Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

for

Selective aqueous acetylation controls the photoanomerization of α-cytidine-5'-phosphate

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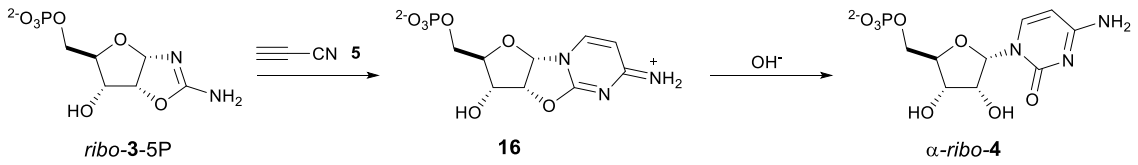
General Experimental

Reagents and anhydrous organic solvents were obtained and used without further purification, unless specified. Cytidine potassium thioacetate, ammonium formate and potassium ferricyanide were purchased from *Sigma Aldrich*. Sodium dihydrogen phosphate, *N*-acetyl imidazole and pyridine were purchased from *Acros Organics*. Uridine was purchased from *Calbiochem*. Cytidine 5'-phosphate and cytosine β-D-arabinofuranoside 5'-monophosphate were purchased from *Carbosynth*. Acetic anhydride was purchased from *Merck*. Ammonia solution 28% was purchased from *Fischer Scientific*. Dowex® 50W×8 resin was purchased from *Acros Organics* and regenerated with HCl solution. Deionized water was obtained from an Elga Option 3 purification system. Automated flash column chromatography was carried out using a Biotage Isolera Four purification system and Biotage KP-C18-HS Snap Cartridge. Solution pH values were measured using a Mettler Toledo Seven Compact pH meter with a Mettler Toledo InLab semi-micro pH probe. The readings for D_2O solutions are reported as pD, and corrected according to Covington et al.[1] The readings for H_2O and H_2O/D_2O solutions are reported uncorrected. NMR Spectra were recorded on a Bruker Avance III (400) equipped with a gradient probe or Bruker Avance III (600) spectrometer, equipped with a cryoprobe. Chemical shifts (δ) are reported in parts per million (ppm) relative to the residual solvent peak, and ^{1}H chemical shifts relative to TMS were calibrated using the residual solvent peak: HOD (δ_H 4.75 ppm) or $CDCl_3$ (δ_H 7.26 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Spin multiplicities are indicated by symbols: s (singlet); d (doublet); t (triplet); q (quartet); ABX (geminal (AB) spin system coupled to one other nuclei (X)); ABXY (germinal (AB) spin system coupled to two other nuclei (X, Y)); obs. (obscured/coincidental signals), or a combination of these. NMR data are reported as follows: chemical shift (multiplicity, coupling constants (*J*), number of protons, nuclear assignment). Spectra were recorded at 298 K. Signal assignments are made by correlation of COSY, HMBC, HSQC and DEPT experiments. ^{1}H NMR spectra (H_2O/D_2O) are solvent suppressed (noesygppr1d) with presaturation and spoil gradients. Infrared (IR) spectra were recorded with a *Shimadzu* 100 FTIR spectrometer. Absorption maxima are reported in wavenumbers (cm^{-1}). Mass spectra were determined by the University College London mass spectrometry service by electrospray ionisation (ESI) using a Waters LCT Premier XE or Thermo Finnigan MAT 900XP instrument. UV irradiations were performed using a UVP Pen-Ray mercury lamp (λ=254 nm) and a quartz micro-

photochemical reaction assembly (Ace glassware). Cooling was provided by recirculated water flowing through an internal quartz water jacket between the lamp and the irradiated solution. All solutions were sparged with Ar or N_2 for 1 h prior to irradiation, and then continuously agitation by continuous Ar or N_2 flow through a sinter glass frit at the bottom of the reaction vessel during irradiation.

*Preparative synthesis of α-ribo-**4** from ribo-**3**-5P.*



ribo-**3**-5P **16** α-*ribo*-**4**

*D-Ribofuranosyl-2,2'-anhydrocytidine-5'-phosphate (**16**)*

Ribose aminooxazoline-5′-phosphate (*ribo*-**3**-5P; 0.046M, 760 mg, 2.99 mmol) and sodium dihydrogen phosphate (1.1 eq.) were dissolved in water and the solution was adjusted to pH 6.5 with 4M NaOH. Cyanoacetylene (**5**; 1M, 7 eq.) was added (to give total volume of 65 mL) and the solution was stirred at r.t. for 16 h then lyophilized. The lyophilite was purified by ion-exchange chromatography (Dowex® 50W×8, H^+-form cation-exchange resin), eluting with water (100 mL), then 0.125M HCl (100 mL), 0.25M HCl (100 mL), 0.5M HCl (100 mL), 1M HCl (100 mL), 2M HCl (100 mL) and 4M HCl (100 mL). D-Ribofuranosyl-2,2'-anhydrocytidine-5'-phosphate (**16**) was obtained in 0.5–1M HCl fractions, and was confirmed by ^{1}H NMR. These fractions were concentrated and lyophilized to afford D-ribofuranosyl-2,2′-anhydrocytidine-5′-phosphate (**16**; 729 mg, 80%) as a white powder.

^{1}H NMR (600 MHz, D_2O) δ 8.19 (d, J = 7.4 Hz, 1H, (C6)-H), 6.69 (d, J = 7.4 Hz, 1H, (C5)-H), 6.62 (d, J = 5.4 Hz, 1H, (C1′)-H), 5.68 (dd, J = 5.6, 5.4 Hz, 1H, (C2′)-H), 4.58 (dd, J = 8.7, 5.6 Hz, 1H, (C3′)-H), 4.28 (ABXY, J = 11.0, 5.6, 2.6 Hz, 1H, (C5′)-H). 4.07 - 4.17 (m, 2H, (C4′)-H, (C5″)-H). ^{13}C NMR (151 MHz, D_2O) δ 168.2 (C4), 161.6 (C2), 141.0 (C6), 103.9 (C5), 90.9 (C1′), 84.1 (C2′), 80.1 (d, C4′), 70.1 (C3′), 63.4 (d, C5′). ^{31}P NMR (162 MHz, D_2O, ^{1}H-decoupled) δ 0.49.

The spectroscopic properties of this compound were consistent with the data reported in the literature.[2]

*α-Cytidine-5'-phosphate (α-ribo-**4**)*

D-Ribofuranosyl-2,2′-anhydrocytidine-5′-phosphate (**16**, 600 mg, 1.97 mmol) was dissolved in water (50 mL) and 4M NaOH was added to adjust the solution to pH 8.5. The solution pH was monitored periodically and readjusted with 4M NaOH until the pH

stabilized at pH 8.5. The solution was then lyophilized and the lyophilite was purified by ion-exchange chromatography (Dowex® 50W×8 H^+-form cation-exchange resin), eluting with HCl_{aq} in the following concentration gradient: water (100 mL), 0.125M (100 mL), 0.25M (100 mL), 0.5M (100 mL), 1M (100 mL), 2M (100 mL) and 4M (100 mL). The fractions with product were concentrated *in vacuo* and lyophilized to obtain α-cytidine-5′-phosphate (α-*ribo*-**4**, 498 mg, 78%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 7.88 (d, J = 7.6 Hz, 1H, (C6)-H), 6.04–6.15 (m, 2H, (C1′)-H, (C5)-H), 4.44 (t, J = 4.5 Hz, 1H, (C2′)-H), 4.31–4.36 (m, 1H, (C4′)-H), 4.28 (dd, J = 6.3, 4.5 Hz, 1H, (C3′)-H), 4.08 (ABXY, J = 11.7, 5.6, 2.3 Hz, 1H, (C5′)-H), 3.94 (ABXY, J = 11.7, 6.3, 5.0 Hz, 1H, (C5″)-H). ^{13}C NMR (151 MHz, D_2O) δ 159.8 (C4), 148.8 (C2), 146.2 (C6), 94.4 (C5), 87.8 (C1′), 83.2 (d, C4′), 71.2 (C2′), 70.8 (C3′), 65.5 (d, C5′). ^{31}P NMR (162 MHz, D_2O, ^{1}H-decoupled) δ 0.35.

