

Supporting information

An improved method for the synthesis of nucleoside triphosphate analogues

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General Methods.

All reactions, unless otherwise specified, were performed under an inert atmosphere of nitrogen. All chemicals were purchased commercially and used without further purification unless otherwise specified. Dry acetonitrile was stored over 4 Å activated molecular sieves. Thin

layer chromatography was performed on 0.20 mm thick aluminium-backed silica gel plates. Components were visualized by spraying with potassium permanganate solution (3.00 g KMnO_4 , 20.00 g K_2CO_3 , 5 ml 5 % aqueous NaOH , 300 mL distilled water) or ultraviolet light ($\lambda=254$ nm). Flash chromatography was performed using 230-400 mesh Ultra Pure Silica Gel 60. ^1H and ^{13}C NMR spectra were recorded in deuterated chloroform and deuterium oxide at 25°C in 5 mm NMR tubes on a 500 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to the trimethylsilane (TMS) internal standard at 0 ppm for deuterated chloroform, while ^1H NMR spectra in deuterium oxide were referenced to the solvent peak (4.7 ppm). Proton decoupled ^{31}P spectra were referenced relative to external aqueous 85 % H_3PO_4 at a spectrometer operating frequency of 101.260 MHz (AC-250) and 202.466 MHz (AV-500). Proton decoupled ^{19}F spectra were referenced relative to external 0.5 % $\text{CF}_3\text{C}_6\text{H}_5$ in CDCl_3 (-63.7 ppm), at a spectrometer operating frequency of 235.36 MHz. proton decoupled ^{13}C spectra were recorded at a spectrometer operating frequency of 125.769 MHz. US spectra were recorded on a spectrophotometer at 25°C in water, using quartz cuvettes with a 1 cm pathlength. The molar concentrations of adenine-containing compounds in a given solution were determined from absorbance measurements at 259 nm (pH 7) using a molar extinction coefficient of $15.4 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ (*i.e.*, that reported for AMP, ADP, and ATP).²³ The molar concentrations of uridine-containing compounds in a given solution were determined from absorbance measurements at 262 nm (pH 7), using a molar extinction coefficient of $10 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.²⁴ Automated flash column chromatography purifications were performed on a high performance flash chromatography system using C18 cartridges, eluting with a linear gradient (0-100 %, 30 CV) of buffer A (water / 10 mM tributylamine / 30 mM acetic acid) and buffer B (methanol / 10 mM

tributylamine). The flow rate was 25 mLmin⁻¹. The purification was monitored with an UV detector at two wavelengths, $\lambda = 215$ nm, $\lambda = 254$ nm. Fractions were collected every 9 mL.

Preparation of the activated AMP-*N*-methylimidazolid, Method A

5'-AMP free acid monohydrate (100 mg, 0.27 mmol) was suspended in a mixture of acetonitrile (1 mL) and triethylamine (348 mg, 480 μ L, 3.44 mmol), cooled to 0°C in an ice-bath and stirred under nitrogen. In a separate flask, a solution of trifluoroacetic anhydride (713 mg, 480 μ L, 3.4 mmol) in acetonitrile (300 μ L) was cooled to 0°C and added drop-wise by a syringe to the flask containing 5'-AMP. The reaction was allowed to stir for 10 min at room temperature, whereby a pale orange solution was obtained. Excess trifluoroacetic anhydride and the *in situ*-produced trifluoroacetic acid were then removed *in vacuo*. The reaction mixture was then cooled to 0°C in an ice-bath. In a separate flask, a mixture of *N*-methylimidazole (74 mg, 72 μ L, 0.9 mmol) in acetonitrile (300 μ L), and triethylamine (229 mg, 315 μ L, 2.26 mmol) was prepared, cooled to 0°C in an ice-bath, and then added to the flask containing the trifluoroacyl phosphate. The reaction was allowed to stir for 10 min at 0°C, after which time a bright yellow solution was obtained. Preparation of the activated AMP-*N*-methylimidazolid was confirmed by TLC (10:10:1 CHCl₃/MeOH/ 1 M ammonium acetate pH 7, R_f 0.51), and δ_P -10.24 (s).¹⁶⁻¹⁷

Method B

The activated AMP-*N*-methylimidazolid was prepared according to Method A, using 5'AMP monosodium salt dihydrate (111 mg, 0.27 mmol). Identical spectroscopic data for AMP-*N*-methylimidazolid were obtained.

