Section A-Research paper

Optimized synthesis of a key intermediates of Phenyl hydrogen (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methyl phosphonate

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ABSTRACT:

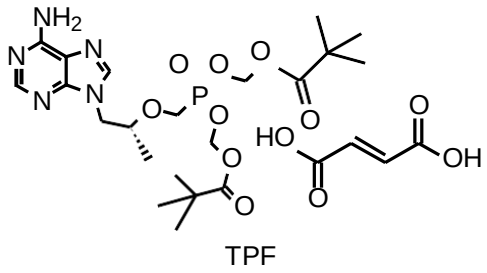
In this study, the development of a simple, scalable, green and concise alternative process for the manufacturing of Phenyl hydrogen (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methyl phosphonate (TPF), which is a promising potent antiviral agent discovered and developed by Cipla Ltd. This study found that carrying a reaction in an aprotic organic solvent or mixture of aprotic organic solvents led to a scalable but economically feasible green process. This process also involved desirable yield, purity and scalability from 10 to 30kg scale of operation.

KEYWORDS: Antiviral, Anti-HIV drugs, resistance, aprotic organic solvents.

INTRODUCTION

Many challenges are encountered while developing antiviral agents, including adverse events and developing drug-resistant viruses[1], which necessitate chemists, biologists, and pharmacologists to develop improved, more potent and less toxic medicines. Antiretroviral drugs target major steps in the HIV lifecycle, which include viral attachment to the host cell, fusion, reverse transcription, integration, protein processing and maturation[2].

Due to the threat of resistance posed by the known drugs, there is a constant need to update the development pipeline and consider different antiviral drugs[3-5]. As a part of our drive to explore and investigate different ARV drugs, we recently initiated a systematic investigation to develop a novel drug candidate. One such work has provided a promising anti-HIV drug, which could be a promising Nucleoside reverse transcriptase class of drugs[6], TPF compound, by CIPLA LTD. The compound TPF and its pharmaceutically acceptable salts of disclose in US9.227,930BM[7].



TPF

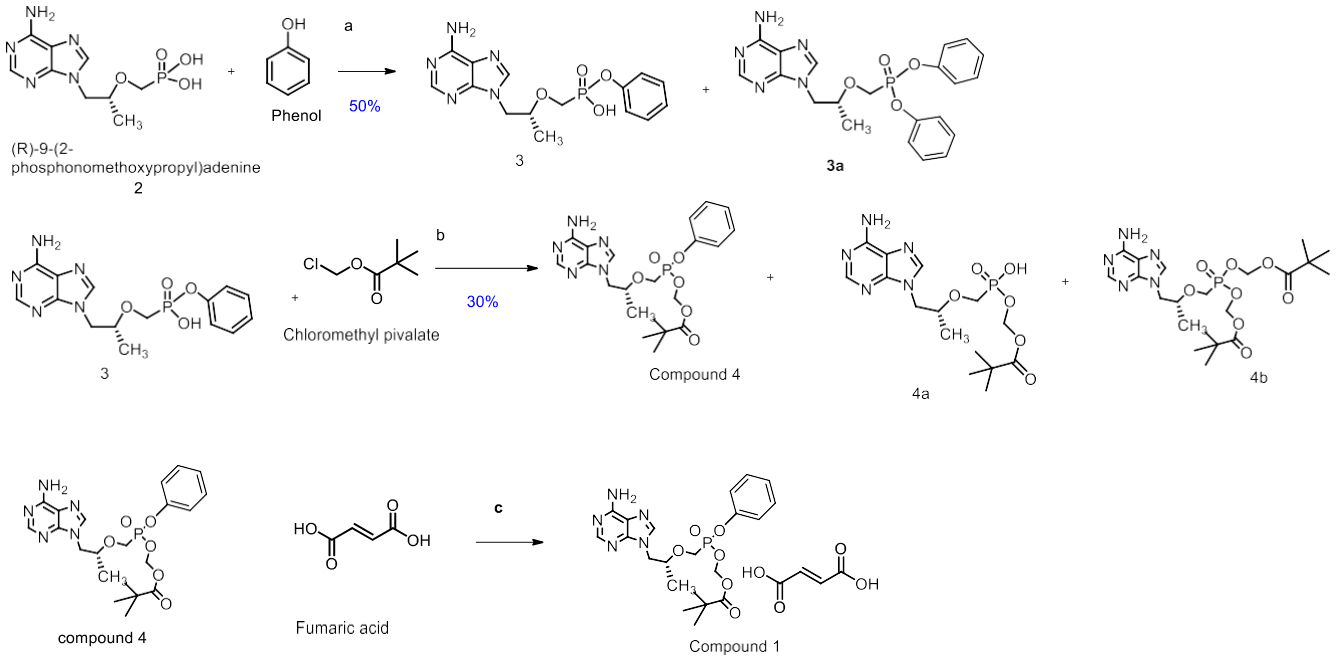
Figure 1: Structure of Phenyl hydrogen (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methyl phosphonate (TPF)

The TPF can exist as a diastereomer with the configurations (R, R), (S, S), (R, S), or (S, R). The compound in Figure 1 or its acid salt should preferably be the (R, R) diastereomer. As a result, the salts of the current study include all diastereomers found in the salts. This TPF

compound has valuable pharmacological characteristics. For example, it can be used as a nucleotide reverse transcriptase inhibitor in treating diseases that respond to protein kinase inhibition. TPF (**Figure 1**) has several structural features that make synthesising difficult. While a discovered synthesised method seems appropriate for synthesising the TPF compound, there are several issues with producing this active pharmaceutical component in larger quantities.

