Mechanism-Based Inactivation of Ribonuclease A

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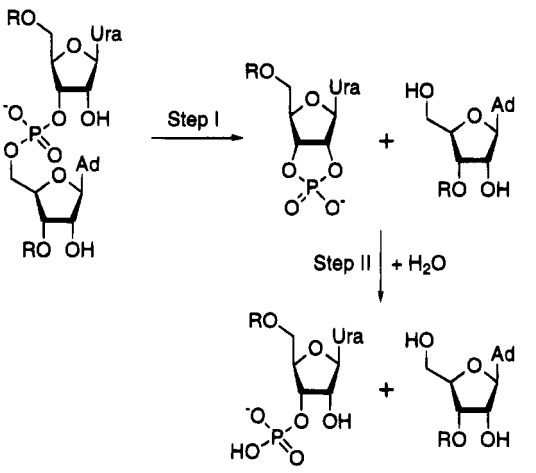
The first example of a mechanism-based inhibitor of a phosphodiesterase is reported. Although the inactivation brought about by the fluoride **4** is not complete, this compound provides a useful starting point for the synthesis of other more potent inhibitors of ribonuclease A, as well as inhibitors of other nucleases. In addition, an inexpensive method is described for the synthesis of phosphate diesters that cannot be synthesized using standard phosphoramidite methodology. Phosphitylation of the target alcohol with a dialkyl chlorophosphite, followed by activation of the resulting trialkyl phosphite with I_2, yields an iodophosphate. The resulting iodophosphate can then be coupled to a second alcohol, phenol, or enolate to give a phosphate triester, which after subsequent deprotection affords the desired phosphate diester. The novel phosphorylation chemistry presented should greatly facilitate the synthesis of other similar mechanism-based phosphodiesterase inhibitors.

Phosphodiesterases comprise an important group of enzymes whose biological roles include such diverse functions as signal transduction,[1] DNA processing and replication,[2] phospholipid metabolism,[3] and viral reproduction.[4] Ribonucleases are among the best understood of all the phosphodiesterases. In particular, ribonuclease A (RNase A; EC 3.1.27.5) has been the object of landmark work on enzymology; on the folding, stability, and chemistry of proteins; and on molecular evolution.[5] RNase A is a small protein (124 amino acid residues; 13.7 kDa) that catalyzes the two-step hydrolysis of the P–$O^{5'}$ bond of RNA. Scheme 1 depicts a catalytic sequence that is widely-accepted and consistent with all known data.[6,7]

In the enzyme-catalyzed reaction shown in Scheme 1, the side chain of His12 acts as a general base that abstracts a proton from the 2′-hydroxyl of a substrate molecule, thereby facilitating attack on the phosphorus atom.[8] This attack proceeds in-line to displace a nucleoside.[9] The side chain of His119 acts as a general acid that protonates the 5″-oxygen to assist its departure.[8]

Scheme 1



Both products are then released to solvent. The slow hydrolysis of the 2′,3′-cyclic phosphate occurs in a separate step that resembles the reverse of transphosphorylation.[10]

Despite our relatively advanced understanding of how RNase A works, the design of inhibitors for this enzyme is still in its infancy. Indeed, there are relatively few rationally designed phosphodiesterase inhibitors of any kind and no published examples of mechanism-based phosphodiesterase inhibitors.

We recently published several strategies for the design and synthesis of mechanism-based phosphatase inactivators.[11] The implementation of one of these strategies has led to the successful development of phosphotyrosine phosphatase inactivators.[12] These approaches are illustrated by the chemical "motifs" shown in Scheme 2.

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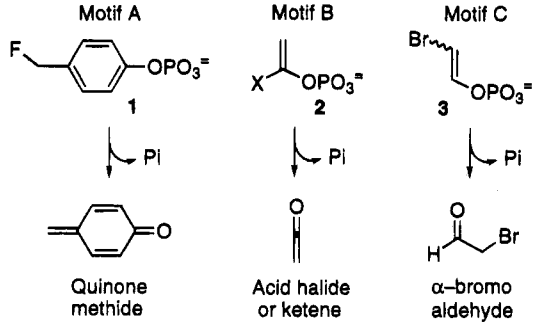
Scheme 2



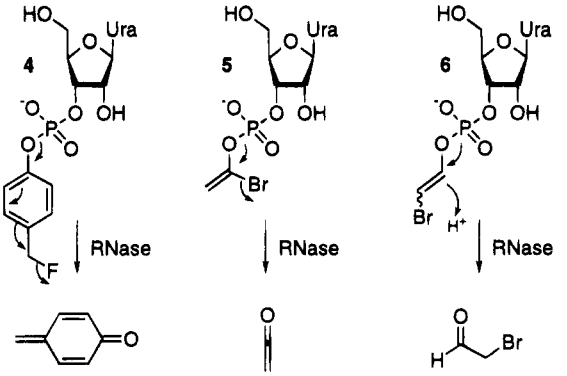
An integral design consideration for these inhibitory motifs was that they be applicable to the development of phosphodiesterase or phosphotriesterase inhibitors simply by incorporating the appropriate chemical functionalities into suitable substrates. In this paper, we describe a chemical strategy for the introduction of these inhibitory motifs into substrates for RNase A. We then examine the inactivation of the enzyme brought about by use of inhibitory motif A.

Results and Discussion

Inhibitor Design. RNase A binds the bases of adjacent RNA residues in three enzymic subsites: B1, B2, and B3.[13] Catalysis by RNase A results in the cleavage of the P–O$^{5'}$ bond specifically on the 3′-side of pyrimidine nucleotides that are bound in the B1 subsite.[14] Indeed, the B1 subsite appears to bind[14,15] only residues having a pyrimidine base. Although the B2 subsite has a preference for residues having an adenine base and the B3 subsite has a preference for residues having a purine base, the B2 and B3 subsites can accommodate all residues. Indeed, the enzyme readily catalyzes the transphosphorylation of other phosphodiesters, such as the uridine 3′-phosphate diester with *p*-nitrophenol.[8]

A number of important phosphodiesterases such as phospholipases D and C show a similar proclivity to RNase A in that enzyme specificity is conferred largely by the alcohol that remains phosphorylated.[16] In these cases, this type of selectivity permits the design of potential mechanism-based enzyme inactivators based on the motifs shown in Scheme 2. Starting with the appropriate phosphate ester, simply appending one of the latent reactive functionalities shown in Scheme 2 gives rise to a potential mechanism-based enzyme inactivator. Using this principle, we envisioned the structures of potential mechanism-based ribonuclease inactivators (**4**–**6**) shown below (Scheme 3). Inhibitor **4** would induce the enzyme-catalyzed liberation of a quinone methide. Inhibitor **5** gives rise to an acylating agent, and inhibitor **6** would yield an α-bromo aldehyde, which is a reactive alkylating agent. The three motifs shown below represent just a few of the many possible ways in which our strategy could be used to generate the structures of potential enzyme inactivators.

