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Nine-Step Stereoselective Synthesis of Islatravir from Deoxyribose

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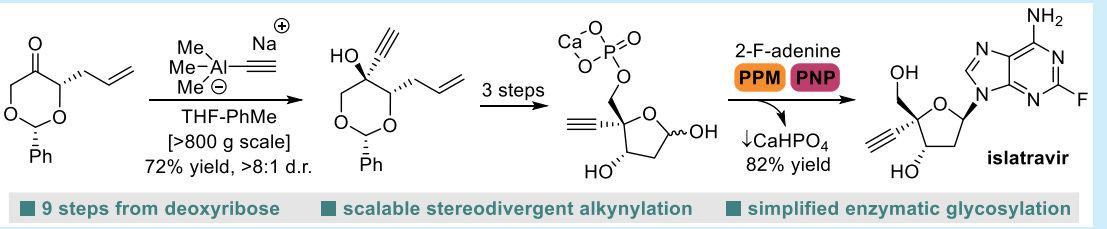

ABSTRACT: A stereoselective nine-step synthesis of the potent HIV nucleoside reverse transcriptase translocation inhibitor (NRTTI) islatravir (EfdA, MK-8591) from 2-deoxyribose is described. Key findings include a diastereodivergent addition of an acetylide nucleophile to an enolizable ketone, a chemoselective ozonolysis of a terminal olefin and a biocatalytic glycosylation cascade that uses a unique strategy of byproduct precipitation to drive an otherwise-reversible transformation forward.

The World Health Organisation (WHO) estimates that over 37 million people worldwide are currently living with HIV.[1] Although modern antiretroviral drugs are highly effective and the death rate from HIV/AIDS is at its lowest since the 1980s, the number of infected individuals continues to rise by almost 2 million in 2016 alone. Today, nucleoside reverse transcriptase inhibitors (NRTIs) are a mainstay of modern antiretroviral therapy, and five drugs in this class are currently approved HIV treatment by the FDA.[2a] However, with no cure in sight, patients often take such drugs for decades; therefore new medicines that address the issues of side effects, efficacy, resistance, and dosing with existing treatments are still highly sought after.[2b,c]

Islatravir (EfdA, MK-8591, **1**) is an adenosine-based inhibitor of reverse transcriptase translocation (i.e., an NRTTI)[2d] discovered by researchers at the Yamasa Corporation and their academic collaborators in 2004 (see Scheme 1).[3,4] It is the first NRTTI reported to date and has shown great promise in preclinical studies, including remarkable potency and a long intracellular half-life. As befits its promise as a drug candidate, several total syntheses of **1** have been reported in the literature thus far.[5]

In 2017, researchers at Merck & Co., Inc. disclosed a scalable route amenable to the production of multikilogram quantities of islatravir from inexpensive dihydroxyacetone.[5d] Although this route is highly efficient (17% overall yield), it is relatively long (14 steps) and suffers from a low yielding and poorly diastereoselective penultimate glycosylation step. More recently, the development of an efficient and highly stereoselective biocatalytic glycosylation solved this latter problem, allowing **1** to be prepared from ribose 5′-phosphate **2** with near-perfect stereoselectivity (Scheme 1).[6] Although ultimately it was found that **2** could also be prepared enzymatically, before this biocatalytic route became available, we sought a chemical synthesis of **2** that would allow us to leverage this new enzyme-mediated glycosylation method. Unfortunately, attempts to adapt published syntheses of **1** or similar nucleosides for the preparation of phosphate **2** resulted in lengthy, inefficient synthetic sequences, and highlighted the need for a completely new approach to this key intermediate.

With this goal in mind, we envisioned a chiral pool approach, predicated on the use of the known 2-deoxy-D-ribose-derived ketone **5** as starting material (see Scheme 1). Achieving an efficient and diastereoselective acetylide addition to **5** to correctly set the stereochemistry at the fully substituted stereogenic center in masked triol **4a** would be a key challenge of this approach, because of the well-known difficulty of achieving axial addition to α-substituted cyclohexanones.[7] If this could be accomplished, then acetal **4a** contains all the necessary carbon atoms and stereogenic centers found in ribose **2**. From here, acetal deprotection, phosphorylation of the primary alcohol, selective ozonolysis of the terminal olefin and deprotection were expected to give the target ribose-5-phosphate **2**, whose biocatalytic conversion to **1** has been demonstrated.[6]

