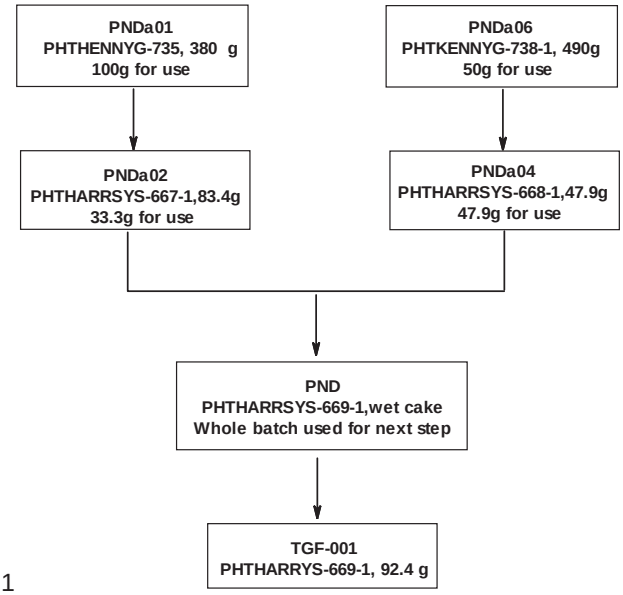
Abbreviation	Description
ADS	Analytical datasheet
MeCN	Acetonitrile
aq.	Aqueous
CuI	Copper(I) iodide
CP	Chinese Pharmacopoeia
DOE	Design Of Experiment
DIPEA	N, N-Diisopropylethylamine
eq.	Equivalent (s)
EA	Ethyl acetate
EtOH	Ethanol
h	Hour (s)
g	Gram (s)
HPLC	High performance liquid chromatography
HCl	Hydrogen chloride
H_3PO_4	Phosphoric acid
H_2O	Water
IPA	propan-2-ol
IPC	In process control
kg	kilogram (s)
L	Liter (s)
LOD	Loss on dry
Max.	Maximum
MT	More than
MeOH	Methanol
mL	Milliliter (s)
N/A	Not available
No.	Number
NMT	Not more than
Na_2CO_3	Sodium carbonate
NH_4OH	Ammonium hydroxide
NaOH	Sodium hydroxide
N_2	Nitrogen
N.D.	Not detected
$POCl_3$	phosphorus oxychloride
pH	Potential of hydrogen
ppm	Phases per million
RRT	Relative retention time
Res.	Residue
SM	Starting material
Spec.	Specification
Temp.	Temperature
THF	Tetrahydrofuran
U.I.	Unknown impurity
V	Volume (s)
v/w	Volume/ weight
w/w	Weight/weight
^oC	Celsius degree

3. Result and Conclusion

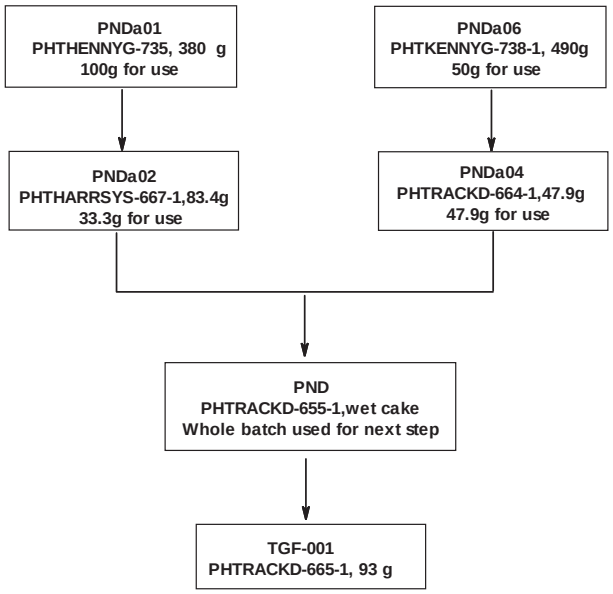
3.1. List of Starting Materials

Name	CAS	Quantity	Purity [%area]
6-methoxypyridin-3-amine	6628-77-9	300g/ bucket	98%
2,4-dichlorobenzoic acid	50-84-0	1000g/Bottle	98%
Na_2CO_3	497-19-8	500 g/Bottle	99%
CuI	7681-65-4	100g/Bottle	99.9%
$POCl_3$	10025-87-3	500mL/Bottle	95%
DIPEA	7087-68-5	500mL/Bottle	99%
Propylene carbonate	108-32-7	6kg/Bottle	97%
Pyrrolidine	123-75-1	1kg/Bottle	98%
Paraformaldehyde	30525-89-4	1kg/ Bottle	96%
2M HCl in EA	7647-01-0	1L/ Bottle	---
H_3PO_4	7664-38-2	500mL/Bottle	85%

3.2. Batch tracking for the synthesis of TGF-001on approx. 100g scale (Phase II)



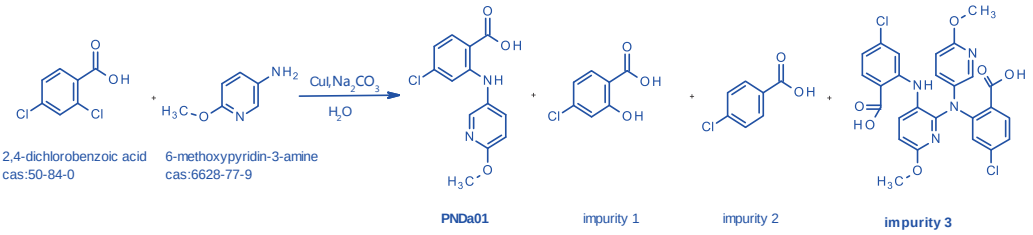
Batch 1



Batch 2

3.3. PNDa01 step

3.3.1. Reaction scheme



3.3.2. Results of PNDa01

- The results of batches made in phase II are shown below. The results consisted with phase 1 work (see ref 10)
- The process of PNDa01 did not change from the phase I procedure doing the second phase of development.
- The assay of PNDa01 is very important (see spec in table 2) for the next step, the HPLC purity has little influence for the next step.

Table 0. Analytical data of PNDa01 5A2MP used in 3 batches.

Batch	Appearance	Water content, %w/w	Purity, %area	Assay, %w/w
2401001	dark brownish color liquid	0.55%	96.18% (HPLC, 254nm)	95.6%
2401002	dark brownish color liquid	0.72%	96.17% (HPLC, 254nm)	95.5%
2401003	dark brownish color liquid	0.56%	96.16% (HPLC, 254nm)	95.0%

Table 1. Results of PNDa01 reaction

No.	**6-methoxypyridin-3-amine**	**2,4-dichlorobenzoic acid (SM1, assay: 99.7%)**	**Na_2CO_3**	**CuI**	**Water**	**Reaction Temp.**	**IPC_M1 (16h)**	**Isolated PNDa01**
PHTKENNYG-735	200g (1.0eq) (assay: 95.6%)	1.4 eq.	2.2 eq.	0.05 eq.	6v/w	95°C	SM2: 2.48%	Amount: 380.0g Yield: 81.8%
PHTKENNYG-736	200g (1.0eq) (assay: 95.5%)	1.4 eq.	2.2 eq.	0.05 eq.	6v/w	95°C	SM2: 2.42%	Amount: 381.0g Yield: 80.6%
PHTKENNYG-737	200g (1.0eq) (assay: 95.0%)	1.4 eq.	2.2 eq.	0.05 eq.	6v/w	95°C	SM2: 2.33%	Amount: 375.0g Yield: 81.4%

3.3.3. The procedure for the preparation of PNDa01 in experiment PHTKENNYG-735

- A suspension of 2,4-dichlorobenzoic acid (435.2g, 2.23 mol, 1.4 eq.), 6-methoxypyridin-3-amine (200g, 1.60 mol, 1.0 eq.), Na_2CO_3 (375.7 g, 3.51 mol, 2.2 eq.) and Copper (I) iodide (15.2 g, 79.7 mmol, 0.05 eq.) in water (1.2L,6v/w) was heated to 95°C and stirred for 16 hrs.
- HPLC showed 6-methoxypyridin-3-amine was 2.48% **(limit: NMT 3.0%)**.

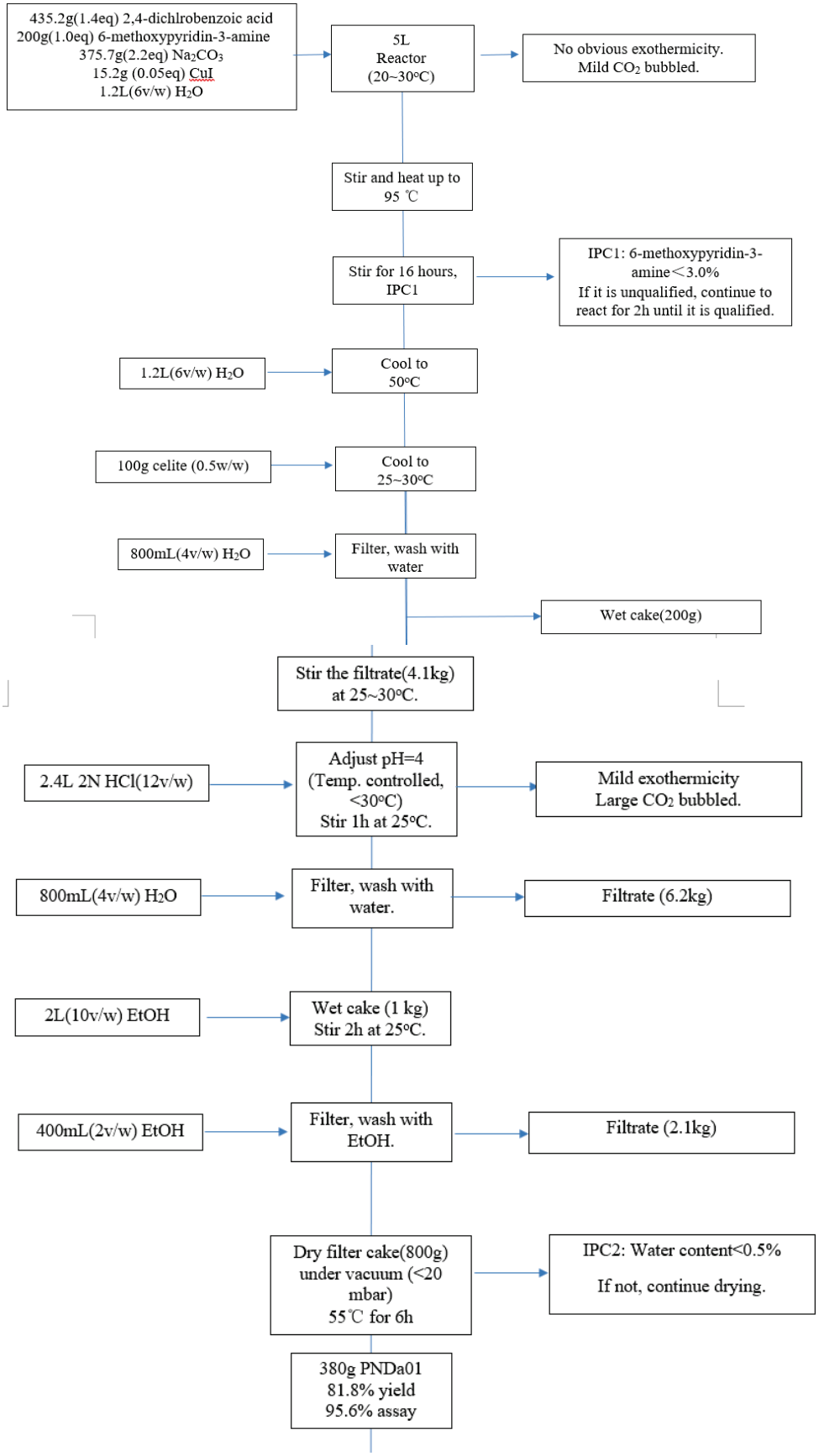
- The reaction solution was added with water (1.2 L, 6v/w).
- The solution was cooled to room temperature(25°C), and charged celite (100g,0.5 w/w) then stirred at 25°C for 0.5 h.
- The suspension was filtered, and the cake was washed with water (400 mL*2,4v/w).
- The combined filtrate was acidified to **pH=4.0** with 2N HCl (approx. 2.4 L, 12v/w, mild exothermicity).
- The suspension was stirred at room temperature (25°C) for 1 h then filtered, and cake was washed with water (400 mL*2,4v/w).
- The crude product was purified by re-slurry in EtOH (2 L,10v/w) at room temperature(25°C) for 2 hrs.
- The suspension was filtered, and cake was washed with EtOH (400 mL,2v/w).
- The collected solid was dried in vacuo at 55°C for 6 h (water: NMT 0.5%w/w) to give 380g PNDa01(Yield 81.8%)**.**

Note: v/w was based on 6-methoxypyridin-3-amine.

Table 2. Analytical data of isolated PNDa01

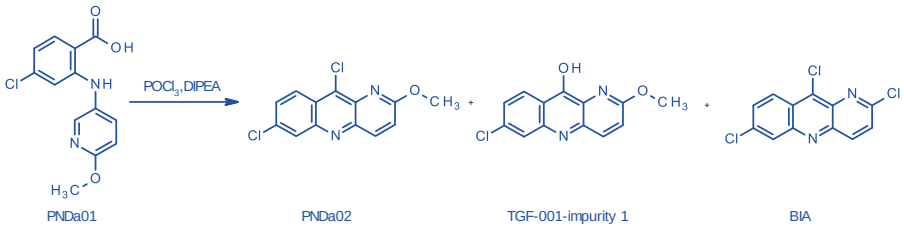
Items	**Specification**	**PHTKENNYG-735**	**PHTKENNYG-736**	**PHTKENNYG-737**
Appearance	Brown solid	Brown solid	Brown solid	Brown solid
Identity by HPLC	Similar retention time for sample and reference solution peak	Complies	Complies	Complies
HPLC purity, %area	NLT 92.0%	93.6%	95.9%	98.1%
HPLC assay, %w/w	NLT92.0%	95.6%	94.1%	96.5%
Water content, %w/w	NMT 0.35%	0.22%	0.09%	0.20%

3.3.4. Flow chart for PNDa01



3.4. PNDa02 step

3.4.1. Reaction scheme



3.4.2. Results of PNDa02

- In phase II development work, it was found that using 15% NaOH aqueous to quench the reaction instead of NH3 H2O gave advantages: (lower production cost, better filtration, no nitrogen-containing wastewater).
- Higher assay is obtained (~95%w/w) compared to the old process(~90%w/w) and the yield was comparable.

Table 3. Results of PNDa02

No.	PNDa01	$POCl_3$	DIPEA	Propylene carbonate	Reaction temperature	IPC_M1 (2h)	Isolated PNDa02
PHTHARRYS-659	Batch PHTKENNYG-735 Assay: 95.6% 100g (1.0eq)	4.0 eq.	4.4 eq.	8v/w	100°C	PNDa01: 0.03% TGF-001 impurity 1:0.5% BIA:0.13% PNDa02:96.2%	Amount: 79.5g Yield: 80.4%
PHTHARRYS-667	Batch PHTKENNYG-735 Assay: 95.6% 100g (1.0eq)	4.0 eq.	4.4 eq.	8v/w	100°C	PNDa01: 0.02% TGF-001-impurity 1:0.16% BIA: 0.22% PNDa02:96.4%	Amount: 83.4g Yield: 82.3%

3.4.3. The procedure for the preparation of PNDa02 in experiment PHTHARRYS-667

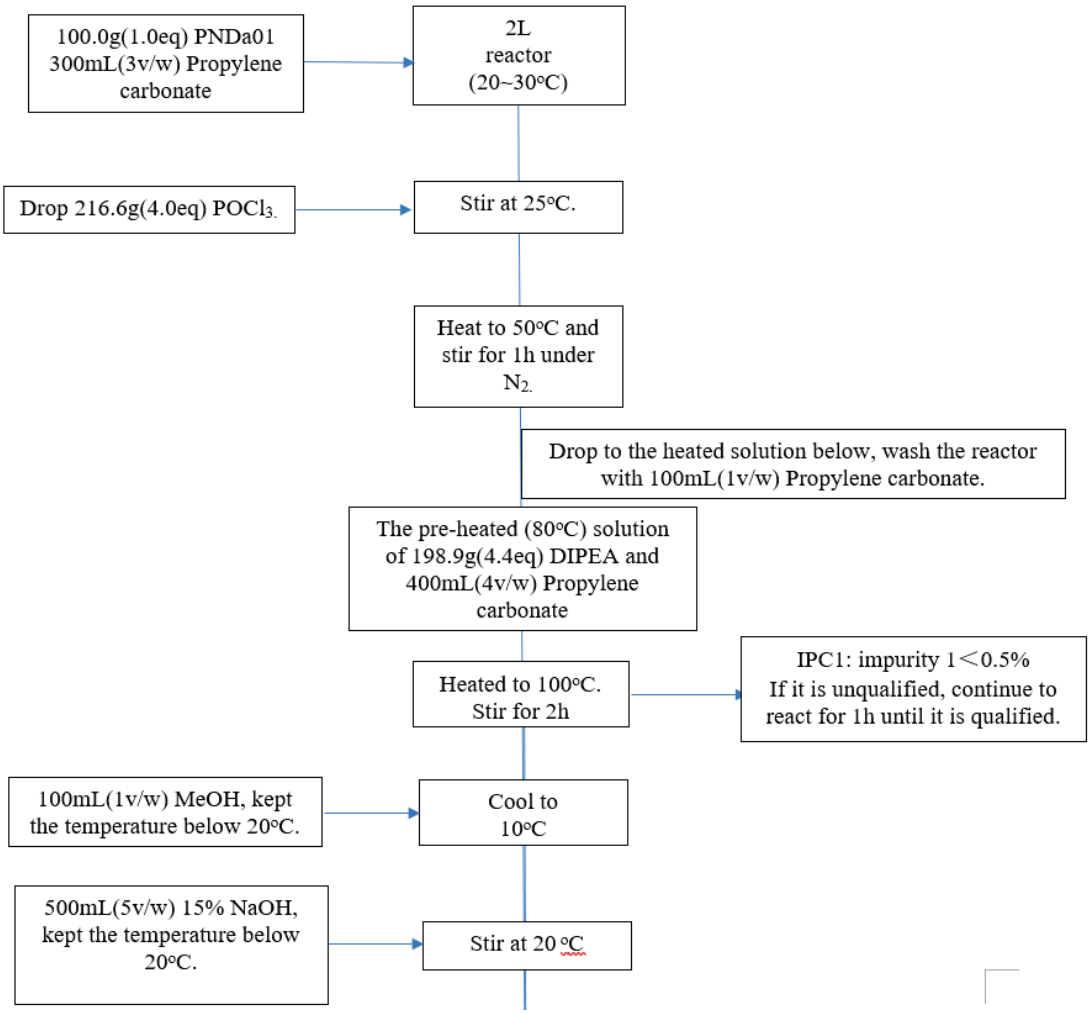
- Charge PNDa01 (100g, 346.2mmol,1.0eq) and Propylene carbonate (300mL, 3v/w) into a 1L flask. $POCl_3$ (216.6g, 1384.8mmol,4.0eq) was then added dropwise into the mixture.
- The mixture was stirred at 50°C for 1h under N_2 atmosphere.
- Charge DIPEA (198.9g, 1523.2mmol, 4.4eq) and Propylene carbonate (400 mL, 4v/w) into another 2L flask and the mixture was heated to 80°C under N_2 atmosphere.
- The prepared acyl chloride was then added dropwise into the mixture (significant increase in temperature during addition: 80°C raised to 87°C).
- After addition, the dropping funnel was washed with Propylene carbonate (100mL, 1V/W).
- The mixture was then reacted at 100°C for 2h. HPLC showed PND01 was 0.03% (**NMT 0.2%**) and TGF-001 impurity1 (PNDa02 intermediate) at RRT 0.75 was 0.16% **(NMT 0.5%)**.
- The mixture was then cooled with an ice bath.

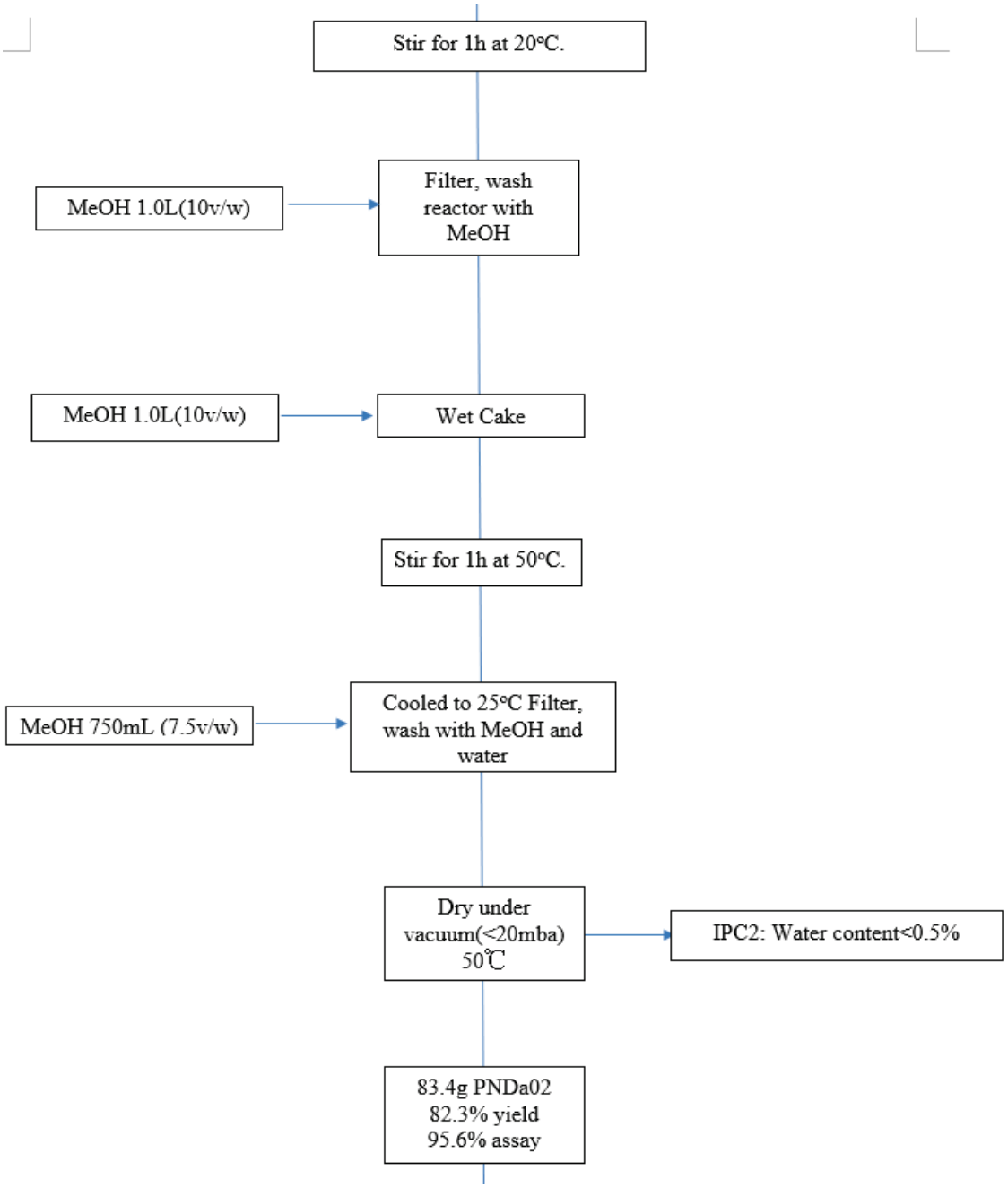
- MeOH (100mL,1v/w) was then added dropwise into mixture and the temperature kept below 20°C. (Significant increase in temperature during addition)
- 500mL 15% NaOH aqueous was then added dropwise into the suspension at ice bath and kept the temperature below 20°C.
- After addition, the mixture was stirred at 20°C for 1h.
- Crude PNDa02 was obtained by filtration.
- The residual PNDa02 on the flask wall was washed with MeOH(1000mL,10v/w).
- MeOH(1000mL,10v/w) was added into the crude PNDa02, and the mixture was stirred at 50°C for 1h.
- The mixture was then filtered under vacuum and the filter cake was washed with (MeOH (750mL,7.5v/w).
- 83.4g PNDa02(Yield:82.3%) as a grey solid was obtained after drying (water: **NMT 0.5%w/w**)

Table 4. Analytical data of isolated PNDa02

Items	**Specification**	**PHTHARRYS-659**	**PHTHARRYS-667**
Appearance	Grey solid	Grey solid	Grey solid
Identity by HPLC	Similar retention time for sample and reference solution peak	Complies	Complies
HPLC purity, %area	NLT 99.0%	99.8%	99.8%
PND01	NMT 0.2%	n. d.	n. d.
BIA	NMT 0.5%	0.13%	0.2%
TGF-001 impurity	NMT 0.5%	n. d.	n. d.
HPLC assay, %w/w	NLT 94.0%	96.3%	95.3%
Water content, %w/w	NMT 0.2%	0.09%	0.08%

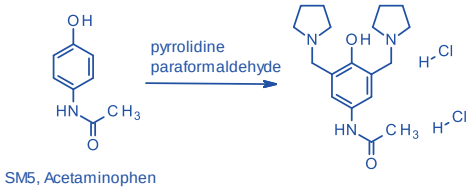
3.4.4. Flow chart for PNDa02





3.5. PNDa06-HCl step

3.5.1. Reaction scheme



SM5, Acetaminophen
CAS:103-90-2
Formula Weight: 151.06

PNDa06-HCl
Formula Weight: 389.16

3.5.2. Results of PNDa06-HCl (using a phase II process showing the following changes)

- Using 2.4eq. Pyrrolidine/paraformaldehyde instead of 2.5eq in PNDa06 reaction.
- Using EtOH(3V) instead of IPA(5V) in salt formation process.
- The results of the modified process were consistent with the phase I work.

Table 5. Results of PNDa06-HCl

No.	Acetaminophen （SM5）	Pyrrolidine/ paraformaldehyde	EtOH	Reaction temperature	IPC by area%(16h)	Isolated PNDa06-HCl
PHTHARRYS-653	200g(1.0eq)	2.4 eq.	5v/w	70°C	SM5:0.07%; PNDa06:99.1%	485.8g (91.3% yield)
PHTKENNYG-738	200g(1.0eq)	2.4 eq.	5 v/w	70°C	SM5:0.02%; PNDa06:99.1%	490.0g (94.5% yield)

3.5.3. The procedure for the preparation of PNDa06-HCl in experiment PHTHARRYS-653

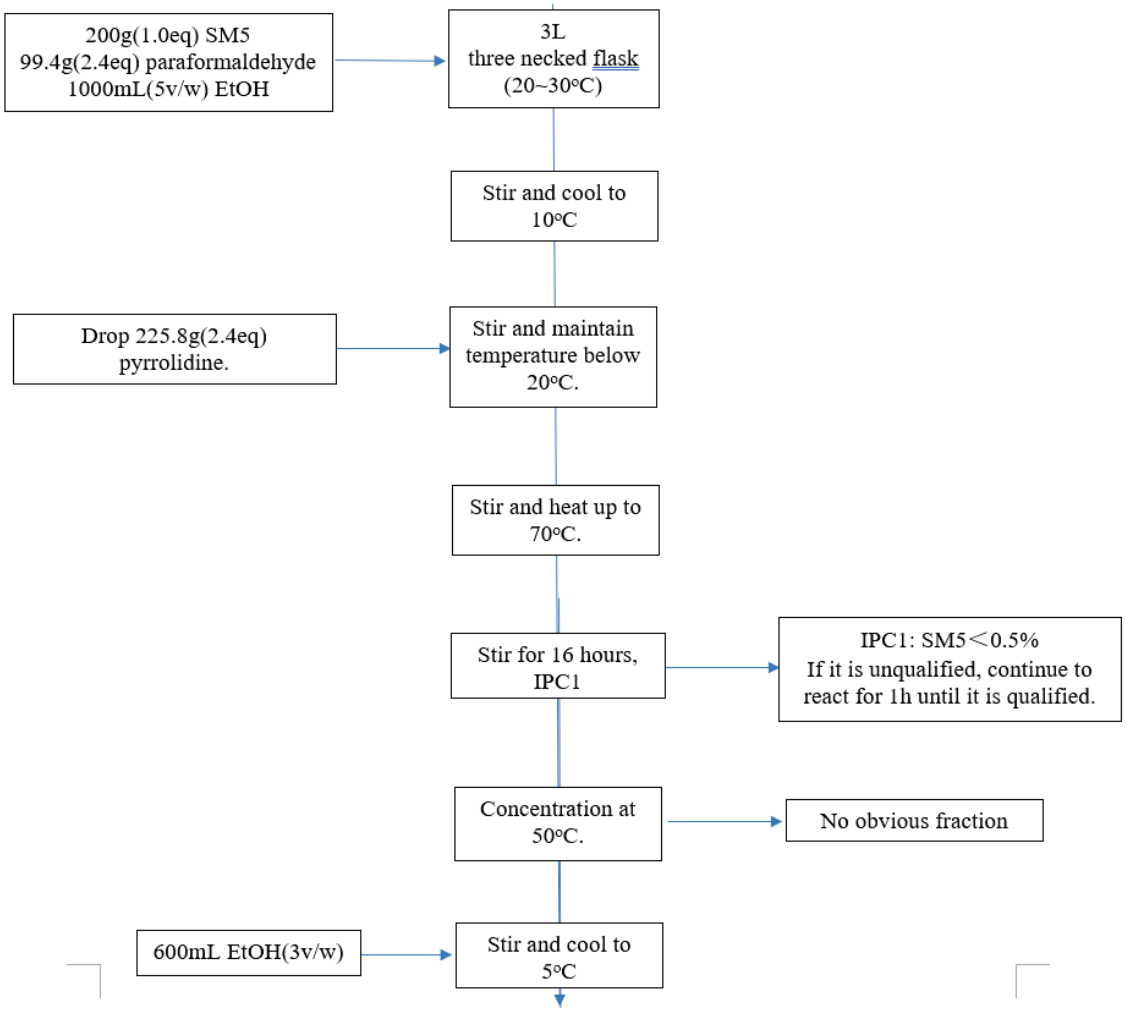
- Charge Acetaminophen (SM5, 200g, 1296.6mmol, 1.0eq), Paraformaldehyde (99.4g, 3111.9mmol, 2.4eq) and Ethanol (1000mL,5v/w) into a 3000mL flask.
- The mixture was then stirred at 30°C for 0.5h.
- Then Pyrrolidine (225.8g, 3111.9mmol, 2.4eq) was added dropwise for 0.5h at 10~20 °C.
- The reaction was raised to 70°C and stirred for 16h under N_2 atmosphere.
- HPLC showed SM5 was 0.07% **(NMT 0.5%)**.
- The solvent was evaporated (50°C) to dryness under reduced pressure to give 455g crude as an orange oil (no obvious fraction).
- EtOH (600mL,3v/w) was added to the residue (orange oil) and the flask was cooled to 5 °C.
- 2M HCl in EA (2400mL,12v/w) was added dropwise to the reaction. EA (1000mL,5v/w) was added to the solution.
- The mixture was heated to 50°C for 10min.
- Lots of solids precipitated out from the solvent, then continued stirring for 1 hour at 25°C.

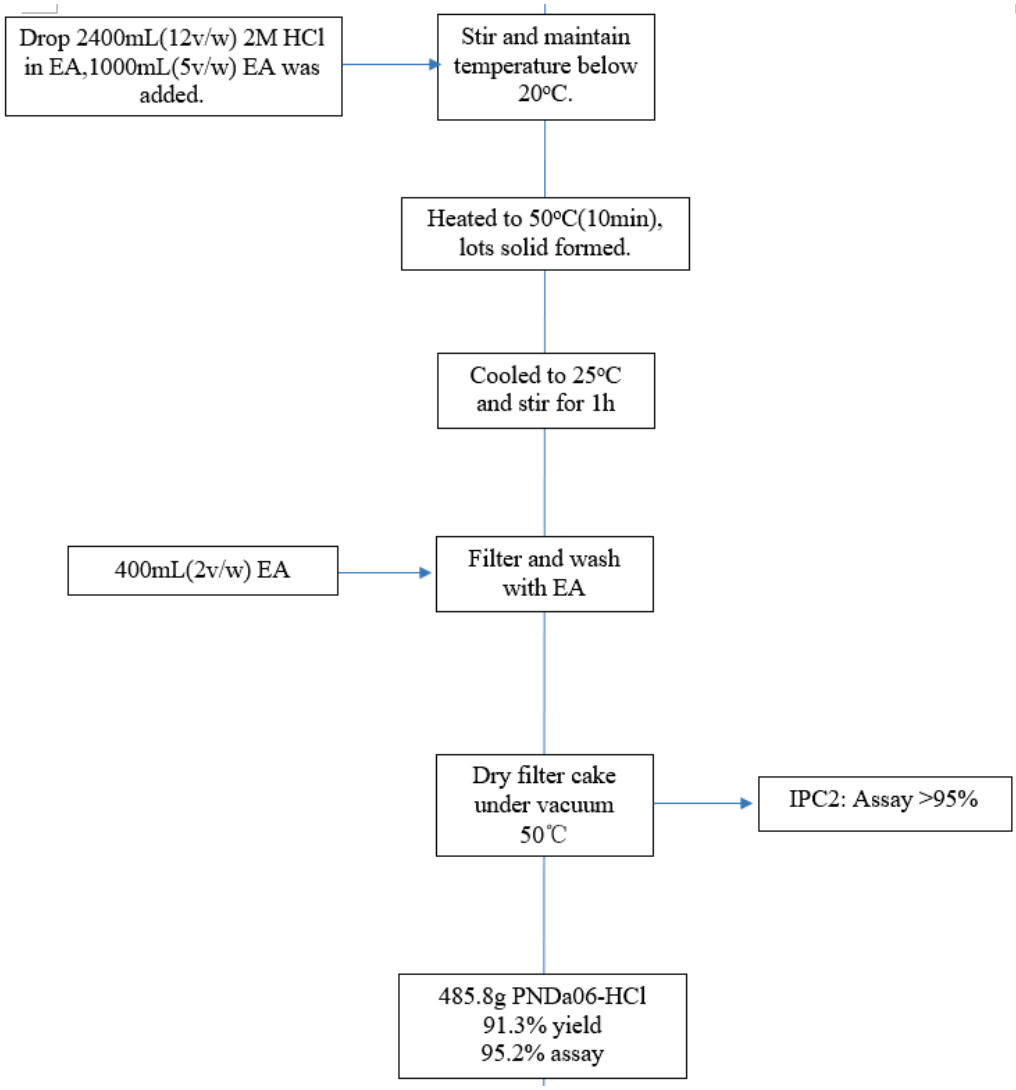
- The solid was filtered and washed with EA (400mL,2v/w).
- The solid was dried under vacuum at 50°C for 3hs to furnish PNDa06-HCl (485.8g, 91.3% yield,95.2% assay).

Table 6. Analytical data of isolated PNDa06-HCl

Items	**Specification**	**PHTHARRYS-653**	**PHTKENNYG-738**
Appearance	Yellow to off-white solid	Yellow to off-white solid	Yellow to off-white solid
Identity by HPLC	Similar retention time for sample and reference solution peak	Complies	Complies
HPLC purity, %area	NLT 98.0%	99.2%	98.3%
Acetaminophen	NMT 0.50%	0.09%	n.d.
HPLC assay, %w/w	NLT 95.0%	95.2%	97.7%

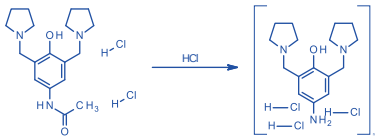
3.5.4. Flow chart for PNDa06-HCl





3.6. PNDa04-HCl step

3.6.1. Reaction scheme



PNDa06-HCl
Formula Weight: 389.16

PNDa04-HCl (not isolated)
Formula Weight: 384.77

3.6.2. Results of PNDa04-HCl

- The results (assay and yield) of 2 batches were consistent with former process in phase I.
- The process has no change in this step.

Table 7. Results of PNDa04-HCl

No.	PNDa06-HCl	HCl aqueous (6mol)	Reaction temperature	IPC_M1 (1 h)	Yield (not isolated)
PHTHARRYS-668	Batch PHTKENNYG-738 Assay: 97.7%, 50g (1.0eq)	4V	100°C	PNDa06: n.d. PNDa04:99.6%	Use for next step as 100% yield
PHTRACKD-664	Batch PHTKENNYG-738 Assay: 97.7%, 50g (1.0eq)	4V	100°C	PNDa06: n.d. PNDa04:99.6%	Use for next step as 100% yield

3.6.3. The procedure for the preparation of PNDa04 in experiment PHTHARRYS-668

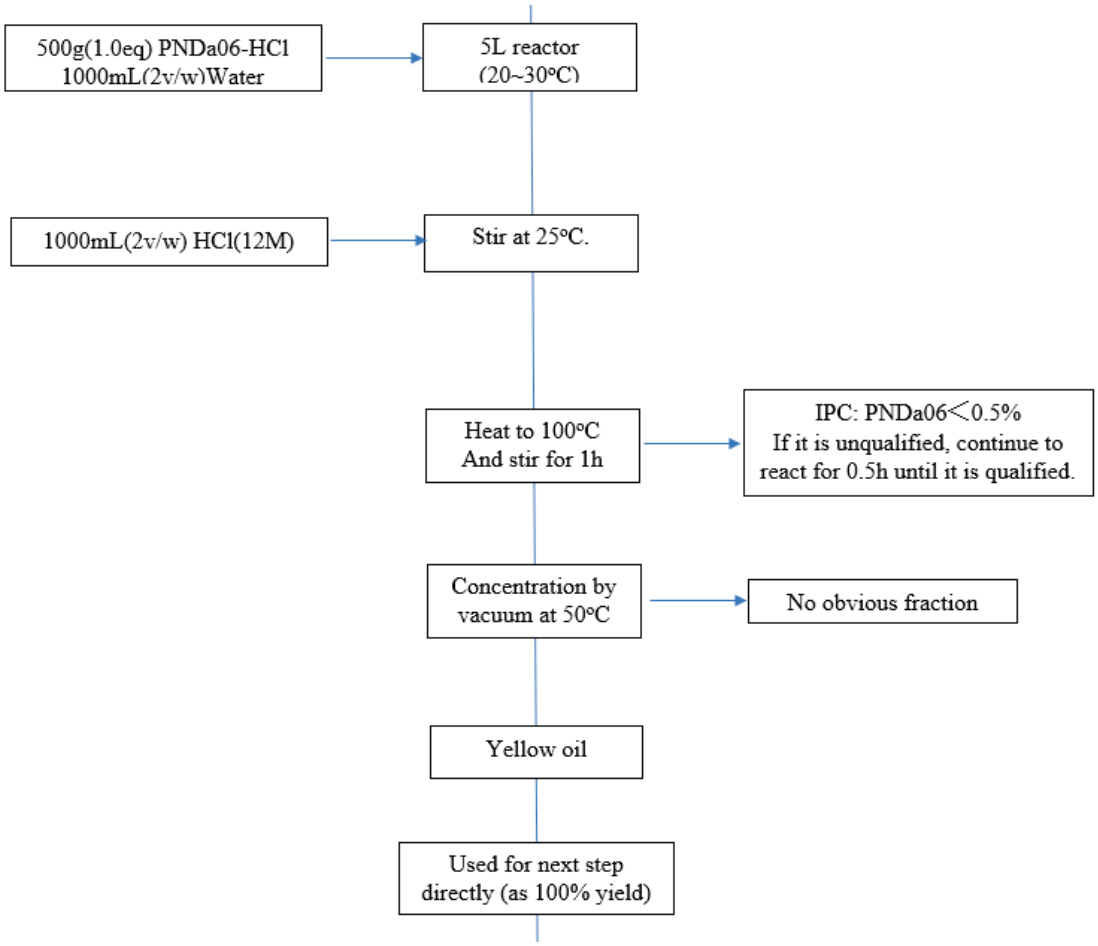
1 Hydrochloric acid (12M/L, 100mL,2v/w) was added to the solution of PNDa06-HCl (50g, 125.1mmol, 1.0eq) in water (100mL).

2 The mixture was stirred at 100°C for 1h.

3 HPLC showed PNDa06=0.0% **(NMT 0.5%)**.

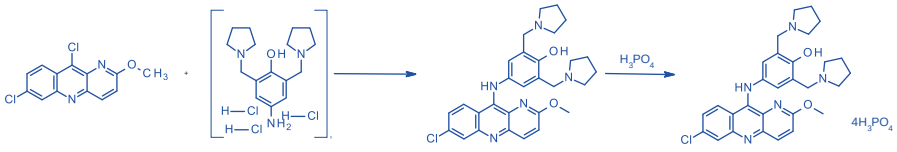
4 The solvent evaporated to dryness (no obvious fraction) at 50°C, the residue was used directly for the next step.

3.6.4. Flow chart for PNDa04-HCl



3.7. PND and TGF-001 steps

3.7.1. Reaction scheme



3.7.2. Results of TGF-001(using a phase II process showing the following changes)

- THF was replaced by acetone in purification step. Acetone can give a stable crystal form. And acetone was easier to dry.
- TGF-001 impurity 1 can be controlled better when reacted at 10℃.
- Residual Cu can meet the specification in modified process. PNDa02 and TGF-001 were easier to filter, maybe the reason.
- The assay of H_3PO_4 was higher in 2 batches. To meet the assay and crystal form requirements need further investigation.

Table 8. Results of TGF-001

No.	PNDa02	PNDa04-HCl	PND IPC (1.5h)	H_3PO_4	Salt formation		Purification	Results
PHTHARRYS-669	PHTHARRYS-667 (Assay: 95.3%w/w) 33.3g (1eq.)	PHTHARRYS-668 (IPC purity:99.6%) (1.1eq)	Batch PHTHARRYS-669 PNDa02:0.10%, PNDa04:0.46% Impurity 1:0.31%, PND:97.7%	6 eq.	Water (5v)	EtOH (10v)	Water(5v)/Acetone (10v) 1eq. H_3PO_4 Wash cake by Acetone	92.4g Yield:86.1%
PHTRACKD-665	PHTHARRYS-667 (Assay: 95.3%w/w) 33.3g (1eq.)	PHTRACKD-664 (IPC purity:99.6%) (1.1eq)	Batch PHTRACKD-665 PNDa02:0.07%, PNDa04:0.53% Impurity 1:0.29%, PND:98.1%	6 eq.	Water (5v)	EtOH (10v)	Water(5v)/Acetone (10v) 1eq. H_3PO_4 Wash cake by Acetone	93g Yield:85.6%

Note: The yield was calculated based on PNDa02.

3.7.3. The procedure for the preparation of TGF-001 in experiment PHTHARRYS-669

- To the solution of PNDa04-HCl (47.9g,124.5mmol,1.1eq) in EtOH (250mL,5v/w) was added PNDa02 (33.3g, 113.7mmol, 1.0eq).
- The suspension was then stirred at 10°C under N_2 atmosphere for 16hs.
- HPLC showed PNDa02=0.10%<0.5%. The solvent was evaporated (50°C) to dryness under reduced pressure to give a semi-solid.
- The solid was dissolved in water(500mL,10v/w). The solution was filtered to remove mechanical impurities. The filtrate was adjusted the pH to 12 with 15% NaOH(70mL).

- Lots of solids precipitated out from the solvent, then continued stirring for 1 hour at 25°C. Collect the solid by filtration, washed with water (250mL,5v/w).
- The solid was transferred to a 1L flask, water (250mL, 5v/w) was added.
- Then H_3PO_4 (85%,78.7g, 682.3mmol, 6.0eq) was added. The mixture was heated to 45°C until a clear solution was found (0.5h).
- EtOH(500mL,10v/w) was added, lots solid was formed.
- The mixture was cooled to 25°C and stirred for 1h. Collect the solid by filtration, washed with 100mL EtOH(2v/w).
- The solid was dried under vacuum at 50°C to furnish TGF-001 (103g).
- Charge TGF-001 crude (103g, 1125.9mmol) into water (500mL,5v/w).
- Then H_3PO_4 (85%,13.1g, 113.7mmol, 1.0eq) was added.
- The suspension was then stirred at 45°C for 0.5h to get a clear solution.
- Acetone (1L,10v/w) was added dropwise. Solid formed as added.
- The mixture was cooled to 25°C and stirred for 1h.
- Collect the solid by filtration, the cake was washed by Acetone (200mL). The cake was dried under vacuum (water: NMT 0.5%w/w, EtOH: NMT 5000ppm, Acetone: NMT 5000ppm) at 50°C to give TGF-001 (92.4g, 86.1% yield for 3 steps).