Preparation of the activated UMP-*N*-methylimidazolid

The activated UMP-*N*-methylimidazolid was prepared according to Method A, using 5'-UMP disodium salt dihydrate (110 mg, 0.27 mmol). Preparation of the activated UMP-*N*-

methylimidazolide was confirmed by TLC (10:10:1 CHCl₃/MeOH/ 1 M ammonium acetate pH 7, R_F 0.45), and δ_P -10.35 (s)).¹⁷

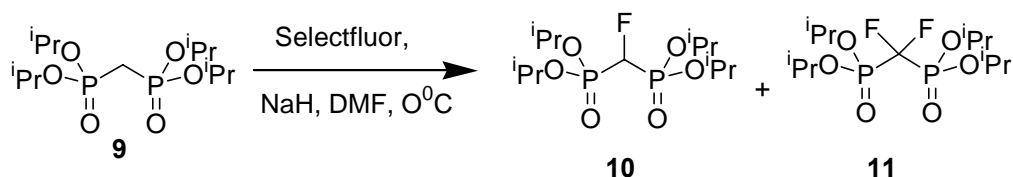
Tetraisopropyl monofluoromethylenediphosphonate (10), Method C

Tetraisopropyl methylenediphosphonate (**9**) (5.00 g, 14.5 mmol) in DMF (40 mL) was cooled to 0°C in an ice-bath. In a separate flask NaH (0.38 g, 16 mmol) was suspended in DMF (20 mL), cooled to 0°C for 5 minutes, and added drop-wise by cannula to the flask containing **9**. The mixture was stirred 0°C for a further 10 min, then at room temperature for one hour, then cooled to 0°C for 10 min. In a separate flask, selectfluor® (1-chloromethyl-4-fluoro-1,4-diazabicyclo[2.2.2]octane bis(tetrafluoroborate) (5.14 g, 14.5 mmol) was dissolved in DMF (20 mL), cooled in an ice-bath for 15 min, and added all at once by a cannula to the flask containing the anion of **9**. The reaction mixture was stirred at 0°C for 10 min, and then at room temperature for 1 hr. The reaction progress was monitored by taking 0.6 mL aliquots for ³¹P NMR spectroscopy [**9** 19.38; **10** 11.04, (d), **11** 3.21, (t)], when a ratio of 1:2:0.45 was observed, the reaction mixture was cooled in an ice-bath for 10 min, diluted with CH₂Cl₂ (100 mL) and quenched by the addition of saturated aqueous ammonium chloride (50 mL). The bottom layer was removed and the upper layer was extracted with CH₂Cl₂ (2 x 50 mL). The combined organic extracts were dried over anhydrous sodium sulfate, then concentrated to a syrup, which upon purification by repeated silica gel column chromatography (1 % MeOH in EtOAc) afforded 3.05 g of **10** (58 %) and 0.72 g of **11** (13 %). δ_P **10** 11.04 (d, ²J_{P,F} 61.46 Hz); **11** 3.21 (t, ²J_{P,F} 85.67 Hz).²²

Method D

The reaction was completed according to Method C, using **9** (5 g, 14.5 mmol) and selectfluor® (7.71 g, 21.78 mmol). After 1 hr, ³¹P NMR spectroscopy showed 50 %, and 31 % conversion of

9 to **10** and **11**, respectively. The workup was completed according to Method C and afforded 2.7 g of **10** (50 %) and 1.71 g of **11** (31 %).



Methylenediphosphonic acid (**12**)

This reaction was not conducted under nitrogen. Tetraisopropyl methylenediphosphonate **9** (5.00 g, 14.5 mmol) in aqueous HCl (100 mL, 6 M) was refluxed for 24 hours, after which time ^{31}P NMR spectroscopy indicated complete hydrolysis of **9** (19.4) into **12** (16.7). The mixture was concentrated *in vacuo*. Methanol (5 x 10 mL) was added, and the volatiles were then removed *in vacuo*, to yield, after drying, 2.50 g (100 %). δ_{P} (D_2O) 16.7.²⁷

Monofluoromethylenediphosphonic acid (**13**)

