JOC The Journal of Organic Chemistry

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Chemical Synthesis of the Antiviral Nucleotide Analogue ddhCTP

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Cite This: J. Org. Chem. 2021, 86, 8843−8850 Read Online

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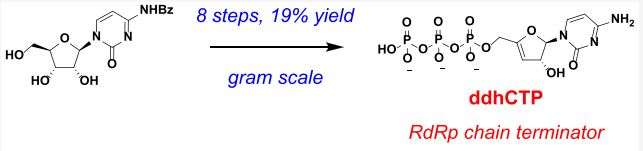
ABSTRACT: 3′-Deoxy-3′,4′-didehydro-cytidine triphosphate (ddhCTP) is a novel antiviral molecule produced by the enzyme viperin as part of the innate immune response. ddhCTP has been shown to act as an obligate chain terminator of flavivirus and SARS-CoV-2 RNA-dependent RNA polymerases; however, further biophysical studies have been precluded by limited access to this promising antiviral. Herein, we report a robust and scalable synthesis of ddhCTP as well as the mono- and diphosphates ddhCMP and ddhCDP, respectively. Identification of a 2′-silyl ether protection strategy enabled selective synthesis and facile purification of the 5′-triphosphate, culminating in the preparation of ddhCTP on a gram scale.



INTRODUCTION

The frequent occurrence of epidemics in the past two decades (SARS-CoV-1, MERS-CoV, EBOV) and the emergence of the COVID-19 pandemic has brought widespread attention to the impacts viruses can have on human life and healthcare systems. Viruses are implicated in the majority of epidemic and pandemic diseases, which reflects their high transmissibility and the paucity of effective antiviral therapies.[1] In the absence of vaccines, treatment of emergent and re-emergent viruses relies on the repurposing of existing drugs, and therefore, the development of broad-spectrum antiviral drugs is of great value.[2] Attainment of this goal is challenging as viruses are obligate parasites which employ host cell machinery and present fewer druggable targets compared to other pathogens. Nevertheless, a number of nucleotide analogues which target viral proteins such as RNA-dependent RNA polymerases (RdRp) and DNA polymerases do exhibit broad-spectrum activity and constitute an important class of antiviral drugs (Figure 1a).[3]

In 2018, Gizzi et al. reported that viperin (Virus Inhibitory Protein, Endoplasmic Reticulum-associated, Interferon-inducible), also known as RSAD2 (radical SAM domain-containing 2), catalyzes the formal dehydration of cytidine triphosphate (CTP, **1**) to 3′-deoxy-3′,4′-didehydro-cytidine triphosphate (ddhCTP, **2**) (Figure 1b).[4] Viperin expression is induced by interferon pathways[5] and has been shown to inhibit replication of both RNA and DNA viruses by diverse mechanisms, including protein−protein interactions and modulation of immune signaling, thereby contributing to the innate antiviral response.[6] The role of ddhCTP (**2**) in the antiviral activity of viperin is still not completely understood; however, it has been shown to compete with CTP (**1**) for incorporation by RdRps of the *Flaviviridae* (dengue virus, West Nile virus, Zika virus, and hepatitis C virus), resulting in obligate chain termination and inhibition of viral genome replication.[4] A recent study has also shown that ddhCTP (**2**) is efficiently utilized by the SARS-CoV-2 replicase.[7] Importantly, ddhCTP (**2**) production does not affect the viability or growth rate of Vero and HEK293T cells and is specific for non-native RNA polymerases.[4,8]

In light of these findings, ddhCTP (**2**) represents a promising platform for the development of broad-spectrum antiviral agents; however, elucidation of its antiviral activity is ongoing. Previous studies have been conducted with small quantities of ddhCTP (**2**) prepared enzymatically from CTP (**1**) using isolated viperin; however, purification of ddhCTP (**2**) to remove unconverted CTP (**1**) poses a significant challenge.[4] To circumvent complications arising from CTP (**1**) impurities, we sought to establish a chemical synthesis of ddhCTP (**2**) that would provide sufficiently pure material for biophysical studies and evaluation of antiviral activity. We envisioned ddhCTP (**2**) would be accessible by phosphorylation of an appropriately protected ddh-nucleoside **3**, which in turn could be prepared by formal dehydration of 4-*N*-benzoylcytidine (**4**). We anticipated chemical synthesis of ddhCTP (**2**) from a ddh-nucleoside **3** would be more scalable than the existing bioenzymatic synthesis and would also provide the opportunity to prepare differently phosphorylated forms of the parent nucleoside for elucidation of biochemical pathways. Herein, we report the development of a protecting group strategy to provide convenient access to the ddhC nucleotide derivatives ddhCMP, ddhCDP, and ddhCTP (**2**).

Received: March 31, 2021
Published: June 14, 2021

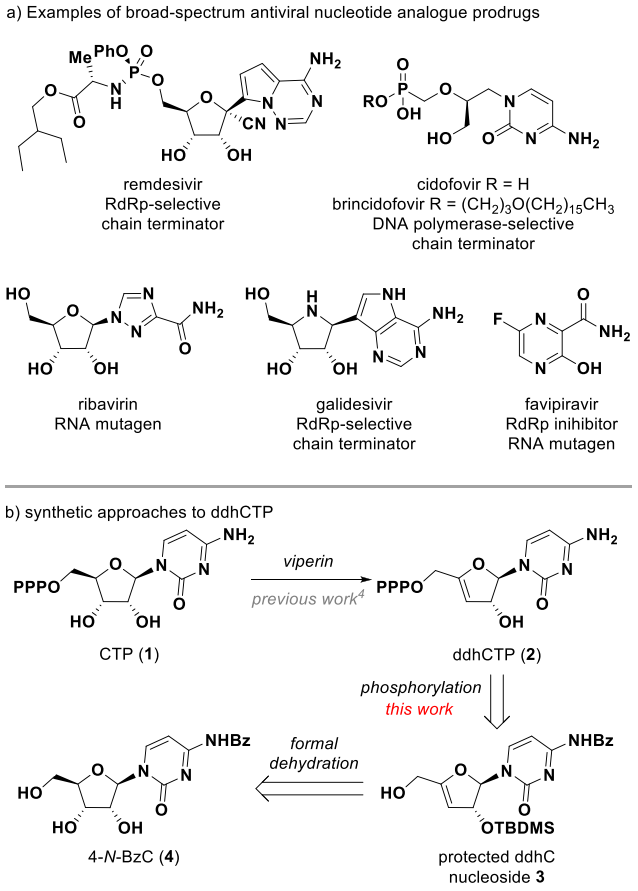
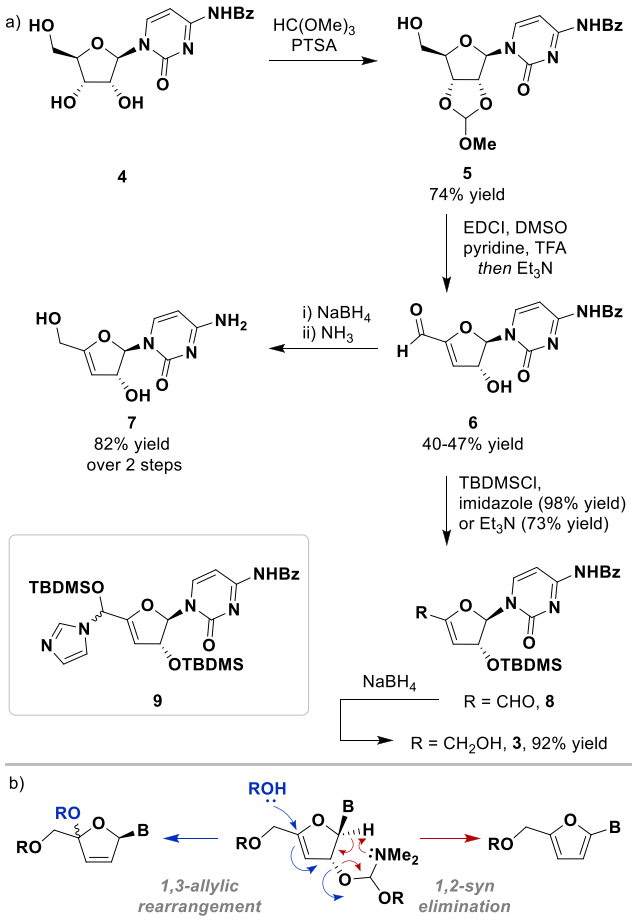