Scheme 3



Inhibitor Synthesis. Although there are a variety of useful methods available for the synthesis of nucleoside phosphodiesters, it was clear that the synthesis of enol phosphate diesters such as **5** and **6** would not be possible using standard phosphoramidite chemistry.[17] Moreover, the expense of the reagents used for phosphoramidite chemistry prompted us to examine the possibility of developing other methods suitable for the synthesis of phosphodiesters. Following up on earlier reports on the oxidative activation of phosphite triesters,[18] we recently developed a protocol for the synthesis of phosphate esters from trialkyl phosphites, I_2, pyridine, and the target alcohol (eq 1).[19]

$$P(OR)_3 \xrightarrow{I_2} (RO)_2P(=O)I \xrightarrow{R'OH,\ Py} (RO)_2P(=O)OR' \qquad (1)$$

As shown below (eq 2), this methodology can easily be used for the synthesis of phosphodiesters simply by starting with a dialkyl chlorophosphite. Phosphitylation

$$Cl\text{–}P(OR)_2 \xrightarrow{R'OH} R'O\text{–}P(OR)_2 \xrightarrow[2.\ R''OH,\ Py]{1.\ I_2} R'O\text{–}P(=O)(OR'')OR \qquad (2)$$

of the target alcohol gives a trialkyl phosphite that can be oxidized *in situ* with iodine. The resulting activated ester (the phosphoryl iodide is probably formed initially and then reacts with chloride ion to give the chlorophosphate) can then be treated with a second alcohol, phenol, or enolate to give the requisite triester.

For the synthesis of complex phosphodiesters, the chemistry outlined in eq 2 provides a useful and inexpensive alternative to phosphoramidite-type syntheses. We have applied this methodology to the synthesis of two initial target compounds as shown below (Schemes 4 and 5). Starting with doubly-protected uridine (**7**), treatment with diethyl chlorophosphite gives the trialkylphosphite, which was not isolated. *In situ* oxidation of this material, followed by the addition of *p*-hydroxybenzaldehyde (for the synthesis of compound **4**), led to the desired triester **8** (53.8% from **7**) in reasonable overall yield. Compound **8** is formed as a mixture of two diastereomers owing to the newly created chiral center at phosphorus. Since final deblocking of the phosphate triester destroys this chirality, the mixture was not separated but carried on

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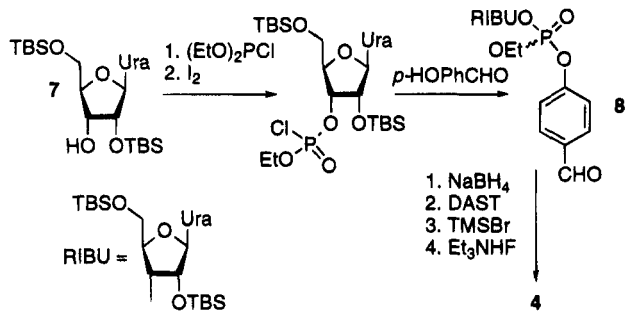
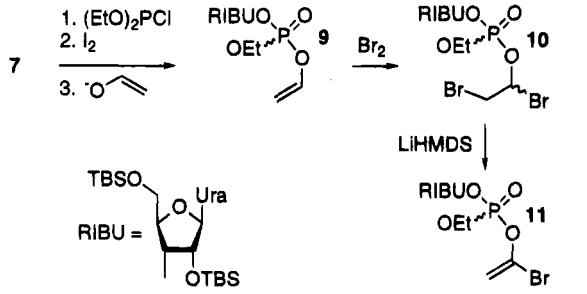
Scheme 4



Scheme 5



in the synthesis. Inhibitor **4** was then obtained by reduction of **8** with sodium borohydride (94.9%), followed by fluorination with (diethylamino)sulfur trifluoride (78.6%) and deblocking with TMSBr and fluoride ion as shown (72.8%).

Although our most immediate concerns centered on the enzymology of the benzylic fluoride **4**, we were interested in ascertaining whether the phosphorylation chemistry we had developed would be suitable for construction of the enol phosphate diesters ultimately required for the synthesis of compounds **5** and **6**. A second question we wished to address concerned the possibility of regioselective dehydrobromination of the dibromide derived from enol phosphate **9** (Scheme 5). Recently, we had reported that good regioselectivity in the elimination of halides from 1,2-dihalo phosphates could be obtained by taking advantage of complex-induced proximity effects.[11b] In the current case, we were worried that the highly oxygenated nature of the nucleoside might preclude the use of such effects to guide the elimination.

Enol phosphate **9** was synthesized in a manner analogous to that described for triester **8**. Phosphitylation of **7** followed by oxidation and trapping of the activated phosphate ester with the enolate of acetaldehyde gave the expected enol phosphate **9** (67.4% from **7**, mixture of two diastereomers) in reasonable overall yield. No attempt was made to separate these diastereomers. Bromination of **9** gave the dibromide **10** as an inseparable mixture of all four diastereomers. Gratifyingly, we found that treatment of these dibromides with LiHMDS in toluene gave the 1-bromo enol phosphate **11** (56.8% from **9**, mixture of two diastereomers) as the sole product, indicating that complete regioselective control of the dehydrobromination is possible.

Although the phosphitylation/oxidation/phosphorylation sequence shown in Schemes 4 and 5 provides a relatively facile method to construct phosphate esters with three different substituents (*e.g.*, vinyl phosphate **9**), an intrinsic drawback to this methodology is that the last coupling reaction proceeds via a phosphorochloridate, an acid halide that often couples poorly to alcohols. In contrast, we have found that phosphoryl iodides give

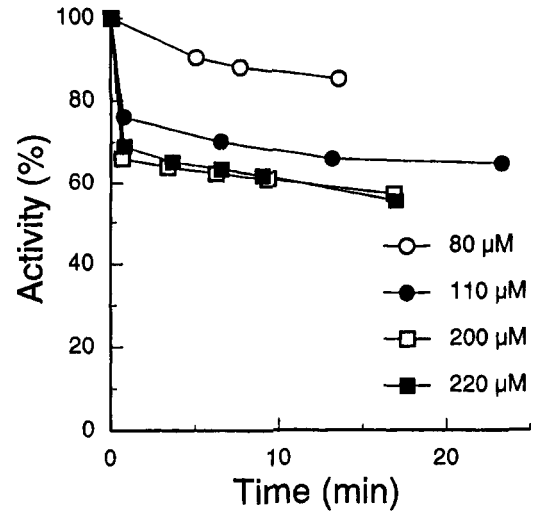
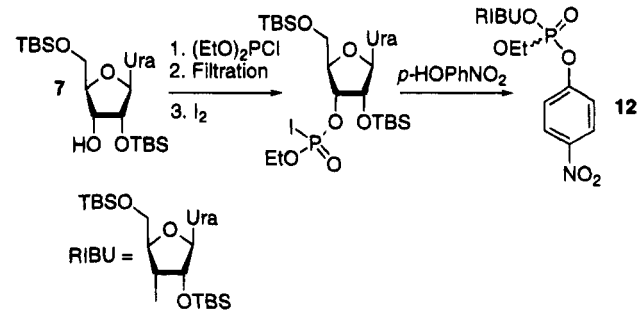


Figure 1. Loss of enzymatic activity of RNase A during incubation with different concentrations of **4**.