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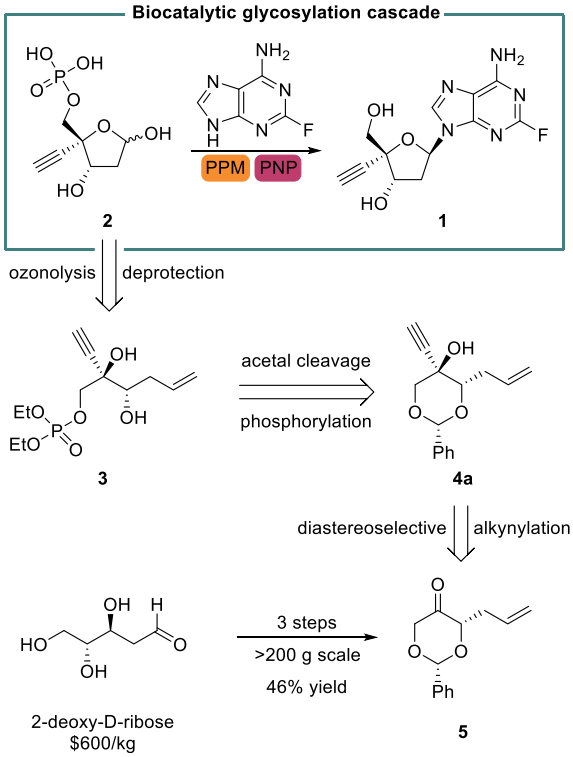
Scheme 1. Retrosynthetic Analysis of 1[a]

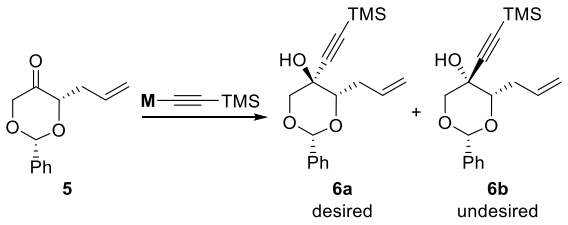


[a]PNP = purine nucleoside phosphorylase and PPM = phosphopentomutase.

Although ketone **5** has been used as a synthetic intermediate in the literature,[8] it was impractical to obtain the quantities necessary to develop our route using the reported procedures, which required three chromatographic purifications. Thus, significant development of the literature chemistry was performed, in order to enable preparation of multihundred-gram quantities of **5** (see the Supporting Information for details). Crucially, although ketone **5** is a low-melting and difficult-to-handle wax, we found that it readily forms a stable crystalline hydrate. This enabled us to purify this material by crystallization from aqueous acetonitrile, which, along with other changes, allowed us to eliminate two chromatographic purification steps from the reported sequence.[9] With ketone **5** now readily available, we began to study the key acetylide addition reaction, the stereochemical outcome of which was uncertain at the outset of these studies. First, we examined the use of TMS-protected acetylide nucleophiles, as these can be conveniently prepared from inexpensive trimethylsilylacetylene without the need for handling or generating acetylene, which is extremely hazardous on scale,[10] and allowed us to screen a range of alkynylmetals (see Table 1). Disappointingly, although a range of protected acetylide nucleophiles was found to react with **5** in good yield, none of these favored the required diastereomer. This is perhaps unsurprising, since formation of the desired diastereomer would require the axial approach of the acetylide, which is a trajectory that is generally disfavored for larger nucleophiles on steric grounds (see Scheme 2).[7,11]

In view of these findings, we decided to investigate the use of smaller acetylide nucleophiles. Encouragingly, the addition of sodium acetylide to 4-*tert*-butylcyclohexanone has long been known to favor the product of axial addition—corresponding

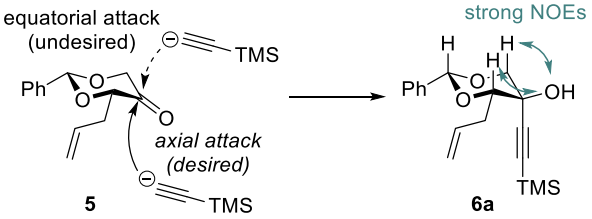
Table 1. Addition of Metalated Ethynyltrimethylsilanes



entry	alkyne nucleophile	yield (%)	ratio of **6a**:**6b**[a]
1	Cl_2Ce—TMS	93	1.0:8.0
2	Li—TMS	53	1.0:3.0
3	MeZn—TMS	81	1.0:1.6
4	BrMg—TMS	95	1.0:1.5

[a]Diastereomeric ratio determined by ^{1}H NMR.