Table 9. Analytical data for TGF-001 Crude

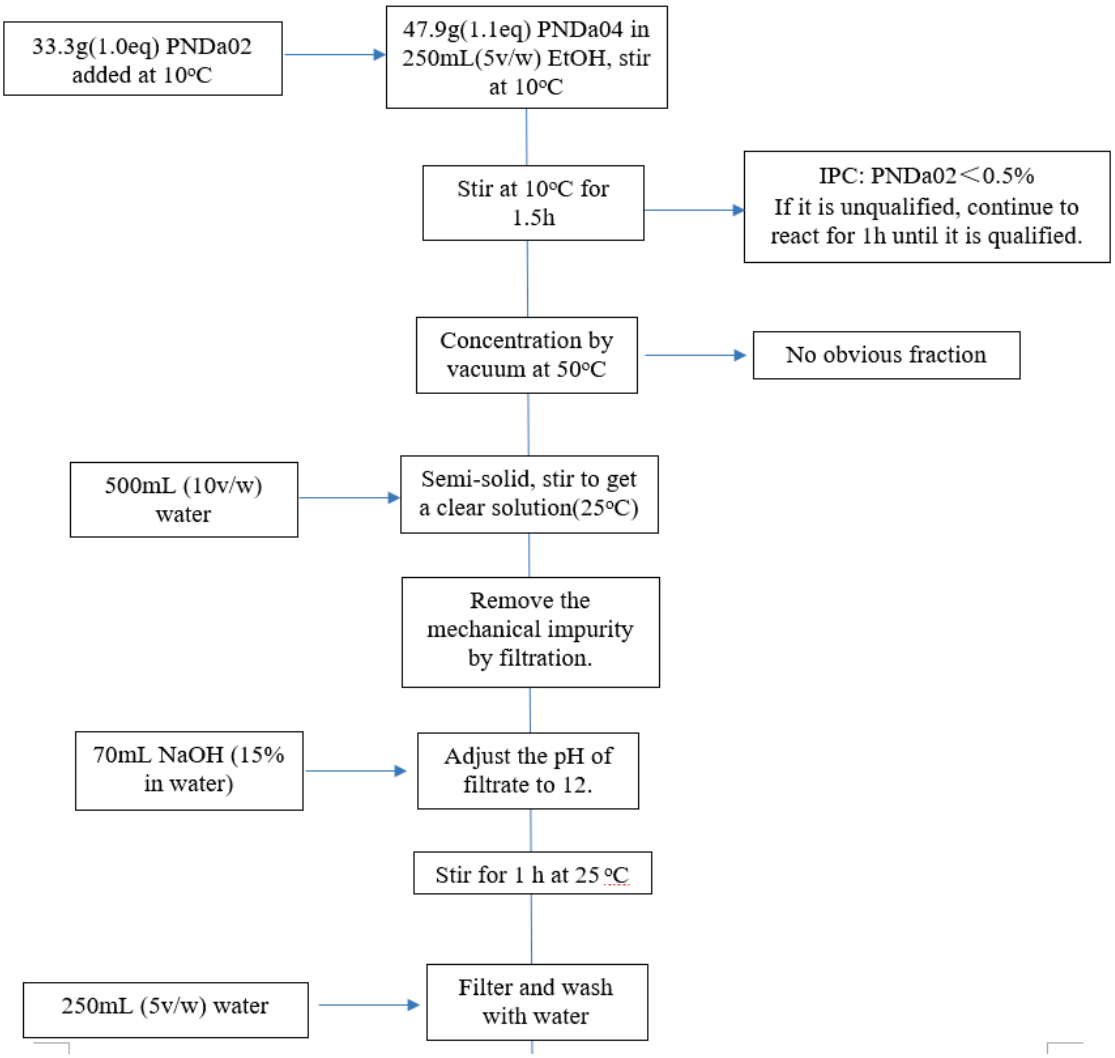
No.	Related substances (HPLC, INV_054926_HPLC_M4)	Residue of EtOH	Water content	Assay (HPLC, INV_054926_HPLC_M4)
PHTHARRYS-669	DIA: 0.11% area DIN: N. D. Impurity 1: 0.16% Total impurities: 0.54% area	0.25% (HNMR)	4.75%(KF)	93.8%
PHTRACKD-665	DIA: 0.07% area DIN: 0.02% area Impurity 1: 0.11% area Total impurities: 0.50% area	1.10% (HNMR)	4.80%(KF)	95.3%

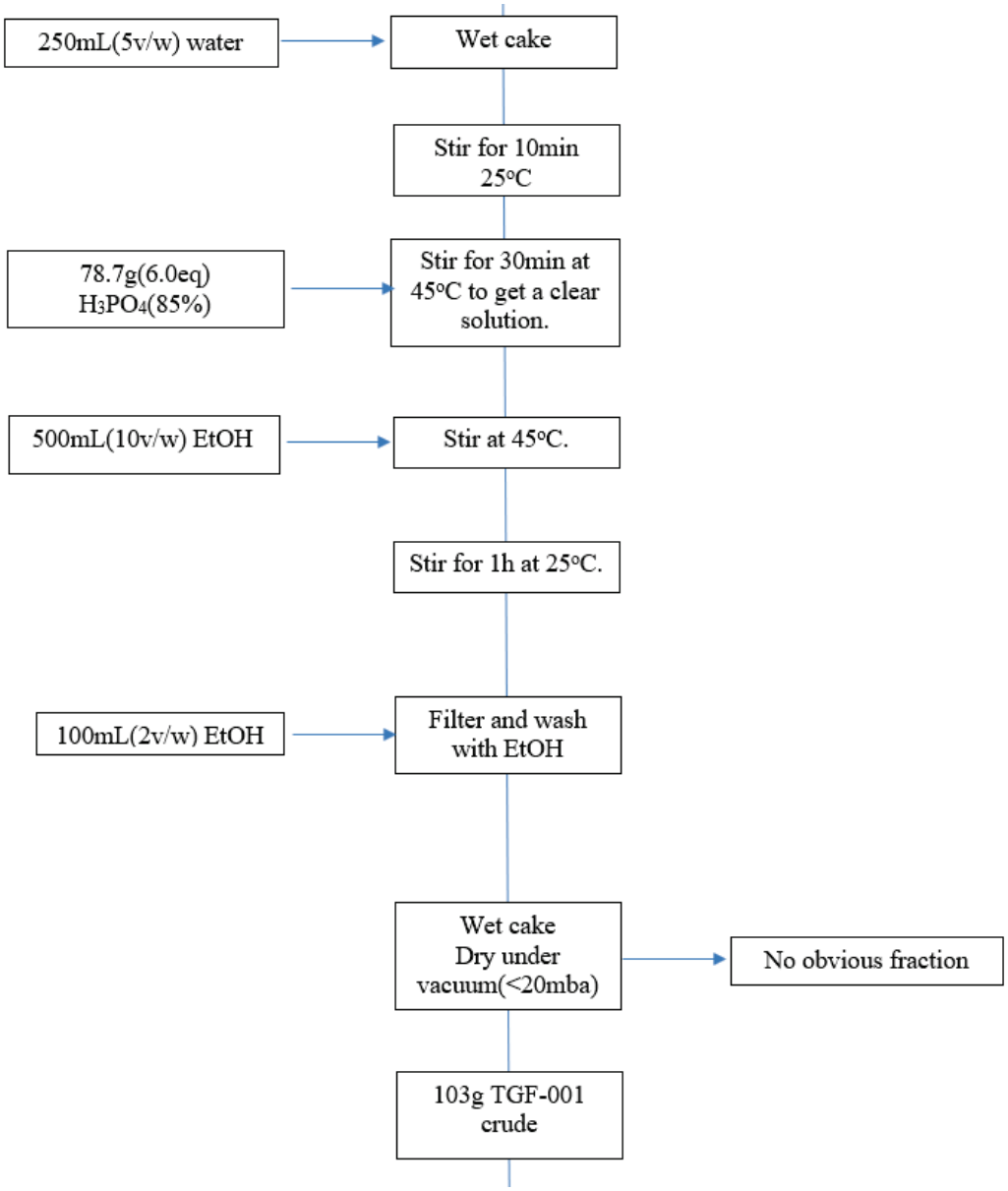
Note: The assay of TGF-001 crude met the specification (exclusive with EtOH and water).

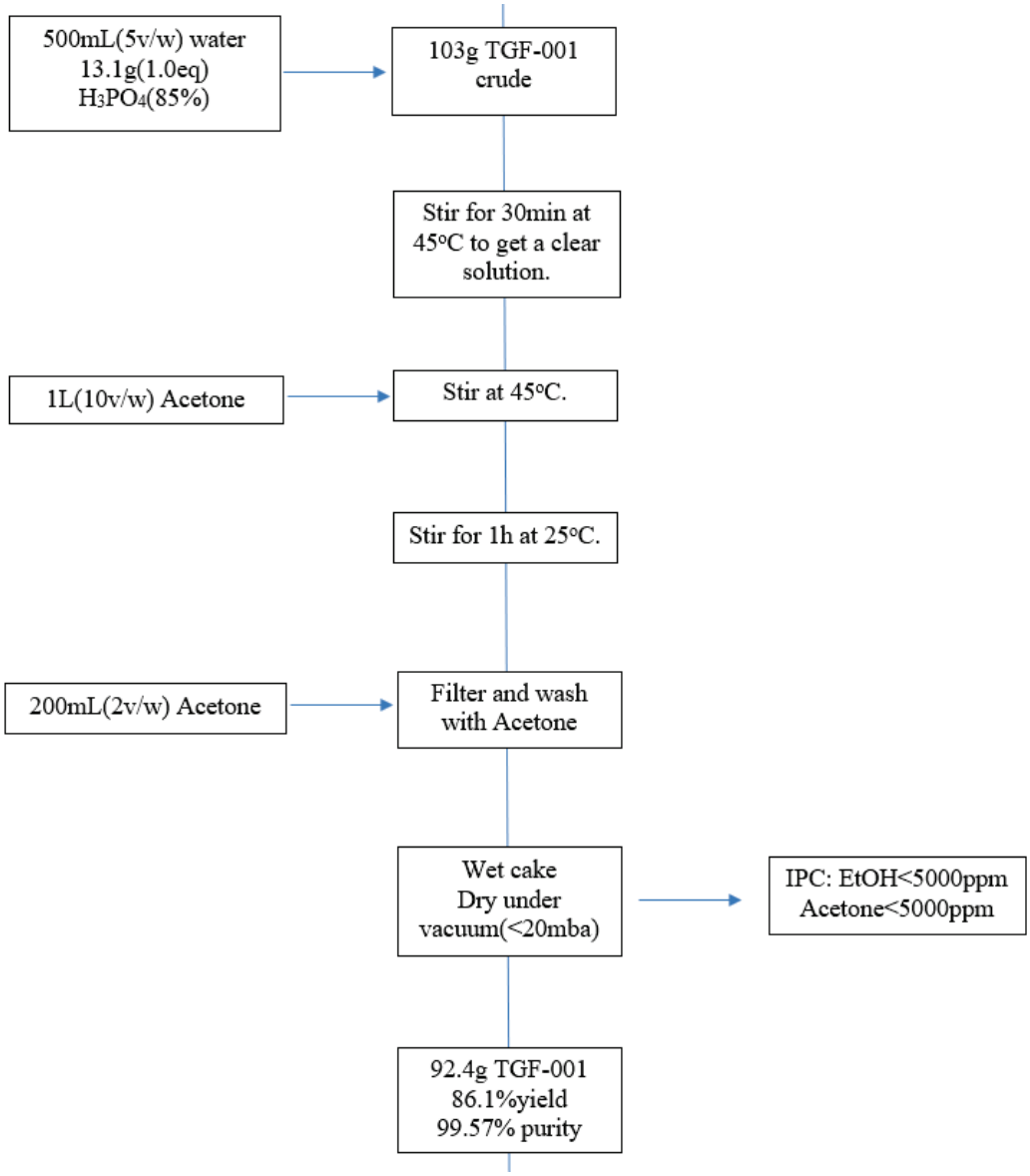
Table 10. Analytical data for final TGF-001

Items	Procedure	PHTHARRYS-669-1	PHTRACKD-665-1
Appearance	Visual inspection	Complies	Complies
Identification	HPLC, INV_054926_HPLC_M4	Complies	Complies
	IR, USP <197>, ATR	Complies	Complies
	Phosphate identification, CP <Malaridine Phosphate>	Positive	Positive
pH value	CP <Malaridine Phosphate>	2.33	2.35
Chloride content	CP <Malaridine Phosphate>	< 0.03%	< 0.03%
Insoluble substances in water	CP <Malaridine Phosphate>	0.2 mg	0.7 mg
Related substances	HPLC, INV_054926_HPLC_M4	DIA: 0.04%w/w DIN: N. D. Impurity 1: 0.02%w/w Total impurities: 0.43% area	DIA: 0.04%w/w DIN: N. D. Impurity 1: 0.02%w/w Total impurities: 0.35% area
Formaldehyde content	CP <Malaridine Phosphate>	< 0.02%	< 0.02%
Pyrrolidine content	CP <Malaridine Phosphate>	Complies	Complies
Loss on drying	CP <Malaridine Phosphate>	0.4%	1.0%
Assay (anhydrous)	HPLC, INV_054926_HPLC_M4	96.1%	95.0%
Residual solvents	INV_054926_GC_M1	225ppm <15 ppm N. D. <15 ppm	217ppm <15 ppm N. D. 132ppm
Elemental impurity (Cu)	ICP-MS, USP <233>	1.8 ppm	2.5 ppm

3.7.4. Flow chart for TGF-001



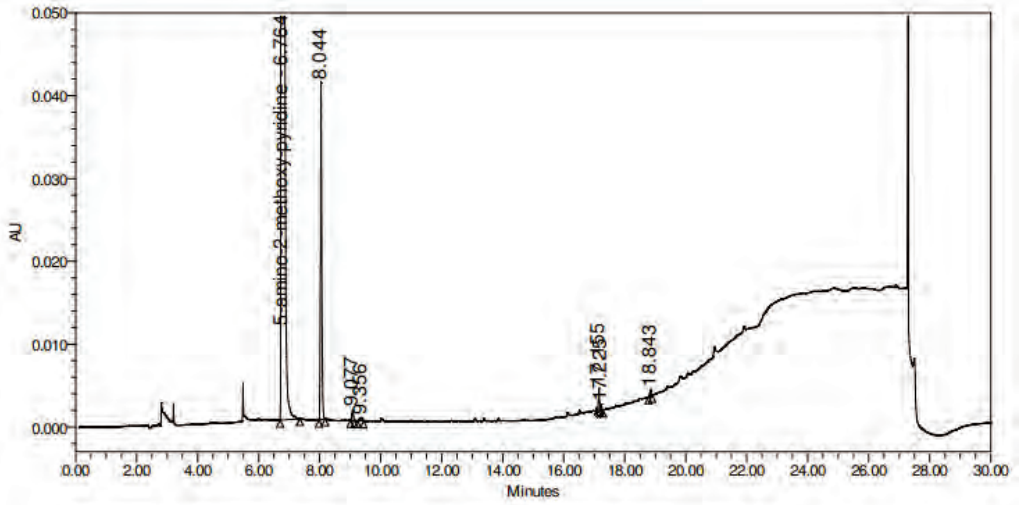




4. Attachments:

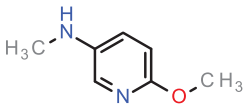
Chromatograms:

Figure 1: HPLC chromatogram of 5A2MP batch 2401001



	Name	RT	RRT	Area	% Area	Resolution
1	5-amino-2-methoxy-pyridine	6.764	1.00	3427998	96.18	
2		8.044	1.19	119314	3.35	
3		9.077	1.34	4096	0.11	
4		9.356	1.38	2259	0.06	
5		17.155	2.54	6433	0.18	
6		17.225	2.55	1812	0.05	
7		18.843	2.79	2053	0.06	

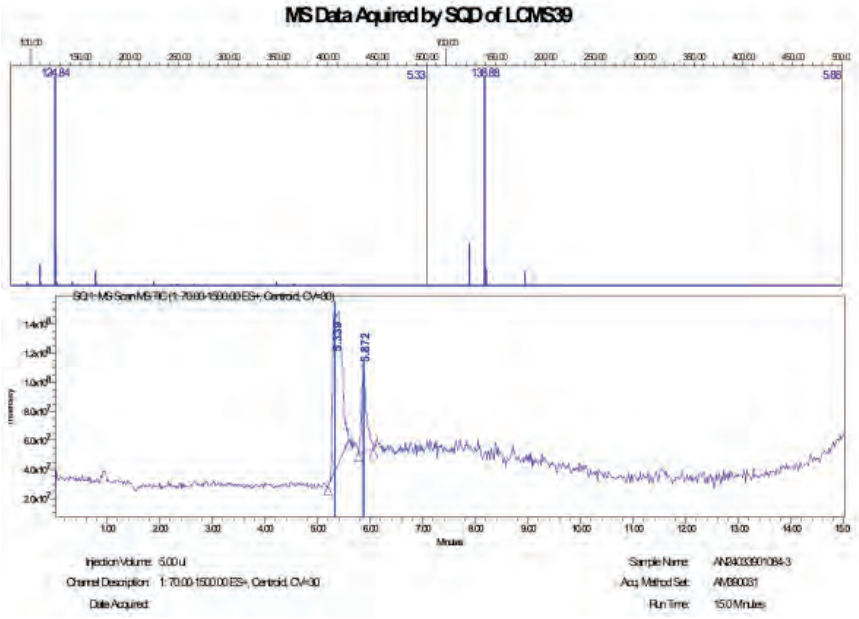
The proposed structure of impurity at RRT=1.19:



Molecular Weight: 138.17
Molecular Formula: $C_7H_{10}N_2O$

The structure of impurity was inferred from LC-MS and ^{1}HNMR.

LC-MS:


MS Data Aquired by SQD of LCMS39
124.84
5.33
138.88
5.88
5.339
5.872
Injection Volume: 5.00 ul
Channel Description: 1: 70.00-1500.00 ES+, Centroid, CV=30
Date Acquired:
Sample Name:
Acq Method Set:
Run Time: 15.0 Minutes

^{1}HNMR:

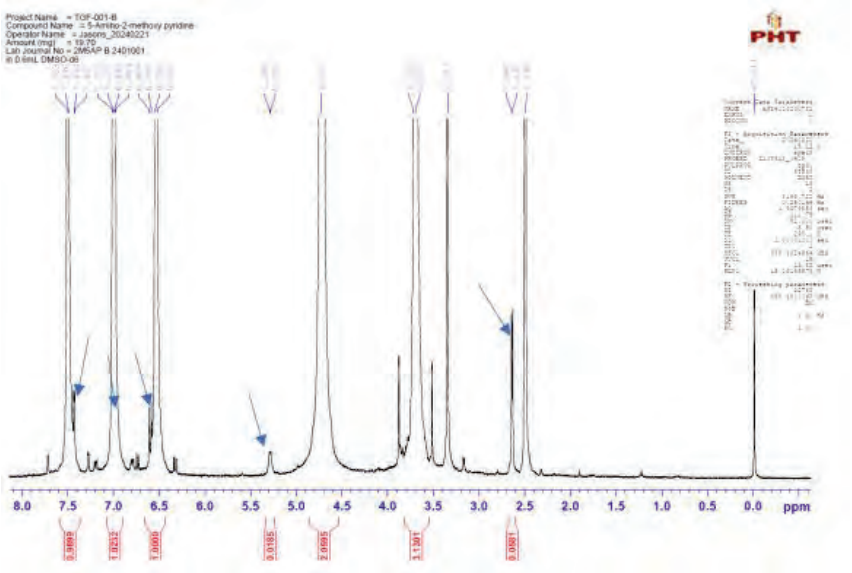
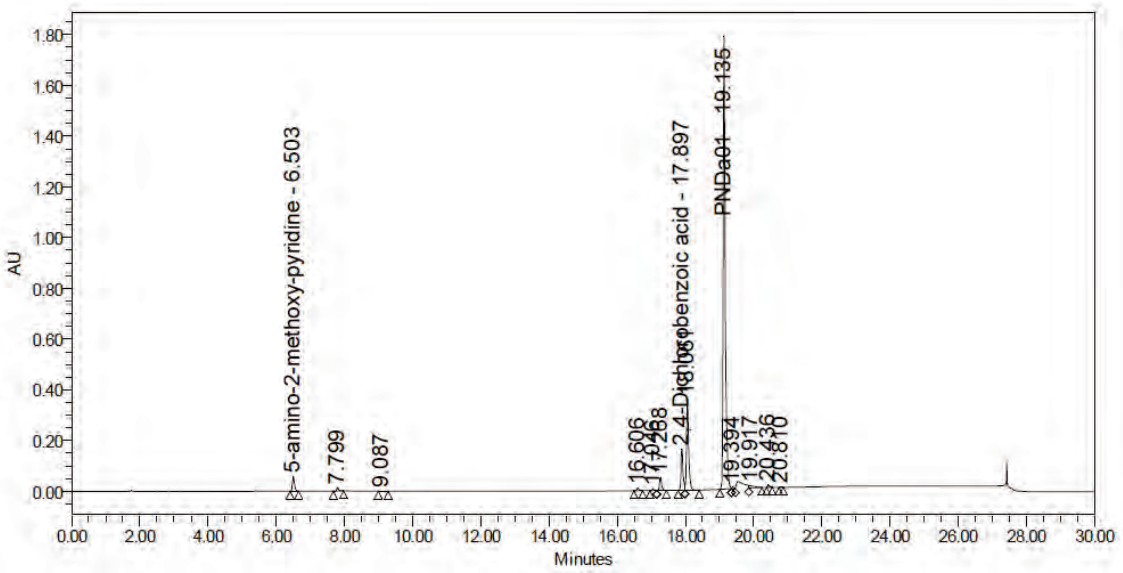

PHT
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

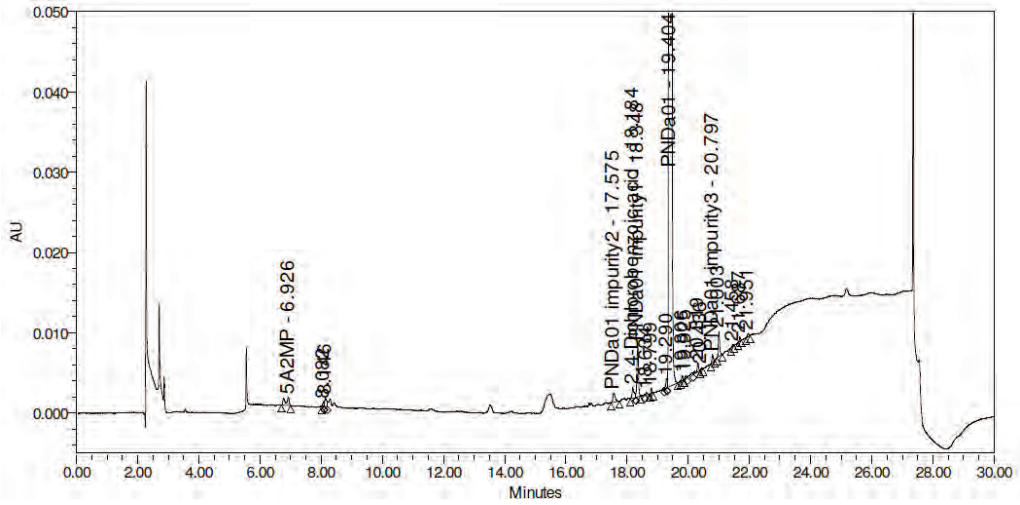
Figure 2: HPLC chromatogram of PNDa01 batch PHTKENNYG-737 (IPC)



	Name	RT	RRT	Area	% Area	Resolution
1	5-amino-2-methoxy-pyridine	6.503	0.34	242027	2.33	
2		7.799	0.41	52849	0.51	
3		9.087	0.47	3210	0.03	
4		16.606	0.87	44442	0.43	
5		17.046	0.89	20419	0.20	
6		17.268	0.90	220029	2.12	
7	2,4-Dichlorobenzoic acid	17.897	0.94	662185	6.39	
8		18.061	0.94	1543410	14.89	
9	PNDa01	19.135	1.00	7408328	71.45	

	Name	RT	RRT	Area	% Area	Resolution
10		19.394	1.01	29221	0.28	
11		19.917	1.04	97955	0.94	
12		20.436	1.07	34991	0.34	
13		20.810	1.09	9328	0.09	

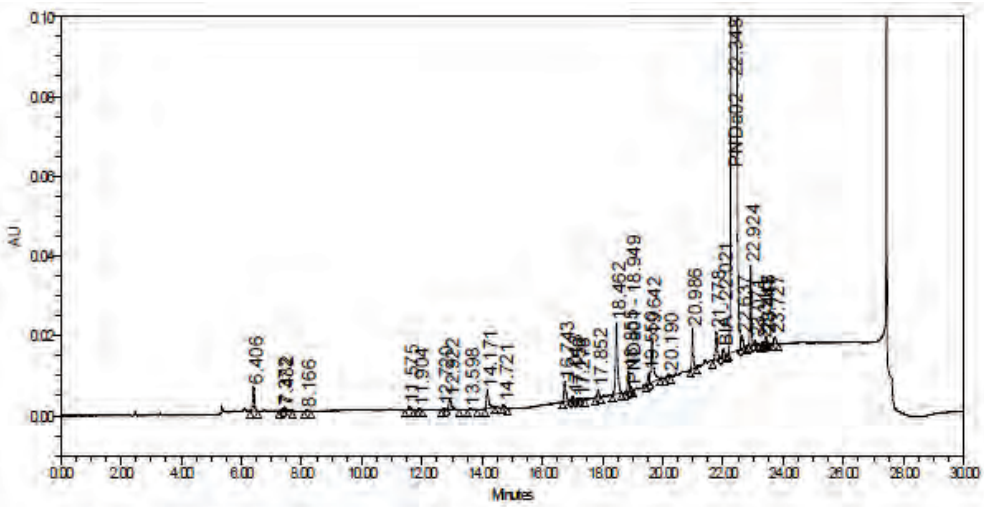
Figure 3: HPLC chromatogram of PNDa01 batch PHTKENNYG-737 (isolated)



	Name	RT	RRT	Area	% Area	Resolution
1	5A2MP	6.926	0.36	8309	0.20	
2		8.082	0.42	1714	0.04	
3		8.146	0.42	4743	0.11	
4	PNDa01 impurity2	17.575	0.91	6056	0.14	
5	2,4-Dichlorobenzoic acid	18.184	0.94	6523	0.15	
6	PNDa01 impurity1	18.348	0.95	21076	0.50	
7		18.634	0.96	1270	0.03	
8		18.799	0.97	1751	0.04	
9		19.290	0.99	3494	0.08	

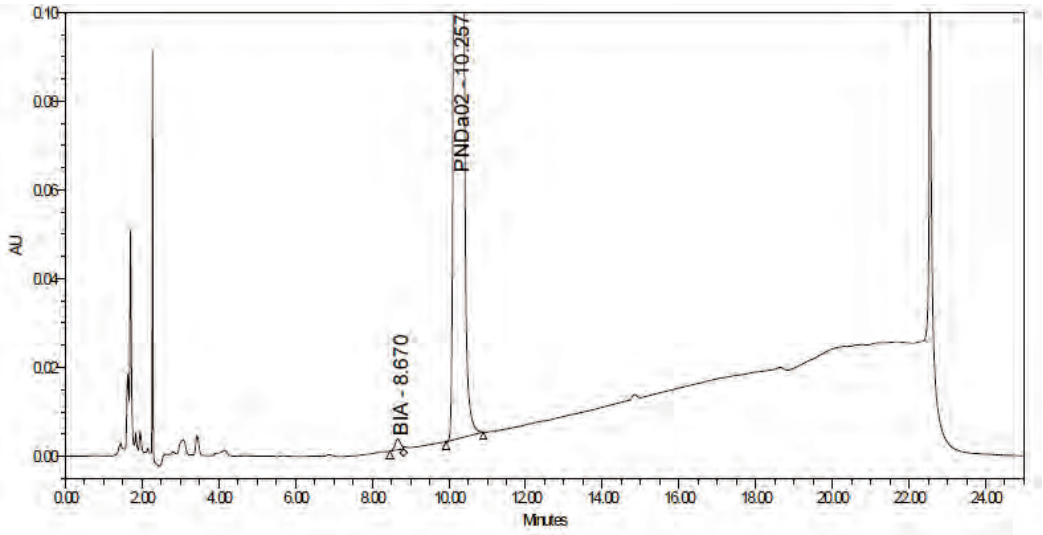
	Name	RT	RRT	Area	% Area	Resolution
10	PNDa01	19.404	1.00	4157205	98.13	
11		19.806	1.02	1590	0.04	
12		19.925	1.03	1216	0.03	
13		20.319	1.05	4387	0.10	
14		20.436	1.05	693	0.02	
15	PNDa01 impurity3	20.797	1.07	3058	0.07	
16		21.003	1.08	9791	0.23	
17		21.458	1.11	700	0.02	
18		21.687	1.12	1544	0.04	
19		21.951	1.13	1498	0.04	

Figure 4: HPLC chromatogram of PNDa02 batch PHTHARRYS-667 (IPC)



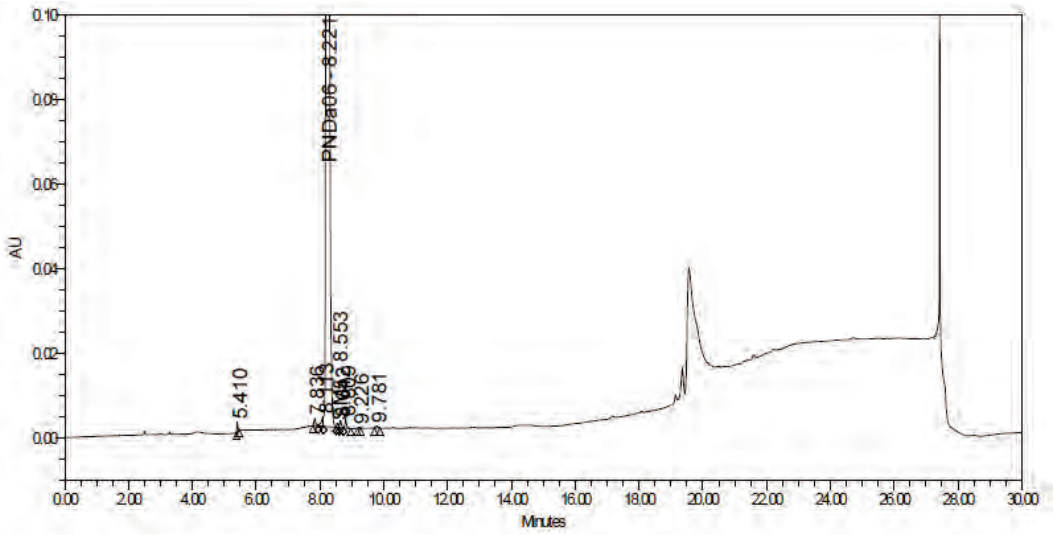
	Name	RT	RRT	Area	%Area	Resolution
1		6.406	0.29	24712	0.19	
2		7.372	0.33	2608	0.02	
3		7.432	0.33	6704	0.05	
4		8.166	0.37	1385	0.01	
5		11.575	0.52	5054	0.04	
6		11.904	0.53	1216	0.01	
7		12.720	0.57	2521	0.02	
8		12.922	0.58	15400	0.12	
9		13.598	0.61	1923	0.01	
10		14.171	0.63	25108	0.19	
11		14.721	0.66	2297	0.02	
12		16.743	0.75	21847	0.16	
13		17.013	0.76	3707	0.03	
14		17.150	0.77	1949	0.01	
15		17.278	0.77	1153	0.01	
16		17.852	0.80	7686	0.06	
17		18.462	0.83	84770	0.64	
18		18.855	0.84	18098	0.14	
19	PNDa01	18.949	0.85	3382	0.03	
20		19.550	0.87	13477	0.10	
21		19.642	0.88	59328	0.45	
22		20.190	0.90	3239	0.02	
23		20.986	0.94	32704	0.25	
24		21.778	0.97	30966	0.23	
25	BIA	22.021	0.99	8842	0.07	
26	PNDa02	22.348	1.00	1282504	96.38	
27		22.637	1.01	14723	0.11	
28		22.924	1.03	59607	0.45	
29		23.071	1.03	3115	0.02	
30		23.315	1.04	1788	0.01	
31		23.415	1.05	7463	0.06	
32		23.447	1.05	4591	0.03	
33		23.727	1.06	9881	0.07	

Figure 5: HPLC chromatogram of PNDa02 batch PHTHARRYS-667 (isolated)



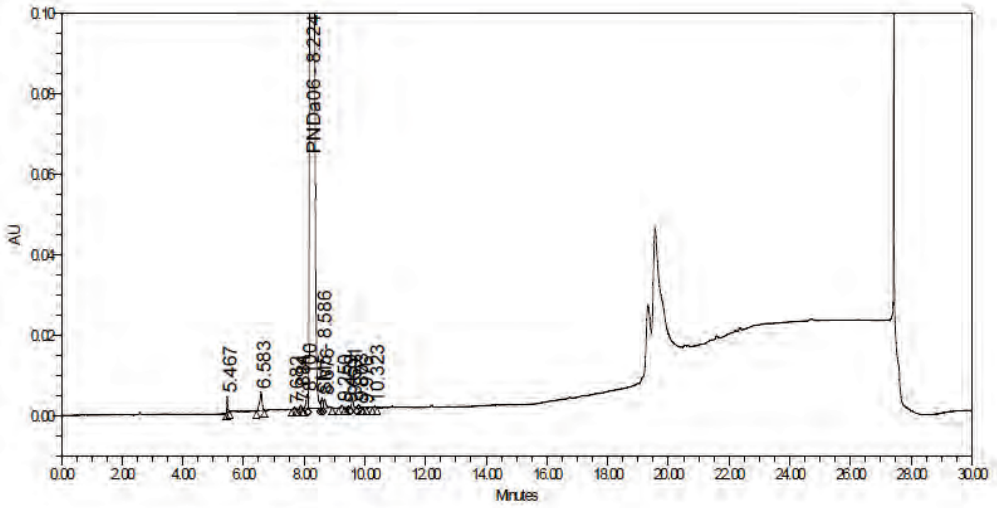
	Name	RT	RRT	Area	%Area	Resolution
1	BIA	8.670		24502	0.20	
2	PNDa02	10.257		11933647	99.80	

Figure 6: HPLC chromatogram of PNDa06-HCl batch PHTHARRYS-653 (IPC)



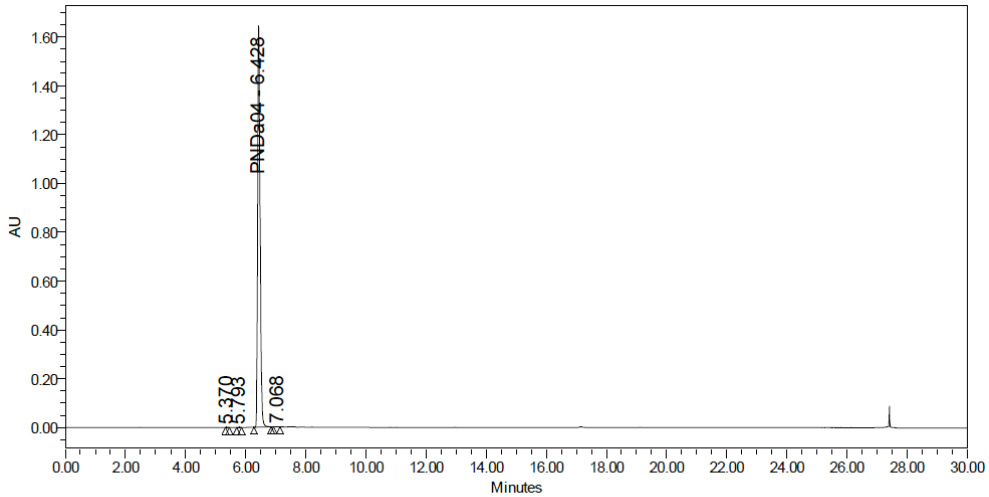
	Name	RT	RRT	Area	%Area	Resolution
1		5.410	0.66	4754	0.10	
2		7.836	0.95	6676	0.14	
3		8.113	0.99	10451	0.22	
4	PNDa06	8.221	1.00	4716758	99.11	
5	SM5	8.553	1.04	3475	0.07	
6		8.642	1.05	6407	0.13	
7		8.809	1.07	7371	0.15	
8		9.226	1.12	844	0.02	
9		9.781	1.19	2263	0.05	

Figure 7: HPLC chromatogram of PNDa06-HCl batch PHTHARRYS-653 (isolated)



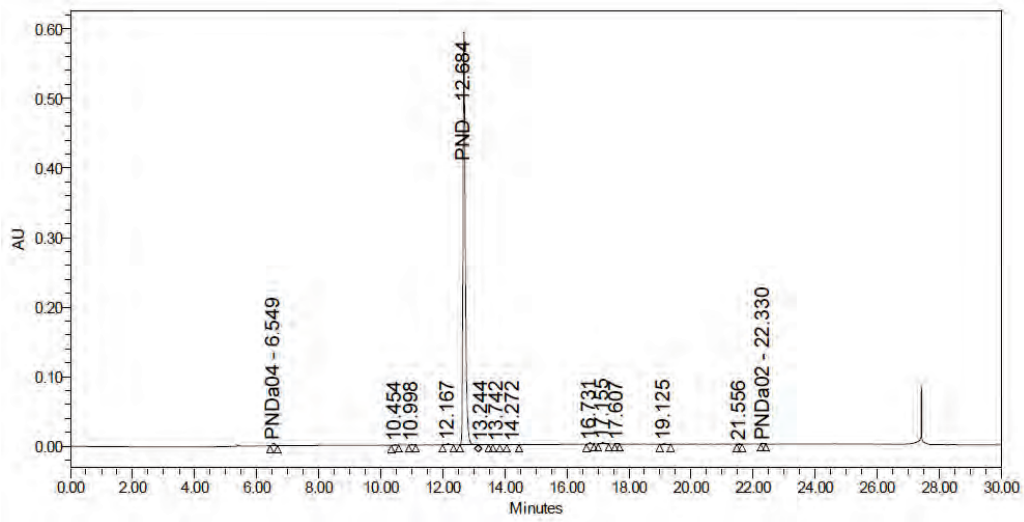
	Name	RT	RRT	Area	%Area	Resolution
1		5.467	0.66	7712	0.07	
2		6.583	0.80	24118	0.21	
3		7.682	0.93	1392	0.01	
4		7.884	0.96	2803	0.02	
5		8.100	0.98	14160	0.13	
6	PNDa06	8.224	1.00	11158602	99.18	
7	SM5	8.586	1.04	10251	0.09	
8		8.676	1.05	12963	0.12	
9		9.250	1.12	2441	0.02	

Figure 8: HPLC chromatogram of PNDa04-HCl batch PHTRACKD-664 (IPC)



	Name	RT	RRT	Area	% Area	Resolution
1		5.370	0.84	4933	0.05	
2		5.793	0.90	3128	0.03	
3	PNDa04	6.428	1.00	9436473	99.85	
4		7.068	1.10	6029	0.06	
5	PNDa06	8.200				

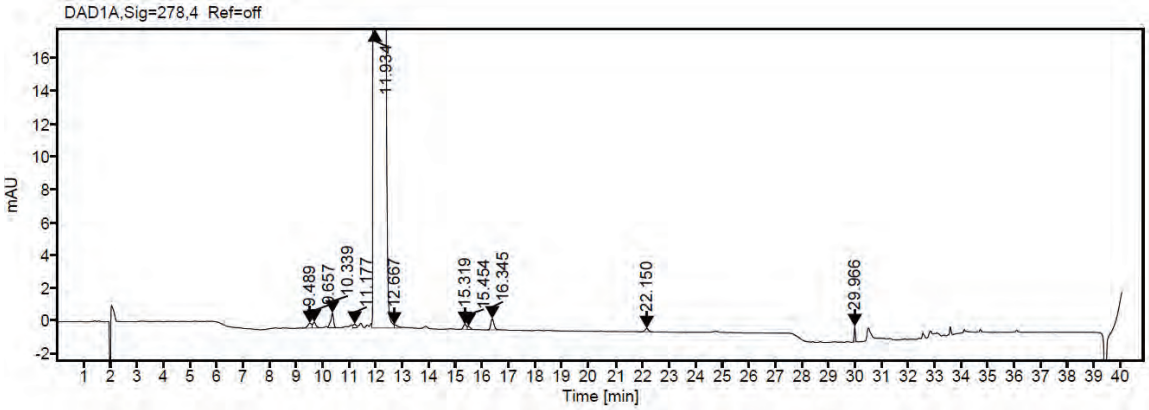
Figure 9: HPLC chromatogram of PND batch PHTRACKD-665 (IPC)



	Name	RT	RRT	Area	% Area	Resolution
1	PNDa04	6.549	0.52	16231	0.51	
2		10.454	0.82	2861	0.09	
3		10.998	0.87	1536	0.05	
4		12.167	0.96	11548	0.36	
5	PND	12.684	1.00	3098797	97.74	
6		13.244	1.04	3544	0.11	
7		13.742	1.08	1030	0.03	
8		14.272	1.13	2832	0.09	
9		16.731	1.32	7963	0.25	

	Name	RT	RRT	Area	% Area	Resolution
10		17.155	1.35	14150	0.45	
11		17.607	1.39	1426	0.04	
12		19.125	1.51	4080	0.13	
13		21.556	1.70	2755	0.09	
14	PNDa02	22.330	1.76	1845	0.06	

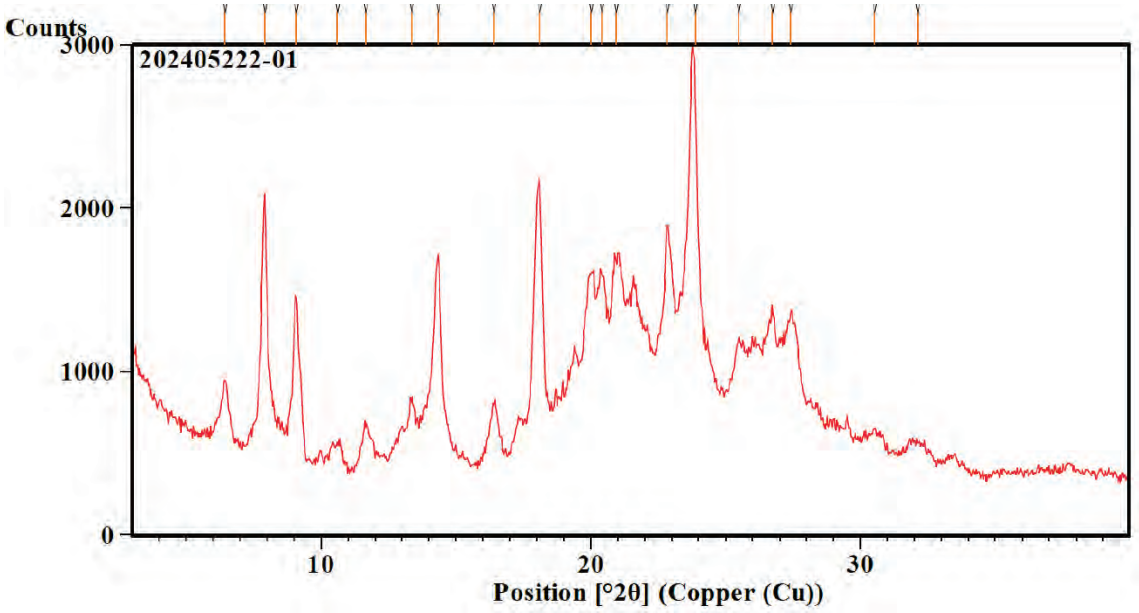
Figure 10: HPLC chromatogram of TGF-001(API) batch PHTRACKD-665



Signal: DAD1A,Sig=278,4 Ref=off

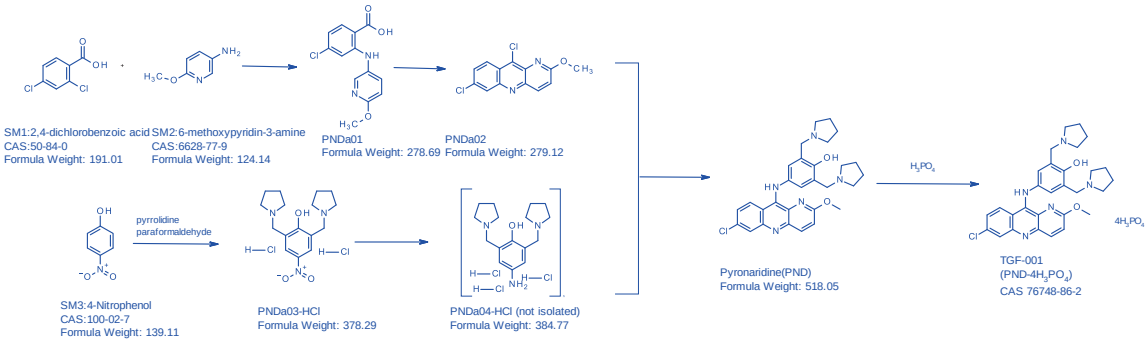
Peak	Peak Relative Ret Time	Peak Signal To Noise	RT [min]	Width [min]	Area	Height	Area%
			9.489	0.2910	2.8028	0.2891	0.03
			9.657	0.3367	2.9551	0.3562	0.03
			10.339	0.3586	6.6607	0.8962	0.08
			11.177	0.1768	1.7317	0.2234	0.02
			11.934	0.8428	8593.3062	591.2730	99.65
			12.667	0.2703	1.8066	0.1984	0.02
			15.319	0.2257	2.4313	0.2962	0.03
			15.454	0.2265	1.3839	0.1887	0.02
			16.345	0.4425	5.5680	0.6736	0.06
			22.150	0.5554	1.8167	0.1996	0.02
			29.966	0.1587	2.9053	0.9325	0.03
				Sum	**8623.3683**		

Figure 11: XRPD for TGF-001 batch PHTRACKD-665

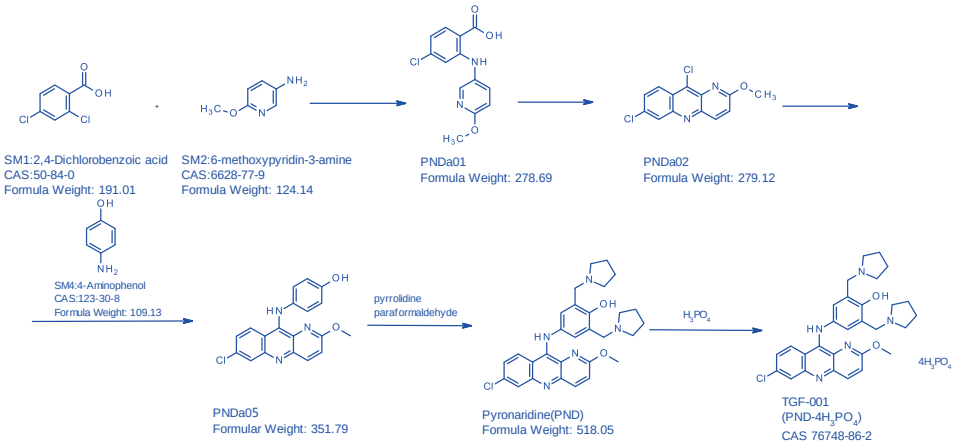


5. Process development for TGF-001 (by three routes)

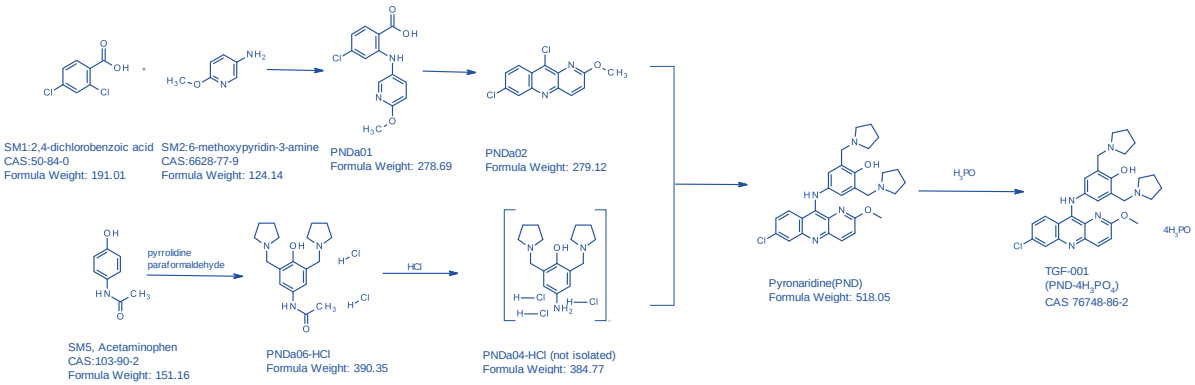
5.1. Synthetic scheme of TGF-001



Scheme 1. Synthesis of TGF-001 by route 1(convergent)



Scheme 2. Synthesis of TGF-001 by route 2(linear)

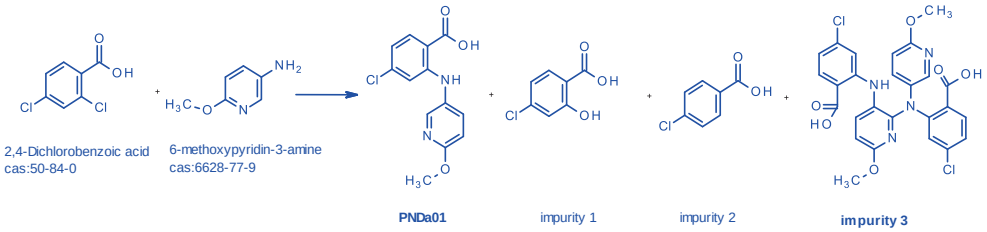


Scheme 3. Synthesis of TGF-001 by route 3 (modified convergent)

5.2. Progress development of TGF-001(route 1)

5.2.1. PNDa01 step (used in 3 routes)

5.2.1.1. Reaction scheme



5.2.1.2. Process and results of PNDa01

- The yield of PNDa01 step could be up to 80% (verified by two scale batches). The residue of 6-methoxypyridin-3-amine and the formation of impurity 3 affect the yield.
- Water can be used as reaction solvent directly without surfactants such as TPGS-750M or Savie.
- The quality of PNDa01 impacts the next step reaction, it must be purified before using for the next step.

