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Process Improvements for the Manufacture of Tenofovir Disoproxil Fumarate at Commercial Scale

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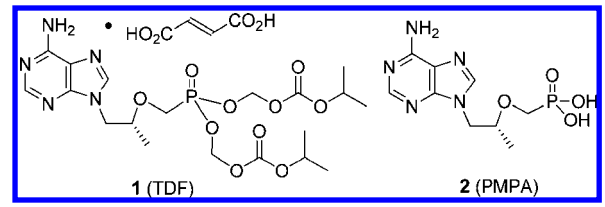
Abstract:

The three-step manufacturing process used in the synthesis of tenofovir disoproxil fumarate (1) was studied and optimized, leading to a more productive and robust process. The yield was improved from about 13% overall to 24%. Key process improvements identified included implementation of a telescoped process for the second stage that obviated the need for an extraction and solvent exchange, and significant optimization of the final reaction, including the beneficial effect of adding a quaternary ammonium salt to the alkylation reaction and development of a nonaqueous process for removal of NMP and triethylamine from the product mixture to decrease the level of decomposition of product during the isolation.

Introduction

Tenofovir disoproxil fumarate (**1**, or TDF) is a nucleoside analogue reverse transcriptase inhibitor (nRTI) that is used in the treatment of HIV/AIDS and hepatitis B.[1] TDF is a prodrug of tenofovir (**2**, also known as PMPA) designed to improve absorption and cell permeability of the active moiety. See Scheme 1. There are a number of advantages to the use of tenofovir in HIV therapy over the alternatives zidovudine (AZT) and stavudine (d4T), including a long half-life (allowing for once-daily dosing), a lower rate of resistance development, and improved side-effect profiles when compared to the older medications.[2]

Scheme 1. **Tenofovir disoproxil fumarate and its active metabolite, tenofovir**



From a manufacturing perspective, the diminished stability in water that makes **1** an effective pro-drug also adds to the difficulty in manufacturing and isolating this product. Hydrolysis of the disoproxil side chains occurs readily under a range of conditions, complicating the workup and isolation of the tenofovir disoproxil free base. As a result, the design of the reaction and isolation to form the API represents the most critical portion of the manufacturing process.

TDF (**1**) is manufactured in a three-stage, four-reaction process as shown in Scheme 2. Reaction of readily available adenine (**3**) with *R*-propylene carbonate (**4**) is followed by base-promoted alkylation of the secondary alcohol with tosylated hydroxymethylphosponate diester, **6**. Hydrolysis of the diethyl phosphonate esters produces tenofovir (**2**). An alkylative esterification completes the process to produce crude free base, **9**, which is then treated with fumaric acid to crystallize TDF (**1**).

The yields for this process[3] are fair to modest, with an overall yield from **3** of about 13%. The third stage, for the reason described above, is particularly challenging, with isolated yields of **1** of only about 35% based upon **2**. This contribution reports improvements throughout the process, with a particular focus on the stage 3 chemical transformation and isolation procedures.

Results and Discussion

Stage 1. Synthesis of Hydroxypropyl-adenine (5). Adenine (**3**) and *R*-propylene carbonate (**4**) react readily at 120 °C in the presence of sodium hydroxide in DMF to form alcohol **5** in high yield. *R*-propylene carbonate (**4**) is used in excess (1.3

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(1) (a) Arimilli, M. N.; Kim, C. U.; Dougherty, J.; Mulato, A.; Oliyai, R.; Shaw, J. P.; Cundy, K. C.; Bischofberger, N. *Antiviral Chem. Chemother.* **1997**, *8*, 557. (b) Naessens, L.; Bischofberger, N.; Augustijns, P.; Annaert, P.; Van Den Mooter, G.; Arimilli, M. N.; Kim, C. U.; De Clerq, E. *Antimicrob. Agents Chemother.* **1998**, *42*, 1568. (c) Robbins, B. L.; Srinivas, R. V.; Kim, C.; Bischofberger, N.; Fridland, A. *Antimicrob. Agents Chemother.* **1998**, *42*, 612.
(2) Balzarini, J.; Holy, A.; Jindrich, J.; Naesens, L.; Snoeck, R.; Schols, D.; De Clerq, E. *Antimicrob. Agents Chemother.* **1993**, *37*, 332.
(3) Arimilli, M. N.; Cundy, K. C.; Dougherty, J. P.; Kim, C. U.; Oliyai, R.; Stella, V. J. U.S. Patent 5,922,695, 1998.

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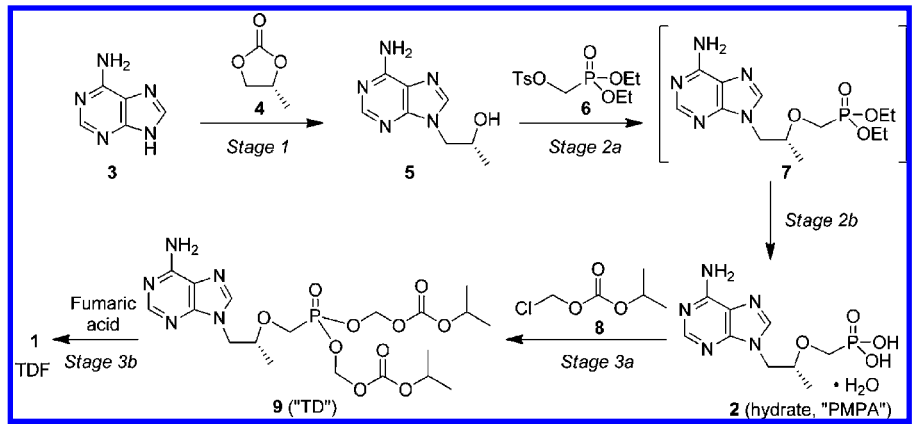
Scheme 2. **Manufacturing route to tenofovir disoproxil fumarate**