Figure 1. (a) Broad-spectrum antiviral nucleotide analogue prodrugs and (b) proposed synthesis of ddhCTP (**2**).

RESULTS AND DISCUSSION

Our development of a suitable synthetic strategy toward the ddhC-nucleotides commenced with preparation of ddhC (**7**) from commercially available 4-*N*-benzoylcytidine (**4**) following a method reported by Petrová et al.[9] This approach involved oxidation of the 5′-alcohol to an aldehyde, which facilitates base-catalyzed elimination of a 2′,3′-orthoformate at the 3′-position to establish the 3′-deoxy-3′,4′-didehydroribo-ring system (Scheme 1a). While following this work, we identified a number of procedural modifications which improved the practicality of the synthesis on a large scale, including the replacement of two chromatographic purification steps; orthoester **5** and alcohol **7** were both obtained cleanly by trituration. The reduction of aldehyde **6** was best performed with only 0.5 equiv of sodium borohydride to prevent reduction of the cytosine ring, while benzoyl cleavage with ammonia avoided a 4-*N*-methylcytidine side product that arose when the deprotection was carried out using the prescribed methylamine. More significantly, the Moffatt−Pfitzner oxidation to aldehyde **6** was poorly reproducible in our hands. Conversion to the aldehyde was efficient, yet it proved unstable during workup despite being stable once isolated; typical isolated yields ranged from 40−47%, compared with the literature reported yield of 69%.[9a] Exploration of alternative oxidation methodologies was largely precluded by the limited solubility profile of alcohol **5**. However, through optimization we found the reaction went to completion with fewer equivalents of the EDC-DMSO reagent system. This in turn facilitated purification of aldehyde **6** but failed to improve isolated yields.

Scheme 1. (a) Synthesis of ddhC (7) and TBDMS-Protected Derivative 3 via Aldehyde 6 and (b) Previously Reported Reactivity of 2′-*O*-DMF Acetals of 3′,4′-Didehydroribo Ring Systems[9b]



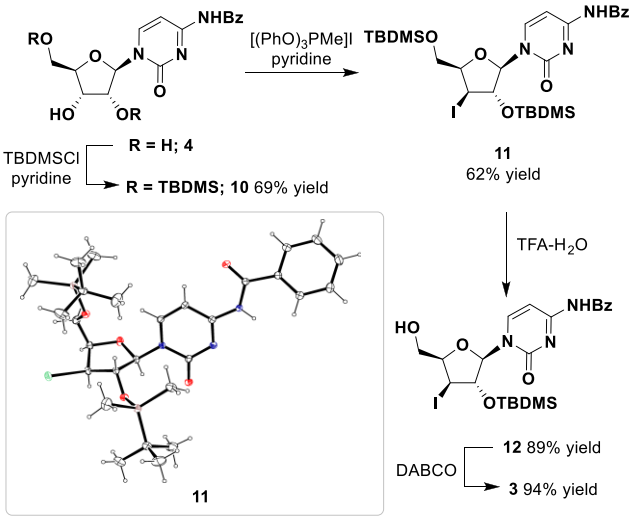
During our optimization of the Moffat−Pfitzer oxidation, we identified aldehyde **6** (Scheme 1) as a potentially convenient intermediate to attempt selective protection of the 2′-alcohol. It was anticipated the didehydroribo system would be incompatible with the deprotection conditions most commonly used in benzyl- or allyl-based protecting group strategies. Additionally, Petrová et al. have reported that 2′-*O*-DMF acetals of didehydroribo systems readily undergo Ferrier-type allylic rearrangement as well as *syn*-elimination across C1′−C2′ to form furan products (Scheme 1b).[9b] An acyl or carbonate protecting group therefore presented the risk of undesirable reactivity. A *tert*-butyldimethylsilyl protecting group strategy was ultimately adopted, with the view that a lipophilic silyl group might aid purification of intermediates by both normal and reverse-phase chromatography. Standard protection conditions of silyl chloride and imidazole afforded the 2′-*O*-TBDMS ether **8** in high yield; however, careful control of reagent stoichiometry was required to avoid formation of *N*,*O*-acetal **9**, and the reaction was capricious, particularly when attempting to scale up. Recovery of the aldehyde from *N*,*O*-acetal **9** required prohibitively harsh conditions,[10] and so imidazole was substituted by triethylamine in the synthesis of silyl ether **8** to deliver more

https://doi.org/10.1021/acs.joc.1c00761
J. Org. Chem. 2021, 86, 8843−8850

consistent results. Clean reduction of the aldehyde could be achieved with 0.5 equiv of borohydride, affording alcohol **3** in 92% yield.

Having identified **3** as a suitable intermediate to attempt phosphorylation reactions toward ddhC-nucleotides, an alternative, reliable, and scalable synthesis of this intermediate was sought. Accordingly, we envisioned a strategy involving elimination of a 3′-iodide in the *xylo* configuration to construct the 3′-deoxy-3′,4′-dehydroribose ring system.[11] Regioselective silyl protection[12] of 4-*N*-benzoylcytidine (**4**) and then iodination with methyltriphenoxyphosphonium iodide[13] afforded 3′-iodide **11** (Scheme 2). A more obvious reagent

Scheme 2. Synthesis of TBDMS-Protected ddhC 3 by Elimination of Iodide 11[a]



[a]ORTEP diagram of **11** shown with 30% probability ellipsoids.

system of PPh_3, I_2, and imidazole was also investigated for this iodination,[14] but necessitated the use of high temperatures at which the 4-*N*-benzoyl group was labile. A crystal structure obtained for **11** confirmed the iodination proceeded with stereoinversion, setting the stage for base-mediated elimination of HI. Accordingly, 5′-*O*-silyl cleavage and then treatment with DABCO provided 4-*N*-benzoyl-2′-*O*-TBDMS ddhC (**3**). This intermediate formed the linchpin for the synthesis of all ddhC-based targets.

