Organic LETTERS

Letter

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Enantioselective Synthesis of 4′-Ethynyl-2-fluoro-2′-deoxyadenosine (EFdA) via Enzymatic Desymmetrization

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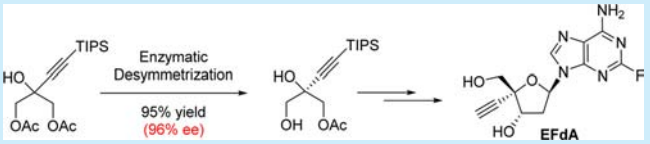
Supporting Information

ABSTRACT: An enantioselective synthesis of the potent anti-HIV nucleoside EFdA is presented. Key features of stereocontrol include construction of the fully substituted 4′-carbon via a biocatalytic desymmetrization of 2-hydroxy-2-((triisopropylsilyl)ethynyl)propane-1,3-diyl diacetate and a Noyori-type asymmetric transfer hydrogenation to control the stereochemistry of the 3′-hydroxyl bearing carbon. The discovery of a selective crystallization of an *N*-silyl nucleoside intermediate enabled isolation of the desired β-anomer from the glycosylation step.



Nucleoside reverse transcriptase inhibitors (NRTIs) remain a cornerstone of highly active antiretoviral therapy (HAART) for human immunodeficiency virus (HIV).[1] According to the World Health Organization (WHO) there are approximately 37 million people living with HIV at present, with 2 million of those newly infected in 2015 alone. Though there are a number of FDA-approved drugs in this class[2] (emtricitabine, lamivudine, zidovudine, didanosine, tenofovir, stavudine, and abacavir), improved NRTIs are highly sought after as new first-line therapeutics to address persisting issues of safety, resistance, long-term efficacy, and ease of administration. EFdA (4′-ethynyl-2-fluoro-2′-deoxyadenosine, Figure 1) is an adenosine-based NRTI that was discovered via collaborative studies among the Ohrui group, Mitsuya group, and Yamasa Corporation.[3] In preclinical studies, EFdA has shown high promise as a next-generation NRTI, displaying exceptional potency, no acute toxicity, and a long intracellular half-life. These favorable anti-HIV properties combined with an attractive molecular architecture provide strong motivation for total synthesis of EFdA, and the Kuwahara−Ohrui groups have published several strategically distinct approaches.[4] In 2012, Merck & Co., Inc. (Kenilworth, NJ, USA) licensed EFdA from Yamasa Corporation and sought to develop this important drug candidate through more in-depth preclinical studies and human clinical trials. To support these goals, an expedient synthesis amenable to the production of multikilogram quantities of EFdA was required. Herein, we describe a novel enantioselective synthesis of EFdA that meets these predefined goals and enables flexible access to related antiviral pharmacophores.

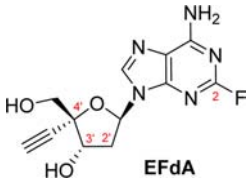


Figure 1. Structure of anti-HIV molecule EFdA.

A key structural feature of EFdA is the fully substituted alkyne-bearing stereocenter at the 4′-position. Defining an effective, stereocontrolled method for the construction of this stereocenter was recognized as a priority objective from the outset, and this would likely necessitate a de novo nucleoside synthesis. Additional synthetic challenges include control of the 3′-hydroxy-bearing stereocenter and the nontrivial problem of stereoselective *N*-glycosylation in the absence of a neighboring substituent in the 2′-position.[5,6] Taken together, these elements of synthetic complexity make EFdA a densely functionalized and uniquely interesting target.

As shown in Figure 2, our synthetic strategy was premised on an observation of latent molecular symmetry[7] within the EFdA structure. Accordingly, disconnection at the glycosidic bond reveals commercially available 2-fluoroadenine and the activated 2′-deoxyribose fragment **A**, which itself may be simplified through retrosynthetic scission of a two-carbon acetate fragment **B** to reveal the key glycerol derivative **C**. We envisaged **C** could be synthesized via desymmetrization of achiral intermediate **D**, itself derived via addition of an acetylide anion to an appropriately protected 1,3-dihydroxyacetone starting material **E**.