equiv) to ensure that the reaction goes to completion; the reaction proceeds relatively quickly, while **4** and the desired product **5** were confirmed to be quite stable under the reaction conditions. The only significant impurity formed is a regioisomer, assigned structure **10** (Figure 1), in an approximately 10:1 product:impurity ratio. Precipitation of solids from the reaction mixture by addition of toluene provides an 84% yield of crude **5**, contaminated with 7−8 area % of **10**. However, we found that the product can be crystallized in 66% yield from the DMF reaction solvent by addition of a 1:1 MeOH/*i*-PrOH mixture to provide product of 97.8 area % purity, with only 1.7% regioisomer remaining. This impurity can be carried forward in the higher amount to the next stage of the process, but we elected to reduce the impurity level at this point in the process, in order to maximize utilization of reagents and reduce the amount of potential impurities in the second reaction stage.

Stage 2. Preparation of Phosphonic Acid 2. The alcohol **5** is converted into **2** in a two-reaction sequence involving alkylation with the tosylate **6** followed by hydrolysis. The two reactions are optimally telescoped together, as the intermediate product, the diethylphosphonate, **7**, is water-soluble, prone to hydrolysis to the corresponding monoester, and difficult to crystallize. The alkylation reaction can be run in a number of polar aprotic solvents, with DMF and NMP giving best results. The presence of DMF in the second stage of the sequence (de-esterification reaction) leads to some dimethylphosphamide impurity formation as a result of the presence of free dimethylamine as a contaminant in DMF, leading to the selection of NMP for the two-stage process.

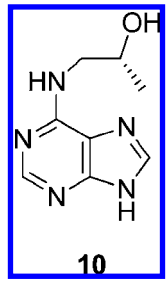


Figure 1. **Stage 1 key impurity.**

Table 1. **Selection of base for stage 2a**[a]

base	conversion to **7** (%)
LiO-*t*-Bu	61
LiO-*i*-Pr	35
LiO-$CH_2C(CH_3)_3$	59
NaO-*t*-Bu	39
NaO-*i*-Pr	0
NaO-$CH_2CH(Et)C_4H_9$	68
KO-*t*-Bu	10
Mg(O-*t*-Bu)$_2$(MTB)	90
LiHMDS	52

[a] Standard conditions: **5** (1 equiv), base (3 equiv), NMP (5 vol), **6** (1.5 equiv), 70 °C.

Stage 2a. The alkylation can, in principle, be effected with a number of bases other than the reported lithium *tert*-butoxide[3,4] and magnesium *tert*-butoxide (MTB).[5] Our trials with various hindered alkoxide bases and with lithium hexamethyldisilazide (Table 1) show that magnesium *tert*-butoxide (MTB) provides a significantly higher *in situ* conversion to **7** than any alternative investigated. Other bases including carbonates, DBU, tetramethylguanidine, and phosphazene bases provided less than 50% conversion.

We noted, however, variation in results dependent upon the batch or supplier of MTB. A number of additives (potential ionic contaminants) were studied in this reaction in an effort to determine what might affect the performance of the MTB (Table 2), and analytical tests (including base titration, KF, IR, TGA, and a determination of *tert*-butyl alcohol levels) were employed. While some correlations were observed, none of these tests proved predictive of reaction success, although control of halide content seems the most likely factor to consider. The authors recommend use-testing of all material to ensure successful reactions.

Stage 2b. The hydrolysis of diester **7** to tenofovir (**2**) proceeds via a Lewis acid-promoted nucleophilic dealkylation.[6] The previous reported conditions for this reaction are the use of a substantial excess (6 equiv) of TMSBr. In addition to being

(4) Schultze, L. M.; Chapman, H. H.; Dubree, N. J. P.; Jones, R. J.; Kent, K. M.; Lee, T. T.; Louie, M. S.; Postich, M. J.; Prisbe, E. J.; Rohloff, J. C.; Yu, R. H. *Tetrahedron Lett.* **1998**, *39*, 1853.
(5) Becker, M. W.; Chapman, H. H.; Cihlar, T.; Eisenberg, E. J.; He, G.-X.; Kernan, M. R.; Lee, W. A.; Prisbe, E. J.; Rohloff, J. C.; Sparacino, M. L. PCT Intl. WO/0208241A32002.
(6) Houghton, S. R.; Melton, J.; Fortunak, J.; Brown Ripin, D. H.; Boddy, C. N. Manuscript in preparation.

Table 2. **Effect of additives on conversion in 5 + 6 with MTB**

additive	conversion with 0.1 equiv (%)	conversion with 0.5 equiv (%)	conversion with 1.0 equiv (%)
$MgBr_2$	65	33	66
$MgCl_2$	82	80	30
$Mg(OH)_2$	84	83	84
NaBr	69	59	38
NaCl	86	85	79
NaOH	84	62	46
NaOTs	88	85	82

expensive and variable in quality, TMSBr requires special handling to minimize the destructive impact of this reagent on the materials of reactor construction. Two practical alternatives to TMSBr for the execution of this process are available. Either aqueous HBr[7] or the use of TMSCl in the presence of NaBr in organic solvent[6] was preferred for process stage 2b. The use of aqueous HBr or other mineral acid for the de-esterification requires first a high-vacuum distillation to remove organic solvent (e.g., DMF) or a high-volume dichloromethane (DCM) extraction of the highly water-soluble intermediate (**7**) from the water/NMP (or DMF) layer following the alkylation reaction (stage 2a).

