Organic LETTERS

Letter

pubs.acs.org/OrgLett

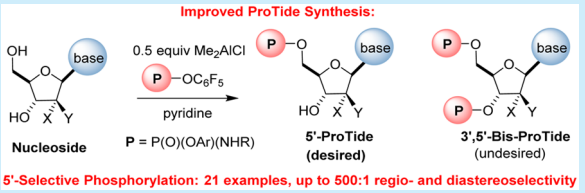
Mechanism-Based Solution to the ProTide Synthesis Problem: Selective Access to Sofosbuvir, Acelarin, and INX-08189

Bryon Simmons,* Zhuqing Liu, Artis Klapars, Ana Bellomo, and Steven M. Silverman*

Department of Process Chemistry, Merck & Co., Inc., P.O. Box 2000, Rahway, New Jersey 07065, United States

Supporting Information

ABSTRACT: A general and efficient method for the synthesis of pronucleotide (ProTide) 5′-phosphoramidate monoesters is reported. This method consists of a highly stereoselective 5′-phosphorylation mediated by dimethylaluminum chloride to afford the desired target ProTides in excellent yields without employing 3′-protection strategies. The application of this methodology to the synthesis of a number of pharmaceutically relevant compounds currently marketed or under investigation in clinical research is demonstrated.



For over 40 years, nucleoside analogues have occupied a privileged status within medicine for the treatment of viral disease and cancer.[1] This is evidenced by the fact that almost half of all antivirals currently on the market are nucleoside derivatives.[2] One challenge encountered in the development of nucleoside analogues is their limited capacity to undergo in vivo phosphorylation to their biologically active nucleotide triphosphate forms.[3] In the early 1990s, McGuigan and co-workers introduced the 5′-aryloxyphosphoramidate or "ProTide" moiety as a novel nucleoside prodrug strategy capable of dramatic enhancement of cellular permeability and phosphorylation rates.[4] The general utility of this approach is evidenced by the approval of sofosbuvir (**1**)[5] for treatment of HCV (vs inactive PSI-6206, Figure 1) as well as ongoing advanced clinical investigation of the ProTides Acelarin (solid tumors)[6] and tenofovir alafenamide (HIV).[7]

ProTides now generate over $10 billion USD in patient value annually, and although methods that allow access to the active pharmaceutical ingredients have been practiced for over 20 years, they remain suboptimal for large-scale production.[8] Synthetically, two main challenges exist: (1) the exclusive generation of the desired phosphorus diastereomer (S_p vs R_p) and (2) discrimination in the phosphorylation event between 5′ and 3′-nucleoside hydroxy groups of similar chemical reactivity (Figure 1, eqs 1 and 2).

Advances in ProTide clinical development have highlighted the need for improved stereoselective phosphorylations. Initially, **1** and its R_p-diastereomer PSI-7976 (vide infra, Table 3) were isolated as a 1:1 product mixture through unselective phosphorylation of PSI-6206.[5a] Upon discovery of a dramatic potency difference between 1 and PSI-7976, a diastereoselective approach was developed by Ross and co-workers employing a chiral phenolic phosphorylation agent that, unlike traditional chlorophosphoramidate reagents, is configurationally stable at phosphorus.[9] More recently, concerns over uniformity, crystallinity, and stability have led researchers to investigate

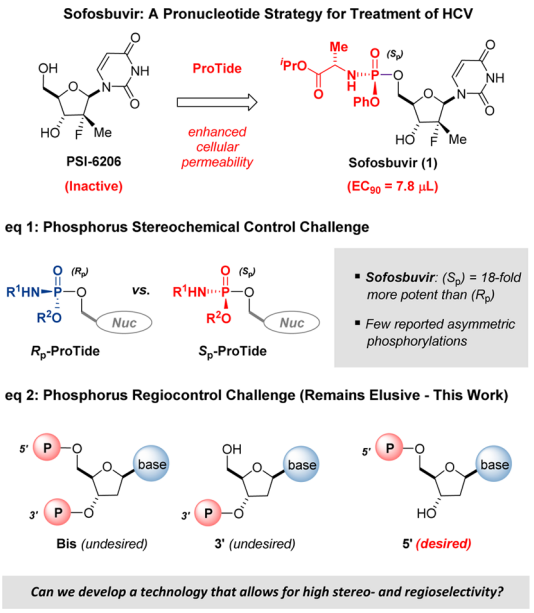


Figure 1. Nucleoside 5′-phosphorylation challenge.