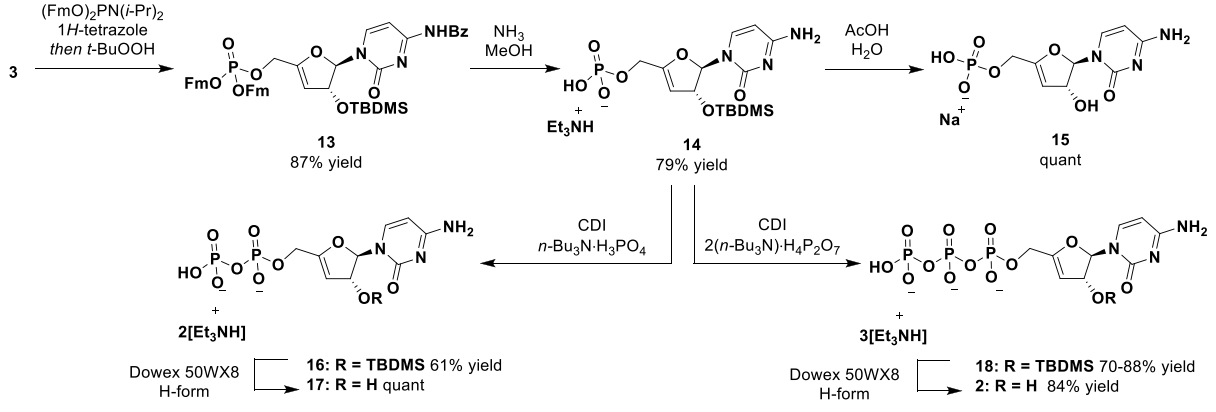
Synthesis of ddhCMP (**15**) was first attempted by applying the Yoshikawa method ($POCl_3$ in trimethylphosphate) to intermediate **3**;[15] however, a complex mixture of products was obtained, implying the phosphorodichloridate intermediate was unstable (Scheme 3). Phosphoramidite chemistry was instead employed to access the monophosphate,[16] delivering difluorenylmethyl phosphate **13** in 87% yield. Advancement of phosphate ester **13** to ddhCMP (**15**) required global deprotection: both the 4-*N*-benzoyl and fluorenylmethyl groups were readily removed using ammonia in methanol. Traceless deprotection of the TBDMS ether under mild acidic conditions gave ddhCMP (**15**) quantitatively, which was converted to its sodium salt by ion exchange.

ddhCDP (**17**) and ddhCTP (**2**) were both prepared from TBDMS-protected monophosphate **14** using the method reported by Hoard and Ott.[17] Adoption of this strategy over direct di- or triphosphorylation of alcohol **3** was motivated by the scalability of the reaction[18] as well as the simplicity of the reagent system, which would streamline purification. Activation of monophosphate **14** as the imidazolidate was performed using CDI, the excess of which was quenched using water. *In situ* treatment of the imidazolidate with the bis-(tributylammonium) salt of pyrophosphate gave triphosphate **18** in 70−88% yield. When MeOH was used as a CDI quenching agent instead of water, formation of a methyl carbamate at C4 of the cytosine nucleobase was observed. Triphosphate **18** exhibits good retention on C18 silica, a feature we attribute to the lipophilic TBDMS protecting group.[19] This enabled rapid and scalable purification of the triphosphate by reverse-phase flash chromatography using an aqueous Bu_3N-AcOH and MeOH ion-pairing eluent system, whereas oligophosphates usually require purification by strong ion-exchange chromatography.[20] Traceless TBDMS cleavage was effected by treatment with Dowex resin in water, affording ddhCTP (**2**) in excellent yield. This synthesis was used to prepare approximately 1 g of ddhCTP (**2**) in a single pass and therefore provides ample access to this compound for biological evaluation. ddhCDP (**17**) was also readily accessed from monophosphate **14** by activation with CDI, treatment with orthophosphate, and Dowex-mediated TBDMS cleavage.

CONCLUSION

In conclusion, a synthetic route utilizing a 2′-*O*-TBDMS protected ddhC derivative has enabled facile synthesis of biologically relevant phosphates of ddhC (**7**). The benefits of

Scheme 3. Synthesis of ddhCMP (15), ddhCDP (17), and ddhCTP (2)



https://doi.org/10.1021/acs.joc.1c00761
J. Org. Chem. 2021, 86, 8843−8850

the TBDMS protecting group are twofold: it provides a lipophilic handle that enables reverse-phase flash chromatographic purification of highly charged compounds that would otherwise require more intensive purification methods, and its traceless removal under mild conditions provides the deprotected targets in good purity. Taken together, these properties have facilitated a robust and scalable synthesis of ddhCTP (**2**), providing useful quantities of this antiviral metabolite and its prodrugs for biological studies.

EXPERIMENTAL SECTION

Silyl ether **10**,[12] methyltriphenoxyphosphonium iodide,[21] difluorenyl *N*,*N*-diisopropylphosphoramidite,[22] tributylammonium phosphate,[23] and bis(tributylammonium) pyrophosphate[24] were prepared according to literature procedures. Reactions requiring anhydrous conditions were carried out in flame-dried glassware under a positive pressure of argon in anhydrous solvents using standard Schlenk techniques. Reaction temperatures above room temperature (22−23 °C) were carried out in heating mantles with an internal temperature probe. Reaction progress was monitored by thin layer chromatography (TLC) on Merck Aluminum-backed silica gel coated TLC plates (60 Å, F254 indicator). TLC plates were visualized by exposure to ultraviolet light (254 nm) and/or $KMnO_4$. Flash column chromatography was performed either manually in glass columns using Sigma-Aldrich silica gel (35−75 μm particle size) or with a Büchi Pure C-815 Flash automated flash chromatography system using prepacked FlashPure cartridges containing either silica gel (50 μm irregular) or C18 silica gel (50 μm spherical), using ACS grade solvents. All yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C{^{1}H} NMR) pure material. NMR spectra were recorded using a Bruker 500 MHz spectrometer. All chemical shifts (δ) are reported in parts per million (ppm) and referenced to residual protium or the carbon resonance of the NMR solvent, respectively. ^{31}P{^{1}H} NMR spectra were referenced to H_3PO_4 as an external standard. Data are represented as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (J) in Hertz (Hz), integration. High resolution electrospray ionization (ESI) mass spectra were undertaken on a Waters Q-TOF Premier Tandem Mass spectrometer fitted with a Waters 2795 HPLC. Optical rotations were measured on a Rudolph Research Analytical Autopol IV automatic polarimeter. Crystallographic data for compound **11** were collected on an Agilent SuperNova diffractometer fitted with an EOS S2 detector.