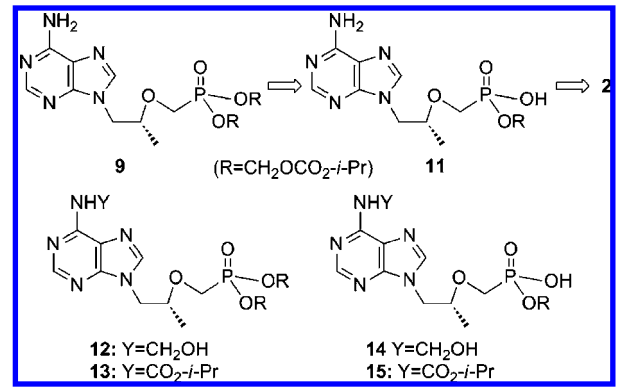
As a result, we chose to optimize a telescoped process wherein the alkylation reaction is run in NMP, taken through nonaqueous workup to remove salts, and hydrolyzed in the remaining NMP using TMSCl and NaBr. Workup and pH adjustment precipitates phosphonic acid **2** as its hydrate. This process precludes the need for an intermediate product extraction.

The mass balance of the process was monitored to determine where product loss occurs. The alkylation with **6** reproducibly provides an 85−90% *in situ* yield of the intermediate product **7**, with the remainder of the mass being residual starting material and a number of lower-level impurities. In the magnesium salt cake, a 15% loss of **7** is observed; some of this loss can be recovered in a manufacturing setting with a stirred filter but can be difficult in a lab setting where the salt cake quickly absorbs atmospheric water. This intermediate is hydrolyzed to **2** via the intermediate monoethyl phosphonate ester in 95% conversion. Following the addition of water, little product is lost in the subsequent organic extractions to remove residual organic material, and a 55% yield of product **2** (from **5**) was isolated in the experimental run, with 13% of product remaining in the aqueous mother liquors.

Stage 3a Process Analytical Technology. Assay for Formation of Tenofovir Disoproxil (9). Before work could be done to optimize the formation and isolation of **1** from **2**, an effective analytical method for the assay of the stage 3a process had to be developed. Several key impurities (including persistent reaction intermediate **11** along with *N*-hydroxymethylated impurities **12** and **14**, and isopropylcarbamate impurities **13** and **15**) are consistently observed in the stage 3a process (Scheme 3).

The ability to separate the *N*-hydroxymethyl impurities proved to be highly dependent on the pH of the mobile phase;

Scheme 3. **Key impurities formed in the stage 3a process**



an aqueous component at pH 2.5 was ideal. Using these conditions the major impurities are well resolved. In the chromatogram of a reaction (not optimized) shown in Figure 2, **2** elutes at a retention time of 0.82 min followed by **11** and **14** at 0.97 and 1.17 min. The diester **9** elutes at 5.60 min, well separated from the main *N*-hydroxylmethyl impurity **12** at 6.26 min. It was observed during development that use of a higher pH buffer results in less effective separation of impurities and column performance that deteriorates rapidly over multiple injections. At pH 5.5 no resolution is observed. In addition to its use in reaction monitoring, this method has been used successfully for assaying process streams as well as evaluation of isolated API. This method allowed us to improve the throughput, reproducibility, and scalability of the stage 3 process.

Stage 3. Research Objectives. Stage 3 of the TDF process comprises three operations. The first is the reaction of **2** to yield diester **9** in solution. The second is the workup of diester **9** to partially remove impurities and provide **9** suitable for conversion to the fumarate salt **1**. The third operation is formation of **1** itself. This discussion primarily focuses on optimization of the first and second of these operations.

Stage 3a: Synthesis of 9. The conversion of **2** into **9** by base-promoted alkylation with excess chloromethyl isopropyl carbonate (**8**) is accompanied by physical realities that complicate the chemical reaction and workup. Due to the poor solubility of **2** and its salts, the stage 3a reaction must be run in a polar, aprotic solvent, with non-nucleophilic amine bases being preferred (triethylamine, DIPEA, TMG, and 2,6-lutidine are notable). The phosphonic acid **2** is not initially soluble in NMP (or other solvents such as DMF, DMSO, or acetonitrile). Addition of triethylamine to a stirred suspension of **2** in NMP at 40−60 °C causes the rapid dissolution of **2** to give a solution. After a short period of stirring, however, a thick suspension is formed by precipitation of a mixture of mono- and bis-triethylammonium salts of **2**. Upon addition of **8**, alkylative esterification gradually occurs; monoester **11** and diester **9** are soluble in the reaction medium, and the suspension of salts gives way to a solution as the reaction proceeds. The conversion of **11** to **9** is significantly slower than the initial alkylation of **2** to give **11**. As conversion to **9** proceeds, the reverse reaction (de-esterification of **9** to **11**) becomes competitive with the rate of the forward reaction itself. We have confirmed that this is purely an effect of the slow conversion of **11** to **9**, while reagent **8**

(7) Vasireddy, U. M. R.; Vellanki, S. R. P.; Balusu, R. B.; Bandi, N. D. R.; Jujjavarapu, P. K.; Ginjupalli, S. R.; Pilli, R. K. PCT Intl. WO2008/007392A2, 2008.