The spectroscopic properties of this compound were consistent with the data reported in the literature.[2]

Protocol A: Nucleotide (**4**; 100mM) was dissolved in D_2O/H_2O (1:1) and the pH adjusted to the desired value by addition of 4M NaOH. The solution was purged with argon for 15 min, potassium ferricyanide (8 eq.) was added followed by potassium thioacetate (10 eq.). The pH was stabilised by addition of 4M HCl/NaOH and the reaction monitored by NMR spectroscopy.

Protocol B: Nucleotide (**4**; 100mM) and *N*-acetyl imidazole (10 eq.) were dissolved in D_2O/H_2O (1:1). The solution pH was stabilised at the desired value through addition of 4M HCl/NaOH and the reaction monitored by NMR spectroscopy. The product was purified by reverse-phase (C18) flash column chromatography (eluted at pH 4 with 100mM NH_4HCO_2/MeCN 98:2 to 80:20). The fractions containing **7** were lyophilised to yield a white powder.

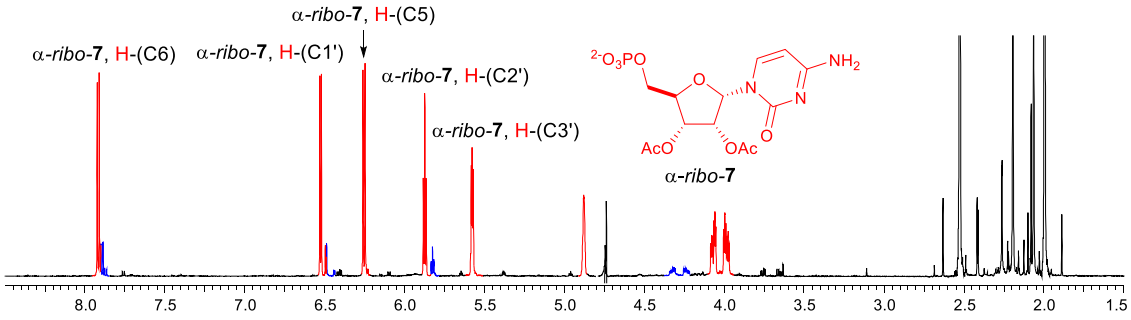


Figure S1: ^{1}H NMR spectrum (600 MHz, D_2O/H_2O 1:1, 1.5 – 8.5 ppm) for the acetylation of α-*ribo*-**4** with thioacetate (10 eq.) and ferricyanide (8 eq.) after 1 h in D_2O/H_2O 1:1 at pH 8.

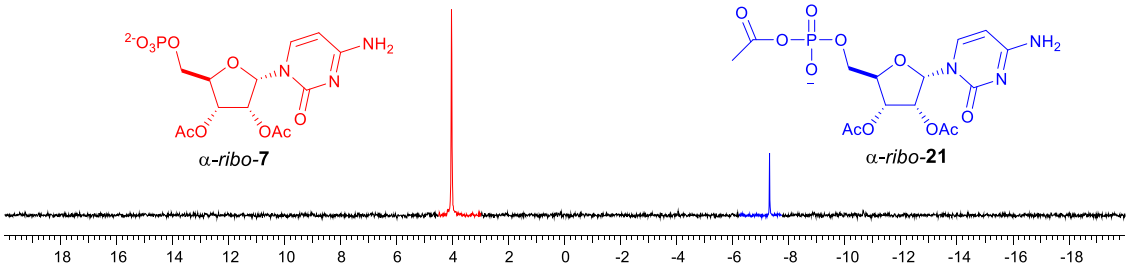


Figure S2: ^{31}P NMR spectrum (162 MHz, D_2O/H_2O 1:1, -20 – 20 ppm) showing 5'-phosphate α-*ribo*-**7** (3.78 ppm, 81%) and acetyl phosphate α-*ribo*-**21** (-7.08 ppm, 14%) observed upon the reaction of α-*ribo*-**4** with thioacetate (10 eq.) and ferricyanide (8 eq.). Note: partially acetylated derivatives are observed in 5% yield.

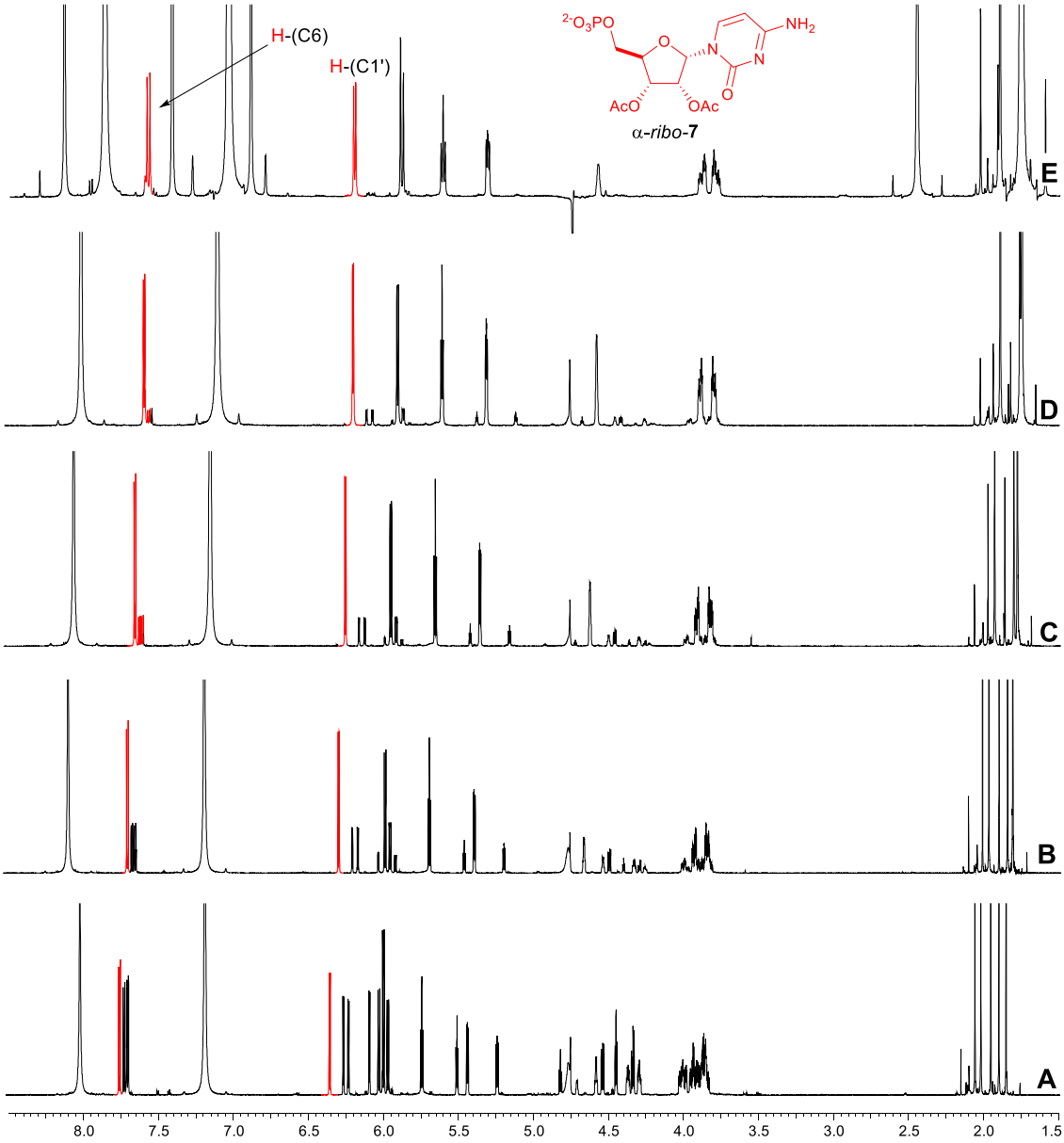
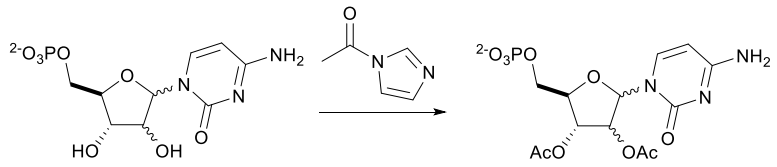


Figure S3: ^{1}H NMR spectra (700 MHz, D_2O/H_2O 1:1, 1.5–8.5 ppm) to show the acetylation of α-*ribo*-**4** with: **A**) 2 eq. of *N*-acetyl imidazole (yield α-*ribo*-**7**: 29%) ; **B**) 4 eq. of *N*-acetyl imidazole (yield α-*ribo*-**7**: 60%); **C**) 6 eq. of *N*-acetyl imidazole (yield α-*ribo*-**7**: 74%); **D**) 8 eq. of *N*-acetyl imidazole (yield α-*ribo*-**7**: 83%) and **E**) 10 eq. of *N*-acetyl imidazole (yield α-*ribo*-**7**: 91%) after 4 h in D_2O/H_2O (1:1) at pH 8.