Scheme 2. Axial Approach of Alkyne That Is Required To Give the Desired Diastereomer 6a[a]



[a]The stereochemical outcome was confirmed by NoE analysis.

to the desired stereochemistry in our case.[12] Unfortunately, we were not able to obtain any of the desired product from the reaction of sodium acetylide with ketone **5**,[13] and lithium acetylide ethylenediamine complex was similarly unsuccessful (see Table 2, entries 1 and 2).[14] Although we found that ethynylmagnesium bromide underwent addition in reasonable yield, this again favored the undesired equatorial addition product (Table 2, entry 3). Given the promising literature precedent for the use of sodium acetylide, we again returned to this reagent. Since sodium acetylide is highly insoluble and seemingly too basic to be an effective nucleophile in our system, a way to modify its reactivity was sought. After some experimentation, we were delighted to find that sodium trimethylethynylaluminate—formed by premixing equimolar amounts of sodium acetylide and trimethylaluminum in a mixture of THF and toluene—was a singularly effective acetylide source, giving the desired product in high yield and with better than 19:1 diastereoselectivity (Table 2, entry 4).[15]

Upon further exploration, this aluminate addition reaction exhibits many surprising features. Interestingly, the diastereoselectivity and reaction profile were found to be highly sensitive toward the ratio of trimethylaluminum to sodium acetylide, with the maximum yield and dr being obtained with precisely equimolar quantities of both (Table 2, entry 4). Even a small excess of sodium acetylide was found to result in lower yields, accompanied by the formation of numerous unidentified byproducts, underscoring the incompatibility of **5** with this reagent. Conversely, the use of excess trimethylaluminum was found to greatly decrease the diastereoselectivity of the

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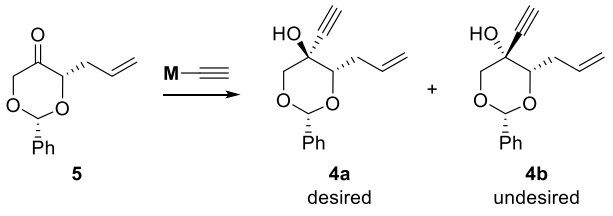
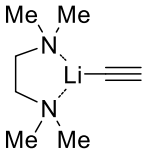
Table 2. Addition of Unprotected Acetylides to 5



entry	alkyne nucleophile	yield (%)	ratio of **4a**:**4b**[a]
1	Na—	N/A	N/A
2		N/A	N/A
3	BrMg—	38	1.0:2.5
4	$NaMe_3Al$—	85	19:1.0[b]
5	$NaMe_3Al$—	100	3.0:2.0[c]
6	$NaMe_3Al$—	100	1.0:3.0[d]
7	$NaMe_3Al$—	72[e]	8.4:1.0[f]

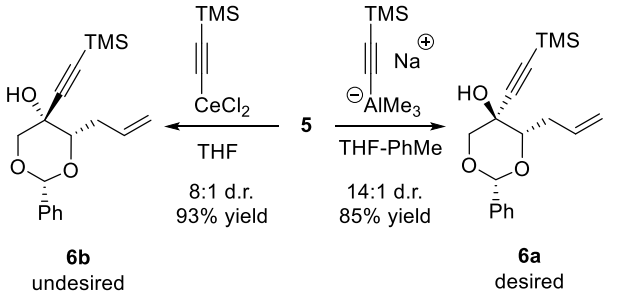
[a]Diastereomeric ratio determined by ^{1}H NMR. [b]Using $AlMe_3$ (2.0 equiv) and NaCCH (2.0 equiv). [c]Using $AlMe_3$ (2.5 equiv) and NaCCH (2.0 equiv). [d]Using $AlMe_3$ (3.0 equiv) and NaCCH (2.0 equiv). [e]Isolated yield on 822 g scale (see the Supporting Information for details). [f]Using $AlMe_3$ (1.92 equiv) and NaCCH (1.55 equiv).

reaction, eventually even causing the unwanted epimer **4b** to be favored (Table 2, entries 5 and 6). The origin of this reversal is not immediately clear, but it may be that the excess trimethylaluminum coordinates to the carbonyl oxygen in a fashion that disfavors the axial approach of the nucleophile, encouraging unwanted equatorial attack—essentially performing the opposite function to the much bulkier Yamamoto aluminum Lewis acids that have been employed to block equatorial attack upon cyclohexanones.[16] The aluminate is a far weaker Lewis acid than trimethylaluminum and likely cannot perform this role alone. Unfortunately, attempts to study the reaction by NMR spectroscopy were complicated by higher-order aggregates and further investigation of this unusual effect is ongoing.