This reaction was not conducted under nitrogen. Tetraisopropyl monofluoromethylenediphosphonate (**10**) (2.80 g, 7.36 mmol) in aqueous HCl (100 mL, 6 M) was refluxed for 24 hours, after which time ^{31}P NMR spectroscopy indicated complete hydrolysis of the starting ester **10** (11.04 (d, $^2J_{\text{P,F}}$ 61.46 Hz)) into **12** (13.06 (d $^2J_{\text{P,F}}$ 65.1 Hz)), the mixture was concentrated *in vacuo*. Methanol (5 x 10 mL) was added, and the volatiles removed were then removed *in vacuo*, to yield, after drying, 1.43 g (100 %). δ_{P} (D_2O) 12.83 (d, $^2J_{\text{P,F}}$ 65.1 Hz); δ_{C} (D_2O) 86.16 (dt, $-\underline{\text{C}}\text{HF}-$, $J_{\text{C,F}}$ 179.4 Hz, $J_{\text{C,P}}$ 146.9 Hz), negative ion ESI-MS, m/z 192.9 $[\text{M-H}]^-$.²⁶

Difluoromethylenediphosphonic acid (**14**)

This reaction was not conducted under nitrogen. The title compound was prepared by following the same procedure as for compound **12**. Tetraisopropyl

difluoromethylenediphosphonate **11** (0.60 g, 1.58 mmol), furnished 0.33 g of **14** (100 %). δ_P (D_2O) 6.711 (t, $^2J_{P,F}$ 85.67 Hz).²⁶

Tris(tetra-*n*-butylammonium) hydrogen methylenediphosphonate (15)

A solution of **12** (1.00 g, 5.68 mmol) in water (20 mL) was titrated to pH 10.0 with aqueous tetra-*n*-butylammonium hydroxide (30 % w/w). The resulting solution was lyophilized to yield 4.97 g (97 %). δ_P (D_2O) 16.67.²⁵

Tris(tetra-*n*-butylammonium) monofluoromethylenediphosphonate (16)

A solution of **13** (1.00 g, 5.16 mmol) in water (20 mL) was titrated to pH 8.0 with aqueous tetra-*n*-butylammonium hydroxide (30 % w/w). The resulting solution was lyophilized to yield 4.45 g (94 %). δ_P (D_2O) 10.87 (d, $^2J_{P,F}$ 59.8 Hz), δ_F (D_2O) -217.38 (dt, $^2J_{F,P\beta}$ 117.7 Hz, $^2J_{F,P\gamma}$ 47.1 Hz).²⁵

Tris(tetra-*n*-butylammonium) difluoromethylenediphosphonate (17)

A solution of **14** (1.00 g, 4.72 mmol) in water (20 mL) was titrated to pH 7.3 with aqueous tetra-*n*-butylammonium hydroxide (30 % w/w). The resulting solution was lyophilized to yield 4.2 g (95 %). δ_P (D_2O) 6.1 (t, $^2J_{P,F}$ 81 Hz), δ_F (D_2O) -120.45 (t, $^2J_{F,P}$ 79.1 Hz).²⁵

Adenosine 5'- β,γ -methylenetriphosphate, AppCH₂p (1), Method E

A solution of AMP-*N*-methylimidazolidine was prepared according to Method A, and was then added drop-wise by a syringe to a flask containing **15** (492 mg, 0.55 mmol) and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. The reaction was stirred at 0°C under nitrogen. Reaction progress was monitored by TLC (4:4:1 EtOH/NH₃/H₂O) and by ³¹P NMR spectroscopy by removing 100 µL aliquots of the reaction mixture every 5 min and quenching with 600 µL of 250 mM aqueous ammonium acetate, pH 7 in an NMR tube [AMP 1.73; AppCH₂p -10.89 (d), 8.96 (dd), 14.6 (d); **15** 16.76]. The reaction was complete after 10 min, and was then quenched