diastereoselective ProTide syntheses in the context of INX-08189 (vide infra, see Table 3).[10,11]

In contrast to the efforts to improve the phosphorus stereochemical control challenge (Figure 1, eq 1), the 3′ vs 5′ regioselectivity challenge (Figure 1. eq 2) has gone largely unaddressed. Unwanted 3′-phosphorylation greatly reduces reaction efficiency, leading to high levels of byproducts such as the commonly observed 3′,5′-bisphosphorylation impurity (Bis).[12] As we further investigated the method of Ross et al. with various nucleosides, we noted it to be complicated by the

Received: March 6, 2017

DOI: 10.1021/acs.orglett.7b00469
Org. Lett. XXXX, XXX, XXX−XXX

generation of high levels of Bis impurities (typically 10−30%), thereby reducing chemical efficiency and increasing the difficulty of separation and isolation.[13] Although not always explicitly mentioned, this lack of 5′-selectivity is often the root cause of low reported synthetic yields and frequently forces redundant synthetic protection of the 3′-hydroxy group beforehand in order to eliminate the significant formation of Bis byproducts.[9,14]

Given the rise of 5′-ProTides as preferred nucleoside prodrugs, the need for development of an improved general methodology for their selective and high-yielding construction is evident. As part of our efforts in the preparation of this motif, we began an investigation toward a general approach to the 5′-selectivity challenge. We envisioned reaction conditions that could be highly diastereo- and regioselective and that also could avoid the use of protecting group manipulations so as to obtain maximize efficiency. In this paper, we describe the successful design and execution of that plan and outline a general method for direct and selective nucleoside 5′-phosphorylation.

Traditional approaches to the synthesis of phosphoramidate prodrugs have heavily relied on the activation of the nucleophile, typically using a strong base (Figure 2). While this method has

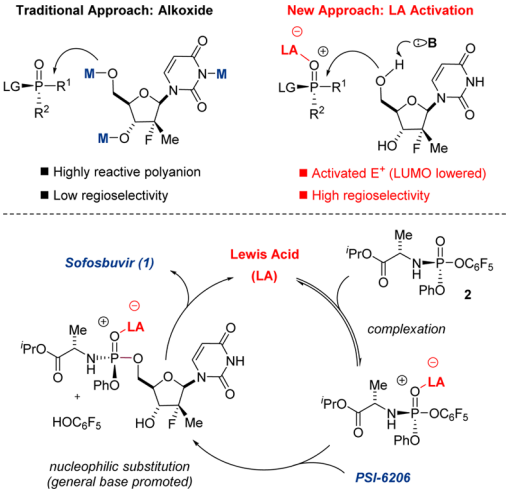
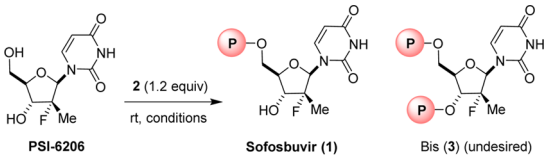


Figure 2. Design plan.

shown some success, it proceeds through a highly reactive polyanion. This often results in low regioselectivity and the need to carefully control the reaction conditions to maximize yield of the desired product, which is consumed to form the Bis product. As depicted in our design plan, we envisioned alternatively activating the electrophilic phosphorylating reagent isopropyl ((*S*)-(perfluorophenoxy)(phenoxy)phosphoryl)-L-alaninate (**2**). This would lower the phosphorus LUMO, such that the reaction could proceed through a general base-promoted pathway. This inversion of the reaction parameters would likely result in a change in selectivity parameters, potentially allowing for greater regio- and diastereselectivity as compared to traditional methods.

