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Communication

Development and Implementation of an Aluminum-Promoted Phosphorylation in the Uprifosbuvir Manufacturing Route

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Cite This: https://dx.doi.org/10.1021/acs.oprd.0c00487

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ABSTRACT: A novel application of the synthesis of pronucleotide (ProTide) 5′-phosphoramidate monoesters promoted by aluminum-based Lewis acids is described. In the multikilogram synthesis of uprifosbuvir (MK-3682, **1**), a clinical candidate for the treatment of hepatitis C, this methodology provided >100:1 diastereoselectivity at the phosphorus stereocenter and >100:1 selectivity for the 5′-mono phosphorylation over undesired bisphosphorylation side products. The high diastereoselectivity and mono/bis ratio achieved enabled elimination of the tedious workup associated with the *tert*-butyl magnesium chloride protocol commonly used to install this functionality in similar nucleotide prodrugs, achieving a near doubling of the isolated yield from 45% to 81%. The process development and purity control strategy of MK-3682, as well as handling of the pyrophoric reagent on scale, will also be discussed.

KEYWORDS: *ProTide synthesis, Lewis acid-mediated phosphorylation, dimethylaluminum chloride, uprifosbuvir, MK-3682, pyrophoric reagents*

INTRODUCTION

Hepatitis C virus (HCV) infection is a disease of global impact, with more than 170 million infected and over 70 million presenting chronic hepatitis C and requiring treatment.[1] Chronic hepatitis C can lead to a spectrum of conditions affecting the liver, ranging from inflammation to cirrhosis and cancer.[2] Uprifosbuvir (MK-3682) is a nucleoside-based prodrug for the treatment of HCV, which, along with other clinical candidates and approved drugs,[3] utilizes the 5′-aryloxyphosphoramidate or "ProTide" moiety introduced by McGuigan et al. in the early 1990s as a strategy to enable dramatic enhancement of cellular permeability and phosphorylation rates.[4] The original phosphorylation of uprifosbuvir penultimate was performed under basic conditions using *tert*-butylmagnesium chloride following the conditions for the preparation of sofosbuvir (Scheme 1).[5] While this process was sufficient to provide material for early clinical studies, there were several challenges to address to develop a process suitable to provide material for late stage clinical studies and commercial supplies, including the following: (1) The reaction was stopped at 85% conversion to avoid increased levels of bis-phosphorylation, wasting 15% of valuable penultimate and increasing the purification burden. (2) The selectivity of mono- vs bisphosphorylation was low (~6:1). (3) A tedious workup procedure was applied to remove a series of impurities. And, (4) even after this workup, multiple crystallizations were required to remove the bis-phosphorylation byproduct and obtain the desired form. These complications led to a 45% overall yield and long cycle times. Here, we present the invention and process development of a Lewis-acid-promoted phosphorylation approach to the preparation of uprifosbuvir.