The existing method for preparing TPF use multistep reactions starting from the ®-9-(2-phosphonomethoxypropyl)adenine (**1**) (**Scheme 1**). Initially, the compound phenyl hydrogen ((R)-1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methylphosphonate (**3**) started from **1** at the phenol, NMP, TEA at 95-100 °C for 2 hrs. The next step was as follows; compound **3**, N, N-diisopropylethylamine, tetrabutylammonium bromide and chloromethyl pivalate at 58-62°C for 4 hrs. and the final step was the addition of fumaric acid in the presence of acetonitrile under heating.

This article describes the challenges we encountered in developing a practical, scalable and reproducible manufacturing process for the first scale-up of the TPF compound. Previously in the lead optimisation, a few hundred grams were prepared using synthesis[7] shown in Scheme 1. Starting from (R)-9-(2-Phosphonomethoxypropyl)adenine (2), a literature procedure was used to prepare Intermediate (3) via a coupling reaction using N, N-Dicyclohexylcarbodimide as a coupling agent and Phenol as a reactant in solvent N-methyl pyrrolidone. Intermediate 1 was isolated and reacted with chloromethyl pivalate in solvent N-methyl pyrrolidone, and the TPF compound was isolated as a fumarate salt.



Standard reaction conditions: [a] The reaction was carried out with NMP, Triethylamine at 95-100^{0}C for 2hrs [b] N, N-diisopropylethylamine, NMP at 58-62^{0}C for 4hrs. [c] Fumaric acid, acetonitrile, heating.

Scheme 1: Previous approach for Synthesis of TPF compound

Over the three steps, the reported synthetic yields of the TPF ranged from 40% to 60%. The main drawbacks of the previous methods for synthesising TPF compounds are as follows: 1) N-methyl pyrrolidone was used as the solvent in both stages; NMP is a high-boiling solvent that is known to be hazardous to humans and the environment; it is difficult to remove completely, so replacement of this solvent was required; 2) Reaction mechanism to be studied to control impurity formation and ultimately increase yield; and Lower yield in each stage; inconsistent reaction conversion. The combination of these issues reduces the effectiveness of larger-scale processes. A scalable, economically viable green process must be developed to avoid the drawbacks above.

This study aims to provide an improved process for preparing TPF compounds or pharmaceutically acceptable salts in high diastereomeric purity. Yet another object of the present study is to provide a green process for the TPF synthesis and pharmaceutically acceptable salts that is simple, sensitive, economical, and suitable for industrial scale-up. In this study, compound 2 was reacting with triphenylphosphite; compound 3 was reacting with chloromethyl pivalate, compound 4 was optionally isolated as phosphate salts, and compound 5a was coveting the phosphate salt compound 5a to an acid addition salt of the TPF compound.

EXPERIMENTAL SECTION

***General information*:** Unless otherwise noted, all reagents were used as received from commercial suppliers. Reactions were monitored using thin-layer chromatography (SiO_2). TLC plates were visualised with UV light (254 nm), iodine treatment, or ninhydrin stain. Column chromatography was carried out using silica gel (60-120 mesh & 100-200 mesh) packed in glass columns. NMR spectra were recorded at 300, 400, and 500 MHz (H) and 75, 101, and 126 MHz (C), respectively. Chemical shifts (δ) are reported in ppm, using the residual solvent peak in $CDCl_3$ (H: δ = 7.26 and C: δ = 77.1 ppm) as internal standard, and coupling constants (*J*) are given in Hz. HRMS were recorded using ESI-TOF techniques.

EXPERIMENTAL SECTION

Phenyl hydrogen (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methyl phosphonate (3): PMPA ((100 g,0.348 mmol) 1,4-dioxane (350 ml, 1.2V) Phenol (62.2 g, 0.661 mmol) and triethylamine (42.22 g, 0.418 mmol) were added to a 2 L reaction vessel equipped with a mechanical stirrer. The reactor contents were agitated at 80-85 °C to give a solution. To this

solution was charged N, N-dicyclohexyl carbodiimide (111.25 g, 0.540 mmol) dissolved in 1,4-dioxane (50 ml, 0.5 V) at 80-85 °C. The reactor contents were then heated to 100-105 °C for a complete solution. The reactor contents were agitated at 100-105 °C for 5 hrs. The reaction mass was sampled and monitored by HPLC. After reaction completion, Water (200 ml) was charged to the solution to obtain a slurry. Reaction mixture as agitated at 25-30 °C for 1 hr. Further, the Reaction mixture was filtered at 25-30 °C to remove inorganics; the clear filtrate was collected in a 3 L reactor, pH of the filtrate was adjusted to 11-12 using 25% sodium hydroxide solution (25 ml), stirring continued for 15 mins at 25-30 °C. To this basic solution, ethyl acetate (200 ml, 2V) was added and stirred for 15 mins at 25-30 C, further layers separated, and an aqueous layer was collected in a 2 L reactor. In contrast, an organic layer containing impurities was discarded ed. pH of the Aqueous layer was adjusted to 2.3-3 using conc. Hydrochloric acid (30 ml), white solid precipitate. The reaction mixture is agitated for 8-10 h at 25-30 °C to ensure complete precipitation, then filtered to collect solid. This solid is slurred in methanol (1000 ml, 10 V) at 25-30 °C and filtered, washing with Methanol (250 ml, 2.5 V). The solid obtained was dried at 50-55 °C for 8-10 h till k_f NMT 1.0 %, and 75 g of compound 3 was obtained.