Commercially available 1,3-diacetoxyacetone (**1**) provided a convenient starting material for our synthesis. Addition of lithium TIPS-acetylide to **1** generated the carbinol product **2** in 92% yield (Scheme 1). The acetate groups in **2** were somewhat labile toward nucleophiles/bases, so the reaction was conducted at −60 °C and a slight undercharge of base (0.99 equiv of *n*-BuLi to TIPS-acetylene) was employed for optimal results.

Received: January 10, 2017
Published: February 6, 2017

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DOI: 10.1021/acs.orglett.7b00091
Org. Lett. 2017, 19, 926−929

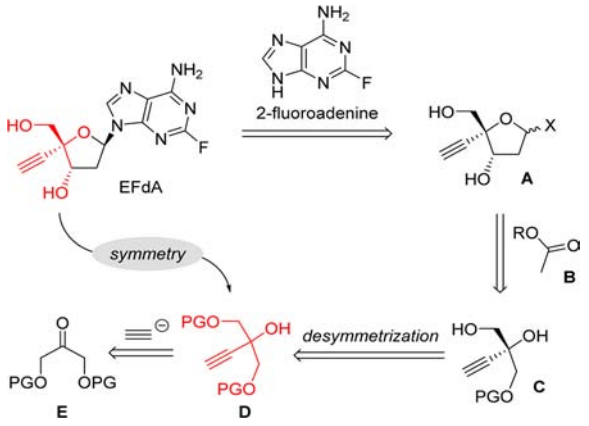
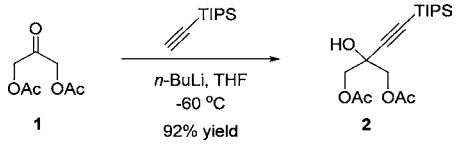


Figure 2. Synthetic strategy for EFdA.

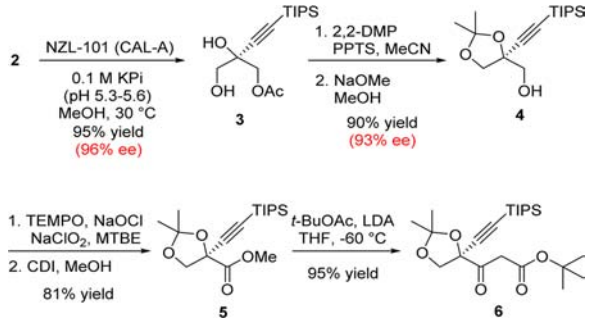
Scheme 1. Synthesis of Diacetate 2



It was postulated that desymmetrization of diacetate **2** could be achieved using a biocatalytic process to selectively hydrolyze a single acetate functional group. Explorative studies quickly identified that a commercially available enzyme NZL-101-(CAL-A) produced the desired (*R*)-diol **3** with moderate conversion and good enantioselectivity (Table 1, entry 1). Significantly, when using NZL-101-(CAL-A) no appreciable over-reaction to the achiral triol **3a** (*symmetric*) was observed, which would ultimately diminish the enantiopurity of the subsequent intermediate **4** if carried forward (vide infra).

Building on this initial result, continued development of the desymmetrization reaction resulted in a better understanding of the impact of solution pH and choice of organic cosolvent on the reaction kinetics. Under carefully optimized conditions, using MeOH as a cosolvent and operating within a pH range 5.3−5.6, it was possible to drive the reaction to very high conversion (95−97%) within 20 h at 30 °C with only 2% of triol **3a** observed (Scheme 2). Under these optimized conditions, the desired diol **3** was obtained in 95% yield and 96% enantiomeric excess.[8] Protection of diol **3** as the corresponding acetonide required careful selection of conditions to avoid stereochemical erosion, presumably the result of acid-catalyzed 1,3-acyl migration. For example, exposure of 96% ee diol to catalytic CSA in acetone