RESULTS AND DISSCUSSION

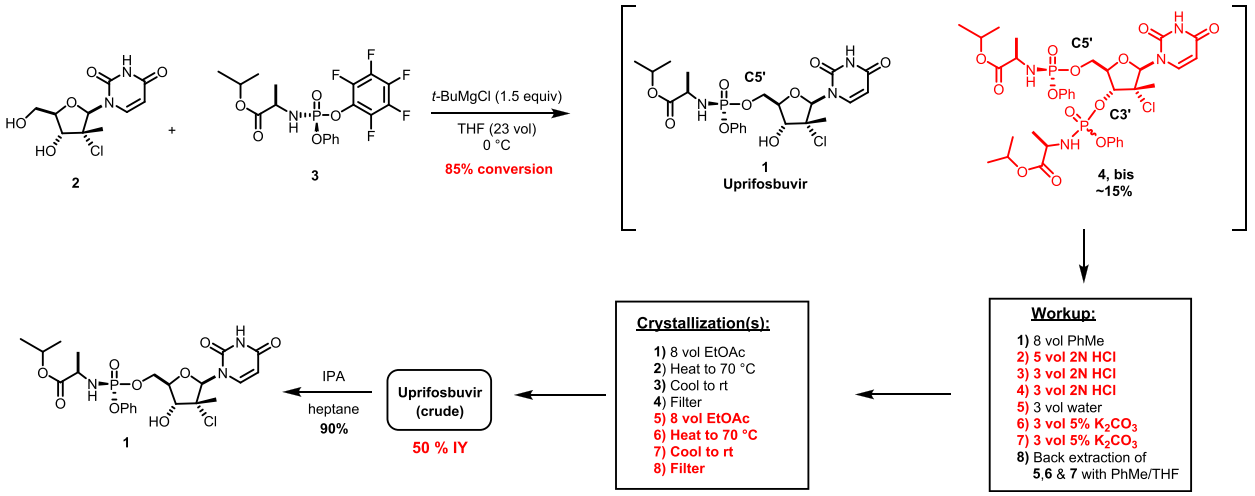
Reaction Optimization. We previously identified general conditions demonstrating the utility of dimethylaluminum chloride as a Lewis acid promoter of highly chemo- and diastereoselective phosphorylation reactions.[6] The scope of the transformation was demonstrated to be quite broad, and the method could be applied to the synthesis of uprifosbuvir (Scheme 2), but the use of pyridine as a solvent and the required chromatography initially rendered the developed conditions unsuitable for use in the uprifosbuvir API step, requiring significant reaction development to render the process suitable for the production of kilogram quantities of **1**.

Our initial investigation of alternative phosphorylation conditions in the synthesis of API **1** revealed multiple aluminum-based Lewis acids to be effective in promoting the reaction, including Et_2AlCl, *i*-Bu_2AlCl, $AlCl_3$, $Al(Ot\text{-}Bu)_3$, and DABCO-$(AlMe_3)_2$, all affording the product in >50:1 diastereoselectivity and mono- to bis-chemoselectivity at >95% conversion. Upon further analysis of the reaction profile and API isolation with this series of reagents, we concluded that Me_2AlCl was the most suitable reagent for large scale synthesis of MK-3682.[7] While $Al(Ot\text{-}Bu)_3$ and DABCO-$(AlMe_3)_2$ were more air stable than Me_2AlCl, the limited supply could not meet the projected commercial demand.

Special Issue: Celebrating Women in Process Chemistry

Received: October 30, 2020

Scheme 1. Uprifosbuvir First Generation Supply Route



Scheme 2. Selective Aluminum-Promoted Phosphorylation in the Synthesis of Uprifosbuvir

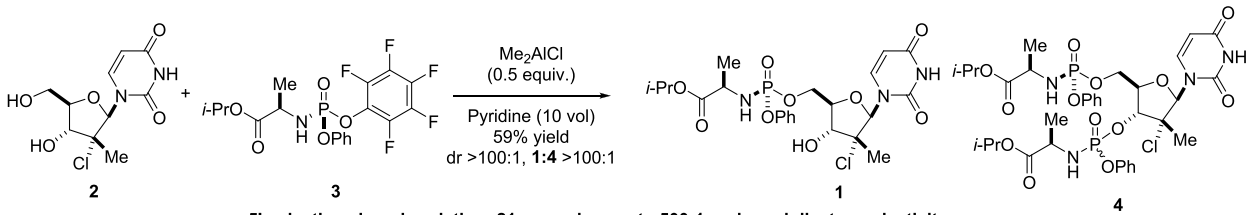
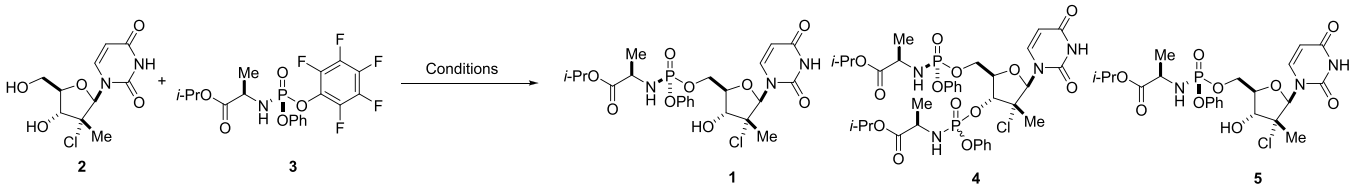