4-*N*-Benzoyl-2′,3′-di-*O*-methoxymethylidene-cytidine (5).[25] To a suspension of 4-*N*-benzoylcytidine (**4**) (15.0 g, 43.2 mmol) in anhydrous DMF (100 mL) at room temperature were added trimethyl orthoformate (14 mL, 130 mmol) and PTSA monohydrate (750 mg, 4.32 mmol). The reaction mixture was stirred at 50 °C for 2 h, after which a homogeneous solution was obtained. The reaction mixture was neutralized by addition of MeOH-washed Amberlyst A21 (6 g) and stirred for 5 min, then filtered and concentrated *in vacuo*. The crude solid obtained was suspended in EtOAc (75 mL) and heated at 70 °C for 30 min and cooled to room temperature, and the solid product was collected by filtration. The mother liquor was left to stand for 24 h, upon which further product precipitated and was collected by filtration. The two crops of product were combined and dried under high vacuum to afford the title compound (12.5 g, 74%) as a colorless powder. The product was a 1.9:1 mixture of diastereomers at the orthoformate stereocenter, designated as *A* (major) and *B* (minor). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.26 (s, 1H, *A*+*B*), 8.31−8.26 (m, 1H, *A*+*B*), 8.01 (d, J = 7.3 Hz, 2H, *A*+*B*), 7.63 (t, J = 7.4 Hz, 1H, *A*+*B*), 7.52 (t, J = 7.8 Hz, 2H, *A*+*B*), 7.38−7.33 (m, 1H, *A*+*B*), 6.11 (s, 0.35H, *B*), 6.01 (s, 0.65H, *A*), 5.97 (d, J = 2.4 Hz, 0.65H, *A*), 5.85 (d, J = 2.0 Hz, 0.35H, *B*), 5.12 (t, J = 5.2 Hz, 0.35H, *B*), 5.07 (t, J = 5.3 Hz, 0.65H, *A*), 5.04−5.00 (m, 1H, *A*+*B*), 4.89 (dd, J = 6.3, 3.4 Hz, 0.35H, *B*), 4.83 (dd, J = 7.1, 3.4 Hz, 0.65H, *A*), 4.31 (q, J = 4.6 Hz, 0.65H, *A*), 4.22 (q, J = 4.3 Hz, 0.35H, *B*), 3.71−3.58 (m, 2H, *A*+*B*), 3.33 (s, *A*, obscured by H_2O), 3.22 (s, 1.05H, *B*); ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 167.3 (*A*+*B*), 163.5 (*A*+*B*), 154.4 (*A*+*B*), 147.0 (*A*), 146.7 (*B*), 133.1 (*A*+*B*), 132.8 (*A*+*B*), 128.4 (*A*+*B*), 118.0 (*A*), 116.7 (*B*), 96.1 (*A*+*B*), 94.3 (*A*), 93.4 (*A*), 88.1 (*A*), 87.0 (*B*), 84.4 (*A*), 83.8 (*B*), 80.8 (*A*), 80.2 (*B*), 61.5 (*A*), 61.1 (*B*), 51.7 (*A*), 50.3 (*B*). The spectroscopic properties of these diastereomers were consistent with the data available in the literature.[25]

4-*N*-Benzoyl-3′-deoxy-3′,4′-didehydro-cytidine-5′-aldehyde (6).[9a] The title compound was prepared according to a procedure adapted from Petrová et al.[9a] Alcohol **5** (12.2 g, 31.3 mmol) and EDC·HCl (12.6 g, 62.4 mmol) were placed under argon and then suspended in DMF (65 mL). To this suspension at room temperature was added DMSO (4.6 mL, 64 mmol), followed by a solution of pyridine (2.6 mL, 32 mmol) and TFA (1.2 mL, 16 mmol) in DMF (13 mL). The reaction mixture was stirred for 1 h, after which it became a pale yellow homogeneous solution. The reaction mixture was cooled to 0 °C, and Et_3N (17.5 mL, 125 mmol) was added, resulting in immediate precipitation of Et_3N·HCl. Then, the mixture was stirred for 10 min. The reaction was quenched by addition of oxalic acid dihydrate (3.95 g, 31.3 mmol) and stirred for a further 5 min at room temperature. The reaction mixture was filtered through a pad of Celite, washed twice with DMF (5 mL), and then the filtrate was carefully concentrated *in vacuo* at room temperature. The crude residue was subjected to flash chromatography (silica gel, 0−20% MeOH−$CHCl_3$), which afforded partially purified product contaminated with DMSO. Trituration of this mixture with EtOAc provided the title compound (4.07 g, 40%) as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.33 (s, 1H), 9.57 (s, 1H), 8.05−7.93 (m, 3H), 7.65−7.61 (m, 1H), 7.55−7.48 (m, 2H), 7.40−7.28 (m, 1H), 6.51 (d, J = 2.8 Hz, 1H), 6.23 (d, J = 3.3 Hz, 1H), 6.16 (d, J = 6.6 Hz, 1H), 5.18 (ddd, J = 6.4, 3.3, 2.8 Hz, 1H); ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ 183.3, 167.4, 163.6, 155.6, 154.0, 146.4, 133.0, 132.8, 128.5, 128.4, 121.7, 96.9, 96.1, 77.0. The spectroscopic properties were consistent with the data available in the literature.[9a]

3′-Deoxy-3′,4′-didehydro-cytidine (7).[9a] To a solution of aldehyde **6** (1.10 g, 3.36 mmol) in MeOH (70 mL) at 0 °C was added $NaBH_4$ (67 mg, 1.8 mmol). The reaction was allowed to warm to room temperature and stirred for 10 min, during which time a colorless precipitate formed. Vacuum filtration afforded *N*-protected ddhC nucleoside (1.02 g, 92%) as a colorless solid. The *N*-protected ddhC nucleoside (890 mg, 2.70 mmol) was treated with NH_3 (7 M in MeOH, 40 mL), stirred at 50 °C for 3 h, and then concentrated *in vacuo*. The crude residue was suspended in MeOH-Et_2O (1:1) and then collected by vacuum filtration and washed with MeOH-Et_2O (1:1, 3 × 1 mL) to afford the title compound (543 mg, 89%) as an off-white solid. ^{1}H NMR (500 MHz, D_2O) δ 7.45 (d, J = 7.5 Hz, 1H), 6.34 (d, J = 2.0 Hz, 1H), 6.06 (d, J = 7.5 Hz, 1H), 5.44−5.39 (m, 1H), 5.01−4.98 (m, 1H), 4.38−4.30 (m, 2H); ^{13}C{^{1}H} NMR (126 MHz, D_2O) δ 166.3, 161.2, 157.2, 140.9, 100.3, 96.4, 93.8, 78.7, 56.3. The spectroscopic properties were consistent with the data available in the literature.[9a]