Balancing these competing scenarios, it was found that, because of difficulties in accurately charging the heterogeneous slurry of sodium acetylide that is available commercially, it was best to use a 5% excess of trimethylaluminum in toluene to ensure reproducibly high yields with acceptable diastereoselectivity. Under these optimized conditions, the reaction was run on 822 g scale, giving 72% yield and 8.4:1 dr. Aside from its unique diastereoselectivity in this reaction, note that, compared to more common acetylide anion sources, such as ethynylmagnesium bromide, sodium trimethylethynylaluminate possesses additional advantages in cost and large-scale availability. Indeed, we could only identify one U.S.-based supplier able to provide ton-scale quantities of the former (and only as a 0.5 M solution), whereas sodium acetylide and trimethylaluminum are inexpensive and widely available in bulk, because of their industrial importance.

Returning to silylated alkynes with this result in mind, we were curious about how the aluminate generated from ethynyltrimethylsilane would behave in the addition reaction and how its diastereoselectivity would compare to the reagents already screened (see Scheme 3). Although this aluminate

Scheme 3. Unexpected Stereodivergence in the Addition of Alkyne Nucleophiles to Ketone 5



reagent has not been previously reported, we found that it could be formed as expected by deprotonation with NaHMDS, followed by treatment of the sodiated alkyne with trimethylaluminum. To our surprise, we found that addition of this aluminate nucleophile strongly favored the desired diastereomer **6a**, in contrast to all other metalated alkynes of this type, which all predominantly produce unwanted alcohol **6b**. This unexpected stereodivergence in the addition of acetylides therefore allows preparation of both epimers of **6** with good diastereoselectivity, a feature that may be useful in the preparation of other 4′-substituted nucleosides. The origin of the unique axial selectivity of aluminate reagents is currently unknown, and it is not clear if these reagents represent a general solution to this problem. Regardless, we suspect that the general difficulty in accesing organosodium species may limit this tactic to alkyne nucleophiles, unless aluminates generated from other more readily prepared organometallic nucleophiles display similar reactivity.

With a scalable and highly diastereoselective acetylide addition in hand, we now turned our attention to advancing alcohol **4a** toward the target (see Scheme 4). First, deprotection of the benzylidene acetal was accomplished using hydrochloric acid in methanol.[17] Next, we studied the regioselective phosphorylation of the primary alcohol in triol **7** using diethyl chlorophosphate. Initially, it was found that the reaction suffered from poor selectivity and produced cyclic phosphate byproducts when aged in a pyridine solvent or run with high loading of nucleophilic catalysts (e.g., 20 mol % DMAP).[18] A favorable balance of reaction rate and selectivity was eventually obtained with 1 mol % DMAP in dichloromethane, delivering phosphate **3** in good yield.

Ozonolysis of substrate **3** invoked concerns surrounding chemoselectivity between the alkyne and olefin, tolerance of sensitive functional groups such as a tertiary propargylic alcohol and primary phosphate, and the isolation of a potentially unstable product.[19] Initial experiments generated a mixture of products that were characterized as the lactol and the related methyl glycoside **8**. We were pleased to discover that either product could be selectively formed by varying the pH and solvent, allowing us to isolate the more stable methyl glycoside from an acidic mixture of 1:3 MeOH:$CHCl_3$. Under

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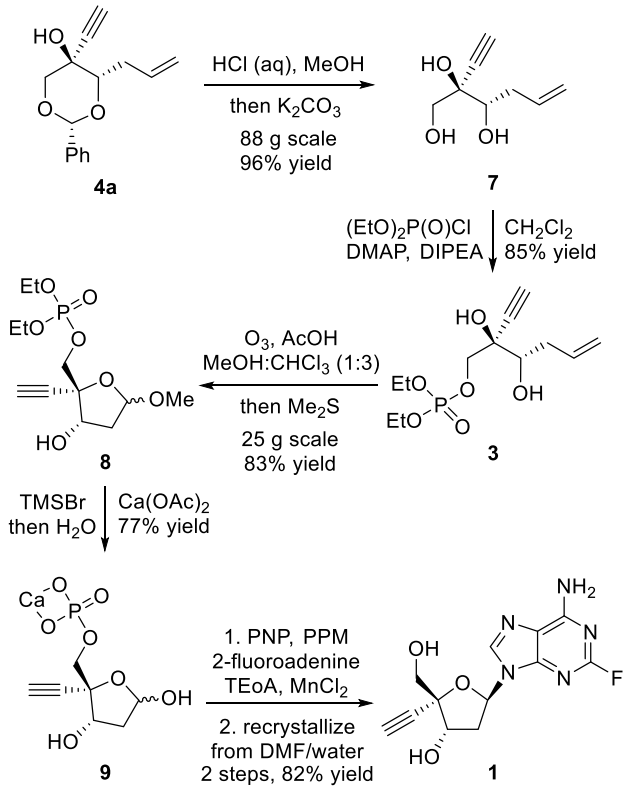
Scheme 4. Completion of Islatravir