Phenyl hydrogen (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methyl phosphonate (3): PMPA (100 g, 0.348 mmol), Acetonitrile (150 ml, 1.5 V), Triphenylphosphite (162.2 g, 0.5226 mmol), 4-N, N-dimethylaminopyridine (42.56 g, 0.3484 mmol), Toluene (150 ml, 1.5 V) and triethylamine (7.05 g, 0.6969 mmol) were added to a 2 L reaction vessel equipped with a mechanical stirrer. The reactor contents were then heated to 80-85 °C for a complete solution. The reactor contents were agitated at 80-85 °C for 40 hrs. The reaction mass was sampled and monitored by HPLC. After reaction completion, Toluene (200 ml, 2 V) and water (200 ml, 2 V)

were charged to the reaction solution to obtain a slurry. The reaction mixture was agitated at 25-30 °C for 30 mins, further layers separated, and an aqueous layer was collected in a 2 L reaction, whereas the organic layer containing impurities was discarded. The aqueous layer was again washed with Toluene (200 ml, 2 V) and further layers separated, and the aqueous layer was collected in a 2 L reactor. In contrast, the organic layer containing impurities was discarded. pH of the Aqueous layer is adjusted to 2-2.5 using conc. Hydrochloric acid (30 ml), white solid precipitate. The reaction mixture is agitated for 1 h. at 25-30 °C to ensure complete precipitation, further chilled to 2-8 °C and filtered to collect solid. This solid is slurred in methanol (1000 ml, 10 V) at 25-30 °C and filtered, washing with Methanol (250 ml, 2.5 V). The solid was dried at 50-55 °C for 8-10 hrs. till kf NMT 1.0%, and 106 g of compound 3 were obtained.

(((1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methyl) (phenoxy) phosphoryloxy) methyl pivalate phosphate (4'): Phenyl hydrogen (((R)-1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methyl phosphonate (3) (100gm,0.275mmol), N, N-diisopropyl ethylamine (72 ml, 52.12 g, 0.404 mmol), tetrabutyl ammonium bromide (30 g, 30%, 0.0930 mmol) and dimethyl carbonate (500 ml, 5 V) were charged to 3 L four-neck flask provided with a thermometer, a dropping funnel and a mechanical stirrer.. The contents of the reactor were agitated at 25-30 °C to give a slurry mixture. This mixture was charged with Chloromethyl pivalate (62 g, 0.4133 mmol) at 25-30 °C. The reactor contents were then heated to 55-60 °C for a complete solution. The reactor contents were agitated at 55-60 °C for 5 h. The reaction was sampled and monitored by HPLC. Evaporation of the solvent under vacuum gave Oil approx. 120gms.The reactor contents were cooled to 25-30 and dissolved in dichloromethane (500 ml, 5 V). The organic layer was washed with 10% Sodium dihydrogen orthophosphate (200 ml, 2 V, twice). The layers were settled and separated.

Evaporation of the organic layer under vacuum at 40-45 °C gave oil. Charged isopropyl alcohol (500 ml, 5 V) to residual oil in the reactor. The reactor jacket was cooled to 25-30 °C. Charged O-phosphoric acid (32.5 g, 0.3316 mmol) to the isopropyl alcohol solution, and reactor contents were agitated at 25-30 °C for 30 mins. The reactor jacket was heated to 70-75 °C, and the reaction mixture was stirred at 70-75 °C for 15 mins to obtain a clear solution. The reactor jacket was cooled to 25-30 °C; the reaction solution was stirred at 25-30 °C for 2 h to obtain a slurry. The reactor contents were filtered, and the cake was washed with isopropyl alcohol (100 ml, 1 V). The wet cake (160 g) was used as such for purification. The wet cake was charged to a 2 L reactor with Methanol (200 ml) and Acetone (500 ml) at 25-30 °C. The reactor jacket was heated at 65-70 °C, and the reaction mixture was stirred at 65-70 °C for 15 mins to get a clear solution. The reactor jacket was then cooled to 25-28 °C and stirred for 2 h at 25-28 °C. The reactor contents were filtered, and the cake was washed with acetone (100 ml, 1 V). Analysis of Compound 4 phosphate on HPLC shows Impurity 4a and Impurity 4b less than 0.5%. Purification is repeated if these two impurities are more than 0.5%. The solid was dried in a vacuum tray below 45 °C to obtain 100 g of compound 4'.

(((1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methyl) (phenoxy) phosphoryloxy) methyl pivalate fumarate (TPF): Compound 4', (100 g, 0.173 mmol) along with dichloromethane (500 ml) and water (200 ml) were charged to a four-necked 2 L reactor with a mechanistic stirrer. The reaction mixture was allowed to stir at 20-25 °C to obtain a clear solution. Liquor ammonia (100 ml, 1 V) was charged to the reaction solution at 25 °C under stirring. After stirring the solution for 30 mins, the layers are allowed to separate. The product was extracted in an organic dichloromethane layer; the aqueous layer was discarded. The organic layer was washed with 10%

sodium dihydrogen phosphate solution (200 ml, 2 V), the organic layer was collected, dried over sodium sulfate, and the solvent was evaporated under a vacuum below 40 °C to obtain the oil. Residue oil was dissolved in a mixture of Isopropyl alcohol (500 ml, 5 V) and water (1000 ml, 2 V). To this solution obtained, charged Fumaric acid (18.15 g,0.156 mmol) and slurry were heated at 45-50 °C under stirring for 30 minutes. The solution was further cooled to 0-5 °C and stirred at this temperature for 2 h. The reaction mixture was then filtered at 0-5 °C, washing to wet cake with water (200 ml, 2 V). The wet cake was dried in a vacuum oven below 45 °C or 5hrs to obtain TPF (60 g) LOD checked below 1%. TPF obtained showed more than 99.5% purity as per the HPLC method.