We chose sofosbuvir as a prototypical substrate for the optimization of 5′-selective phosphorylation conditions (Table 1). Starting with near-stoichiometric amounts of Lewis acid, we observed only trace reactivity in the presence of titanium species (entry 1). Boron, calcium, and iron species were similarly unreactive. Fortunately, when diethylzinc was tested, we saw coupling reactivity restored (entry 2). When trimethylaluminum was employed, we saw vastly improved regioselectivity (55:1), albeit at the expense of diastereoselectivity (entry 3). We

Table 1. Optimization of 5′-Selective Phosphorylation



entry	conditions	ratio **1**/**3**[a]	yield (%)[b]	*dr* **1**[c]
1	0.75 equiv $TiCl_4$, THF	ND	trace	ND
2	0.75 equiv Et_2Zn, THF	8.0	51	14
3	0.50 equiv Me_3Al, THF	55	36	6.5
4	0.50 equiv $AlCl_3$, 2,6-lutidine, THF	241	64	102
5	0.50 equiv Me_2AlCl, 2,6-lutidine, THF	128	86	89
6	0.50 equiv Me_2AlCl, pyridine[f]	110	84[d]	>500
7	2.1 equiv ^{t}BuMgCl, THF	3.0	60[d]	298
8[e]	2.1 equiv ^{t}BuMgCl, THF **−5 to 5 °C**	8.5	68	>100
9	1.0 equiv ^{n}Bu_2Mg, THF	18	ND	>100
10	1.5 equiv NaH, THF	1.6	2	1
11	1.5 equiv Et_3N, THF	1	2	1

[a]Ratio of **1**/**3** determined by HPLC. [b]Assay yield of **1** as determined by HPLC. [c]Diastereoselectivity (S_p/R_p) determined by HPLC. [d]Isolated yield. [e]Data for entry 8 comes from ref 9. [f]Pyridine used as reaction solvent (0.2 M).

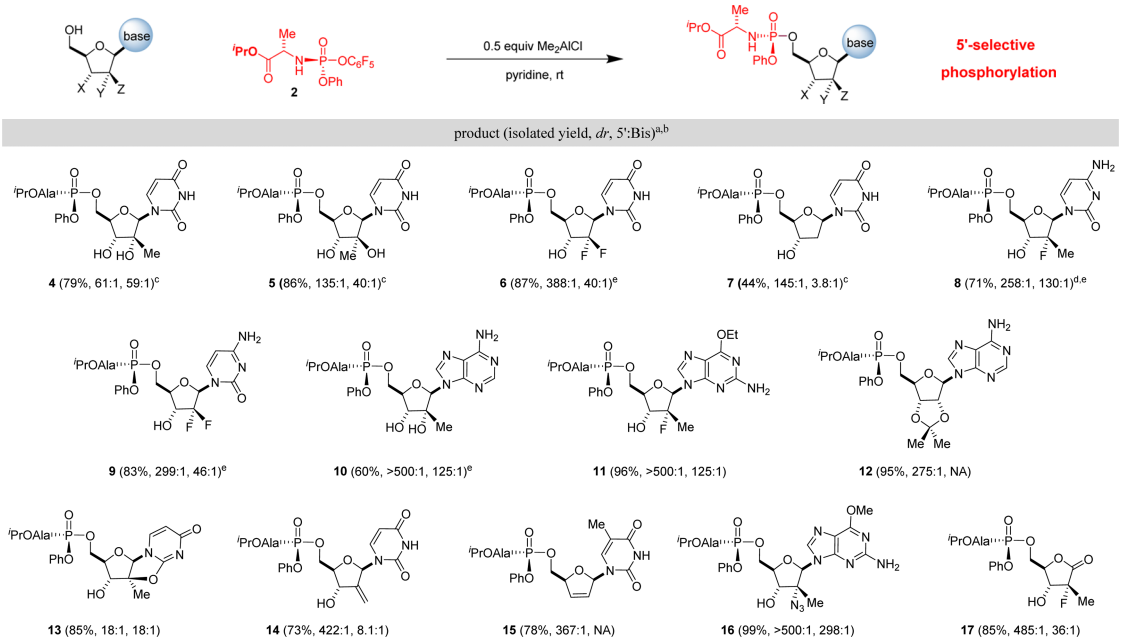
hypothesized that the loss of diastereoselectivity was caused by epimerization of **2** in the presence of strong base. We therefore employed aluminum trichloride with the mild base 2,6-lutidine (entry 4). We were excited to discover that this not only increased reactivity but also gave excellent regio- and diastereoselectivity, both >100:1. Due to the sluggish reaction rate when insoluble aluminum trichloride was used, we sought to increase reactivity through the use of an alternate aluminum reagent. However, trimethylaluminum gave rise to significant product degradation when we attempted to drive the reaction to completion, and therefore, we tested dimethylaluminum chloride as a milder alternative. This reagent showed impressive levels of reactivity and 5′-phosphorylation selectivity (entry 5).