Preparative synthesis of O-acetylated nucleotides in water



α-*ribo*-**4**
β-*ribo*-**4**
β-*arabino*-**4**

α-*ribo*-**7**
β-*ribo*-**7**
β-*arabino*-**7**

Nucleotide (100mM) and *N*-acetyl imidazole (10 eq.) were dissolved in water and the solution was adjusted to pH 8 by addition of 4M NaOH. The reaction mixture was incubated for 4 h at r.t. The solution was then adjusted to pH 4 and the product was immediately purified by C18 column (100mM NH_4HCO_2 in H_2O/MeCN 98:2 to 80:20). The fractions with product were lyophilized to obtain white powders.

2′,3′-Di-*O*-acetyl-α-cytidine-5′-phosphate (α-*ribo*-**7**)

Starting from α-*ribo*-**4** (free acid, 122 mg, 0.38 mmol), yield = 124 mg (81%) as a white powder. $[\alpha]_D^{20}$= -23.7 (c 1.0, H_2O); M. p. 169–175 °C (decomp.). ^{1}H NMR (600 MHz, D_2O) δ 7.90 (d, *J* = 7.7 Hz, 1H, (C6)-H), 6.37 (d, *J* = 5.5 Hz, 1H, (C1′)-H), 6.10 (d, *J* = 7.7 Hz, 1H, (C5)-H), 5.73 (dd, *J* = 5.5, 5.1 Hz, 1H, (C2′)-H), 5.45 (dd, *J* = 5.1, 3.7 Hz, 1H, (C3′)-H), 4.75-4.80 (m, 1H, (C4′)-H, *partially obscured by HOD signal*); 4.04 (ABXY, *J* = 11.7, 5.7, 2.9 Hz, 1H, (C5′)-H), 3.95 (ABXY, *J* = 11.7, 5.7, 3.4 Hz, 1H, (C5′′)-H), 2.01 (s, 3 H, (C3′)-OAc), 1.93 (s, 3 H, (C2′)-OAc). ^{13}C NMR (151 MHz, D_2O) δ 173.3 (3′-OAc), 172.5 (2′-OAc), 167.0 (C4); 158.1 (C2), 142.8 (C6), 95.7 (C5), 85.6 (C1′), 83.4 (d, C4′), 72.8 (C3′), 71.4 (C2′), 64.4 (d, C5′), 20.6 (OAc), 20.3 (OAc). ^{31}P NMR (162 MHz, D_2O, ^{1}H-decoupled) δ 3.92. IR (neat; cm^{-1}) 1742, 1642, 1484, 1213. HRMS (ESI) (*m/z*): [M+H$^+$] calcd. for formula $C_{13}H_{19}N_3O_{10}P$, 408.0803; found, 408.0806.

2′,3′-Di-*O*-acetyl-β-cytidine-5′-phosphate (β-*ribo*-**7**)

Starting from β-*ribo*-**4** (free acid, 160 mg, 0.50 mmol), yield = 172 mg (85%) as a white powder. ^{1}H NMR (600 MHz, D_2O) δ 8.08 (d, *J* = 7.9 Hz, 1H, (C6)-H), 6.22 (d, *J* = 7.9 Hz, 1H, (C5)-H), 6.11 (d, *J* = 5.1 Hz, 1H, (C1′)-H), 5.41 (dd, *J* = 5.4, 5.1 Hz, 1H, (C2′)-H), 5.38 (dd, *J* = 5.4, 4.3 Hz, 1H, (C3′)-H), 4.48 (ddd, *J* = 4.9, 4.3, 2.4 Hz, 1H, (C4′)-H),

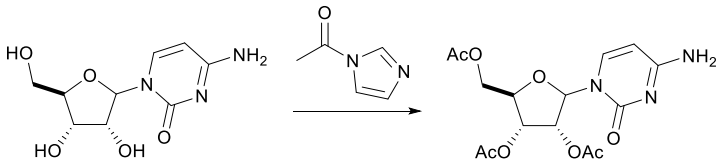
4.13 (ABXY, J = 11.9, 4.4, 2.4 Hz, 1H, (C5′)-H), 4.02 (ABXY, J = 11.9, 4.9, 2.4 Hz, 1H, (C5″)-H), 2.09 (s, 3H, (C3′)-OAc), 2.05 (s, 3H, (C2′)-OAc). ^{13}C NMR (151 MHz, D_2O) δ 173.4 (3′-OAc), 173.1 (2′-OAc), 160.7 (C4), 150.1 (C2), 144.3 (C6), 96.5 (C5), 88.2 (C1′), 82.5 (d, C4′), 74.5 (C2′), 71.5 (C3′), 64.4 (d, C5′), 20.6 (3′-OAc), 20.5 (2′-OAc). ^{31}P NMR (162 MHz, D_2O, ^{1}H-decoupled) δ 0.30. HRMS (ESI) (*m/z*): [M+H$^+$] calcd. for formula $C_{13}H_{19}N_3O_{10}P$, 408.0803; found, 408.0810.

The spectroscopic properties of this compound were consistent with the data reported in the literature.[3]

2′,3′-Di-*O*-acetyl-β-arabinocytidine-5′-phosphate (β-*arabino*-**7**)

Starting from β-*arabino*-**4** (free acid, 120 mg, 0.37 mmol), yield = 99 mg (66%) as a white solid. $[\alpha]_D^{20}$ = +82.8 (c 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 7.83 (d, J = 7.6 Hz, 1H, (C6)-H), 6.28 (d, J = 4.4 Hz, 1H, (C1′)-H), 5.99 (d, J = 7.6 Hz, 1H, (C5)-H), 5.42 (dd, J = 4.4, 2.2 Hz, 1H, (C2′)-H), 5.21 (dd, J = 3.5, 2.2 Hz, 1H, (C3′)-H), 4.30 - 4.36 (m, 1H, (C4′)-H), 4.07–4.13 (m, 1H, (C5′)-H), 4.00–4.07 (m, 1H, (C5″)-H), 2.08 (s, 3H, (C3′)-OAc), 1.92 (s, 3H, (C2′)-OAc). ^{13}C NMR (151 MHz, D_2O) δ 173.5 (3′-OAc), 172.4 (2′-OAc), 166.5 (C4), 157.2 (C2), 143.1 (C6), 96.2 (C5), 85.7 (C1′), 81.5 (d, C4′), 76.7 (C3′), 75.3 (C2′), 64.4 (d, C5′), 20.9 (3′-OAc), 20.4 (2′-OAc). ^{31}P NMR (162 MHz, D_2O, ^{1}H-decoupled) δ 0.68. IR (neat, cm^{-1}) 1745, 1650, 1485, 1429, 1373, 1233, 1054. HRMS (ESI) (*m/z*): [M+H$^+$] calcd. for formula $C_{13}H_{19}N_3O_{10}P$, 408.0803; found, 408.0810.

Preparative synthesis of O-acetylated nucleosides in water



α-*ribo*-**10**
β-*ribo*-**10**

α-*ribo*-**11**
β-*ribo*-**11**

Nucleosides (100mM) and *N*-acetyl imidazole (10 eq.) were dissolved in water and the solution was adjusted to pH 8 by addition of 4M NaOH. The reaction mixture was left standing for 4 h at r.t., and then lyophilized. The residue was purified by C18 column (H_2O/MeOH 98:2 to 0:100). The fractions with product were lyophilized to obtain white powders.