Scheme 2. Synthesis of Ketoester 6



provided the desired acetonide in only 10% ee. This is attributable to the relatively slow rate of acetonide formation using acetone and the use of a relatively strong acid. Alternatively, using 2,2-dimethoxypropane as the reagent led to faster kinetics for the desired protection and catalytic PPTS (weaker acid) in MeCN allowed smooth conversion to the desired acetonide with minimal stereochemical erosion (93% ee), securing the stereochemical integrity of the carbinol for the remainder of the synthesis. Subsequent methanolysis of the remaining acetate (NaOMe, MeOH) then provided alcohol **4** in 90% overall yield from **3**. Using a one-pot NaOCl/TEMPO-Pinnick process, alcohol **4** was oxidized to the corresponding carboxylic acid,[9] which was then converted to methyl ester **5** via activation with CDI and quenched with MeOH (81% yield from **4**). Incorporation of the requisite acetate subunit was then achieved smoothly (95% yield) through Claisen condensation with the lithium enolate of *tert*-butyl actate to provide the ketoester **6**.[10]

Ketoester **6** was considered an ideal juncture to establish the 3′-OH stereochemistry through asymmetric transfer hydrogenation (Scheme 3). Accordingly, high-throughput experimentation

Scheme 3. Synthesis of *Bis*-tolyl-lactone 10

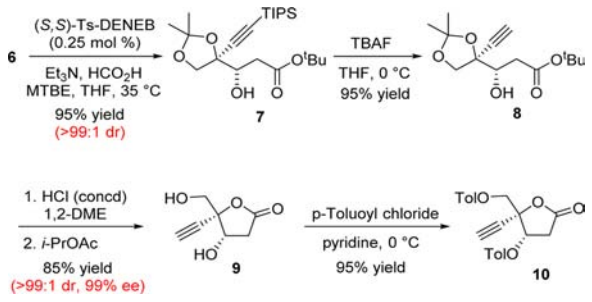
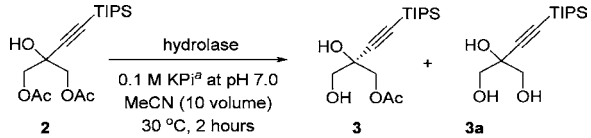


Table 1. Early Screening of Hydrolase for Desymmetrization of Diacetate 2



entry	hydrolase	**2** (%)	**3** (%)	**3a** (%)	ee[b] (%)
1	NZL-101-(CAL-A)	62	38	0	84 (*R*)
2	NZL-102-(CAL-B)	65	11	24	70 (*S*)
3	NZL-103-(M. miehei)	76	18	6	75 (*R*)
4	NZL-105-(T. lanuginosus mutant)	100	0	0	na
5	Amano lipase AK	81	19	0	83 (*R*)

[a]Potassium phosphate buffer. [b]Determined by HPLC analysis on a chiral phase (see the Supporting Information).

DOI: 10.1021/acs.orglett.7b00091
Org. Lett. 2017, 19, 926−929

(HTE) identified (*S*,*S*)-Ts-DENEB, formic acid/triethylamine as an excellent reagent combination, proceeding with essentially complete stereoselectivity. Under optimized conditions with only 0.25 mol % catalyst, the desired *β*-hydroxyester **7** was obtained in 95% yield and extremely high diastereoselectivity (>99:1 dr).[11]

Desilylation of TIPS-alkyne **7** was achieved under standard conditions using TBAF.[12] Subsequent acid-mediated deprotection/lactonization was conducted as a single pot process using concentrated HCl in DME at 45 °C followed by distillative solvent exchange with IPAc, which conveniently induced direct crystallization of lactone diol **9**. This enabled isolation of **9** in good yield (85%) and purity, thus providing an excellent control point for stereochemical purity in the synthesis (>99:1 dr and >99% ee for the major diastereomer). Finally, diol protection was carried out with *p*-toluoyl chloride in pyridine at 0 °C to cleanly provide *bis*-toluoyl lactone **10**, which was isolated directly from the reaction mixture through water-induced crystallization (95% yield). The relative and absolute stereochemistry of **10** was confirmed via single crystal X-ray crystallography (Figure 3).