Scheme 6



superior yields in coupling reactions with a variety of nucleophiles.[19] When the reaction protocol shown in Schemes 4 and 5 is used, a phosphoryl iodide is generated as an obligate intermediate. Under the assumption that generating and using this iodide in the absence of chloride ion would improve the yield of phosphate esters, the initial phosphitylation reaction was run in ether. The pyridine hydrochloride that formed was filtered off and the reaction mixture evaporated to remove excess pyridine. The resulting phosphite was then dissolved in dichloromethane or tetrahydrofuran (the iodophosphate is unstable in tetrahydrofuran and must be used immediately) and the oxidation/phosphorylation run as previously described. This simple change in procedure resulted in substantially better reaction yields. Aldehyde **8** was obtained in 72.7% yield (vs 53.8% for the procedure run in the presence of chloride ion), and vinyl phosphate **9** was obtained in 74.2% yield (vs 67.4% for the procedure run in the presence of chloride ion). In addition, the nitrophenyl ester **12**, an intermediate in the synthesis of a ribonuclease substrate, was obtained in 79.3% yield (Scheme 6).

Inactivation of Ribonuclease A. Compound **4** is an irreversible inactivator of RNase A. The kinetics of inactivation appear to be multiphasic (Figure 1). An initial phase appears to reach completion within 60 s, while other phases reach completion on the time scale of min. The rate and extent of inactivation does not increase when the concentration of **4** is greater than approximately 200 μM. These data are consistent with the steady-state kinetic parameters for the enzymatic cleavage of the uridine 3′-phosphate diester with *p*-nitrophenol, a substrate that resembles **4**. For that

substrate, $k_{cat} = 19\ s^{-1}$ and $K_m = 330\ \mu M$.[8] Enzyme that had been inactivated by **4** is not reactivated by prolonged dialysis, suggesting that the enzyme is indeed modified covalently.

The inactivation of RNase A by **4** is not complete. After prolonged (2 h) exposure of RNase A to saturating **4**, the enzyme still retains 33% of its ability to catalyze the cleavage of UpA. Addition of a fresh aliquot of inhibitor caused no additional inactivation, suggesting that inhibition is not caused by an advantageous impurity present at low concentrations. The fact that inactivation is incomplete suggests that the covalent modification does not occur to His12, His119, or Lys41, because mutation of any one of these residues to alanine causes a $>10^4$-fold decrease in the catalytic activity of the enzyme.[8,20]

Structural and site-directed mutagenesis studies reveal which residues are likely to be candidates for covalent modification by **4**. The three-dimensional structure of a crystalline RNase A·d(ApTpApApG) complex indicates that **4** may modify a residue in the enzymic B2 or P2 subsite.[21] The B2 subsite, which binds to the adenosine base in the UpA substrate, consists of Gln69, Asn71, and Glu111. The P2 subsite, which binds to the phosphoryl group in an RNA substrate proceeding the scissile P–$O^{5'}$ bond, consists of Lys7 and Arg10. Replacing Lys7 or Arg10 with an alanine or glutamine residue,[22,23] or Gln69 or Glu111 with an alanine residue,[24] results in a <2-fold decrease in the ability of RNase A to catalyze the cleavage of a dinucleotide substrate. In contrast, mutation of Asn71 to alanine decreases the value of k_{cat}/K_m for CpA cleavage by 40-fold.[24] Since covalent modification of a residue by **4** imposes a greater obstacle to catalysis than does mutation to alanine, **4** is unlikely to modify Asn71. We therefore believe that the most likely targets for **4** are the side chains of Lys7, Arg10, Gln69, and Glu111. The existence of several possible targets is consistent with the observed multiphasic kinetics of inactivation (Figure 1), which suggest that more than one residue becomes covalently modified by **4**.

Finally, inactivation of RNase A occurs before the alkylating agent leaves the active site. This conclusion follows from the insensitivity of the rate of inactivation to the presence of cysteine, a scavenger nucleophile. If the reactive species had left the active site and then alkylated the enyzme, it would have been intercepted by the cysteine, with a consequent reduction in the rate of inactivation.

Summary

We have shown that it is possible to use motifs originally envisioned for the construction of mechanism-based phosphatase inhibitors for the design of RNase A inhibitors. This is the first reported example of a mechanism-based inhibitor of an enzyme that cleaves phosphate diesters. Although the inactivation brought about by the fluoride **4** is not complete and is not yet fully characterized, this inhibitor provides a useful starting point for the design of more active inhibitors of RNase A, as well as inhibitors of other nucleases. In addition, the new phosphorylation chemistry we have described should greatly facilitate the synthesis of these types of inhibitors.

Experimental Section

Inactivation of Ribonuclease A. Materials. Uridyl-(3′→5′)adenosine (UpA) was synthesized as described.[25] RNase A (type X-A) and adenosine deaminase were from Sigma Chemical (St. Louis, MO).

General Methods. Ultraviolet absorbance measurements were made on a Cary Model 3 spectrophotometer equipped with a Cary Temperature controller. UpA concentration was determined by ultraviolet absorption using $\epsilon_{260} = 24\ 600\ M^{-1}\ cm^{-1}$ at pH 7.0.[26]

Enzyme Assays. Ribonuclease activity was assessed by monitoring the cleavage of UpA with an adenosine deaminase coupled assay.[27] Assays were performed at 25 °C in 0.1 M Mes·HCl buffer, pH 6.0, containing NaCl (0.1 M) and UpA (0.20 mM). Under these conditions, the $\Delta\epsilon$ for the conversion of UpA to uridine 2′,3′-cyclic phosphate and inosine is $-6000\ M^{-1}\ cm^{-1}$ at 265 nm.[28]

Inactivation Kinetics. At time zero, RNase A (0.3 μg) was added to a solution of **4** (0.077–0.32 mM) in 50 mM Mes·HCl buffer, pH 6.0, containing NaCl (0.10 M). The resulting solution was incubated at 25 °C. At known times, aliquots (20 μL) were removed and assayed for enzymatic activity. These inactivation experiments were also performed in the presence of cysteine (20 mM).