with cold aqueous ammonium acetate (3 mL, 250 mM, pH 7). The resultant solution was then washed with chloroform (3 x 5 mL), freeze dried, diluted with buffer A (5 mL) and purified by reversed-phase automated flash column chromatography using a linear gradient of buffer A and buffer B (0-100 %, 30 CV). The appropriate fractions were pooled and adjusted to pH 7 using dilute ammonia solution, the volume was adjusted with water to 500 mL in a volumetric flask, from which 1 mL was diluted with water to 10 mL and the absorbance reading of the resultant solution was measured at $\lambda_{\text{max}} = 259$ nm, and found to be 0.732, yield 87 %. The solution was concentrated, lyophilized and then dissolved in water (10 mL) and passed through an Amberlite IR-120 H^+ column, the fractions collected from the column were monitored by measuring the UV activity at $\lambda_{\text{max}} = 259$ nm. The fractions possessing UV activity were combined and the pH was adjusted to 9.5 by dilute ammonia solution and lyophilized. The product was then dissolved in water (0.5 mL) and precipitated by the drop-wise addition of ethanol (3 mL) where it was maintained at -10°C for 20 min, and then centrifuged for 10 min at 4°C . The aqueous ethanolic solution was decanted out and the residue dried under vacuum. A few drops of water were added to dissolve the residue which was lyophilized again to yield 121 mg of the diammonium salt (82 %). δ_{P} (D_2O) -10.89 (d, P_α , $^2J_{\alpha\beta}$ 25.9 Hz), 8.96 (dd, P_β , $^2J_{\alpha\beta}$ 25.9 Hz, $^2J_{\beta\gamma}$ 8.5 Hz), 14.6 (d, P_γ , $^2J_{\beta\gamma}$ 8.5 Hz); δ_{H} (D_2O , 500.133 MHz) 2.25 (t, 2H, $\text{CH}_{2\beta\gamma}$, $^2J_{\text{H,P}}$ 20.5 Hz), 4.19 (m, 2H, H_5'), 4.36 (br., 1H, H_4'), 4.53 (dd, 1H, H_3' , $J_{3',2'}$ 4.5 Hz, $J_{3',4'}$ 4 Hz) 6.1 (d, 1H, H_1' , $J_{1',2'}$ 6 Hz), 8.23 (s, 1H, H_2), 8.48 (s, 1H, H_8); δ_{C} (D_2O) 29.8 (dd, $\text{CH}_{2\beta\gamma}$, $J_{\text{C,P}\beta}$ 129.9 Hz, $J_{\text{C,P}\gamma}$ 122 Hz), 65.0 (d, C_5' , $J_{\text{C}_5',\text{P}\alpha}$ 3.4 Hz), 70.2 (C_3'), 74.2 (C_2'), 83.8 (d, C_4' , $J_{\text{C}_4',\text{P}\alpha}$ 8.3 Hz), 86.9 (C_1'), 118.1 (C_5), 139.5 (C_2), 148.4 (C_4), 152.4 (C_8), 155.0 (C_6). Negative ion ESI-MS, m/z 504 $[\text{M-H}]^-$.¹⁸

Method F

A solution of AMP-*N*-methylimidazolidine was prepared according to Method A, and was then added drop-wise by a syringe to a flask containing **12** (97 mg, 0.55 mmol), triethylamine (0.2 mL), and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. Reaction monitoring and workup proceeded according to Method E. After chromatography $Abs_{259} = 0.75$ (89 %). The yield of the purified diammonium salt was 125 mg (85 %).

Preparation of AppCH₂p by Method B

A solution of AMP-*N*-methylimidazolidine was prepared according to Method B, and was then added drop-wise by a syringe to a flask containing **15** (492 mg, 0.55 mmol), and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. Reaction monitoring and workup proceeded according to Method E. After chromatography $abs_{259} = 0.75$, yield 89 %. The yield of the purified diammonium salt was 127 mg, 86 %.

Adenosine 5'-β,γ-dichloromethylenetriphosphate, AppCCl₂p (2)

The title compound was prepared according to Method A, using tributylammonium dichloromethylenediphosphonate (350 mg, 0.815 mmol), triethylamine (0.2 mL), and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. Reaction progress was monitored by TLC (4:4:1 EtOH/NH₃/H₂O) and by ³¹P NMR spectroscopy by removing 100 μL aliquots of the reaction mixture every 15 minutes [AMP 1.73; AppCCl₂p -10.33 (d), 1.46 (dd), 8.4 (d), Chlodronic acid 10.57]. The reaction was complete after 40 min. Workup and chromatographic purification were performed according to Method E. $Abs_{259} = 0.78$, yield 92 %. The yield of the purified diammonium salt was 144 mg, 87 %, δ_P (D₂O) -10.80 (d, P_α, ²J_{αβ} 29.9 Hz), 0.62 (dd, P_β, ²J_{αβ} 29.9 Hz, ²J_{βγ} 18.6 Hz), 7.59 (d, P_γ, ²J_{βγ} 18.6 Hz); δ_H (D₂O) 4.14 (m, 2H, H_{5'}), 4.24 (br., 1H, H_{4'}), 4.44 (dd, 1H, H_{3'}, ³J_{3',2'} 4.5 Hz, ³J_{3',4'} 4 Hz), 5.94 (d, 1H, H_{1'}, ¹J_{1',2'} 5.5 Hz), 7.99 (s, 1H, H₂),