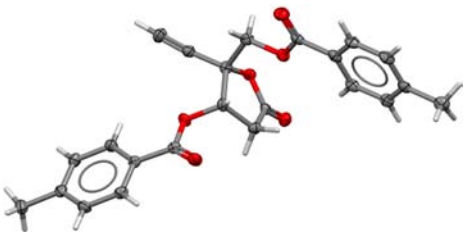


Figure 3. X-ray crystal structure of **10**.

For reduction of *bis*-tolyl-lactone **10**, both $LiAlH(Ot\text{-}Bu)_3$[13] and DIBAL-H[14] were found to be ineffective reagents; the former was poorly reactive at −20 °C and gave multiple products upon warming while the latter was too reactive and easily over-reduced the OTol protecting groups. We suspected the Lewis acidity of DIBAL-H was responsible for undesirable activation of the relatively electron-rich OTol groups. Accordingly, it was found that the anionic reagent Red-Al was more selective and gave relatively clean lactone reduction at −60 °C, providing the lactol intermediate (Scheme 4). The resultant toluene solution of the lactol was acylated with acetic anhydride to yield a mixture of anomeric acetates **11** (80% overall yield from lactone **10**).

Based on existing literature[4a] and preliminary internal experience, the glycosylation step was expected to present a significant challenge with respect to both anomeric selectivity and ability to isolate the desired anomer in pure form. The glycosylation reaction was conducted under Vorbrüggen-type conditions[15] where the (poorly soluble) 2-fluoroadenine base was per-silylated in situ using an excess of bis(trimethylsilyl)-acetamide (BTMSA) and then exposed to the presumed oxocarbenium species arising from treatment of acetate **11** with TMSOTf. After a significant optimization effort, it was determined that the combination of MeCN as a solvent and a reaction temperature of 80 °C afforded the highest anomeric selectivity of 1.8:1, favoring the desired *β*-anomer.[16] Initial attempts to crystallize the product, *after aqueous workup*, were rather discouraging. The undesired *α*-anomer was observed to have lower solubility in multiple solvents and consequently crystallized preferentially. A breakthrough was achieved when the standard aqueous workup was omitted and an attempt was made to directly isolate the *N*-silyl reaction product **12** without hydrolytic quench. Remarkably, retention of the silicon group had a significant impact on the relative solubility of the two anomers, making it possible to crystallize the desired compound **12** in very high purity (>99:1 anomer ratio) and with good recovery (48% isolated yield).[17] The absolute stereochemistry of *N*-silyl derivative **12** was unambiguously determined via single crystal X-ray crystallography (Figure 4).

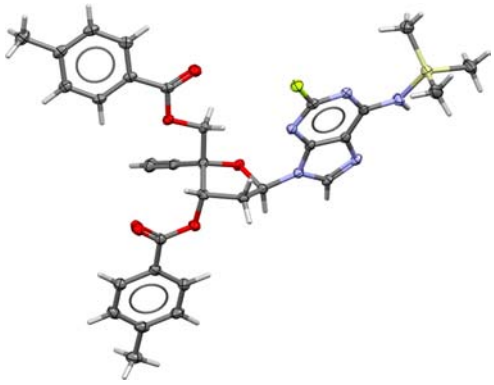
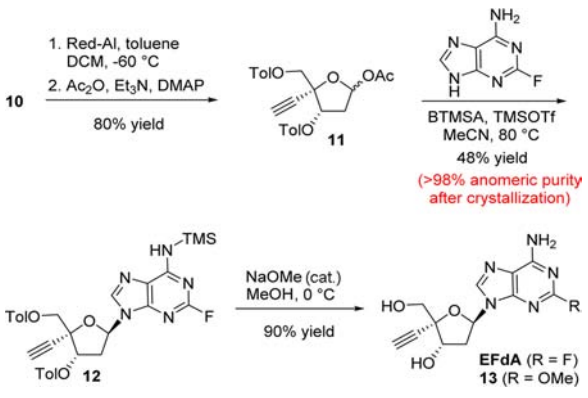


Figure 4. X-ray crystal structure of *N*-silyl glycoside **12**.

