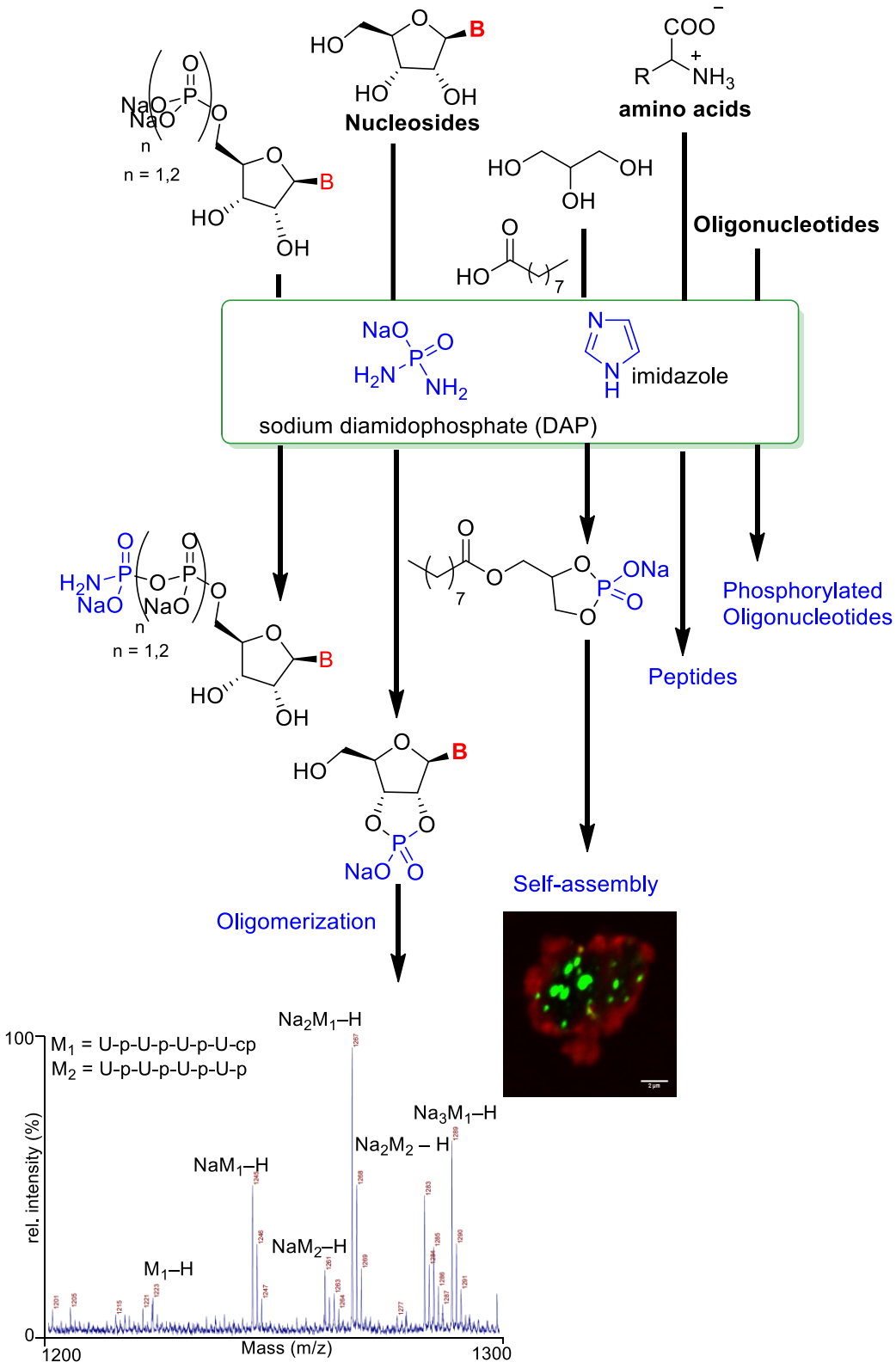
Supplementary Information

Phosphorylation, Oligomerization and Self-assembly in Water Under Potential Prebiotic Conditions

Clémentine Gibard‡, Subhendu Bhowmik‡, Megha Karki‡, Eun-Kyong Kim, Ramanarayanan Krishnamurthy*

correspondence to: rkrishna@scripps.edu

‡ These authors contributed equally to this work.