Table 1. Optimization of Phosphorylation Conditions[a]



entry	base	Me_2AlCl (equiv)	solvent	conversion (%)	dr (1:5)	ratio 1:4
1	NEt_3	0.5	THF	91	10	>100
2	pyridine	0.5	THF	59	>100	>100
3[b]	2,6-lutidine	0.5	THF	73	>100	>100
4	NMM	0.5	THF	87	39	>100
5[b]	DBU	0.5	THF	91	11	67
6[b]	NMI	0.5	THF	97	58	>100
7	2,4,6-collidine	0.5	THF	93	41	>100
8	2,6-lutidine	0.2	THF	84	4.3	121
9	2,6-lutidine	0.3	THF	94	14.3	186
10	2,6-lutidine	0.4	THF	95	46	460
11	**2,6-lutidine**	**0.5**	**THF**	**98**	**101**	**320**
12	2,6-lutidine	0.7	THF	98	96	192
13	2,6-lutidine	0.9	THF	97	95	72
14	2,6-lutidine	0.5	heptane	1	nd	nd
15	2,6-lutidine	0.5	CPME	88	40	44
16	2,6-lutidine	0.5	*i*-PrOAc	90	45	145
17	2,6-lutidine	0.5	DME	96	37	94
18	2,6-lutidine	0.5	MeCN	96	63	49
19	2,6-lutidine	0.5	2-MeTHF	97	58	67
20	2,6-lutidine	0.5	DMF	93	31	232

[a]Nucleoside **2** (1.0 equiv), **3** (1.2 equiv), base (1.5 equiv), Me_2AlCl (0.5 equiv. unless otherwise indicated), 0.2 M, room temperature for 24 h, then 50 °C for 4 h, THF as solvent unless otherwise indicated. [b]Aged for 72 h at room temperature.

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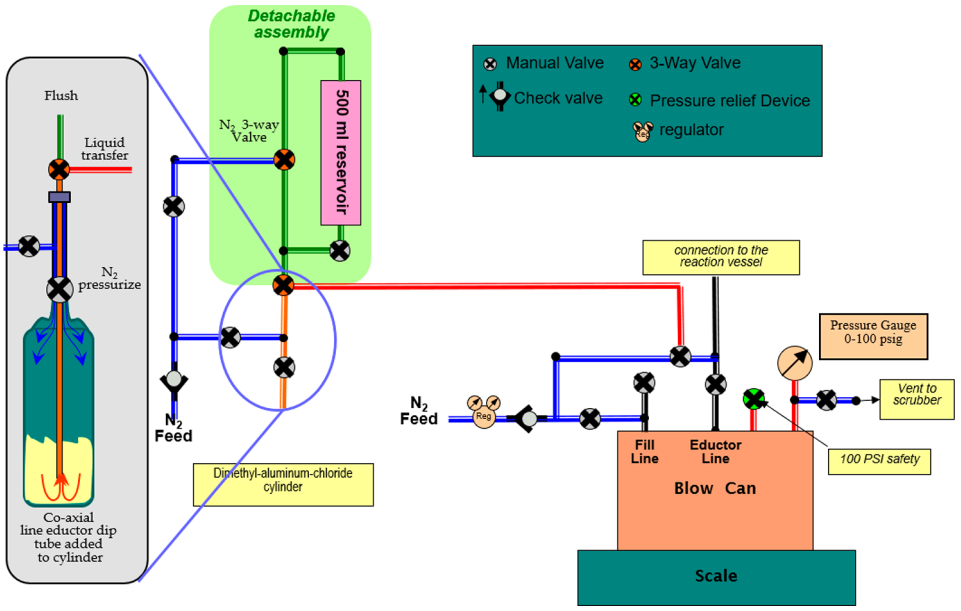

Figure 1. Diagram of dimethylaluminum chloride transfer procedure.