8.32 (s, 1H, H₈); δ_C (D₂O) 65.2 (d, C_{5'}, $J_{C5',P\alpha}$ 5.23 Hz), 70.2 (C_{3'}), 74.28 (C_{2'}), 79.1 (dd, CCl₂ $\beta\gamma$, $J_{C,P\beta}$ 146.1 Hz, $J_{C,P\gamma}$ 118.9 Hz), 83.8 (d, C_{4'}, $^3J_{C,P\alpha}$ 9.1 Hz), 86.8 (C_{1'}), 118.36 (C₅) 139.8 (C₂), 148.8 (C₄), 152.6 (C₈), 155.3 (C₆). Negative ion ESI-MS, m/z (^{35}Cl) 572 [M-H]⁻, isotopic peak (^{37}Cl) m/z 574.¹⁸

Adenosine 5'- β,γ -fluoromethylenetriphosphate, AppCHFp (3)

The title compound was prepared according to Method E, using **16** (503 mg, 0.55 mmol) and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. Reaction progress was monitored by TLC (4:4:1 EtOH /NH₃ /H₂O) and by ^{31}P NMR spectroscopy by removing 100 μL aliquots of the reaction mixture every 15 min [AMP 1.72; AppCHFp -10.43 (d), 4.96 (ddd), 7.4 (dd), **16**; 10.62 (d)]. The reaction was complete after 1 hr. Workup and chromatographic purification were performed according to Method E. Abs₂₅₉ = 0.64, yield 76 %. The yield of the purified diammonium salt was 110 mg, 72 %, δ_P (D₂O) -10.92 (d, P _{α} , $^2J_{\alpha\beta}$ 28 Hz), 1.39 (ddd, P _{β} , $^2J_{P\beta F}$ 62.8 Hz, $^2J_{\alpha\beta}$ 28, $^2J_{\beta\gamma}$ 15 Hz), 8.26 (dd, P _{γ} , $^2J_{P\gamma F}$ 59.8 Hz, $^2J_{\beta\gamma}$ 15 Hz); δ_H (D₂O, 500.133 MHz) 4.10 (m, 2H, H_{5'}), 4.26 (br., 1H, H_{4'}), 4.42 (dd, 1H, H_{3'}, $J_{3',2'}$ 4.5 Hz, $J_{3',4'}$ 3.5 Hz), 4.8 (ddd, CHF _{$\beta\gamma$} , $J_{H,F}$ 45.5 Hz, $^2J_{H,P\beta}$ 12.5 Hz, $^2J_{H,P\alpha}$ 12 Hz) 5.99 (d, 1H, H_{1'}, $J_{1',2'}$ 5.5 Hz), 8.08 (s, 1H, H₂), 8.36 (s, 1H, H₈). δ_C (D₂O) 65.1 (C_{5'}), 70.3 (C_{3'}), 74.3 (C_{2'}), 83.8 (C_{4'}), 86.8 (C_{1'}), 88.0 (CHF _{$\beta\gamma$}), 118.1 (C₅), 139.8 (C₂), 148.8 (C₄), 152.7 (C₈), 155.5 (C₆). Negative ion ESI-MS, m/z 545 [M+Na-H]⁻, 566 [M+2Na-2H]⁻.¹⁸

Adenosine 5'- β,γ -difluoromethylenetriphosphate, AppCF₂p (4)

The title compound was prepared according to Method A, using **17** (512 mg, 0.55 mmol) and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. Reaction progress was monitored by TLC (4:4:1 EtOH/NH₃/H₂O) and by ^{31}P NMR spectroscopy by removing 100 μL aliquots of the reaction mixture every 15 minutes [AMP 1.72; AppCF₂p -10.41 (d), -2.52 (m), 3.69 (m); **17** 6.49