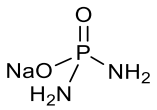

Nucleosides
amino acids
Oligonucleotides
sodium diamidophosphate (DAP)
imidazole
n = 1,2
Phosphorylated
Oligonucleotides
Peptides
Oligomerization
Self-assembly
M1 = U-p-U-p-U-p-U-cp
M2 = U-p-U-p-U-p-U-p
Na2M1–H
Na3M1–H
Na2M2 – H
NaM1–H
NaM2–H
M1–H
rel. intensity (%)
Mass (m/z)
100
0
1200
1300

General Experimental:

Reagents and solvents from Sigma-Aldrich, VWR International, Fischer Scientific, Acros. Oligonucleotides phosphates from IDT and used without further purification. Thin layer chromatography (TLC) performed on silica gel 60 $Å_{F254}$ from Angela Technologies, and visualized by UV lamp and/or a stain solution of phosphomolybdic acid (PMA) in ethanol. Flash Chromatography performed on biotage isolera. NMR recorded at 298 K: Bruker DRX-600 or AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C). ^{31}P-NMR spectra were acquired using a Bruker DPX-400; chemical shifts (δ) in parts per million (ppm), spin multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants (*J*) in Hertz (Hz), number of protons. Excitation sculpting with gradients spectra will be referred as "water suppression" spectra. Mass spectra from Agilent ESI-TOF or ThermoElectron Finnigan LTQ ion trap mass spectrometer. Ion-exchange chromatography: DEAE-A-25-sephadex, 40-120 mesh, HCO^{3-} form, 2.5 x 3.5 cm. Quantification of UV active species: NanoDrop 2000c spectrometer. Light-scattering measurements: DLS instrument Serial # 489-DPN, Model NanoStar, polarized laser at 663.92 nm, 25°C, 10 µL sample in 10 mm diameter glass cuvette, 10 acquisitions of 5 sec total, in triplicate. FPLC: AKTAPURE, 260 nm, DNApacTMPA200 column, flow rate: 1 mL/min. The samples were filtered through 0.22 µm syringe tip filter units (Milliex-GV; PVDF). Buffers for FPLC: Condition 1, Buffer A: 5 mM Tris base buffer, pH 8.2. Buffer B: 5 mM Tris base, 330 mM $NaClO_4$ buffer, pH 8.2. Condition 2, Buffer A: 10 mM Na_2HPO_4, pH 10.5. Buffer B: 10 mM Na_2HPO_4, 1 M NaCl, pH 10.5. The gradient is specified under each profile. MALDI-TOF: Voyager-DE PRO, THAP matrix. LCMS: ThermoScientific Vanquish UHPLC system, elution from a XBridge BEH Amide 5µm column, Buffer A: 10 mM NH_4OAc, pH 6, Buffer B: acetonitrile, flow rate 0.5 ml/min, elution gradient: using 75% buffer B for 5 min then 75 to 55 % buffer B in 17 min. The pH instrument: pH electrode, Hanna instrument from Spectrum Chemicals and Laboratory products. Electron microscopy: Model FEI/Philips CM100, operated at 80KV. The images are taken using Radius 1.3 software with a Megaview G2 CCD camera manufactured by EMSIS GmbH, Germany. Confocal Microscopy: Model Zeiss LSM 780 and 710 equipped with 63x oil Plan Apo, 1.4na DIC. Microwave synthesizer (Initiator Classic, Biotage), 2.5 mL reaction vials.

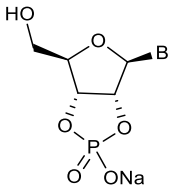
Preparation of the phosphorylating reagent

Sodium diamidophosphate – DAP [S1]



Phenyl phosphorodiamidate (10 g, 58 mmol, 1 equiv.) was suspended in a 4 M sodium hydroxide solution (30 mL, 120 mmol, 2 equiv.) and stirred at 110 °C for 10 min. The clear yellow solution was concentrated and 140 mL of ethanol was added at 0 °C. The precipitate was filtered, dissolved in 100 mL of water and washed consecutively twice with dichloromethane and ethyl acetate (the organic phase should be check by TLC to ensure that no phenol is present). The aqueous phase was concentrated to 5 mL and was added dropwise 100 mL cold ethanol under vigorous stirring to afford a precipitate. The precipitate was filtered, rinsed 3 times with 20 mL of ethanol (at room temperature) and dried under vacuum for 2 days to afford 6.250 g (52.9 mmol, 91 %) of DAP as a white powder. The pH of DAP in water (at 0.1 M) is 8.1 and its ^{31}P NMR chemical shift in D_2O is 14.5 ppm.