Additionally, the corrosive nature of $AlCl_3$ increased the difficulty in implementing this reagent on scale. Of the alkylaluminum chloride options, we observed a small amount of alkyl group transfer to phosphorus in the case of *i*-Bu_2AlCl and Et_2AlCl, but no methyl transfer was observed with Me_2AlCl. Therefore, we chose to optimize the reaction further with this reagent knowing that implementation of appropriate procedural and engineering controls would be required to allow safe handling of the reagent.

In optimizing the aluminum-promoted phosphorylation for production of API **1**, we examined several factors key to obtaining a suitable reaction profile on scale (Table 1). Trends around diastereoselectivity and mono/bis chemoselectivity were observed to correlate with base strength. Lower diastereoselectivity was observed with stronger bases such as NEt_3 and DBU (entries 1, 4, 5), likely due to the presence of additional pentafluorophenoxide, which was observed in control experiments to mediate epimerization of unreacted **3**. In contrast, higher diastereoselectivity was obtained using weaker bases such as pyridine and lutidine (entries 2−3). The reaction rate was also found to correlate with basicity, with faster rates observed with a stronger base. Both NMI and 2,6-lutidine offered an appropriate balance of rate and reaction profile, with the latter being selected for further development due to superior reaction properties as NMI produced a gum-like precipitate during lab runs, which we anticipated would cause challenges on scale. A brief survey of lutidine stoichiometry indicated that the presence of at least 0.75 equiv was necessary for the reaction to achieve full conversion. We selected 1.5 equiv as the preferred charge since this amount safely provided full conversion of starting material with only a slight decrease in diastereoselectivity relative to the starting phosphoramidate.

The effect of equivalents of Me_2AlCl added on the reaction was next studied (entries 8−13). We observed an increase in the reaction rate and diastereoselectivity with a concomitant increase in the quantity of Me_2AlCl employed up to 0.5 equiv. The highest chemoselectivity was achieved at 0.4 equiv. Me_2AlCl, with a decrease observed in the presence of additional Lewis acid (entries 11−13). No benefit to reaction performance was observed when more than 0.5 equiv was used, where diastereoselectivity was observed to be highest, leading to its selection for further optimization.

To further optimize the reaction, we performed a broad solvent screen. Low conversion was observed in heptane, likely due to the exceedingly low solubility of nucleoside **2** under these conditions (entry 14). In many other polar and nonpolar solvents, thick slurries were observed (entries 15−18) due to the low solubility of the reactants and product, which, despite the good selectivity achieved, was deemed problematic from a processing perspective. Nearly homogeneous solutions were observed in solvents such as THF, 2-MeTHF, and DMF (entries 11, 19−20), with THF (entry 11) chosen for further development as the reaction showed the cleanest overall profile.

Me_2AlCl Workup Process. Aluminum salts are prone to form precipitates or emulsions under basic conditions, rendering standard aqueous workup very challenging. Indeed, our initial isolation attempts on the lab scale resulted in substantial product loss attributable to entrainment of the API. Aqueous sodium potassium tartrate solutions have been used for aluminum workups due to the strong chelating effect on the Lewis acid.[8] For this process, we observed similar results using the more cost-effective tartaric acid, with a rapid phase split observed when an aqueous solution of the acid was added to the THF reaction stream. Accordingly, at the end of reaction, a 25−30 wt % tartaric acid aqueous solution (approximately 30% by volume relative to the total amount of solution) was added to the reaction mixture, leading to rapid phase separation and no emulsion. Unfortunately, we observed approximately 5% product loss in a subsequent aqueous wash to remove residual tartaric acid remaining in the organic layer, and it took several hours to achieve complete phase separation; neither of these observations is surprising given the aqueous miscibility of THF. The addition of isopropyl acetate at this stage facilitated phase separation of the aqueous wash and minimized the overall API loss to approximately 1%. After crystallization,

https://dx.doi.org/10.1021/acs.oprd.0c00487
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

uprifosbuvir could be obtained in high purity with less than 10 ppm aluminum in the isolated solid.