Scheme 4. Synthesis of EFdA



It was envisaged that global deprotection to reveal EFdA could be achieved via basic methanolysis. Although high reactivity was observed using NaOMe (the *N*-TMS and *O*-Tol groups are readily removed), a significant side product was observed when reagent stoichiometry or reaction conditions were not adequately controlled. This side product was identified as the 2-methoxy derivative **13**, presumably formed via S_NAr chemistry on the 2-fluoroadenine ring. With this knowledge, the reagent stoichiometry and reaction conditions were fine-tuned to suppress formation of **13** consistently below 1% (catalytic NaOMe was used and temperature was maintained at 0−5 °C). Upon completion of the deprotection, EFdA was isolated in 90% yield and high purity via crystallization.

In summary, we have described an asymmetric synthesis of EFdA that comprises three key aspects. First, leveraging our internal biocatalysis capabilities to exploit the latent symmetry present in EFdA, we efficiently established the fully substituted 4′-carbinol stereocenter from the readily available achiral glycerol derivative **2**. This enzyme-mediated process is economical and robustly delivers the target intermediate in very high yield and enantiomeric excess. Second, controlled installation of 3′-hydroxyl stereochemistry was achieved via an extremely selective Noyori-type asymmetric transfer hydrogenation of *β*-ketoester **6**,

DOI: 10.1021/acs.orglett.7b00091
Org. Lett. 2017, 19, 926−929

for which the optimal catalyst was identified via utilization of contemporary HTE techniques. Last, the challenge to isolate the desired anomer from the difficult glycosylation was overcome following the discovery that *N*-silyl derivative **12** could be directly crystallized from the reaction mixture in high purity. Taken together, these components of stereocontrol enable an effective synthesis of EFdA without recourse to chromatography at any stage in the process.[18] The synthesis described here will ably support future development of EFdA and, more importantly, facilitate a structure−activity relationship investigation of 4′-substituted-2′-deoxyribose-containing pharmaceuticals including anti-HIV NRTIs in medicinal chemistry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00091.

Experimental details, characterization data, and NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors wish to thank Merck & Co., Inc. (Kenilworth, NJ, USA) colleagues Wendy Zhong, Xiaoxia Qian, Vitali Coltuclu, Misha Reibarkh, Richard Ball, Andrew Brunskill, Timothy Nowak, Simon Hamilton, T. J. Novak, and Claire Lee for analytical assistance; Jeremy Scott for helpful suggestions; and Steven Dalby, Steven Miller, and Aaron Whittaker for editorial assistance. The authors also wish to thank Wuxi collaborators Jiahui Chen, Fang Wang, Kaihua Sheng, Di Wang, Fei Jiang, Jian Zhao, and Lu Chen for experimental assistance.

■ REFERENCES

(1) (a) Prajapati, D. G.; Ramajayam, R.; Yadav, M. R.; Giridhar, R. *Bioorg. Med. Chem.* **2009**, *17*, 5744. (b) de Bethune, M.-P. *Antiviral Res.* **2010**, *85*, 75. (c) Lohse, N.; Hansen, A.-B. E.; Pedersen, G.; Kronborg, G.; Gerstoft, J.; Sørensen, H. T.; Væth, M.; Obel, N. *Ann. Intern. Med.* **2007**, *146*, 87.

(2) Flexner, C. *Nat. Rev. Drug Discovery* **2007**, *6*, 959.