Using dimethylaluminum chloride along with 2,6-lutidine as base and THF as solvent, we obtained sofosbuvir in 86% yield with a 128:1 preference for 5′-selectivity. A dimethylaluminum chloride charge of 0.5 equiv proved optimal in terms of conversion to the desired product and the reaction rate. Furthermore, we observed excellent 5′-selectivity and yield when we streamlined the process, by employing pyridine in place of THF as solvent (entry 6). This required no addition of 2,6-lutidine as exogenous base. This protocol proved to be highly diastereoselective while also increasing the reaction rate and solubility, a critical feature in order to expand the substrate scope to less soluble nucleosides.

Comparing the results of our proposed strategy to methods traditionally utilized, we observed a stark contrast. In our hands, the coupling of PSI-6206 and **2** mediated by 2.1 equiv of ^{t}BuMgCl in THF at room temperature afforded sofosbuvir in 60% isolated yield while also generating 20% yield of byproduct **3** (entry 7).[15] We also confirmed a report that demonstrated that sofosbuvir could be isolated in 68% yield while minimizing the level of byproduct **3** to 8% by cooling the reaction temperature to between −5 and +5 °C and running the reaction for precisely 18 h, stopping short of full conversion (entry 8).[16] We noted that upon warming or aging the reaction beyond 18 h the level of impurity **3** increased markedly.[17] Investigation of ^{n}Bu_2Mg as an alternate reagent showed improved 5′-regioselectivity (entry 9), but it was

DOI: 10.1021/acs.orglett.7b00469
Org. Lett. XXXX, XXX, XXX−XXX

Table 2. Evaluation of 5′-Selective Phosphorylation Nucleoside Scope*



*Reaction conditions: nucleoside (1.0 equiv), **2** (1.1−1.2 equiv), Me_2AlCl (0.5 equiv) in pyridine, 20−48 h reaction time. [a]Ratio of **5′**/Bis determined by HPLC or UHPLC. [b]Diastereoselectivity (S_p/R_p) determined by HPLC or UHPLC. [c]Temperature = rt to 50 °C. [d]Temperature = 4 °C. [e]Added 5 equiv of DMPU to enhance solubility.

significantly lower than our aluminum conditions. When other typical strong bases such as sodium hydride (as well as KO^{t}Bu and LDA) were employed, only trace amounts of the desired product **1** were afforded (entry 10). Weak amine bases such as triethylamine also gave only trace amounts of **1** (entry 11), confirming the hypothesized synergistic relationship between Lewis acid and weak base. As additional evidence of the coordination between aluminum and the phosphorylating reagent, we studied the ^{31}P NMR spectra of **2** in the presence of aluminum trichloride. Gratifyingly, it was observed that a chemical shift of nearly 6 ppm was observed as the aluminum reagent was added to a solution of **2**.[18] This result was in agreement with literature observations in similar systems.[19]