Demonstration of Kilogram Scale Process. On the lab scale, a 1 M solution of Me_2AlCl in heptane can be handled using typical safety precautions inherent to flammable organometallic solutions. However, due to commercial availability on scale, it was necessary to utilize neat Me_2AlCl for our kilo lab runs, and given its pyrophoric nature and water reactivity, it was necessary to implement additional procedural and engineering controls. The risks associated with this process were minimized by transferring neat Me_2AlCl from a cylinder into a blow can containing the desired amount of heptane to reduce its interaction with air and moisture as shown in Figure 1 (red line). This transfer allowed for an accurate mass measurement while keeping the entire system inert. The Me_2AlCl solution could then be charged slowly to the reactor containing a THF solution of the other reaction components under moderate pressure at 0 °C (Figure 1, black line), and after transfer, the line was flushed with heptane to prevent any buildup of dimethylaluminum chloride.[9] After addition of the Lewis acid, the reaction mixture was aged at room temperature for 12 h before the temperature was raised to 50 °C for 3 h, which we found to substantially improve overall conversion while maintaining high diastereoselectivity, giving 97.7% conversion (95 LCAP **1**) with a >100:1 diastereoselectivity and mono/bis ratio.

The improved reaction purity profile attributed to the Lewis acid-promoted phosphorylation allowed for API to be crystallized much more easily than the previous generation process. After tartaric acid workup, high purity product could be obtained from multiple crystallization solvent systems. This flexibility was of additional benefit as over 20 forms of uprifosbuvir have been identified, some showing dramatically reduced bioavailability. We knew that crystallization from isopropanol/heptane provided the desired API form, and fortunately, a single crystallization provided MK-3682 in the desired form and in high purity. Under these conditions, a second crystallization was not needed for purity upgrade or form turnover. On the kilogram scale, we observed the growth of large crystals unsuitable for formulation studies (Figure 2a).

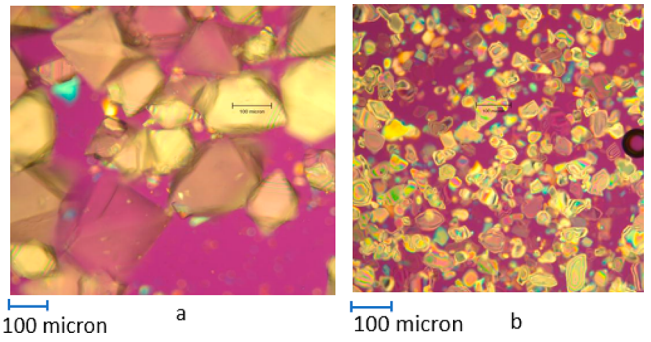

Figure 2. Control of uprifosbuvir particle size through wet milling.

Fortunately, a wet milling process was developed to readily decrease the particle size to meet our D50 < 50 μm specification (Figure 2b). From this campaign, uprifosbuvir was isolated in 82% yield and 99.7 LCAP.