(3) (a) Kohgo, S.; Ohrui, H.; Kodama, E.; Matsuoka, M.; Mitsuya, H. 4′-*C*-Substituted-2-halo-adenosine derivative. Can. Pat. CA 2502109, 2005. (b) Kohgo, S.; Yamada, K.; Kitano, K.; Iwai, Y.; Sakata, S.; Ashida, N.; Hayakawa, H.; Nameki, D.; Kod ma, E.; Matsuoka, M.; Mitsuya, H.; Ohrui, H. *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 671. (c) Ohrui, H. *Chem. Rec.* **2006**, *6*, 133. (d) Ohrui, H.; Hayakawa, H.; Kohgo, S.; Matsuoka, M.; Kodama, E.; Mitsuya, H. J. *Yuki Gosei Kagaku Kyokaishi* **2006**, *64*, 716. (e) Ohrui, H.; Kohgo, S.; Hayakawa, H.; Kodama, E.; Matsuoka, M.; Nakata, T.; Mitsuya, H. *Nucleosides, Nucleotides Nucleic Acids* **2007**, *26*, 1543. (f) Ohrui, H. *Proc. Jpn. Acad., Ser. B* **2011**, *87*, 53.

(4) (a) Kageyama, M.; Nagasawa, T.; Yoshida, M.; Ohrui, H.; Kuwahara, S. *Org. Lett.* **2011**, *13*, 5264. (b) Fukuyama, K.; Ohrui, H.; Kuwahara, S. *Org. Lett.* **2015**, *17*, 828. (c) Kageyama, M.; Miyagi, T.; Yoshida, M.; Nagasawa, T.; Ohrui, H.; Kuwahara, S. *Biosci., Biotechnol., Biochem.* **2012**, *76*, 1219. (d) For other approaches to 4′-substituted nucleosides, see the following review article: Betson, M.; Allanson, N.; Wainwright, P. *Org. Biomol. Chem.* **2014**, *12*, 9291.

(5) (a) Liu, Z.; Li, D.; Yin, B.; Zhang, J. *Tetrahedron Lett.* **2010**, *51*, 240. (b) Brodszki, M.; Backstrom, B.; Horvath, K.; Larsson, T.; Malmgren, H.; Pelcman, M.; Wahling, H.; Wallberg, H.; Wennerberg, J. *Org. Process Res. Dev.* **2011**, *15*, 1027. (c) Komatsu, H.; Umetani, H. *Org. Process Res. Dev.* **2002**, *6*, 847. (d) Yang, F.; Zhu, Y.; Yu, B. *Chem. Commun.* **2012**, *48*, 7097. (e) Henschke, J.; Zhang, X.; Huang, X.; Mei, L.; Chu, G.; Hu, K.; Wang, Q.; Zhu, G.; Wu, M.; Kuo, C.; Chen, Y. *Org. Process Res. Dev.* **2013**, *17*, 1419.

(6) For *N*-glycosylation with 2′-oxy substituents, see: (a) Michigami, K.; Uchida, S.; Adachi, M.; Hayashi, M. *Tetrahedron* **2013**, *69*, 595. (b) Caddell, J. M.; Chapman, A. M.; Cooley, B. E.; Downey, B. P.; LeBlanc, M. P.; Jackson, M. M.; O′Connell, T. M.; Phung, H.; Roper, T. D.; Xie, S. *J. Org. Chem.* **2004**, *69*, 3212. (c) Seela, F.; Peng, X. *J. Org. Chem.* **2006**, *71*, 81. (d) Li, N.; Piccirilli, J. A. *J. Org. Chem.* **2006**, *71*, 4018.