Table 11. Initial research based on 0.5 eq K_2CO_3. (HPLC method: initial method)

Entry	2,4-dichlorobenzoic (SM1)/ 6-methoxypyridin-3-amine (SM2)	Base	Catalyst	Temp.	Solvent	Results (HPLC, area%)
PHTRACKD-239	1.0eq./ 1.07eq.	K_2CO_3(0.5eq)	CuI(0.01eq.)	85°C	2%TPGS-750-M in H_2O(8V)	83.3% SM1
PHTRACKD-240	1.0eq./ 1.07eq.	K_2CO_3(0.5eq)	CuI(0.1eq.)	85°C	2%TPGS-750-M in H_2O(8V)	63.6% SM1
PHTRACKD-242	1.0eq./ 1.07eq.	K_2CO_3(0.5eq)	CuI(0.01eq.)	85°C	2%TPGS-750-M in H_2O(4V)	20.3% SM1
PHTRACKD-243	1.0eq./ 1.07eq.	K_2CO_3(0.5eq)	CuI(0.01eq.)	100°C	2%TPGS-750-M in H_2O(4V)	18.9% SM1
PHTRACKD-248	1.0eq./ 1.07eq.	K_2CO_3(0.5eq)	CuI(0.1eq.)	100°C	2%TPGS-750-M in H_2O(4V)	7.6% SM1
PHTRACKD-256	1.0eq./ 1.07eq.	K_2CO_3(0.5eq)	CuI(0.1eq.)	100°C	2%Savie in H_2O(4V)	92.2% SM1

Note: At the beginning, the initial method was used. Only SM1 and PNDa01 were focused.

At the beginning, 0.5 eq. of K_2CO_3 was used because PNDa01 could be isolated directly after reaction. Otherwise, additional pH adjustment procedure is needed when higher equivalent e.g.: 1.0 eq., 1.8eq.... K_2CO_3 is used. The conversation of SM1 was too low when used 0.5 eq K_2CO_3.

Table 12. Research based on 1.8 eq K_2CO_3. (HPLC method: INV_054926_HPLC_M1)

Entry	2,4-dichlorobenzoic (SM1)/ 6-methoxypyridin-3-amine (SM2)	Base	Catalyst	Temp.	Solvent (8V)	Observation (after 15hours), %area				
						SM1	Impurity 1	Impurity 2	impurity 3	PNDa01
PHTRACKD-257	1.0eq./ 1.07eq.	K_2CO_3(1.8eq.)	CuI(0.1eq.)	100°C	2%Savie in H_2O	0.3%	9.3%	5.4%	4.9%	76.2%
PHTRACKD-258	1.0eq./ 1.07eq.	K_2CO_3(1.8eq.)	CuI(0.1eq.)	100°C	2%TPGS-750-M in H_2O	0.2%	9.3%	4.9%	3.5%	78.0%
PHTRACKD-259	1.0eq./ 1.07eq.	K_2CO_3(1.8eq.)	CuI(0.1eq.)	100°C	H_2O	0.3%	9.4%	4.5%	5.1%	76.3%
PHTHARRYS-388	1.0eq./ 1.07eq.	K_2CO_3(1.8eq.)	CuI(0.1eq.)	85°C	H_2O	0.7%	9.9%	4.4%	2.1%	79.4%
PHTRACKD-264	1.0eq./ 1.07eq.	K_2CO_3(1.8eq.)	CuI(0.1eq.)	70°C	H_2O	23.0%	7.0%	2.5%	1.8%	63.3%
PHTRACKD-263	1.0eq./ 1.07eq.	K_2CO_3(1.8eq.)	CuI(0.1eq.)	50°C	H_2O	43.0%	4.1%	1.2%	0.2%	50.2%
PHTRACKD-262	1.0eq./ 1.07eq.	K_2CO_3(1.8eq.)	CuI(0.1eq.)	25°C	H_2O	92.2%	0.3%	0.2%	N/D	5.5%
PHTRACKD-265	1.0eq./ 1.0eq.	K_2CO_3(1.8eq.)	CuI(0.01eq.)	85°C	H_2O	50.4%	7.6%	1.1%	N/D	38.1%
PHTRACKD-266	1.0eq./ 1.0eq.	K_2CO_3(1.8eq.)	CuI(0.05eq.)	85°C	H_2O	14.7%	9.6%	2.5%	0.9%	69.7%
PHTRACKD-267	1.0eq./ 1.0eq.	K_2CO_3(1.8eq.)	CuI(0.12eq.)	85°C	H_2O	1.2%	10.7%	5.7%	10.5%	67.6%
PHTRACKD-270	1.0eq./ 1.0eq.	K_2CO_3(1.8eq.)	CuO(0.1eq.)	85°C	H_2O	34.9%	14.7%	1.1%	N/D	47.1%
PHTRACKD-271	1.0eq./ 1.0eq.	K_2CO_3(1.8eq.)	Cu_2O(0.1eq.)	85°C	H_2O	1.7%	21.3%	4.0%	4.0%	66.3%

The equivalent of SM1, catalyst, temperature, solvent based on 1.8eq. K_2CO_3 were screened. Impurity 1, impurity 2 were sourced from SM1, they cannot be avoided in water. Impurity 3 was sourced from PNDa01. The residue of SM2 and the formation of impurity 3 can both impact the yield.

Table 13. Research on base and catalyst (HPLC method: INV_054926_HPLC_M1)

Entry	2,4-dichlorobenzoic (SM1)/ 6-methoxypyridin-3-amine (SM2)	Base	Catalyst	Temp.	H_2O (8V)	Observation (after 15hours), %area				
						SM1	Impurity 1	Impurity 2	impurity 3	PNDa01
PHTRACKD-298	1.3eq./ 1.0eq.	Na_2CO_3(2.0eq)	CuI(0.1eq)	85°C	H_2O	2.1%	8.2%	4.7%	3.0%	75.7%
PHTRACKD-299	1.3eq./ 1.0eq.	Na_2CO_3(2.0eq)	CuBr(0.1eq)	85°C	H_2O	8.1%	11.5%	2.8%	0.5%	62.3%
PHTRACKD-300	1.3eq./ 1.0eq.	Na_2CO_3(2.0eq)	CuCl(0.1eq)	85°C	H_2O	8.8%	12.1%	3.7%	0.5%	60.0%
PHTRACKD-303	1.3eq./ 1.0eq.	Na_2CO_3(2.0eq)	CuBr(0.12eq)	100°C	H_2O	7.8%	11.9%	4.2%	0.8%	62.7%
PHTRACKD-304	1.3eq./ 1.0eq.	Na_2CO_3(2.0eq)	CuCl(0.12eq)	100°C	H_2O	6.9%	12.7%	5.1%	1.0%	65.2%
PHTRACKD-285	1.1eq./ 1.0eq.	K_2CO_3(1.8eq)	CuI(0.1eq)	85°C	H_2O	2.8%	9.0%	4.7%	4.4%	74.6%
PHTRACKD-286	1.2eq./ 1.0eq.	K_2CO_3(1.8eq)	CuI(0.1eq)	85°C	H_2O	1.6%	10.6%	4.9%	7.6%	71.2%
PHTRACKD-287	1.2eq./ 1.0eq.	Na_2CO_3(1.8eq)	CuI(0.1eq)	85°C	H_2O	4.3%	7.3%	4.3%	2.8%	77.4%
PHTRACKD-288	1.2eq./ 1.0eq.	AcONa(2.5eq)	CuI(0.1eq)	85°C	H_2O	8.9%	2.2%	11.8%	0.8%	51.0%
PHTRACKD-289	1.2eq./ 1.0eq.	NaOH(2.5eq)	CuI(0.1eq)	85°C	H_2O	28.1%	15.5%	0.5%	ND	36.0%
PHTRACKD-290	1.2eq./ 1.0eq.	KOH(2.5eq)	CuI(0.1eq)	85°C	H_2O	28.1%	18.6%	0.5%	ND	17.8%
PHTANWARL-530	1.4eq./ 1.0eq.	Na_2CO_3(2.2eq)	CuI(0.05eq)	95°C	H_2O(6v)	2.1%	12.9%	3.4%	0.8%	72.0%

The equivalent of SM1, catalyst, temperature, base was screened. PHTRACKD-287 gives the highest reaction conversion. DOE was conducted based on this condition.

Table 14. DOE results (HPLC method: INV_054926_HPLC_M1)

No.	Regular two-level design			D: Volume of water	E: Reaction temp. (℃)	Residue SM2 ,%mol	Impurity 3 ,%area	Yield%, based solution assay
	A: eq. of SM1	B: eq. of Na_2CO_3	C: eq. of CuI					
PHTANWARL-488	1	1.4	0.05	12	75	40.4%	0.7%	57.8% (7.1% assay)
PHTANWARL-489	1	2.2	0.05	6	75	41.9%	0.1%	53.3% (6.5% assay)
PHTANWARL-501	1.4	2.2	0.15	12	95	7.1%	3.6%	74.1% (9.8% assay)
PHTANWARL-502	1	1.4	0.05	6	95	21.6%	1.0%	71.2% (9.4% assay)
PHTANWARL-503	1.2	1.8	0.1	9	85	12.4%	3.0%	81.1% (9.8% assay)
PHTANWARL-504	1	2.2	0.15	6	95	19.4%	1.9%	71.7% (8.9% assay)
PHTANWARL-494	1	1.4	0.15	6	75	28.1%	0.7%	64.9% (8.2% assay)
PHTANWARL-500	1.4	1.4	0.05	12	95	20.1%	2.1%	73.5% (8.9% assay)
PHTANWARL-505	1	2.2	0.05	12	95	30.2%	0.6%	65.2% (7.9% assay)
PHTANWARL-490	1.4	1.4	0.15	12	75	23.2%	1.8%	67.0% (8.9% assay)
PHTANWARL-491	1.4	2.2	0.15	6	75	18.3%	1.0%	72.2% (9.2% assay)
PHTANWARL-506	1	1.4	0.15	12	95	27.7%	3.0%	62.8% (8.1% assay)
PHTANWARL-507	1.4	2.2	0.05	6	95	15.4%	0.4%	84.0% (9.7% assay)
PHTANWARL-508	1.4	1.4	0.15	6	95	9.7%	2.6%	83.2% (9.9% assay)
PHTANWARL-495	1.4	1.4	0.05	6	75	20.0%	0.6%	77.8% (9.7% assay)
PHTANWARL-498	1.2	1.8	0.1	9	85	11.5%	2.8%	74.8% (9.6% assay)
PHTANWARL-492	1	2.2	0.15	12	75	37.6%	0.6%	58.1% (6.7% assay)
PHTANWARL-493	1.4	2.2	0.05	12	75	45.0%	0.2%	52.5% (5.9% assay)
PHTANWARL-496	1.2	1.8	0.1	9	85	14.9%	1.5%	77.0% (9.7% assay)
PHTANWARL-497	1.2	1.8	0.1	9	85	16.1%	1.2%	74.5% (9.4% assay)

PHTANWARL-507 gives the highest reaction conversion. The yield of 500g batch with this condition is 80.1%.

The results of DoE showed that eq of SM1 1.2~ 1.4, 1.4~2.2eq of Na_2CO_3, 0.05~0.15eq of CuI, 6~9 Volume of water, Reaction temperature 85~95 ^oC are operation range.

Table 15. Research on ligand (HPLC method: INV_054926_HPLC_M1)

Entry	2,4-dichlorobenzoic (SM1)/ 6-methoxypyridin-3-amine (SM2)	Base	Catalyst	Ligand	Temp.	H_2O (8V)	Observation (after 15hours), %area				
							SM1	Impurity 1	Impurity 2	impurity 3	PNDa01
PHTRACKD-276	1eq./ 1.0eq.	K_2CO_3(1.8 eq.)	CuI(0.1 eq.)	L-proline(0.2eq)	85°C	H_2O	18.2%	9.9%	1.9%	0.15%	68.0%
PHTRACKD-277	1eq./ 1.0eq.	K_2CO_3(1.8 eq.)	CuI(0.1 eq.)	DMEDA(0.1eq)	85°C	H_2O	9.6%	27.3 %	3.6%	N/A	55.4%
PHTRACKD-278	1eq./ 1.0eq.	K_2CO_3(0.5 eq.)	CuI(0.1 eq.)	L-proline(0.2eq)	85°C	H_2O	64.6%	7.9%	14.0%	N/A	9.8%
PHTRACKD-279	1eq./ 1.0eq.	K_2CO_3(0.5 eq.)	CuI(0.1 eq.)	DMEDA(0.1eq)	85°C	H_2O	33.6%	30.9 %	16.3%	N/A	11.4%

L-proline or DMEDA did not help the conversion of the reaction and reduction of impurities.

5.2.1.3. Purification of PNDa01

Table 16. Purification of PNDa01 (HPLC method: INV_054926_HPLC_M1)

No.	Test Item	Test Element	Result	Remarks
PHTRACKD-295	ICP-MS	Cu	8808.6 ppm	Crude PNDa01, controlled experiment
PHTRACKD-295-EDTA	ICP-MS	Cu	3468.2 ppm	Crude PNDa01 was Washed with EDTA for 15hs
PHTRACKD-295-1N HCl	ICP-MS	Cu	1151.8 ppm	Crude PNDa01 was Washed with 1N HCl for 15hs
PHTHARRYS-389-1N HCl	ICP-MS	Cu	1642.9 ppm	Crude PNDa01 was Washed with 1N HCl for 30min

Purging of Cu was tried at the beginning. We suspected the residue of Cu may lead to the bad results of PNDa02. We did not test it anymore when we found the new process for PNDa02. So, the residue of Cu in PNDa01 and TGF-001 are not comparable.

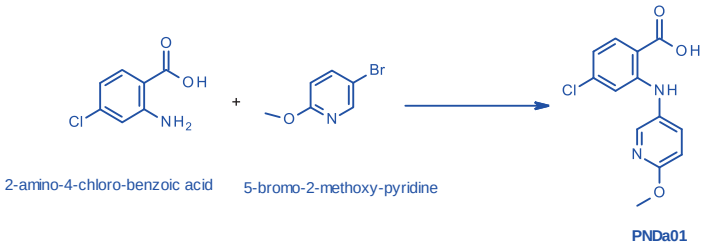
Table 17. Purification of PNDa01 (HPLC method: INV_054926_HPLC_M1)

No.	Level	Assay or purity (crude)	Procedure	Assay or purity (purified)	Obtained amount	Purification yield, %	Remark
PHTHARRYS-425	5.0 g	86.7%area	Adjust pH to 1.16, stir 1h at 30°C, filtrate/ dry	87.3%area	4.47 g	90%	No Assay available, HPLC purity was used for evaluation.
PHTHARRYS-426	5.0 g	86.7%area	adjust pH to 0.60, stir 20h at 30°C, filtrate/ dry	86.1%area	4.57 g	91%	
PHTHARRYS-427	5.0 g	86.7%area	Dissolve in 50mL water and 10mL MeOH, stir 1h at 30°C, filtrate/ dry	87.7%area	4.26 g	86%	
PHTHARRYS-431	5.0 g	86.7%area	Dissolve in 50mL water, stir 1h at 50°C, filtrate/ dry	86.7%area	4.42 g	88%	
PHTHARRYS-440	5.0 g	75.6%w/w	Adjust to alkalinity, then use activated carbon to adsorb impurities, and	79.4%w/w	3.95 g	83%	N/A

			then adjust to acidity, filtrate/ dry				
PHTHARRYS-435-1	5.0 g	81.5%w/w	Washed by 5v/w EtOH at 50°C for 1h, filtrate/ dry	90.9%w/w	3.96 g	88%	N/A
PHTHARRYS-435-2	24.2 g	75.6%w/w	Washed by 10v/w water at 50°C for1h, filtrate/ dry	81.5%w/w	22.4 g	99%	N/A
PHTHARRYS-437	12.0 g	75.6%w/w	Washed by 5v/w EtOH at 50°C for 1h, then washed by 10v/w water at 50°C for1h, filtrate/ dry	88.0%w/w	9.56 g	93%	N/A
PHTHARRYS-442	46.6 g	75.6%w/w		89.4%w/w	35.4 g	90%	N/A
PHTHARRYS-443	36.5 g	79.5%w/w	Washed by 5v/w EtOH at 30°C for 2h, filtrate/ dry	89.6%w/w	30.6g	94%	total yield: 75%

PNDa01 crude could be effectively purified by washing with EtOH and water (at least 10% assay increase with MT 90% yield).

5.2.1.4. New route for PNDa01



2-amino-4-chloro-benzoic acid 5-bromo-2-methoxy-pyridine

PNDa01

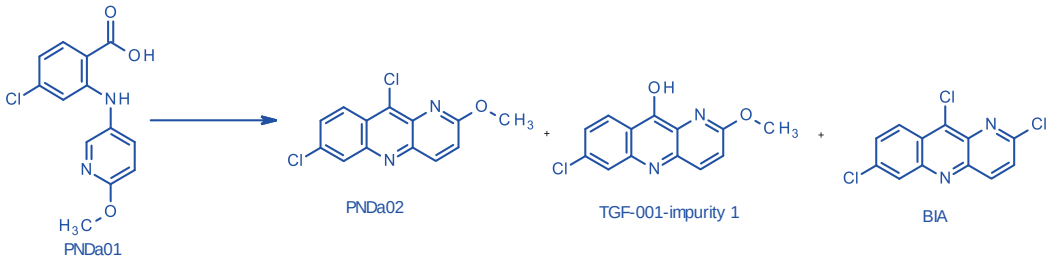
Table 18. Results of new route for PNDa01 (HPLC method: INV_054926_HPLC_M1)

Entry	2-amino-4-chlorobenzoic acid/ 5-bromo-2-methoxy pyridine	Base	Catalyst	Ligand	Temp.	Solvent	HPLC(PNDa01,area%)
PHTRACKD-245	1eq./ 1.1eq.	K_2CO_3(1.2eq.)	CuI(0.1eq.)	N/A	130°C	DMF	2.6%
PHTRACKD-241	1eq./ 1.1eq.	K_2CO_3(1.8eq.)	CuI(0.1eq.)	N/A	85°C	H_2O(8V)	1.0%
PHTRACKD-244	1eq./ 1.1eq.	K_2CO_3(2.0eq.)	CuI(0.2eq.)	N/A	130°C	Isoamyl alcohol	16.9%
PHTRACKD-249	1eq./ 1.1eq.	$NaHCO_3$(2.0eq.)	N/A	N/A	R.T.	DCM	N.D.
PHTRACKD-253	1eq./ 1.1eq.	K_2CO_3(2.0eq.)	Cu(0.2eq.)	N/A	85°C	Propan-2-ol	N.D.
PHTRACKD-272	1eq./ 1.1eq.	K_2CO_3(2.0eq.)	CuI(0.1eq)	Proline(0.2eq)	130°C	DMF	23.7%
PHTRACKD-273	1eq./ 1.1eq.	K_2CO_3(2.0eq.)	CuI(0.1eq)	DMEDA(0.1eq)	130°C	DMF	4.4%
PHTRACKD-274	1eq./ 1.1eq.	N/A	Cu_2O(0.1eq)	Cu(0.2eq)	110°C	DMF	17.8%
PHTRACKD-275	1eq./ 1.1eq.	K_2CO_3(1.0eq.)	Cu_2O(0.1eq)	Cu(0.2eq)	130°C	2-Methoxyethanol	13.2%

All tried reactions failed to get a promising result. The product was too little to optimize. Theoretically, this reaction is difficult to conduct, we just want to see the feasibility of this reaction.

5.2.2. PNDa02 step (used in 3 routes)

5.2.2.1. Reaction scheme



5.2.2.2. Process and results of PNDa02

- The yield could be obviously improved when using purified PNDa01 whose assay (HPLC, w/w%) consisted with purity (HPLC, area%). The crude PNDa01with assay (HPLC, w/w%) lower than purity (HPLC, area%) can inhibit the reaction.
- The solubility of PNDa01 and PNDa02 in reaction solvent is important for this step reaction which impacts the reaction conversion. Propylene carbonate has the best solubility for PNDa01 and PNDa02 after screening.
- The feed sequence is very important for this reaction. The yield and quality of PNDa02 using procedure in PHTHARRYS-513 was higher than one pot reaction.
- The reason was not clear why the IPC (area%) was good but the isolated yield was low using dioxane or toluene.
- The quenching temperature was important (below 10℃) to control BIA.

Table 19. The results for the preparation of PNDa02(with crude PNDa01) (HPLC method: INV_054926_HPLC_M1)

Entry	DIPEA	$POCl_3$	Reaction solvent	Reaction temperature	IPC(4h), %area	Isolation
PHTHARRYS-395	4.4 eq.	1.24v/w	Toluene (6.5v/w)	85°C	PNDa01: N/D PNDa02: 69.0% Other impurities: 31.0%	HPLC purity: 99.6%area Yield (based on HPLC purity): 34.4% Black precipitates were observed.
PHTHARRYS-396	4.4 eq.	1.24v/w	1,4-Dioxane (6.5v/w)	85°C	PNDa01: N/D PNDa02: 77.9% Other impurities: 22.1%	HPLC purity: 99.6%area Yield (based on HPLC purity): 29.1% Black precipitates were observed.
PHTHARRYS-388	N/A	3v/w	Toluene (6.5v/w)	110°C	Failed because many black precipitates were formed.	
PHTHARRYS-392	N/A	3v/w	1,4-Dioxane(15v/w)	100°C	PNDa01: 2.2% PNDa02: 86.3% Other impurities: 11.5%	HPLC purity: 91.0%area Yield (based on HPLC purity): 52.2% Black precipitates were observed.
PHTHARRYS-381	N/A	10v/w	N/A	130°C	PNDa01: N/D PNDa02: 96.7% Other impurities: 3.3%	HPLC purity: 97.7%area Yield (based on HPLC purity): 55.3% Black precipitates were observed.
PHTHARRYS-391	N/A	3v/w	DMF (15v/w)	100°C	No PNDa02 formed	

Note: One-pot reaction.

At the beginning, crude PNDa01 was used for PNDa02 step. Black precipitates were observed, and the yield was low. All the results were negative. But the reaction had a better result when used dioxane as solvent without DIPEA (PHTHARRYS-392).

Table 20. The results for the preparation of PNDa02(with different PNDa01) (HPLC method: INV_054926_HPLC_M1)

Entry	DIPEA/ $POCl_3$	Reaction solvent	Reaction temperature	IPC(3h), %area	Isolation	Remark
PHTHARRYS-401	10.8eq./9.8eq	1,4-Dioxane(15v/w)	100°C	Impurity 1: n.d. PNDa01: 0.9% PNDa02: 93.4%	HPLC purity: 99.3%area Yield (based on HPLC purity): 50.1%	Use crude PNDa01 directly
PHTHARRYS-402	10.8eq./9.8eq	1,4-Dioxane(15v/w)	130°C	Impurity 1: n.d. PNDa01: ND PNDa02: 90.8%	HPLC purity: 91.2%area Yield (based on HPLC purity): 50.7%	Use crude PNDa01 directly
PHTHARRYS-406	10.8eq./9.8eq	1,4-Dioxane(15v/w)	100°C	Impurity 1: 0.2%. PNDa01: 0.1% PNDa02: 96.1%	HPLC purity: 99.4%area Yield (based on HPLC purity): 68.3%	Crude PNDa01 was Washed with 1N HCl for 30min
PHTHARRYS-410	10.8eq./9.8eq	1,4-Dioxane(15v/w)	100°C	Impurity 1: 8.1% PNDa01: ND PNDa02: 90.1%	HPLC purity: 99.8%area Yield (based on HPLC purity): 51.6%	Crude PNDa01 was Washed with 1N HCl for 15hs
PHTHARRYS-411	10.8eq./9.8eq	1,4-Dioxane(15v/w)	100°C	Impurity 1:3.0% PNDa01: 0.5% PNDa02:87.8%	HPLC purity: 97.7%area Yield (based on HPLC purity): 24.8%	Crude PNDa01 was Washed with EDTA for 15hs
PHTHARRYS-412	10.8eq./9.8eq	1,4-Dioxane(15v/w)	100°C	Impurity 1:3.2% PNDa01: 0.3% PNDa02:90.8%	HPLC purity: 98.5%area Yield (based on HPLC purity): 42.0%	Crude PNDa01 was Washed with 1N HCl for 15hs
PHTHARRYS-417	10.8eq./9.8eq	1,4-Dioxane(15v/w)	100°C	Impurity 1:10.2% PNDa01: 0.1% PNDa02: 87.2%	HPLC purity: 99.4%area Yield (based on HPLC purity): 54.3%	Crude PNDa01 was Washed with 1N HCl for 2hs

Note: One-pot reaction.

The yields were different. This might be due to the different purification process for PNDa01. So, purifying PNDa01 with a proper process is important for this reaction (see table 6).

Table 21. The results for the preparation of PNDa02(with purified PNDa01) (HPLC method: INV_054926_HPLC_M1)

Entry	$POCl_3$/DIPEA	Reaction solvent	Reaction temperature	IPC(3h), %area	Isolation
PHTHARRYS-465	4eq./4.4eq (TEA)	1,4-Dioxane(15v/w)	105°C	Impurity 1: 0.1% PNDa01: 0.2% PNDa02: 92.3%	Assay: 70.6%w/w, Yield (based on assay): 64%
PHTHARRYS-468	4eq./4.4eq	1,4-Dioxane(15v/w)	105°C	Impurity 1: 0.1% PNDa01: 0.9% PNDa02:91.7%	Assay: 66.1%w/w, Yield (based on assay): 65.5%
PHTHARRYS-473	4eq./4.4eq (TEA)	Toluene(10v/w)	105°C	Impurity 1: 0.1% PNDa01: 1.3% PNDa02: 84.8%	N/A
PHTHARRYS-474	4eq./4.4eq (TEA)	Toluene(10v/w)	105°C	Impurity 1: 0.1% PNDa01: 2.9% PNDa02: 80.7%	N/A
PHTHARRYS-475	4eq./4.4eq	Toluene(10v/w)	105°C	Impurity 1: 0.1% PNDa01: 0.3% PNDa02: 83.2%	N/A
PHTHARRYS-477	4eq./4.4eq	Toluene(10v/w)	105°C	Impurity 1: 0.2% PNDa01: 1.2% PNDa02: 82.6%	N/A
PHTHARRYS-479	4eq./4.4eq	Toluene(10v/w)	105°C	Impurity 1: n.d. PNDa01: 1.0% PNDa02:89.5%	Assay: 75.7%w/w, Yield (based on assay): 48.4%
PHTHARRYS-480	4eq./4.4eq	1,4-Dioxane (12.5v/w)	105°C	Impurity 1: 1.0% PNDa01: 0.2% PNDa02:91.1%	Assay: 74.7%w/w, Yield (based on assay): 68.1%

Note: procedure II. Procedure II was the procedure in PHTHARRYS-513

PNDa01 was washed with EtOH. Yields were from crude based on assay. Toluene was tried again because dioxane was forbidden to use in industry. Lower reaction conversion using toluene as reaction solvent compared to dioxane with procedure II.

Table 22. The results for the preparation of PNDa02(with propylene carbonate) (HPLC method: INV_054926_HPLC_M1)

Entry	$POCl_3$/DIPEA	Reaction solvent	Reaction temperature	IPC(1h), %area	Isolation	Remark
PHTHARRYS-486	4eq./4.4eq	Propylene carbonate(12.5v/w)	105°C	Impurity 1: 0.1% PNDa01: 0.1% PNDa02:95.6%	Assay: 90.7% Yield (based on assay): 70.8%	Procedure II Drop at 100°C
PHTHARRYS-487	4eq./4.4eq	Propylene carbonate(6.5v/w)	105°C	Impurity 1: 0.5% PNDa01: 0.4% PNDa02:64.9%	N/A	One-pot reaction
PHTHARRYS-488	4eq./4.4eq	Propylene carbonate(8v/w)	105°C	Impurity 1: 0.2% PNDa01: 0.02% PNDa02: 96.7%	Assay: 86.9% Yield (based on assay): 70.6%	Procedure II Drop at 100°C
PHTHARRYS-489	4eq./4.4eq	Propylene carbonate(8v/w)	105°C	Impurity 1: 0.1% PNDa01: 0.1% PNDa02: 95.4%	Assay: 93.0% Yield (based on assay): 76.0%	Procedure II Drop at 100°C
PHTHARRYS-490	4eq./4.4eq	Propylene carbonate(8v/w)	105°C	Impurity 1: 0.1% PNDa01: 0.8% PNDa02: 93.6%	N/A	Procedure II Drop at 100°C
PHTHARRYS-491	2.5eq./2.75eq	Propylene carbonate(8v/w)	105°C	Impurity 1: 0.2% PNDa01: 0.1% PNDa02: 59.2%	N/A	Procedure II Drop at 100°C
PHTHARRYS-492	4eq./4.4eq	Propylene carbonate(8v/w)	105°C	Impurity 1: 0.1% PNDa01: 0.1% PNDa02: 89.6%	N/A	Procedure II Drop at 100°C
PHTHARRYS-493	4eq./4.4eq	Propylene carbonate(8v/w)	100°C	Impurity 1: 0.1% PNDa01: 0.02% PNDa02:95.6%	Assay: 95.6% Yield (based on assay): 78.0%	Procedure II Drop at 80°C
PHTHARRYS-495	4eq./4.4eq	Propylene carbonate(5v/w)	100°C	Impurity 1: 1.5% PNDa01: 0.06% PNDa02:93.4%	Assay: 95.9% Yield (based on assay): 78.2%	Procedure II Drop at 80°C
PHTHARRYS-513	4eq./4.4eq	Propylene carbonate(8v/w)	100°C	Impurity 1: 0.3% PNDa01: 0.03% PNDa02:94.7	Assay: 94.9% Yield (based on assay): 80.8%	Procedure II Drop at 80°C

$POCl_3$/DIPEA (4eq./4.4eq) gives the best result. In PHTHARRYS-492: The acyl chloride has not been completely added yet, and there are already solid precipitates in the reaction bottle, the reaction was worse. Adding acyl chloride at 80°C can solve this problem.

Propylene carbonate gives a better result (reaction conversion, yield) than dioxane.

The stirring status was worse when using 5v/w solvent.

Table 23. The results for the quenching of PNDa02 reaction

PNDa02, IPC_M1, %area	Workup procedure	Isolated PNDa02, HPLC_M3	TGF-001 crude HPLC_M4, %area
PHTHARRYS-582 BIA:0.19%	Quenched with MeOH, then added to water; NH3.H2O was added to the resulted mixture (below 20℃)	PHTHARRYS-582 BIA:0.08%area Assay: 93.4%w/w	PHTRACKD-596 DIA:0.06% PND:99.7%
PHTHARRYS-602 BIA:0.10%	Quenched with MeOH+ 15% NaOH aqueous (below 20℃)	PHTHARRYS-602 BIA:0.16%area Assay: 93.9%w/w	PHTHARRYS-611 DIA:0.17% PND:98.9%
PHTHARRYS-606 BIA:0.08%	Quenched with MeOH+15% NaOH aqueous (below 20℃)	PHTHARRYS-606 BIA: 0.57%area Assay: 98.1%	PHTHARRYS-622 DIA:0.60% PND:98.9%
PHTHARRYS-630 BIA:0.09%	Quenched with MeOH, then added to water; 15% NaOH aqueous was added to the resulted mixture (below 20℃)	PHTHARRYS-630 BIA: 0.26%area Assay: 95.9%	PHTHARRYS-633 DIA:0.26% PND:99.1%
PHTKENNYG-724 BIA:0.08%	Quenched with MeOH+ 15% NaOH aqueous (below 20℃)	PHTKENNYG-724 BIA:0.27%area	N/A
PHTHARRYS-646 BIA:0.11%	Quenched with MeOH, then added to 15% NaOH aqueous solution (below 10℃)	PHTHARRYS-646 BIA: 0.08%area Assay: 94.0%	PHTRACKD-643 DIA:0.03% PND:99.6%
PHTHARRYS-647 BIA:0.09%	Quenched with MeOH and water; then15% NaOH aqueous was added to the resulted mixture (below 10℃)	PHTHARRYS-647 BIA: 0.07%area Assay: 94.0%	PHTRACKD-644 DIA:0.04% PND:99.6%
PHTHARRYS-648 BIA:0.13%	Quenched with MeOH and water; then NH_3H2O was added to the resulted mixture (below 10℃)	PHTHARRYS-648 BIA: 0.13% Assay: 94.1%	PHTHARRYS-652 DIA:0.05% PND:99.4%

Using 15% NaOH aqueous to quench the reaction (advantages: lower production cost, better filtration, no nitrogen-containing wastewater).

The quenching temperature was important to control BIA.

PNDa02 was easier to filter when using modified process. The assay was higher too.

Table 24. The results for the purification of PNDa02

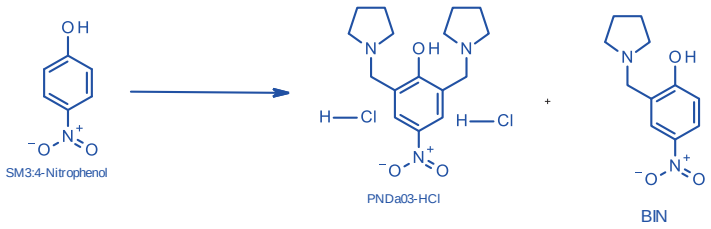
Crude PNDa02(HPLC_M3)	Purification solvent	Isolated PNDa02(HPLC_M3)
PHTKENNYG-724 (BIA:0.27%)	MeOH (ambient)	PHTKENNYG-727 BIA:0.27%area
PHTKENNYG-724 (BIA:0.27%)	EtOH (ambient)	PHTKENNYG-728 BIA:0.30%area
PHTKENNYG-724 (BIA:0.27%)	DCM (ambient)	PHTKENNYG-729 BIA:0.15%area
PHTKENNYG-724 (BIA:0.27%)	Water (ambient)	PHTKENNYG-730 BIA:0.28%area

Note: PNDa02 was slurred in solvent at room temperature for 1hs.

DCM has effect to purge BIA which will be used as a backup (not applied in current process).

5.2.3. PNDa03-HCl step (Route 1)

5.2.3.1. Reaction scheme



5.2.3.2. Process and results of PNDa03-HCl

- The yield was 93% and purity was about 98% after simple condition screening (equivalent of pyrrolidine/ paraformaldehyde, solvent, temperature).
- This intermediate will not be used in the future (see summary).

Table 25. The results for the preparation of PNDa03(solvent screening) (HPLC method: INV_054926_HPLC_M1)

Entry	Pyrrolidine/ paraformaldehyde	Reaction solvent	Reaction temperature	IPC, %area	Isolation
PHTHARRYS-384	5.21eq/5.35eq	IPA(4v/w)	90°C(1h)	SM3: N/D. BIN: 3.3%. PNDa03: 89.7%	N/A
PHTRACKD-320	4.eq./ 4eq.	IPA(4v/w)	50°C(4h)	SM3: 0.44%. BIN: 4.7%. PNDa03: 91%	N/A
PHTRACKD-321	4eq./4eq.	IPA(4v/w)	70°C(1h)	SM3: N/D. BIN: 0.5%. PNDa03: 92.5%	HPLC purity: 95.7%area Yield (based on HPLC purity):80.0%
PHTRACKD-322	4eq./4eq.	IPA(4v/w)	80°C(1h)	SM3: N/D. BIN: 0.9%. PNDa03: 94.0%	HPLC purity: 95.3%area Yield (based on HPLC purity):85.0%
PHTRACKD-323	4eq./4eq.	IPA(4v/w)	90°C(1h)	SM3: N/D. BIN: 0.5%. PNDa03: 92.8%	N/A
PHTRACKD-326	4eq./4eq.	IPA(4v/w)	60°C(6h)	SM3: N/D. BIN: 3.3%. PNDa03: 93.8%	N/A
PHTRACKD-327	4eq./4eq.	2-MeTHF(4v/w)	60°C(2h)	SM3: N/D. BIN: 0.8%. PNDa03: 98.3%	HPLC purity: 97.4%area Yield (based on HPLC purity):90.5%
PHTRACKD-328	4eq./4eq.	CPME(4v/w)	60°C(6h)	SM3: N/D. BIN: 1.4%. PNDa03: 98.0%	N/A

IPA was the reported solvent in literature. 2-MeTHF gives the best result (reaction conversion, purity and yield) among the 3 solvents.

Table 26. The results for the preparation of PNDa03(2-MeTHF) (HPLC method: INV_054926_HPLC_M1)

Entry	Pyrrolidine/ paraformaldehyde	Reaction solvent	Reaction temperature	IPC, %area	Isolation
PHTRACKD-335	3.5eq./3.5eq.	2-MeTHF(4v/w)	60°C(3h)	SM3: N/D.BIN: 0.7%. PNDa03: 96.5%	Purity:97.8%area Yield:92.6%
PHTRACKD-336	3.0eq./3.0eq.	2-MeTHF(4v/w)	60°C(4h)	SM3: N/D.BIN: 0.5%. PNDa03: 96.3%	Purity:97.7%area Yield:92.9%
PHTRACKD-337	2.5eq./2.5eq.	2-MeTHF(4v/w)	60°C(4h)	SM3: N/D. BIN: 0.5%. PNDa03: 97.3%	Purity:97.8%area Yield:93.0%
PHTRACKD-349	2.5eq./2.5eq.	2-MeTHF(4v/w)	70°C(4h)	SM3: N/D. BIN: 1.0%. PNDa03: 96.0%	Purity:98.1%area Yield:94.7%
PHTRACKD-341	2.5eq./2.5eq.	2-MeTHF(4v/w)	70°C(3h)	SM3: N/D. BIN: 0.7%. PNDa03: 98.3%	N/A
PHTRACKD-342	2.5eq./2.5eq.	2-MeTHF(4v/w)	80°C(2h)	SM3: N/D. BIN: 0.1%. PNDa03: 97.7%	N/A

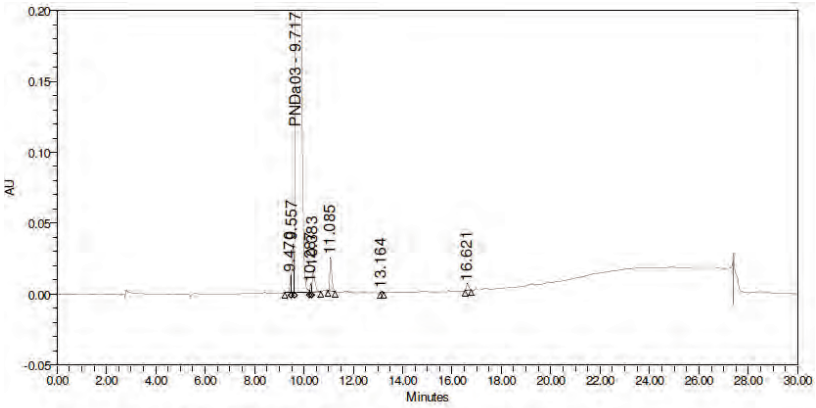
The equivalent of Pyrrolidine/ paraformaldehyde could be decreased to at least 2.5 eq..

The increase of reaction temperature has the improvement on this step (higher conversion or shorter reaction time).

5.2.3.3. Typical procedure for preparation of PNDa03 in experiment PHTRACKD-349

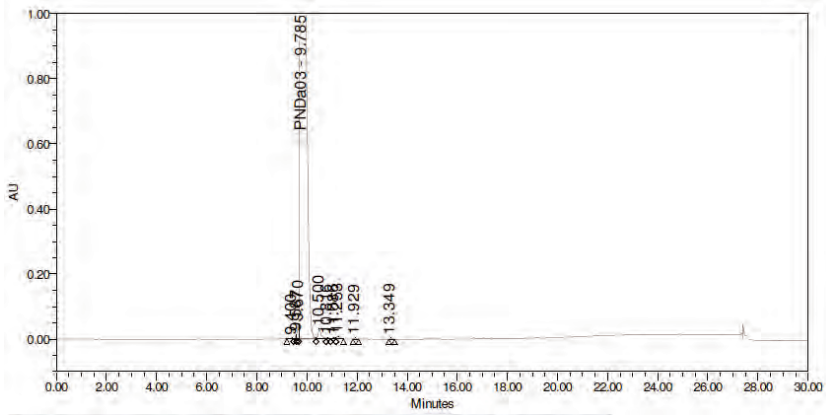
1. Charge 4-Nitrophenol (30g, 213.5mmol,1.0eq), Paraformaldehyde (17.05g, 533.75mmol,2.5eq) and 2-MeTHF(120mL,4v/w) into 500mL three-neck round-bottom flask.
2. Then Pyrrolidine (38.34g, 533.75mmol,2.5eq) was added dropwise for 0.5h at 10~15 °C.
3. Then the reaction temperature was raised to 50°C and stirred for 0.5h under N2 atmosphere.
4. The reaction was raised to 70°C and stirred for 4h under N2 atmosphere.
5. HPLC showed that 96.01% of PND-a03 was formed and no raw material was left. The intermediate (DIN-PNDa03) was 0.99%.
6. The solvent was evaporated (45°C) to dryness under reduced pressure to give 84g crude as an orange oil.
7. IPA (360mL,12v/w) was added to the residue (orange oil) and the flask was cooled to 5 °C.
8. 2M HCl in EA (420mL,14v/w) was added dropwise to the reaction(pH=1~2).
9. Lots of solids precipitated out from the solvent, then continued stirring for 1 hour at 5°C.
10. The solid was filtered and washed with IPA (90mL,3v/w).
11. The solid was dried under vacuum at 50°C furnish PNDa03-HCl (78g,202.27mmol, 94.74% yield,98.1% purity).