Synthesis of Inhibitors. General Methods. All reactions were conducted in oven-dried (120 °C) or flame-dried glassware under an atmosphere of dry argon or nitrogen. All solvents were purified before use. Et_2O and THF were distilled from sodium benzophenone ketyl. CH_2Cl_2 and toluene were distilled from CaH_2.

^1H NMR spectra were recorded at 400 MHz using $CHCl_3$ (δ 7.26 ppm) or CHD_2OD (δ 3.30 ppm) as an internal reference. ^{13}C NMR spectra were recorded at 100 MHz using $CDCl_3$ (δ 77.0 ppm) or CD_3OD (δ 49.0 ppm) as an internal reference. ^{31}P NMR spectra (^1H decoupled) were recorded at 146 MHz using H_3PO_4 as an external reference. ^{19}F NMR spectra were recorded at 340 MHz using $CHFCl_2$ as an external reference. High resolution mass spectra were recorded at 70 eV.

Analytical thin-layer chromatography (TLC) was performed by using 2.5 cm × 10 cm Kieselgel 60 F_{254} plates with a 0.25-mm thickness of silica gel. Compounds were visualized using anisaldehyde stain (93.67 mol % EtOH, 3.99 mol % H_2SO_4, 1.25 mol % anisaldehyde, 1.09 mol % AcOH). Flash chromatography was performed using Kieselgel 60 (230–400 mesh). Unless otherwise noted, all compounds purified by chromatography were sufficiently pure (>95% by ^1H NMR analysis) for use in subsequent reactions.

2′,5′-Di(*tert*-butyldimethylsilyl)-3′-(ethyl 4-formylphenyl phosphate)uridine (8). Method A (via the Chlorophosphate). Diethyl chlorophosphite (1.683 mL, 11.63 mmol) was added dropwise to a solution of 2′,5′-di(*tert*-butyldimethylsilyl)uridine (**7**) (5.000 g, 10.58 mmol) and pyridine (4.277 mL, 52.88 mmol) in CH_2Cl_2 (100.0 mL) at 0 °C. After 10 min, I_2 (2.685 g, 10.58 mmol) was added to the reaction mixture. After an additional 10 min, the reaction mixture was allowed to warm to 25 °C, filtered to remove any precipitate, and concentrated *in vacuo* and the resulting solid dissolved in pyridine (15.0 mL). 4-Hydroxybenzaldehyde (6.458 g, 52.88 mmol) was then added to the solution. After 16 h, the reaction mixture was concentrated *in vacuo*, diluted

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with Et_2O (200 mL), washed with 25% aqueous $NaHSO_4$ (4 × 15 mL) and 10% aqueous sodium phosphate buffer (pH = 7) (1 × 15 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (80% CH_2Cl_2/Et_2O) afforded the diastereomeric mixture of the 4-formylphenyl phosphate **8a/8b** (3.918 g, 54.1% yield) as a white solid. On the basis of the integral of ^1H NMR, the ratio of the diastereomers **8a/8b** was determined to be 69:31.

Method B (via the Iodophosphate). Diethyl chlorophosphite (84 μl, 0.58 mmol) was added dropwise to a solution of 2′,5′-di(*tert*-butyldimethylsilyl)uridine (**7**) (0.250 g, 0.529 mmol) and pyridine (85 μL, 1.1 mmol) in Et_2O (2.0 mL). After 30 min, the reaction mixture was filtered and the resulting liquid concentrated *in vacuo* to remove any solvent and trace amounts of pyridine present. The resulting solid was redissolved in Et_2O (2.0 mL), filtered, and again concentrated *in vacuo* to afford the trialkyl phosphite **13**. The phosphite **13** was then dissolved in CH_2Cl_2 (5.0 mL), and I_2 (0.128 g, 0.504 mmol) was added to the solution at 0 °C. After 5 min, the solution was allowed to warm to 25 °C. The solution was then added dropwise, over a period of 10−15 min, to another flask containing 4-hydroxybenzaldehyde (96.9 mg, 0.793 mmol) and pyridine (0.171 mL, 2.11 mmol) in CH_2Cl_2 (5.0 mL) at −30 °C. After an additional 15 min, the reaction mixture was warmed to 25 °C, diluted with Et_2O (100 mL), and subjected to the workup described above. Flash chromatography afforded the 4-formylphenyl phosphate **8a/8b** (0.263 g, 72.7% yield) as a white solid. The ratio of the diastereomers **8a/8b** was 69:31. **Major isomer 8a**: ^1H NMR ($CDCl_3$, 400 MHz) δ 9.98 (s, 1 H), 8.43 (s, 1 H), 7.91 (d, 1 H, J = 8.6 Hz), 7.83 (d, 2 H, J = 8.1 Hz), 7.40 (d, 2 H, J = 8.1 Hz), 6.06 (d, 1 H, J = 6.2 Hz), 5.71 (dd, 1 H, J = 8.1, 2.4 Hz), 4.82 (m, 1 H), 4.35−4.21 (m, 4 H), 3.87 (dd, 1 H, J = 11.8, 1.8 Hz), 3.72 (dd, 1 H, J = 11.8, 1.8 Hz), 1.35 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.83 (4s, 18 H), 0.13−0.03 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.7, 163.8, 155.8 (d, J = 6 Hz), 151.2, 140.3, 134.2, 132.4, 121.3 (d, J = 6 Hz), 103.3, 87.7, 83.9 (d, J = 3 Hz), 78.0, 75.3 (d, J = 5 Hz), 66.0 (d, J = 6 Hz), 63.0, 26.0, 25.6, 25.5, 18.4, 18.1, 16.0 (d, J = 7 Hz), −5.1, −5.2, −5.6, −5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −6.82. **Minor isomer 8b**: ^1H NMR ($CDCl_3$, 400 MHz) δ 9.98 (s, 1 H), 8.46 (s, 1 H), 7.91 (d, 1 H, J = 8.6 Hz), 7.85 (d, 2 H, J = 8.1 Hz), 7.41 (d, 2 H, J = 8.1 Hz), 6.09 (d, 1 H, J = 6.4 Hz), 5.72 (dd, 1 H, J = 8.1, 2.4 Hz), 4.82 (m, 1 H), 4.40 (d, 1 H, J = 2.2 Hz), 4.35−4.21 (m, 3 H), 3.95 (dd, 1 H, J = 11.8, 1.8 Hz), 3.86 (dd, 1 H, J = 11.8, 1.8 Hz), 1.34 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.83 (4s, 18 H), 0.13−0.03 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.8, 163.8, 155.9 (d, J = 6 Hz), 151.2, 140.3, 134.2, 132.4, 121.2 (d, J = 6 Hz), 103.3, 87.7, 84.1 (d, J = 3 Hz), 78.0, 75.3 (d, J = 5 Hz), 65.7 (d, J = 6 Hz), 63.0, 26.0, 25.6, 25.5, 18.4, 18.1, 16.0 (d, J = 7 Hz), −5.1, −5.2, −5.6, -5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −7.02; LRMS *m/e* (relative intensity) 685.3 (1.5), 628.2 (12.0), 627.2 (29.8), 343.2 (24.5), 287.1 (31.2), 265.1 (10.1), 259.0 (12.4), 211.1 (30.9), 191.0 (10.8), 73.1 (21.4); HRMS for $C_{30}H_{49}N_2O_{10}PSi_2$ [M + H^+] calcd 685.2741, found 685.2728.