(((1-(6-amino-9H-purin-9-yl)propan-2-yloxy)methyl) (phenoxy) phosphoryloxy) methyl pivalate methane sulfonate: Compound 4' (20 g, 0.034 mmol) along with dichloromethane (200 ml) and water (100 ml) were charged to a four-necked 2 L reactor with a mechanistic stirrer, and the reaction mixture was allowed to stir at 20-25 °C to obtain a clear solution. Liquor ammonia (100 ml, 1 V) was charged to the reaction solution at 25 °C under stirring. After stirring the solution for 30 mins, the layers are allowed to separate. The product was extracted in an organic dichloromethane layer; the aqueous layer was discarded. The organic layer was washed with 10% sodium dihydrogen phosphate solution (40 ml, 2 V), the organic layer was collected, dried over sodium sulfate, and the solvent was evaporated under a vacuum below 40 °C to obtain other il. Residue oil was dissolved in a mixture of ethyl acetate (100 ml, 5 V) and cooled to 15-20 °C. To this solution, charged Methane sulfonic acid (3.5 g, 0.0364 mmol) and the slurry were stirred initially at 15-2 °C and then at 25-30 °C under stirring for 30 minutes. The reaction mixture was then filtered at 25-30 °C, and the wet cake was washed with ethyl acetate (20 ml, 1 V). The wet

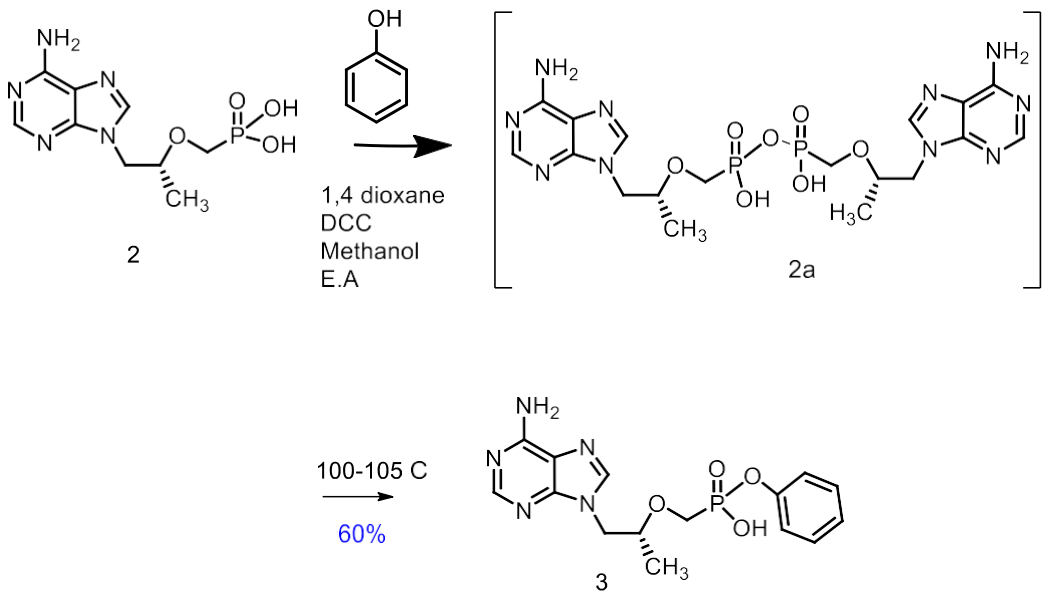
cake was dried in a vacuum oven below 40 °C or 5 h to obtain compound 5 (15 g) LOD checked below 1%. Compound 5 obtained more than 99.5% purity per the HPLC method.

Results & Discussion:

Our first approach was to select starting material, 9-[2-(phosphonyl methoxy)propyl]adenine (**2**) was readily available in good yield and chiral purity; it is also used in the synthesis of other Anti-HIV drugs like Tenofovir disoproxil[9] and tenofovir alafenamide fumarate[10] hence it was well established starting material and so was selected as starting material for initial development and scale-up process.