HPLC chromatogram of PNDa03(IPC)



	Name	RT	RRT	Area	% Area	Resolution
1		9.470	0.97	45143	0.34	
2		9.557	0.98	188314	1.42	
3	PNDa03	9.717	1.00	12736670	96.01	
4		10.287	1.06	18971	0.14	
5		10.383	1.07	109902	0.83	
6		11.085	1.14	131672	0.99	
7		13.164	1.35	3476	0.03	
8	p-Nitrophenol	15.545				
9		16.621	1.71	31617	0.24	

HPLC chromatogram of PNDa03(isolated)

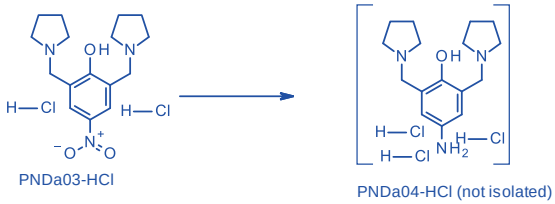


	Name	RT	RRT	Area	% Area	Resolution
1		9.400	0.96	16460	0.05	
2		9.587	0.98	42581	0.13	
3		9.670	0.99	147359	0.45	
4	PNDa03	9.785	1.00	32272056	98.07	
5		10.500	1.07	254690	0.77	
6		10.816	1.11	27122	0.08	
7		11.086	1.13	36024	0.11	
8		11.253	1.15	68225	0.21	
9		11.929	1.22	18140	0.06	

	Name	RT	RRT	Area	% Area	Resolution
10		13.349	1.36	23894	0.07	
11	p-Nitrophenol	15.545				

5.2.4. PNDa04-HCl step (Route 1)

5.2.4.1. Reaction scheme



5.2.4.2. Process and results of PNDa04

- The quality of PNDa04-HCl is important for the quality of TGF-001.
- Failed to isolate free base of PNDa04, it is unstable reported in literature.
- PNDa04-HCl was an oil after concentration, it was hard to solidify. So, purification of PNDa04-HCl is hard.

Table 27. The results for the preparation of PNDa04(1.5%eq Pd) (HPLC method: INV_054926_HPLC_M1)

Entry	10% Pd/C (50% water)	Reaction solvent	37% HCl	Reaction temperature	Reaction pressure	IPC (~15 hours), %area
PHTRACKD-346	1.5%. (Pd)	2% TPGS-750-M aqueous (5V)	N/A	30°C	0.4M Pa	PNDa03: 1.3%, PNDa04: 86.5% Max. impurity @RRT 1.36: 5.9%
PHTRACKD-350	1.5%. (Pd)	MeOH(5V)	N/A	30°C	0.4M Pa	PNDa03: 2.0%, PNDa04: 87.0% Max. impurity @RRT 1.36: 2.7%
PHTRACKD-316	1.5%. (Pd)	Water(5V)	1.0 eq.	30°C	0.4M Pa	PNDa03: 4.8%, PNDa04: 92.3% Max. impurity @RRT 1.46: 1.2%
PHTHARRYS-404	1.5%. (Pd)	2% TPGS-750-M aqueous (5V)	1.0 eq.	30°C	0.4M Pa	PNDa03: 1.9%, PNDa04: 94.5% Max. impurity @RRT 1.18: 1.3%
PHTRACKD-357	1.5%. (Pd)	MeOH(5V)	1.0 eq.	30°C	0.4M Pa	PNDa03: N. D., PNDa04: 93.2% Max. impurity @RRT 1.08: 2.2%
PHTRACKD-361	1.5%. (Pd)	EtOH(5V)	1.0 eq.	30°C	0.4M Pa	PNDa03: N. D., PNDa04: 93.6% Max. impurity @RRT 1.15: 3.4%

The reaction solution was used for the next step directly after removing catalyst.

Water system was not suitable for next step (see table 17). EtOH was more environmentally than MeOH.

An additional 1.0 eq. of HCl could inhibitor the formation of impurities.

Table 28. The results for the preparation of PNDa04(screen Pd amount) (HPLC method: INV_054926_HPLC_M1)

No.	Reaction solvent	37% HCl	Pd (Pd/C)	Reaction temperature	Pressure	IPC (~15 hours), %area
PHTRACKD-376	TPGS-750-M/ water(5V)	1.0 eq.	1.5% eq.	30°C	0.4M Pa	PNDa03: 0.3%, PNDa04: 93.3%
PHTRACKD-318	TPGS-750-M/ water(5V)	1.0 eq.	0.2% eq.	30°C	0.4M Pa	PNDa03: 7.6%, PNDa04: 84.3%
PHTRACKD-370	MeOH(5V)	1.0 eq.	1.5% eq.	30°C	0.4M Pa	PNDa03: 0.5., PNDa04: 96.3%
PHTRACKD-379	MeOH(5V)	1.0 eq.	0.4% eq.	30°C	0.4M Pa	PNDa03: 0.5%, PNDa04: 94.4%
PHTRACKD-383	MeOH(5V)	1.0 eq.	0.4% eq.	30°C (~6 hours)	0.4M Pa	PNDa03: 7.4%, PNDa04: 86.3%
PHTRACKD-387	MeOH(5V)	1.0 eq.	0.4% eq.	50°C (~6 hours)	0.4M Pa	PNDa03: 2.9%, PNDa04: 90.3%
PHTRACKD-374	MeOH(5V)	1.0 eq.	0.2% eq.	30°C	0.4M Pa	PNDa03: 16.4%, PNDa04: 78.9%
PHTRACKD-361	EtOH(5V)	1.0 eq.	1.5% eq.	30°C	0.4M Pa	PNDa03: n. d., PNDa04: 93.6%
PHTRACKD-366	EtOH(5V)	1.0 eq.	0.4% eq.	30°C	0.4M Pa	PNDa03: 0.5%, PNDa04: 94.1%
PHTRACKD-378	EtOH(5V)	1.0 eq.	0.2% eq.	30°C	0.4M Pa	PNDa03: 91.8%, PNDa04: 6.3%
PHTRACKD-421	EtOH(5V)	1.0 eq.	0.4% eq.	30°C	0.4M Pa	PNDa03: 0.1%, PNDa04: 95.1%

Pd catalyst could be decreased to at least 0.4% eq. in alcohol.

All solvents listed in table16 are all acceptable for this step. The selection of solvent for this non-isolation step needs to be considered in the next step reaction.

Table 29. The results for the preparation of PNDa04(investigation on Nickel) (HPLC method: INV_054926_HPLC_M1)

No.	Starting material	Raney Nickel	Reaction solvent	Reaction temperature	Reaction pressure	IPC (8 hours), %area
PHTRACKD-344	PNDa03 free base	10%w/w	MeOH(10V)	60°C	0.4M Pa	PNDa03: 80.8%, PNDa04: 9.0% Major impurity: 7.1%
PHTRACKD-345	PNDa03 free base	10%w/w	MeOH(10V)	60°C	2M Pa	PNDa03: N.D., PNDa04: 23.0% Major impurity: 56.8%
PHTRACKD-348	PNDa03 free base	10%w/w	MeOH(10V)	60°C	4M Pa	PNDa03: N.D., PNDa04: 28.2% Major impurity: 40.1%

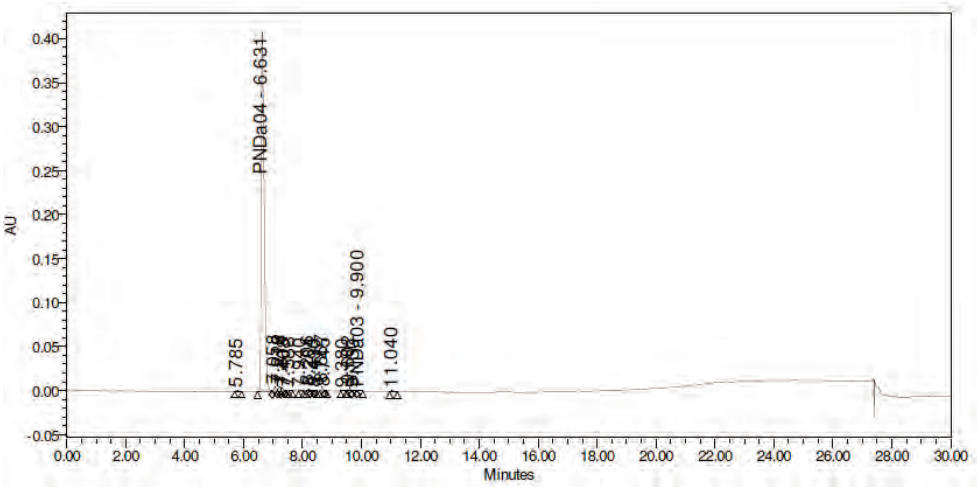
A new unknown major impurity (RRT 0.98) was formed when using Raney Nickel.

Raney Nickel seems not suitable for this reaction.

5.2.4.3. Typical procedure for the preparation of PNDa04 in experiment PHTRACKD-421

1. Charge PNDa03-HCl (5g, 12.97mmol, 1.0eq) and Hydrochloric acid (12M, 1.28g, 1eq) into a 100 mL tube.
2. Charge EtOH (25mL,5v/w) into the tube.
3. Charge 10% Pd/C (55.2mg, 0.052mmol, 0.004eq) into the tube.
4. The light-green solution was stirred for 16h under H_2 atmosphere (4bar pressure) at 30°C.
5. HPLC showed 95.1% PNDa04 formed and 0.1% PNDa03 left.
6. The solution was filtered with celite.
7. The filtrate was used for the next step directly. The yield was as 100% for next step.

HPLC chromatogram of PNDa04(IPC)

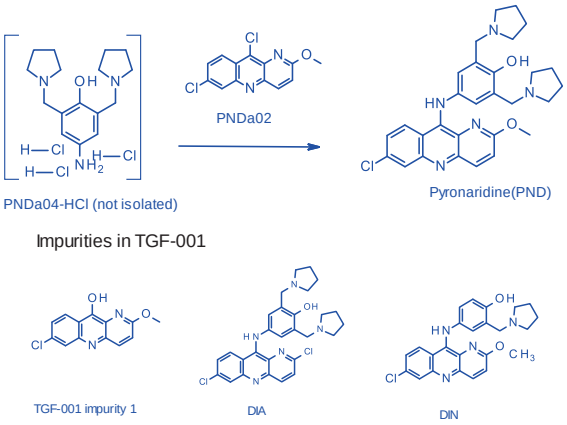


	Name	RT	RRT	Area	% Area	Resolution
1		5.785	0.87	5418	0.18	
2	PNDa04	6.631	1.00	2883132	95.13	
3		7.058	1.06	28398	0.94	
4		7.218	1.09	23816	0.79	
5		7.314	1.10	18255	0.60	
6		7.408	1.12	939	0.03	
7		7.588	1.14	3108	0.10	
8		7.940	1.20	2733	0.09	
9		8.206	1.24	9023	0.30	

	Name	RT	RRT	Area	% Area	Resolution
10		8.284	1.25	5043	0.17	
11		8.430	1.27	2089	0.07	
12		8.667	1.31	21738	0.72	
13		8.745	1.32	2882	0.10	
14		9.380	1.41	1616	0.05	
15		9.602	1.45	10848	0.36	
16		9.730	1.47	2210	0.07	
17	PNDa03	9.900	1.49	3178	0.10	
18		11.040	1.66	6317	0.21	

5.2.5. PND step (route 1)

5.2.5.1. Reaction scheme



5.2.5.2. Process and results of PND

- EtOH was the best solvent among water, EtOH, MeOH.
- The impurity cannot be controlled in this route.
- The quality of PNDa04 was important for this step.

Table 30. The results for the preparation of PND (HPLC method: INV_054926_HPLC_M1)

No.	Reaction solvent	Reaction temperature	IPC(1h), %area	Remark
PHTRACKD-372	2% TPGS-750-M aqueous (5V)	75°C(20h)	PNDa02:2.6%, PNDa04: n. d., PND:79.3% DIA:0.59%, DIN: 5.95%, TGF-001 impurity 1: 6.61%	1g scale
PHTRACKD-377	2% TPGS-750-M aqueous (5V)	75°C(20h)	PNDa02:30.7%, PNDa04:0.5%, PND:56.1% DIA: 0.94%, DIN:0.89%, TGF-001 impurity 1: 5.81%	10 scale
PHTRACKD-360	MeOH(5V)	50°C(2h)	PNDa02: n. d., PNDa04:1.39%, PND:92.0% DIA:0.71%, DIN:0.18%, TGF-001 impurity 1: 4.37%	1 g level
PHTRACKD-391	MeOH(5V)	50°C(2h)	PNDa02: n. d., PNDa04:0.2%, PND:91.5% DIA:1.31%, DIN:0.74%, TGF-001 impurity 1:5.5%	10 g level
PHTRACKD-362	EtOH(5V)	50°C(2h)	PNDa02: n. d., PNDa04:0.8%, PND:95.6% DIA:1.36%, DIN:0.36%, TGF-001 impurity 1:1.7%	1 g level
PHTRACKD-424	EtOH(5V)	50°C(2h)	PNDa02: n. d., PNDa04:0.4%, PND: 96.4% DIA: 0.66%, DIN: 0.25%, TGF-001 impurity 1:1.35%	10 g level

PNDa02 was consumed completely when used 1.1eq PNDa04(PNDa04 was cheaper).

The reaction is slower when using TPGS-750-M compared to that of alcohol.

PNDa02 is not dissolved in 2% TPGS aqueous and high level of PNDa02 was observed by HPLC IPC after reaction. This is the disadvantage compared to alcohol.

EtOH was more environmentally friendly than MeOH. They had similar results.

Table 31. The results for the preparation of PND (HPLC method: INV_054926_HPLC_M1)

No.	PNDa04	IPC_M1 (2 hours), %area
PHTHARRYS-498	*Batch PHTHARRYS-497* PNDa03：3.35%, PNDa04：91.0%	PNDa02: 0.11%, PNDa04: 0.46% PND: 95.8%, DIA: 1.41%, U.I. @RRT 0.97: 0.75%
PHTRACKD-424	*Batch PHTRACKD-421* PNDa03：0.1%, PNDa04：95.1%	PNDa02: n.d., PNDa04: 0.36% PND: 96.5%, DIA: 0.76%, U.I. @RRT 0.97: 0.66%
PHTRACKD-442	*Batch PHTRACKD-438 (isolated solid)* PNDa03：n. d., PNDa04：92.1%	PNDa02: n.d., PNDa04: 0.57% PND:96.9%, DIA: 0.34%, U.I. @RRT 0.97: 0.53%
PHTRACKD-391	*Batch PHTRACKD-390* PNDa03：0.1%, PNDa04：94.7%	PNDa02: n.d., PNDa04: 0.15% PND: 91.8%, DIA:1.30%, U.I. @RRT 0.97: 0.70%
PHTRACKD-382	*Batch PHTRACKD-379* PNDa03：0.45%, PNDa04：94.4%	PNDa02: n.d., PNDa04: 1.11% PND:94.0%, DIA: 0.91%, U.I. @RRT 0.97: 0.29%

Reaction using same batch of PNDa02 and different batches of PNDa04 will lead quite different purity profile of PND IPC solution, especially for DIA and unknown impurity at RRT 0.97.

The unknown impurity (>0.10%) in TGF-001 which is difficult to be purged probably come from PNDa04.

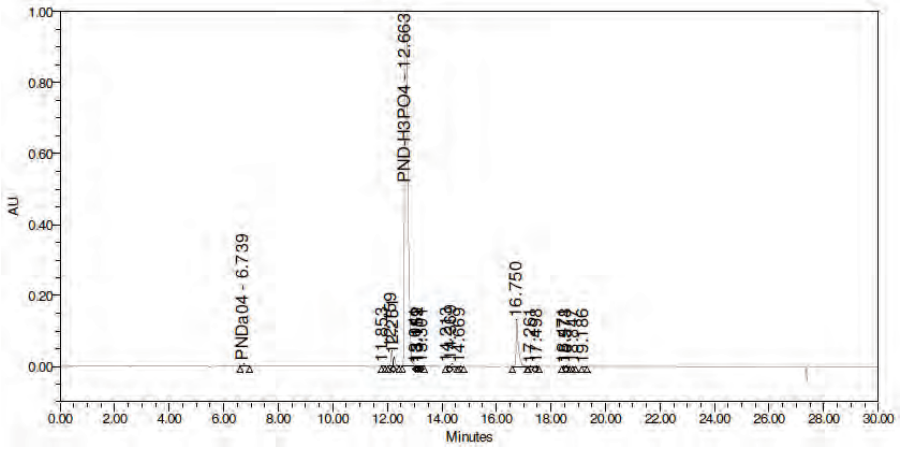
PNDa04 was not stable and hard to purify. A new route for PNDa04 synthesis (route 3) was investigated.

5.2.5.3. Typical procedure for the preparation of PND in experiment PHTRACKD-391

1. Add PNDa02(3.18g,10.38mmol,0.8eq) to the filtrate of PNDa04-PHTRACKD-390.
2. The suspension was stirred at 50°C for 2hs. HPLC showed PNDa02 was consumed completely.
3. The solvent was evaporated (50°C) to dryness under reduced pressure to give a brown solid.
4. The solid was dissolved in 50ml water, adjusted the pH to 12 with 15% NaOH(2mL).
5. Lots of solids precipitated out from the solvent, then continued stirring for 1 hour at 25℃.
6. Collect the solid by filtration, washed with 20mL water. The solid was dried under vacuum at 50℃ furnish PND (5.5g,9.51mmol, 91.72%

   yield,89.62% purity).

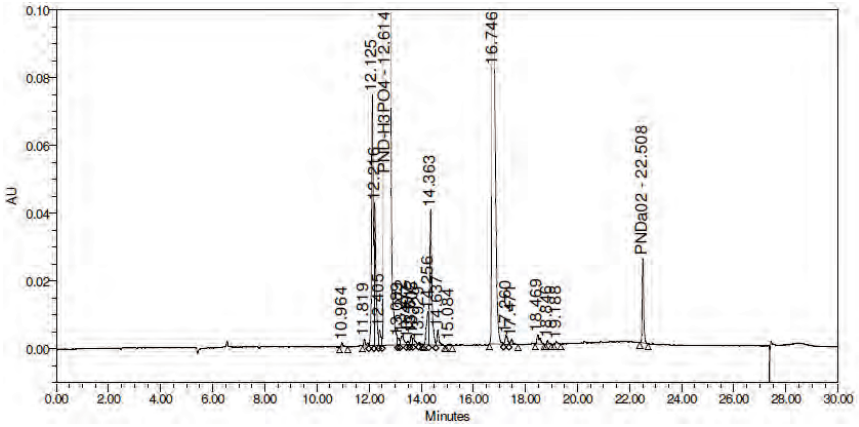
HPLC chromatogram of PND (IPC)



	Name	RT	RRT	Area	% Area	Resolution
1	PNDa04	6.739	0.53	29094	0.20	
2		11.853	0.94	3512	0.02	
3		12.159	0.96	189901	1.31	
4		12.251	0.97	107261	0.74	
5	PND-H3PO4	12.663	1.00	13291588	91.53	
6		13.041	1.03	4731	0.03	
7		13.119	1.04	134	0.00	
8		13.152	1.04	921	0.01	
9		13.301	1.05	2111	0.01	

	Name	RT	RRT	Area	% Area	Resolution
10		14.213	1.12	12433	0.09	
11		14.369	1.13	39703	0.27	
12		14.669	1.16	5521	0.04	
13		16.750	1.32	798448	5.50	
14		17.261	1.36	17457	0.12	
15		17.498	1.38	2323	0.02	
16		18.471	1.46	9210	0.06	
17		18.578	1.47	3268	0.02	
18		18.847	1.49	1136	0.01	
19		19.186	1.52	2348	0.02	
20	PNDa02	22.423				

HPLC chromatogram of PND (isolated)

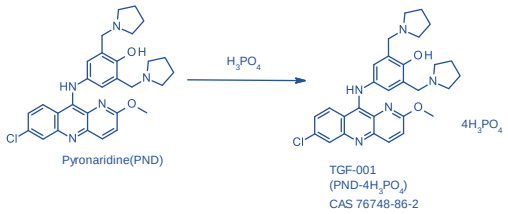


	Name	RT	RRT	Area	% Area	Resolution
1	PNDa04	6.629				
2		10.964	0.87	6163	0.03	
3		11.819	0.94	11175	0.05	
4		12.125	0.96	339861	1.42	
5		12.216	0.97	205365	0.86	
6		12.405	0.98	26698	0.11	
7	PND-H3PO4	12.614	1.00	21386976	89.62	
8		13.089	1.04	9060	0.04	
9		13.272	1.05	23651	0.10	

	Name	RT	RRT	Area	% Area	Resolution
10		13.497	1.07	4776	0.02	
11		13.612	1.08	12583	0.05	
12		13.705	1.09	11096	0.05	
13		13.929	1.10	1368	0.01	
14		14.256	1.13	53207	0.22	
15		14.363	1.14	221011	0.93	
16		14.637	1.16	22854	0.10	
17		15.084	1.20	2891	0.01	
18		16.746	1.33	1377686	5.77	
19		17.260	1.37	16798	0.07	
20		17.471	1.39	9470	0.04	
21		18.469	1.46	20420	0.09	
22		18.846	1.49	4169	0.02	
23		19.188	1.52	3682	0.02	
24	PNDa02	22.508	1.78	92412	0.39	

5.2.6. Salt formation and purification step (route 1)

5.2.6.1. Reaction scheme



Pyronaridine(PND)

TGF-001
(PND-$4H_3PO_4$)
CAS 76748-86-2

5.2.6.2. Process and analytical data of TGF-001

- TGF-001 synthesis by this route(route1) can meet the CP specification, but it cannot meet the requirement of BGMF (version 1).
- All the trials to meet the requirement of BGMFon this route failed.
- The results of PNDa04, PND, TGF-001 indicated the impurity that hard to purge was sourced from PNDa04, so another route for PNDa04 was investigated.

Table 32. The results for the preparation of TGF-001(CP Spec.)

No.	TGF-001 crude (IPC_M1), %area	TGF-001 (IPC_M1), %area	TGF-001 (CP method), %area	Yield% (based on purity)	CP specification
PHTHARRYS-405-2	From 2% TPGS-750-M aqueous (5V) HPLC purity: 97.8% TGF-001 impurity 1: 0.43% Max. single impurity: 0.43%	HPLC purity: 98.0% TGF-001 impurity 1: 0.24% Max. single impurity: 0.63%	N/A	63% (2 steps)	Single impurity: NMT 1.0% Total impurities: NMT 2.0%
PHTRACKD-363	From MeOH(5V) HPLC purity: 97.6% TGF-001 impurity 1: 0.35% Max. single impurity: 0.74%	HPLC purity: 98.0% TGF-001 impurity 1: 0.21% Max. single impurity: 0.63%	HPLC purity: 98.2% Possible TGF-001 impurity 1: 0.18% Max. single impurity: 0.45%	86% (2 steps)	
PHTRACKD-364	From EtOH(5V) HPLC purity: 97.9% TGF-001 impurity 1: 0.66% Max. single impurity: 0.71%	HPLC purity: 97.9% TGF-001 impurity 1: 0.17% Max. single impurity: 0.69%	HPLC purity: 98.2% Possible TGF-001 impurity 1: 0.14% Max. single impurity: 0.54%	85% (2 steps)	

All the results met the CP specification (purity).

A requirement from BGMF (version 1) was received, more purification shall be investigated.

Table 33. The results for the purification of TGF-001(organic solvent only) (HPLC method: INV_054926_HPLC_M1)

No.	TGF-001 crude (IPC_M1), %area	Solvent	TGF-001 (IPC_M1), %area
PHTRACKD-384	DIA: 0.90% UI@RRT 0.97: 0.33% TGF-001 impurity 1: 0.23% Total impurities: 1.7%	Acetone	DIA: 0.76% UI@RRT 0.97: 0.28% TGF-001 impurity 1: 0.19% Total impurities: 1.5%
PHTRACKD-385	DIA: 0.65% UI@RRT 0.97: 0.61% TGF-001 impurity 1: 0.20% Total impurities: 1.8%	MeOH	DIA: 0.52% UI@RRT 0.97: 0.70% TGF-001 impurity 1: 0.24% Total impurities: 2.4%
PHTRACKD-386	DIA: 0.64% UI@RRT 0.97: 0.66% TGF-001 impurity 1: 0.2 % Total impurities: 2.1%	EA	DIA: 0.67% UI@RRT 0.97: 0.72% TGF-001 impurity 1: 0.18% Total impurities: 2.7%

Purification with organic solvent e.g.: Acetone, MeOH and EA is helpless. TGF-001 cannot dissolved in organic solvent, so water/organic solvent were tried.

Table 34. The results for the purification of TGF-001(HPLC method: INV_054926_HPLC_M1)

No.	TGF-001 crude (IPC_M1), %area	Solvent	TGF-001 (IPC_M1), %area
PHTRACKD-402	DIA: 0.88% UI@RRT 0.97: 0.59% TGF-001 impurity 1: 2.92% Total impurities: 5.5%	Acetone(40v) / water(10v)	DIA: 0.88%, UI@RRT 0.97: 0.69% TGF-001 impurity 1: 0.47%, Total impurities: 2.9%
PHTRACKD-403		EtOH(40v) / water(10v)	DIA:0.77%, UI@RRT 0.97: 0.67% TGF-001 impurity 1: 0.39%, Total impurities:2.4%
PHTRACKD-404		MeOH(40v) / water(10v)	DIA:0.65%, UI@RRT 0.97:0.78% TGF-001 impurity 1: 0.24%, Total impurities: 2.2%
PHTRACKD-405		IPA (40v) / water(10v)	DIA: 1.11%, UI@RRT 0.97: 0.80% TGF-001 impurity 1:0.56%, Total impurities: 3.5%
PHTRACKD-406		THF (40v) / water(10v)	DIA:0.64%, UI@RRT 0.97: 0.66% TGF-001 impurity 1:0.07%, Total impurities: 1.9%
PHTRACKD-407		CAN (40v) / water(10v)	DIA:0.87%, UI@RRT 0.97: 0.75% TGF-001 impurity 1:1.62%, Total impurities: 3.9%
PHTRACKD-408		MIBK (40v) / water(10v)	No solid formed
PHTRACKD-409		Butyl acetate (40v) / water(10v)	No solid formed
PHTRACKD-410		EA (40V) / water(10v)	No solid formed

Purification with organic solvent/ water could remove TGF-001 impurity 1, however, it is no benefit to purge impurity DIA and UI@RRT 0.97.

THF/water system was the best system to purge impurities.

Table 35. The results for the purification of TGF-001(HPLC method: INV_054926_HPLC_M4)

No.	TGF-001 crude (Method: M4, %area)	Solvent	TGF-001 (Method: M4, %area)
PHTRACKD-435	*Batch PHTRACKD-431* DIA: 0.07% U. I. @RRT 0.80: 0.33% U. I. @RRT 0.81: 0.39% Total impurities: 1.7%	THF (10v) / water(10v)	DIA: <0.05% U. I. @RRT 0.80: 0.32% U. I. @RRT 0.81: 0.41% Total impurities: 0.8%
PHTRACKD-436		THF (20v) / water(10v)	DIA: <0.05% U. I. @RRT 0.80: 0.27% U. I. @RRT 0.81: 0.37% Total impurities: 0.7%

A new HPLC method(M4) was developed who can worked well for all impurities.

Purification procedure: dissolve crude TGF-001 in water, heat solution to 60 °C, and then add THF (clear solution), lower solution temperature to 40 °C, then to 25 °C. Collect the solid by filtration.

U. I. @RRT 0.80 and U. I. @RRT 0.81 cannot purged under this condition.

Table 36. The results for the salification of TGF-001(HPLC method: INV_054926_HPLC_M4)

No.	PND (method: M4, %area)	H_3PO_4	Solvent 1	Solvent 2	TGF-001 crude (method: M4, %area)
PHTRACKD-428	*Batch PHTRACKD-424-1* DIA: 0.18% U. I. @RRT 0.80: 0.41% U. I. @RRT 0.81: 0.40% Total impurities: 3.3%	3 eq.	Water(6v)	Acetone(12v)	DIA: 0.10%, U. I. @RRT 0.80: 0.38% U. I. @RRT 0.81: 0.39%, Total impurities: 1.5%
PHTRACKD-429		5 eq.	Water(6v)	Acetone(12v)	DIA: 0.10%, U. I. @RRT 0.80: 0.39% U. I. @RRT 0.81: 0.39%, Total impurities: 1.6%
PHTRACKD-430		5 eq.	Water(6v)	IPA (12v)	DIA: 0.10%, U. I. @RRT 0.80: 0.35% U. I. @RRT 0.81: 0.38%, Total impurities: 1.7%
PHTRACKD-431		5 eq.	Water(6v)	THF (12v)	DIA: 0.07%, U. I. @RRT 0.80: 0.33% U. I. @RRT 0.81: 0.39%, Total impurities: 1.7%
PHTRACKD-432		5 eq.	Water(6v)	EtOH(12v)	DIA: 0.13%, U. I. @RRT 0.80: 0.43% U. I. @RRT 0.81:0.40%, Total impurities: 2.1%

U. I. @RRT 0.80 and U. I. @RRT 0.81 cannot purged under this condition.

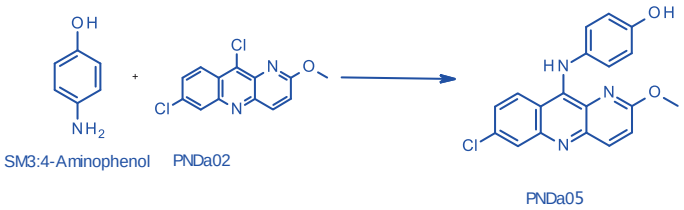
5.2.6.3. The procedure for the preparation of TGF-001

The procedure for salification and purification was presented in route 3.

5.3. Progress development of TGF-001(route 2)

5.3.1. PNDa05 step (route 2)

5.3.1.1. Reaction scheme



5.3.1.2. Process and results of PNDa05

- A few optimizations for this step were done, repeated the literature condition and feasibility study for this route.

Table 37. The results for the preparation of PNDa05(HPLC method: INV_054926_HPLC_M1)

Entry	4-Aminophenol (SM4)	H_2SO_4	Reaction solvent	Reaction temperature	IPC (16h), area%	Isolation PND
PHTHARRYS-407	2.0eq	1.0eq	2% TPGS-750-M aqueous (10V)	75°C	PNDa02: 1.5% PNDa05: 87.5%	HPLC purity:96.4% Yield (based on HPLC purity): :82.3%
PHTRACKD-399	1.1 eq.	1.0eq.	2% TPGS-750-M aqueous (10V)	75°C	PNDa02: 1.60% PNDa05: 93.8%	HPLC purity: 94.0% Yield (based on HPLC purity): 96.0%
PHTRACKD-400	1.1 eq.	1.0eq.	2% TPGS-750-M aqueous (10V)	75°C	PNDa02: 1.56% PNDa05: 91.6%	HPLC purity: 95.4% Yield (based on HPLC purity): 95.3%
PHTRACKD-437	1.5 eq.	1.0eq.	2% TPGS-750-M aqueous (10V)	75°C	PNDa02: 1.92% PNDa05: 96.0%	HPLC purity: 97.0% Yield (based on HPLC purity): 97.3%

The intermediate PNDa02 is the key compound considering the cost for this project.

Route 1could save ca. 8~10% of PNDa02 than Route 2 for per kg final product.

Therefore, this route wasn't applied for process development.

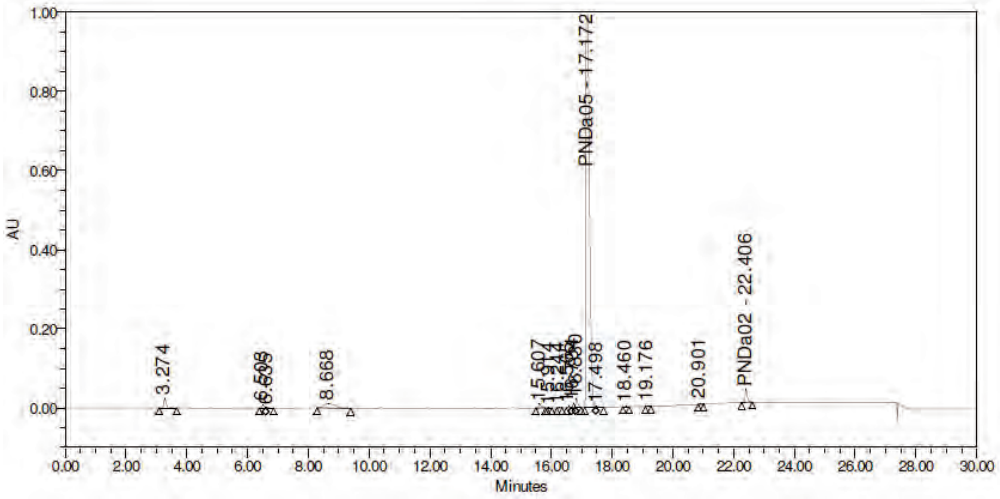
5.3.1.3. Typical procedure for preparation of PNDa05 in the experiment of PHTRACKD-400

- Charge 4-Aminophenol (0.78g, 7mmol,1.1eq), PNDa02 (2.0g, 6.4mmol,1.0eq) and 2% TPGS-750-M/H_2O (15mL,7.5v/w) into 100mL three-neck round-bottom flask.
- Then H_2SO_4 (0.63g, 6.4mmol,2.5eq,98%) was added to the stirred mixture at 25 °C.
- Then the reaction temperature was raised to 75°C and stirred for 16h under N_2 atmosphere.
- HPLC showed that 91.59% of PND-a05 was formed and 1.56% PNDa02 was left.
- The reaction mixture was neutralized with 25% aqueous ammonium solution(2mL) to form

the orange solid.

- The solid was filtered and washed with IPA (20mL,10v/w).
- The solid was dried under vacuum at 50°C furnish PNDa05 (2.25g,202.27mmol, 95.3% yield,95.4% purity).

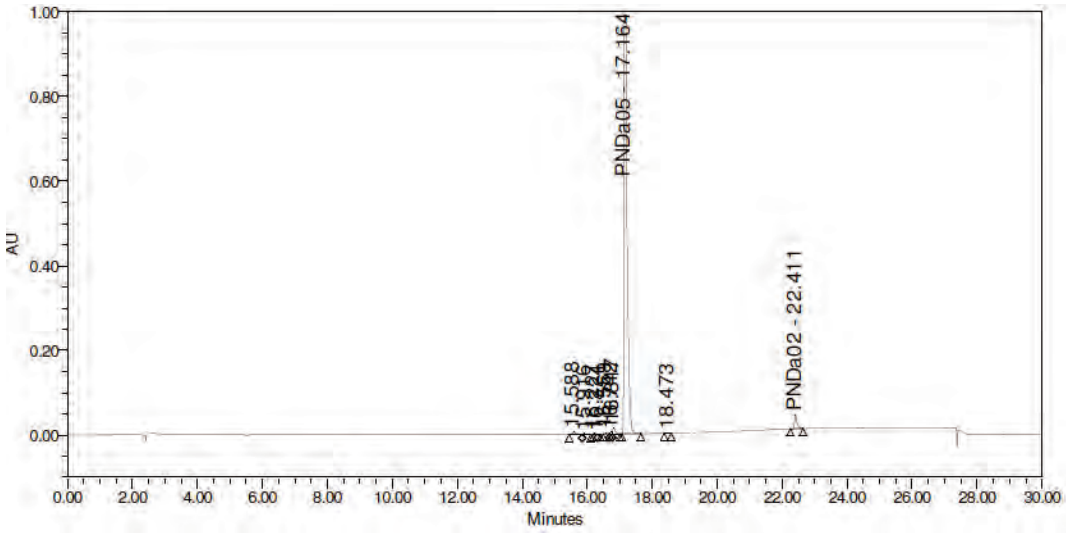
HPLC chromatogram of PNDa05 (IPC)



	Name	RT	RRT	Area	% Area	Resolution
1	4-Aminophenol	2.745				
2		3.274	0.19	135700	1.25	
3		6.503	0.38	40545	0.37	
4		6.635	0.39	17671	0.16	
5		8.668	0.50	289474	2.67	
6		15.607	0.91	60510	0.56	
7		15.914	0.93	6514	0.06	
8		16.244	0.95	5915	0.05	
9		16.565	0.96	15810	0.15	

	Name	RT	RRT	Area	% Area	Resolution
10		16.734	0.97	39864	0.37	
11		16.830	0.98	97008	0.89	
12	PNDa05	17.172	1.00	9932765	91.59	
13		17.498	1.02	10229	0.09	
14		18.460	1.07	7187	0.07	
15		19.176	1.12	6184	0.06	
16		20.901	1.22	10961	0.10	
17	PNDa02	22.406	1.30	168751	1.56	

HPLC chromatogram of PNDa05 (isolated)

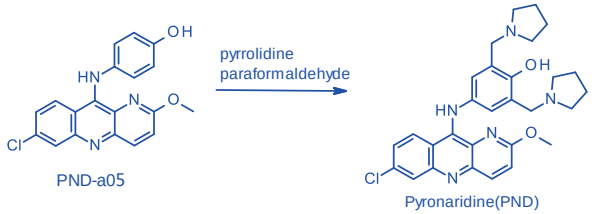


	Name	RT	RRT	Area	% Area	Resolution
1	4-Aminophenol	2.745				
2		15.588	0.91	38481	0.52	
3		15.916	0.93	7793	0.11	
4		16.227	0.95	4807	0.07	
5		16.324	0.95	1282	0.02	
6		16.569	0.97	17901	0.24	
7		16.742	0.98	17478	0.24	
8		16.817	0.98	75214	1.02	
9	PNDa05	17.164	1.00	7021625	95.43	

	Name	RT	RRT	Area	% Area	Resolution
10		18.473	1.08	6520	0.09	
11	PNDa02	22.411	1.31	166518	2.26	

5.3.2. PND step (route 2)

5.3.2.1. Reaction scheme



5.3.2.2. Process and results of PND

o This route doesn't have advantages in controlling impurities.

Table 38. The results for the preparation of PND (HPLC method: INV_054926_HPLC_M1)

No.	Pyrrolidine/ paraformaldehyde	TEA	Reaction solvent	Reaction temperature	IPC (16h), %area
PND-PHTRACKD-425	5.0eq./ 5.0eq.	3.0eq	2% TPGS-750-M aqueous (10V)	75°C	PND: 90.4%, PNDa05: n. d., DIN: 6.9%
PND-PHTRACKD-433	5.0eq./ 5.0eq.	N/A	2% TPGS-750-M aqueous (10V)	75°C	PND:66.6%, PNDa05: 16.4%, DIN:17.4%
PND-PHTRACKD-427	5.0eq./ 5.0eq.	3.0eq	2-MeTHF	75°C	PND:89.9%, PNDa05: n. d., DIN: 6.2%
PND-PHTRACKD-434	5.0eq./ 5.0eq.	N/A	2-MeTHF	75°C	PND:90.8%, PNDa05: n.d., DIN: 6.3%
PND-PHTHARRYS-504	6.0eq./6.0eq.	N/A	EtOH	50°C	PND:42.5%, PNDa05: 31.5%, DIN: 24.0%
PND-PHTRACKD-415	10.0eq./10.0eq.	N/A	EtOH	50°C	PND:93.1%, PNDa05: n. d., DIN:2.5%
PND-PHTRACKD-416	10.0eq./ 10.0eq.	N/A	2-MeTHF	70°C	PND:95.0%, PNDa05: n. d., DIN:1.1%
PND-PHTHARRYS-503	20.0eq./ 20.0eq.	N/A	EtOH	70°C	PND:96.6%, PNDa05: 0.1%, DIN:1.4%

TEA could inhibit the formation of impurity DIN in TPGS aqueous (but 6% is still too high).

High amount of pyrrolidine/ paraformaldehyde could inhibit the formation of impurity DIN (but high cost).

Table 39. The results for the purification of TGF-001

No.	PND (IPC_M1, %area)	TGF-001 crude (IPC_M1, %area)	Solvent	TGF-001 (IPC_M1, %area)	TGF-001 (Method: M4, %area)
PHTRACKD-419	DIA: 0.31%, DIN: 2.24% UI@RRT 0.96: 1.20% UI@RRT 0.97: 0.25% Total impurities: 6.22%	DIA: 0.23%, DIN: 0.66% UI@RRT 0.96: 0.94% UI@RRT 0.97: 0.13% Total impurities: 3.11%	THF (20v)/ water(10v)	DIA:0.25%, DIN:0.27% UI@RRT 0.96: 0.76% UI@RRT 0.97: 0.16% Total impurities: 2.11%	DIA:0.20%, DIN:0.20% U. I. @RRT 0.78:0.13% U. I. @RRT 0.80:0.39% U. I. @RRT 0.99:0.18% Total impurities:1.5%
PHTRACKD-420	DIA: 0.25%, DIN: 1.09% UI@RRT 0.96: 1.05% UI@RRT 0.97: 0.17% Total impurities: 4.42%	DIA: 0.25%, DIN: 0.45% UI@RRT 0.96: 0.63% UI@RRT 0.97: 0.18% Total impurities: 2.23%	THF (20v)/ water(10v)	DIA:0.28%, DIN:0.16% UI@RRT 0.96:0.77% UI@RRT 0.97:0.11% Total impurities: 1.6%	DIA:0.16%, DIN:0.09% U. I. @RRT 0.80:0.28% U. I. @RRT 0.90: 0.18% U. I. @RRT 0.99: 0.58% Total impurities: 1.7%
PHTHARRYS-507	N/A	DIA: 0.13%, DIN:0.38% UI@RRT 0.96: 0.56% UI@RRT 0.97: 0.09% Total impurities: 1.32%	THF (20v)/ water(10v)	DIA:0.08%, DIN:0.08% UI@RRT 0.96:0.48% UI@RRT 0.97:0.10% Total impurities:0.86%	DIA:0.05%, DIN:0.10% U. I. @RRT 0.80: 0.34% U. I. @RRT 0.99: 0.12% Total impurities: 0.8%

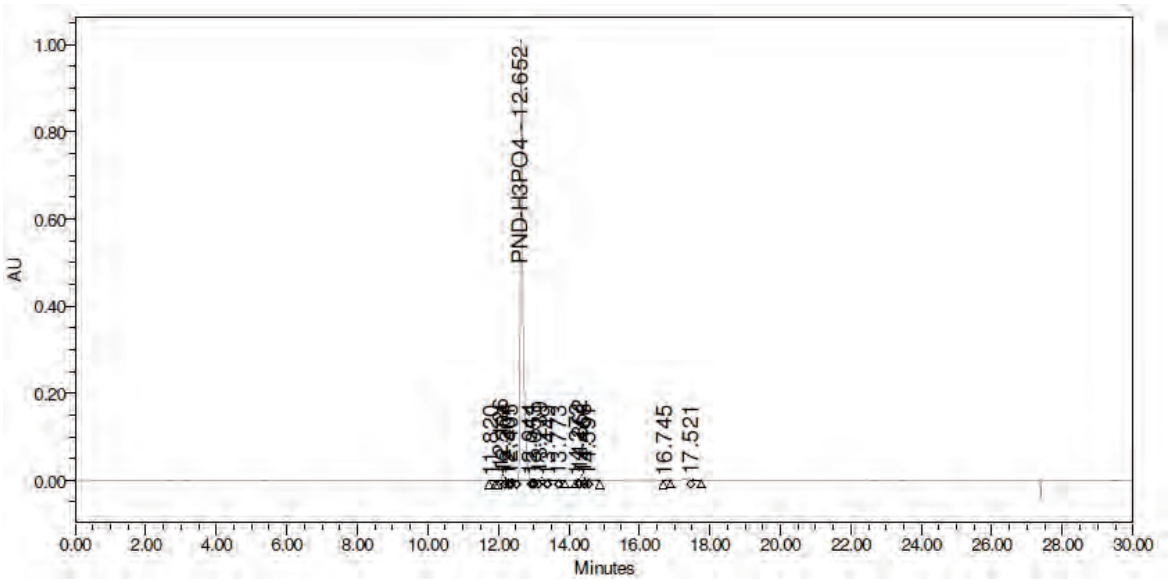
DIN could be controlled if a higher amount of pyrrolidine/ paraformaldehyde and organic solvent was used during PND synthesis, but more other impurities will be formed.

IPC method 'INV_054926_HPLC_M1' was used at the beginning. U.I. @RRT0.97 and DIA peaks might be coelution peaks for several impurities by this IPC method.

5.3.2.3. Typical procedure for preparation of PND in the experiment of PHTRACKD-416

1. Charge 4-Aminophenol (0.78g, 7mmol,1.1eq), PNDa02 (2.0g, 6.4mmol,1.0eq) and 2% TPGS-750-M/H_2O (15mL,7.5v/w) into 100mL three-neck round-bottom flask.
2. Charge PNDa05(1g, 2.71mmol, 1.0eq), Pyrrolidine (0.98g, 13.56mmol,5.0eq) and Paraformaldehyde (0.43g, 13.56mmol,5.0eq),2-MeTHF(5mL) into a 100 mL three-neck round-bottom flask.
3. The mixture was then stirred at 70°C for 2hs under N_2.
4. HPLC showed 95.03% of PND formed and an intermediate (DIN) with an area of 1.14%.
5. Water(5mL,5v/m) was added to the mixture. The mixture was stirred at 30 °C for 1h.
6. Collect the solid by filtration. Washed the cake with water(10mL,10v/m).
7. The solid was dried under vacuum at 50°C furnish PND (1.3g,2.4mmol, 88.4% yield,95.58% purity).