With our hypothesis validated, we began an examination of the practical utility of our phosphorylation method by submitting a variety of pharmaceutically relevant nucleosides to our optimized phosphorylation conditions (Table 2). We were pleased to find that the 2′-hydroxy functionality was well tolerated. For example, 2′-methyluridine derivatives **4** and **5** were obtained in high yields and selectivities despite being −OH epimers at 2′. Difluorouridine derivative **6** also furnished the desired product in high yield and 40:1 5′/Bis selectivity. DMPU was added as a cosolvent to further improve the solubility of the nucleoside in the reaction mixture and improve the rates (products **6** and **8**−**10**). A key observation was that 2′-disubstitution was critical to achieving high levels of selectivity. For example, deoxyuridine provided a 3.8:1 preference of 5′-monophosphorylation product **7** to bisphosphorylation impurity. More complex purine and pyrimidine substrates were also effective coupling partners (products **8**−**12**). We were pleased to find that sensitive functionality such as the acetonide moiety of compound **12** and cyclouridine **13** were compatible with our protocol. Compounds containing unsaturation such as **14** and **15** also behaved well in the coupling chemistry. Finally, azide **16** and lactone **17** were well tolerated under these mild reaction conditions.

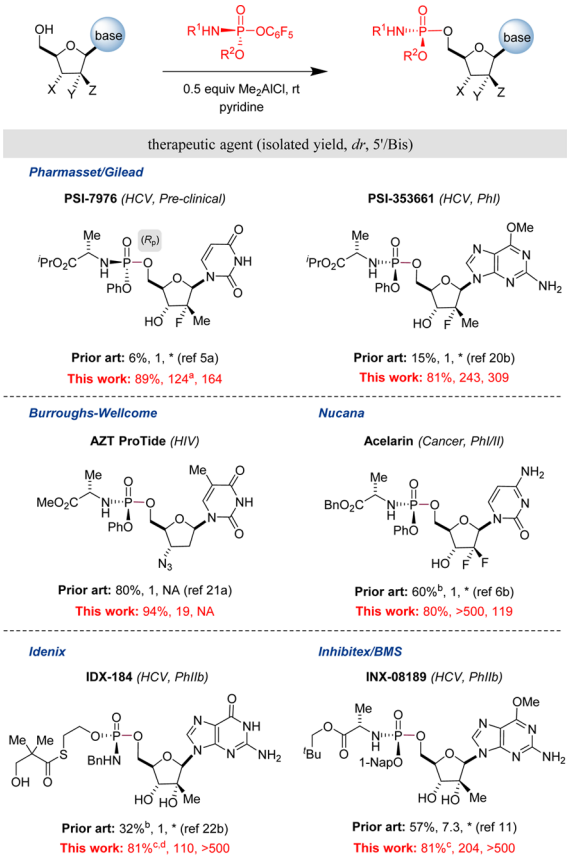
Finally, we extended our method to the synthesis of six pharmaceutically relevant ProTide targets investigated for treatment of viral infections or cancer (Table 3). In detail, we achieved the efficient and selective coupling of PSI-6206 and ***ent*-2** (R_p enantiomer) to generate PSI-7976, the first reported direct synthesis of this R_p-ProTide. PSI-353661,[20] a guanosine nucleoside, was delivered in 81% yield using our method. The known methyl and benzyl ester derivatives of **6** were used to access AZT ProTide[8a,21] and Acelarin[6a] in 94% and 81% yields, respectively. Finally, we investigated IDX-184[22] and INX-08189,[23] both liver-targeted prodrugs of the mononucleotide 2-methylguanosine monophosphate that were investigated through phase II clinical studies. Both showed suboptimal reactivity under the standard reaction conditions due to low solubility. We envisioned that transient formation of the 2′,3′-boronate ester using a slight excess of phenylboronic acid might provide a solution entirely compatible with our standard conditions. Gratifyingly, this was validated in practice, and both modified ProTide functionalities could be installed using our method. In this manner, INX-08189 was prepared as a single diastereomer matching the recently disclosed S_p compound.[11]

As anticipated, application of our selective coupling protocol to the six compounds in Table 3 was achieved with unprecedented levels of efficiency. Perhaps most striking was that the average isolated yield for these six ProTide targets using our coupling method was 84% versus an average of 42% using previously existing methodology.