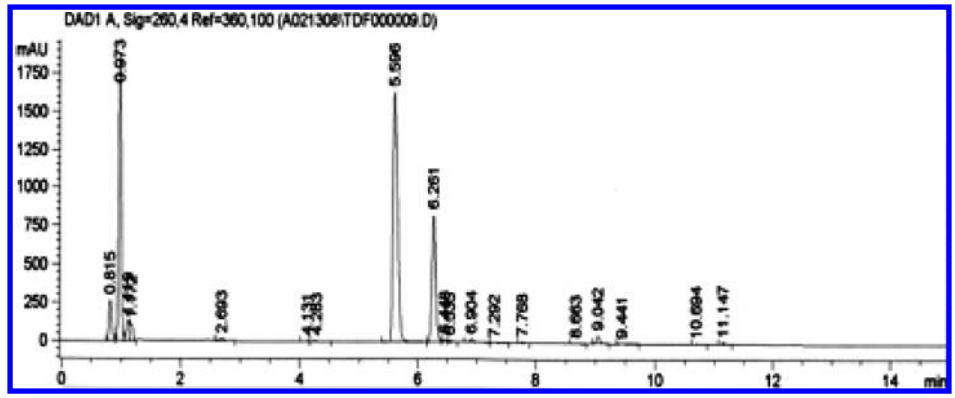


Figure 2. **HPLC of reaction mixture with pH 2.5 aqueous eluent.**

and triethylamine do not react together or otherwise undergo significant decomposition under the reaction conditions.

Running the reaction using the conditions published by Gilead[3] (4 vols of NMP, 4 equiv of TEA, 4.7 equiv of **8**,[8] 60 °C, 4 h), a typical reaction result is approximately 50−65% *in situ* yield, with 5−10% of the *N*-hydroxymethylated impurity **12** and 10−15% of the monoester intermediate **11** (and 2−5% its *N*-hydroxymethylated analogue **14**) present along with other lower level impurities, including **13** and **15**. Efforts to push the reaction further to completion lead to product decomposition at a greater rate than product formation. Indeed, the product quality degrades steadily once the reaction reaches maximum conversion, leading inevitably to lower yields as the process is scaled (due to increased process time with scale). Isolated yields for the reported procedure rarely exceed 35%.

The isolation of product also proved to be problematic, with significant degradation observed throughout the procedure. Product decomposition during workup is primarily a result of aqueous hydrolysis, with NMP bringing water into the organic layer of the extractions and taking product into the aqueous layer. Additionally, the presence of TEA in the workup leads to a basic water layer (pH = 10), promoting this hydrolysis. With several back-extractions required to maximize recovery of product, the process proved to be extremely sensitive to scale.

Therefore, an intensive investigation was undertaken to improve conversion, product stability during isolation, and overall robustness of the process. Several critical processing parameters were identified that affect the outcome of both the reaction to make **9** and its isolation. These include base selection, reaction concentration, water content in the starting material and reaction mixture, and reaction temperature. As the starting material mixture is sparingly soluble in the reaction solvent, addition of phase-transfer reagents was also investigated. Once the above parameters were largely optimized, a follow-up investigation considered the reaction solvent chosen, in the event that the improved conditions might allow a solvent choice making for easier product isolation. For the workup and isolation, solvent considerations (e.g., extractions), pH, and processing time were seen as critical to maximizing quality and throughput.

For comparison of reaction conditions, reaction mixtures were sampled during the alkylation stage at various time points,

(8) For studies in this paper, 5.0 equiv of **8** were used. Further optimization is possible.

Table 3. **Effect of solvent volume on stage 3a conversion**[a]

volumes NMP	product (**9**) (%)	monoester (**11**) (%)	N-CH_2OH impurity (**12**) (%)	time to max *in situ* yield of **9** (h)
1.5	58.3	7.4	18.6	6
3	63.7	10	12.1	6
4	63.3	15.3	9.9	7
6	64.0	12.4	10.1	7
8	67.1	13.2	7.7	9
8[b]	69.9	6.3	11.5	3

[a] Standard conditions: 5 equiv of **8**, 4 equiv of TEA base, 50° C. [b] Reaction run with 8 equiv of **8**.

and the relative success of the reaction was based on the maximum conversion (area % of **9** by HPLC) found. As well, area percent levels of key intermediates and process impurities were also monitored, and are reported below.

Optimization of Formation and Isolation of Tenofovir Disoproxil (9). Base. Bases screened included inorganic bases Na_2CO_3, K_2CO_3, and Li_2CO_3, and organic bases triethylamine (TEA), *N*,*N*-diisopropylethylamine, tetramethylguanidine, and 2,6-lutidine. Inorganic bases produced insoluble phosphonic acid salts and negligible conversions. Among organic bases, TEA provided the best performance in the reaction. Use of three or more equivalents provided optimum conversion. Running the reaction with prepared lithium, sodium, or potassium salts of **2** in the presence of TEA also led to low conversions, presumably due to the poor solubility of the salt.

Reaction Concentration. Selecting an optimal reaction concentration (solvent volume) is a balancing act, as illustrated by the results in Table 3: more *N*-hydroxymethylated impurity is formed at higher concentrations, therefore favoring more solvent/higher dilution. However, there is a practical upper limit to the amount of NMP that can be used, with respect to the aqueous workup, that favors lower solvent amounts. At higher dilution the reaction is enhanced by additional quantities of **8**, but at significant additional cost. These experiments led to selection of four volumes of NMP as the optimum; this provides a relatively thick, but stirrable, reaction mixture.