optimized conditions, we successfully performed the ozonolysis on 25 g scale to deliver the desired methyl glycoside **8** in 83% yield.

Methyl glycoside **8** could subsequently be fully dealkylated using bromotrimethylsilane, and after quenching with water, the aqueous solution of lactol could be used directly in the enzymatic glycosylation step. Unfortunately, this protocol generated product streams containing high levels of Br ions that adversely affected the efficiency of the enzymatic glycosylation—particularly the purine nucleoside phosphorylase—requiring high dilution and high enzyme loading in order to overcome the negative impact of halide ions on this biocatalyst.[6] This result, taken in combination with a notable lack of solid intermediates throughout the described synthesis, caused us to target an isolation at the lactol stage utilizing the phosphate group as a handle. Initial results indicated that salts with Ca^{2+}, Mn^{2+}, Mg^{2+}, or NH^{4+} were isolable, but purity, stability, and ease of isolation guided us toward the Ca^{2+} salt. After some optimization, it was found that we could isolate the stable calcium phosphate lactol **9** in high yield by addition of $Ca(OAc)_2$ to the crude aqueous lactol solution, followed by trituration and filtration of the highly insoluble solid with acetonitrile.

To our delight, isolated calcium lactol phosphate **9** far outperformed the aqueous stream of **2** from the deprotection that had originally been used in the final enzymatic glycosylation cascade. Furthermore, the use of **9** as an intermediate provided an additional advantage, because it allowed us to simplify the glycosylation protocol. As originally published,[6] using the parent phosphoric acid **2** (instead of its calcium salt **9**), the biocatalytic glycosylation requires the addition of sucrose phosphorylase and sucrose in order to sequester the phosphate ions produced in the reaction (as glucose 1-phosphate).[20,21] Without the driving force provided by this phosphate-scavenging system, the otherwise-reversible glycosylation cascade from **2** to **1** does not proceed to high conversion. However, when **9** is used as starting material, the phosphate produced in the reaction is precipitated as a highly insoluble calcium salt, driving the reaction to completion even in the absence of sucrose/sucrose phosphorylase.[22] The crude API obtained from this reaction could be dissolved in dimethylformamide (DMF), filtered away from the inorganic calcium phosphate byproducts and recrystallized upon the addition of water to deliver the product in >99% purity and 82% isolated yield.

We have reported a highly stereoselective synthesis of islatravir in six steps (36% overall yield) from known ketone **5** (nine steps overall from inexpensive 2-deoxyribose). Of particular interest is the highly diastereoselective addition of sodium trimethylethynylaluminate, which is an inexpensive and underutilized acetylide equivalent whose use was demonstrated on a >800 g scale, as well as a selective ozonolysis of the terminal olefin, and an enzymatic glycosylation cascade driven forward by the precipitation of calcium phosphate. This sequence represents an expedient approach to 4′-substituted ribose derivatives that may be generalizable for other non-natural nucleosides.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c00239.

Experimental procedures, characterization data, and ^1H and ^{13}C NMR spectra of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) *HIV/AIDS Data and Statistics*. Available via the Internet at: http://www.who.int/hiv/data/en/ (accessed Nov 15, 2018).

(2) (a) *FDA-Approved HIV Medicines*. Available via the Internet at: https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/21/58/fda-approved-hiv-medicines (accessed Jan 16, 2020). For information on NRTIs, see: (b) De Clercq, E. The Nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase tnhibitors, and protease inhibitors in the treatment of HIV infections (AIDS). *Adv. Pharmacol.* **2013**, *67*, 317−358. For a broader view of anti-viral drugs, see: (c) De Clercq, E. Antiretroviral drugs. *Curr. Opin. Pharmacol.* **2010**, *10*, 507−515. Conversely, for a discussion of the NRTTI mechanism of action, see: (d) Michailidis, E.; Marchand, B.; Kodama, E. N.; Singh, K.; Matsuoka, M.; Kirby, K. A.; Ryan, E. M.; Sawani, A. M.; Nagy, E.; Ashida, N.; Mitsuya, H.; Parniak, M. A.; Sarafianos, S. G. DNA: Replication, repair, recombination, and chromosome dynamics. *J. Biol. Chem.* **2009**, *284*, 35681−35691.