2′,5′-Di(*tert*-butyldimethylsilyl)-3′-(ethyl 4-(hydroxymethyl)phenyl phosphate)uridine (14). $NaBH_4$ (0.541 g, 14.3 mmol) was added portionwise to a solution of the 4-formylphenyl phosphate **8** (3.918 g, 5.726 mmol) in MeOH (11.0 mL) and CH_2Cl_2 (11.0 mL) at 0 °C. After 30 min, the reaction mixture was warmed to 25 °C and added to an aqueous 1.0 M HCl solution (150 mL). The aqueous layer was extracted with CH_2Cl_2 (5 × 75 mL). The combined organic layers were washed with phosphate buffer (pH = 7) (2 × 50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography in (100% Et_2O) afforded the diastereomeric mixture of the 4-(hydroxymethyl)phenyl phosphate **14a/14b** (3.729 g, 94.9% yield) as a white solid. On the basis of the integral of ^1H NMR, the ratio of the diastereomers **14a/14b** was determined to be 69:31. **Major isomer 14a**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.47 (s, 1 H), 7.84 (d, 1 H, J = 8.1 Hz), 7.35 (d, 2 H, J = 8.3 Hz), 7.21 (d, 2 H, J = 8.3 Hz), 6.01 (d, 1 H, J = 6.2 Hz), 5.74 (dd, 1 H, J = 8.1, 2.2 Hz), 4.77 (m, 1 H), 4.67 (d, 2 H, J = 5.9 Hz), 4.31−4.20 (m, 4 H), 3.84 (dd, 1 H, J = 11.6, 1.9 Hz), 3.70 (dd, 1 H, J = 11.6, 1.9 Hz), 1.34 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.84 (4s, 18 H), 0.13−0.04 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.9, 151.3, 150.4 (d, J = 7 Hz), 140.4, 139.0, 129.1, 120.7 (d, J = 5 Hz), 103.3, 87.6, 84.0, 77.6, 75.3 (d, J = 5 Hz), 65.6 (d, J = 6 Hz), 64.7, 63.1, 26.0, 25.6, 18.4, 18.0, 16.0 (d, J = 8 Hz), −5.1, −5.2, −5.6, −5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −6.19. **Minor isomer 14b**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.58 (s, 1 H), 7.87 (d, 1 H, J = 8.1 Hz), 7.35 (d, 2 H, J = 8.3 Hz), 7.23 (d, 2 H, J = 8.3 Hz), 6.06 (d, 1 H, J = 6.2 Hz), 5.75 (dd, 1 H, J = 8.1, 2.2 Hz), 4.81 (m, 1 H), 4.67 (d, 2 H, J = 5.9 Hz), 4.38 (d, 1 H, J = 2.7 Hz), 4.31−4.20 (m, 3 H), 3.94 (dd, 1 H, J = 11.6, 1.9 Hz), 3.85 (dd, 1 H, J = 11.6, 1.9 Hz), 1.33 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.84 (4s, 18 H), 0.13−0.04 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.9, 151.3, 150.5 (d, J = 6 Hz), 140.4, 139.0, 129.1, 120.6 (d, J = 5 Hz), 103.3, 87.6, 84.1, 77.6, 75.3 (d, J = 5 Hz), 65.4 (d, J = 6 Hz), 64.7, 63.1, 26.0, 25.6, 18.4, 18.0, 16.0 (d, J = 8 Hz), −5.1, −5.2, −5.6, −5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −6.56; LRMS *m/e* (relative intensity) 631.2 (24.7), 630.2 (39.3), 629.2 (100.0), 343.2 (67.4), 289.1 (75.3), 211.1 (79.8), 151.0 (34.8), 73.0 (59.6); HRMS for $C_{30}H_{51}N_2O_{10}PSi_2$ [M − tBu]$^+$, calcd 629.2116, found 629.2107.