Water. Water is a major factor in impurity formation and in product stability. When water is present in the stage 3a reaction, formation of the *N*-hydroxymethylated impurities **12** and **14** is enhanced. If the stable, hydrated, form of **9** is used directly, resulting in water content of >6000 ppm in the reaction mixture, these impurities may comprise >25% of the product mixture. With reaction mixtures at <500 ppm water, 5−10% impurity

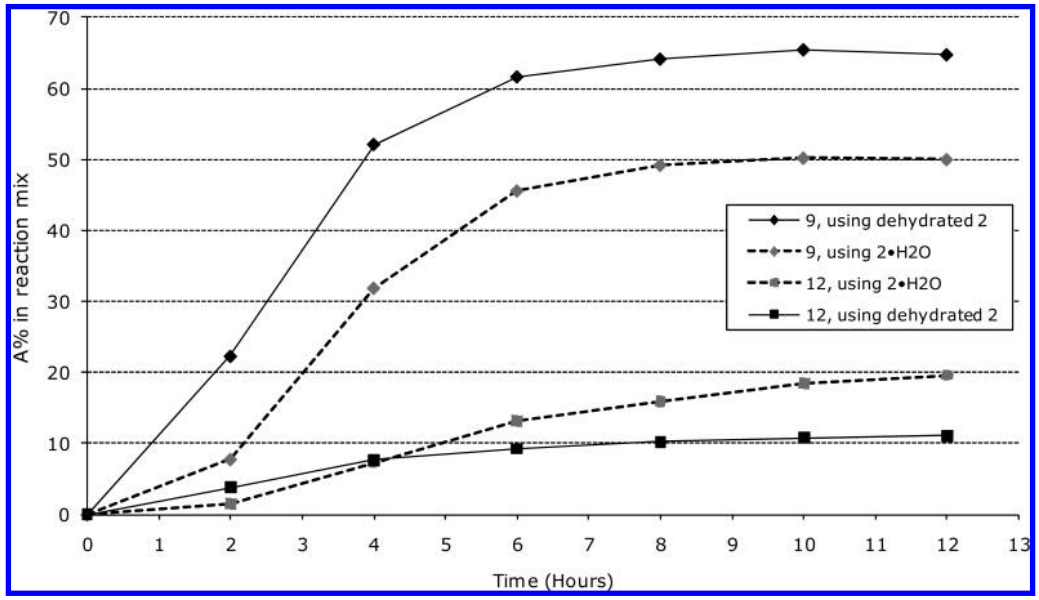


Figure 3. **Effect of water on stage 3a: hydrated vs dehydrated 2. Standard conditions: 4 volumes NMP, 5 equiv 8, 4 equiv TEA, 50 °C.**

formation is observed (see Figure 3). There are a number of possible mechanisms for the formation of these impurities, and the fact that there is still *N*-hydroxymethylated byproduct formation even in the driest reactions (<50 ppm water) seems to indicate that more than one mechanism for the formation of formaldehyde or a reactive equivalent are at work. Lower amounts of hydroxymethlyated impurity are formed at higher dilution, supporting this hypothesis.

Two methods for controlling water levels were identified: drying **2** at high temperature to drive off crystalline water, and using a solvent for azeotropic drying of the suspension of **2** in NMP prior to addition of reagents in the stage 3a process. The optimal and most efficient process for drying may be to dehydrate **2** at 70−90 °C under vacuum in an agitated dryer, to provide the anhydrous form of **2** prior to the subsequent reaction. Anhydrous **2** is hygroscopic, forming again the stable monohydrate, and care must be taken to prevent any rehydration prior to the stage 3a process. Alternatively, azeotropic distillation of a suspension of **2** in NMP using either cyclohexane or toluene is effective for producing a reaction mixture of low water content. In practice, in order to ensure low water content, both high-temperature drying of the solid and azeotropic drying of the NMP suspension were used for scale-up studies.

Additive. As the TEA salt of **2** (as initially formed in the reaction) has limited solubility in NMP, and the reaction is heterogeneous until conversion nears its maximum, it was postulated that a phase-transfer catalyst might enhance the rate and conversion in the reaction. Additionally, the *in situ* generation of a more reactive derivative of **8**, such as the bromide or iodide, might also improve the reaction profile. To test this, a number of additives were tested in the reaction (Table 4). A significant enhancement of the reaction rate was observed when tetrabutylammonium bromide (TBAB) was included, with the reaction reaching maximum conversion at 4−6 h with additive vs 8−10 h without (Figure 4). The lack of improvement on addition of Bu_4NCl and Bu_4NOTs indicates that the improvements are likely a result of the presence of bromide, with a likely positive effect due to enhanced solubility of the salts of **2**. However, we note that the bromo and iodo analogues of **8**—which may be prepared but are unstable to isolation as neat substances—do not give better reactions than does **8** itself. There is some enhancement at substoichiometric levels of additive, but the best results were obtained with one equivalent of tetrabutylammonium bromide. Other tetraalkylammonium bromides may also be suitable; the reaction was comparably successful with $BnBu_3NBr$.

As shown in Table 4, the use of a phase transfer reagent not only improved the reaction rate but also appeared to stabilize the product in the reaction mixture, maintaining a low rate of formation of impurities (such as **12**, cf. Figures 3 and 4) under

Table 4. **Effect of phase transfer reagent additives on stage 3a conversion[a]**

additive	equiv of additive	product (**9**) (%)	monoester (**11**) (%)	N-CH_2OH (**12**) (%)	time to max in situ yield of **9** (h)
none	0	63.3	15.3	9.9	10 h
Bu_4NCl	1	64.8	15.3	12.0	8 h
Bu_4NBr	1.5	74.5	4.8	10.0	3 h
Bu_4NBr	1	75.6	5.8	10.5	4 h
Bu_4NBr	0.5	71.5	8.6	8.8	6 h
Bu_4NBr	0.1	67.6	11.9	11.4	7 h
Bu_4NBr + NaI	0.5 each	38.9	13.7	12.5	8 h
$BnBu_3NBr$	1	72.0	9.4	9.4	6 h
Bu_4NOTs	1	66.3	9.3	13.7	8 h
NaOTs	3	0	7.0	0	n/a

[a] Standard conditions: 4 volumes NMP vs **2**, 5 equiv **8**, 4 equiv of TEA base, 50 °C.