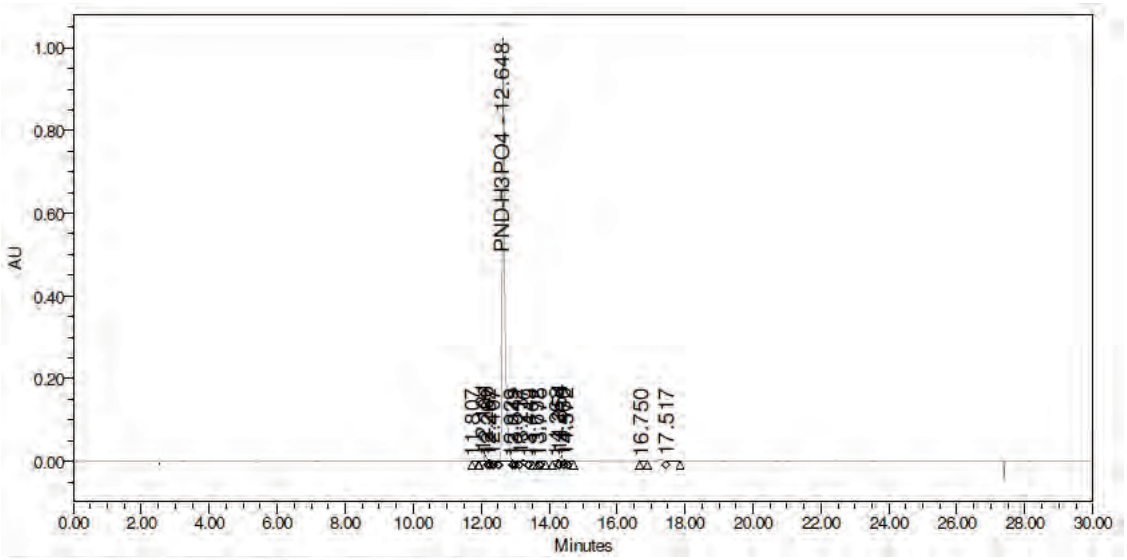
HPC chromatogram of PND (IPC)



	Name	RT	RRT	Area	% Area	Resolution
1	PNDa04	6.629				
2		11.820	0.93	1911	0.03	
3		12.126	0.96	61646	1.11	
4		12.204	0.96	19362	0.35	
5		12.301	0.97	5924	0.11	
6		12.405	0.98	10515	0.19	
7	PND-H3PO4	12.652	1.00	5290181	95.03	
8		12.944	1.02	5895	0.11	
9		13.053	1.03	7787	0.14	

	Name	RT	RRT	Area	% Area	Resolution
10		13.239	1.05	41429	0.74	
11		13.449	1.06	7604	0.14	
12		13.773	1.09	3941	0.07	
13		14.272	1.13	12021	0.22	
14		14.362	1.14	63690	1.14	
15		14.468	1.14	17961	0.32	
16		14.591	1.15	5335	0.10	
17		16.745	1.32	3216	0.06	
18		17.521	1.38	8252	0.15	
19	PNDa02	22.423				

HPLC chromatogram of PND (isolated)



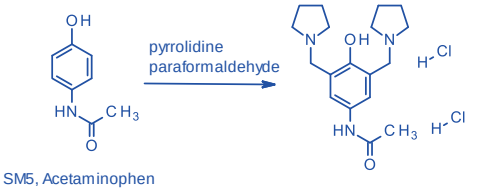
	Name	RT	RRT	Area	% Area	Resolution
1	PNDa04	6.629				
2		11.807	0.93	2081	0.04	
3		12.121	0.96	57833	1.05	
4		12.206	0.97	13788	0.25	
5		12.262	0.97	9182	0.17	
6		12.407	0.98	8990	0.16	
7	PND-H3PO4	12.648	1.00	5275975	95.58	
8		12.929	1.02	5701	0.10	
9		13.042	1.03	5497	0.10	

	Name	RT	RRT	Area	% Area	Resolution
10		13.237	1.05	28179	0.51	
11		13.449	1.06	2443	0.04	
12		13.692	1.08	723	0.01	
13		13.775	1.09	1691	0.03	
14		14.268	1.13	12881	0.23	
15		14.354	1.13	59981	1.09	
16		14.460	1.14	15647	0.28	
17		14.572	1.15	4943	0.09	
18		16.750	1.32	4728	0.09	
19		17.517	1.38	9974	0.18	
20	PNDa02	22.423				

5.4. Progress development of TGF-001(route 3)

5.4.1. PNDa06 step (route 3)

5.4.1.1. Reaction scheme



SM5, Acetaminophen
CAS:103-90-2
Formula Weight: 151.06

PNDa06-HCl
Formula Weight: 389.16

5.4.1.2. Process and results of PNDa06

Table 40. The results for the preparation of PNDa06 (HPLC method: INV_054926_HPLC_M1)

No.	Pyrrolidine/ paraformaldehyde	Solvent(5v/w)	Reaction temperature	IPC (16h), %area
PHTRACKD-440	5.0eq/5.0eq.	EtOH	50℃	SM5:0.27%. PNDa06:98.8%
PHTRACKD-441	5.0eq/5.0eq.	2-Me-THF	70℃	SM5:0.32%. PNDa06:98.4%
PHTRACKD-449	3.0eq/3.0eq.	EtOH	50℃	SM5:1.2%. PNDa06:98.3%
PHTRACKD-450	3.0eq/3.0eq.	EtOH	70℃	SM5:0.22%. PNDa06:98.5%
PHTRACKD-451	3.0eq/3.0eq.	2-Me-THF	70℃	SM5:0.35%. PNDa06:98.6%
PHTRACKD-452	2.5eq/2.5eq.	EtOH	70℃	SM5:0.14%. PNDa06:99.1%
PHTRACKD-456	2.5eq/2.5eq.	EtOH	60℃	SM5:0.28%. PNDa06:98.8%
PHTHARRYS-514	2.5eq/2.5eq.	EtOH	60℃	SM5:1.9%. PNDa06:97.8%
PHTHARRYS-515	2.5eq/2.5eq.	EtOH	70℃	SM5:0%. PNDa06:99.4%
PHTHARRYS-585	2.4eq./ 2.4eq.	EtOH	70℃	SM5:0.36%. PNDa06:99.4%
PHTHARRYS-584	2.3eq./ 2.3eq.	EtOH	70℃	SM5:1.0%. PNDa06:98.4%
PHTHARRYS-583	2.1eq./ 2.1eq.	EtOH	70℃	SM5:8.7%. PNDa06:91.2%

2.4 equivalent of pyrrolidine/ paraformaldehyde is sufficient for the reaction.

The yield of this step is about 95% based on assay.

Table 41. The results for work up of PNDa06 (HPLC method: INV_054926_HPLC_M1)

No.	Pyrrolidine/ paraformaldehyde	Solvent(5v/w)	Reaction temperature	IPC (16h), %area	Salt formation process	Isolated PNDa06-HCl
PHTHARRYS-520	2.5eq/2.5eq.	EtOH	70℃	SM5:0.1%. PNDa06:99.5%	IPA(5v) and HCl/EtOAc (2M, 12v), EA(8v)	Assay: 96.7% Yield:93.0%
PHTHARRYS-586	2.4eq./ 2.4eq.	H_2O(5V)	70°C	SM5:0.24%. PNDa06:99.35%	PNDa06 was collected by filtration directly	Assay: 95.3% (free base) Yield:71.3%
PHTHARRYS-588	2.5eq./ 2.5eq.	H_2O(5V)	70°C	SM5:0.1%. PNDa06:99.72%	PNDa06 was collected by filtration directly	Assay: 90.1% (free base) Yield:76.8%
PHTKENNYG-689	2.4eq./ 2.4eq.	EtOH	70℃	SM5:0.08%. PNDa06:99.85%	Added HCl/EtOAC to the reaction solution directly.	No solid formed
PHTKENNYG-690	2.4eq./ 2.4eq.	EtOH	70℃	SM5:0.08%. PNDa06:99.75%	EtOH(5v) and HCl/EtOAc (2M, 12v), EA(5v)	Assay: 98.8% Yield:92.1%

EtOH (same as reaction solvent) is used for salifying instead of IPA.

The reaction of PNDa06 in water was OK(IPC), but the yield was lower for PNDa06(free base) dissolved in water too.

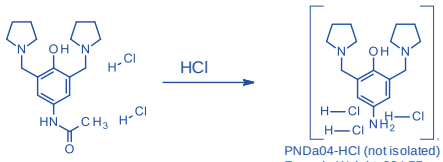
Table 42. The results for solvent recycling of PNDa06

SM5	Pyrrolidine/ paraformaldehyde	Reaction solvent	Reaction temp.	PNDa06 IPC_M1 (16h)	water content (KF)	Remark
1.0eq.	2.5eq./ 2.5eq.	EtOH(5V)	70°C	PHTKENNYG-700 SM5: 0.06% PNDa06: 98.72%	Crude PNDa06(free):0.88%	There was no problem of salt formation.
1.0eq.	2.5eq./ 2.5eq.	EtOH (5V, recycled from PHTKENNYG-700)	70°C	PHTKENNYG-702 SM5: 0.08% PNDa06: 99.75%	Crude PNDa06(free):0.59%	There was no solid formed in salt formation.
1.0eq.	2.5eq./ 2.5eq.	EtOH (5V, recycled from PHTKENNYG-702)	70°C	PHTKENNYG-703 SM5: 0.08% PNDa06: 98.63%	Crude PNDa06(free):1.03%	There was no solid formed in salt formation.

Using recycled EtOH by distillation directly failed. The reason cause is unclear at this moment.

5.4.2. PNDa04 step (route 3)

5.4.2.1. Reaction scheme



5.4.2.2. Process and results of PNDa04

Table 43. The results for the preparation of PNDa04 (HPLC method: INV_054926_HPLC_M1)

No.	HCl aqueous (6mol)	Reaction temperature	IPC, %area
PHTRACKD-471	10V	100°C(1h)	PNDa06: n. d., PNDa04: 98.9%
PHTRACKD-493	4V	100°C(1h)	PNDa06: n. d., PNDa04: 99.9%
PHTRACKD-494	6V	100°C(1h)	PNDa06: n. d., PNDa04: 99.9%
PHTHARRYS-494	8V	100°C(1h)	PNDa06: n. d., PNDa04: 98.6%
PHTRACKD-490(70g)	8V	100°C(1h)	PNDa06: n. d., PNDa04: 99.6%
PHTRACKD-461	6V (2mol/L, HCl)	100°C(16h)	PNDa06: 17.2%., PNDa04: 82.8%
PHTRACKD-460	5V (6mol/L, HCl)	70°C(16h)	PNDa06: n.d., PNDa04: 96.4%
PHTRACKD-459	7.5V (4mol/L, HCl)	70°C(16h)	PNDa06: n.d., PNDa04: 97.6%
PHTRACKD-463	$SOCl_2$(1.0eq) MeOH(5V)	70°C(7h)	PNDa06: n. d., PNDa04: 97.3%

PHTRACKD-455	10V	100°C(16h)	PNDa06: n. d., PNDa04: 93.84%

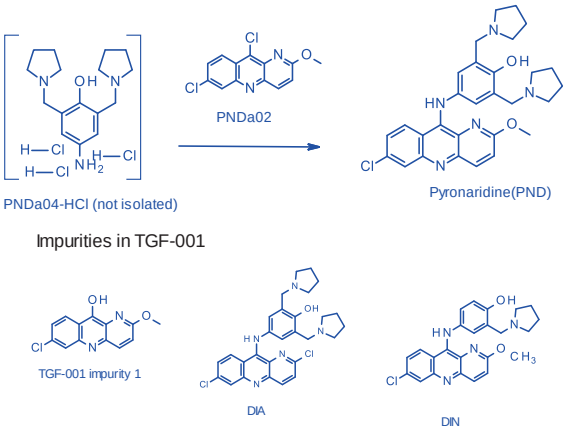
PHTRACKD-455 showed PNDa04 will be degraded if reacted too long.

PNDa04-HCl was used for the next step directly after concentration.

The quality of PNDa04 was better than that of route 1.

5.4.3. PND step (route 3)

5.4.3.1. Reaction scheme



5.4.3.2. Process and results of PND

Table 44. The results for the preparation of PND (HPLC method: INV_054926_HPLC_M1)

No.	PNDa02	PNDa04-HCl	Reaction solvent	Reaction temperature	IPC (1.5h), %area
PHTRACKD-495	1.0eq	1.1eq	EtOH (5V)	50°C	PNDa02: n.d., PNDa04: 0.7%, PND:96.1% TGF-001 impurity:2.3%.
PHTRACKD-496	1.0eq	1.1eq	EtOH (5v)	50°C	PNDa02: n.d., PNDa04: 1.0%, PND:96.4% TGF-001 impurity:1.8%.
PHTRACKD-500	1.0eq	1.1eq	EtOH (5v)	50°C	PNDa02: n.d., PNDa04: 0.6%, PND:95.7% TGF-001 impurity:2.8%.
PHTHARRYS-522	1.0eq	1.1eq	EtOH (5v)	50°C	PNDa02: 0.02%., PNDa04: 1.2%, PND:93.3% TGF-001 impurity:2.0%.
PHTHARRYS-526	1.0eq	1.1eq	EtOH(5V)	50°C	PNDa02: 0.14%, PNDa04: 0.6%, PND:96.0% TGF-001 impurity:2.0%.
PHTHARRYS-529	1.0eq	1.1eq	EtOH(5V)	50°C	PNDa02: 0.09%, PNDa04: 0.7%, PND:95.4% TGF-001 impurity:2.15%.

Note: Remove the solvent of PNDa04, add 5v ethanol as PND reaction solvent

The biggest impurity in IPC was TGF-001 impurity 1(2%-3%).

Optimizing the procedure to reduce TGF-001 impurity 1 is listed below.

PND was not dried. The yield was calculated for 3 step3 (PND, salification, purification).

Table 45. TGF-001 impurity 1 control at PNDa04-HCl and PND steps (HPLC method: INV_054926_HPLC_M1)

PNDa06-HCl	Reaction solvent	Reaction temp.	PNDa04-HCl IPC_M1	PNDa02	PND IPC_M1	Remark
PHTHARRYS-520 (original)	6M HCl(4v)	100°C (1h)	PHTHARRYS-535 PNDa06: n. d. PNDa04: 99.0%	0.91 eq.	PHTHARRYS-536 (1.5h,50°C) PNDa02: 0.05%, PNDa04: 0.35% Impurity 1: 2.4%, PND: 95.9%	remove water, add 5v ethanol as solvent
PHTHARRYS-520	$SOCl_2$ (1.0 eq.) MeOH(5V)	70°C (7h)	PHTRACKD-527 PNDa06: 0.5% PNDa04: 98.4%	0.91 eq.	N/A	No downstream reaction.
PHTHARRYS-520	$SOCl_2$ (3.0 eq.) MeOH(5V)	70°C (3h)	PHTRACKD-538 PNDa06: 0.08% PNDa04: 99.0%	0.91 eq.	PHTRACKD-541 (1.5h, 50°C) PNDa02: n.d., PNDa04: 3.1% Impurity 1: 5.8%, PND: 89.5%	PNDa02 was added to the PNDa04 reaction directly
PHTHARRYS-520	HCl in MeOH (5v)	70°C (3h)	PHTRACKD-539 PNDa06: 0.17% PNDa04: 97.8%	0.91 eq.	PHTRACKD-542 (1.5h, 50°C) PNDa02: n.d., PNDa04: 2.0% Impurity 1: 5.0%, PND :91.0%	PNDa02 was added to the PNDa04 reaction directly
PHTHARRYS-588 (Free base)	8M HCl(9v)	100°C (2h)	PHTHARRYS-590 PNDa06: 0.46% PNDa04: 92.2%	0.91 eq.	PHTRACKD-591 (1.5h, 50°C) PNDa02: n.d., PNDa04: 0.7% Impurity 1: 1.7%, PND:95.5%	Free base of PNDa06 is used as percussor
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-545 PNDa06: n. d. PNDa04: 99.5%	0.91 eq.	PHTRACKD-548 (4h, 50°C) PNDa02: 2.6%, PNDa04: 1.4% Impurity 1: 0.31%, PND: 93.5%	Water as solvent directly
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-546 PNDa06: n. d. PNDa04: 99.6%	0.91 eq.	PHTRACKD-549 (4h, 50°C) PNDa02: 1.9%, PNDa04: 0.3% Impurity 1: 0.6%, PND: 94.7%	Water as solvent directly, but addition of EtOH (1v) before PND reaction.
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-547 PNDa06: n. d. PNDa04: 98.7%	0.83 eq.	PHTRACKD-550 (1.5h, 50°C) PNDa02: 0.05%., PNDa04: 0.9% Impurity 1: 2.3%, PND: 95.8%	Remove water, add 5v ethanol as solvent
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-551 PNDa06: n. d. PNDa04: 99.6%	0.91 eq.	PHTRACKD-555 (16h, 20°C) PNDa02: 0.2%, PNDa04: 0.8% Impurity 1: 0.6%, PND: 97.7%	Remove water, add 5v ethanol as solvent
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-552 PNDa06: n. d. PNDa04: 99.8%	0.91 eq.	PHTRACKD-556 (16h, 50°C) PNDa02: 0.5%, PNDa04: 1.9% Impurity 1: 1.8%, PND: 83.2%	Add THF(1v) to PNDa04 solution directly (do not remove water).
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACD-553 PNDa06n. d. PNDa04: 99.7%	0.91 eq.	PHTRACKD-557 (16h, 50°C) PNDa02: n.d., PNDa04: 1.0% Impurity 1: 1.8%, PND: 91.5%	Add ACN(1v) to PNDa04 solution directly (do not remove water).
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-554 PNDa06: n. d. PNDa04: 99.6%	0.91 eq.	PHTRACKD-558 (16h, 50°C) PNDa02: 0.6%, PNDa04: 2.0% Impurity 1: 1.9%, PND :83.4%	Add Acetone(1v) to PNDa04 solution directly (do not remove water).
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-560 PNDa06: n. d. PNDa04: 99.7%	0.91 eq.	PHTRACKD-563 (2h, 50°C) PNDa02: 0.8%, PNDa04: 1.0% Impurity 1: 0.3%, PND: 96.8%	Remove water for PNDa04 solution, then add water(4v)/THF(1v) as PND reaction solvent.
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-565 PNDa06: n. d. PNDa04: 99.7%	0.91 eq.	PHTRACKD-567 (16h, 50°C) PNDa02: 0.1%, PNDa04: n.d. Impurity 1: 0.8%, PND: 98.2%	Remove water for PNDa04 solution, then add water(4v)/THF(1v) as PND reaction solvent.
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-561 PNDa06: n. d. PNDa04: 99.5%	0.91 eq.	PHTRACKD-564 (2h, 50°C) PNDa02: 0.7%, PNDa04: 0.9% Impurity 1: 0.2%, PND: 95.6%	Remove water for PNDa04 solution, then add water(4v)/ACN(1v) as PND reaction solvent.
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-566 PNDa06: n. d. PNDa04: 99.8%	0.91 eq.	PHTRACKD-568 (16h, 50°C) PNDa02: 0.1%, PNDa04: n.d. Impurity 1: 1.1%, PND: 97.6%	Remove water for PNDa04 solution, then add water(4v)/ACN(1v) as PND reaction solvent.
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-579 PNDa06: n. d. PNDa04: 99.8%	0.91 eq.	PHTRACKD-582 (16h, 10°C) PNDa02: 0.07%, PNDa04: 0.6% Impurity 1: 0.35%, PND: 98.4%	Remove water, add 5v ethanol as solvent.
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-580 PNDa06: n. d. PNDa04: 99.6%	0.91 eq.	PHTRACKD-583 (16h, 30°C) PNDa02: 0.14%, PNDa04: 0.4% Impurity 1: 0.9%, PND:97.9%	Remove water, add 5v ethanol as solvent.
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-591 PNDa06: n. d. PNDa04: 99.6%	0.91 eq.	PHTRACKD-593 (22h, 0°C) PNDa02: 0.1%, PNDa04: 0.6% Impurity 1: 0.3%, PND: 98.5%	Remove water, add 5v ethanol as solvent. It took longer time to complete the reaction.

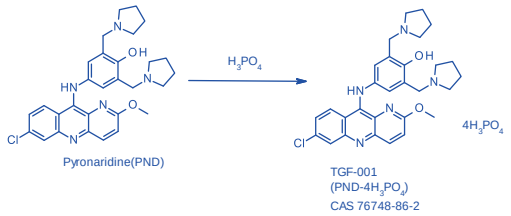
PHTHARRYS-520	6M HCl(4v)	100°C (1h)	PHTRACKD-592 PNDa06: n. d. PNDa04: 99.8%	0.91 eq.	PHTRACKD-594 (16h, -10°C) PNDa02: 22.5%, PNDa04: 5.3% Impurity 1: 0.12%, PND:64.5%	There was too much PNDa02 remained when reacted at -10℃.
PHTHARRYS-587	6M HCl(4v)	100°C (1h)	PHTRACKD-649 PNDa06: n. d. PNDa04: 99.7%	0.91 eq.	PHTRACKD-650 (16h, 10°C) PNDa02: 0.41%, PNDa04: 0.69% Impurity 1: 0.10%, PND:98.3%	Remove water, add 10v IPA as solvent.
PHTHARRYS-587	6M HCl(4v)	100°C (1h)	PHTHARRYS-663 PNDa06: n. d. PNDa04: 99.5%	0.91 eq.	PHTHARRYS-664(3h, 30°C) PNDa02: 0.14%, PNDa04: 0.58% Impurity 1: 0.54%, PND: 98.4%	Remove water, add 10v IPA as solvent.
PHTHARRYS-587	6M HCl(4v)	100°C (1h)	PHTRACKD-657 PNDa06: n. d. PNDa04: 99.5%	0.91 eq.	PHTRACKD-659(2h, 50°C) PNDa02: 0.10%, PNDa04: 0.15% Impurity 1: 1.02%, PND: 97.9%	Remove water, add 10v IPA as solvent.
PHTHARRYS-587	6M HCl(4v)	100°C (1h)	PHTHARRYS-665 PNDa06: n. d. PNDa04: 99.3%	0.91 eq.	PHTHARRYS-666(16h, 10°C) PNDa02: 12.2%, PNDa04: 2.4% Impurity 1: 0.34%, PND: 82.6%	Remove water, add 20v IPA as solvent.

Impurity 1 can be controlled below 0.4% in PND reaction when reacted at 10℃.

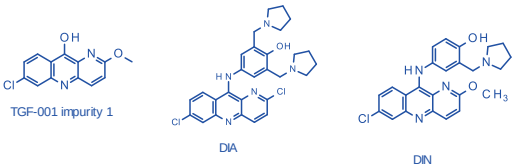
Lots of PNDa02 remained when using IPA as solvent for a big scale(50g). IPA was not ta suitable solvent for PND reaction.

5.4.4. Salt formation and purification step (route 3)

5.4.4.1. Reaction scheme



Impurities in TGF-001



5.4.4.2. Process and results of PND

Table 46. The results for the salification of TGF-001 (HPLC method: INV_054926_HPLC_M4)

No.	PND (method: M4, %area)	H_3PO_4	Solvent 1	Solvent 2	TGF-001 crude (method: M4, %area)
PHTRACKD-476	*Batch PHTRACKD-473* DIA: 0.14% Impurity 1: 3.37% U. I. @RRT 0.80/0.81: 0.18% U. I. @RRT 0.99: 0.14% Total impurities: 5.7%	5 eq.	Water (10v)	EtOH (20v)	DIA: 0.06%, Impurity 1: 0.43% U. I. @RRT 0.80/0.81: 0.14%, U. I. @RRT 0.99: 0.09% Total impurities: 1.4%
PHTRACKD-477		5 eq.	Water (10v)	Acetone (20v)	DIA: 0.09%, Impurity 1: 0.34% U. I. @RRT 0.80/0.81: 0.15%, U. I. @RRT 0.99:0.12% Total impurities:1.6%
PHTRACKD-478		5 eq.	Water (10v)	ACN (20v)	DIA: 0.10%, Impurity 1: 0.46% U. I. @RRT 0.80/0.81: 0.16%, U. I. @RRT 0.99: 0.11% Total impurities:1.8%
PHTRACKD-479		5 eq.	Water (10v)	IPA (20v)	DIA: 0.11%, Impurity 1: 0.48% U. I. @RRT 0.80/0.81: 0.15%, U. I. @RRT 0.99:0.12% Total impurities:2.0%

PHTRACKD-480		5 eq.	Water (10v)	MeOH (20v)	DIA:0.06%, Impurity 1:0.24% U. I. @RRT 0.80/0.81: 0.08%, U. I. @RRT 0.99:0.09% Total impurities:1.0%
PHTRACKD-475		5 eq.	Water (10v)	THF (20v)	DIA: 0.11%, Impurity 1: 0.20% U. I. @RRT 0.80/0.81: 0.18%, U. I. @RRT 0.99: 0.08% Total impurities:1.6%

EtOH and MeOH give the best impurity profile (less impurity close to 0.10%) after salt formation. However, the salt formation yield is only ~48% when using MeOH.

The %w/w of impurities could be below 0.10% (except TGF-001 impurity 1) for TGF-001 crude.

Prospect: If TGF-001 impurity 1 could be controlled well at PND step reaction, the additional purification procedure might be removed which could greatly reduce the cost.

Table 47. The results for the purification of TGF-001 (HPLC method: INV_054926_HPLC_M4)

No.	TGF-001 crude (method: M4, %area)	Purification	TGF-001 (method: M4, %area)
PHTRACKD-481	Batch PHTRACKD-475: DIA: 0.11% Impurity 1: 0.20% U. I. @RRT 0.80/0.81: 0.18% U. I. @RRT 0.99: 0.08% Total impurities: 1.6%	Water(10v)/ Acetone(20v)	DIA: 0.07%, Impurity 1: n. d. U. I. @RRT 0.80/0.81: 0.16%, U. I. @RRT 0.99:0.09% Total impurities: 0.9%
PHTRACKD-482		Water(10v)/ EtOH(20v)	DIA: 0.07%, Impurity 1: 0.09% U. I. @RRT 0.80/0.81: 0.15%, U. I. @RRT 0.99: 0.09% Total impurities: 1.0%
PHTRACKD-483		Water(10v)/ ACN (20v)	DIA: 0.07%, Impurity 1: 0.06% U. I. @RRT 0.80/0.81: 0.11%, U. I. @RRT 0.99: 0.08% Total impurities: 0.9%
PHTRACKD-484		Water(10v)/ IPA (20v)	DIA: 0.07%, Impurity 1:0.06% U. I. @RRT 0.80/0.81: 0.10%, U. I. @RRT 0.99:0.10% Total impurities: 0.9%
PHTRACKD-486		Water(10v)/ THF (20v)	DIA:<0.05%, Impurity 1: n. d. U. I. @RRT 0.80/0.81: 0.14%, U. I. @RRT 0.99:0.06% Total impurities:0.8%
PHTRACKD-485	Batch PHTRACKD-476: DIA: 0.06%, Impurity 1: 0.43%, U. I. @RRT 0.80/0.81: 0.14% U. I. @RRT 0.99: 0.09% Total impurities: 1.4%	Water(10v)/ THF (20v)	DIA: <0.05%, Impurity 1: n. d. U. I. @RRT 0.80/0.81: 0.10%, U. I. @RRT 0.99: 0.05% Total impurities: 0.6%

The product (PHTRACKD-485) using EtOH as salt formation solvent and THF as purification solvent has the highest purity and less impurities.

Prospect: THF is a class 2 solvent and more expensive than EtOH, perhaps it could be replaced by a cheaper class 3 solvent.

The volume of solvent used for purification was too much, it needs to be reduced.

Table 48. The results for the purification of TGF-001 (HPLC method: INV_054926_HPLC_M4)

No.	H_3PO_4	Salt formation	TGF-001 crude (method: M4, %area)	Purification	TGF-001 (method: M4, %area)	TGF-001 (method: M4, %w/w)	HPLC Assay LOD Res. Solvent
PHTRACKD-489 (10g)	5 eq.	Water(10v)/ EtOH(20v)	DIA: 0.08% DIN: <0.05% Impurity 1: 0.67% U. I. @RRT 0.93: 0.09% Total impurities: 1.3%	Water(10v)/THF (20v)	DIA: <0.05% DIN: <0.05% Impurity 1: <0.05% U. I. @RRT 0.93: 0.13% Total impurities: 0.5%	DIA:<0.05%, DIN: <0.05% Impurity 1:<0.05% U. I. @RRT 0.93: 0.08% Total impurities: 0.3%	Assay: 99.4% LOD: 1.7% THF: 5699 ppm
PHTHARRYS-518 (10g)	5 eq.	Water(10v)/ EtOH(20v)	DIA: 0.09% DIN: 0.30% Impurity 1: 0.44% Total impurities: 1.1%	Water(10v)/THF (20v)	DIA: <0.05% DIN: <0.05% Impurity 1: <0.05% Total impurities: 0.2%	DIA:<0.05% DIN: <0.05% Impurity 1:<0.05% Total impurities:0.06%	Assay: 98.6% LOD: 4.9% THF: 3737 ppm
PHTRACKD-492 (100g)	5 eq.	Water(10v)/ EtOH(20v)	DIA: 0.10% DIN: <0.05% Impurity 1: 1.46% Total impurities: 1.7%	Water(10v)/THF (20v)	DIA: <0.05% DIN: <0.05% Impurity 1: <0.05% Total impurities: 0.1%	DIA:<0.05% DIN:<0.05% Impurity 1:<0.05% Total impurities: 0.09%	Assay: 103.1% LOD: 0.7% THF: 2900 ppm

All impurities are controlled well.

Residual solvent THF is more than 720 ppm (ICH limit) and is difficult to purge after drying. Continued drying is does not help

It seems the amount of H_3PO_4 is not fixed from batch to batch and is less than 4 eq.

Table 49. The results for the purification of TGF-001 (HPLC method: INV_054926_HPLC_M4)

No.	H_3PO_4	Salt formation	Purification	TGF-001 (Method: M4, %area)	TGF-001 (Method: M4, %w/w)	HPLC Assay Water Res. Solvent pH
PHTRACKD-497	6 eq.	Water(10v)/ EtOH(20v)	Water(10v)/THF (20v)	DIA: 0.05% DIN: <0.05% Total impurities: 0.1%	DIA: <0.05% DIN: <0.05% Total impurities: 0.06%	Assay: 101.2% Water: 0.4% THF: 3132 ppm
PHTRACKD-498	6 eq.	Water(10v)/ EtOH(20v)	Water(10v)/THF (20v) Wash cake by EtOH	DIA:0.07% DIN: <0.05% Total impurities: 0.1%	DIA: <0.05% DIN: <0.05% Total impurities: <0.05%	Assay: 103.1% Water: 0.2% EtOH: 3950 ppm
PHTRACKD-504-1	7 eq.	Water(10v)/ EtOH(20v)	Water(10v)/THF (20v)	DIA: <0.05% DIN: <0.05% Total impurities: 0.07%	DIA: <0.05% DIN: <0.05% Total impurities: 0.04%	Assay: 100.9% Water: 0.3% THF: 8973 ppm pH: 2.7
PHTRACKD-504-2	7 eq.	Water(10v)/ EtOH(20v)	Water(10v)/THF (20v) Wash cake by Acetone	DIA:<0.05% DIN:<0.05% Total impurities: 0.07%	DIA: <0.05% DIN: <0.05% Total impurities: 0.04%	Assay: 102.1% Water: 0.6% THF: 2024 ppm pH: 2.7
PHTRACKD-504-3	7 eq.	Water(10v)/ EtOH(20v)	Water(10v)/THF (20v)	DIA:<0.05% DIN:<0.05%	DIA: <0.05% DIN: <0.05%	Assay: 100.3% Water: 0.7%

			Wash cake by EtOH	Total impurities: 0.07%	Total impurities: 0.04%	EtOH: 15386 ppm pH: 2.7
PHTHARRYS-530	6 eq.	Water(10v)/ EtOH(20v)	Water(10v)/THF (20v) 1eq. H3PO4 Wash cake by EtOH	DIA: <0.05% DIN: <0.05% Total impurities: 0.08%	DIA: <0.05% DIN: <0.05% Total impurities: 0.05%	Assay: 99.3% Water: 0.5% EtOH: 9306 ppm pH: 2.6
PHTHARRYS-527	6 eq.	Water(10v)/ EtOH(20v)	Water(10v)/THF (20v) 2eq. H3PO4 Wash cake by EtOH	DIA:<0.05% DIN:<0.05% Total impurities: 0.1%	DIA:<0.05% DIN: <0.05% Total impurities: 0.05%	Assay: 99.5% Water: 0.3% EtOH: 2425 ppm pH: 2.3

6+1 eq. H_3PO_4 (cake washed by EtOH) gives the best results, and all analytical data meet requirement (version 1) except for residual EtOH, however, it could be purged blew 5000ppm after drying more time.

Table 50. The results for the purification of TGF-001 (HPLC method: INV_054926_HPLC_M4)

PND (wet)	H3PO4	Salt formation solvent	TGF-001 crude	Purification	TGF-001
PHTHARRYS-536 (2023 phase 1) PND:95.9% Impurity 1: 2.4 %area	6 eq.	Water(10v)/ EtOH (20v)	N/A	Water(10v)/THF (20v) 1eq. H_3PO_4 Wash cake by EtOH	PHTHARRYS-537 DIA: <0.05 %area DIN: n. d. Impurity 1: 0.05 %area
PHTRACKD-562 PND:98.2% Impurity 1: 0.60 %area	6 eq.	Water(10v)/ EtOH (20v)	PHTRACKD-562 PND:99.77% DIA: 0.06 %area DIN: n. d. Impurity 1: 0.10 %area	Water(10v)/EtOH (20v) 1eq. H_3PO_4 Wash cake by EtOH	PHTRACKD-575 PND:99.80% DIA: 0.04 %area DIN: n. d. Impurity 1: 0.05 %area
PHTRACKD-578 (dry) PND:97.8% Impurity 1: 0.67 %area	6 eq.	Water(5v)/ EtOH (10v)	PHTRACKD-585 PND:99.78% DIA: 0.05 %area DIN: 0.01%area. Impurity 1: 0.12 %area	Water(5v)/EtOH (10v) 1eq. H_3PO_4 Wash cake by EtOH	PHTRACKD-588 PND:99.86% DIA: 0.04%area. DIN: n.d. Impurity 1: 0.03 %area
PHTRACKD-578 (dry) PND:97.8% Impurity 1: 0.67 %area	6 eq.	Water(5v)/ THF (10v)	PHTRACKD-586 PND:99.89% DIA: 0.03%area. DIN: n.d. Impurity 1: 0.03 %area	Water(5v)/EtOH (10v) 1eq. H_3PO_4 Wash cake by EtOH	PHTRACKD-589 PND:99.91% DIA: 0.03%area. DIN: n.d. Impurity 1: 0.01 %area
PHTRACKD-578 (dry) PND:97.8% Impurity 1: 0.67 %area	6 eq.	Water(5v)/Acetone (10v)	PHTRACKD-587 PND:99.77% DIA: 0.07 %area DIN: 0.01%area. Impurity 1: 0.08 %area	Water(5v)/EtOH (10v) 1eq. H_3PO_4 Wash cake by EtOH	PHTRACKD-590 PND:99.91% DIA: 0.06%area. DIN: n.d. Impurity 1: 0.03 %area
PHTRACKD-596 PND:98.4% Impurity 1: 0.30 %area	6 eq.	Water(4v)/ EtOH (8v)	PHTRACKD-596 PND:99.69% DIA: 0.06 %area DIN: n.d. Impurity 1: 0.10 %area	N/A	N/A
PHTRACKD-601 PND:97.9% Impurity 1: 0.33 %area	6 eq.	Water(5v)/ EtOH (10v)	PHTRACKD-601 PND:99.78% DIA: 0.05 %area DIN: n.d. Impurity 1: 0.07 %area	N/A	N/A

TGF-001 crude has a good purity enough. But crystal form needs more research with different purification process.

Table 51. Salt formation process (HPLC method: INV_054926_HPLC_M4)

PND	Solvent	Salt formation process	TGF-001 crude, HPLC M4, %area	pH (4% in water)	Water Solvents, %w/w	Impurity in %w/w	Assay, HPLC M4, %w/w	Yield
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43%	Water(5v) / EtOH (10v)	Add 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool to 30 ℃ and stir for 1h. Filter.	PHTRACKD-622 PND:99.6% DIA: 0.06% DIN: 0.01% Impurity 1: 0.09%	2.44	Water:0.8% EtOH:1.18%	DIA: 0.035% DIN: 0.004% Impurity 1: 0.054%	97.3%	88.1%
PHTRACKD-596 (wet) PND:98.4% Impurity 1: 0.30%	Water(4v) / EtOH (8v)	Added 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool to 20 ℃ and stir for 1h. Filter.	PHTRACKD-596 PND:99.7% DIA: 0.06% DIN: n.d. Impurity 1: 0.11%	2.49	Water:1.6% EtOH:0.08%	DIA: 0.034% DIN: 0.004% Impurity 1:0.073%	98.9%	89.7%
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43 %area	Water(5v) / EtOH (10v)	Added 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool to 20 ℃ and stir for 1h. Filter.	PHTRACKD-623 PND:99.5% DIA: 0.07% DIN: 0.01 Impurity 1: 0.09%	2.52	Water:0.9% EtOH:2.91%	DIA: 0.038% DIN: 0.003% Impurity 1: 0.048%	97.1%	92.3%
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43 %area	Water(5v) / EtOH (10v)	Added 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added dropwise. Stir at 45℃ for 1h. Cool to 20 ℃ and stir for 1h. Filter.	PHTRACKD-625 PND:99.5% DIA: 0.07 % DIN: 0.01% Impurity 1: 0.10%	2.56	Water:0.9% EtOH:2.75%	DIA: 0.035% DIN: 0.004% Impurity 1: 0.057%	96.6%	91.3%
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43 %area	Water(5v) / EtOH (10v)	Added 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool	PHTRACKD-624 PND:99.4% DIA: 0.08% DIN: 0.01% Impurity 1: 0.11%	2.42	Water:1.1% EtOH:0.72%	DIA: 0.048% DIN: 0.004% Impurity 1: 0.064%	96.0%	93.6%

		to 10 ℃ and stir for 1h. Filter.						
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43 %area	Water(5v) / EtOH (10v)	Added 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool to 35 ℃ in1h. Cool to 20 ℃ in1h and stir for another 1h. Filter.	PHTRACKD-626 PND:99.5% DIA: 0.06% DIN: 0.01%. Impurity 1: 0.10%	2.52	Water:0.9% EtOH:1.53%	DIA: 0.035% DIN: 0.003% Impurity 1: 0.057%	96.4%	91.7%

The EtOH addition procedure has no obvious impact on TGF-001 quality and crystal form.

Table 52. Salt formation process (HPLC method: INV_054926_HPLC_M4)

PND	Solvent	Salt formation process	TGF-001 crude, HPLC M4, %area	Yield
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43%	Water(10v)/ EtOH (10v)	Add 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool to 20 ℃ and stir for 1h. Filter. Wash cake with EtOH.	PHTRACKD-631 PND:99.52% DIA: 0.05%, DIN: n.d. Impurity 1: 0.11%	92.0%
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43 %area	Water(10v)/ EtOH (10v)	Added 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool to 10 ℃ and stir for 1h. Filter. Wash cake with EtOH.	PHTRACKD-632 PND:99.38% DIA: 0.06%, DIN: n.d. Impurity 1: 0.20%	93.3%
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43 %area	Water(10v)/ EtOH (7.5v)	Added 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool to 20 ℃ and stir for 1h. Filter. Wash cake with EtOH.	PHTRACKD-633 PND:99.55% DIA: 0.03 %, DIN: n.d. Impurity 1: 0.11%	90.6%
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43 %area	Water(10v)/ EtOH (5v)	Added 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. No solid formed. Cooled to 35℃, solid formed. Cool to 20 ℃ and stir for 1h. Filter.	PHTRACKD-634 PND:99.55% DIA: 0.03%, DIN: n.d. Impurity 1: 0.12%	84.7%
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43 %area	Water(10v)/ EtOH (5v)	Added 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. Cooled to 20 ℃, no solid form. EtOH was added dropwise. Stir at 20 ℃ for1h. Filter. Wash cake with EtOH.	PHTRACKD-635 PND:99.58% DIA: 0.03%, DIN: 0.01%. Impurity 1: 0.11%	81.8%
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43%	Water(5v)/ EtOH (10v)	Add 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool to 20 ℃ and stir for 1h. Filter. Wash cake with EtOH(10v)/water(5v).	PHTRACKD-645 PND:99.15% DIA: 0.05%, DIN: 0.04. Impurity 1: 0.15%	91.0%
PHTRACKD-619 (dry) PND:98.8% Impurity 1: 0.43%	Water(5v)/ EtOH (10v)	Add 6eq. H3PO4 into the PND aqueous solution. Increase the solution temp. (45℃) to get a clear solution. EtOH was added in one portion. Stir at 45℃ for 1h. Cool to 20 ℃ and stir for 1h. Filter. Wash cake with EtOH(10v)/water (2.5v).	PHTRACKD-646 PND:99.19% DIA: 0.04%, DIN: 0.04% Impurity 1: 0.14%	90.5%

The purity of TGF-001 doesn't have obvious difference between EtOH(10v)/ water(10v) and EtOH(10v)/ water(5v) for Salt formation.

EtOH gives better results on filtration& material color compared to mixed solvent water/ethanol for cake washing.

Table 53. Impurity control and crystal form:(HPLC method: INV_054926_HPLC_M4)

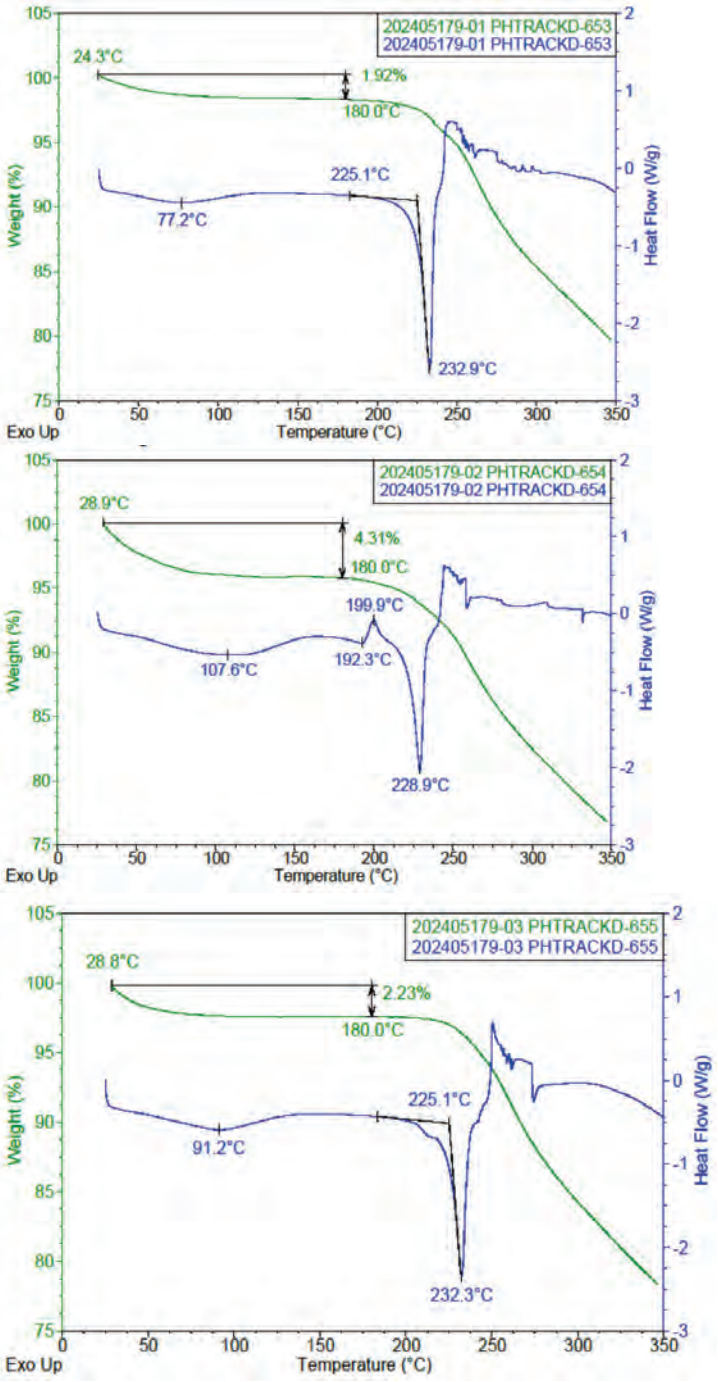
TGF-001 crude	Purified TGF-001	XRPD
PHTRACKD-648 (EtOH as reaction solvent) - 270 nm Total impurities: 0.75%area Impurity 1: 0.32%area - 278 nm Total impurities: 0.44%area Impurity 1: 0.10%area	PHTRACKD-651(EtOH slurry at R.T.) - 270 nm Total impurities: 0.71%area, Impurity 1: 0.32%area - 278 nm Total impurities: 0.44%area, Impurity 1: 0.10%area	Similar to crude (using dried PND, EtOH as reaction solvent)
	PHTRACKD-652 (Acetone slurry at R.T.) - 270 nm Total impurities: 0.69%area, Impurity 1: 0.32%area - 278 nm Total impurities: 0.52%area, Impurity 1: 0.10%area	Similar to crude (using dried PND, EtOH as reaction solvent)
	PHTRACKD-653(75% EtOH reflux 80°C to R.T.) - 270 nm Total impurities: 0.44%area, Impurity 1: 0.02%area - 278 nm Total impurities: 0.43%area, Impurity 1: 0.01%area	Similar to form E (CN 11220926 A)
	PHTRACKD-654 (10v water, 20v EtOH 45°C to R.T.) - 270 nm Total impurities: 0.41%area, Impurity 1: 0.09%area - 278 nm Total impurities: 0.32%area, Impurity 1: 0.03%area	Similar to crude (using wet PND, EtOH as reaction solvent)
	PHTRACKD-655 (10v water, 20v Acetone 45°C to R.T.) - 270 nm Total impurities: 0.45%area, Impurity 1: 0.06%area - 278 nm Total impurities: 0.43%area, Impurity 1: 0.02%area	Meet the requirement of BGMF

Four crystal forms were gotten with different purification systems. The Acetone/water system was chosen as the purification system.