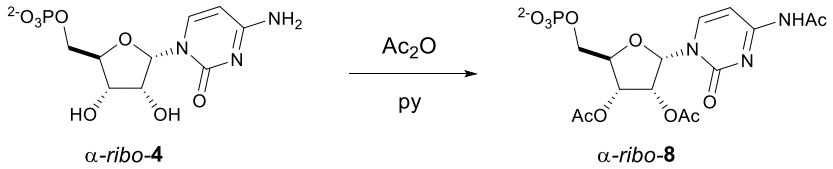
2',3',5'-Tris-*O*-acetyl-α-cytidine (α-*ribo*-**11**)

Starting from α-*ribo*-**10** (240 mg, 1.0 mmol), yield = 236 mg (65%) as a white powder. $[\alpha]_D^{20}$= -39.8 (c 1.0, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 7.73 (d, *J* = 7.6 Hz, 1H, (C6)-H), 6.33 (d, *J* = 5.0 Hz, 1H, (C1′)-H), 6.00 (d, *J* = 7.6 Hz, 1H, (C5)-H), 5.69 (t, *J* = 5.0 Hz, 1H, (C2′)-H), 5.42 (t, *J* = 5.0 Hz, 1H, (C3′)-H), 4.71 - 4.78 (m, 1H, (C4′)-H, *partially obscure by HOD signal*), 4.33 (ABX, *J* = 12.3, 3.0 Hz, 1H, (C5′)-H), 4.21 (ABX, *J* = 12.3, 4.4 Hz, 1H, (C5″)-H), 2.09 (s, 3H, (C5′)-OAc), 2.01 (s, 3H, (C3′)-OAc), 1.93 (s, 3H, (C2′)-OAc). ^{13}C NMR (151 MHz, D_2O) δ 174.4 (5′-OAc), 173.1 (3′-OAc), 172.4 (2′-OAc), 166.6 (C4), 157.5 (C2), 142.6 (C6), 95. 9 (C5), 85.7 (C1′), 80.6 (C4′), 71. 8 (C3′), 71.2 (C2′), 64.2 (C5′), 20.8 (OAc), 20.5 (OAc), 20.2 (OAc).

2',3',5'-Tris-*O*-acetyl-β-cytidine (β-*ribo*-**11**)

Starting from α-*ribo*-**10** (120 mg, 0.5 mmol), yield = 141 mg (77%) as a white powder. ^{1}H NMR (600 MHz, D_2O) δ 7.59 (d, *J* = 7.5 Hz, 1H, (C6)-H), 5.97 (d, *J* = 7.5 Hz, 1H, (C5)-H), 5.93 (d, *J* = 4.5 Hz, 1H, (C1′)-H), 5.43 (dd, *J* = 5.7, 4.5 Hz, 1H, (C2′)-H), 5.35 (dd, *J* = 5.9, 5.7 Hz, 1H, (C3′)-H), 4.41–4.47 (m, 1H, (C4′)-H), 4.35 (ABX, *J* = 12.5, 2.9 Hz, 1H, (C5′)-H), 4.30 (ABX, *J* = 12.5, 4.4 Hz, 1H, (C5″)-H), 2.08 (s, 3H, (C3′)-OAc), 2.07 (s, 3H, (C5′)-OAc), 2.06 (s, 3H, (C2′)-OAc). ^{13}C NMR (151 MHz, D_2O) δ 174.3 (OAc), 173.3 (OAc), 173.2 (OAc), 166.9 (C4), 157.7 (C2), 142.6 (C6), 97.1 (C5), 90.0 (C1′), 79.9 (C4′), 74.2 (C2′), 70.9 (C3′), 63.8 (C5′), 20.8 (OAc), 20.6 (OAc), 20.5 (OAc). *The spectroscopic properties of this compound were consistent with the data reported in the literature.*[4]

*Peracetylation of α-cytidine-5′-phosphate (α-ribo-**4**)*



α-*ribo*-**4** α-*ribo*-**8**

α-Cytidine-5′-phosphate (α-*ribo*-**4**, 300 mg, 0.93 mmol) was dissolved in water (200 µL) and added dropwise to stirred pyridine/acetic anhydride (1:1, 10 mL) at r.t. After 3 d the solution was evaporated *in vacuo* by co-evaporation with toluene (10 mL). The residue was dissolved in pyridine/water (1:5, 12 mL), stirred for 2 h at r.t. and concentrated *in*

vacuo. The residue was purified by reverse phase (C18) column chromatography (100mM NH_4HCO_2 (pH 4)/MeCN 98:2 to 0:1) to afford N^4-acetyl-2',3'-di-*O*-acetyl-α-cytidine-5'-phosphate (α-*ribo*-**8**, 259 mg, 62%) as a white powder.

M. p. 174–179 °C (decomp.). ^{1}H NMR (600 MHz, D_2O) δ 8.21 (d, J = 7.6 Hz, 1H, (C6)-H), 7.33 (d, J = 7.6 Hz, 1H, (C5)-H), 6.39 (d, J = 5.5 Hz, 1H, (C1′)-H), 5.79 (dd, J = 5.5, 5.2 Hz, 1H, (C2′)-H), 5.46 (dd, J = 5.2, 3.6 Hz, 1H, (C3′)-H), 4.75–4.80 (m, 1H, (C4′)-H, *partially obscured by HOD signal*), 4.06 (ABXY, J = 11.7, 5.5, 3.0 Hz, 1H, (C5′)-H), 3.98 (ABXY, J = 11.7, 5.5, 3.4 Hz, 1H, (C5″)-H), 2.17 (s, 3H, NHAc); 1.97 (s, 3H, (C3′)-OAc), 1.87 (s, 3H, (C2′)-OAc). ^{13}C NMR (151 MHz, D_2O) δ 174.8 (NHAc), 173.1 (3′-OAc), 172.3 (2′-OAc), 163.5 (C4), 157.37 (C2), 146.9 (C6). 97.9 (C5), 86.5 (C1′), 83.3 (d, C4′), 72.4 (C3′), 71.2 (C2′), 65.2 (d, C5′), 24.7 (NHAc), 20.5 (3′-OAc), 20.2 (2′-OAc). ^{31}P NMR (162 MHz, D_2O, ^{1}H-decoupled) δ 0.43. IR (cm^{-1}) 1743, 1650, 1619, 1566, 1492, 1223. HRMS (ESI) (*m/z*): [M+H$^+$] calcd. for formula $C_{15}H_{21}N_3O_{11}P$, 450.0908; found, 450.0910.

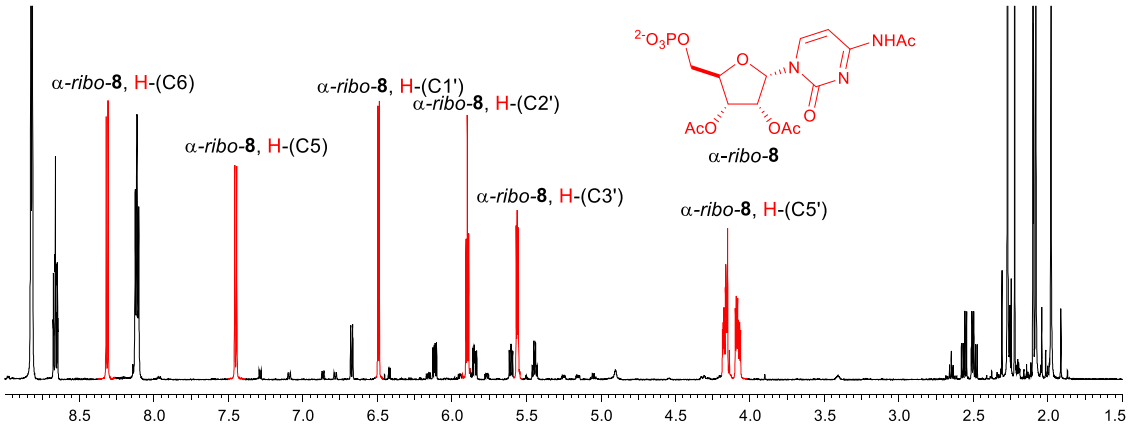


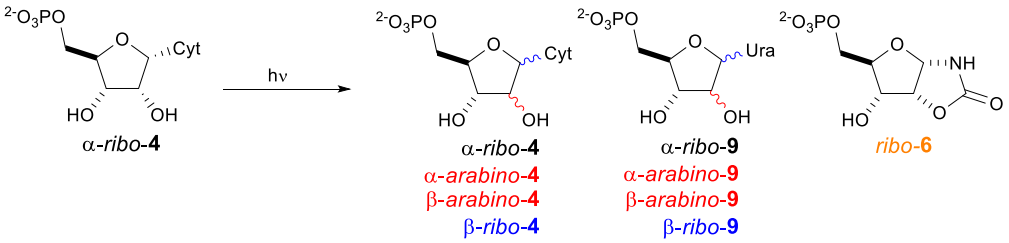
Figure S4: ^{1}H NMR spectrum (600 MHz, D_2O, 1.5 – 9.0 ppm) to show the crude products of the acetylation of α-*ribo*-**4** with acetic anhydride in pyridine after 3 d at r.t.