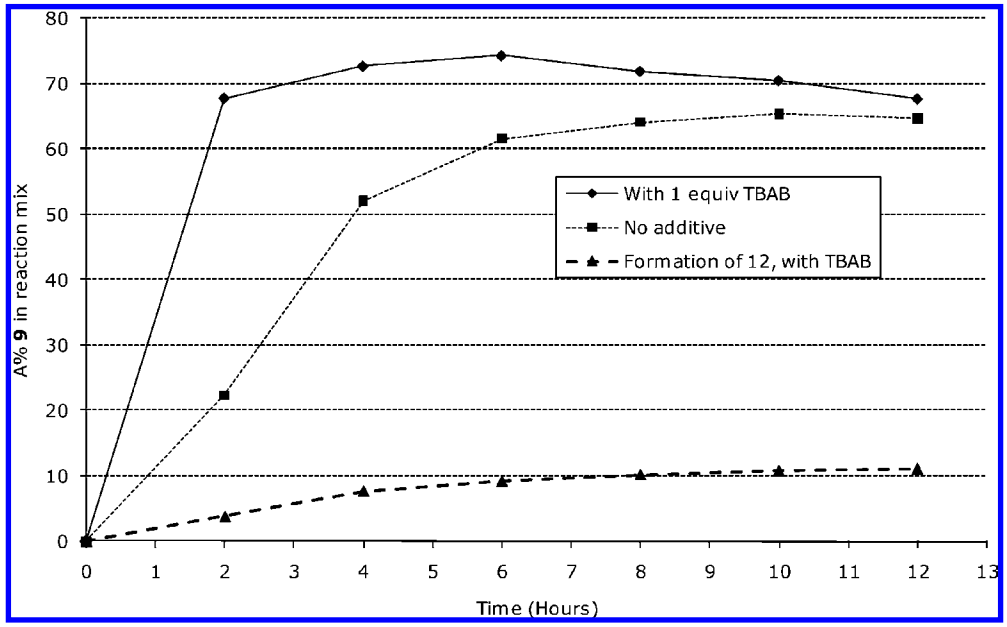


Figure 4. **Reaction profile with and without added TBAB.**

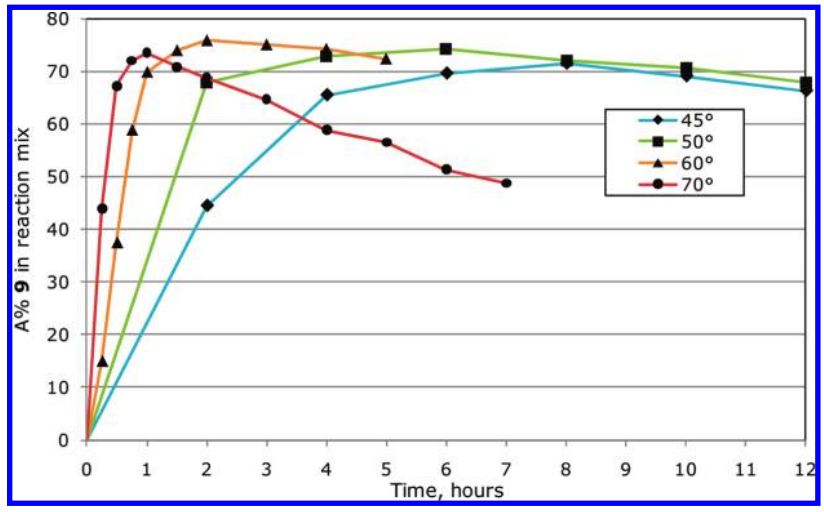


Figure 5. **Temperature optimization, with phase transfer reagent. Standard conditions: 4 volumes NMP, 5 equiv 8, 4 equiv TEA, 1 equiv TBAB.**

the reaction conditions. In a separate experiment, when **9** is subjected to the reaction conditions, the reverse reaction (hydrolysis) as well as formation of impurities **12** and **14** is slower in the presence of TBAB than in its absence.

Temperature. The combination of water removal and addition of TBAB allowed for the reaction temperature to be lowered from the reported reaction temperature of 65−70 °C. As can be seen from Figure 5, reducing the reaction temperature from 70 to 60 °C or lower leads to a significant improvement in the stability of the product under the reaction conditions. When the phase transfer reagent is used, a temperature range of 50−60 °C is indicated, maximizing conversion, stability, and reduced reaction time.

Solvent. A polar aprotic solvent is necessary for the alkylation reaction to proceed adequately, primarily driven by solubilities of the starting material, its salts, and salts of the monoalkylated intermediate.[9] The reported best solvent for the reaction is NMP, but workup of reactions using this solvent can be more difficult when compared with other polar aprotic solvent options. Therefore, once the process improvements above had been identified, we undertook a cursory examination of solvent selection using the improved conditions (Table 5). The reaction proceeds at a reasonable rate in acetonitrile, but the formation of new impurities is observed, presumably from reaction of acetonitrile with **8**, followed by reaction of the

Table 5. **Stage 3a conversion in different solvents**[a]

solvent	product (**9**) (%)	monoester (**11**) (%)	N-CH_2OH (**12**) (%)	time to max in situ yield of **9** (h)
ethyl acetate	14.9	12.0	7.4	9 h
acetonitrile	48	17.5	10.2	8 h
DMSO	52.5	13.9	13.1	3 h
DMF	63.6	11.2	14.5	4 h
NMP	75.6	5.8	10.5	4 h

[a] Standard conditions: 5 volumes solvent, 5 equiv **8**, 1 equiv TBAB, 4 equiv TEA, 50 °C.

(9) Poor sampling technique can lead to the impression that the reaction is proceeding cleanly in less polar solvents, while in actuality the conversion is low.

activated intermediate (based on MS data). In DMF, higher levels of *N*-hydroxymethylated impurities as well as solvent-derived *N*,*N*-dimethylphosphamide impurities are observed. The use of DMSO leads to significant impurity formation, including oxidation byproduct. As well, the reverse reaction rate of **9** to **2** is enhanced in DMF and DMSO relative to NMP. Thus, NMP was confirmed as the optimal solvent for the stage 3a process.