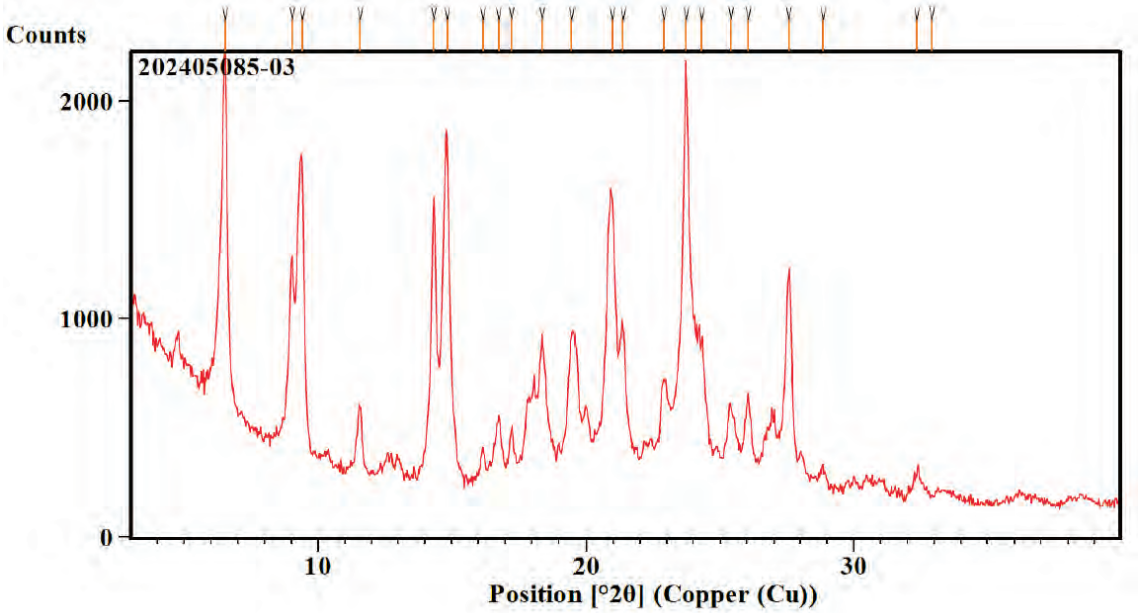
Table 54. DSC-TGA results of 3 crystal forms

Purified TGF-001	TGA weight loss, %	DSC endotherm (peak temp., °C)
PHTRACKD-653 (75% EtOH reflux 80℃ to R.T.)	1.92 (180.0 °C)	77.2, 225.1*
PHTRACKD-654 (10v water, 20v EtOH 45℃ to R.T.)	4.31 (180.0 °C)	107.6, 192.3, 199.9#, 228.9
PHTRACKD-655 (10v water, 20v Acetone 45℃ to R.T.)	2.23 (180.0 °C)	91.2, 225.1*

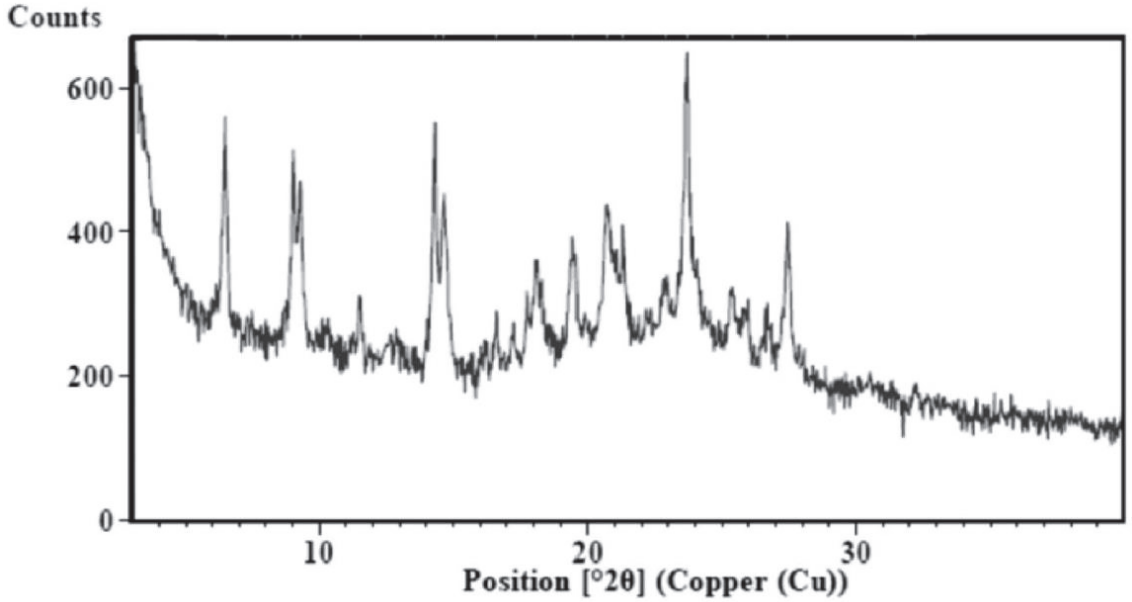
DCS-TGA curves:



XRPD of PHTRACKD-653

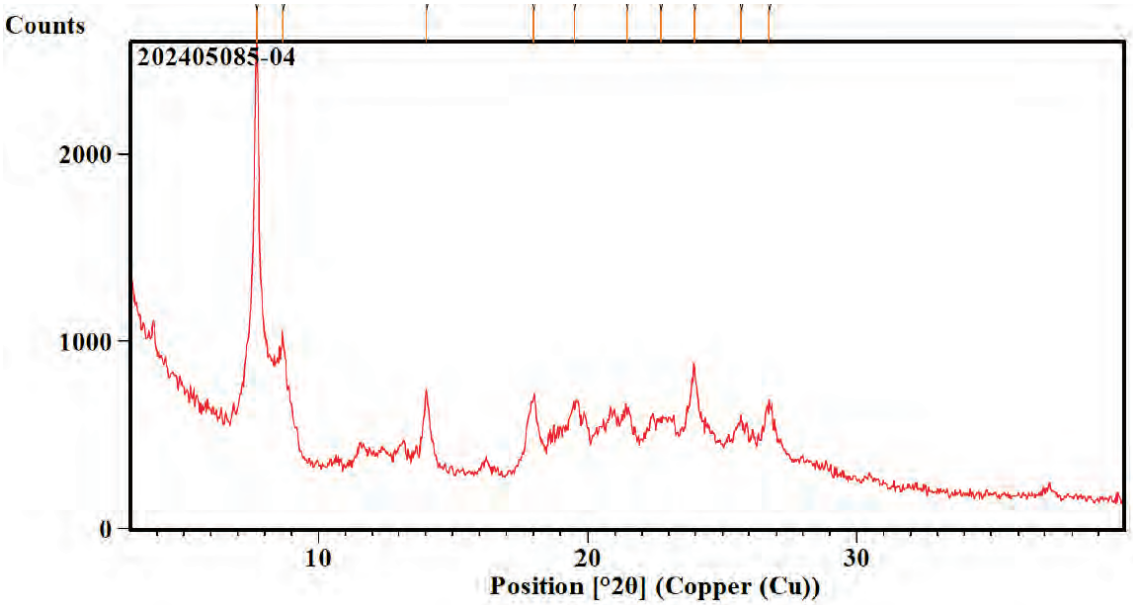


XRPD of Form E(CN112209926A)

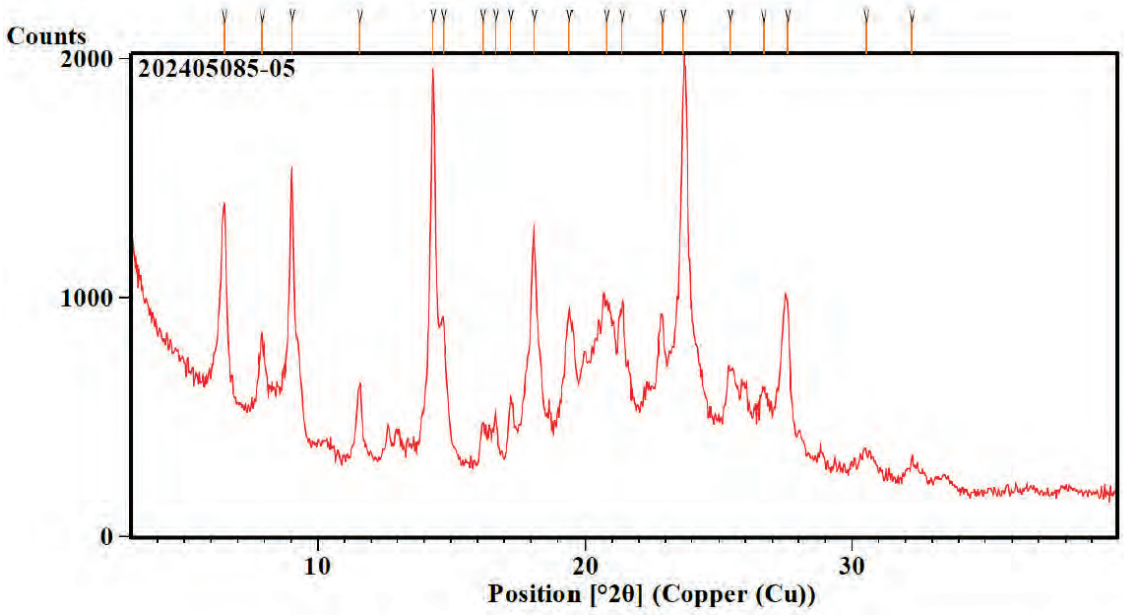


XRPD of PHTRACKD-653 is similar to form E repotted in CN112209926A

XRPD of PHTRACKD-654



XRPD of PHTRACKD-655



5.5. Use test of 5A2MP

5.5.1. PNDa01 and PNDa02 steps:

Table 55. Results of PNDa01 and PNDa02

5A2MP (SM2)	SM1 (assay: 99.7%)	PNDa01 IPC_M1 (SM2: <3.0%)	Isolated PNDa01	PNDa02 IPC_M1 (PNDa01: <0.2%)	Isolated PNDa02
Supplier 1 500g (1.0 eq.) (assay: 91.1%)	1.4 eq.	SM2: 2.43%	PHTANWARL-530 Amount: 920 g Assay: 89.0% Yield: 80.1%	PHTHARRYS-519 PNDa01: 0.02% TGF-001 impurity 1: 0.45% BIA: 0.07% PNDa02: 96.3%	PHTHARRYS-519 Amount: 390g Assay:90.1% Yield: 78.9%
Supplier 2 50g (1.0 eq.) (assay: 95.6%%)	1.4 eq.	SM2: 2.76%	PHTKENNYG-682 Amount: 97.9g Assay:92.3% Yield: 84.2%	PHTKENNYG-686 PNDa01: 0.06% TGF-001 impurity 1: 0.06% BIA: 0.18% PNDa02: 96.3%	PHTKENNYG-686 Amount: 37.2g Assay:93.5% Yield: 75.3%
Supplier 3 50g (1.0 eq.) (assay: 83.6%)	1.4 eq.	SM2: 4.91%	PHTKENNYG-687 N/A	N/A	N/A
In house 10g (1.0 eq.) (assay: 99.5%)	1.4 eq.	SM2: 2.76%	PHTKENNYG-740 Amount: 19g Assay:96.4% Yield: 82.0%	PHTKENNYG-741 PNDa01: 0.03% TGF-001 impurity 1: 0.48% BIA: 0.14% PNDa02: 95.6%	PHTKENNYG-741 Amount: 8.5g Assay:94.1% Yield: 82.8%

5.5.2. PNDa04-HCl, PND and TGF-001 Steps:

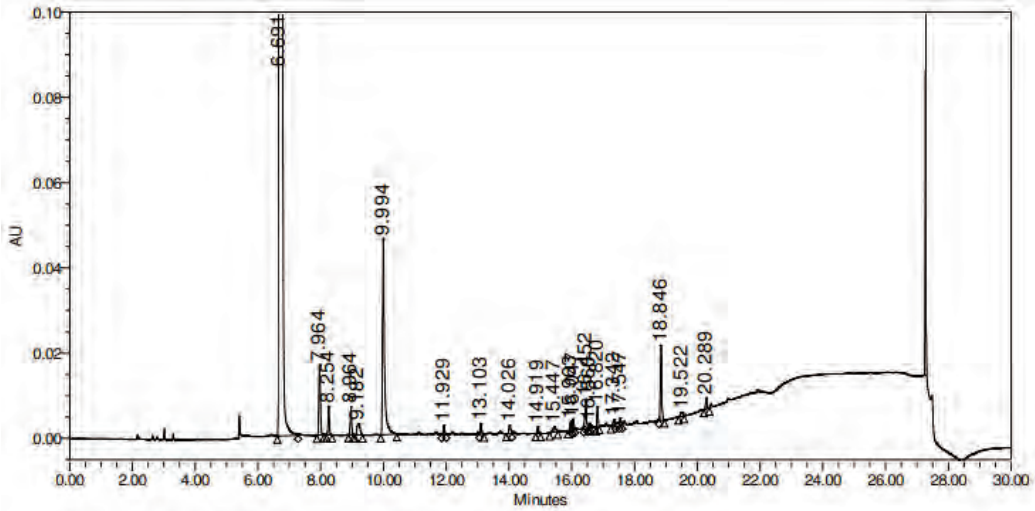
Table 56. Results of PNDa04, PND and TGF-001 step

PNDa06-HCl	PNDa04-HCl IPC_M1 (1h) (PNDa06: <0.5%)	PNDa02	PND IPC_M1	TGF-001 (API)
PHTHARRYS-520 500g (1.1 eq.) (assay: 96.7%)	PHTHARRYS-535 PNDa06: n. d. PNDa04: 99.0%	PHTHARRYS-519 (from supplier 1's 5A2MP) 341g (1 eq.) (Assay: 90.1%)	PHTHARRYS-536 PNDa02: 0.05% PNDa04: 0.35% Impurity 1: 2.4% PND: 95.9%	PHTHARRYS-537 DIA: <0.05 %area DIN: n. d. Impurity 1: 0.05 %area Assay: 100.2% Yield: 85.7%
PHTHARRYS-587 44.5g (1.1 eq.) (assay: 96.5%)	PHTKENNYG-691 PNDa06: n. d. PNDa04: 98.3%	PHTKENNYG-686 (from supplier 2's 5A2MP) 30g (1 eq.) (Assay: 93.5%)	PHTKENNYG-692 PNDa02: 0.05% PNDa04: 0.7% Impurity 1: 3.5% PND: 94.2%	PHTKENNYG-693 DIA: <0.05 %area DIN: n. d. Impurity 1: <0.05 %area Assay: 100.2% Yield: 82.5%
PHTKENNY-738 10.8g(1.1eq) (assay: 97.6%)	PHTKENNYG-691 PNDa06: n. d. PNDa04: 99.5%	PHTKENNYG-741 (from in house 5A2MP) 8g (1 eq.) (Assay: 94.1%)	PHTKENNYG-743 PNDa02: 0.32% PNDa04: 0.21% Impurity 1: 0.43% PND: 97.5%	PHTKENNYG-743 DIA: <0.05 %area DIN: n. d. Impurity 1: <0.05 %area Assay: 96.6% Yield: 80.2%

The use test result in terms of impurity profile showed that 5A2MP obtained by the in-house developed process could be used for TGF0-001 synthesis.

Chromatograms of 5A2MP:

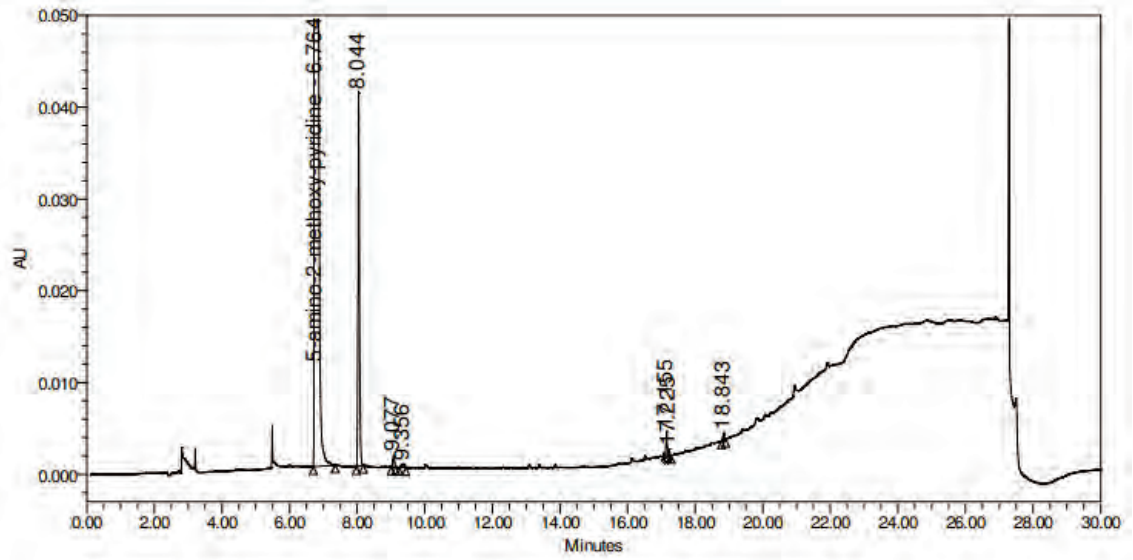
Figure 1: HPLC chromatogram of 5A2MP (supplier 1)



	Name	RT	RRT	Area	% Area	Resolution
1		6.691		6027510	92.81	
2		7.964		51081	0.79	
3		8.254		19347	0.30	
4		8.964		18807	0.29	
5		9.182		17390	0.27	
6		9.994		193701	2.98	
7		11.929		7490	0.12	
8		13.103		8569	0.13	
9		14.026		7701	0.12	

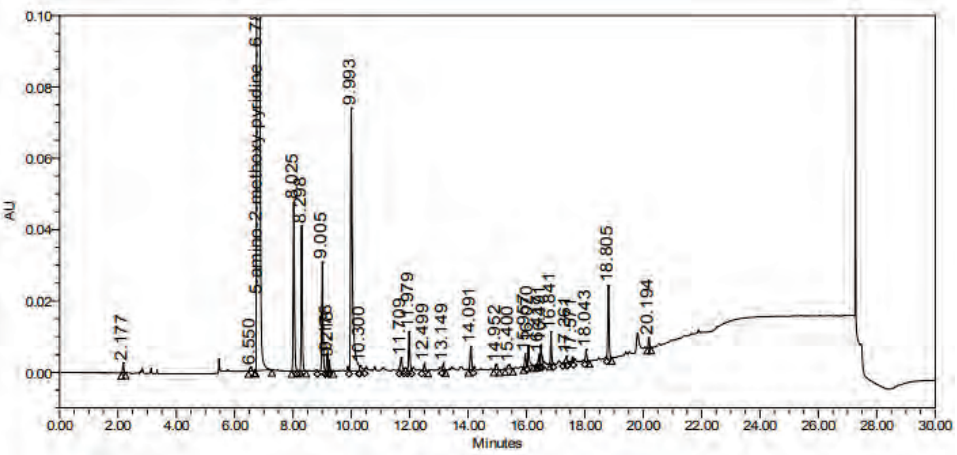
	Name	RT	RRT	Area	% Area	Resolution
10		14.919		5526	0.09	
11		15.447		8052	0.12	
12		15.963		6290	0.10	
13		16.047		7629	0.12	
14		16.452		22946	0.35	
15		16.560		3630	0.06	
16		16.820		11215	0.17	
17		17.342		6273	0.10	
18		17.547		5915	0.09	
19		18.846		52607	0.81	
20		19.522		5009	0.08	
21		20.289		7902	0.12	

Figure 2: HPLC chromatogram of 5A2MP (supplier 2)



	Name	RT	RRT	Area	% Area	Resolution
1	5-amino-2-methoxy-pyridine	6.764	1.00	3427998	96.18	
2		8.044	1.19	119314	3.35	
3		9.077	1.34	4096	0.11	
4		9.356	1.38	2259	0.06	
5		17.155	2.54	6433	0.18	
6		17.225	2.55	1812	0.05	
7		18.843	2.79	2053	0.06	

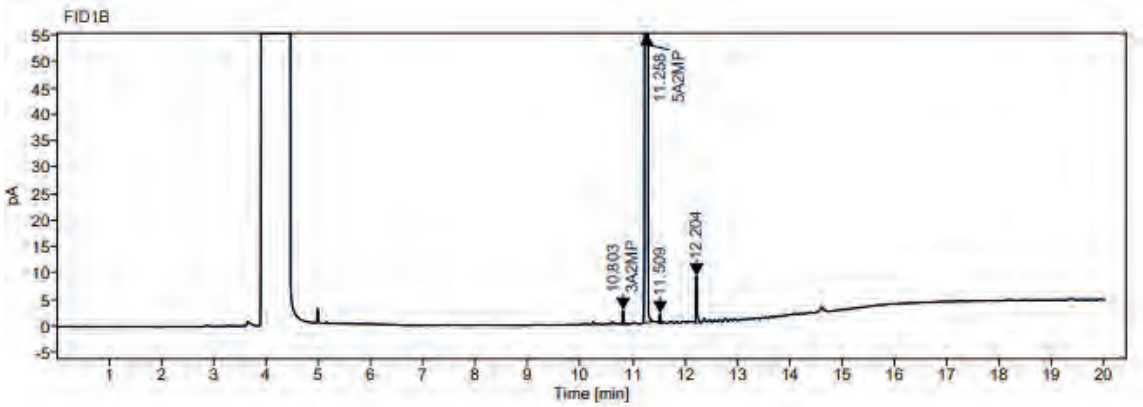
Figure 3: HPLC chromatogram of 5A2MP (supplier 3)



	Name	RT	RRT	Area	% Area	Resolution
1		2.177	0.32	7476	0.14	
2		6.550	0.97	5613	0.11	
3	5-amino-2-methoxy-pyridine	6.782	1.00	4322523	81.40	
4		8.025	1.18	137909	2.60	
5		8.298	1.22	110849	2.09	
6		9.005	1.33	88473	1.67	
7		9.178	1.35	17204	0.32	
8		9.218	1.36	9231	0.17	
9		9.993	1.47	333545	6.28	

	Name	RT	RRT	Area	% Area	Resolution
10		10.300	1.52	8510	0.16	
11		11.709	1.73	16636	0.31	
12		11.979	1.77	33602	0.63	
13		12.499	1.84	6972	0.13	
14		13.149	1.94	6579	0.12	
15		14.091	2.08	23781	0.45	
16		14.952	2.20	6442	0.12	
17		15.400	2.27	7891	0.15	
18		15.957	2.35	12301	0.23	
19		16.070	2.37	20395	0.38	
20		16.417	2.42	7185	0.14	
21		16.491	2.43	14698	0.28	
22		16.841	2.48	21603	0.41	
23		17.361	2.56	7862	0.15	
24		17.571	2.59	5746	0.11	
25		18.043	2.66	10592	0.20	
26		18.805	2.77	58780	1.11	
27		20.194	2.98	7849	0.15	

Figure 4: GC chromatogram of 5A2MP (in house)



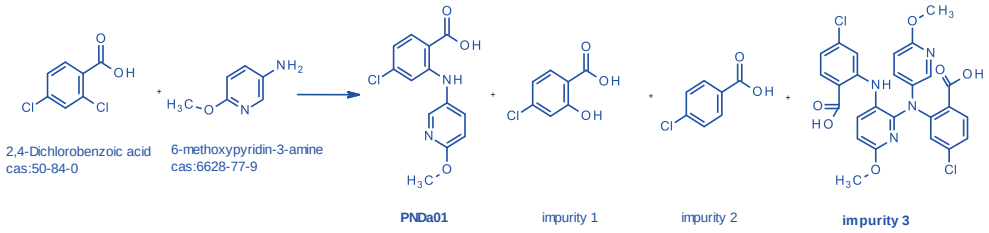
Signal: FID1B

RT [min]	Name	Type	Width [min]	Area	Height	Area%
10.803	3A2MP	BM m	0.1331	4.2073	2.6758	0.0695
11.258	5A2MP	MM m	0.2240	6034.6434	3285.8718	99.6512
11.509		VV	0.0702	3.1388	1.8948	0.0518
12.204		VB	0.1318	13.7746	8.8092	0.2275
			Sum	**6055.7640**		

5.6. Synthesis of Impurities

5.6.1. Impurity 1, impurity 2 and impurity 3

5.6.1.1. Reaction scheme



5.6.1.2. The procedure for the preparation of impurity 3 in experiment PHTRACKD-301

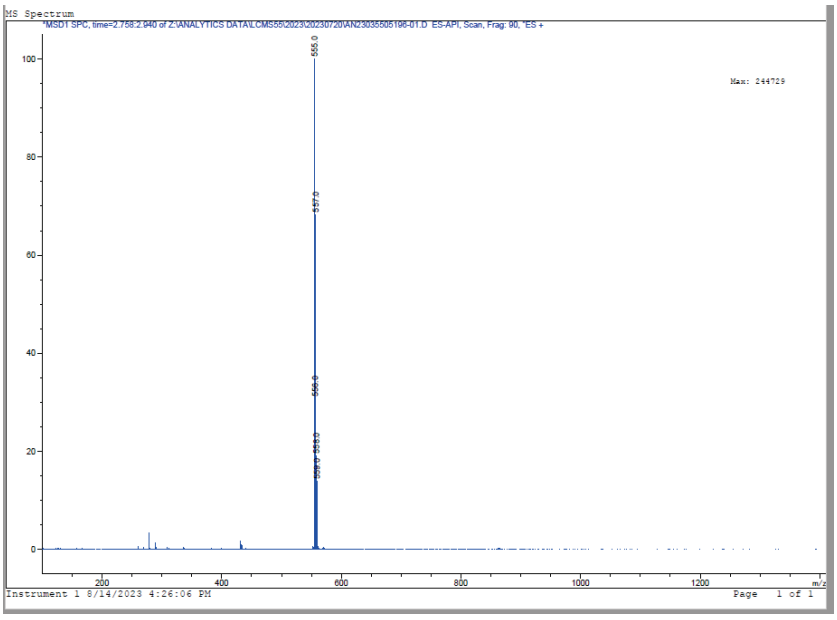
1. The suspension of 2,4-dichlorobenzoic acid (9.23g, 47.37mmol), 6-methoxypyridin-3-amine (5g, 39.472mmol), Potassium carbonate (10.02g, 71.05mmol5) Copper (I) iodide (1.15g, 5.92mmol) in water (40mL) was stirred at 100°C for 18hs.

2. HPLC(PNDa01-PHTRACKD-301-IPC) showed impurity 3 at 20.5min was 12.8%.

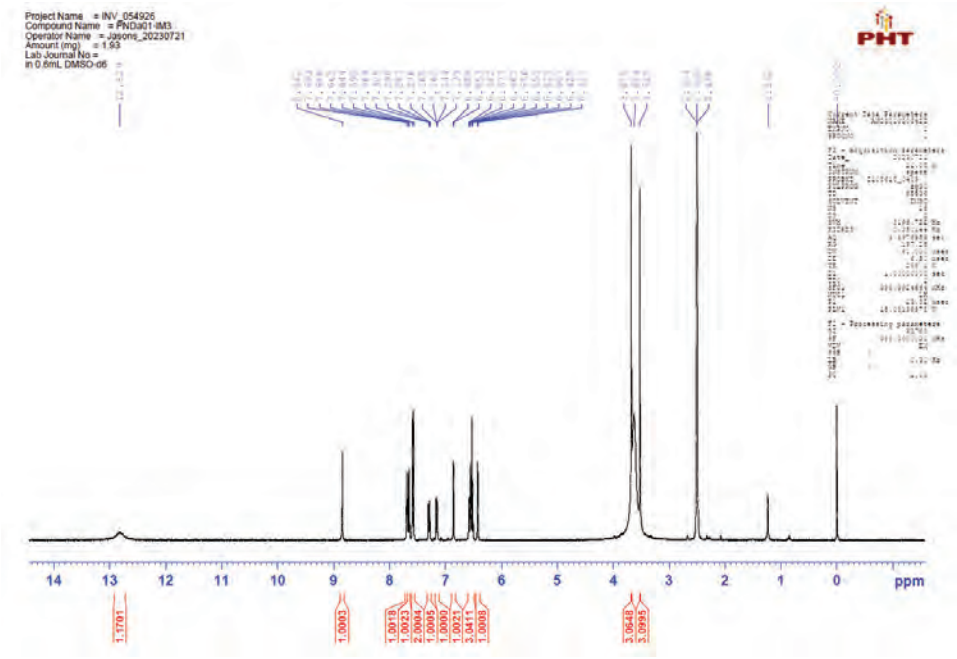
3. Cooled the reaction to 30°C，removed the undissolved materials by filtration. Adjusted the pH of the filtrate to 2. The filter cake was dried to give a 10g black solid.

4. The impurity 3 was isolated by pre-HPLC to give 180mg yellow solid. Only 1.5g of crude was used to isolate the impurity.

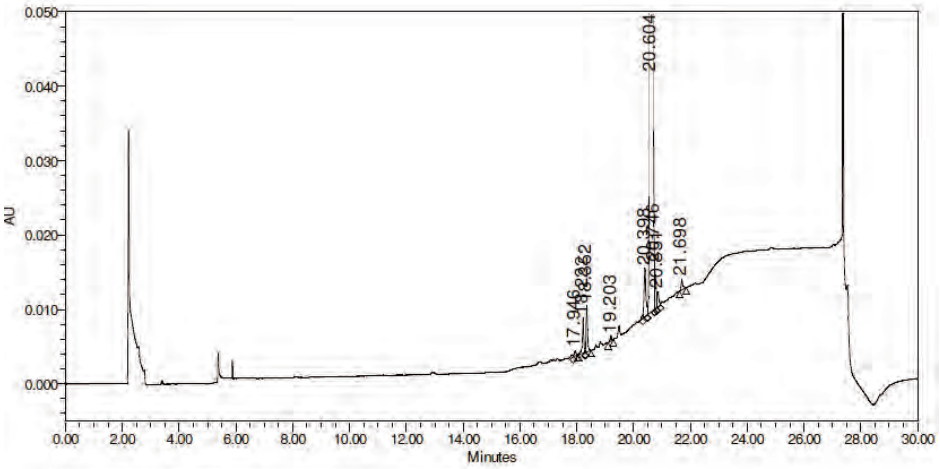
MS of impurity 3



HNMR of impurity 3



HPLC chromatogram of impurity 3

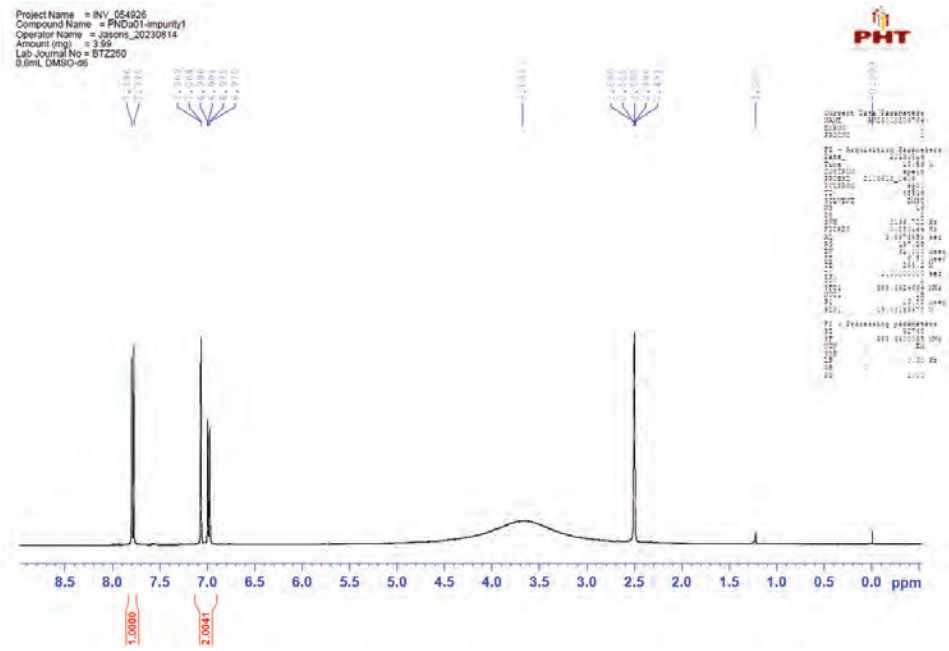


	Name	RT	RRT	Area	% Area	Resolution
1		17.946		2915	0.05	
2		18.222		16762	0.29	
3		18.352		20364	0.35	
4		19.203		2779	0.05	
5		20.398		29098	0.50	
6		20.604		5682233	98.22	
7		20.746		16439	0.28	
8		20.851		8785	0.15	
9		21.698		5849	0.10	

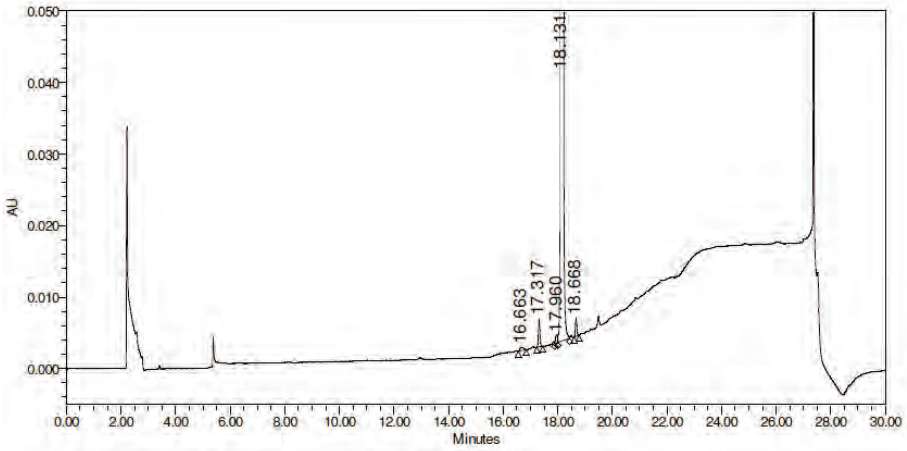
Impurity1 and Impurity 2 were bought by a commercial company.

MS of impurity 1

HNMR of impurity 1



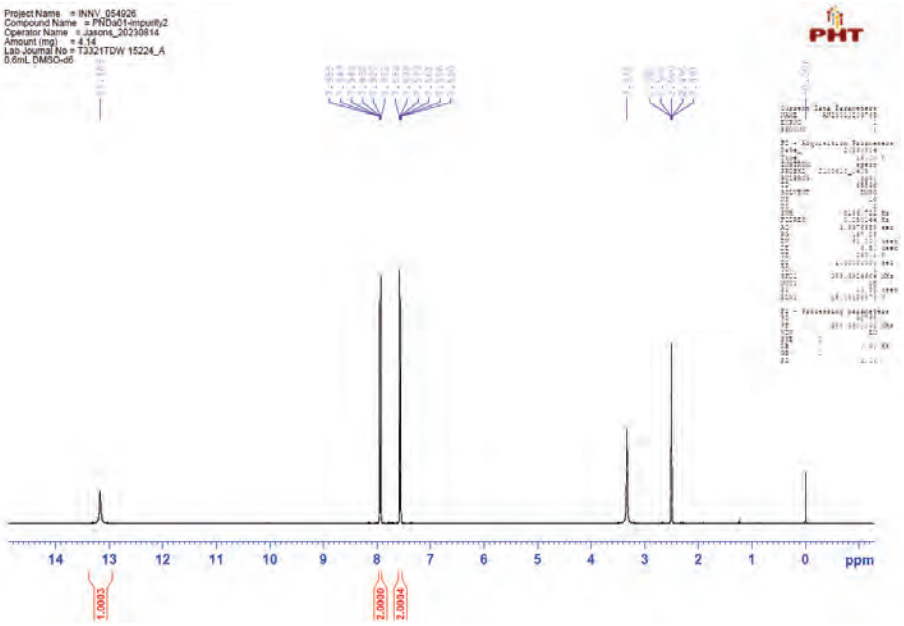
HPLC chromatogram of impurity 1



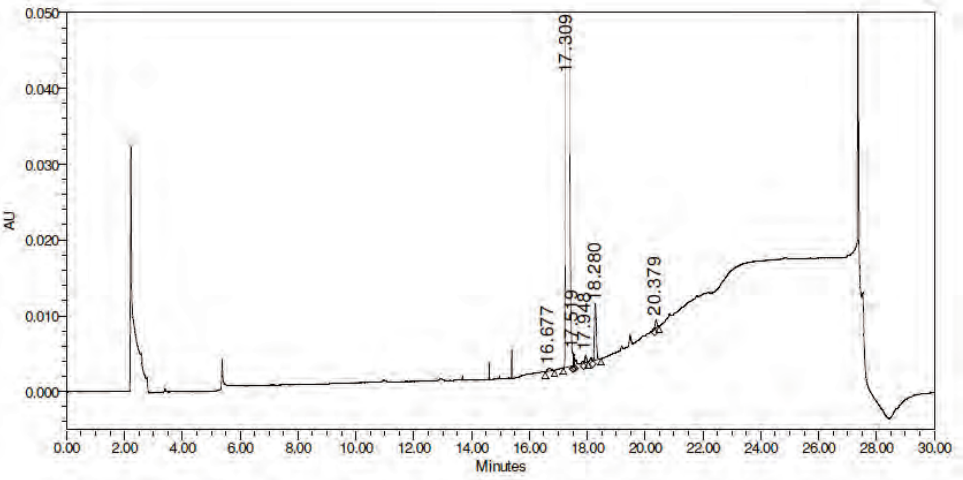
	Name	RT	RRT	Area	% Area	Resolution
1		16.663		3952	0.07	
2		17.317		15220	0.28	
3		17.960		5368	0.10	
4		18.131		5476240	99.38	
5		18.668		9501	0.17	

MS of impurity 2

HNMR of impurity 2



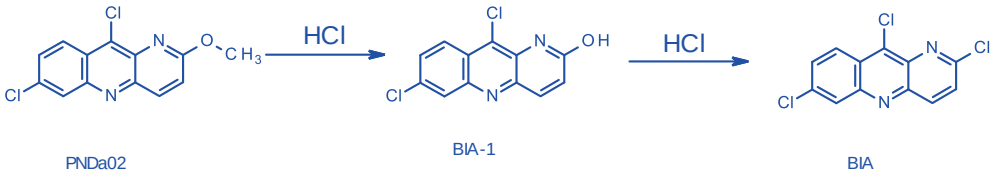
HPLC chromatogram of impurity 2



	Name	RT	RRT	Area	% Area	Resolution
1		16.677		3081	0.05	
2		17.309		6068690	99.19	
3		17.519		5775	0.09	
4		17.948		4245	0.07	
5		18.280		32449	0.53	
6		20.379		4280	0.07	

5.6.2. BIA in PNDa02

5.6.2.1. Reaction scheme



PNDa02 BIA-1 BIA

5.6.2.2. The procedure for the preparation of BIA in experiment PHTHARRYS-476，478

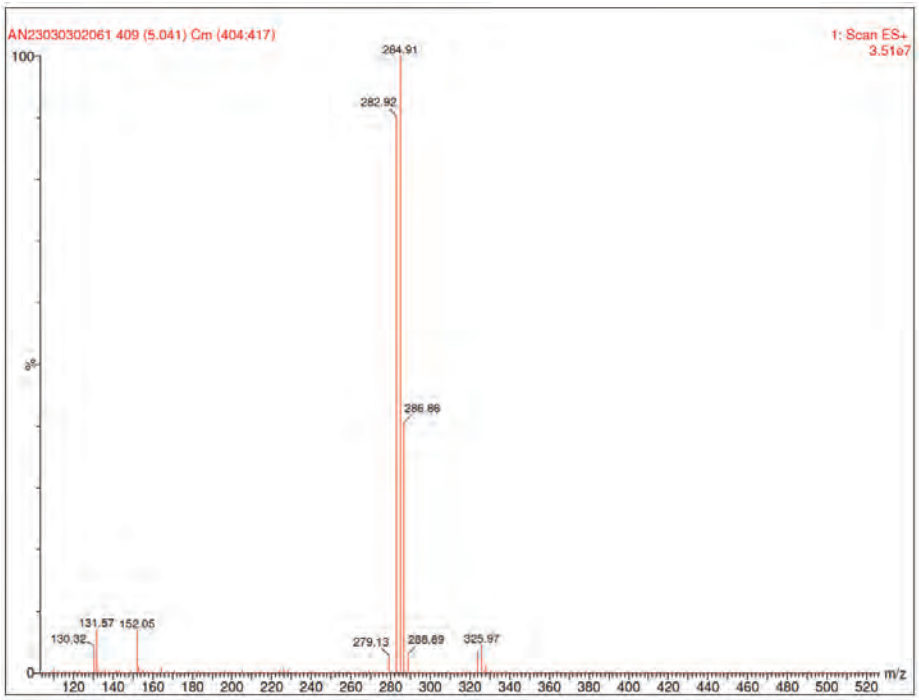
PHTHARRYS-476

- Charge PNDa02(1g, 3.2mmol) and THF (5mL) into a 100mL Flask.
- Charge Hydrochloric acid (12N，5mL); water (5mL) into the flask.
- The suspension was then stirred at 100°C for 1h
- HPLC （PNDa02-IM-PHTHARRYS-476-IPC） showed the reaction was completed,1.74% PNDa02 was left. 73.6% of BIA was formed in HLPC.
- The solvent was concentrated under a vacuum.
- 10mL H_2O was then added into the residue.
- The pH of the mixture was then adjusted to 9 with 15% NaOH.
- BIA-1 (716mg, 62.156% yield) was obtained after filtration and drying.

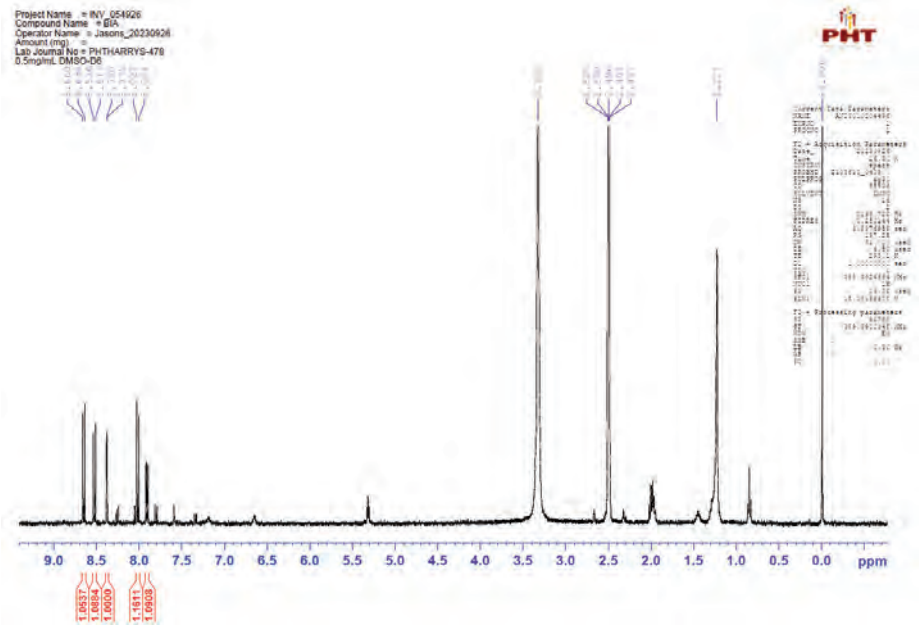
PHTHARRYS-478

- Charge BIA-1 (716mg, 1.99mmol), toluene (10mL) into a 100mL flask.
- Charge Phosphorus (V) trichloride oxide (8.2g, 50.96mmol,5mL) into the flask.
- The mixture was then stirred at 100°C for 1h.
- HPLC(PNDa02-IM-PHTHARRYS-478-ipc) showed 95.8% BIA was formed.
- The mixture was then concentrated under a vacuum.
- 10mL Water was added into the residue.
- The pH was adjusted to 9 with 15% NaOH.
- BIA (520mg,1.7762mmol, 89.289% yield) was obtained after filtration and drying.