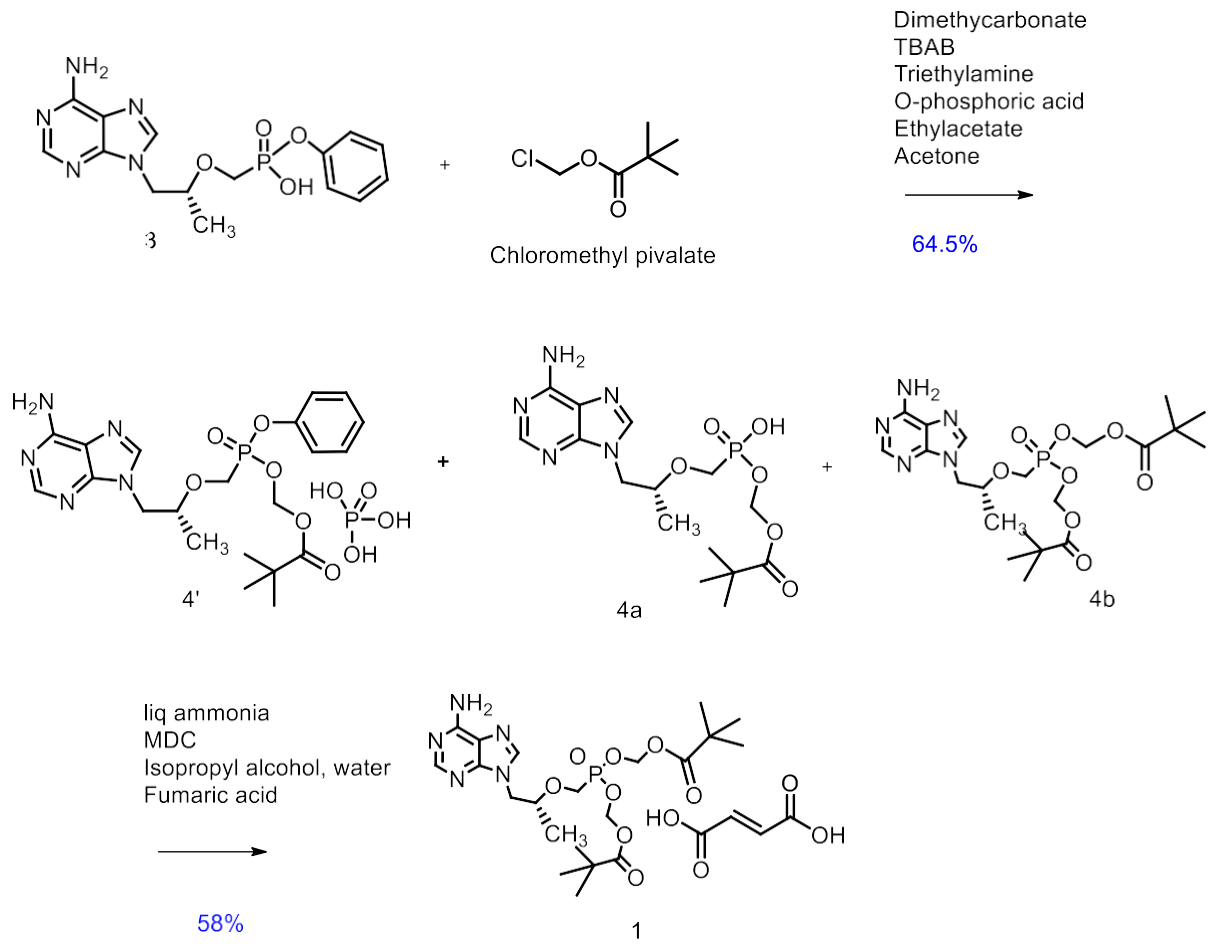
After selecting starting material, we explored the red the reaction mechanism for **stage 1** (conversion of Starting material 2 to Compound 3) and stage 2 (conversion of compound 3 to compound 4) Scheme 1.

Reaction mechanism stage 1: HPLC monitoring of **stage 1** reaction showed that Starting material 2 is first converted to intermediate 2a, which is then converted to Intermediate 3, Scheme 2. Thus, monitoring the complete conversion of Intermediate 2a to Intermediate 3 becomes important.



Scheme 2: Stage 1 Route of synthesis

Reaction mechanism stage 2: Conversion of Intermediate 3 to TPF is crucial. When the reaction was monitored closely, it was observed that during the reaction, Intermediate 3 degrades back to starting material 2, which couples with chloromethyl pivalate to give compound 4a and compound 4b impurity(dipivalate). Scheme 3; these impurities are difficult to be removed and lead to lesser yield. Understanding these two stages' mechanisms helped in good optimisation design and reaction monitoring to achieve the final goal of a robust process. For process optimisation, different process parameters were selected and further explored.



Scheme 3: Stage 2 Route of synthesis

Reagent Optimization:

Moles of DCC & Phenol for Stage 1: Conversion of 1 to 2 was accompanied using DCC as a coupling agent with better yield than all known coupling agents. After working on different equivalents ranging from 0.8 to 2 equivalent, we found that a 1.55 equivalent of DCC would allow for the full conversion of 1 to 2. Similarly, 1.9 moles of Phenol were required to achieve good conversion, purity, and Yield.

Table 1: Optimization condition for Moles of DCC and Moles of Phenol in Stage 2

Entry	Moles of DCC	Moles of Phenol	Observation
1	0.8	1.4	Incomplete reaction
2	1	1.6	40%
3	1.5	1.9	60%
4	2	2	More impurities

Screening of temperature: Stage 1 reaction didn't proceed at a lower temperature; hence it was carried out at an initial 80-85 °C and then further heated to 100-105 °C, the boiling point for 1,4-dioxane. Addition of N, N-dicyclohexycarbodimide was tried at 25-30°C and 80-85 °C, but additionally, at lower temperatures gave lower yield; hence the addition of N, N-dicyclohexylcarbodimide was fixed at the 80-85 °C.

Solvent Optimization:

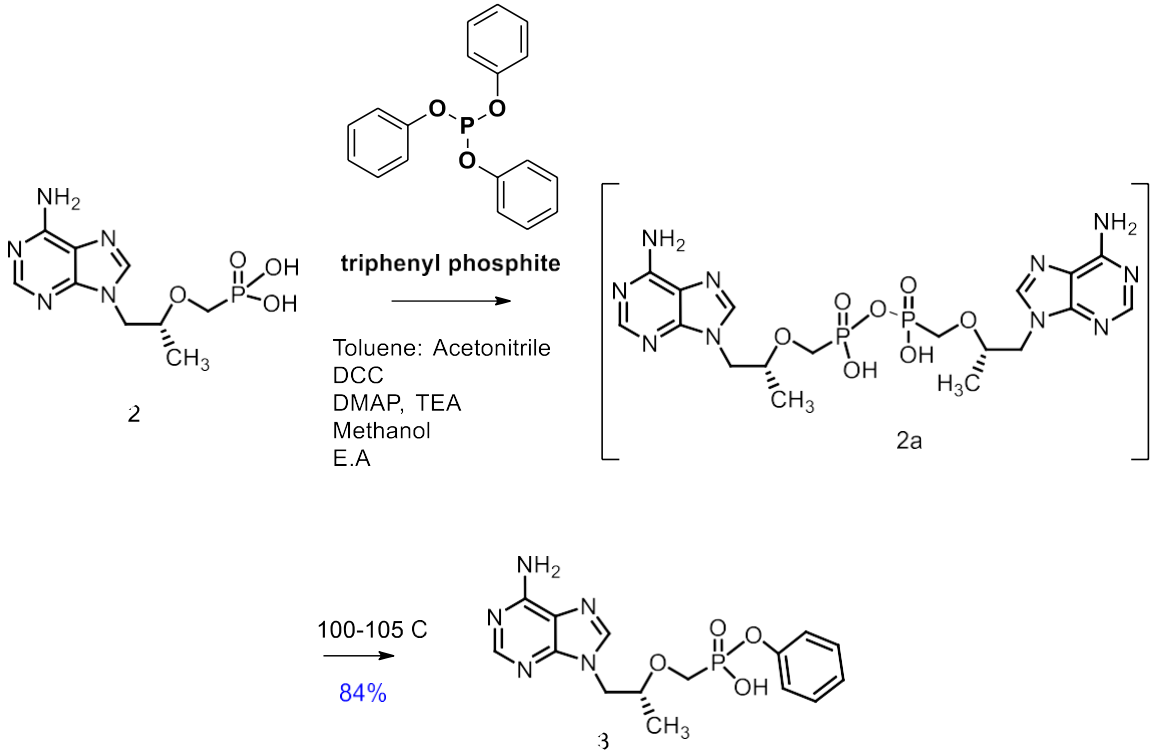
Stage 1: Replacement of N-methyl pyrrolidinone in stage 1 with other solvents was considered. We tried reactions in different solvents, and the results are depicted in **Table 2**. It shows that a better yield was obtained using 1,4-dioxane as a solvent. Since 1,4-dioxane is not an environmentally friendly solvent, we further optimised the process and cut its volume to 1.2V only; this gave us good yield, reaction conversion and lesser environmental impact.

Table 2: Optimization condition for Solvent selection in Stage 1

Entry	**Solvent**	**Solvent volume**	**Observation**

1	Toluene	4	The sticky non-stirrable reaction mass
2	NMP	3	30-40%
3	1,4-Dioxane	3	55-65%
4	MIBK	3	30-40%

Though the use of 1,4-dioxane increased the yield and purity of Stage 1, we explored replacing phenol reagent with Triphenylphosphite, which was reported by Colby et al. The reaction shown in Scheme 4 was reported in different solvents and s.



Scheme 4: Preparation of Compound 3

Re-optimization on Stage 1, replacing Phenol with Triphenylphosphite:

With this change in reagent, optimisation of stage 1 was reinitiated.

Solvent selection with changed reagent:

Using reagent Triphenylphosphite, the reaction mass was carried out in polar aprotic solvents like N-methylpyrrolidine, acetonitrile and non-polar aprotic solvents like cyclohexane, Toluene as well as a mixture of solvents. Expected yield and purity were obtained when a mixture of Toluene & acetonitrile was tried, as shown in **Table 4**.

Table 4: Optimization condition for Solvent selection in Stage 1 using Triphenyl phosphite

Sr. No.	**01**	**02**	**03**
Input	100 gm	50 gm	100 gm
Solvent	NMP	Dean Stark in Cyclohexane & then reaction in NMP	Toluene: Acetonitrile (1:1)
Output	101.2 gm	41.3 gm	106 gm
Yield	80 %	65.30 %	83.7%
Quality (Purity)	99.05%	98.53 %	99.71%
Remarks	**Sticky reaction mass**	**Yield less**	**Good Yield & Purity**

Screening of moles of Reagents:

After the finalisation of the solvent as Toluene: Acetonitrile mixture, the base was narrowed down to Triethylamine. The design of experiments was planned to obtain the operating range for moles of Reagent and optimal temperature. As shown in **Table 5**, maximum conversion and purity were obtained in run 11, which helped us finalise Triphenyl phosphite moles, 4-dimethylaminopyridine w.r.t to Triphenylphosphite, Triethylamine and temperature.

Table 5: DOE Stage 1

Std	Run	A: Triphenyl Phosphite	B: DMAP (W.r.t. Triphenyl Phosphite)	C: TEA	D: Temp.	Isolated-Purity	Remark
		Equi.'s	Equi.'s	Equi.'s	°C	%	
3	1	1.2	1.2	1.5	85	95.97	
5	2	1.2	0.8	2.5	85	94.24	
4	3	2	1.2	1.5	60	92.86	
11	4	1.6	1	2	72.5	97.83	
8	5	2	1.2	2.5	85	98.68	
1	6	1.2	0.8	1.5	60	8.8	
7	7	1.2	1.2	2.5	60	57.31	
10	8	1.6	1	2	72.5	98.47	
6	9	2	0.8	2.5	60	77.9	
9	10	1.6	1	2	72.5	97.93	
2	11	2	0.8	1.5	85	99.22	Gives maximum yield and purity

Isolation & workup:

Workup and isolation are critical to obtaining a scalable process and the intended quality of compound 3. To achieve this, different trials of isolation & workup were carried out and, as Tabulated in **Table 6.**

Table 6: Trials on work up

Entry	Workup & isolation	Yield	Purity	Remark

1.	Work done adding water to RM & giving back wash of Toluene to Aq. Layer and Further Isolation from Aqueous Layer.	83.7%	99.71%	Distillation is avoided, impurities removed in an organic solvent, and crystallisation in the presence of acetonitrile and water is achieved
2.	Workup did by distilling RM after the reaction & adding water to the residue. Adjusting pH to 11-12 using aq NaoH & giving EA wash to Aq. Layer & further isolation from the aqueous layer.	55.55%	99.5%	Distillation of solvent to obtain residue is difficult to achieve during scaleup.