(t)]. The reaction was complete after 1.25 hr. Workup and chromatographic purification were performed according to Method E. $\text{Abs}_{259} = 0.69$, yield 82 %. The yield of the purified diammonium salt was 124 mg, 79 %, δ_{P} (D_2O) -10.43 (d, P_{α} , $^2J_{\alpha\beta}$ 29.77 Hz), -3.1 (tdd, P_{β} , $^2J_{\text{P}\beta\text{F}}$ 89 Hz, $^2J_{\alpha\beta}$ 29.77, $^2J_{\beta\gamma}$ 22.38 Hz), 3.619 (td, P_{γ} , $^2J_{\text{P}\gamma\text{F}}$ 81 Hz, $^2J_{\beta\gamma}$ 22.38 Hz); δ_{H} (D_2O , 500.133 MHz) 4.10 (m, 2H, H_5'), 4.24 (br., 1H, H_4'), 4.41 (dd, 1H, H_3' , $J_{3',2'}$ 4.5 Hz, $J_{3',4'}$ 3.5 Hz), 5.94 (d, 1H, H_1' , $J_{1',2'}$ 5.5 Hz), 8.00 (s, 1H, H_2), 8.32 (s, 1H, H_8); δ_{C} (D_2O) 65.1 (C_5'), 70.2 (C_3'), 74.3 (C_2'), 83.8 (d, C_4' , $^3J_{\text{C},\text{P}\alpha}$ 8.68 Hz), 86.8 (C_1'), 118.4 (C_5), 139.8 (C_2), 148.8 (C_4), 152.6 (C_8), 155.3 (C_6). δ_{C} (D_2O , ^{19}F decoupled) 120.00 (dd, $\text{CF}_{2\beta\gamma}$, $J_{\text{C},\text{P}\beta}$ 179.2 Hz, $J_{\text{C},\text{P}\gamma}$ 159.1 Hz).¹⁸

Uridine 5'- β,γ -methylenetriphosphate, UppCH₂p (5)

A solution of UMP-*N*-methylimidazolidine was prepared according to Method A, and was then added drop-wise by a syringe to a flask containing **15** (492 mg, 0.55 mmol) and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. The reaction was stirred at 0°C under nitrogen. Reaction progress was monitored by TLC (4:4:1 EtOH/ NH_3 / H_2O) and by ^{31}P NMR spectroscopy by removing 100 μL aliquots of the reaction mixture every 5 minutes and quenching with 600 μL of 250 mM ammonium acetate, pH 7 in an NMR tube [UMP 4.3; UppCH₂p -10.4 (d), 12.0 (d), 13.57 (dd); **15** 16.8]. The reaction was complete after 15 min, and was then quenched with cold aqueous ammonium acetate (3 mL, 250 mM, pH 7), the resultant solution was then washed with chloroform (3 x 5 mL), freeze dried, diluted with buffer A (5 mL) and purified by reversed-phase automated flash column chromatography using a linear gradient of buffer A and buffer B (0-100 %, 30 CV). The appropriate fractions were pooled and adjusted to pH 7 using dilute ammonia solution. The volume was adjusted with water to 250 mL in a volumetric flask, from which 0.5 mL was diluted with water to 10 mL and the absorbance readings of the resultant solution were measured at $\lambda_{\text{max}} = 262$ nm, and found to be 0.46 (84 %). The solution was

concentrated, lyophilized and then dissolved in water (10 mL) and passed through an Amberlite IR-120 H⁺ column. The fractions collected from the column were monitored by measuring the UV activity at $\lambda_{\text{max}} = 262$ nm, the fractions possessing UV activity were combined and the pH was adjusted to 9.5 by dilute ammonia solution and lyophilized. The product was then dissolved in water (0.5 mL) and precipitated by the drop-wise addition of ethanol (3 mL) where it was left in freezer for 20 min, and then centrifuged for 10 min at 4°C. The aqueous ethanolic solution was decanted out and the residue was dried under vacuum. A few drops of water were added to dissolve the residue which was lyophilized again to yield 117 mg of the diammonium salt (83 %). δ_{P} (D₂O) -10.4 (d, P _{α} , $^2J_{\alpha\beta}$ 27.5 Hz), 9.5 (dd, P _{β} , $^2J_{\alpha\beta}$ 27.5 Hz, $^2J_{\beta\gamma}$ 5.1 Hz), 15.2 (d, P _{γ} , $^2J_{\beta\gamma}$ 5.1 Hz); δ_{P} (D₂O, 500.133 MHz) 2.11 (t, 2H, CH₂ $\beta\gamma$, $^2J_{\text{H,P}}$ 20.0 Hz), 4.06 (m, 2H, H_{5'}), 4.13 (br., 1H, H_{4'}), 4.23 (m, 2H, H_{2'}, H_{3'}) 5.818 (br., 1H, H_{1'}), 5.822 (d, 1H, H₅, $J_{5,6}$ 8 Hz), 7.8 (d, 1H, H₆, $J_{6,5}$ 8 Hz); δ_{C} (D₂O) 29.8 (dd, CH₂ $\beta\gamma$, $J_{\text{C,P}\beta}$ 129.3 Hz, $J_{\text{C,P}\alpha}$ 120.89 Hz) 64.5 (d, C_{5'}, $^2J_{\text{C,P}\alpha}$ 5.35 Hz), 69.47 (C_{3'}), 73.7 (C_{2'}), 83.1 (d, C_{4'}, $^3J_{\text{C,P}\alpha}$ 8.93 Hz), 88.4 (C_{1'}), 102.6 (C₅), 141.6 (C₆), 151.9 (C₄), 166.3 (C₂). Negative ion ESI-MS, m/z 481 [M-H]⁻.