4-*N*-Benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-3′,4′-didehydrocytidine-5′-aldehyde (8). Method 1: Aldehyde **6** (400 mg, 1.22 mmol) and imidazole (170 mg, 2.44 mmol) were dissolved in dry DMF (10 mL), to which was added TBDMSCl (250 mg, 1.61 mmol) at room temperature. The mixture was stirred for 3 h before more TBDMSCl (80 mg, 0.51 mmol) was added. The mixture was stirred for a further 2 h before the reaction was quenched by addition of a few drops of water, and the mixture was stirred for 20 min. Water and EtOAc were added, and the aqueous layer was extracted with EtOAc; then, the combined organic phases were washed once with water, then brine, dried over $MgSO_4$, and concentrated to dryness. To the residue was added toluene (20 mL), and the toluene solution was concentrated to dryness to remove residual DMF; this process was repeated three times before the crude was purified by column chromatography (silica gel, 0−5% MeOH−$CHCl_3$) to afford the title compound (528 mg, 98%) as a colorless solid. $[\alpha]_D^{20}$ − 67.5 (0.20, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.56 (s, 1H), 9.08 (s, 1H), 7.86 (d, J = 7.5 Hz, 2H), 7.57−7.50 (m, 2H), 7.50−7.38 (m, 3H), 6.16−6.12 (m, 2H), 5.25 (t, J = 2.6 Hz, 1H), 0.86 (s, 9H), 0.12 (s,

https://doi.org/10.1021/acs.joc.1c00761
J. Org. Chem. 2021, 86, 8843−8850

3H), 0.09 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 181.7, 166.9, 163.0, 156.1, 154.1, 144.6, 133.2, 132.9, 129.0, 127.8, 119.5, 97.2, 79.2, 25.7, 18.0, −4.6, −4.8; HRMS (ESI+): Calculated for $C_{22}H_{28}N_3O_5Si$: 442.1798. Found $[M + H]^+$: 442.1797.

Method 2: To a solution of aldehyde **6** (3.02 g, 6.46 mmol) in DMF (30 mL) at room temperature was added Et_3N (2.7 mL, 19 mmol), followed by TBDMSCl (1.50 g, 9.95 mmol). The reaction mixture was heated at 50 °C for 3 h and then concentrated *in vacuo*. The crude residue was suspended in EtOAc (150 mL) and filtered, and then the filtrate was washed with sat. aq $NaHCO_3$ (30 mL), deionized H_2O (30 mL), and brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude oil was purified by flash chromatography (silica gel, 0−30% MeCN−CH_2Cl_2) to afford the title compound (2.08 g, 73%) as a colorless foam.

4-*N*-Benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-3′,4′-didehydrocytidine (3). From aldehyde **8**: To a solution of aldehyde **8** (1.53 g, 3.45 mmol) in MeOH (35 mL) at 0 °C was added $NaBH_4$ (67 mg, 1.8 mmol) in one portion. The reaction mixture was stirred at 0 °C for 30 min and then quenched by addition of acetone (1 mL). The reaction was warmed to room temperature, stirred for 5 min, and then concentrated *in vacuo*. The crude residue was purified by flash chromatography (silica gel, 1−10% MeOH−CH_2Cl_2) to afford the title compound (1.41 g, 92%) as a colorless foam. $[\alpha]_D^{20}$ −60.0 (0.25, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.72 (s, 1H), 7.94−7.81 (m, 2H), 7.68−7.57 (m, 2H), 7.56−7.42 (m, 3H), 6.36 (d, J = 1.5 Hz, 1H), 5.21 (d, J = 2.4 Hz, 1H), 4.86 (br s, 1H), 4.41−4.30 (m, 2H), 0.90 (s, 9H), 0.16 (s, 3H), 0.11 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 166.9, 162.8, 161.4, 154.9, 143.8, 133.2, 133.0, 129.0, 127.8, 101.5, 97.3, 94.3, 80.8, 57.4, 25.8, 18.2, −4.5, −4.7; HRMS (ESI+): Calculated for $C_{22}H_{30}N_3O_5Si$: 444.1955. Found $[M + H]^+$: 444.1953.

From iodide **12**: To a suspension of iodide **12** (2.75 g, 4.81 mmol) in PhMe (96 mL) was added DABCO (1.91 g, 16.8 mmol), and then the resulting suspension was heated to 75 °C and stirred for 18 h. The reaction mixture was cooled to room temperature and filtered to remove precipitated salts, and then the filtrate was concentrated *in vacuo*. The crude product was purified by flash column chromatography (silica gel, 40−100% EtOAc-Hex) to afford the title compound (2.00 g, 94%) as a colorless solid.

4-*N*-Benzoyl-2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-3′,4′-didehydro-5′-(1*H*-imidazol-1-yl)cytidine (9). To a solution of aldehyde **6** (1.50 g, 4.58 mmol) and imidazole (950 mg, 14.0 mmol) in DMF (4.6 mL) at room temperature was added TBDMSCl (1.55 g, 10.2 mmol) in a single portion. The reaction mixture was stirred for 45 min and then quenched by addition of approximately 20 mL of crushed ice. The reaction mixture was partitioned between CH_2Cl_2 (20 mL) and H_2O (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL), and then the combined organic layers were washed with sat. aq $NaHCO_3$ (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, 1−15% MeOH−$CHCl_3$) to afford the title compound (2.13 g, 75%) as a pale yellow foam. The product was a 3:2 mixture of diastereomers at C5′, designated as *A* (major) and *B* (minor). ^1H NMR (500 MHz, $CDCl_3$) δ 9.31 (s, 1H, *A*+*B*), 7.91−7.84 (m, 2H, *A*+*B*), 7.75 (br s, 0.6H, *A*), 7.72 (br s, 0.4H, *B*), 7.59−7.52 (m, 1H, *A*+*B*), 7.47−7.39 (m, 2.8H, *A*+*B*), 7.23 (br s, 0.6H, *A*) 7.09−7.06 (m, 2H, *A*+*B*), 6.76 (d, J = 7.5 Hz, 0.6H, *A*), 6.24 (d, J = 1.5 Hz, 0.4H, *B*), 6.22 (d, J = 1.6 Hz, 0.6H, *A*), 6.17 (s, 0.4H, *B*), 6.10 (s, 0.6H, *A*), 5.43 (dd, J = 2.4, 1.1 Hz, 0.6H, *A*), 5.00 (dd, J = 2.5, 1.1 Hz, 0.4H, *B*), 4.86 (dd, J = 2.5, 1.6 Hz, 0.6H, *A*), 4.82 (ddd, J = 2.4, 1.6, 0.7 Hz, 0.4H, *B*), 0.88 (s, 5.4H, *A*), 0.87 (s, 3.6H, *B*), 0.86 (s, 5.4H, *A*), 0.83 (s, 3.6H, *B*), 0.14 (s, 1.8H, *A*), 0.13 (s, 1.2H, *B*), 0.12 (s, 1.8H, *A*), 0.10 (s, 1.2H, *B*), 0.06 (s, 1.8H, *A*), 0.02 (s, 1.2H, *B*), −0.01 (s, 1.2H, *B*), −0.03 (s, 1.8H, *A*); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 167.0 (*A*+*B*), 162.8 (*B*), 162.6 (*A*), 158.9 (*B*), 158.8 (*A*), 154.2 (*A*+*B*), 143.1 (*B*), 142.6 (*CA*), 136.1 (*A*), 135.8 (*B*), 133.2 (*A*+*B*), 133.1 (*A*+*B*), 130.2 (*A*), 129.9 (*B*), 129.0 (*B*), 128.9 (*A*), 127.8 (*A*+*B*), 116.8 (*B*), 116.3 (*A*), 103.0 (*B*), 102.3 (*A*), 97.0 (*A*+*B*), 95.24 (*B*), 95.21 (*A*), 80.1 (*A*), 80.0 (*B*), 76.1 (*B*), 76.0 (*A*), 25.7 (*A*+*B*), 25.44 (*B*), 25.40 (*A*), 18.0 (*A*+*B*), −4.54 (*A*), −4.6 (*B*), −4.8 (*B*), −4.9 (*A*), −5.28 (*A*+*B*), −5.31 (*B*), −5.5 (*A*); HRMS (ESI+): Calculated for $C_{31}H_{46}N_5O_5Si_2$: 624.3032. Found $[M + H]^+$: 624.3040.