(3) Discovery: (a) Kohgo, S.; Ohrui, H.; Kodama, E.; Matsuoka, M.; Mitsuya, H. 4′-C-Substituted-2-halo-adenosine derivative. Can. Patent No. CA 2502109, March 22, 2005. (b) Kohgo, S.; Yamada, K.; Kitano, K.; Iwai, Y.; Sakata, S.; Ashida, N.; Hayakawa, H.; Nameki, D.; Kodama, E.; Matsuoka, M.; Mitsuya, H.; Ohrui, H. Design, efficient synthesis, and anti-HIV activity of 4′-C-cyano- and 4′-C-ethynyl-2′-deoxy purine nucleosides. *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 671−690. (c) Ohrui, H. 2′-deoxy′4′-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, is highly potent against all human immunodeficiency viruses type 1 and has low toxicity. *Chem. Rec.* **2006**, *6*, 133−143. (d) Ohrui, H.; Hayakawa, H.; Kohgo, S.; Matsuoka, M.; Kodama, E.; Mitsuya, H. Creation of low toxic reverse-transcriptase inhibitory nucleosides that prevent the emergence of drug-resistant HIV variants. *Yuki Gosei Kagaku Kyokaishi* **2006**, *64*, 716−721. (e) Ohrui, H.; Kohgo, S.; Hayakawa, H.; Kodama, E.; Matsuoka, M.; Nakata, T.; Mitsuya, H. 2′-Deoxy-4′-C-ethynyl-2-fluoroadenosine: A nucleoside reverse transcriptase inhibitor with highly potent activity against wide spectrum of HIV-1 strains, favorable toxic profiles, and stability in plasma. *Nucleosides, Nucleotides Nucleic Acids* **2007**, *26*, 1543−1546. (f) Ohrui, H. Development of modified nucleosides that have supremely high anti-HIV activity and low toxicity and prevent the emergence of resistant HIV mutants. *Proc. Jpn. Acad., Ser. B* **2011**, *87*, 53−58.

(4) In 2012, Merck & Co., Inc. (Kenilworth, NJ, USA) licensed the compound from Yamasa for further development.

(5) Previous syntheses of **1**: (a) Kageyama, M.; Nagasawa, T.; Yoshida, M.; Ohrui, H.; Kuwahara, S. Enantioselective total synthesis of the potent anti-HIV nucleoside EfdA. *Org. Lett.* **2011**, *13*, 5264−5266. (b) Kageyama, M.; Miyagi, T.; Yoshida, M.; Nagasawa, T.; Ohrui, H.; Kuwahara, S. Concise synthesis of the anti-HIV nucleoside EfdA. *Biosci., Biotechnol., Biochem.* **2012**, *76*, 1219−1225. (c) Fukuyama, K.; Ohrui, H.; Kuwahara, S. Synthesis of EFdA via a diastereoselective aldol reaction of a protected 3-keto furanose. *Org. Lett.* **2015**, *17*, 828−831. (d) McLaughlin, M.; Kong, J.; Belyk, K. M.; Chen, B.; Gibson, A. W.; Keen, S. P.; Lieberman, D. R.; Milczek, E. M.; Moore, J. C.; Murray, D.; Peng, F.; Qi, J.; Reamer, R. A.; Song, Z. J.; Tan, L.; Wang, L.; Williams, M. J. Enantioselective synthesis of 4′-ethynyl-2-fluoro-2′-deoxyadenosine (EFdA) via enzymatic desymmetrization. *Org. Lett.* **2017**, *19*, 926−929. (e) Kamata, M.; Takeuchi, T.; Hayashi, E.; Nishioka, K.; Oshima, M.; Iwamoto, M.; Nishiuchi, K.; Kamo, S.; Tomoshige, S.; Watashi, K.; Kamisuki, S.; Ohrui, H.; Sugawara, F.; Kuramochi, K. Synthesis of nucleotide analogues, EFdA, EdA and EdAP, and the effect of EdAP on hepatitis B virus replication. *Biosci., Biotechnol., Biochem.*, *84*, 217−227.