Screening and optimisation of Stage 1 was completed, and then Stage 2 screening and optimisation was initiated.

Selection of solvent for Stage 2:

Replacement of reprotoxic N-methyl pyrrolidinone in stage 2 was our primary requirement. As shown in Scheme 3, reducing compound 4a and compound 4 b formation was the target. We tried different polar aprotic and nonpolar solvents; the results are depicted in **Table 7**. It shows the required conversion and minimum impurities in the Dimethyl carbonate solvent, also a green solvent.

Table 7: Solvent screening for Stage 2

Entry	Solvent	Remark
1.	Acetonitrile	0.94% dipivalate formation
2.	1,4-dioxane	0.93% dipivalate formation
3.	DMA	Slow conversion
4.	Dimethyl carbonate	0.65% dipivalate formation

5.	Water	Incomplete reaction
6.	DMC/water 0.5%	Incomplete reaction
7.	DMF	1.2% Dipivalate formation

Selection of Base for Stage 2:

Before finalising N, N-diisopropylamine as a base, screening of different organic & inorganic bases was done, as shown in **Table 8**. Reaction conversion and targeted purity were obtained using N, N-diisopropylethylamine as a base.

Table 8: Optimization condition for Selection of bases in Stage 2

Entry	Bases	*Remark
1	TEA	1.4% dipivalate impurity formation
2	K_2CO_3	The reaction does not initiate
3	DBU	1.19% dipivalate impurity formation
4	DIPEA	0.61% dipivalate impurity
5	Potassium t-butoxide	The reaction does not initiate
6	NaOH	The reaction does not initiate
7	Sodium methoxide	The reaction does not initiate

*Chloromethylpivalate was fixed at 1.5 moles

Screening of Temperature along with Moles of Chloromethyl pivalate & N, N-diisopropyl ethylamine base Stage 2:

Screening different moles of chloromethyl pivalate reagent and N, N-diisopropyl ethylamine base at varying temperatures was done to obtain the conversion of compound 3 to compound 4. Results

are depicted in **Table 9** and show the best results are obtained by 1.5 moles of N, N-diisopropyl amine and 1.5 moles of chloromethyl pivalate used, keeping the reaction mass temperatures at 60 °C. Though the reaction remains incomplete at 60 °C, dipivalate formation is less, and earlier work has shown that removing starting material compound 3 is easier than removing dipivalate impurity.

After finalising moles of N, N-diisopropylethylamine, chloromethyl pivalate and the temperature required for maximum reaction conversion with minimum yield, it was important to look into the workup and isolation of compound 4.

Table 9: Screening of Moles of Chloromethyl pivalate and Moles of N, N-diisopropyl ethylamine in Stage 2

Entry	Process condition			Yield in %	Purity In %	Remark
	Moles of DIPEA	Moles of Chloromethyl pivalate	Temp. in °C			
1	1.2	3.0	80°C	56%	96.97	At 80°C, HPLC Shows 13.05 % un-reacted compound 3 and 2.15% of dipivalate Imp.
2	3.0	1.2	40°C	20%	95.34	At 40°C, HPLC Shows 62.87 % un-reacted compound 3 and shows Lower 0.41% of dipivalate Imp, so, at 40°C, Reaction conversion is Less.
3	1.5	1.5	60°C	53%	96.20	At 60°C HPLC Shows 22.97 % un-reacted Ten Phenol and shows higher % of Dipivalate Imp,i.e.e 0.64 %

Screening of different salts of COMPOUND 4:

In the earlier process, fumarate salt was directly isolated after reaction conversion; when we followed the same, it was observed that the purity of fumarate, when directly isolated from reaction mass, is very less, and this process cannot be used for scaleup. Hence another approach of isolating a different salt and then converting it to fumarate was tried. This gave us better results & yield. After screening several pharmaceutically accepted salts, as shown in **Table 10**, Phosphate salt was selected, which gave better yield and purity than others.

Table 10: Optimization condition for Selection of different salts of Compound 4

Entry	Salts	Remarks*
1	Citric acid	93% Purity of citrate salt of compound 4
2	Salicylic acid	No solid obtained
3	Acetic acid	No solid obtained
4	O-Phosphoric acid	98.43% purity and Good yield of phosphate salt of compound 4
5	Succinic acid	99.26% purity and Less yield w.r.t. to phosphate salt.
6	D(-)tartaric acid	99.39% purity and Less yield w.r.t. to phosphate salt.
7	Oxalic acid	98.54% purity and Less yield w.r.t. to phosphate salt.
8	Methane sulfonic acid	98.3% purity and good yield of Methane sulfonate salt of compound 4

*Isolation solvents varied based on crystallisation.

Selection of solvent for Compound 4 isolation & Purification:

After finalising O-Phosphoric acid as the salt of compound 4, it was necessary to screen different solvents for isolation, **Table 11**. Based on the results, MEOH: Acetone (2:7 respectively) combination was selected, which gave targeted purity and removed maximum impurity in the Mother liquor.