General procedure for UV irradiation

Nucleotides (2.5–5mM) were dissolved in water at pH 6.5, sparged with Ar or N_2 (1 h) and irradiated continuously at 254 nm. A flow of water (through a quartz water-jacket between the lamp and solution) maintained the solution temperature (25–35 ºC) and the solution was continuously agitated by continuous Ar or N_2 flow. After irradiation (λ=254 nm) the solution was allowed to stand at r.t. for 1 h and lyophilized. The lyophilite was dissolved in water and NMR spectra were acquired. The solution was then lyophilized. The lyophilite was dissolved in water (2 mL) and heated for 16 h at 90 °C to eliminate photohydrates.[2,5–10] The solution was then lyophilized, the lyophilite was dissolved in D_2O and analyzed by NMR in D_2O. In the case of the acetylated nucleotides, the residue was lyophilized again and subsequently treated with NH_4OH (28% in H_2O, 2 mL) for 16 h at r.t. Then, the reaction mixture was lyophilized and the residue analyzed by NMR. Yields were calculated by integration of H-(C6) for nucleotides, nucleosides and nucleobases and by integration of H-(C2') for oxazolidinones.

Irradiation of free nucleotides

Irradiation of α-ribocytidine-5'-phosphate (α-ribo-4)



Irradiation (λ=254 nm) of α-ribocytidine-5′-phosphate (α-*ribo*-**4**, 2.5mM) for 16 h affords a mixture: α-ribocytidine-5′-phosphate (α-*ribo*-**4**, 17%), β-ribocytidine-5′-phosphate (β-*ribo*-**4**, 5%), β-arabinocytidine-5′-phosphate (β-*arabino*-**4**, 5%), α-ribouridine-5′-phosphate (α-*ribo*-**9**, 9%), β-ribouridine-5′-phosphate (β-*ribo*-**9**, 5%), β-arabinouridine-5′-phosphate (β-*arabino*-**9**, 11%), ribose oxazolidinone-5'-phosphate (*ribo*-**6**, 22%), uracil (14%), cytosine (12%).

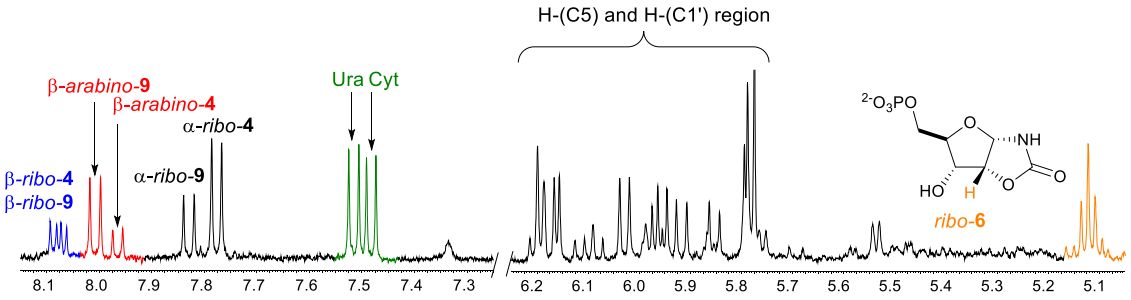


Figure S5. ^{1}H NMR spectrum (400 MHz, D_2O, 5.00–8.15 ppm) to show products of the irradiation (λ=254 nm) of α-ribocytidine-5′-phosphate (α-*ribo*-**4**, 2.5mM) for 16 h followed by heating at 90 °C to eliminate uridine photohydrates. Cyt=cytosine; Ura=uracil.

Irradiation of β-ribocytidine-5'-phosphate (β-ribo-4) and β-arabinocytidine-5'-phosphate (β-arabino-4).

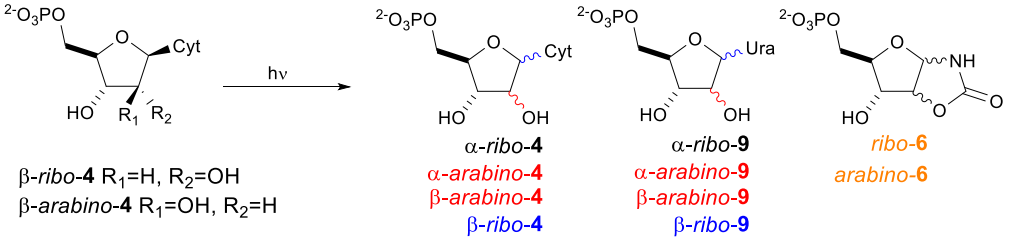


Table S1. Yields of products observed following the irradiation (λ=254 nm) of free nucleotides (conc. = 5mM).

Compound	t (h)	Yield of photoproducts (%)								
		β-*ribo*-**4**	β-*ribo*-**9**	β-*arabino*-**4**	β-*arabino*-**9**	α-*ribo*-**4**	α-*ribo*-**9**	Cyt + Ura	*ribo*-**6**	*arabino*-**6**
β-*ribo*-**4**	16	20	tr.	9	12	14	8	16	21	-
β-*arabino*-**4**	72	10	tr.	44	25	tr.[a]	tr. [a]	10	4	7
β-*arabino*-**4**	16	10	-	54	26	tr.[a]	tr. [a]	7	-	tr.

tr. = trace. [a] α-anomers (*ribo*- and *arabino*-).

Irradiation of acetylated nucleotides

Irradiation of 2',3'-di-O-acetyl α-ribocytidine-5'-phosphate (α-ribo-7)

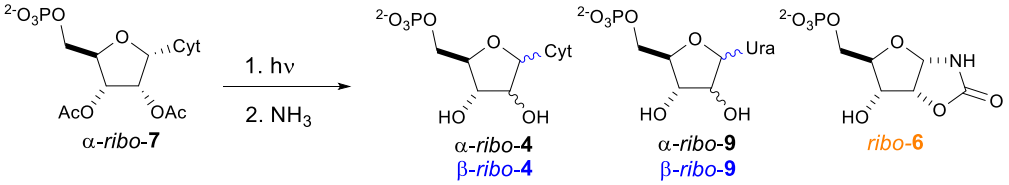


Table S2. Yields of products observed following the irradiation (λ=254 nm) of 2',3'-di-O-acetyl-α-cytidine-5'-phosphate (α-ribo-7) and deacetylation with aqueous ammonia.

Conc. (mM)	t (h)	pH	Yield of photoproducts (%)					
			β-*ribo*-**4**	β-*ribo*-**9**	α-*ribo*-**4**	α-*ribo*-**9**	Cyt + Ura	*ribo*-**6**
5	16	6.5	14	-	59	15	7	5
5	24	6.5	10	tr.	36	26	21	7
2.5	16	6.5	18	4	41	19	11	7
2.5	24	6.5	9	6	35	31	18	11
5	16	8.0	16	tr.	41	12	20	11
2.5	16	8.0	17	tr.	49	15	11	8

tr. = trace.