1-(2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-3′-iodo-β-D-*threo*-pentofuranosyl)-4-*N*-benzoylcytosine (11). Methyltriphenoxyphosphonium iodide (9.04 g, 16.0 mmol) and alcohol **10** (5.70 g, 9.90 mmol) were placed under argon and then dissolved in DMF (65 mL). Pyridine (1.60 mL, 19.7 mmol) was added, and then the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was quenched by addition of MeOH (5 mL) and Et_3N (5 mL), stirred for 15 min, and then partitioned between H_2O (600 mL) and EtOAc (100 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL), and then the combined organic layers were washed with brine (3 × 50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude oil was purified by flash chromatography (silica gel, 0−30% EtOAc-Hex) to afford the title compound (4.24 g, 62%) as a colorless solid. Needle-shaped crystals suitable for X-ray crystallography were grown by slow evaporation of a solution of iodide **10** in Et_2O-Hex. mp (Et_2O-Hex) 72.0−74.3 °C; $[\alpha]_D^{20}$ +35.6 (0.090, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, J = 7.5 Hz, 1H), 7.89 (d, J = 7.8 Hz, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.50 (t, J = 7.7 Hz, 3H), 5.75 (d, J = 1.7 Hz, 1H), 4.79 (t, J = 1.8 Hz, 1H), 4.13 (dd, J = 4.1, 1.8 Hz, 1H), 4.07−3.99 (m, 2H), 3.82 (dd, J = 10.3, 5.1 Hz, 1H), 0.94 (s, 9H), 0.92 (s, 9H), 0.20 (s, 3H), 0.16 (s, 6H), 0.14 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 166.3, 162.4, 154.6, 145.3, 133.3, 129.2, 127.7, 95.7, 93.7, 83.8, 81.9, 68.0, 30.5, 26.0, 25.8, 18.5, 18.0, −4.6, −4.8, −5.0, −5.1; HRMS (ESI+): Calculated for $C_{28}H_{45}N_3O_5Si_2I$: 686.1942. Found $[M + H]^+$: 686.1950.

1-(2′-*O*-(*tert*-Butyldimethylsilyl)-3′-iodo-β-D-*threo*-pentofuranosyl)-4-*N*-benzoylcytosine (12). To a solution of silyl ether **11** (5.64 g, 8.23 mmol) in THF (24 mL) at 0 °C was added a mixture of TFA-H_2O (1:1, 7.2 mL) dropwise over 3 min. The reaction mixture was warmed to room temperature and stirred for 3.5 h. The reaction was neutralized by addition of sat. aq $NaHCO_3$ (50 mL) and then extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with sat. aq $NaHCO_3$ (20 mL), brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude oil was purified by flash chromatography (silica gel, 10−100% EtOAc-Hex) to afford the title compound (4.17 g, 89%) as a colorless solid. $[\alpha]_D^{20}$ +13.7 (0.088, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.20 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.62−7.47 (m, 4H), 5.67 (d, J = 1.5 Hz, 1H), 4.88 (t, J = 1.7 Hz, 1H), 4.18−4.04 (m, 3H), 3.88 (dd, J = 11.7, 4.3 Hz, 1H), 0.91 (s, 9H), 0.20 (s, 3H), 0.16 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 166.6, 162.7, 155.1, 145.7, 133.3, 129.2, 127.7, 96.0, 94.6, 83.7, 82.0, 68.0, 29.3, 25.8, 18.0, −4.6, −4.8; HRMS (ESI+): Calculated for $C_{22}H_{31}N_3O_5SiI$: 572.1078. Found $[M + H]^+$: 572.1084.

Difluorenylmethyl-4-*N*-benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-3′,4′-didehydrocytidine-5′-phosphate (13). To a solution of alcohol **3** (1.39 g, 3.13 mmol) in anhydrous MeCN (21 mL) at room temperature was added 1*H*-tetrazole (0.45 M in MeCN, 18 mL, 8.1 mmol), followed by difluorenyl *N*,*N*-diisopropylphosphoramidite (1.0 M in benzene, 4.95 mL, 4.95 mmol) dropwise. The reaction mixture was stirred for 1 h and then cooled to 0 °C, and *tert*-butyl hydroperoxide (70 *w*/*w*% in H_2O, 1.10 mL, 7.95 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature, stirred for 90 min, and then quenched by addition of 10% aq $Na_2S_2O_3$ (5 mL) and sat. aq $NaHCO_3$ (100 mL). The reaction mixture was extracted with EtOAc (3 × 50 mL), and the combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude oil was purified by flash column chromatography (silica gel, 5−50% EtOAc-PhMe) to afford the title compound (2.39 g, 87%) as a colorless foam. $[\alpha]_D^{20}$ −36.8 (0.26, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.88 (s, 1H), 7.92 (d, J = 7.6 Hz, 2H), 7.75−7.68 (m, 4H), 7.59 (t, J = 7.4 Hz, 1H), 7.54 (d, J = 7.5 Hz, 2H), 7.52−7.46 (m, 4H), 7.44−7.23 (m, 10H), 6.30 (d, J = 2.0 Hz, 1H), 5.12 (d, J = 2.7 Hz, 1H), 4.80 (t, J = 2.2 Hz, 1H), 4.50−4.38 (m, 2H), 4.37−4.28 (m, 4H), 4.13 (td, J = 6.4, 2.9 Hz, 2H), 0.89 (s, 9H), 0.15 (s, 3H), 0.09 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 166.7, 162.4, 156.0 (d, J =

https://doi.org/10.1021/acs.joc.1c00761
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6.9 Hz), 155.9, 154.1, 143.1, 142.9, 141.4, 133.2, 129.0, 128.0, 127.7, 127.2, 125.03, 124.99, 120.10, 120.06, 104.3, 97.2, 94.5, 80.5, 69.46 (d, J = 5.2 Hz), 69.39 (d, J = 5.2 Hz), 61.2 (d, J = 5.8 Hz), 47.9 (d, J = 7.5 Hz), 25.8, 18.1, −4.5, −4.8; $^{31}P\{^1H\}$ NMR (202 MHz, $CDCl_3$) δ −1.6; HRMS (ESI+): Calculated for $C_{50}H_{51}N_3O_8SiP$: 880.3183. Found $[M + H]^+$: 880.3185.