Reoptimization of Reaction Conditions: In-process Control of Impurity 6. While the typical end-of-reaction profile was very clean, containing only residual starting materials, bis-phosphorylated byproduct **4**, and diastereomer **5** at levels that were easily purged during crystallization, during our initial scaleups, we observed a structurally related impurity in ~1.5 LCAP. We inferred it to be cyclic phosphoramidate **6** (Figure 3) based on its mass, and upon standing for

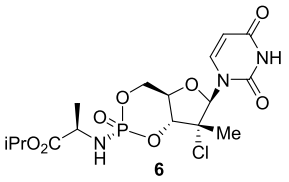

Figure 3. Structure of observed cyclic phosphoramidate impurity **6**.

approximately 3 days, we observed precipitation of the impurity from the mother liquor that enabled us to isolate and fully characterize it to confirm its identity. It also allowed additional crystallization studies necessary to formulate a control strategy. The solubility of impurity **6** in the final crystallization solvent system was measured to be only 0.9 mg/mL. As a result, this impurity could pose a risk to the isolated API purity on scale due to the longer cycle times required. To address this concern, we designed a control strategy through deeper understanding of the impurity formation, thereby ensuring that the level remained low during the reaction and mitigating risk to batch quality associated with potential crystallization of the impurity.

The slow crystallization kinetics of impurity **6** were confirmed with seeding studies (Table 2). Elevated levels of

Table 2. LCAP of Impurity 6 Present in Isolated Product

time	seeded with pure MK-3682	seeded with MK-3682 and 1.5% **6**
15 h	<0.02	<0.02
2 days	0.03	0.03
3 days	0.5	0.73
4 days	1.08	0.78

the impurity were observed in the isolated product after aging the crystallization mixture for 3−4 days; these levels were observed to be similar to those observed when MK-3682 was coseeded with 1.5 wt % phosphoramidate **6**. This observation is consistent with a slow, unseeded crystallization of this impurity from the solvent system being used to crystallize uprifosbuvir and caused a concern on scale due to the processing time.

Reinvestigating our optimized conditions, we observed the level of the cyclic impurity **6** to increase linearly with the amount of lutidine used in the reaction (Figure 4).[10] The reaction temperature, however, played a more profound role in formation of the impurity, as only a moderate increase in the reaction temperature led to a significant increase in impurity formation during the reaction. For example, when 1.25 equiv of 2,6-lutidine was used, a change in internal temperature from 35 to 45 °C increased the impurity level from 1.7% to 4.8% at 36 h under the reaction conditions. We found that the impurity level in the reaction could be controlled at 0.5−0.6 LCAP at temperatures not exceeding 35 °C for a period of 14−16 h. These conditions provided formation of uprifosbuvir in approximately 95% conversion and 92 LCAP. These finalized conditions (Scheme 3) were employed in multiple pilot plant campaigns and provided high quality material on the multikilogram scale.