Uridine 5'- β,γ -dichloromethylenetriphosphate, UppCCl₂p (6)

The title compound was prepared as for compound **5**, using tributylammonium dichloromethylenediphosphonate (350 mg, 0.815 mmol), triethylamine (0.2 mL), and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. Reaction progress was monitored by TLC (4:4:1 EtOH/NH₃/H₂O) and by ³¹P NMR spectroscopy by removing 100 μ L aliquots of the reaction mixture every 15 min [UMP 4.3; UppCCl₂p -10.29 (d), 0.81 (dd), 8.23 (d); Chlodronate 10.72]. The reaction was complete after 50 min. Workup and chromatographic purification were performed according to compound **5**. Abs₂₆₂ = 0.47 (86 %). The yield of the purified diammonium salt was 133 mg (83 %). δ_{P} (D₂O) -10.94 (d, P _{α} , $^2J_{\alpha\beta}$ 31 Hz), 1.1 (dd, P _{β} , $^2J_{\alpha\beta}$ 31

Hz, $^2J_{\beta\gamma}$ 17.6 Hz), 7.7 (d, P_γ , $^2J_{\beta\gamma}$ 17.6 Hz); δ_H (D₂O, 500.133 MHz) 4.13 (m, 3H, H_{5'}, H_{4'}, $J_{5',4'}$), 4.23 (br., 1H, H_{3'}), 4.28 (br., 1H, H_{2'}), 5.81 (m, 2H, H_{1'}, H₅), 7.85 (d, 1H, H₆, $J_{6,5}$ 8 Hz). δ_C (D₂O) 64.9 (d, C_{5'}, $^2J_{C,P\alpha}$ 5.28 Hz), 69.5 (C_{3'}), 73.7 (C_{2'}), 83.2 (d, C_{4'}, $^3J_{C,P\alpha}$ 9.18 Hz), 78.7 (dd, $\underline{C}Cl_{2\beta\gamma}$, $J_{C,P\beta}$ 144.43 Hz, $J_{C,P\alpha}$ 121.54 Hz), 88.4 (C_{1'}), 102.6 (C₅), 141.7 (C₆), 151.8 (C₄), 166.2 (C₂). Negative ion ESI-MS, (^{35}Cl) m/z 549 [M-H]⁻, isotopic peak (^{37}Cl) m/z 551.

Uridine 5'-β,γ-fluoromethylenetriphosphate, UppCHFp (7)