In summary, we have developed a novel and efficient synthetic tool for the selective preparation of 5′-nucleotide phosphoramidates and have demonstrated its use on representative derivatives of purine and pyrimidine nucleosides. We have further demonstrated this method by applying it to the preparation of a number of pharmaceutically relevant ProTide targets. Investigations into the mechanism of this unprecedented aluminum-

DOI: 10.1021/acs.orglett.7b00469
Org. Lett. XXXX, XXX, XXX−XXX

Table 3. Application to Selected Therapeutic Agents[**]



[**]Reaction conditions: nucleoside (1.0 equiv), phosphorylating agent (1.2 equiv), Me_2AlCl (0.5 equiv) in pyridine, rt, 10−24 h reaction time. Ratio of S_p/R_p and 5′/Bis determined by HPLC or UHPLC. *5′/Bis ratio was not given in original report. [a]Ratio of R_p/S_p. [b]Yield over two steps. [c]Nucleoside was precondensed with 1.05 equiv of phenylboronic acid; see the Supporting Information for details. [d]Product isolated as *O*-trityl precursor.

mediated phosphorylation protocol are underway and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00469.

Experimental procedure/data and discussion (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: bryon_simmons@merck.com.
*E-mail: steven.silverman@merck.com.

ORCID

Bryon Simmons: 0000-0003-3073-6978

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge Kevin R. Campos, L.-C. Campeau, John Limanto, Paul G. Bulger, and Ian W. Davies (Merck & Co., Inc.) for helpful discussions.