(6) Huffman, M. A.; Fryszkowska, A.; Alvizo, O.; Borra-Garske, M.; Campos, K. R.; Canada, K. A.; Devine, P. N.; Duan, Da.; Forstater, J. H.; Grosser, S. T.; Halsey, H. M.; Hughes, G. J.; Jo, J.; Joyce, L. A.; Kolev, J. N.; Liang, J.; Maloney, K. M.; Mann, B. F.; Marshall, N. M.; McLaughlin, M.; Moore, J. C.; Murphy, G. S.; Nawrat, C. C.; Nazor, J.; Novick, S.; Patel, N. R.; Rodriguez-Granillo, A.; Robaire, S. A.; Sherer, E.; Truppo, M. D.; Whittaker, A. M.; Verma, D.; Xiao, L.; Xu, Y.; Yang, H. Design of an in vitro biocatalytic cascade for the manufacture of islatravir. *Science* **2019**, *366*, 1255−1259.

(7) The reaction of dioxanones with methylmagnesium chloride and lithium aluminum hydride has long been known to be highly selective for the desired axial addition products, as a result of the greater torsional strain that develops in the transition state for equatorial addition (in fact, addition to dioxanones is generally more axial-selective than for the parent cyclohexanones, because of the shorter C−O bonds). See: (a) Urbansky, M.; Davis, C. E.; Surjan, J. D.; Coates, R. M. Synthesis of enantiopure 2-C-methyl-d-erythritol 4-phosphate and 2,4-cyclodiphosphate from d-arabitol. *Org. Lett.* **2004**, *6*, 135−138. (b) Wu, Y. D.; Houk, K. N. Electronic and conformational effects on pi-facial stereoselectivity in nucleophilic additions to carbonyl compounds. *J. Am. Chem. Soc.* **1987**, *109*, 908−910. However, addition of larger nucleophiles such as ethynylmagnesium bromide has been reported to be relatively unselective in a similar system, highlighting the challenge of achieving selective axial attack of larger carbon nucleophiles under substrate control: (c) Carda, M.; Casabo, P.; Gonzalez, F.; Rodriguez, S.; Domingo, L. R.; Marco, J. A. Diastereoselectivity of the reactions of organometallic reagents with protected d-and l-erythrulose 1, 3-O-ethylidene acetals. *Tetrahedron: Asymmetry* **1997**, *8*, 559−577.

(8) Urosa, A.; Marcos, I. S.; Diez, D.; Padron, J. M.; Basabe, P. Synthesis of luffarin L and 16-epi-luffarin L using a temporary silicon-tethered ring-closing metathesis reaction. *J. Org. Chem.* **2015**, *80*, 6447−6455.

(9) The parent 2-phenyldioxanone has been reported to form a similar hydrate: Carlsen, P. H. J.; Sørbye, K.; Ulven, T.; Aasbø, K.; Klinga, M.; Romerosa, A. Synthesis of benzylidene-protected dihydroxyacetone. *Acta Chem. Scand.* **1996**, *50*, 185−187. [Note that, prior to the alkyne additions, the ketone hydrate was dehydrated by azeotropic concentration from toluene to give a dry solution of the ketone (see the Supporting Information for details).]

(10) Miller, S. A.; Penny, E. Hazards in Handling Acetylene in Chemical Processes Particularly under Pressure. In *Symposium on Chemical Process Hazards*; IChemE Symposium Series No. 7, 1960; pp 87−94.