The title compound was prepared as for compound **5**, using **16** (503 mg, 0.55 mmol) and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. Reaction progress was monitored by TLC (4:4:1 EtOH/NH₃/H₂O) and by ^{31}P NMR spectroscopy by removing 100 μL aliquots of the reaction mixture every 15 min [UMP 4.3; UppCHFp -10.43 (d), 5.15 (ddd), 7.56 (dd); **16** 10.88 (d)]. The reaction was complete after 1 hr. Workup and chromatographic purification were performed according to compound **5**. Abs₂₆₂ = 0.46 (84 %). The yield of the purified diammonium salt was 120 mg (82 %). δ_P (D₂O) -11.04 (d, P_α , $^2J_{\alpha\beta}$ 26.9 Hz), 1.39 (ddd, P_β , $^2J_{P\beta F}$ 62.8 Hz, $^2J_{\alpha\beta}$ 26.9 Hz, $^2J_{\beta\gamma}$ 15 Hz), 8.17 (dd, P_γ , $^2J_{P\gamma F}$ 59.8 Hz, $^2J_{\beta\gamma}$ 15 Hz); δ_F (D₂O) 213.33 (dd, $J_{F,P\beta}$ 85.56, $J_{F,P\alpha}$ 62.14); δ_H (D₂O) 4.17 (br., 2H, H_{5'}), 4.22 (br., 1H, H_{4'}), 4.33 (br., 2H, H_{2'}, H_{3'}), 4.8 (ddd, $\underline{C}HF_{\beta\gamma}$, $J_{H,F}$ 46.23 Hz, $^2J_{H,P\beta}$ 12.4 Hz, $^2J_{H,P\alpha}$ 12.1 Hz), 5.92 (br., 2H, H_{1'}, H₅), 7.9 (d, 1H, H₆, $J_{6,5}$ 7.93 Hz); δ_C (D₂O) 64.5 (d, C_{5'}, $^2J_{C,P\alpha}$ 5 Hz), 69.5 (C_{3'}), 73.67 (C_{2'}), 83.3 (d, C_{4'}, $^3J_{C,P\alpha}$ 8.8 Hz), 88.3 (C_{1'}), 89.5 (ddd, $\underline{C}HF_{\beta\gamma}$, $J_{C,F}$ 46.1, $J_{C,P\beta}$ 25.1, $J_{C,P\gamma}$ 13.8), 102.6 (C₅), 141.6 (C₆), 151.8 (C₄), 166.2 (C₂). Negative ion ESI-MS, m/z 499 [M-H]⁻.

Uridine 5'-β,γ-difluoromethylenetriphosphate, UppCF₂p (8)

The title compound was prepared as for compound **5**, using **17** (512 mg, 0.55 mmol) and 4 Å molecular sieves (ca. 10-15) in acetonitrile (1 mL) at 0°C. Reaction progress was monitored by TLC (4:4:1 EtOH /NH₃ /H₂O) and by ^{31}P NMR spectroscopy by removing 100 μL aliquots of the

reaction mixture every 15 min [UMP 4.3; UppCF₂p -10.29 (d), -2.5 (m), 4.29 (m); **17** 6.38 (t)]. The reaction was complete after 1.25 hr. Workup and chromatographic purification were performed according to compound **5**. Abs₂₆₂ = 0.44 (81 %). The yield of the purified diammonium salt was 116 mg (77 %). δ_P (D₂O) -10.99 (d, P _{α} , $^2J_{\alpha\beta}$ 30.17 Hz), -5.12 (tdd, P _{β} , $^2J_{P\beta F}$ 89.9 Hz, $^2J_{\alpha\beta}$ 30.17 Hz, $^2J_{\beta\gamma}$ 58.2 Hz), 3.10 (td, P _{γ} , $^2J_{P\gamma F}$ 73.43 Hz, $^2J_{\beta\gamma}$ 58.2 Hz); δ_F (D₂O) -114.99 (dd, $J_{F,P\beta}$ 89.9, $J_{F,P\gamma}$ 73.43); δ_H (D₂O) 4.09 (dd, 2H, H_{5'}, $J_{5',P\alpha}$ 3.1 Hz, $J_{5',4'}$ 2.8 Hz), 4.13 (br., 1H, H_{4'}), 4.25 (br., 2H, H_{2'}, H_{3'}), 5.823 (d, 1H, H_{5'}, $J_{5,6}$ 14 Hz), 5.825 (s, 1H, H_{1'}), 7.8 (d, 1H, H_{6'}, $J_{6,5}$ 8 Hz); δ_C (D₂O) 64.8 (d, C_{5'}, $^2J_{C,P\alpha}$ 5.2 Hz), 69.5 (C_{3'}), 73.7 (C_{2'}), 83.3 (d, C_{4'}, $^3J_{C,P\alpha}$ 9.1 Hz), 88.3 (C_{1'}), 102.6 (C₅), 141.6 (C₆), 151.8 (C₄), 166.2 (C₂). δ_C (D₂O, ¹⁹F decoupled) 120 (dd, CF₂P _{γ} , $J_{C,P\beta}$ 179 Hz, $J_{C,P\gamma}$ 160 Hz). Negative ion ESI-MS, m/z 517 [M-H]⁻.

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