■ REFERENCES

(1) Elion, G. B. *Annu. Rev. Pharmacol. Toxicol.* **1993**, *33*, 1.
(2) Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. *Nat. Rev. Drug Discovery* **2013**, *12*, 447.
(3) (a) Mehellou, Y.; Balzarini, J.; McGuigan, C. *ChemMedChem* **2009**, *4*, 1779. (b) Pertusati, F.; Serpi, M.; McGuigan, C. *Antiviral Chem. Chemother.* **2011**, *22*, 181.
(4) Cahard, D.; McGuigan, C.; Balzarini, J. *Mini-Rev. Med. Chem.* **2004**, *4*, 371.
(5) (a) Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H. R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Steuer, H. M.; Niu, C.; Otto, M. J.; Furman, P. A. *J. Med. Chem.* **2010**, *53*, 7202. (b) Singh, H.; Bhatia, H. K.; Grewal, N.; Natt, N. *J. Pharmacol. Pharmacother.* **2014**, *5*, 278.
(6) (a) McGuigan, C.; Habib, N. A.; Wasan, H. S.; Gabra, H.; Jiao, L. R.; Slusarczyk, M.; Chabot, J. A.; Saif, M. W. *J. Clin. Oncol.* **2011**, *29*, No. e13540. (b) Slusarczyk, M.; Huerta-Lopez, M.; Balzarini, J.; Mason, M.; Jiang, W.-G.; Blagden, S.; Thompson, E.; Ghazaly, E.; McGuigan, C. *J. Med. Chem.* **2014**, *57*, 1531.
(7) Eisenberg, E. J.; He, G. X.; Lee, W. A. *Nucleosides, Nucleotides Nucleic Acids* **2001**, *20*, 1091.
(8) (a) McGuigan, C.; Pathirana, R. N.; Mahmood, N.; Devine, K. G.; Hay, A. J. *Antiviral Res.* **1992**, *17*, 311. (b) McGuigan, C.; Pathirana, R. N.; Mahmood, N.; Hay, A. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 701.
(9) Ross, B. S.; Reddy, P. G.; Zhang, H.-R.; Rachakonda, S.; Sofia, M. J. *J. Org. Chem.* **2011**, *76*, 8311.
(10) Pertusati, F.; McGuigan, C. *Chem. Commun.* **2015**, *51*, 8070.
(11) Tran, K.; Beutner, G. L.; Schmidt, M.; Janey, J.; Chen, K.; Rosso, V.; Eastgate, M. D. *J. Org. Chem.* **2015**, *80*, 4994.
(12) For examples of 3′,5′-bisphosphorylation or the "ProTide Synthesis Problem", see: Pradere, U.; Garnier-Amblard, E. C.; Coats, S. J.; Amblard, F.; Schinazi, R. F. *Chem. Rev.* **2014**, *114*, 9154.
(13) Phosphorylation of PSI-6130 (ref 9) in our hands, under the reported conditions, rendered the desired product in 53% yield as well as the corresponding 3′,5′-bisphosphorylation impurity in 30% yield.
(14) Donghi, M.; Attenni, B.; Gardelli, C.; Di Marco, A.; Fiore, F.; Giuliano, C.; Laufer, R.; Leone, J. F.; Pucci, V.; Rowley, M.; Narjes, F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1392.
(15) Compound **3** was isolated in 20% yield; prior to isolation, the ratio of **1**/**3** was 3:1.
(16) These carefully controlled conditions do not allow the reaction to achieve full conversion; rather, the reaction is quenched at a predetermined conversion that maximizes the product **1** to impurity **3** ratio.
(17) After the product had aged for 36 h, the level of impurity **3** had increased to 14%.
(18) See the Supporting Information (Figure 1) for spectral data.
(19) Burford, N.; Royan, B. W.; Spence, R. E. v. H.; Cameron, T. S.; Linden, A.; Rogers, R. D. J. *J. Chem. Soc., Dalton Trans.* **1990**, *5*, 1521.
(20) (a) Chang, W.; Bao, D.; Chun, B. K.; Naduthambi, D.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Zhang, H. R.; Bansal, S.; Espiritu, C. L.; Keilman, M.; Lam, A. M.; Niu, C.; Steuer, H. M.; Furman, P. A.; Otto, M. J.; Sofia, M. J. *ACS Med. Chem. Lett.* **2011**, *2*, 130. (b) Liao, L.; Wang, G.; Yokokawa, F. WO 2014/082935A, Jun 5, 2014.
(21) (a) Cui, H.; Ruiz-Pérez, L. M.; González-Pacanowska, D.; Gilbert, I. H. *Bioorg. Med. Chem.* **2010**, *18*, 7302. (b) Jiang, P.; Guo, X.; Fu, H.; Jiang, Y.; Zhao, Y. *Synlett* **2005**, *16*, 2537.
(22) (a) Zhou, X. J.; Pietropaolo, K.; Sullivan-Bólyai, J.; Kuca, B.; Liu, W.; Xu, L.; Belanger, B.; Khan, S.; Mayers, D. *J. Hepatol.* **2009**, *50*, S351. (b) Sommadossi, J.-P.; Gosselin, G.; Pierra, C.; Perigaud, C.; Peyrottes, S. US 7902202B2, Mar 08, 2011.
(23) Vernachio, J. H.; Bleiman, B.; Bryant, K. D.; Chamberlain, S.; Hunley, D.; Hutchins, J.; Ames, B.; Gorovits, E.; Ganguly, B.; Hall, A.; Kolykhalov, A.; Liu, Y.; Muhammad, J.; Raja, N.; Walters, C. R.; Wang, J.; Williams, K.; Patti, J. M.; Henson, G.; Madela, K.; Aljarah, M.; Gilles, A.; McGuigan, C. *Antimicrob. Agents Chemother.* **2011**, *55*, 1843.

DOI: 10.1021/acs.orglett.7b00469
Org. Lett. XXXX, XXX, XXX−XXX