Stage 3. Improved Workup of 9 and Isolation of 1. Once the stage 3a reaction is completed, a significant amount of work is required to isolate **9** of sufficient purity to form **1** (TDF) that meets specifications. The published procedure[3] includes precipitation of triethylamine salts by addition of isopropyl acetate, and aqueous workup of the filtered solution to remove the NMP and additional salts from the solution of **9**. It was noted that the pH of the aqueous phase during this workup was approximately 10. Under these conditions, decomposition of the product was measured as the workup progressed. As the reaction process uses excess amine base, which would cause this elevated pH on workup, we examined the removal of excess triethylamine by concentration of the reaction mixture, after addition of the ethyl acetate to precipitate salts. Co-distillation of triethylamine with ethyl acetate provided a mixture that (without filtration of salts) gave an aqueous pH of 4 during workup, with notably lower levels of decomposition products. In an early comparison trial (prior to other optimizations noted above), the concentration process provided a 43% product yield compared with 35% for the filtration process. However, the codistillation process extended the process times, so an alternative procedure was sought.

After significant experimentation, it was found that addition of cyclohexane at the end of the reaction results in a two-phase mixture, provided that four or fewer volumes of NMP are used in the reaction. Under these conditions, only trace amounts of **9** transfer into the cyclohexane layer, but excess TEA and a significant amount of NMP and reagent **8** partition into the cyclohexane. Three extractions with cyclohexane (5−6 volumes each) were found to provide an optimal balance between NMP removal and ability to manipulate the product-containing reaction phase, which becomes rather thick. The fluid cyclohexane layers must be removed from the top of the thick reaction mass, requiring the use of a dip-tube or other decanting technique. In this way, **9** is concentrated in a decreasing amount of NMP, and excess reagents and some byproduct are removed.[10]

Partitioning the residue between ethyl acetate and water provides a solution of crude **9**. Notably, the aqueous pH during these extractions is 5−6, showing that excess TEA has been removed. The product **9** exhibits much greater stability in both the aqueous and organic phases throughout the extraction process, a vital consideration given the expected extended processing times on scale-up. Following solvent exchange to isopropanol, **1** is crystallized by addition of fumaric acid and isolated in acceptable purity. Using this optimized process in comparison to the published procedure, recovered yields for stage 3 improve from about 35% to 60−65%.

(10) In one experiment, the cyclohexane extracts were concentrated in vacuo, and the resulting mixture, containing **8**, was recycled for use in the stage 3 process. Provided that moisture is excluded throughout the process, this recycling process works efficiently.

Conclusion

Several improvements were made to the tenofovir manufacturing process. Key findings included the use of a telescoped procedure for the stage 2 process, identifying high-quality magnesium *tert*-butoxide as the optimum base for that process, and utilizing the lower-cost TMSCl/NaBr reagent mixture in place of TMSBr. In the final stage of the process, the introduction of tetrabutylammonium bromide significantly improved the reaction profile, and the development of a cyclohexane extraction to remove excess reagents in the absence of water significantly reduced the level of product decomposition during the workup and isolation. As a result of these changes, the overall process yield was improved to 24.3% from the reported 13% reported by published procedures, resulting in a significant reduction in the cost of treating patients with HIV/AIDS.

Experimental Section

Unless noted otherwise, purities are reported as HPLC A% results with detection at 260 nm. HPLC conditions for stage 1: Zorbax XDB-C18 or equivalent column (15 cm ×0.46 cm, 3.5 μm particles), isocratic at 1.0 mL/min, mobile phase 900 mL of 10 mM phosphate buffer adjusted to pH 5.5 mixed with 100 mL 1:1 acetonitrile/methanol. HPLC conditions for stage 2: same as stage 1, but using the mobile phase above mixed 89:11 with 1:1 acetonitrile/methanol (to give a net 20% organic mobile phase). HPLC conditions for stage 3: Zorbax RX-C18 column (15 cm ×0.46 cm, 3.5 μm particles), linear 15-min gradient of 15% to 70% acetonitrile vs a pH 2.5 10 mM phosphate buffer, 1.5 mL/min flow, 40 °C.

Preparation of (*R*)-9-(2-Hydroxypropyl)adenine (5). Adenine (**3**, 20.0 g, 0.148 mol) and NaOH (0.47 g, 0.08 equiv) were mixed with DMF (95 mL) at 25−30 °C, and the mixture was stirred for 10 min at 25−30 °C. *R*-Propylene carbonate (**4**, 19.6 g, 1.32 equiv) was added to the reaction mass over 10−15 min at 25−30 °C. The mixture was heated to 120 °C and held for 24 h. Conversion, monitored by HPLC, reached about 90% after about 22 h (with regioisomer content about 8%).

The reaction mass was cooled to 70 °C, and a mixture of methanol (60 mL) and 2-propanol (60 mL) was added over 15−20 min. During addition of solvent, the reaction mass temperature was allowed to cool to about 55 °C, and precipitation was observed. The reaction mass was cooled to 15 °C and held at this temperature for about 1 h. The product was isolated by filtration and the cake washed with a mixture of methanol (10 mL) and 2-propanol (10 mL) chilled to 5 °C. The solids were dried under vacuum at 70−75 °C, providing 18.8 g (65.9%) of alcohol **5**. HPLC purity of **5**: 97.8% with regioisomer **9**, 1.7%. Note that **9** has a significantly different UV spectrum from **3** and **5**, so that A% measurements may not accurately reflect mass ratios of **5** and **9**.