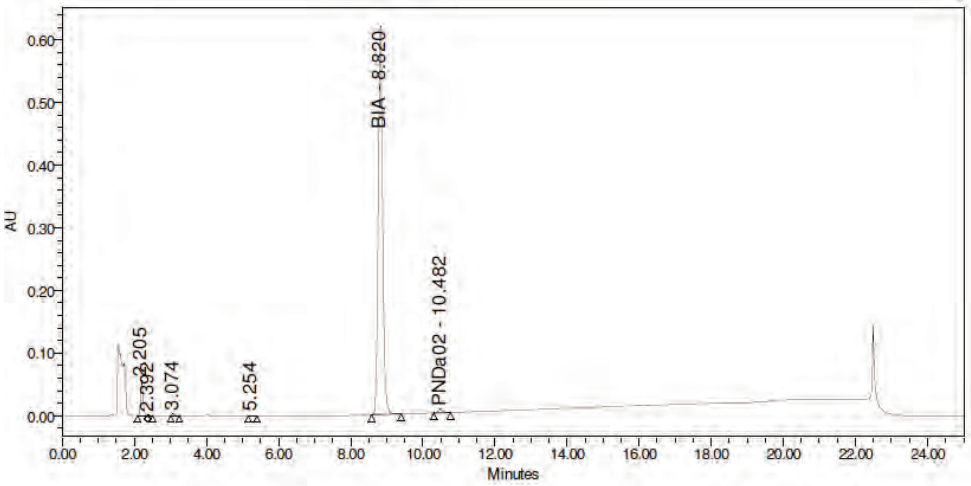
MS of impurity BIA



HNMR of impurity BIA



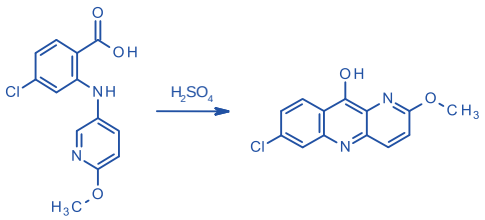
HPLC chromatogram of impurity BIA



	Name	RT	RRT	Area	% Area	Resolution
1		2.205	0.25	255373	4.56	
2		2.392	0.27	2310	0.04	
3		3.074	0.35	14022	0.25	
4	PNDa01	3.300				
5		5.254	0.60	3318	0.06	
6	BIA	8.820	1.00	5274321	94.16	
7	PNDa02	10.482	1.19	52025	0.93	

5.6.3. TGF-001 impurity 1

5.6.3.1. Reaction scheme

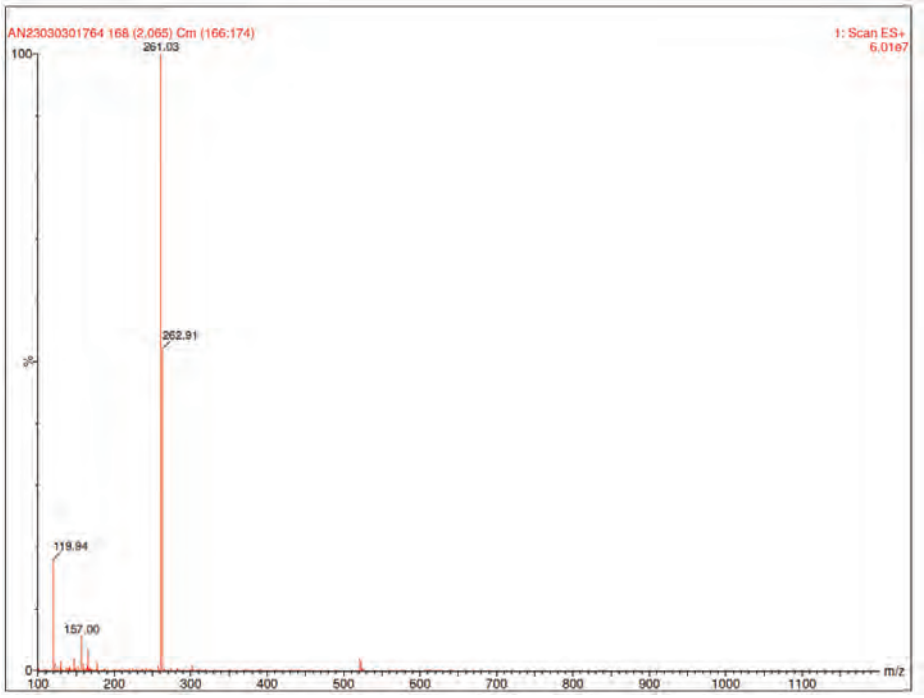


PNDa01 TGF-001 impurity 1

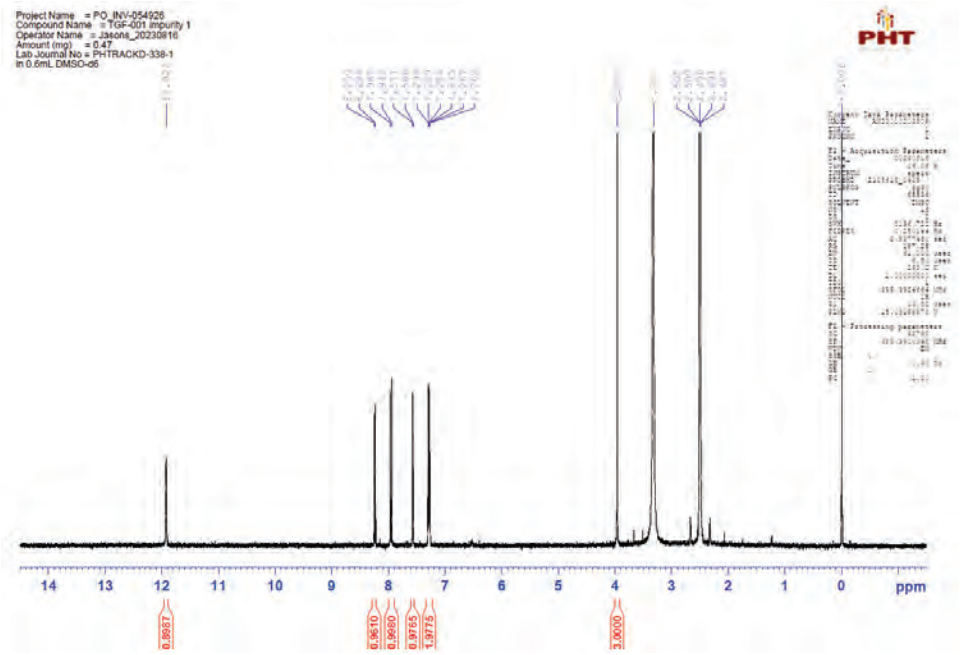
5.6.3.2. The procedure for the preparation of TGF-001 impurity 1 in experiment PHTRACKD-338

- The mixture of PNDa01 (5g, 13.56mmol) in Sulfuric acid (30g) was stirred at 100°C for 1.5h.
- HPLC (PNDa02-IM-PHTRACKD-338-IPC) showed 67.6% TGF-001 impurity 1 was formed.
- The mixture was then cooled to room temperature and then added into crushed ice.
- 4g crude was obtained after filtration and drying under vacuum at 50°C.
- Phase of the crude was isolated by pre-HPLC. 400mg TGF-001 impurity 1was gotten.

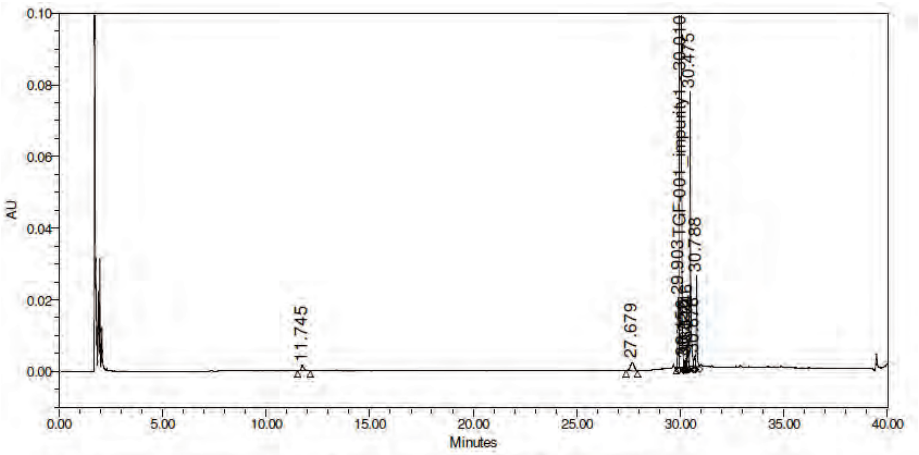
MS of TGF-001 impurity 1



HNMR of TGF-001 impurity 1



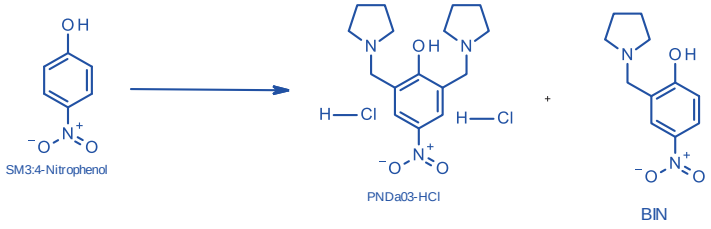
HPLC chromatogram of TGF-001 impurity 1



	Name	RT	RRT	Area	% Area	Resolution
1		11.745	0.39	12961	0.15	
2		27.679	0.92	27834	0.31	
3		29.903	1.00	63057	0.71	
4	TGF-001_impurity1	30.010	1.00	8449682	94.68	
5		30.156	1.00	4242	0.05	
6		30.222	1.01	6657	0.07	
7		30.310	1.01	6140	0.07	
8		30.346	1.01	21066	0.24	
9		30.475	1.02	245318	2.75	

5.6.4. Impurity BIN

5.6.4.1. Reaction scheme



5.6.4.2. The procedure for the preparation of BIN in experiment PHTRACKD-319

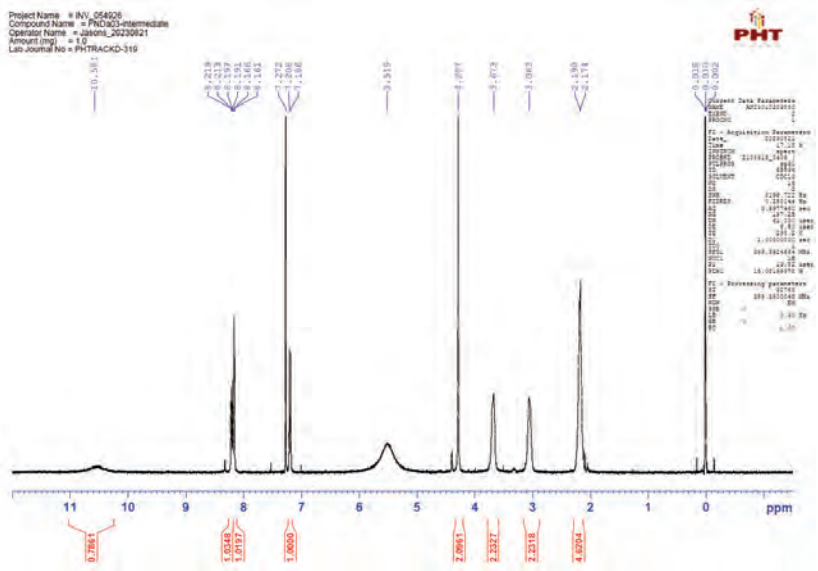
- Charge 4-Hydroxynitrobenzene (2g, 14.23mmol) , Paraformaldehyde (1.81g, 56.93mmol) and IPA (8 mL) into 100mL three-neck round-bottom flask.
- Then Pyrrolidine (4.09g, 56.93mmol) was added dropwise for 0.5h at 10~15 °C.
- Then the reaction temperature was raised to 50℃ and stirred for 0.5h.
- HPLC(PNDa03-PHTRACKD-319-50) showed that 17.7% of PNDa03 was formed and 31.7% raw material was left.
- And the possible intermediate (BIN) was 50.6%.
- The solvent was evaporated to dryness under reduced pressure to give 5.5g crude as an

orange oil.

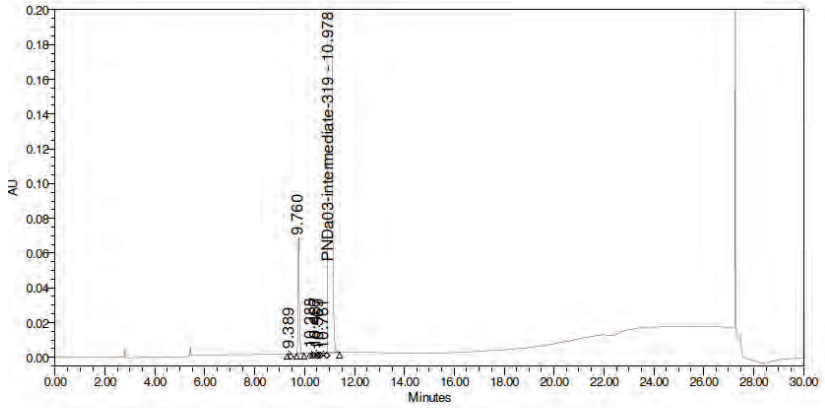
o Transfer the oil to AD to isolate the possible intermediate by pre-HPLC.

MS of impurity BIN

HNMR of impurity BIN



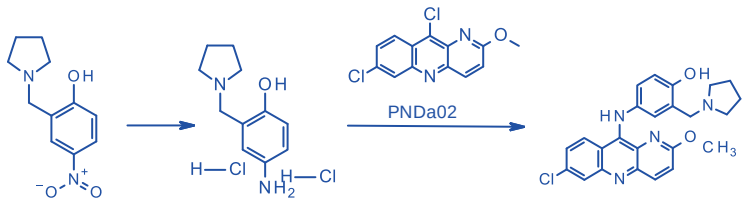
HPLC chromatogram of impurity BIN



	Name	RT	RRT	Area	% Area	Resolution
1		9.389	0.86	6563	0.14	
2		9.760	0.89	246393	5.31	
3		10.288	0.94	2930	0.06	
4		10.467	0.95	5665	0.12	
5		10.569	0.96	2315	0.05	
6		10.761	0.98	8407	0.18	
7	PNDa03-intermediate-319	10.978	1.00	4368080	94.13	

5.6.5. Impurity DIN

5.6.5.1. Reaction scheme

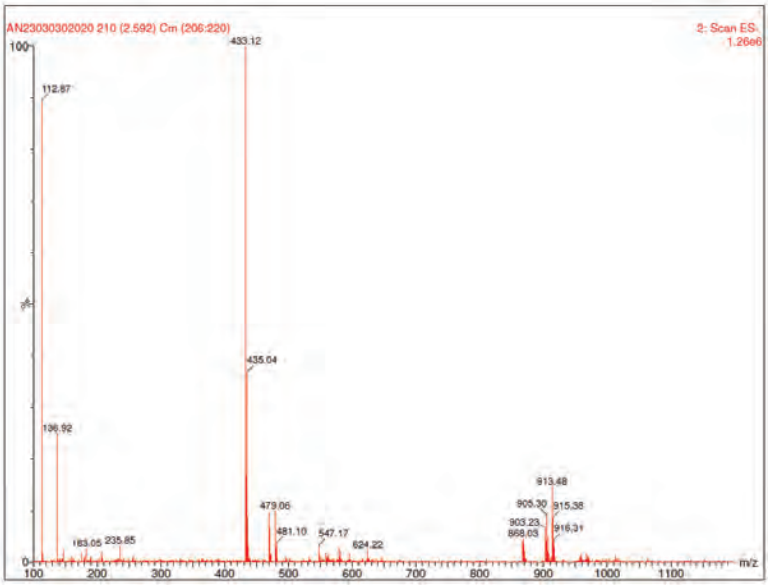


5.6.5.2. The procedure for the preparation of DIN in experiment PHTRACKD-388, PHTRACKD-389

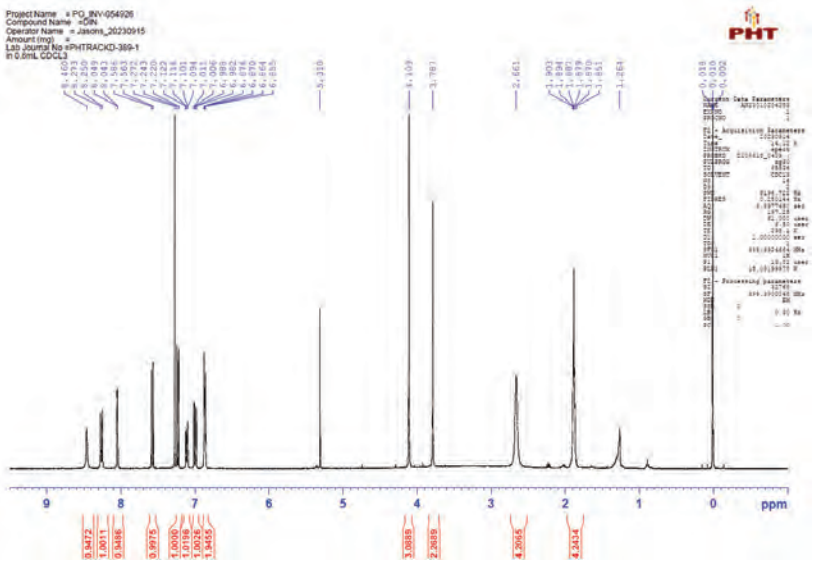
- Charge BIN (500mg, 1.92mmol) and hydrogen chloride (189mg, 1.92mmol) into a 40 mL tube.
- Charge Methanol (2.5mL) into the tube.
- Charge Pd/C (30mg, 0.03mmol) into the tube.
- The light-green solution was stirred for 16h under H_2 atmosphere (4bar pressure) at 30°C.
- HPLC(PNDa04-IM-PHTRACKD-388-IPC) showed that 96.98% of PND-a04-DIN was formed.
- Removed the catalyst by filtration with celite. The filtrate was used for the next step directly.
- Charge PNDa02 (461mg) into the filtrate of PHTRACKD-388.

- The suspension was then stirred at 50°C under N_2 atmosphere for 2h.
- HPLC（DIN-PHTRACKD-389-IPC）showed PNDa04 was consumed completely and 93.97% DIN was formed.
- Removed the solvent by concentration under vacuum at 50°C.
- The residue was dissolved in 20mL water, adjusting the pH of the solution to 12 with 15% NaOH aqueous. Lot solid was precipitated.
- The suspension was stirred at 25°C for 1h. Collected the solid by filtration, washed the cake with 20ml water.
- The solid was dried under vacuum at 50°C to DIN (680mg,1.5122mmol, 98.287% yield).

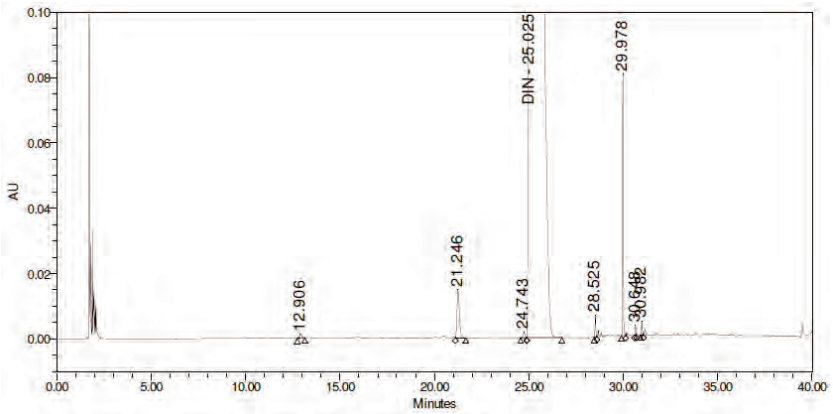
MS of impurity DIN



HNMR of impurity DIN



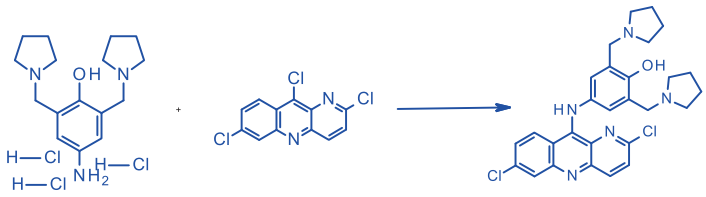
HPLC chromatogram of impurity DIN



	Name	RT	RRT	Area	% Area	Resolution
1		12.906	0.52	8576	0.05	
2		21.246	0.85	129864	0.78	
3		24.743	0.99	12599	0.08	
4	DIN	25.025	1.00	16172261	97.53	
5		28.525	1.14	17501	0.11	
6		29.978	1.20	217969	1.31	
7		30.648	1.22	8887	0.05	
8		30.982	1.24	14187	0.09	

5.6.6. DIA

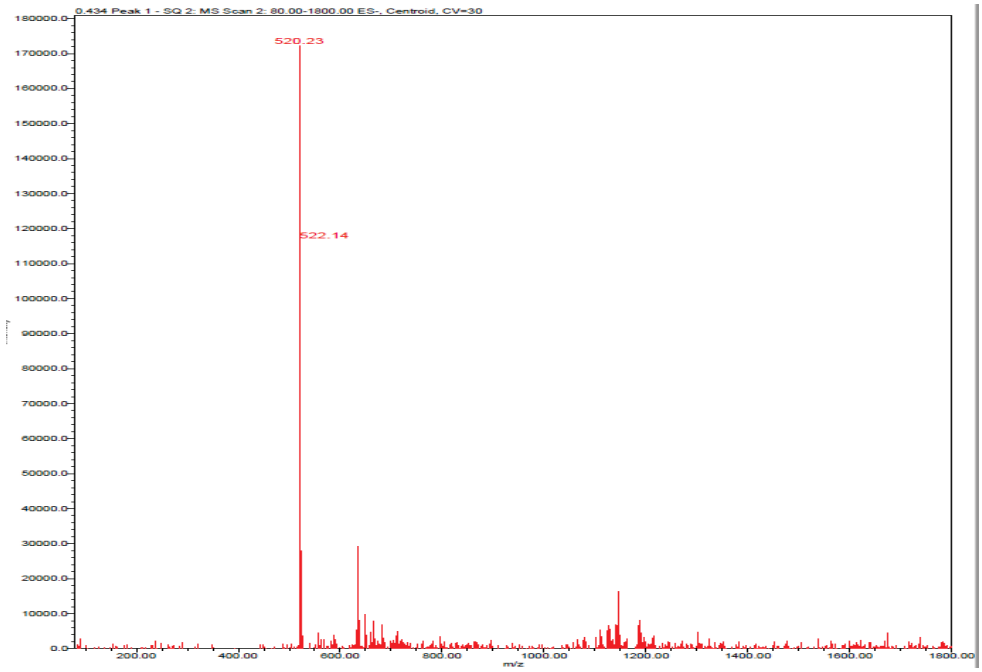
5.6.6.1. Reaction scheme



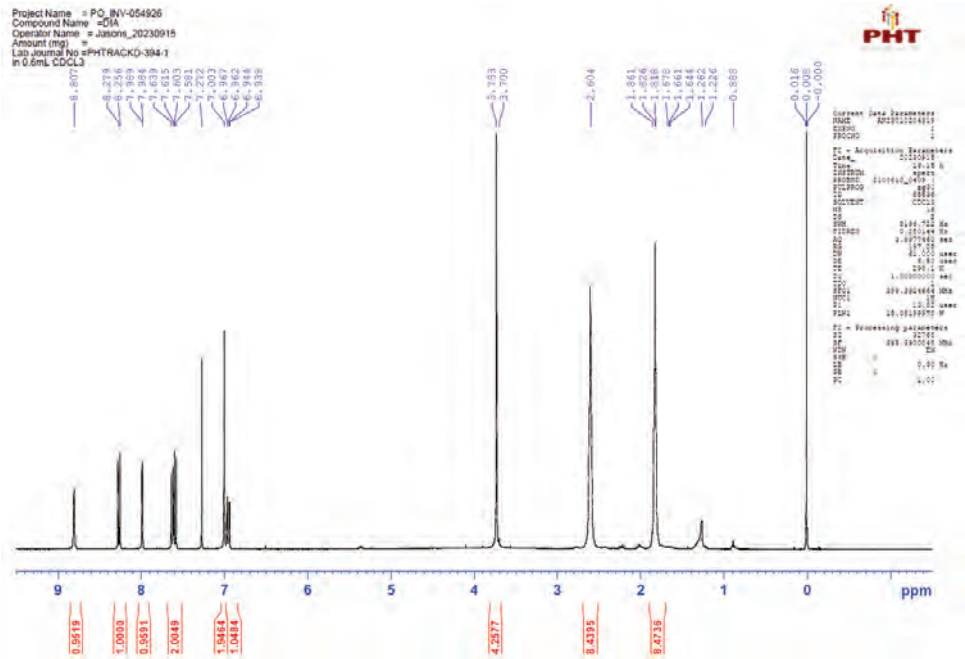
5.6.6.2. The procedure for the preparation of DIA in experiment PHTRACKD-394,

- Charge BIA (303mg, 1.03mmol) into the filtrate of PHTRACKD-392(498mg PNDa04-HCl in 2.5mL EtOH).
- The suspension was then stirred at 50°C under N_2 atmosphere for 2h.
- HPLC（DIA-PHTRACKD-394-IPC）showed 89.96% DIA was formed.
- The solvent was evaporated (50°C) to dryness under reduced pressure to give brown solid.
- The solid was dissolved in 5ml water, adjusted the pH to 12 with 15 NaOH(0.2mL).
- Lots of solids precipitated out from the solvent, then continued stirring for 1 hour at 25°C.
- Collect the solid by filtration, washed with 5mL water. The solid was dried under vacuum at 50°C furnish DIA (500mg,0.8458mmol, 81.685% yield).

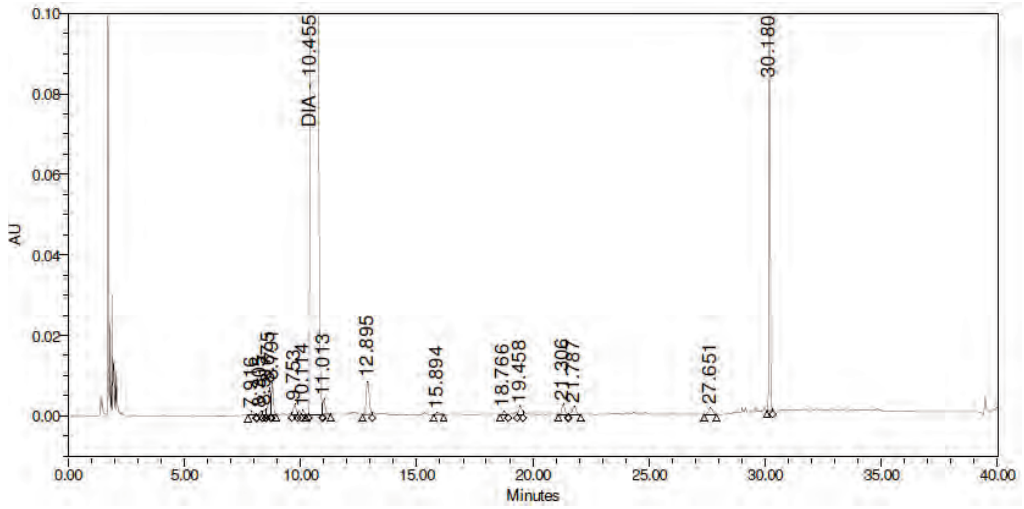
MS of impurity DIA



HNMR of impurity DIA



HPLC chromatogram of impurity DIA



	Name	RT	RRT	Area	% Area	Resolution
1		7.916	0.76	6897	0.06	
2		8.305	0.79	7772	0.06	
3		8.517	0.81	9674	0.08	
4		8.675	0.83	48260	0.40	
5		8.791	0.84	43003	0.36	
6		9.753	0.93	19345	0.16	
7		10.114	0.97	7097	0.06	
8	DIA	10.455	1.00	10764672	89.26	
9		11.013	1.05	25669	0.21	

	Name	RT	RRT	Area	% Area	Resolution
10		12.895	1.23	76925	0.64	
11		15.894	1.52	6039	0.05	
12		18.766	1.79	6507	0.05	
13		19.458	1.86	17868	0.15	
14		21.306	2.04	23444	0.19	
15		21.787	2.08	24095	0.20	
16		27.651	2.64	20290	0.17	
17		30.180	2.89	952802	7.90	

6. Results of DSC and RC1

6.1. Results of DSC

Table 57. Sample information

Sample Name	Sample No.	State of sample	Sample receiving date
PNDa01	231116-01-01	Grey powder	2023.11.24
PNDa02	231116-01-02	White solid	2023.11.24
PNDa06-HCl	231116-01-03	White powder	2023.11.24
TGF-001	231116-01-04	Yellow powder	2023.11.24

Table 58. Test equipment information

Name of equipment	Equipment No.	Calibration expiration date
METTLER DSC 3	BS-02	2025-04-23
METTLER XS205	BS-06	2024-04-23
Test method	30°C~450°C@5K/min	

Table 59. Results of test

Sample	Test method	Temperature (℃)			ΔH (J/g)	Comments
		Onset	Peak	End		
PNDa01	30°C~450°C @5K/min	188.2	194.7	209.9	-85.64	Endotherm
		337.4	358.2	380.4	157.58	Exotherm
PNDa02	30°C~450°C @5K/min	189.5	190.6	207.6	-113.11	Endotherm
		283.5	307.2	329.1	277.65	Exotherm
PNDa06-HCl	30°C~450°C @5K/min	194.4	203.1	218.2	-44.92	Endotherm
		221.1	240.8	259.7	91.03	Exotherm
		341.7	353.3	383.9	64.61	Exotherm
TGF-001	30°C~450°C @5K/min	222.1	226.9	231.4	-28.63	Endotherm
		231.2	242.6	247.9	27.17	Exotherm

Test Standard: GB/T 22232-2008 Test method for the thermal stability of chemicals by differential scanning calorimetry.

Location of testing: 4F, Building 1, No. 778, Huaxi Avenue, Jiancheng Street, Changxing County, Huzhou City, Zhejiang Province.

- During the dynamic scanning, PNDa01 sample shows an Endothermic phenomenon. The Endothermic peak starts from 188.2°C with -85.64J/g heat, which may refer to melting of products. PNDa01 sample also shows an exothermic phenomenon. The exothermic peak starts from 337.4°C with 157.58 J/g heat, The exotherm may be related to material decomposition but further tests such as ARC should be carried out to confirm the result.
- During the dynamic scanning, PNDa02 sample shows an Endothermic phenomenon. The Endothermic peak starts from 189.5°C with -113.11J/g heat, which may refer to melting of products. PNDa02 sample also shows an exothermic phenomenon. The exothermic peak starts from 283.5°C with 277.65 J/g heat, The exotherm may related to material

decomposition but further tests such as ARC should be carried out to confirm the result.

- During the dynamic scanning, PNDa06-HCl sample shows an Endothermic phenomenon. The Endothermic peak starts from 194.4°C with -44,92J/g heat. There is also an exothermic peak coupled with the endothermic peak. The exothermic peak starts from 221.1°C with 91.03 J/g heat. PNDa06-HCl sample also shows an exothermic phenomenon. The exothermic peak starts from 341.7°C with 64.61 J/g heat, which may refer to the main product decomposition behavior. The exotherm may be related to material decomposition but further tests such as ARC should be carried out to confirm the result.
- During the dynamic scanning, TGF-001 sample shows an Endothermic phenomenon. The Endothermic peak starts from 222.1°C with -37.08J/g heat. There is also an exothermic peak coupled with the endothermic peak. The exothermic peak starts from 231.2°C with 27.17 J/g heat.
- As DSC is just used as screening device, the accurate decomposition behavior should be further studied in the adiabatic calorimeter (ARC). For this test, a more precise decomposition behavior can be delivered with pressure behavior during the decomposition.

DSC Test Curve

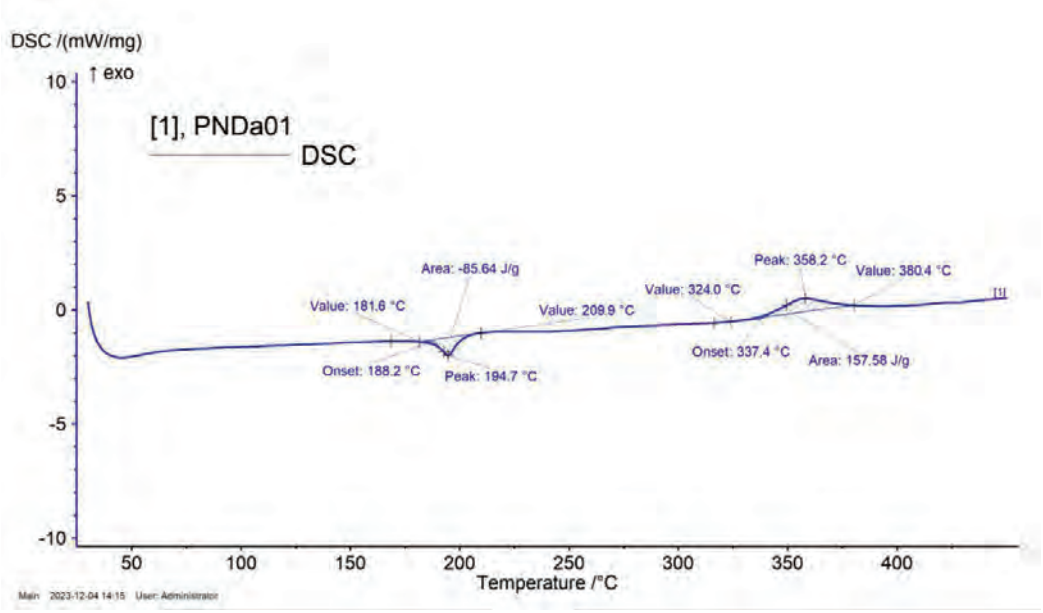


Figure 1: PNDa01 DSC Test Curve

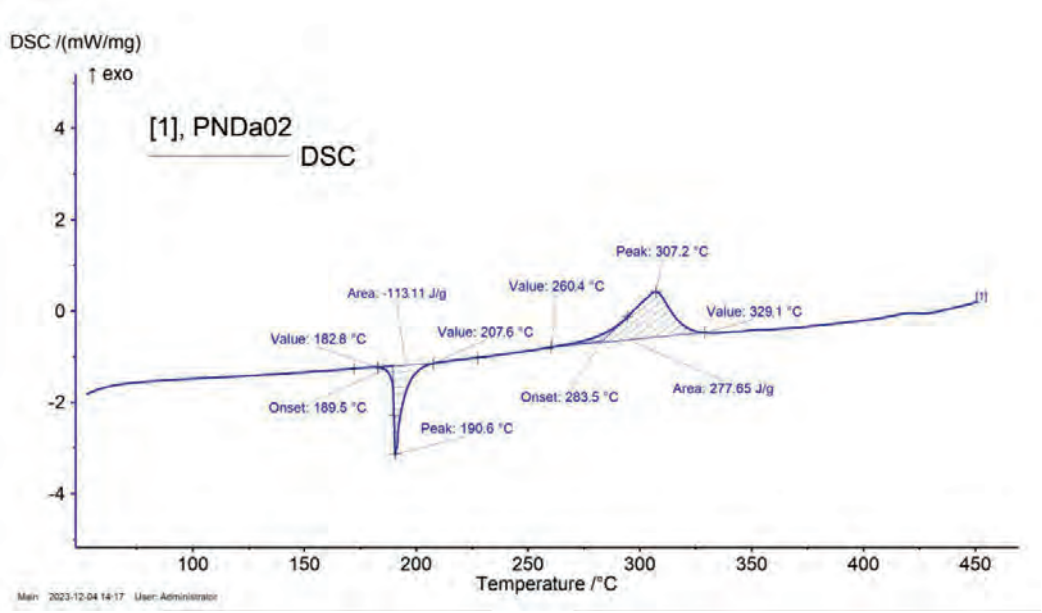


Figure 2: PNDa02 DSC Test Curve

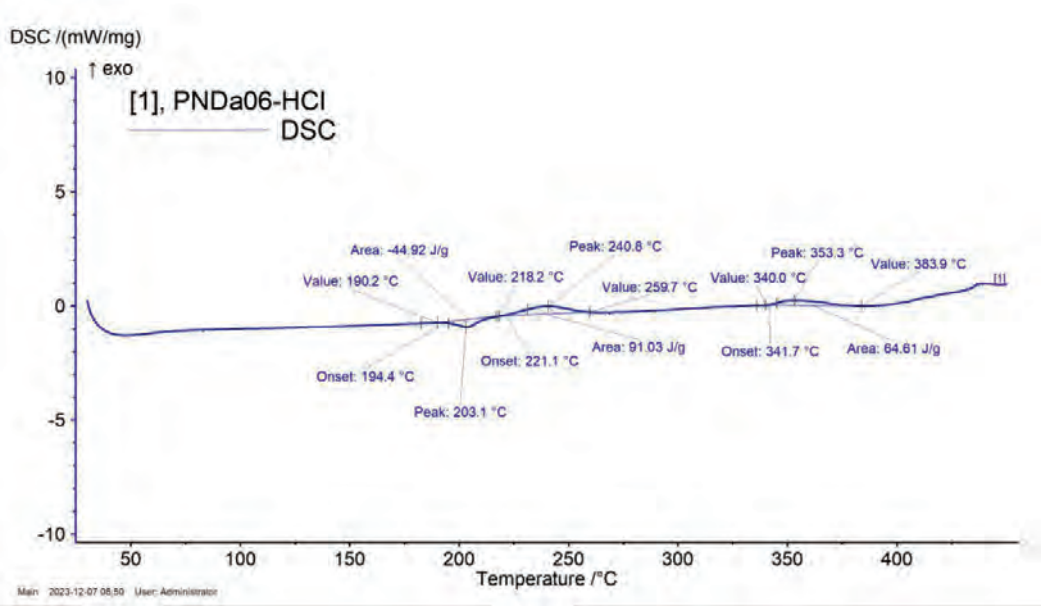


Figure 3: PNDa06-HCl DSC Test Curve

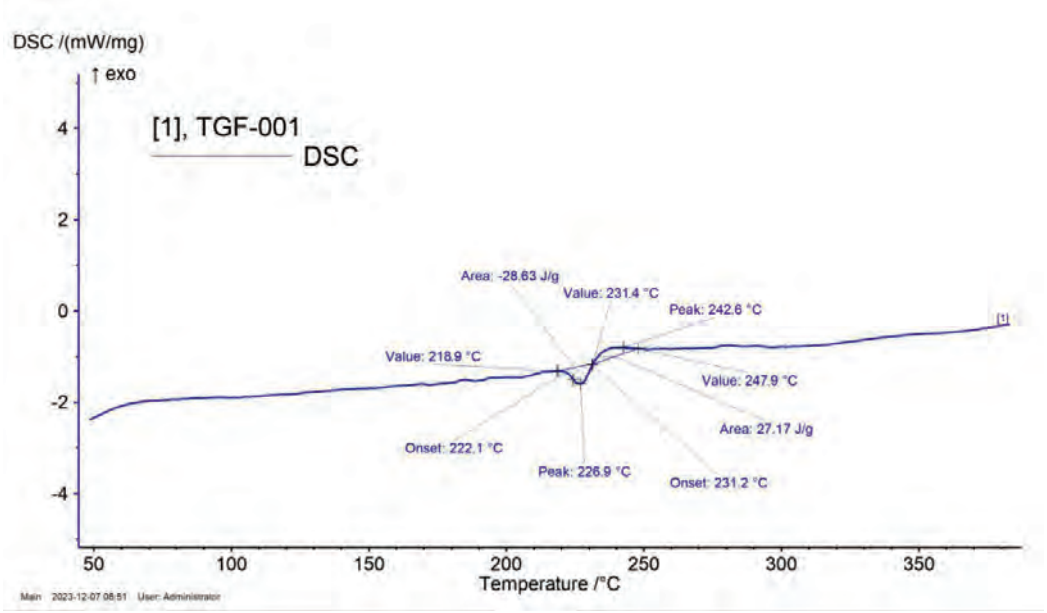


Figure 4: TGF-001 DSC Test Curve

6.2. Results of RC1 Calorimetry

6.2.1. PNDa02 step

Table 60. Sample information

Sample Name	Sample No.	State of sample	Sample receiving date
PNDa01	231116-01-01	Grey powder	2023.11.24
Propylene carbonate	231116-01-05	Colorless liquid	2023.11.24
$POCl_3$	231116-01-06	Colorless liquid	2023.11.24
DIPEA	231116-01-07	Light yellow liquid	2023.11.24

Table 61. Test method

Name of equipment	Equipment No.	Calibration expiration date
METTLER RC1e	BS-23	2024-11-23
METTLER XSR4002S	BS-08	2024-04-23
Test method	**First step reaction** 1. Charge PNDa01 (83.3g) and Propylene carbonate (300.0g) into a 1000mL Reactor. Start the stirrer at a speed of 300 rpm. Heat up the temperature to 25ºC. 2. Start the first calibration. 3. $POCl_3$ (163.4g,) was then added dropwise into the mixture.	

	4. The mixture was stirred at 50°C for 1h under N_2 atmosphere. 5. Start the second calibration. 6. Stop the reaction and discharge the final product. **Second step reaction** 1. Charge DIPEA (104.2g) and Propylene carbonate (275.0g) into another 1000mL Reactor. Start the stirrer at a speed of 300 rpm. Raise the temperature to 80ºC under N_2 atmosphere. 2.Start the first calibration. 3.The prepared acyl chloride (376.6g,) was then added dropwise into the mixture. 4.After dropping, the dropping funnel was washed with Propylene carbonate (7.0g). 5.The mixture was then reacted at 100°C for 1h. 6.The mixture was then cooled to 5°C with an ice bath. 7.Stop the reaction and discharge the final product.

- **First step reaction**

Table 62. Mass balance

In(g)		Out(g)	
PNDa01	83.3	Total out	544.5
Propylene carbonate	300.0		
$POCl_3$	163.4		
Total in	546.7		

Table 63. Specific heat and Exchange coefficients

Cp ($kJ \cdot kg^{-1} \cdot K^{-1}$)	Cp ($kJ \cdot kg^{-1} \cdot K^{-1}$)	U($W \cdot m^{-2} \cdot K^{-1}$)	U($W \cdot m^{-2} \cdot K^{-1}$)
Cp1	Cp2	U1	U2
1.5	1.2	66.6	138.7
RC1,22°C	RC1,50°C	RC1,22°C	RC1,50°C

Heat of reaction

The enthalpies of reaction were calculated using the formula:

Qr = Qflow + Qdos + Qaccu + Qloss

With Qflow: heating exchanged on the glass walls.

Qdos: heating supplied by the additions

Qaccu: heating accumulated by the medium and the immerged inserts (sensor, mixer…)

Qoss: heat lost by the cover of the reactor

The total enthalpy was:

Qr = 14.24 kJ for 163.4 g of POCl3.

The adiabatic temperature rise is then:

$$\Delta Tad = \frac{Qr}{m_{total} \times Cp} = \frac{14.24 \times 1000}{546.7 \times (1.5+1.2) \div 2} = 19.3K$$

Heat flux follow-up

The heat flux information is given in Figure 1. As can be seen, heat started releasing with the addition of $POCl_3$. The heat release speed is most fast at the beginning of $POCl_3$ addition and decrease with the reaction progress. During the heating process first appeared a heat

absorption, This may be caused by material dissolution. And then there's a heat release, when the heating ends, the heat release basically disappeared.

- **Second step reaction**

Table 64. Mass balance

In(g)		Out(g)	
DIPEA	104.2	Total out	760.3
Propylene carbonate	282.0		
The prepared acyl chloride	376.6		
Total in	762.8		

Table 65. Specific heat and Exchange coefficients

Cp (kJ·kg^{-1}·K^{-1})	Cp (kJ·kg^{-1}·K^{-1})	U(W·m^{-2}·K^{-1})	U(W·m^{-2}·K^{-1})
Cp1	Cp2	U1	U2
1.9	-	83.0	-
RC1,80°C	-	RC1,80°C	-

Heat of reaction

The enthalpies of reaction were calculated using the formula:

Qr = Qflow + Qdos + Qaccu + Qloss

With Qflow : heating exchanged on the glass walls

Qdos : heating supplied by the additions

Qaccu : heating accumulated by the medium and the immerged inserts (sensor, mixer…)

Qloss : heat lost by the cover of the reactor

The total enthalpy was:

Qr = 92.83 kJ for 376.6 g of the prepared acyl chloride.

The adiabatic temperature rise is then:

$$\Delta Tad = \frac{Qr}{m_{total} \times Cp} = \frac{92.83 \times 1000}{762.8 \times 1.9} = 64.1K$$

Heat flux follow-up

The heat flux information is given in Figure 2. As can be seen, heat started releasing with the addition of the prepared acyl chloride. The heat release speed is fastest at the beginning of the prepared acyl chloride addition and decreases with the reaction progress. At the end of the prepared acyl chloride addition, the heat release basically disappeared.