2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-3′,4′-didehydrocytidine-5′-phosphate triethylammonium Salt (14). Difluorenylmethyl phosphate ester **13** (2.20 g, 2.50 mmol) was dissolved in a 7 M solution of anhydrous NH_3 in MeOH (25 mL) and stirred at room temperature for 18 h. The crude reaction mixture was then adsorbed onto Celite for purification by dry load. Celite (approximately 6 g) was added to the reaction mixture, and the resulting suspension was concentrated *in vacuo* until a dry, free-flowing solid mixture was obtained. Purification by flash column chromatography (silica gel, 10−100% eluent B-EtOAc, where eluent B is 5% conc aq NH_4OH in MeOH) afforded the product as an ammonium salt, which was then coevaporated three times with MeOH and Et_3N to afford the title compound (1.03 g, 79%) as a colorless solid. ^1H NMR (500 MHz, CD_3OD) δ 7.51 (d, J = 7.6 Hz, 1H), 6.29 (d, J = 1.7 Hz, 1H), 5.95 (d, J = 7.5 Hz, 1H), 5.34 (d, J = 2.4 Hz, 1H), 4.90 (s, 1H), 4.55−4.46 (m, 2H), 3.19 (q, J = 7.3 Hz, 6H), 1.31 (t, J = 7.3 Hz, 9H), 0.90 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, CD_3OD) δ 166.9, 160.7 (d, J = 8.9 Hz), 156.8, 142.3, 103.2, 96.6, 95.1, 82.0, 60.6 (d, J = 3.8 Hz), 47.7, 26.2, 18.9, 9.2, −4.50, −4.55; $^{31}P\{^1H\}$ NMR (202 MHz, CD_3OD) δ 0.9; HRMS (ESI−): Calculated for $C_{15}H_{25}N_3O_7PSi$: 418.1199. Found $[M - H]^-$: 418.1198.

3′-Deoxy-3′,4′-didehydrocytidine-5′-phosphate sodium salt (15). Silyl ether **14** (115 mg, 0.221 mmol) was dissolved in AcOH-deionized H_2O (1:1, 4.4 mL); the resulting solution was stirred at room temperature for 18 h and then concentrated *in vacuo* at or below 40 °C. The residue obtained was coevaporated with deionized H_2O three times to remove residual AcOH, affording the triethylammonium phosphate as a colorless solid. This solid was dissolved in deionized H_2O and passed through an ion-exchange column (1 g Dowex 50W X8 Na-form), eluting with deionized H_2O. Product-containing fractions were combined and lyophilized to afford the title compound (74 mg, quant) as a colorless solid. ^1H NMR (500 MHz, D_2O) δ 7.50 (d, J = 7.5 Hz, 1H), 6.33 (d, J = 2.1 Hz, 1H), 6.07 (d, J = 7.6 Hz), 5.52 (d, J = 2.6 Hz, 1H), 4.98−4.94 (m, 1H), 4.64−4.55 (m, 2H); $^{13}C\{^1H\}$ NMR (126 MHz, D_2O) δ 165.9, 158.8 (d, J = 7.6 Hz), 156.6, 141.2, 101.8, 96.4, 93.7, 78.7, 59.4 (d, J = 4.1 Hz); $^{31}P\{^1H\}$ NMR (202 MHz, D_2O) δ 0.7; HRMS (ESI−): Calculated for $C_9H_{11}N_3O_7P$: 304.0335. Found $[M - H]^-$: 304.0343.

2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-3′,4′-didehydrocytidine-5′-diphosphate bis(triethylammonium) Salt (16). Monophosphate **14** (100 mg, 0.192 mmol) and CDI (160 mg, 0.937 mmol) were dissolved in MeCN (1.9 mL) under argon and stirred at room temperature for 20 h, and then excess CDI was quenched by addition of H_2O (30 μL, 1.7 mmol). The reaction mixture was stirred for 1 h, loaded onto Dowex 50WX8 Et_3NH-form resin, and eluted with MeCN to remove imidazole from the crude mixture. Fractions containing the intermediate imidazolidate were combined and concentrated *in vacuo* to dryness. The crude imidazolidate was dissolved in MeCN (1.0 mL), to which solution was added tributylammonium phosphate (1 M in MeCN, 0.96 mL, 0.96 mmol). The reaction mixture was stirred at room temperature for 24 h and then concentrated *in vacuo*. The crude residue dissolved in deionized H_2O and passed through an ion exchange column (Dowex 50WX8 Et_3NH form) eluting with deionized H_2O to convert the diphosphate product to its di(triethylammonium) salt form. Product-containing fractions were combined and then concentrated *in vacuo*. The crude oil thus obtained was purified by flash chromatography (C18 silica gel, 5−100% MeOH-H_2O) to afford the title compound (82 mg, 61%) as a colorless solid. ^1H NMR (500 MHz, CD_3OD) δ 7.61 (d, J = 7.7 Hz, 1H), 6.22 (d, J = 1.7 Hz, 1H), 6.11 (d, J = 7.7 Hz, 1H), 5.40 (d, J = 2.7 Hz, 1H), 4.94−4.91 (m, 1H), 4.74−4.64 (m, 2H), 3.19 (q, J = 7.3 Hz, 8H), 1.31 (t, J = 7.3 Hz, 12H), 0.90 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, CD_3OD) δ 163.8, 160.2 (d, J = 9.1 Hz), 152.5, 143.4, 103.9, 96.6, 94.9, 81.7, 61.2 (d, J = 4.2 Hz), 47.4, 26.2, 18.9, 9.1, −4.5; $^{31}P\{^1H\}$ NMR (202 MHz, CD_3OD) δ −10.0 (d, J = 19.3 Hz), −11.1 (d, J = 19.3 Hz); HRMS (ESI−): Calculated for $C_{15}H_{26}N_3O_{10}P_2Si$: 498.0863. Found $[M - H]^-$: 498.0872.