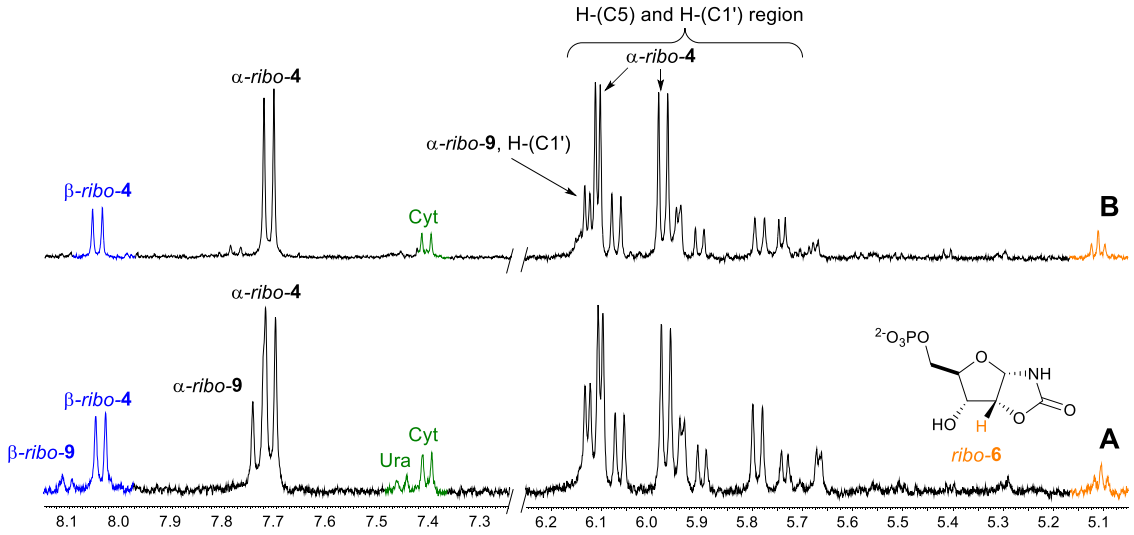


Figure S6. ^{1}H NMR spectrum (400 MHz, D_2O, 5.00–8.15 ppm) to show the products of 16 h irradiation (λ=254 nm) of 2',3'-di-*O*-acetyl-α-cytidine-5'-phosphate (α-*ribo*-**7**, 2.5mM) at: **A**) pH 6.5 and **B**) pH 8.0 followed by elimination of photohydrates and deacetylation with aqueous ammonia. Cyt=cytosine; Ura=uracil

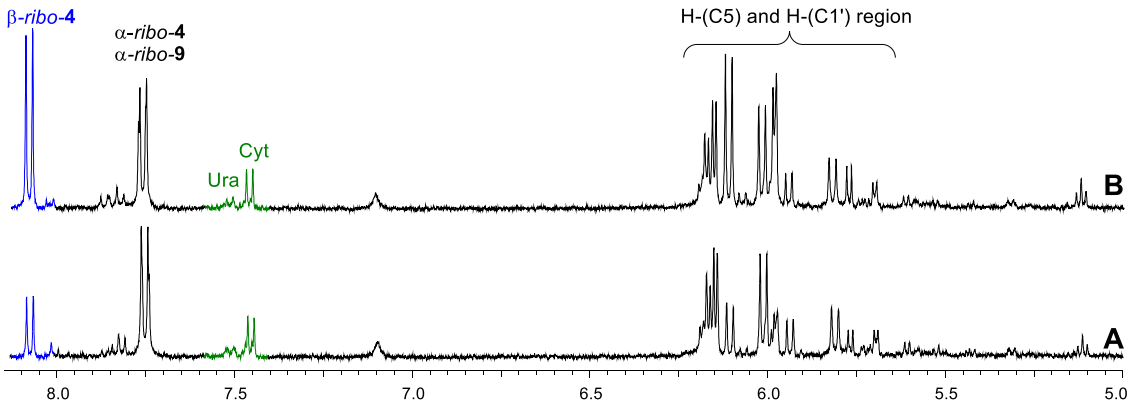


Figure S7. ^{1}H NMR spectra (400 MHz, D_2O, 5.00–8.15 ppm) to show the products of 16 h irradiation (λ=254 nm) of 2',3'-di-*O*-acetyl-α-cytidine-5'-phosphate (α-*ribo*-**7**, 2.5mM) at pH 6.5 after: **A**) elimination of photohydrates and deacetylation with aqueous ammonia; **B**) spiking with β-*ribo*-**4**.

*Acetylation of α-ribo-**4** followed by irradiation in one-pot protocol*

Nucleotide α-*ribo*-**4** (100mM) and *N*-acetyl imidazole (10 eq.) were dissolved in water and the solution was adjusted to pH 8.0 by addition of 4M NaOH. The reaction mixture was left standing for 4 h at r.t., and then diluted to 2.5mM and readjusted to pH 8.0 by addition of 4M NaOH. The resulting solution was sparged with Ar (1 h) and irradiated for 16 h at 254 nm as described above.

Table S3. Yields of products observed following the acetylation then irradiation (λ=254 nm) of 2',3'-di-O-acetyl-α-cytidine-5'-phosphate (α-ribo-7, 2.5mM) for 16 h and deacetylation with aqueous ammonia.

Conc. (mM)	pH	Yield of photoproducts (%)					
		β-*ribo*-**4**	β-*ribo*-**9**	α-*ribo*-**4**	α-*ribo*-**9**	Cyt + Ura	*ribo*-**6**
2.5	8.0	11	tr.	57	10	13	9

tr. = trace.

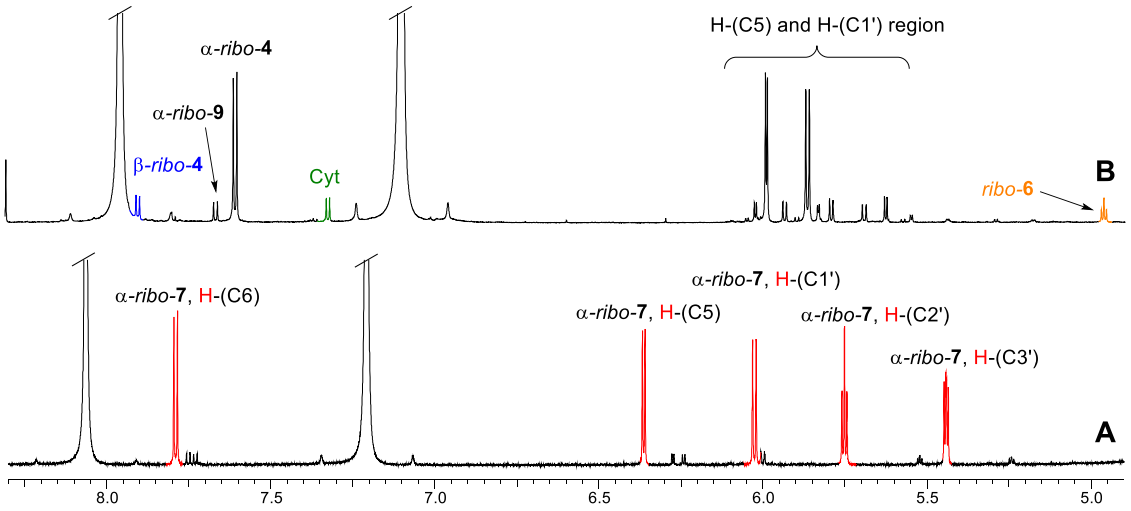


Figure S8. ^{1}H NMR spectrum (700 MHz, D_2O, 4.90–8.30 ppm) to show the products of: **A**) acetylation of α-*ribo*-**4** (100mM) with *N*-acetyl imidazole (10 eq.) after 4 h in H_2O at pH 8 (yield α-*ribo*-**7**: 87%, the rest are partially *O*-acetylated products); **B**) 16 h irradiation (λ=254 nm) of the dilute crude mixture (α-*ribo*-**7**, 2.5mM) at pH 8.0 followed by elimination of photohydrates and deacetylation with aqueous ammonia. Cyt=cytosine.

Role of 5'-phosphate in the irradiation of α-ribo-7: Irradiation of acetylated α/β cytidines with and without phosphate buffer

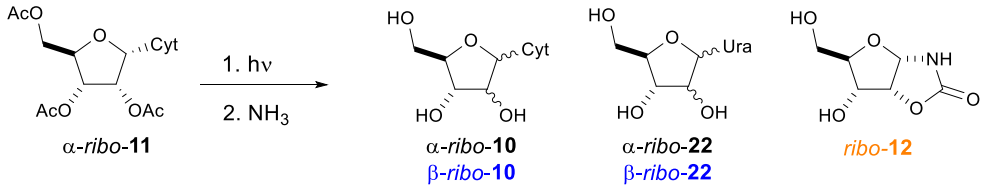


*Table S4. Yields of products observed following the irradiation (λ=254 nm) of 2',3',5'-tri-O-acetyl-α-cytidine (α-ribo-**11**) or 2',3',5'-tri-O-acetyl-β-cytidine (β-ribo-**11**) and deacetylation with aqueous ammonia. Cyt = cytidine, Ura = uridine.*

Compound	Conc. (mM)	t (h)	Yield of photoproducts (%)					
			β-*ribo*-**10**	β-*ribo*-**22**	α-*ribo*-**10**	α-*ribo*-**22**	Cyt + Ura	*ribo*-**12**
α-*ribo*-**11**	10	16	2	0	74	17	2	5
α-*ribo*-**11**	10	72	0	9	tr.	42	16	33
α-*ribo*-**11** + P_i[a]	10	72	0	0	69	22	7	2
β-*ribo*-**11**	10	16	57[b]	28[b]	2	0	3	tr.

tr. = trace. [a] 50 eq. of phosphate buffer. [b] alongside *arabino*-cytidine and *arabino*-uridine (10% yield of *arabino*-nucleosides).