2′,5′-Di(*tert*-butyldimethylsilyl)-3′-(ethyl 4-(fluoromethyl)phenyl phosphate)uridine (15). DAST (0.298 mL, 2.26 mmol) was added dropwise to a solution of the 4-(hydroxymethyl)phenyl phosphate **14** (1.107 g, 1.613 mmol) in CH_2Cl_2 (175 mL) at 0 °C. After 15 min, the reaction mixture was added to a saturated $NaHCO_3$ solution (40 mL). The aqueous layer was extracted with CH_2Cl_2 (5 × 20 mL). The combined organic layers were washed with phosphate buffer (pH = 7) (2 × 10 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography in (75% Et_2O/hexanes) afforded the diastereomeric mixture of the 4-(fluoromethyl)phenyl phosphate **15a/15b** (0.873 g, 78.6% yield) as a white solid. On the basis of the integral of ^1H NMR, the ratio of the diastereomers **15a/15b** was determined to be 69:31. **Major isomer 15a**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.19 (s, 1 H), 7.85 (d, 1 H, J = 8.1 Hz), 7.37 (d, 2 H, J = 8.3 Hz), 7.26 (d, 2 H, J = 8.1 Hz), 6.05 (d, 1 H, J = 6.2 Hz), 5.70 (dd, 1 H, J = 8.1, 2.2 Hz), 5.35 (d, 2 H, J = 47.8 Hz), 4.78 (m, 1 H), 4.33−4.20 (m, 3 H), 4.17 (d, 1 H, J = 2.2 Hz), 3.84 (dd, 1 H, J = 11.6, 1.9 Hz), 3.67 (dd, 1 H, J = 11.6, 1.9 Hz), 1.35 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.84 (4s, 18 H), 0.13−0.04 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.9, 151.4 (d, J = 5 Hz), 151.2, 140.4, 134.0 (d, J = 16.8 Hz), 129.9 (d, J = 6 Hz), 121.0 (d, J = 5 Hz), 103.2, 87.8, 84.2 (d, J = 166 Hz), 84.0 (d, J = 4 Hz), 77.7, 75.4 (d, J = 5 Hz), 65.7 (d, J = 7 Hz), 63.0, 26.0, 25.6, 18.4, 18.1, 16.0 (d, J = 7 Hz), −5.1, −5.2, −5.6, −5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −6.15; ^{19}F NMR ($CFHCl_2$, 340 MHz) δ −205.9 (t, J = 48 Hz). **Minor isomer 15b**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.21 (s, 1 H), 7.87 (d, 1 H, J = 8.1 Hz), 7.37 (d, 2 H, J = 8.3 Hz), 7.27 (d, 2 H, J = 8.1 Hz), 6.08 (d, 1 H, J = 6.2 Hz), 5.71 (dd, 1 H, J = 8.1, 2.2 Hz), 5.35 (d, 2 H, J = 47.8 Hz), 4.81 (m, 1 H), 4.40 (d, 1 H, J = 2.7 Hz), 4.33−4.20 (m, 3 H), 3.95 (dd, 1 H, J = 11.6, 1.9 Hz), 3.86 (dd, 1 H, J = 11.6, 1.9 Hz), 1.33 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.84 (4s, 18 H), 0.13−0.04 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.9, 151.5 (d, J = 5 Hz), 151.2, 140.4, 134.0 (d, J = 16.8 Hz), 129.9 (d, J = 6 Hz), 120.8 (d, J = 5 Hz), 103.2, 87.9, 84.2 (d, J = 166 Hz), 84.0 (d, J = 4 Hz), 77.7, 75.4 (d, J = 5 Hz), 65.5 (d, J = 7 Hz), 63.0, 26.0, 25.6, 18.4, 18.1, 16.0 (d, J = 7 Hz), −5.1, −5.2, −5.6, −5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −6.47; ^{19}F NMR ($CFHCl_2$, 340 MHz) δ −205.9 (t, J = 48 Hz); LRMS *m/e* (relative intensity) 689.3 (2.3), 632.2 (29.6), 631.2 (62.1), 627.2 (23.4), 397.2 (32.3), 344.2 (21.4), 343.2 (79.0), 291.0 (79.8), 265.0 (35.4), 263.0 (38.2), 211.1 (100.0), 73.0 (76.3); HRMS for $C_{30}H_{50}FN_2O_9PSi_2$ [M + H^+] calcd 689.2855, found 689.2842.

3′-(4-(Fluoromethyl)phenyl phosphate)uridine, Triethylammonium Salt (4). TMSBr (0.575 mL, 4.36 mmol) was added to a solution of the 4-(fluoromethyl)phenyl phosphate **15** (0.120 g, 0.174 mmol) in CH_2Cl_2 (1.2 mL). After 24 h, the reaction mixture was concentrated *in vacuo* with toluene (3 × 5 mL) to remove the excess TMSBr. The reaction mixture was then diluted with CH_2Cl_2 (2.4 mL) and quenched at 0 °C with a mixture of NEt_3 (0.5 mL) and MeOH (0.5 mL). All operations were carried out under nitrogen. After concentration *in vacuo*, the reaction mixture was dissolved in a mixture of THF (1.0 mL) and triethylammonium fluoride (0.106 g,

0.875 mmol). After 24 h, the reaction mixture was concentrated *in vacuo*. Flash chromatography on a prewashed silica gel column (washed with 10 column volumes of 98.0% MeOH/2.0% formic acid, and 10 column volumes 35.0% MeOH/64.5% Et_2O/0.5% formic acid) in the solvent system 35.0% MeOH/64.5% Et_2O/0.5% formic acid afforded the 4-(fluoromethyl)-phenyl phosphate salt **4** (67.6 mg, 72.8% yield) as a clear, colorless gum: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.08 (s, 1 H), 8.02 (d, 1 H, J = 8.1 Hz), 7.33 (dd, 2 H, J = 8.6, 1.9 Hz), 7.28 (d, 2 H, J = 8.6 Hz), 5.94 (d, 1 H, J = 5.6 Hz), 5.69 (d, 1 H, J = 8.1 Hz), 5.29 (d, 2 H, J = 48.3 Hz), 4.69 (m, 1 H), 4.29 (t, 1 H, J = 5.1 Hz), 4.21 (dt, 1 H, J = 4.0, 2.4 Hz), 3.77 (dd, 1 H, J = 12.4, 2.4 Hz), 3.71 (dd, 1 H, J = 12.4, 2.7 Hz), 3.18 (q, 6 H, J = 7.3 Hz), 1.29 (t, 9 H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.1, 155.4 (d, J = 6 Hz), 153.3, 143.3, 133.8 (d, J = 17.5 Hz), 131.1 (d, J = 5 Hz), 122.2 (d, J = 4 Hz), 103.4, 90.4, 85.9 (d, J = 4 Hz), 85.6 (d, J = 164 Hz), 76.5, 75.7 (d, J = 5 Hz), 62.5, 48.1, 9.2; ^{31}P NMR (H_3PO_4, 146 MHz) δ −3.92; ^{19}F NMR ($CFHCl_2$, 340 MHz) δ −203.9 (t, J = 46 Hz).

2′,5′-Di(*tert*-butyldimethylsilyl)-3′-(ethyl vinyl phosphate)uridine (9). Method A (via the Chlorophosphate). In order to generate the enolate of acetaldehyde, a solution of *n*-butyllithium (2.60 M solution in hexane, 6.322 mL, 16.4 mmol) in THF (75 mL) was stirred for 30 min at 0 °C, followed by an additional 16 h at 25 °C. In a separate flask, diethyl chlorophosphite (0.747 mL, 5.16 mmol) was added dropwise to a solution of 2′,5′-di(*tert*-butyldimethylsilyl)uridine (**7**) (2.220 g, 4.70 mmol) and pyridine (1.900 mL, 23.49 mmol) in CH_2Cl_2 (90.0 mL) at 0 °C. After 10 min, I_2 (1.192 g, 4.696 mmol) was added to the reaction mixture. After an additional 10 min, the reaction mixture was allowed to warm to 25 °C, filtered to remove any precipitate, and concentrated *in vacuo* and the resulting solid triterated with THF (4 × 100 mL). After the solution was cooled to −60 °C, the enolate of acetaldehyde (16.4 mmol) was added dropwise, over a period of 2 h, to the solution. After an additional 30 min, the reaction mixture was diluted with Et_2O (1000 mL), washed with 25% aqueous $NaHSO_4$ (4 × 75 mL) and 10% aqueous sodium phosphate buffer (pH = 7) (1 × 75 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (75% Et_2O/hexanes) afforded the diastereomeric mixture of the ethyl vinyl phosphate **9a/9b** (1.921 g, 67.4% yield) as a white solid. On the basis of the integral of ^1H NMR, the ratio of the diastereomers **9a/9b** was determined to be 54:46.