3′-Deoxy-3′,4′-didehydrocytidine-5′-diphosphate bis(triethylammonium) Salt (17). To a solution of silyl ether **16** (75 mg, 0.107 mmol) in deionized H_2O (10 mL) was added Dowex 50WX8 H-form (218 mg), which had previously been washed with MeOH and deionized H_2O. The suspension was stirred at room temperature for 1 h, and then the reaction mixture was neutralized by addition of Et_3N (0.3 mL) and stirred for a further 5 min. The reaction mixture was filtered, and the resin was washed with deionized H_2O (3 × 5 mL). The filtrate and combined washings were concentrated *in vacuo* at 40 °C to approximately 5 mL in volume and then lyophilized to afford the title compound (65 mg, quant) as a colorless solid. ^1H NMR (500 MHz, D_2O) δ 7.52 (d, J = 7.6 Hz, 1H), 6.35 (br s, 1H), 6.08 (d, J = 7.6 Hz, 1H), 5.54 (br s, 1H), 4.93 (br s, 1H), 4.75−4.62 (m, 2H), 3.24 (q, J = 7.3 Hz, 12H), 1.32 (t, J = 6.6 Hz, 18H); $^{13}C\{^1H\}$ NMR (126 MHz, D_2O) δ 166.2, 158.6 (d, J = 8.1 Hz), 157.0, 141.1, 101.8, 96.5, 93.5, 78.8, 60.0 (d, J = 4.8 Hz), 46.7, 8.1; $^{31}P\{^1H\}$ NMR (202 MHz, D_2O) δ −9.5 (d, J = 21.8 Hz), −11.2 (d, J = 21.1 Hz); HRMS (ESI−): Calculated for $C_9H_{12}N_3O_{10}P_2$: 383.9998. Found $[M - H]^-$: 384.0007.

2′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-3′,4′-didehydrocytidine-5′-triphosphate tris(triethylammonium) Salt (18). Monophosphate **14** (950 mg, 1.83 mmol) and CDI (1.55 g, 9.08 mmol) were dissolved in MeCN (19 mL) under argon and stirred at room temperature for 20 h, and then excess CDI was quenched by addition of H_2O (0.26 mL, 14 mmol). The reaction mixture was stirred for 1 h, then bis(tributylammonium) pyrophosphate (2.50 g, 4.56 mmol) was added, and the reaction mixture was stirred for a further 48 h, after which the reaction was complete by TLC analysis (silica gel, 6:1:3 *i*-PrOH-H_2O-28% aq NH_4OH). The reaction solvent was removed *in vacuo*, and then the crude triphosphate was purified by flash chromatography (C18 silica gel, 20−100% eluent B-eluent A, where eluent A is 20 mM Bu_3N and 30 mM AcOH in H_2O, and eluent B is 15 mM Bu_3N in MeOH). Fractions containing clean triphosphate, as judged by TLC analysis, were pooled and concentrated *in vacuo* to approximately 15 mL in volume. The triphosphate solution was converted to its triethylammonium salt by passage through an ion exchange column (Dowex 50WX8 Et_3NH form), eluting with deionized H_2O, and then lyophilized to afford the title compound (1.12 g, 70%) as a colorless solid. ^1H NMR (500 MHz, D_2O) δ 7.55 (d, J = 7.6 Hz, 1H), 6.42 (d, J = 1.9 Hz, 1H), 6.13 (d, J = 7.5 Hz, 1H), 5.59 (d, J = 1.6 Hz, 1H), 5.19 (br s, 1), 4.75−4.64 (m, 2H, signal obscured by H_2O), 3.26 (q, J = 7.3 Hz, 18H), 1.34 (t, J = 7.3 Hz, 24H), 0.95 (s, 9H), 0.21 (s, 3H), 0.18 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, D_2O) δ 166.2, 158.2 (d, J = 8.2 Hz), 156.9, 141.4, 102.6, 96.9, 93.6, 80.8, 60.4 (d, J = 4.5 Hz), 46.7, 25.1, 17.5, 8.39, −5.58, −5.65; $^{31}P\{^1H\}$ NMR (202 MHz, D_2O) δ −10.6 (d, J = 19.8 Hz), −11.4 (d, J = 19.9 Hz), −23.2 (t, J = 19.9 Hz); HRMS (ESI−): Calculated for $C_{15}H_{27}N_3O_{13}P_3Si$: 578.0526. Found $[M - H]^-$: 578.0535.

3′-Deoxy-3′,4′-didehydrocytidine-5′-triphosphate tris(triethylammonium) Salt (ddhCTP, 2). To a solution of silyl ether **18** (1.10 g, 1.25 mmol) in deionized H_2O (100 mL) was added Dowex 50WX8 H-form (2.0 g), which had previously been washed with MeOH and deionized H_2O. The suspension was stirred at room temperature for 1 h, and then the reaction mixture was neutralized by addition of Et_3N (2.0 mL) and stirred for a further 5 min. The reaction mixture was filtered, and the resin was washed with deionized water (3 × 15 mL). The filtrate and combined washings were concentrated *in vacuo* at 40 °C to approximately 20 mL in volume and then lyophilized to afford the title compound (991 mg, 83.2% potency, 84% yield) as a colorless solid. Potency was determined by ^1H qNMR using DMSO as an internal standard; the largest impurity present in the sample was residual triethylammonium buffer at 1.5% w/w. ^1H NMR (500 MHz, D_2O) δ 7.52 (d, J = 7.6 Hz, 1H), 6.36 (br s, 1H), 6.09 (d, J = 7.6 Hz, 1H), 5.56 (br s, 1H), 4.93 (br s, 1H), 4.75−4.67 (m, 2H, obscured by H_2O), 3.23 (q, J = 7.3 Hz, 21H), 1.31 (t, J = 7.3 Hz, 31H); $^{13}C\{^1H\}$ NMR (126 MHz, D_2O) δ 166.2, 158.4

https://doi.org/10.1021/acs.joc.1c00761
J. Org. Chem. 2021, 86, 8843−8850

(d, J = 8.1 Hz), 157.0, 141.1, 102.0, 96.5, 93.5, 78.8, 60.3 (d, J = 5.2 Hz),.46.7, 8.1; $^{31}P\{^{1}H\}$ NMR (202 MHz, D_2O) δ −10.2 (d, J = 20.5 Hz), −11.5 (d, J = 19.9 Hz), −23.3 (t, J = 20.2 Hz); HRMS (ESI−): Calculated for $C_9H_{13}N_3O_{13}P_3$: 463.9661. Found $[M - H]^-$: 463.9669. The spectroscopic properties were consistent with the data available in the literature.[4]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00761.

Copies of ^{1}H NMR, $^{13}C\{^{1}H\}$ NMR, and $^{31}P\{^{1}H\}$ NMR spectra and the crystal structure refinement table for compound **11** (PDF)

FAIR data, including the primary NMR FID files, for compounds **2**, **3**, **8**, **9**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, and **18** (ZIP)

Accession Codes

CCDC 2072645 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare the following competing financial interest(s): L.D.H. and J.M.W. are inventors on a provisional patent application (AU.2020904583) that incorporates work described in this manuscript.

■ ACKNOWLEDGMENTS

J.M.W. and L.D.H. thank the Ministry of Business Innovation & Employment for support of this work (Endeavour Fund, Contract UOOX1904 (N.Z.)). S.C.A. receives support from NIH P30AI124414.

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J. Org. Chem. 2021, 86, 8843−8850