Preparation of (*R*)-(((Adenin-9-yl)propan-2-oxy)methyl)phosphonic Acid (7). Alcohol **5** (4.00 g, 0.021 mol) was mixed with NMP (20.0 mL) and magnesium *tert*-butoxide (10.6 g, 3 equiv) at 25−30 °C and heated to 70 °C. Tosylate **6** (10.0 g, 1.5 equiv) was added to the stirred mixture at 70−74 °C over 10−15 min. Progress of the reaction was monitored by HPLC; conversion reached 90% at 7 h.

The mixture was cooled to 15−20 °C. The pH was adjusted to 6−7 with acetic acid (∼8.0 mL) at 15−25 °C, ethyl acetate (100 mL) was added with vigorous stirring to precipitate magnesium salts, and the mixture was heated to 50−60 °C. The mixture was stirred for 30 min at 50−60 °C and cooled, and the precipitated salts were filtered at 45−47 °C. Note that the salts are very hygroscopic, becoming difficult to filter on exposure to a moist atmosphere; at laboratory scale it was more practical to decant the liquors from the solid mass. To extract additional product, the salt cake was taken up in additional ethyl acetate (75 mL) and stirred for 30 min at 55−60 °C, then filtered at 45−47 °C. The salts were washed with ethyl acetate (25 mL), and all filtrates were combined and concentrated on a rotary evaporator under reduced pressure below 55 °C.[11] Care should be taken during this process to avoid taking up adventitious moisture. The moisture content of the final concentrate should be below approximately 0.9% to proceed to the next stage. An azeotropic drying process using cyclohexane was used in the laboratory to ensure dryness, chasing the ethyl acetate with one or two distillations of cyclohexane (30 mL) as needed. In a manufacturing environment, this should not be necessary.

The solution above was transferred to a reaction flask, and sodium bromide (7.5 g, 3.5 equiv based on **5**) was added. The mixture was cooled to 0−5 °C. Trimethylsilyl chloride (12.0 g, 5.3 equiv based on **5**) was added over 10 min, and the reaction mixture was heated to 75 °C. Progress of the reaction was monitored by HPLC; the reaction was considered complete (>90% of **2**) at 16 h.

The reaction mixture was cooled to 20−25 °C, diluted with water (40 mL) and washed twice by extraction with ethyl acetate (2 × 40 mL). The aqueous layer was cooled to about 5 °C, and the pH was adjusted to between 2.8 and 3.2 with 40% NaOH solution at 3−6 °C, crystallizing the product. The resulting mixture was stirred at 5−8 °C for 2.0 h, and the product isolated by filtration. The solids were washed with chilled water (∼5 °C, 15 mL) and dried under vacuum below 65 °C to yield 3.72 g of **2** (PMPA•H_2O, 59.1%). The solids had an HPLC purity of 98.4%, assaying (anhydrous basis) at 91.0% w/w, with a moisture content of 5.2% w/w.

Preparation of 1 and its Fumarate Salt (TD and TDF). Tenofovir (**2**, 53.1 g, 0.174 mol as hydrate) was charged to a reaction flask and dried at about 100 °C under vacuum (25−40 Torr) for 36 h (theoretically providing 50.0 g anhydrous **2**). To this was added dry NMP (200 mL) and cyclohexane (150 mL). The latter was removed by distillation under reduced pressure at not more than 60 °C. Cyclohexane (150 mL) was again added and the distillation repeated. The concentrate was cooled to 45 °C, and triethylamine (70.4 g, 4.0 equiv) was added, causing precipitation of triethylamine salts of **2**. Tetrabutylammonium bromide (56.1 g, 1.0 equiv, water content <1%) was added, and the resulting mixture was heated to 50 °C. Chloromethyl isopropyl carbonate (**8**, 132.8 g, 5.0 equiv) was added at this temperature, and stirring continued. The reaction was monitored for completion; the reaction reached 75 area % of **9** at 5.5 h, at which point the originally thick suspension was nearly clear. The mass was cooled to 25−30 °C, and cyclohexane (300 mL) was added. After the mixture stirred for 15 min and settled for 30 min, the upper phase was removed by suction, providing 280 mL. Cyclohexane (200 mL) was again added and the process repeated, providing an upper layer of 260 mL. Analysis of the cyclohexane washes showed no product. To the remaining reaction mixture were added ethyl acetate (500 mL) and water (250 mL), and the mixture was stirred for 15 min and settled for separation. The aqueous phase from the separation was extracted twice more with ethyl acetate (150 mL each). The combined organic phases were washed three times with chilled (5 °C) water (300 mL each) and once with 10% aqueous NaCl. The resulting organic phase was dried with solid sodium sulfate and concentrated under reduced pressure. Cooling the residue to about 5 °C provided a waxy, solid mass of 90.6 g (100% yield if pure; HPLC purity 84.3 A%).

This crude **9** (90.0 g) was taken up in 2-propanol (315 mL) at ambient temperature. To this solution was added fumaric acid (26.1 g, 1.3 equiv), and the mixture was heated to 50 °C. The resulting solution was stirred at this temperature for 2 h, then cooled in stages to 5 °C, and stirred an additional hour to fully crystallize the product. The resulting crystals were isolated by filtration, washing with a mixture of 2-propanol (75 mL) and cyclohexane (150 mL) chilled to 5 °C. The solids were dried under vacuum at 35 °C to provide 69.0 g of **1** (TDF, 62.3% yield, HPLC purity 98.4%).

A reaction run at the same scale, without use of tetrabutylammonium bromide, provided **1** in 56.0% yield and 98.0% purity.

(11) Given the large volumes of ethyl acetate required for successful workup, recycling of the recovered solvent is indicated.

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Supporting Information Available

HPLC methods with chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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