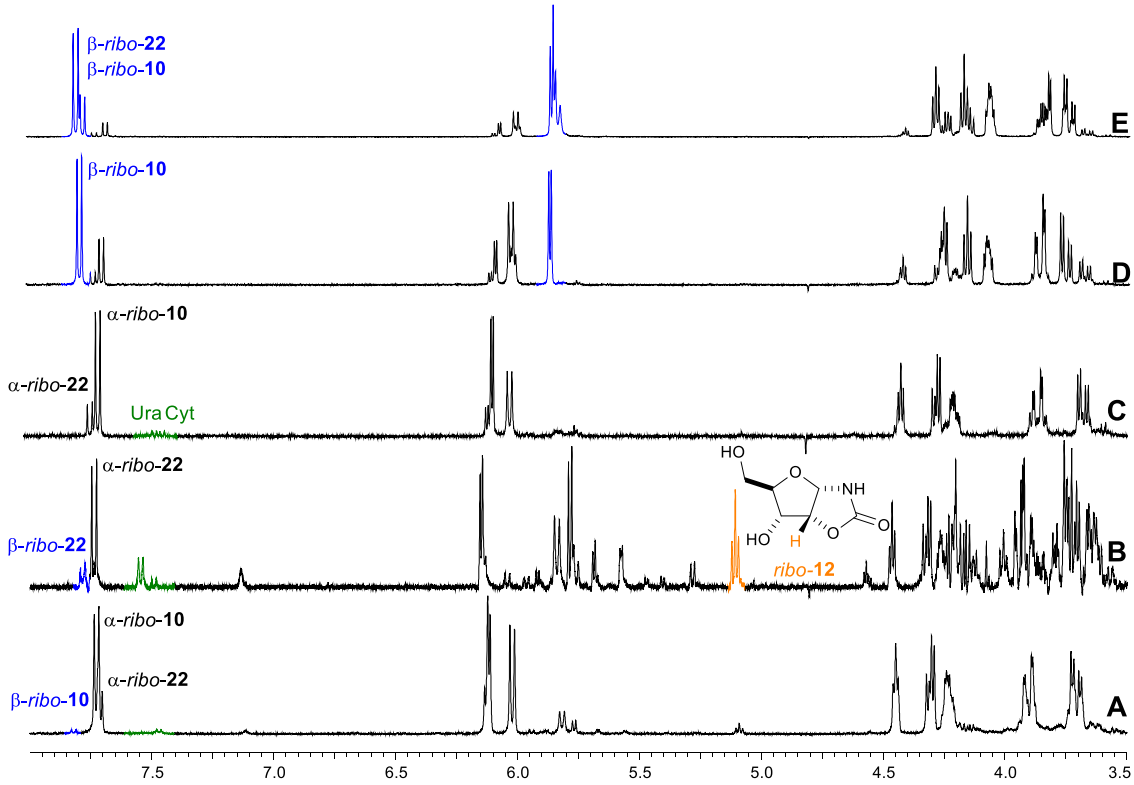


Figure S9: ^{1}H NMR spectra (400 MHz, D_2O, 3.50–8.00 ppm) to show the irradiation (λ=254 nm) of α-cytidine-2',3',5'-tris-*O*Ac (α-*ribo*-**11**) for: **A**) 16 h followed by elimination of photohydrates and deacetylation with aqueous ammonia; **B**) 72 h followed by elimination of photohydrates and deacetylation with aqueous ammonia; **C**) 72 h in the presence of phosphate buffer followed by elimination of photohydrates and deacetylation with aqueous ammonia. **D**) Spiking of spectrum **C** with β-*ribo*-**10**. **E**) Spiking of spectrum D with β-*ribo*-**22**.

*Irradiation (λ=254 nm) of N^4-acetyl-2',3'-di-O-acetyl-α-cytidine-5'-phosphate (α-ribo-**8**).*

N-Acetylation was not observed under aqueous conditions, however to study the effect of pyrimidine *N*-acetylation on pyrimidine photoanomerization, solutions of α/β-*ribo*-**8** were irradiated at 10mM concentration.

*Table S5. Yields of products observed following the irradiation (λ=245 nm) of peracetylated nucleotides (ribo-**8**) and deacetylation with aqueous ammonia.*

Compound	Conc. (mM)	t (h)	Yield of photoproducts (%)					
			β-*ribo*-**4**	β-*ribo*-**9**	α-*ribo*-**4**	α-*ribo*-**9**	Cyt + Ura	*rido*-**6**
α-*ribo*-**8**	10	16	tr.	tr.	24	51	19	6
α-*ribo*-**8**	10	72	-	9	18	9	61	3
β-*ribo*-**8**	10	16	31	46	8	11	4	-

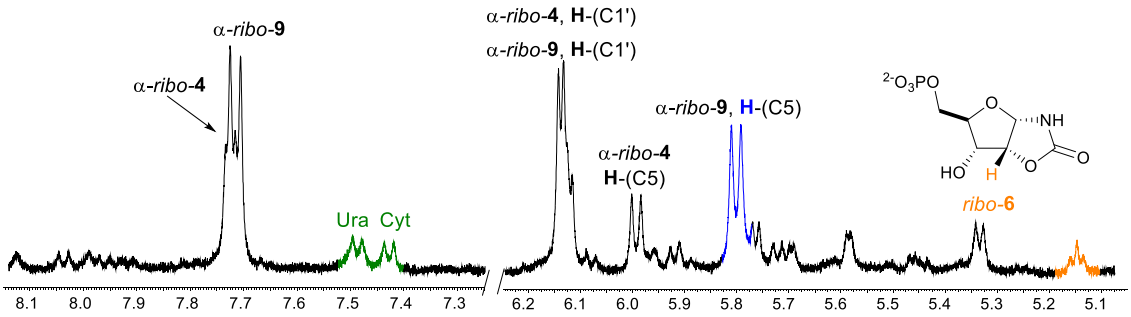


Figure S10. ^{1}H NMR spectrum (400 MHz, D_2O, 5.00–8.15 ppm) to show the products of 16 h irradiation (λ=254 nm) of N^4-acetyl-2',3'-di-*O*-acetyl-α-cytidine-5'-phosphate (α-*ribo*-**8**; 10mM), after elimination of photohydrates and deacetylation with aqueous ammonia.

2′,3′-Di-*O*-acetyl-α-cytidine-5′-phosphate (α-*ribo*-**7**)

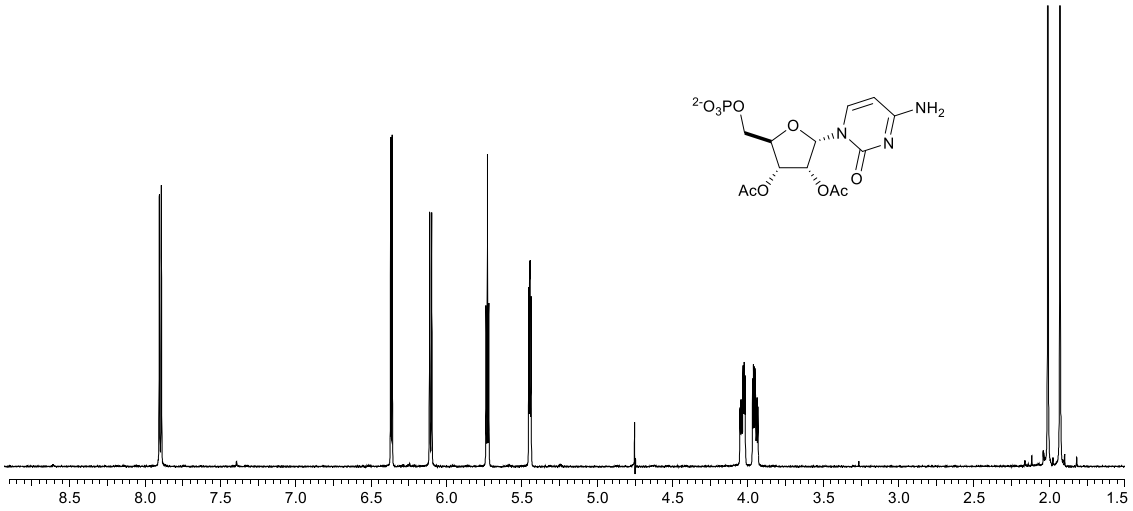


Figure S11: ^{1}H NMR spectrum (600 MHz, D_2O, 1.5–9.0 ppm) to show α-*ribo*-**7**.