2',3'-cyclic nucleotide formation



5 B = U
6 B = C
7 B = A
8 B = G

From the nucleoside:

Method A (reaction in solution at pH 5.5): To an Eppendorf tube containing 0.1 M of the nucleoside (1 equiv.) in D_2O, was added DAP (5 equiv.), imidazole (zero or 1 equiv.) and metal chloride (0.5 or 3 equiv. of zinc chloride or magnesium chloride). The pH of the reaction mixture was adjusted to 5.5 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the pH was adjusted to 5.5 twice a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy. After all the DAP was consumed in about 4 days (^{31}P NMR), an additional 5 equivalents of DAP were added and the reaction was continued.

Note: Upon the addition of zinc, a precipitate is formed (especially when 3 equiv. of zinc chloride were added). The precipitate appears when DAP and zinc were mixed together. In the case of magnesium chloride, a precipitate appeared over time. The ^{31}P NMR of the heterogenic mixture and of the supernatant were compared and showed similar signals. In the case of 1H NMR only the supernatant showed interpretable signals.

Method B (Reactions in solution at pH 7): To an Eppendorf tube containing 0.1 M of the nucleoside (1 equiv.) in D_2O, DAP (5 equiv.), imidazole (1 equiv.) and metal chloride (0.5 or 3 equiv. of zinc chloride or magnesium chloride). The pH of the reaction mixture was adjusted to 7 with hydrochloric acid (4 M) (in the case of 3 equiv. of $ZnCl_2$ the pH was raised to 7 with sodium hydroxide (1 M)). The reactions were kept at room temperature and the pH was adjusted to 7 once a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy. After about 1 week all the DAP was consumed (^{31}P NMR), an additional 5 equivalents of DAP were added and reaction was continued.

Method C (reactions under "paste conditions"): Nucleoside (1 equiv.), DAP (5 equiv.), imidazole (5 equiv.) and about 2 drops of D_2O were placed in an Eppendorf tube and the resulting mixture was ground using a glass rod to obtain a paste. The paste-reaction was kept at room temperature and ground (for 5 min every day). The progress of the reaction was monitored by taking a small portion of the paste-reaction mixture and dissolving it in 500 µL of D_2O. The pH of the solution was adjusted to 7 (from original pH of 8.5 and 9.5 - to ensure consistency in NMR chemical shifts) with hydrochloric acid (4 M) prior to the ^{1}H, ^{13}C and ^{31}P NMR spectroscopy measurement. When all the DAP was consumed (^{31}P NMR, between 10 and 15 days) an additional 5 equivalents of DAP were added and reaction was continued.

From the 3'-nucleoside monophosphate

Method A (Reactions in solution at pH 5.5): To an Eppendorf tube containing 0.1 M of the 3'-nucleoside monophosphate (1 equiv.) in D2O was added DAP (5 equiv.), imidazole (1 equiv.) and metal chloride (0.5 of zinc chloride or magnesium chloride). The pH of the reaction mixture was adjusted to 5.5 with hydrochloric acid (4 M). The reactions were kept at room

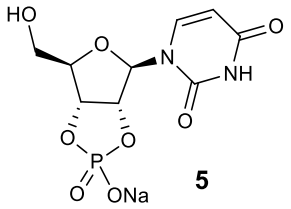
temperature and the pH was adjusted to 5.5 twice a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.

Method B (Reactions in solution at pH 7): To an Eppendorf tube containing 0.1 M of the 3'-nucleoside monophosphate (1 equiv.) in D_2O was added DAP (5 equiv.), imidazole (1 equiv.) and metal chloride (0.5 of zinc chloride or magnesium chloride). The pH of the reaction mixture was adjusted to 7 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the pH was adjusted to 7 once a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.