(11) Mehta, G.; Chandrasekhar, J. Electronic control of facial selection in additions to sterically unbiased ketones and olefins. *Chem. Rev.* **1999**, *99*, 1437−1467.
(12) Hennion, G. F.; O'Shea, F. X. Ethynylation of 4-*t*-Butylcyclohexanone and kinetics of saponification of the ethynylcarbinol esters. *J. Am. Chem. Soc.* **1958**, *80*, 614−617.
(13) Some decomposition of the starting material was observed. We speculate that the greater acidity of the protons adjacent to the ketone **5** in our system may lead to enolate formation and self-condensation. Generally, more basic nucleophiles were found to produce greater levels of side-products with this system.
(14) The parent lithium acetylide was avoided, because of its well-known propensity to disproportionate to lithium carbide and acetylene, which makes its use complicated and hazardous on scale: (a) Mortier, J.; Vaultier, M.; Carreaux, F.; Douin, J.-M. Disproportionation of monolithium acetylide into dilithium carbide and acetylene is a reversible reaction in tetrahydrofuran at 0 °C. *J. Org. Chem.* **1998**, *63*, 3515−3516. (b) Midland, M. M.; Gallou, F. *Lithium Acetylide in Encyclopedia of Reagents for Organic Synthesis* [Online] 2006 (DOI: 10.1002/047084289X.rl035.pub2).
(15) Joung, M. J.; Ahn, J. H.; Yoon, N. M. Sodium trimethylethynylaluminate, a new chemoselective ethynylating agent. *J. Org. Chem.* **1996**, *61*, 4472−4475. Although this reagent was described some time ago, to the best of our knowledge, it has not been applied in a multistep organic synthesis before this report.
(16) A more classical solution to achieve equatorial nucleophilic attack on cyclohexanones involves the use of the bulky MAD aluminum Lewis acids. However, these are generally less selective for α-substituted ketones, presumably because of the steric clash of neighboring groups with the Lewis acid. See: Maruoka, K.; Itoh, T.; Yamamoto, H. Methylaluminum Bis(2,6-di-*tert*-butyl-4-alkylphenoxide). A New Reagent for Obtaining Unusual Equatorial and Anti-Cram Selectivity in Carbonyl Alkylation. *J. Am. Chem. Soc.* **1985**, *107*, 4573−4576.
(17) This seemingly simple transformation was complicated by the extremely high water solubility of triol **7**, which precluded a traditional aqueous workup. An efficient protocol was developed wherein, upon completion, water was added to the methanolic reaction mixture, allowing the benzaldehyde generated to be removed by extraction with heptanes, which is immiscible with the methanol−water phase containing the product. Saturating the aqueous mixture with K_2CO_3 allowed (i) phase separation of the MeOH (where the triol resides) from the water, and (ii) stabilization of the triol intermediate, which was found to decompose if concentrated from low pH solutions. Although unnecessary for downstream chemistry, residual salts could be removed by slurrying the crude product in TBME, followed by filtration. This procedure provided 44 g of triol **7** in 96% isolated yield and 94 wt% purity. For further discussion of the phase separation of miscible liquids, see: Hyde, A. M.; Zultanski, S. L.; Waldman, J. H.; Zhong, Y.-L.; Shevlin, M.; Peng, F. General principles and strategies for salting-out informed by the Hofmeister series. *Org. Process Res. Dev.* **2017**, *21*, 1355−1370.
(18) Phosphate ester migration and cyclization of similar compounds under basic conditions has been reported. See: Brown, D. M.; Todd, A. R. Nucleotides. Part X. Some observations on the structure and chemical behaviour of the nucleic acids. *J. Chem. Soc.* **1952**, 52−58.
(19) For examples of industrial-scale ozonolysis, see: (a) Van Ornum, S. G.; Champeau, R. M.; Pariza, R. Ozonolysis applications in drug synthesis. *Chem. Rev.* **2006**, *106*, 2990−3001. (b) Fisher, T. J.; Dussault, P. H. Alkene ozonolysis. *Tetrahedron* **2017**, *73*, 4233−4258. For chemoselective ozonolysis of alkenes over alkynes, see ref 5a and: McCurry, P. M., Jr.; Abe, K. Selective ozonolysis of an enyne derivative. The synthesis of cis-jasmone. *Tetrahedron Lett.* **1974**, *15*, 1387−1390.
(20) Tischler, W.; Hans-Georg, I.; Barzu, O.; Sakamoto, H.; Pistotnik, E.; Marliere, P.; Pochet, S. *Enzymatic Synthesis of Deoxyribonucleosides*. U.S. Patent No. US07229797B1, 2007.
(21) Hammer-Jespersen, K.; Munch-Petersem, A. Phosphodeoxyribomutase from escherichia coli purification and some properties. *Eur. J. Biochem.* **1970**, *17*, 397−407.
(22) The related tactic of driving a phosphate-producing reaction to completion by precipitating insoluble magnesium phosphate has been reported, for example, for cellodextrin phosphorylase: Zhong, C.; Luley-Goedl, C.; Nidetzky, B. Product solubility control in cellooligosaccharide production by coupled cellobiose and cellodextrin phosphorylase. *Biotechnol. Bioeng.* **2019**, *116*, 2146−2155. In this case, we found magenisum additives to be less effective than either the use of a calcium salt as described here or the sucrose/sucrose phosphorylase system described in ref 6.

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