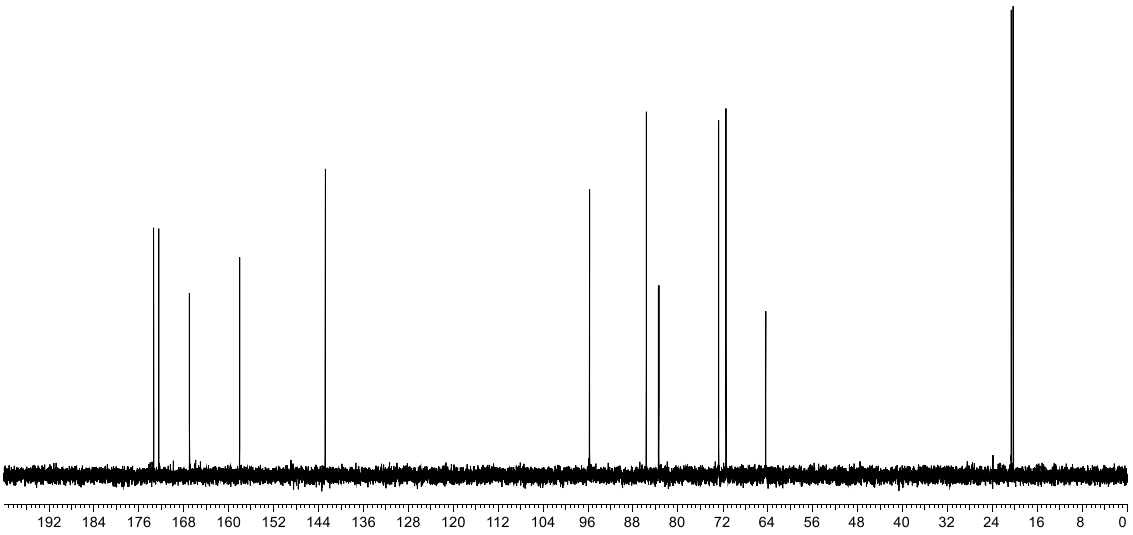


Figure S12: ^{13}C NMR spectrum (600 MHz, D_2O, 0–200 ppm) to show α-*ribo*-**7**.

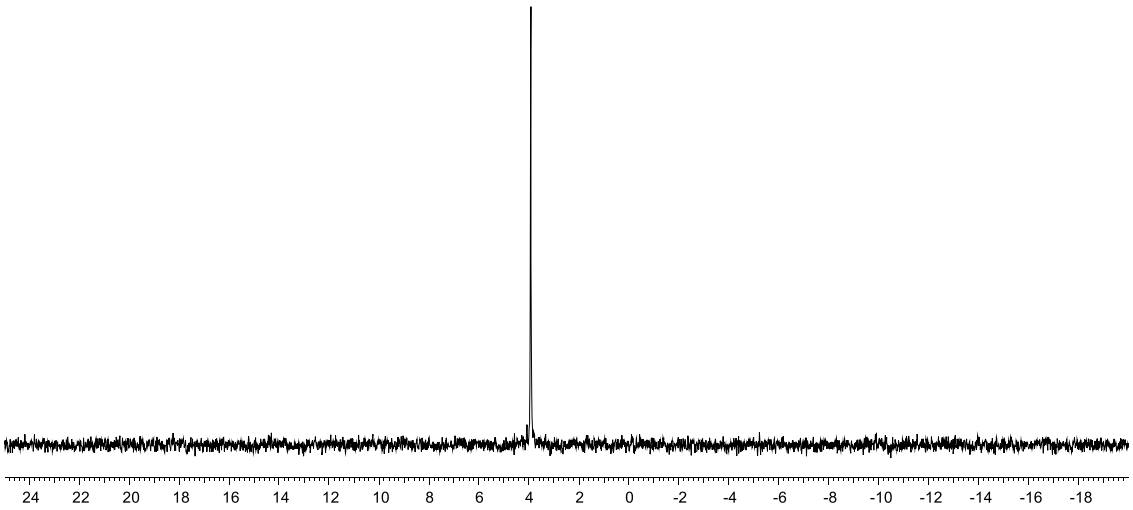


Figure 13: ^{31}P NMR spectrum (162 MHz, D_2O, -20–25 ppm) to show α-*ribo*-**7**.

2',3',5'-tris-*O*-acetyl-α-cytidine (α-*ribo*-**11**)

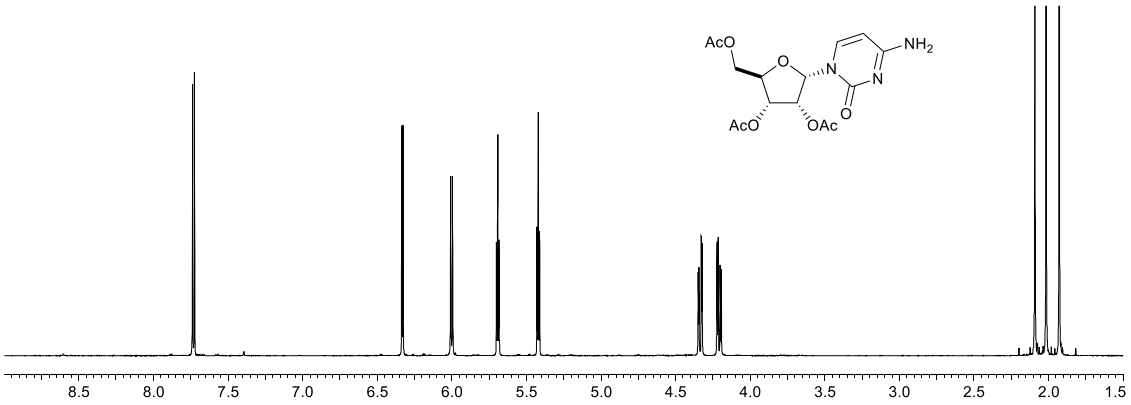


Figure S14: ^{1}H NMR spectrum (600 MHz, D_2O, 1.5–9.0 ppm) to show α-*ribo*-**11**.

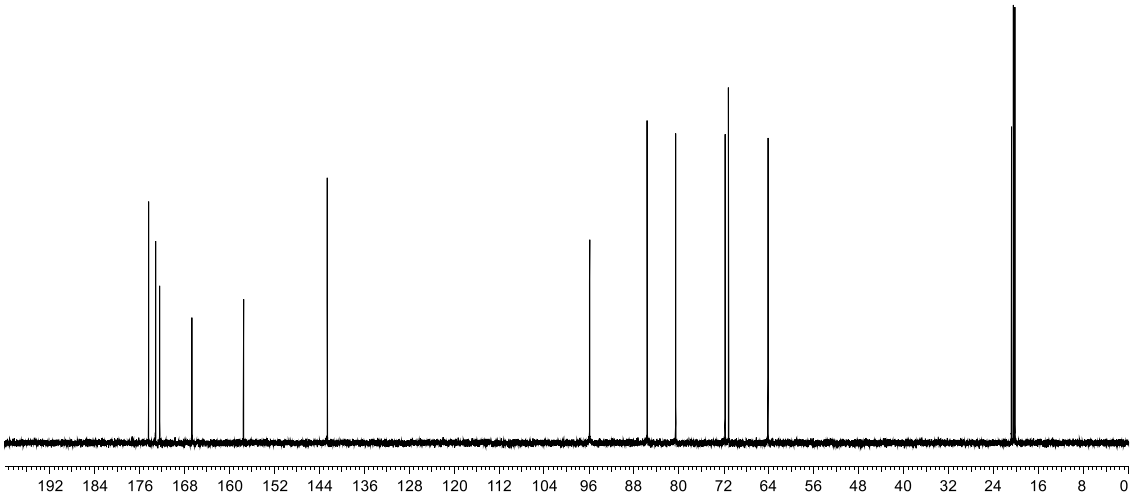


Figure S15: ^{13}C NMR spectrum (600 MHz, D_2O, 0–200 ppm) to show α-*ribo*-**11**.

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