Table 11: Screening of Solvents for isolation of compound 4'.

Entry	Yield %	Solvents	Volume	Remark Target compound 4b is NMT 0.50%
1.	90%	MeOH: EA	2:10	The content of compound 4b is 0.72%
2.	90%	MeOH: Acetone	2:10	The content of compound 4b is 0.64%
3.	16%	MeOH: Toluene	2:10	The content of compound 4b is 0.44%
4.	~ 50%	DMF: Acetone	2:5	The content of compound 4b is 0.66%
5.	~ 50%	MeOH: MIBK	2:5	The content of compound 4b is 0.67%
6.	~ 50%	NMP: Acetone	2:5	The content of compound 4b is 0.60%
7.	~ 50%	MeOH: MDC: Acetone	2:5	The content of compound 4b is 0.41%
8.	76.8%	MeOH: Acetone	2:7	The content of compound 4b is 0.37%

Solvent for isolation of fumarate salt of Compound 4 (compound 4'):

Earlier process in literature mentions fumarate salt of compound 4 isolated in Acetonitrile. During scale-up, TPF, when isolated using acetonitrile solvent, it was observed that the final product didn't meet ICH limits for residual solvents. Hence, we studied the crystallisation of fumarate in different solvents, as shown in **Table 12**. Yield and fumaric acid content were parameters monitored for this experimentation, and the best results were obtained in Isopropyl alcohol. When further scale-up was tried for fumarate salt crystallisation in IPA, we faced the

challenge of slow filtration. Hence further experimentation was carried out, and we finalised the isopropyl alcohol: water (5:10) mixture as the best solvent for obtaining ICH quality TPF with the robust scale-up process.

Table 12: Screening of Solvents for TPF crystallisation.

Process condition			**Remark**
Solvent	**Volume**	**Temp. in °C**	
MeOH	5.0	35	62.90% yield and fumaric acid content do not comply
Acetone	10	50-55	55.64% yield obtained, and fumaric acid content complies
MeOH: Acetone	1:5	35-40	51.48% yield obtained, and fumaric acid content complies
IPA	5	60-65	63.57% yield obtained, and fumaric acid content complies
Ethyl acetate	10	25-30	46.59% yield and more fumaric acid content obtained
EA: Isopropyl acetate	5	25-30	20.46% fails in fumaric acid content
EA: Acetone	5	25-30	24.35% fails in fumaric acid content
Acetone: Isopropyl acetate	5	25-30	26.19% fails in fumaric acid content

Conclusion:

The original synthetic sequence was improved to meet the preclinical demand of multi-hundred grams of TPF. The removal of the NMP solvent in stage 1 increased the efficiency of stage 1 and allowed a much cleaner workup. The replacement of the phenol reagent with triphenylphosphite and the change in workup and isolation solvent of stage 1 helped us to achieve the targeted yield and purity of compound 3. Using Dimethyl carbonate in Stage 2 and isolation as phosphate salt and then its conversion to Fumarate salt gave good purity, yield and consistent results. Methane

sulfonate salt and Phosphate salt also showed good yield and results. The stability of Methane sulfonate salt is under progress.

This improved process is practical and overcomes the disadvantages of earlier methods of harsh reaction conditions and multiple purifications. With the optimised process, Pilot batches of 10kg input and Plant batches of 50kg input were successfully demonstrated. This helped to escalate to support clinical development. Thus, we achieved to develop a more efficient and robust process on a milligram-to-kilogram scale.

Acknowledgement

1. Desai, M.; Iyer, G.; Dikshit, R.K. Antiretroviral drugs: critical issues and recent advances. *Indian Journal of Pharmacology*, **2012**, *44(3)*, 288.
2. Chang, Y. C.; Tyring, S. K. Therapy of HIV infection. Dermatologic Therapy. **2004**, *17(6)*, 449-64.
3. Ghosh, R. K.; Ghosh, S. M.; Chawla, S. Recent advances in antiretroviral drugs. *Expert opinion on pharmacotherapy*. **2011,** *1;12(1)*, 31-46.
4. Hughes, A.; Barber, T.; Nelson, M. New treatment options for HIV salvage patients: an overview of second-generation PIs, NNRTIs, integrase inhibitors and CCR5 antagonists. *Journal of Infection*. **2008,** *1;57(1)*, 1-0.
5. Pillay, D.; Taylor, S.; Richman, D. D. Incidence and impact of resistance against approved antiretroviral drugs. *Reviews in Medical Virology*. **2000**, *10(4)*, 231-53.
6. Malhotra, G.; Joshi, K.; Ghosalkar, J. WO2019/021319A1, 2019.

7. Phull, M. S.; Kankan, R. N.; Dharmaraj, R. Antiviral phosphonate analogues and process for preparation. Patent US9227990B2, 2016.
8. Sherwood, J.; Parker, H. L.; Moonen, K.; Farmer, T. J.; Hunt, A. J. N-Butylpyrrolidinone as a dipolar aprotic solvent for organic synthesis. *Green Chemistry*. **2016**, *18(14)*, 3990-6.
9. Amilli, M., N.; Cundy, K. C; Oliyali, R.; Stella, V. WO98/04569
10. Chapman, H.; Kernan, M.; Prisbe, E.; Rohloff, J.; Sparacino,M.; Terhorst, T. Practical synthesis, separation, and stereochemical assignment of the PMPA pro-drug GS-7340. *Nucleosides Nucleotides and Nucleic acid*. **2001**, *20 (4-7)*, 621-628.