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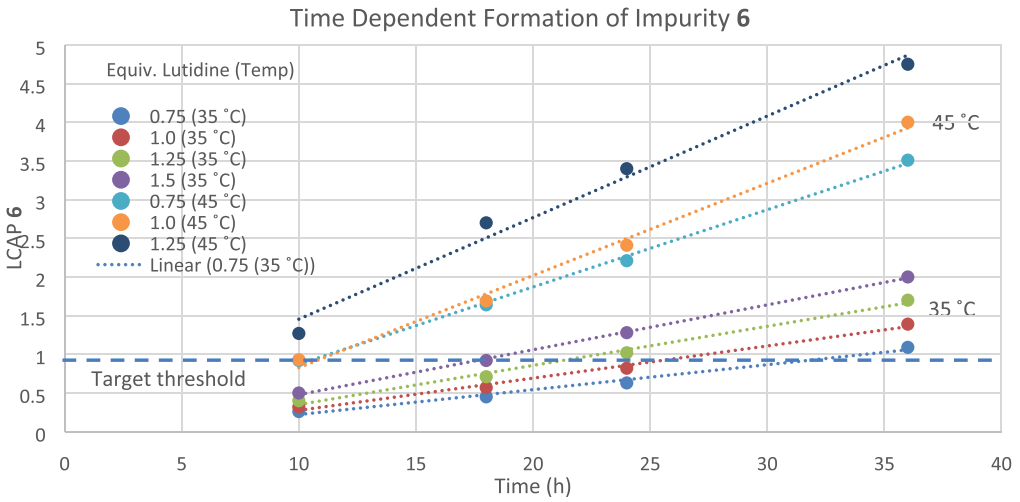
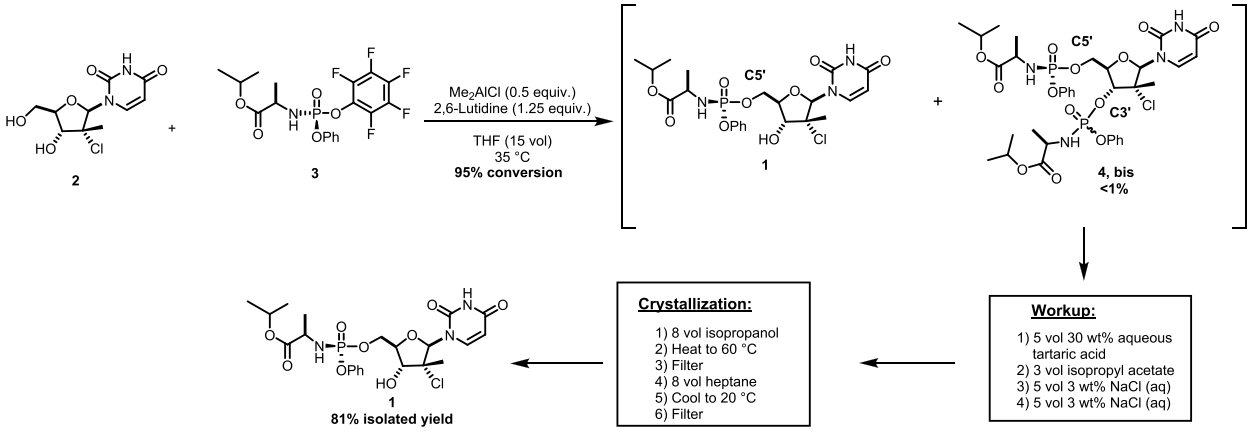


Figure 4. Formation of **6** over time at 35−45 °C. Reaction conditions: nucleoside (1.0 equiv), **2** (1.2 equiv), 2,6-lutidine (as shown), Me_2AlCl (0.5 equiv), 0.2 M in THF.

Scheme 3. Dimethylaluminum Chloride-Promoted Process for the Synthesis of Uprifosbuvir 1



CONCLUSIONS

We have shown the development and implementation of an efficient ProTide synthesis promoted by dimethylaluminum chloride that proceeds with high chemoselectivity for mono- vs bisphosphorylation and with high diastereoselectivity in the uprifosbuvir manufacturing route. The formation of a cyclic phosphoramidation impurity was identified and studied, and a control strategy was developed to minimize any associated risk to the process. When techniques developed to safely handle the pyrophoric Me_2AlCl were used, this process was successfully executed on a multikilogram scale to produce API meeting all required specifications for use in late-stage clinical trials.