(7) For total syntheses of natural products using desymmetrization, see: (a) Poullennec, K.; Romo, D. *J. Am. Chem. Soc.* **2003**, *125*, 6344. (b) Gu, Q.; Rong, Z.; Zheng, C.; You, S. *J. Am. Chem. Soc.* **2010**, *132*, 4056. (c) He, W.; Huang, J.; Sun, X.; Frontier, A. *J. Am. Chem. Soc.* **2007**, *129*, 498. (d) Shimada, K.; Kaburagi, Y.; Fukuyama, T. A. *J. Am. Chem. Soc.* **2003**, *125*, 4048. (e) Inoue, M.; Sato, T.; Hirama, M. *J. Am. Chem. Soc.* **2003**, *125*, 10772. (f) Sharpe, R. J.; Johnson, J. S. *J. Am. Chem. Soc.* **2015**, *137*, 4968. (g) Leverett, C. A.; Purohit, V. c.; Johnson, A. G.; Davis, R. L.; Tantillo, D. J.; Romo, D. *J. Am. Chem. Soc.* **2012**, *134*, 13348.

(8) For more details on the desymmetrization optimization, see the table on p S2 in the Supporting Information.

(9) Oxidation process adapted from: Zhao, M. M.; Li, J.; Eiichi, M.; Song, Z. J.; Tschaen, D. M. *Organic Syntheses* **2005**, *81*, 195. It is possible to isolate the carboxylic acid as an aminoindanol salt, which can provide a stereochemical upgrade to >99% ee if desired. See the Supporting Information for more details.

(10) The product *β*-ketoester **6** is prone to loss of the *tert*-butyl group and subsequent decarboxylation if exposed to acidic conditions for extended periods. For this reason, it is important to conduct a final wash with aqueous $NaHCO_3$ during workup of the Claisen reaction, ensuring there is no trace of acid remaining in the organic solution of **6**.

(11) For an example of an ATH reaction accelerated in the presence of water, see: Wu, X.; Li, X.; Hems, W.; King, F.; Xiao, J. *Org. Biomol. Chem.* **2004**, *2*, 1818.

(12) During workup with MTBE, the tetrabutylammonium salts were easily rejected by aqueous washing with negligible loss of the desired product **8**.

(13) For reduction of lactone with $LiAlH(O^tBu)_3$, see: (a) Wang, P.; Chun, B.; Rachakonda, S.; Du, J.; Khan, N.; Shi, J.; Stec, W.; Cleary, D.; Ross, R. S.; Sofia, M. J. *J. Org. Chem.* **2009**, *74*, 6819. (b) Chen, Z.; Jochmans, D.; Ku, T.; Paeshuyse, J.; Neyts, J.; Seley-Radtke, K. L. *ACS Infect. Dis.* **2015**, *1*, 357. (c) Reddy, P. G.; Chun, B.; Zhang, H.; Rachakonda, S.; Ross, B. S.; Sofia, M. J. *J. Org. Chem.* **2011**, *76*, 3782.

(14) For reduciton of lactone with DIBAL-H, see: (a) Choi, W.; Wilson, L. J.; Yeola, S.; Liotta, D. C. *J. Am. Chem. Soc.* **1991**, *113*, 9377. (b) Takahashi, H.; Iwai, Y.; Hitomi, Y.; Ikegami, S. *Org. Lett.* **2002**, *4*, 2401.

(15) (a) Vorbrüggen, H.; Niedballa, U. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 461. (b) Vorbrüggen, H. *Acc. Chem. Res.* **1995**, *28*, 509.

(16) Glycosylation at room temperature generated mixtures of *N*-7 anomers and *N*-9 anomers. *N*-7 anomers slowly converted to *N*-9 anomers upon heating to 80 °C. Similar experimental results were reported in ref 5e.

(17) The overall efficiency/selectivity for the current glycosylation is similar to that observed for the early Kuwahara synthesis (ref 4a), where chromatography was necessary to isolate the desired anomer. Concurrent with our experimental work on EFdA, a related silyl-group-assisted purification method was reported (ref 5e).

(18) This synthesis proceeds in 17% overall yield and has been used to prepare more than 10 kg of EFdA for use in human clinical trials.

DOI: 10.1021/acs.orglett.7b00091
Org. Lett. 2017, 19, 926−929