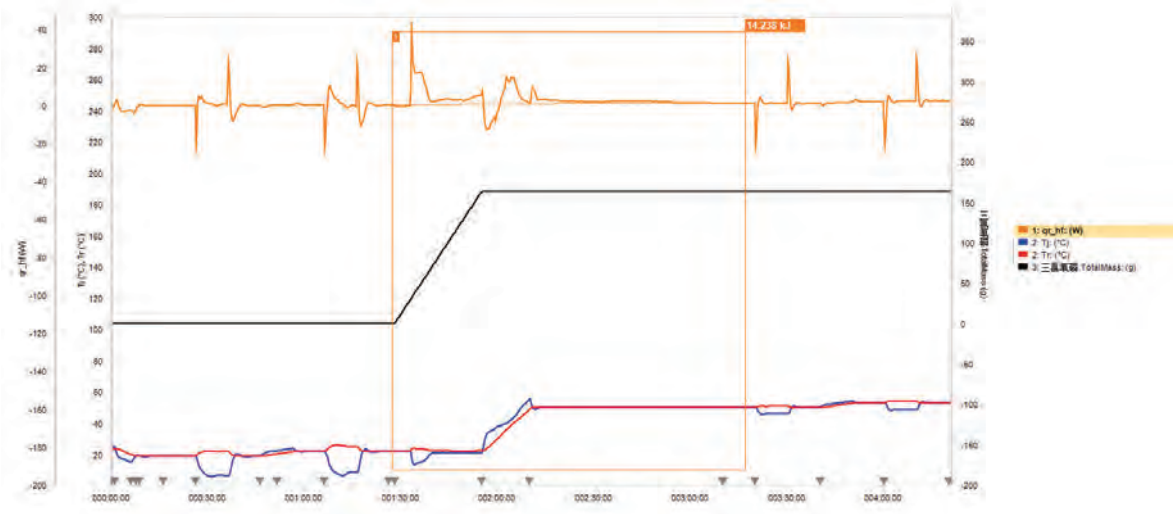


Figure 5:First step reaction RC1 Test Curve

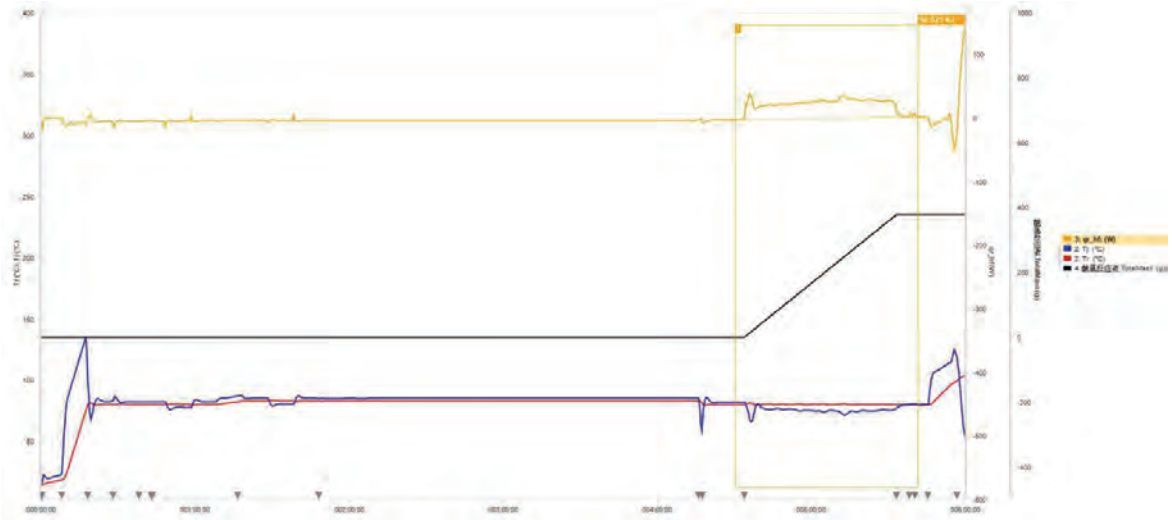


Figure 6:Second step reaction RC1 Test Curve

6.2.2. PNDa06 step

Table 66. Sample information

Sample Name	Sample No.	State of sample	Sample receiving date
SM5	231116-01-08	White solid	2023.11.24
Paraformaldehyde	231116-01-09	White powder	2023.11.24
Ethyl alcohol	231116-01-10	Colorless liquid	2023.11.24
Pyrrolidine	231116-01-11	Light yellow liquid	2023.11.24

Table 67. Test method

Name of equipment	Equipment No.	Calibration expiration date
METTLER RC1e	BS-23	2024-11-23
METTLER XSR4002S	BS-08	2024-04-23
Test method	1. Charge SM5(75.0g)、Paraformaldehyde (38.8g) and Ethanol (296.0g) into a 1000mL Reactor. Start the stirrer at a speed of 300 rpm. Heat up the temperature to 30ºC under N_2 atmosphere. 2. Start the first calibration. 3. Pyrrolidine (86.5g,) was added dropwise for 0.5h at 15 °C. 4. The reaction was raised to 70°C and stirred for 16h under. 5. Start the second calibration. 6. Stop the reaction and discharge the final product.	

Table 68. Mass balance

In(g)		Out(g)	
SM5	75.0	Total out	493.2
Paraformaldehyde	38.8		
Ethanol	296.0		
Pyrrolidine	86.5		
Total in	496.3		

Table 69. Specific heat and Exchange coefficients

Cp (kJ·kg^{-1}·K^{-1})	Cp (kJ·kg^{-1}·K^{-1})	U(W·m^{-2}·K^{-1})	U(W·m^{-2}·K^{-1})
Cp1	Cp2	U1	U2
2.6	2.7	190.3	199.5
RC1,30°C	RC1,70°C	RC1,30°C	RC1,70°C

Heat of reaction

The enthalpies of reaction were calculated using the formula:

Qr = Qflow + Qdos + Qaccu + Qloss

With Q flow : heating exchanged on the glass walls

Q dos : heating supplied by the additions

Q accu : heating accumulated by the medium and the immerged inserts (sensor, mixer...)

Q loss : heat lost by the cover of the reactor

The total enthalpy was:

Qr = 105.19 kJ for 86.5 g of Pyrrolidine.

The adiabatic temperature rise is then:

$$\Delta T\text{ad} = \frac{Q\text{r}}{m_{total} \times Cp} = \frac{105.19 \times 1000}{496.3 \times (2.6 + 2.7) \div 2} = 80.0K$$

Heat flux follow-up

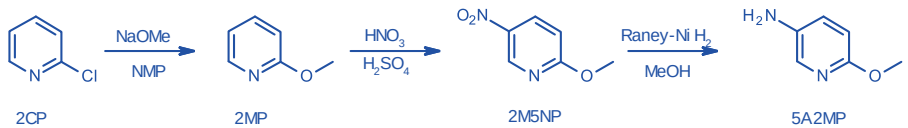
The heat flux information is given in Figure 1. As can be seen, heat started releasing with the addition of Pyrrolidine. The heat release speed is most fast at the beginning of Pyrrolidine

addition and decrease with the reaction progress. During the heating process there's a heat release, when the heating ends, the heat release basically disappears.

Figure 7: RC1 Test Curve

7. Process development for 5A2MP

7.1. Synthetic scheme of 5A2MP



Exact Mass: 113
Molecular Formula: C_5H_4ClN

Exact Mass: 109.05
Molecular Formula: C_6H_7NO

Exact Mass: 154.04
Molecular Formula: $C_6H_6N_2O_3$

Exact Mass: 124.06
Molecular Formula: $C_6H_8N_2O$

7.2. Progress development of 5A2MP

7.2.1. 2MP step

7.2.1.1. Reaction scheme

Exact Mass: 113
Molecular Formula: C_5H_4ClN

Exact Mass: 109.05
Molecular Formula: C_6H_7NO

7.2.1.2. Process and results of 2MP

- The yield of 2MP step could be up to 86%(99.4%area).
- MeONa/MeOH was the best system for this reaction, but high pressure was needed.
- MeONa/NMP system was the final system for this reaction, but distillation condition of the product needs more investigation (30cm column filled with glass spring, fraction temperature:45℃, oil temperature:105℃, 20mmba).

Table 70. Result of MeOH system

No.	2CP	Base	MeOH	Reaction Temp.	IPC_M1	Remark
PHTFORDX-1052	1.0 eq.	MeONa (1.2 eq.)	10v/w	65°C (4h)	2CP: 99.7% 2MP: 0.2%	Ref: CN113979928A
PHTFORDX-1056	1.0 eq.	MeONa (1.2 eq.)	10v/w	130°C (5h) (0.6MPa)	2CP: 87.4% 2MP:12.6%	Ref: WO2007148093A1
PHTFORDX-1060	1.0 eq.	MeONa (1.2 eq.)	8v/w	130°C (24h) (0.6MPa)	2CP: 3.1% 2MP: 96.0%	
PHTFORDX-1061	1.0 eq.	MeONa (1.4 eq.)	8v/w	130°C (24h) (0.6MPa)	2CP: n. d. 2MP: 97.8%	
PHTFORDX-1062	1.0 eq.	MeONa (1.4 eq.)	8v/w	130°C (12h) (0.6MPa)	2CP: 1.8% 2MP: 96.9%	
PHTFORDX-1063	1.0 eq.	MeONa (1.4 eq.)	8v/w	65°C (12h)	2CP: 92.9% 2MP: 7.0%	
PHTFORDX-1066	1.0 eq.	MeONa (1.4 eq.)	10v/w	130°C (24h) (0.6MPa)	2CP: n.d. 2MP: 96.0%	
PHTFORDX-1067	1.0 eq.	MeONa (1.4 eq.)	6v/w	130°C (24h) (0.6MPa)	2CP: n. d. 2MP: 97.8%	

PHTFORDX-1070	1.0 eq.	MeONa (1.4 eq.)	8v/w	120°C (24h) (0.5MPa)	2CP: 0.4% 2MP: 98.6%	
PHTFORDX-1072	1.0 eq.	MeONa (1.4 eq.)	8v/w	120°C (12h) (0.5MPa)	2CP: 3.8% 2MP: 95.4%	
PHTFORDX-1068	1.0 eq.	MeONa (1.4 eq.)	8v/w	65°C (3h)	2CP: 96.4% 2MP:2.2% (3h)	Ref:CN113979928A Add MeOH at reflux temperature
PHTFORDX-1053	1.0 eq.	NaOH (1.2 eq.)	10v/w	65°C (3h)	2CP: 97.8% 2MP: 2.2%	Ref: CN106905229A
PHTFORDX-1073	1.0 eq.	MeONa (1.4 eq.)	8v/w	110°C (24h) (0.29MPa)	2CP: 3.6% 2MP: 96.4%	
PHTFORDX-1076	1.0 eq.	MeONa (1.5 eq.)	6v/w	130°C (12h) (0.6MPa)	2CP: 0.3% 2MP: 96.9%	
PHTFORDX-1077	1.0 eq.	MeONa (1.5 eq.)	6v/w	120°C (12h) (0.4MPa)	2CP: 3.1% 2MP:96.0%	
PHTFORDX-1079	1.0 eq.	NaOH (1.5 eq.)	6v/w	130°C (12h) (0.6MPa)	2CP: n.d. 2MP: 96.8%	
PHTFORDX-1080	1.0 eq.	NaOH (1.5 eq.)	6v/w	110°C (12h) (0.6MPa)	2CP: 3.95% 2MP: 93.2%	
PHTFORDX-1091	1.0 eq.	NaOH (1.5 eq.)	6v/w	120°C (12h) (0.4MPa)	2CP: 0.58% 2MP: 97.84%	

NaOH/MeOH or MeONa/MeOH system can give a good result (IPC), but high pressure was needed.

Table 71. Result of solvent screening

No.	2CP	Base	Sovent	Reaction Temp.	IPC_M1	Remark
PHTFORDX-1087	1.0 eq.	MeONa (1.4 eq.)	CH3CN(6v/w)	82°C (12h)	2CP: 23.2% 2MP: 46.9%	Ref: CN113979928A
PHTFORDX-1088	1.0 eq.	NaOMe (1.4 eq.)	DMF 6v/w	120°C (12h)	2CP: n.d. 2MP: 95.43%	Ref: WO2007148093A1
PHTFORDX-1089	1.0 eq.	NaOMe (1.4 eq.)	NMP 6v/w	120°C (12h)	2CP: 0.08% 2MP: 97.67%	
PHTFORDX-1090	1.0 eq.	NaOMe (1.4 eq.)	DMSO 6v/w	120°C (12h)	2CP: n. d. 2MP: 91.12%	
PHTRACKD-597	1.0 eq.	NaOMe (1.4 eq.)	DMF(6v/w)	120°C (12h)	2CP: n.d. 2MP: 93.96%	The reaction solution was distilled directly, 2MP and DMF were co-distilled (52℃, 20mmba)
PHTRACKD-598	1.0 eq.	NaOMe (1.4 eq.)	DMF(6v/w)	120°C (12h)	2CP: n.d. 2MP: 92.44%	The reaction solution was distilled after filtration (52℃, 20mmba)
PHTRACKD-603	1.0 eq.	NaOMe (1.4 eq.)	NMP(7v/w)	120°C (12h)	2CP: n. d. 2MP: 98.58%	The reaction solution was distilled directly (58℃, 20mmba) HNMR showed there was 20% NMP in 2MP after distillation.
PHTRACKD-604	1.0 eq.	NaOMe (1.4 eq.)	NMP(6v/w) PhMe(1v/w)	120°C (12h)	2CP: n. d. 2MP: 99.31%	Diluted the reaction solution with EtOAc, washed NMP with brine. Much 2MP went into water too.

The reaction worked well in NMF or NMP(IPC). But it is hard to distill 2MP from DMF.

Table 72. Result of NMP system

No.	2CP	Base	Solvent	Reaction Temp.	IPC_M1, %area	Distillation
PHTRACKD-609	1.0 eq.	NaOMe (1.4 eq.)	NMP (7v/w)	80°C	2CP: 17%, 2MP: 82.3% (2h) 2CP: 9.1%, 2MP: 90.5% (4h) 2CP: 2.5%, 2MP: 96.8% (8h)	N/A
PHTRACKD-607	1.0 eq.	NaOMe (1.4 eq.)	NMP (7v/w)	80°C (12h)	2CP: n. d. 2MP: 98.9%	N/A
PHTRACKD-610	1.0 eq.	NaOMe (1.4 eq.)	NMP (7v/w)	100°C	2CP: 0.9%, 2MP: 98.4% (2h) 2CP: n.d., 2MP: 99.2% (4h)	N/A
PHTRACKD-606	1.0 eq.	NaOMe (1.4 eq.)	NMP (7v/w)	100°C (12h)	2CP: n. d. 2MP: 98.9%	N/A
PHTRACKD-605	1.0 eq.	NaOMe (1.4 eq.)	NMP (7v/w)	120°C (12h)	2CP: n. d. 2MP: 95.7%	The reaction solution was distilled directly (20cm Vigreux colum,55℃, 20mmba). 1HNMR showed there was ~20% NMP in 2MP after distillation.
PHTRACKD-608	1.0 eq.	NaOMe (1.4 eq.)	NMP (7v/w)	120°C (12h)	2CP: n. d. 2MP: 98.9%	The reaction solution was distilled directly (30cm column filled with glass spring, fraction temperature:45℃, oil temperature:105℃, 20mmba). There was no NMP remaining in 2MP.
PHTRACKD-611	1.0 eq.	NaOMe (1.4 eq.)	NMP (7v/w)	100°C (4h)	2CP: n. d. 2MP: 98.6%	The reaction solution was distilled directly (30cm column filled with glass spring, fraction temperature:45℃, oil temperature:105℃, 20mmba). There was no NMP remaining in 2MP.
PHTRACKD-620	1.0 eq.	NaOMe (1.2 eq.)	NMP (7v/w)	100°C (4h)	2CP: 9.9% 2MP: 89.2%	N/A
PHTRACKD-621	1.0 eq.	NaOMe (1.3 eq.)	NMP (7v/w)	100°C (4h)	2CP: 1.0% 2MP: 98.3%	N/A

2MP can be purified by distillation with 30cm column filled with glass spring.

The reaction temperature can be reduced to 100℃ (reaction time: 4 hours).

The equivalent of NaOMe cannot be reduced (1.4 eq. is recommended).

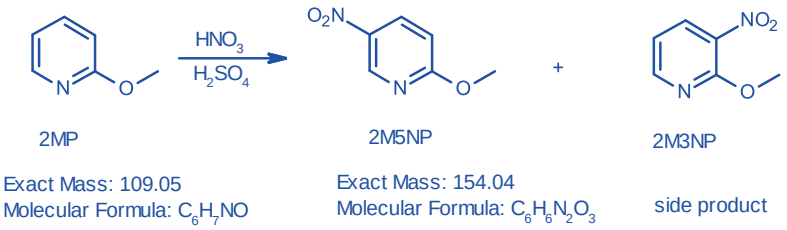
7.2.1.3. Typical procedure for preparation of 2MP in experiment PHTRACKD-627

- Charge MeONa (66.6g, 1221mmol,1.4eq) to the solution of 2-Chloropyridine (100.0g, 871.9mmol,1.0eq) in NMP (700mL).
- Then the reaction temperature was raised to 100°C and stirred for 4h under N_2 atmosphere.
- HPLC showed that 0.19% of 2CP remained.
- The mixture was distilled with column filled with glass spring(30cm) directly (20mmba, oil temperature:105℃, fraction temperature: 45℃).

o 2-methoxypyridine (82g, 85.6% yield,99.4% purity) was gotten after distillation.

7.2.2. 2M5NP step

7.2.2.1. Reaction scheme



2MP

Exact Mass: 109.05
Molecular Formula: C_6H_7NO

2M5NP

Exact Mass: 154.04
Molecular Formula: $C_6H_6N_2O_3$

2M3NP

side product

7.2.2.2. Process and results of 2M5NP

o The yield of 2M5NP step using flow chemistry could achieve to 70%.

o The yield of 2M5NP step using batch condition could achieve to 80%. But it was potentially more dangerous.

o 2M3NP was purged after pH adjusting and re-slurry in water.

Table 73. Results of batch reaction

No.	2MP	HNO_3 (65%)	H_2SO_4 (98%)	Reaction Temp.	IPC_M1	Isolated 2M5N
PHTFORDX-1030	1.0 eq.	1.2 eq.	6v/w	75°C (3h)	2MP: 1.4% 2M5NP: 92.5% 2M3NP:5.5%	HPLC purity: 99.5% 2M3NP: n.d. Yield: 80.3% (based on purity)
PHTFORDX-1054	1.0 eq.	1.2 eq.	5v/w	75°C (3h)	2MP: 0.7% 2M5NP: 86.5% 2M3NP:9.8%	
PHTFORDX-1058	1.0 eq.	1.2 eq.	4v/w	75°C (12h)	2MP: 1.6% 2M5NP: 91.2% 2M3NP:5.0%	
PHTFORDX-1057	1.0 eq.	1.2 eq.	3v/w	75°C (12h)	2MP: 21.4% 2M5NP: 72.4% 2M3NP:5.1%	

Higher amount of H_2SO_4, higher reaction conversion (Low amount of H_2SO_4 needs more time to complete the reaction).

Side product 2M3NP could be removed after workup.

Table 74. Results of flow chemistry study (CORNING G1-2A, 5 glass FM)

No.	Flow rate		Temp.	Retention time	Molar ratio	HPLC_IPC_M1		
	Phase A 2MP in H2SO4	Phase B 65%HNO3 in H2SO4	°C	Second	A/B	2MP	2M3NP	2M5NP
1	19ml/min 32.8g/min	11mL/min 20.1g/min	60	82	1/1.2	88.9%	0.4%	10.4%
2	19ml/min 32.8g/min	11mL/min 20.1g/min	70	82	1/1.2	83.9%	0.7%	15.1%
3	19ml/min 32.8g/min	11mL/min 20.1g/min	80	82	1/1.2	77.0%	1.2%	21.5%
4	19ml/min 32.8g/min	11mL/min 20.1g/min	90	82	1/1.2	65.8%	2.1%	32.1%

5	19ml/min 32.8g/min	11mL/min 20.1g/min	100	82	1/1.2	51.8%	3.1%	44.8%
6	19ml/min 32.8g/min	11mL/min 20.1g/min	110	82	1/1.2	38.3%	4.4%	57.2%
No.	Phase A 2MP in H2SO4 (ml/min)	Phase B 95%HNO3 in H2SO4 (ml/min)	°C	Second	A/B	2MP	2M3NP	2M5NP
7	14ml/min 22.75g/min	15.4mL/min 28.4g/min	50	82	1/1.2	86.2%	0.5%	13.3%
8	14ml/min 22.75g/min	15.4mL/min 28.4g/min	60	82	1/1.2	84.5%	0.6%	14.7%
9	14ml/min 22.75g/min	15.4mL/min 28.4g/min	70	82	1/1.2	80.2%	0.9%	19.0%
10	14ml/min 22.75g/min	15.4mL/min 28.4g/min	80	82	1/1.2	72.3%	1.4%	26.3%
11	14ml/min 22.75g/min	15.4mL/min 28.4g/min	90	82	1/1.2	52.7%	2.9%	44.4%

Higher temperature, higher reaction conversion, but there was still much 2MP left.

Table 75. Results of flow chemistry study (CORNING G1-2A, 5 glass FM)

No.	Flow rate		Temp.	Retention time	Molar ratio	HPLC_IPC_M1		
	Phase A 2MP in H2SO4	Phase B 95%HNO3 in	°C	Second	2MP/HNO3	2MP	2M3NP	2M5NP
1	14ml/min 22.7g/min	15.4mL/min 28.2g/min	90	86	1/1.5	55.6%	2.7%	41.7%
2	14ml/min 22.7g/min	19.3mL/min 35.3g/min	90	76	1/1.2	66.2%	2.2%	31.7%
3	10ml/min 16.2g/min	11mL/min 20.1g/min	90	120	1/1.2	45.6%	3.4%	51.1%
4	7ml/min 11.4g/min	7.7mL/min 14.1g/min	90	171	1/1.2	50.5%	3.3%	46.2%
5	10ml/min 16.2g/min	11mL/min 20.1g/min	90	117	1/1.2	54.3%	2.9%	42.8%
6	10ml/min 16.2g/min	11mL/min 20.1g/min	100	117	1/1.2	50.1%	3.5%	46.4%
7	10ml/min 16.2g/min	11mL/min 20.1g/min	110	117	1/1.2	37.7%	4.5%	57.8%
8	14ml/min 22.7g/min	15.4mL/min 28.2g/min	110	84	1/1.2	24.8%	5.3%	69.9%
9	14ml/min 22.7g/min	15.4mL/min 28.2g/min	110	84	1/1.2	29.1%	5.3%	65.6%
10	14ml/min 22.7g/min	19.3mL/min 35.3g/min	110	74	1/1.5	30.6%	5.4%	64.0%
11	14ml/min 22.7g/min	25.7mL/min 47.0g/min	110	62	1/2.0	20.4%	5.8%	73.8%
12	14ml/min 22.7g/min	32.1mL/min 58.7g/min	110	53	1/2.5	20.6%	5.8%	73.7%
13	14ml/min 22.7g/min	26mL/min 47.7g/min	110	62	1/2.0	17.9%	6.3%	75.8%
14	14ml/min 22.7g/min	26mL/min 47.7g/min	120	62	1/2.0	13.1%	7.0%	79.9%
15	14ml/min 22.7g/min	26mL/min 47.7g/min	130	62	1/2.0	4.3%	8.4%	87.3%
16	14ml/min 22.7g/min	28.6mL/min 52.4g/min	130	58	1/2.2	5.1%	10.7%	84.3%
17	14ml/min 22.7g/min	32.5mL/min 59.5g/min	130	53	1/2.5	3.5%	8.0%	88.5%

At present, temperature is the main influencing factor of this nitration reaction.

DSC of 2MP, 2M5NP, 2M3NP had been tested. These 3 products are stable and no exotherm until 400℃. The nitration at 130℃ seems feasible with flow chemistry.

7.2.2.3. Typical procedure for preparation of 2M5NP in experiment PHTHARRYS-645

The reaction was conducted in flow chemistry. The conditions were as below:

Table 76 the preparation of Materials for 2M5NP

Item	*starting materials*	*density (g/mL)*	*wt%*
Flow A	300g 2MP in 1121.5g 98% H_2SO_4	1.592	20.68%
Flow B	357.63g fuming HNO_3 in 2267.92g 98% H_2SO_4	1.832	11.23%

Entry	Flow A (2MP in H2SO4)		Flow B (HNO3 in H2SO4)		Temp.	Molar ratio	n(FMs)	Rt.	Pressure	IPC %area on 254nm		
	g/min	mL/min	g/min	mL/min	℃	HNO3/2MP	pcs	Second	bar	2MP	2M3NP	2M5NP
1	22.29	14.00	42.69	23.30	130	1.80	5	66.0	5.8	5.0%	7.0%	84.6%

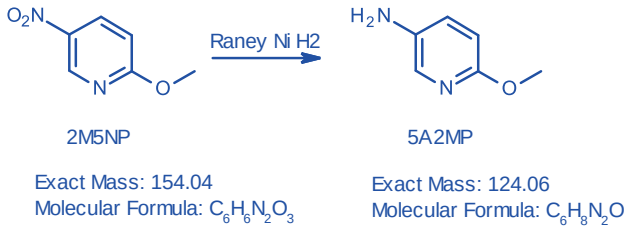
- Preheat the 2MP-H2SO4 solution to 80℃, inject it with pump 1#; the fuming HNO3-H2SO4 solution inject with pump 2#. Control the flow rate and reaction time.
  Note: Sampling in 5 minutes;15min;30min;45min; 60min.The results showed that the process is stable.
- The reaction solution was added into 15kg crushed ice to quench the reaction.
- 2.7kg NaOH was added into the mixture in batches to adjust the pH to 12 and control the temperature below 40℃.
- The mixture was then stirred at room temperature for 1h.
- 580g wet 2M5NP was obtained after filtration.
- The wet 2M5NP was then stirred at 3L H2O for 16h to remove the salts.
- 288.45g 2M5NP (HPLC:99.63%; Yield:69.2% by HPLC area) was obtained after filtration and drying under vacuum at 50℃.

7.2.2.4. Typical procedure for preparation of 2M5NP in experiment PHTHARRYS-603(batch procedure)

- 2MP (100.0g, 898mmol,1.4eq) was added dropwise to H2SO4 (500mL,5v/w) under 20°C.
- Then the reaction solution was stirred for 30min at 10°C.
- HNO3(104.5g,1077.6mmol,1.2eq, 65%) was added to the above mixture under 20°C.
- The mixture was heated to 80°C and stirred for 4hs.
- HPLC showed that 1.67%% of 2CP remained, 5.3% 2M3NP was formed. 91.87% 2M2NP was formed.
- The mixture was cooled to room temperature.
- The mixture was added to the solution of NaOH(750g) in water (3.5L) slowly under 30°C.
- Collected the solid by filtration.
- The filter cake was added to water (1.5L), then stirred at room temperature for 3hs.
- 2M5NP (116.7g, 84.2% yield,99.4% purity) was gotten after filtration and drying.

7.2.3. 5A2MP step

7.2.3.1. Reaction scheme



2M5NP

Exact Mass: 154.04
Molecular Formula: $C_6H_6N_2O_3$

5A2MP

Exact Mass: 124.06
Molecular Formula: $C_6H_8N_2O$

7.2.3.2. Process and results of 5A2MP

- The yield of 5A2MP step using Pd/C or Raney-Ni could be up to 90%. Assay was more than 95%.
- It was found that the reduction (5A2MP step) could be also conducted by flow chemistry if using Pd/C. This reaction needs more investigation if necessary.

Table 77. Results of Raney-Ni as catalyst

No.	Catalyst(w/w)	Solvent	Reaction Temp.	Pressure	IPC_GC	Purity_GC	Result
PHTKENNYG-708	10%Raney-Ni	EtOH 10v/w	50°C (16h)	1.0Mpa	3A5MP:0.09% 2M5NP:0.16% 5A2MP:99.05%	3A5MP:0.09% 2M5NP:0.02% 5A2MP:99.11%	Yield：94.2% Assay:96.86%
PHTKENNYG-709	10%Raney-Ni	EtOH 10v/w	50°C (6h)	1.0Mpa	3A5MP:0.09% 2M5NP:0.05% 5A2MP:99.06%	3A5MP:0.09% 2M5NP:0.04% 5A2MP:99.10%	Yield：92.6% Assay:96.69%
PHTKENNYG-710	5%Raney-Ni	EtOH 10v/w	50°C (16h)	1.0Mpa	3A5MP:0.09% 2M5NP:3.89% 5A2MP:94.62%	N/A	N/A
PHTKENNYG-711	8%Raney-Ni	EtOH 10v/w	50°C (16h)	1.0Mpa	3A5MP:0.09% 2M5NP:0.52% 5A2MP:97.70%	3A5MP:0.08% 2M5NP:0.05% 5A2MP:96.93%	Yield：91.1% Assay:95.69%
PHTKENNYG-713	8%Raney-Ni	EtOH 10v/w	20°C (16h)	1.0Mpa	3A5MP:0.08% 2M5NP:5.32% 5A2MP:91.82%	NA	

The reaction can get a good result (91% yield, 95.7% assay).

Table 78. Results of reduction reaction

No.	Catalyst(w/w)	Solvent	Reaction Temp.	Condition	IPC GC_M2, %area	Purity GC_M2, %area	Yield (based on purity)
PHTKENNYG-714	8%Raney-Ni	EtOH 10v/w	40°C (16h)	1.0Mpa	2M5NP(SM):0.03%(HPLC) 5A2MP:99.3%(HPLC) UI@RRT 3.16: 0.11%(HPLC)	2M5NP:0.03% 3A2MP:0.08% 5A2MP:99.2%	94.7%
PHTKENNYG-715	8%Raney-Ni	EtOH 10v/w	40°C (7h)	1.0Mpa	2M5NP(SM): 0.07% (HPLC) 5A2MP: 98.6% (HPLC) UI@RRT 3.16: 0.60%(HPLC)	2M5NP:0.03% 3A2MP:0.09% 5A2MP:98.9%	96.2%
PHTKENNYG-716	8%Raney-Ni	EtOH 10v/w	40°C (7h)	0.4Mpa	2M5NP(SM):7.98% 3A2MP(Isomer):0.09% 5A2MP:91.3%	N/A	

PHTKENNYG-717	8%Raney-Ni	EtOH 10v/w	40°C (16h)	0.4Mpa	2M5NP(SM):5.09% 3A2MP(Isomer):0.11% 5A2MP:94.3%	N/A	
PHTKENNYG-719	8%Raney-Ni	EtOH 10v/w	40°C (16h)	HCOONH4	2M5NP(SM):82.3% 3A2MP(Isomer): n.d. 5A2MP:2.69%	N/A	
PHTKENNYG-720	8%Pd/C (10%)	EtOH 10v/w	40°C (16h)	HCOONH4	2M5NP(SM): n.d. 3A2MP(Isomer):0.09% 5A2MP:95.9%	2M5NP: n. d. 3A2MP:0.10% 5A2MP:95.9% UI@RRT 1.19: 3.59%	96.2%
PHTKENNYG-721	8%Raney-Ni	EtOH 10v/w	30°C (16h)	1.0Mpa	2M5NP(SM):20.4% 3A2MP(Isomer):0.10% 5A2MP:77.6%	N/A	
PHTKENNYG-723	8%Raney-Ni	EtOH 10v/w	40°C (5h)	1.0Mpa	2M5NP(SM):28.60% 3A2MP(Isomer):0.07% 5A2MP:70.7%	N/A	

$HCOONH_4$ sublimed in the reaction system.

Table 79. Results of reduction reaction

No.	Catalyst(w/w)	Solvent	Reaction Temp.	Condition	IPC GC_M2, %area
PHTKENNYG-726	8%Pd/C (10%)	EtOH10v/w	40°C (2h)	HCOONH4	2M5NP(SM): n.d. 3A2MP(Isomer):0.09% 5A2MP:98.9%
PHTKENNYG-731	8%Pd/C (10%)	EtOH 10v/w	40°C (4h)	H2 balloon	2M5NP(SM):35.5% 3A2MP(Isomer):0.11% 5A2MP:63.7%
PHTKENNYG-732	8%Pd/C (10%)	EtOH 10v/w	20°C (2h)	H2 balloon	2M5NP(SM):59.9% 3A2MP(Isomer):0.09% 5A2MP:39.4%
PHTKENNYG-733	8%Pd/C (10%)	EtOH 10v/w	40°C (16h)	H2 balloon	2M5NP(SM):n.d. 3A2MP(Isomer):0.10% 5A2MP:99.1%
PHTKENNYG-734	8%Pd/C (10%)	EtOH 10v/w	40°C (7h)	H2 (1MPa)	2M5NP(SM):n.d. 3A2MP(Isomer):0.09% 5A2MP:99.4%
PHTKENNYG-715	8%Raney-Ni	EtOH 10v/w	40°C (7h)	1.0Mpa	2M5NP(SM): 0.07% (HPLC) 5A2MP: 98.6% (HPLC) UI@RRT 3.16: 0.60%(HPLC)
PHTKENNYG-739	8%Raney-Ni	EtOH 10v/w	40°C (7h)	1.0Mpa	2M5NP(SM):n.d. 3A2MP(Isomer):0.07% 5A2MP:99.6%

Pd/C system gives similar IPC result (reaction conversion) compared to Raney-Ni system.

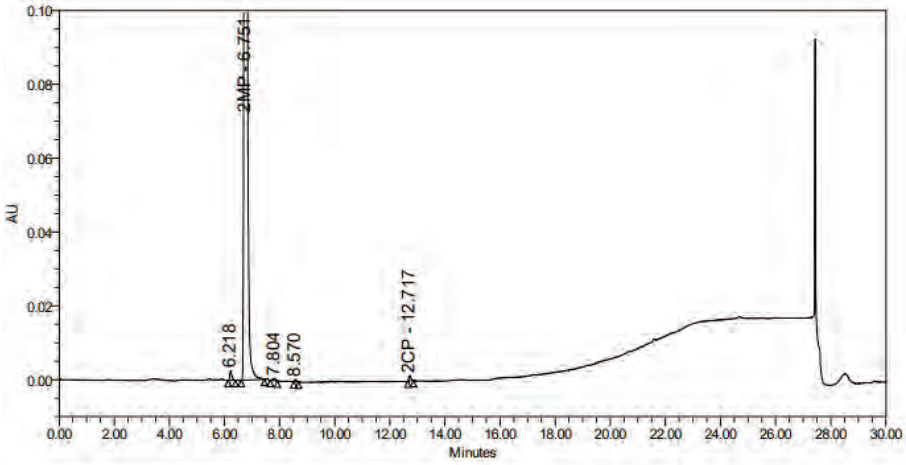
The amount of Pd/C, reaction pressure needs further investigation.

7.2.3.3. Typical procedure for preparation of 5A2MP in experiment PHKENNNYG-739

- To the mixture of 2M5NP (20g, 129.7mmol) in EtOH (150mL) was added Raney-Ni (1.6g,8% w/w, Grace RANEY®2800).
- The mixture was degassed with H_2 for three times.
- The reaction was raised to 40°C and stirred for 7h under H_2 atmosphere (1.0MPa).
- HPLC showed the reaction was completed (2M5NP was NMT 0.5%).
- The mixture was cooled to 25°C.
- Remove the catalyst with celite by filtration.

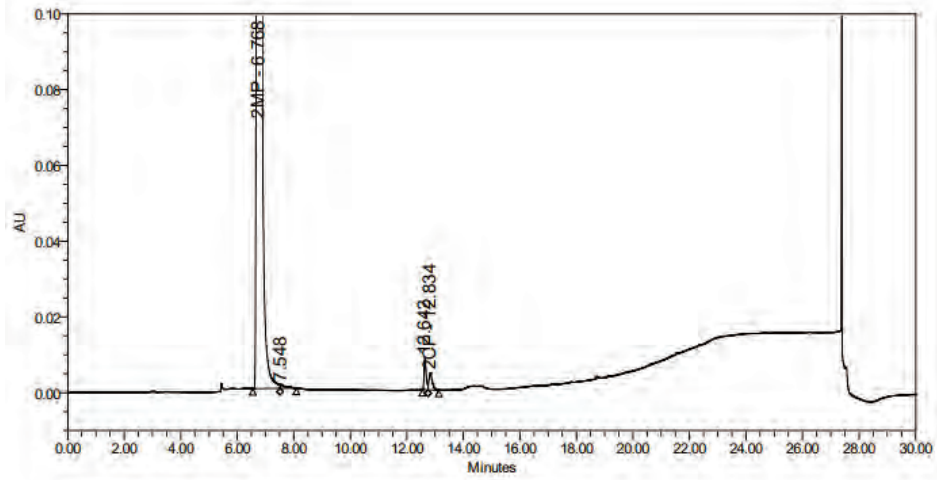
- The filtrate was evaporated (50°C) to dryness under reduced pressure to give a brownish red oil (15.6g,99.5% assay,96.4% yield).
- The oil was used directly for next stop without purification.

Figure 1: HPLC chromatogram of 2MP-PHTRACKD-627(IPC)



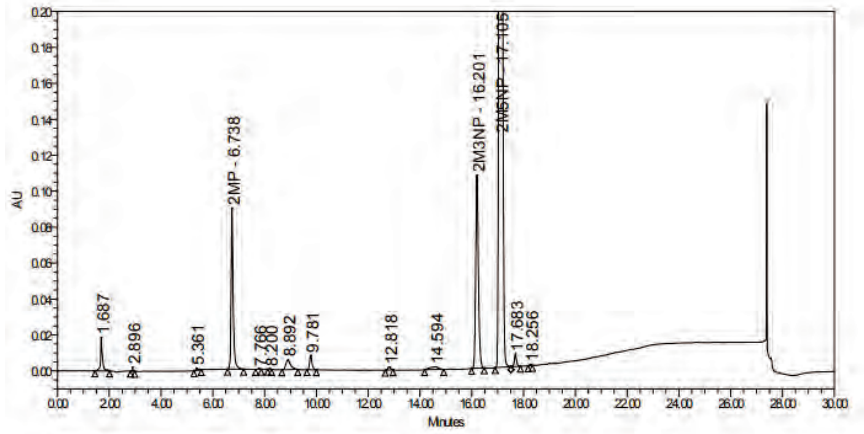
	Name	RT	RRT	Area	% Area	Resolution
1		6.218	0.92	13158	0.36	
2	2MP	6.751	1.00	3604963	99.28	
3		7.804	1.16	3367	0.09	
4		8.570	1.27	2614	0.07	
5	2CP	12.717	1.88	6875	0.19	

Figure 2: HPLC chromatogram of 2MP-PHTRACKD-627(isolated)



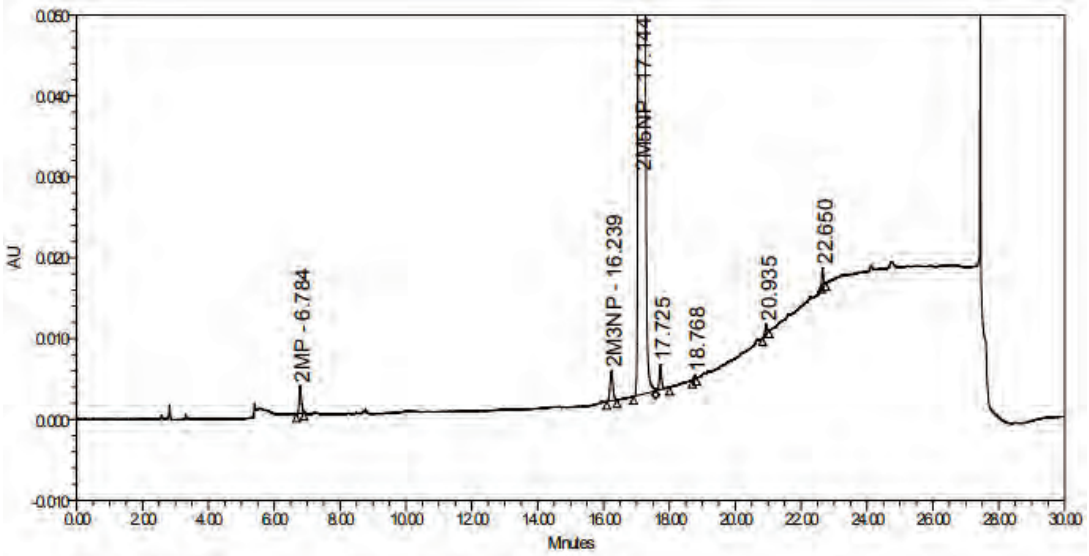
	Name	RT	RRT	Area	% Area	Resolution
1	2MP	6.768	1.00	14676865	99.38	
2		7.548	1.12	12867	0.09	
3		12.642	1.87	41878	0.28	
4	2CP	12.834	1.90	36259	0.25	

Figure 3: HPLC chromatogram of 2M5NP-PHTHARRYS-645(IPC)



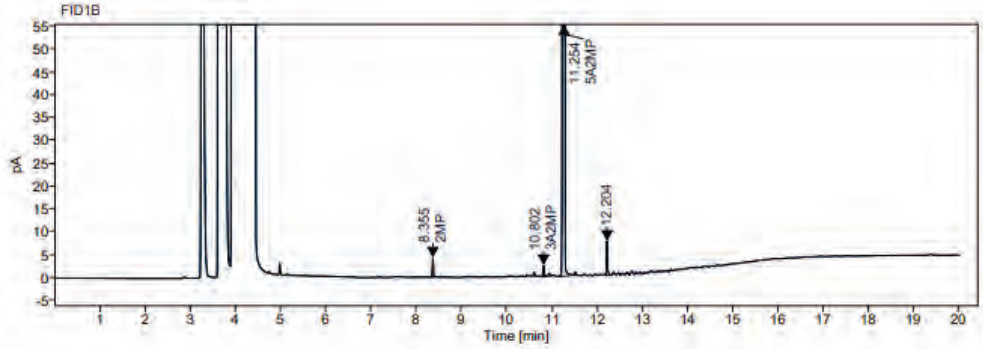
	Name	RT	RRT	Area	%Area	Resolution
1		1.687	0.10	95094	0.85	
2		2.896	0.17	4392	0.04	
3		5.361	0.31	6303	0.06	
4	2MP	6.738	0.39	492842	4.42	
5		7.766	0.45	1691	0.02	
6		8.200	0.48	1511	0.01	
7		8.892	0.52	70520	0.63	
8		9.781	0.57	51142	0.46	
9		12.818	0.75	12187	0.11	

Figure 4: HPLC chromatogram of 2M5NP-PHTHARRYS-645(isolated)



	Name	RT	RRT	Area	%Area	Resolution
1	2MP	6.784	0.40	16271	0.09	
2	2M3NP	16.239	0.95	22599	0.13	
3	2M6NP	17.144	1.00	17678361	99.63	
4		17.725	1.03	15098	0.09	
5		18.768	1.09	1346	0.01	
6		20.935	1.22	4718	0.03	
7		22.650	1.32	6264	0.04	

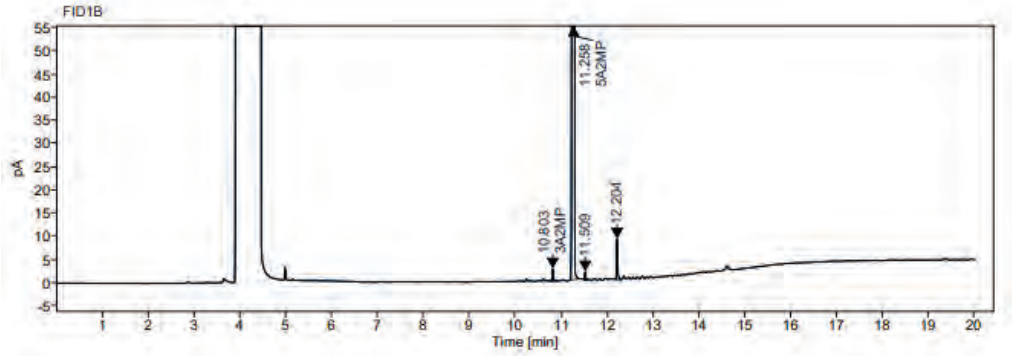
Figure 5: HPLC chromatogram of 5A2MP-PHTKENNYG-739(IPC)



Signal: FID1B

RT [min]	Name	Type	Width [min]	Area	Height	Area%
8.355	2MP	BV	0.1212	6.0963	4.3134	0.1172
10.802	3A2MP	BV	0.0878	3.6719	2.4677	0.0706
11.254	5A2MP	BV	0.2933	5179.7392	2982.6425	99.5819
12.204		VB	0.1252	11.9794	7.5524	0.2303
			Sum	**5201.4868**		

Figure 5: HPLC chromatogram of 5A2MP-PHTKENNYG-739(isolated)



Signal: FID1B

RT [min]	Name	Type	Width [min]	Area	Height	Area%
10.803	3A2MP	BM m	0.1331	4.2073	2.6758	0.0695
11.258	5A2MP	MM m	0.2240	6034.6434	3285.8718	99.6512
11.509		VV	0.0702	3.1388	1.8948	0.0518
12.204		VB	0.1318	13.7746	8.8092	0.2275
			Sum	**6055.7640**		

8. Reference

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21. HPLC Method Limited Verification for PNDa02 (non-GMP)
22. HPLC Method Limited Verification for TGF-001 (non-GMP)