EXPERIMENTAL SECTION

Synthesis of Uprifosbuvir (1). To a 100 L glass lined vessel equipped with a nitrogen inlet, overhead stirrer, and thermocouple was charged THF (21.5 L) followed by 1-((2*R*,3*R*,4*R*,5*R*)-3-chloro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (1.500 kg, 5.42 mol) and (*R*)-isopropyl 2-(((*R*)-(perfluorophenoxy)(phenoxy)phosphoryl)amino)propanoate (2.95 kg, 6.51 mol). The reactor was cooled to an internal temperature of 0 °C. 2,6-Lutidine (0.944 L, 8.13 mol) was charged slowly to the vessel while maintaining a temperature of <5 °C. The jacket temperature was then set to −10 °C, and dimethylaluminum chloride (~1 M solution in heptane premade from neat reagent in the blow can, 1.895 kg) was charged under nitrogen pressure over approximately 1 h. The charge apparatus was rinsed with heptane (2 × 375 mL). The resulting suspension was warmed to 30−35 °C and aged for 15−16 h. The batch was cooled to an internal temperature of 10 °C, and a 30 wt % tartaric acid solution (8.14 kg) was added, followed by isopropyl acetate (4.5 L). The aqueous phase was separated, and the organic layer was washed with a 3 wt % aqueous sodium chloride solution (2 × 7.5L). A constant volume distillation was then performed adding isopropanol to the reactor until the solvent composition reached <5 mol % THF, as determined by ^{1}H NMR spectroscopy. A final concentration of the organic layer was performed to reach an 11.8 L total volume. The solution was filtered at 50 °C, and the batch was heated to an internal temperature of 50−60 °C and seeded (45 g seeds). Heptane (12 L) was charged slowly to the batch while maintaining a constant internal temperature, at which point the batch was slowly cooled to 20 °C and allowed to crystallize over 14 h. The batch was heated to 60 °C and wet milled for 3 h to meet the desired particle size specifications, after which it was cooled to 20 °C, filtered, and washed with isopropanol/heptane (1:1). The wet cake was dried under a vacuum at 40 °C to give **1** (2.41 kg, 81%). ^{1}H NMR (500

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Org. Process Res. Dev. XXXX, XXX, XXX−XXX

MHz, DMSO-d_6): δ (ppm) 11.64−11.40 (s, 1H), 7.74−7.49 (d, J = 8.1 Hz, 1H), 7.48−7.32 (t, J = 7.9 Hz, 2H), 7.32−7.22 (d, J = 8.3 Hz, 2H), 7.24−7.11 (t, J = 7.3 Hz, 1H), 6.33−6.21 (s, 1H), 6.21−6.06 (m, 2H), 5.62−5.52 (dd, J = 8.1, 1.7 Hz, 1H), 4.95−4.68 (hept, J = 6.2 Hz, 1H), 4.50−4.35 (dd, J = 11.4, 3.7 Hz, 1H), 4.35−4.20 (m, 1H), 4.17−4.01 (d, J = 9.2 Hz, 1H), 3.92−3.67 (ddt, J = 24.4, 10.0, 7.0 Hz, 2H), 3.39−3.24 (s, 1H), 1.50−1.31 (s, 3H), 1.31−1.19 (d, J = 7.1 Hz, 3H), 1.18−1.05 (dd, J = 8.5, 6.4 Hz, 6H). ^{13}C NMR (125 MHz, DMSO): δ 172.61, 172.57, 162.62, 150.69, 150.64, 150.50, 129.68, 124.55, 119.90, 119.86, 102.06, 80.03, 77.73, 72.34, 67.99, 63.59, 49.68, 22.37, 21.33, 21.28, 19.74, 19.69. LC-MS calcd for $C_{22}H_{30}ClN_3O_9P$ [M + H]+ m/z: 546.14. Found: 546.2.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.0c00487.

NMR characterization of specified impurities is provided along with spectral data (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Aaron Moment, Morgan Crawford, and Darryl Chang for discussions and experimental contributions. We thank Ryan Cohen and Rose Mathew for help with impurity characterization. We thank Rebecca Ruck, Paul Bulger, Neil Strotman, and L.-C. Campeau for proofreading suggestions.

■ ABBREVIATIONS USED

V = volume
LCAP = HPLC/UPLC area percentage
IY = isolated yield
AY = assay yield

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https://dx.doi.org/10.1021/acs.oprd.0c00487
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

their limited availability or corrosive nature proved prohibitive for multikilogram use.

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(9) To protect the scientists executing the procedure, fire-resistant aluminum lab coats (Fisher Part Number 19-049676) were used as an added precaution.

(10) For instance, the impurity increased from 1% with 0.75 equivalents of lutidine to 2% with 1.5 equivalents of lutidine after aging at 35 °C for 36 h.

https://dx.doi.org/10.1021/acs.oprd.0c00487
Org. Process Res. Dev. XXXX, XXX, XXX−XXX