Method B (via the Iodophosphate). The enolate of acetaldehyde was generated as described above using *n*-butyllithium (2.52 M solution in hexane, 0.683 mL, 1.72 mmol) and THF (7.5 mL). In a separate flask, diethyl chlorophosphite (84 μL, 0.58 mmol) was added dropwise to a solution of 2′,5′-di(*tert*-butyldimethylsilyl)uridine (**7**) (0.250 g, 0.529 mmol) and pyridine (85 μL, 1.1 mmol) in Et_2O (2.0 mL). After 30 min, the reaction mixture was filtered and the resulting liquid concentrated *in vacuo* to remove any solvent and trace amounts of pyridine present. The resulting solid was redissolved in Et_2O (2.0 mL), filtered, and concentrated *in vacuo* to afford the trialkyl phosphite **13**. The phosphite **13** was then dissolved in THF (5.0 mL), and I_2 (0.128 g, 0.504 mmol) was added to the solution at 0 °C. The solution was immediately cooled to −60 °C and the enolate of acetaldehyde (1.72 mmol) added dropwise over a period of 30 min. After an additional 30 min, the reaction mixture was diluted with Et_2O (150 mL) and subjected to the workup described above. Flash chromatography afforded the ethyl vinyl phosphate **9a/9b** (0.238 g, 74.2% yield) as a white solid. The ratio of the diastereomers **9a/9b** was 54:46. **Major isomer 9a**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.17 (s, 1 H), 7.88 (d, 1 H, J = 8.3 Hz), 6.58 (m, 1 H), 6.04 (d, 1 H, J = 5.9 Hz), 5.70 (dd, 1 H, J = 8.1, 2.2 Hz), 4.91 (td, 1 H, J = 13.4, 2.2, 1.1 Hz), 4.70 (m, 1 H), 4.61 (m, 1 H), 4.37 (m, 1 H), 4.28 (dt, 1 H, J = 4.6, 1.6 Hz), 4.20 (m, 2 H), 3.94 (dd, 1 H, J = 11.8, 1.8 Hz), 3.82 (dd, 1 H, J = 11.8, 1.7 Hz), 1.37 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.87 (3s, 18 H), 0.13−0.04 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.8, 151.1, 142.8 (d, J = 7 Hz), 140.5, 103.1, 100.7 (d, J = 10 Hz), 88.1, 84.0 (d, J = 3 Hz), 76.9, 75.3 (d, J = 5 Hz), 65.3 (d, J = 6 Hz), 62.9, 26.0, 25.6, 18.4, 18.1, 16.0 (d, J = 7 Hz), −5.1, −5.6, −5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −4.82. **Minor isomer 9b**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.17 (s, 1 H), 7.88 (d, 1 H, J = 8.3 Hz), 6.58 (m, 1 H), 6.03 (d, 1 H, J = 5.9 Hz), 5.70 (dd, 1 H, J = 8.1, 2.2 Hz), 4.93 (td, 1 H, J = 13.4, 2.2, 1.1 Hz), 4.74 (m, 1 H), 4.61 (m, 1 H), 4.33 (m, 1 H), 4.30 (dt, 1 H, J = 4.6, 1.6 Hz), 4.20 (m, 2 H), 3.94 (dd, 1 H, J = 11.8, 1.8 Hz), 3.85 (dd, 1 H, J = 11.8, 1.7 Hz), 1.36 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.87 (3s, 18 H), 0.13−0.04 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.8, 151.1, 142.7 (d, J = 7 Hz), 140.5, 103.1, 101.1 (d, J = 10 Hz), 88.1, 83.8 (d, J = 3 Hz), 76.9, 75.4 (d, J = 5 Hz), 65.4 (d, J = 6 Hz), 62.8, 26.0, 25.6, 18.4, 18.1, 16.0 (d, J = 7 Hz), −5.1, −5.6, −5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −4.86; LRMS *m/e* (relative intensity) 549.2 (57.9), 397.2 (21.3), 343.2 (40.6), 265.1 (26.5), 211.1 (77.8), 209.0 (100.0), 191.0 (26.0), 183.0 (25.8), 155.0 (31.2), 73.0 (76.0); HRMS for $C_{25}H_{47}N_2O_9PSi_2$ [M − tBu]$^+$ calcd 549.1853, found 549.1875.

2′,5′-Di(*tert*-butyldimethylsilyl)-3′-(1-bromovinyl ethyl phosphate)uridine (11). A solution of Br_2 (19 μL, 0.37 mmol) in CH_2Cl_2 (3.0 mL) was added dropwise to a solution of the ethyl vinyl phosphate **9** (0.224 g, 0.369 mmol) in CH_2Cl_2 (17.0 mL) at −30 °C. After 10 min, the reaction mixture was concentrated *in vacuo* and the resulting oil dissolved in toluene (17.0 mL). LiHMDS (1.00 M, 1.02 mL, 1.02 mmol) was then added dropwise, over a period of 10 min, to the solution at −78 °C. After 15 min, the reaction mixture was warmed to 0 °C, diluted with Et_2O (200 mL), washed with 25% aqueous $NaHSO_4$ (4 × 15 mL) and 10% aqueous sodium phosphate buffer (pH = 7) (1 × 15 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (65% Et_2O/hexanes) afforded the diastereomeric mixture of the ethyl 1-bromovinyl phosphate **11a/11b** (0.144 g, 56.9% yield) as a white solid. On the basis of the integral of ^1H NMR, the ratio of the diastereomers **11a/11b** was determined to be 54:46. **Major isomer 11a**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.40 (s, 1 H), 7.85 (d, 1 H, J = 8.2 Hz), 6.07 (d, 1 H, J = 6.4 Hz), 5.71 (dd, 1 H, J = 8.2, 1.9 Hz), 5.49 (dd, 1 H, J = 3.0, 3.0 Hz), 5.12 (dd, 1 H, J = 3.0, 2.2 Hz), 4.82 (m, 1 H), 4.38 (d, 1 H, J = 1.9 Hz), 4.32−4.24 (m, 3 H), 3.94 (dd, 1 H, J = 11.6, 1.8 Hz), 3.83 (dd, 1 H, J = 11.6, 1.7 Hz), 1.40 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.87 (2s, 18 H), 0.13-0.04 (4s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.8, 151.1, 140.4, 129.3 (d, J = 10 Hz), 106.7 (d, J = 5 Hz), 103.3, 87.6, 84.0 (d, J = 3 Hz), 78.4 (d, J = 4 Hz), 75.2 (d, J = 5 Hz), 66.3 (d, J = 6 Hz), 63.1, 26.0, 25.6, 18.4, 18.1, 16.0 (d, J = 7 Hz), −5.1, −5.2, −5.5, −5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −7.96. **Minor isomer 11b**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.40 (s, 1 H), 7.84 (d, 1 H, J = 8.2 Hz), 6.06 (d, 1 H, J = 6.4 Hz), 5.71 (dd, 1 H, J = 8.2, 1.9 Hz), 5.52 (dd, 1 H, J = 3.0, 3.0 Hz), 5.11 (dd, 1 H, J = 3.0, 2.2 Hz), 4.74 (m, 1 H), 4.41 (d, 1 H, J = 1.9 Hz), 4.32−4.24 (m, 3 H), 3.94 (dd, 1 H, J = 11.6, 1.8 Hz), 3.86 (dd, 1 H, J = 11.6, 1.7 Hz), 1.41 (dt, 3 H, J = 7.0, 1.1 Hz), 0.94−0.87 (2s, 18 H), 0.13-0.04 (4s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.8, 151.1, 140.4, 129.2 (d, J = 10 Hz), 106.6 (d, J = 5 Hz), 103.3, 87.8, 83.8 (d, J = 3 Hz), 78.4 (d, J = 4 Hz), 75.3 (d, J = 5 Hz), 66.4 (d, J = 6 Hz), 63.0, 26.0, 25.6, 18.4, 18.0, 16.0 (d, J = 7 Hz), −5.1, −5.2, −5.5, −5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −8.08; LRMS *m/e* (relative intensity) 629.1 (41.0), 627.1 (36.3), 343.2 (56.2), 289.0 (41.0), 287.0 (50.7), 265.1 (30.6), 211.1 (89.8), 191.0 (10.8), 155.0 (43.0), 151.0 (37.6), 143.0 (39.5), 113.0 (45.2), 73.0 (100.0); HRMS for $C_{25}H_{46}BrN_2O_9PSi_2$ [M − tBu]$^+$ calcd 627.0959, found 627.0955.

2′,5′-Di(*tert*-butyldimethylsilyl)-3′-(ethyl 4-nitrophenyl phosphate)uridine (12). Trialkyl phosphite **13** was prepared as described above (method B, compounds **8** and **9**) using diethyl chlorophosphite (84 μL, 0.58 mmol), 2′,5′-di(*tert*-butyldimethylsilyl)uridine (**7**) (0.250 g, 0.529 mmol), pyridine (85 μL, 1.1 mmol), and Et_2O (2 × 2.0 mL). The phosphite **13** was then dissolved in CH_2Cl_2 (5.0 mL), and I_2 (0.128 g, 0.504 mmol) was added to the solution at 0 °C. After 5 min, the solution was allowed to warm to 25 °C. The solution was then added dropwise, over a period of 10−15 min, to another flask containing 4-nitrophenol (0.110 g, 0.791 mmol) and pyridine (0.171 mL, 2.11 mmol) in CH_2Cl_2 (5.0 mL) at −30 °C. After an additional 15 min, the reaction mixture was warmed to 25 °C, diluted with Et_2O (100 mL), and subjected to the workup described above (method B, compounds **8** and **9**). Flash chromatography (83% CH_2Cl_2/Et_2O) afforded the diastereomeric mixture of the 4-nitrophenyl phosphate **12a/12b** (0.294

g, 79.3% yield) as a white solid. On the basis of the integral of ^{1}H NMR, the ratio of the diastereomers **12a/12b** was determined to be 69:31. **Major isomer 12a**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.59 (s, 1 H), 8.26 (d, 2 H, J = 9.1 Hz), 7.82 (d, 1 H, J = 8.2 Hz), 7.40 (d, 2 H, J = 9.1 Hz), 6.07 (d, 1 H, J = 6.3 Hz), 5.72 (dd, 1 H, J = 8.2, 2.2 Hz), 4.82 (m, 1 H), 4.35–4.23 (m, 4 H), 3.89 (dd, 1 H, J = 11.8, 1.8 Hz), 3.74 (dd, 1 H, J = 11.8, 1.8 Hz), 1.36 (dt, 3 H, J = 7.0, 1.2 Hz), 0.94–0.82 (4s, 18 H), 0.14–0.02 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.9, 155.9 (d, J = 7 Hz), 151.3, 145.6, 140.3, 126.4, 121.3 (d, J = 5 Hz), 103.4, 87.6 (d, J = 3 Hz), 83.8 (d, J = 4 Hz), 78.3, 75.2 (d, J = 5 Hz), 66.2 (d, J = 7 Hz), 63.1, 26.0, 25.6, 25.5, 18.4, 18.1, 16.0 (d, J = 7 Hz), −5.1, −5.6, -5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −6.97. **Minor isomer 12b**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.62 (s, 1 H), 8.26 (d, 2 H, J = 9.1 Hz), 7.83 (d, 1 H, J = 8.2 Hz), 7.42 (d, 2 H, J = 9.1 Hz), 6.10 (d, 1 H, J = 6.3 Hz), 5.73 (dd, 1 H, J = 8.2, 2.2 Hz), 4.84 (m, 1 H), 4.39 (d, 1 H, J = 2.2 Hz), 4.35–4.23 (m, 3 H), 3.95 (dd, 1 H, J = 11.8, 1.8 Hz), 3.86 (dd, 1 H, J = 11.8, 1.8 Hz), 1.36 (dt, 3 H, J = 7.0, 1.2 Hz), 0.94–0.82 (4s, 18 H), 0.14–0.02 (5s, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.9, 156.0 (d, J = 7 Hz), 151.3, 145.6, 140.3, 126.4, 121.3 (d, J = 5 Hz), 103.4, 87.6 (d, J = 3 Hz), 84.0 (d, J = 4 Hz), 78.3, 75.2 (d, J = 5 Hz), 65.9 (d, J = 7 Hz), 63.1, 26.0, 25.6, 25.5, 18.4, 18.1, 16.1 (d, J = 7 Hz), −5.1, −5.6, -5.7; ^{31}P NMR (H_3PO_4, 146 MHz) δ −7.12; LRMS *m/e* (relative intensity) 646.2 (17.8), 645.2 (40.3), 644.2 (100.0), 590.2 (11.2), 400.1 (26.1), 397.2 (31.5), 343.2 (34.9), 304.0 (75.0), 276.0 (44.8), 265.1 (28.8), 211.1 (77.1), 191.0 (32.4), 169.0 (41.6), 73.0 (39.4); HRMS for $C_{29}H_{48}N_3O_{11}PSi_2$ [M − tBu]$^+$ calcd 644.1861, found 644.1869.

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