From the 2'-nucleoside monophosphate

Method B (Reactions in solution at pH 7): To an Eppendorf tube containing 0.1 M of the 3'-nucleoside monophosphate (1 equiv.) in D_2O was added DAP (5 equiv.), imidazole (1 equiv.) and metal chloride (0.5 of magnesium chloride). The pH of the reaction mixture was adjusted to 7 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the pH was adjusted to 7 once a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.

Gram scale preparation of 5



5

Uridine (1 g, 4.11 mmol, 1 equiv.), DAP (2.42 g, 4.1 mmol, 5 equiv.), imidazole (5 equiv.) and about 10 drops of D_2O were placed in a mortar. Then the mixture was ground using a pestle to obtain a paste. The paste-reaction was kept at room temperature and ground (for 15 min every day). The progress of the reaction was monitored by taking an aliquot of the paste reaction mixture and dissolving it in 500 µL of D_2O. The pH of the solution was adjusted to 7 (from original pH of 8.5 and 9.5 - to ensure consistency in NMR chemical shifts) with (4 M) hydrochloric acid prior to the ^{1}H, ^{13}C and ^{31}P NMR spectroscopy measurement. When all the DAP was consumed (^{31}P NMR, every 2 weeks) an additional 5 equivalents of DAP were added and the reaction was continued. After 36 days about 83% of the uridine was converted to phosphorylated species as determined by ^{1}H NMR (Supplementary Figure 17).

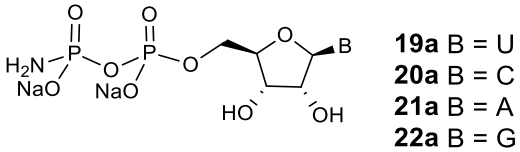
Purification of 5

The above crude mixture was taken with 60 mL of methanol and triturated for 30 min. After centrifugation, the precipitate and supernatant were separated and the supernatant was concentrated to dryness. The residue was dissolved in the minimum amount of water and subjected to ion-exchange chromatography (DEAE-A-25-sephadex, 40-120 mesh, HCO^{3-} form, 2.5 x 35 cm, washed with 500 mL of water, followed by elution with 0.1 M $Et_3N.H_2CO_3$ in 0.05 M increments of 100 mL in 2 x 50 ml installments). The concentration of UV active species in each fraction (50 ml each) was determined by NanoDrop and the ^{31}P-NMR of the most concentrated UV active fractions were recorded. The fractions containing the pure product were combined and concentrated to dryness at 35 °C overnight (rotovap). The resulting 2',3'-cyclic uridine monophosphate triethylammonium salt which contained excess Et_3N was converted to

the sodium salt using the following protocol[S2]. 1 M solution of sodium iodide was prepared by dissolving 75 g of NaI in 500 mL of distilled (dry) acetone. The triethylammonium salt of the c-UMP (containing excess Et_3N) was dissolved in 33 mL of methanol and the sodium iodide solution was added dropwise under vigorous stirring until no additional precipitate was formed. The resulting suspension was filtered and the solid was washed several times with distilled (dry) acetone to remove the remaining trace of triethylamine and sodium iodide. The product **5** was re-dissolved in water and lyophilized two times to afford 888 mg of a white solid (2.7 mmol, 65 %). ^{1}H NMR (600 MHz, D_2O) δ ppm: 3.77 (dd, J = 12.4, 5.6 Hz, 1H, C5'-HH), 3.86 (dd, J = 12.4, 3.8 Hz, 1H, C5'-HH), 4.25 (m, 1H, C4'-H), 4.88 (m, 1H, C3'-H), 5.12 (ddd, J = 7.2, 6.9, 3.0 Hz, C2'-H), 5.81 (d, J = 8.01 Hz, C6-H), 5.87 (d, J = 2.90 Hz, C1'-H), 7.67 (d, J = 8.5 Hz, C5-H); ^{13}C NMR (150 MHz, D_2O) δ ppm : 165.9, 150.7,143.4, 101.7 , 92.4 (d, 3J = 6.3 Hz), 84.7 (3J = 2.3 Hz), 80.2 (2J = 2.3 Hz), 76.6, 60.4; ^{31}P NMR {H-coupled}(162 MHz, D_2O) δ ppm : 20.5 (dd, J_{H-P} = 11.7, 11.5 Hz) ; ^{31}P NMR{H-decoupled} (162 MHz, D_2O) δ ppm: 20.7; HRMS (negative. m/z): [M+Na]–calcd. for $C_9H_{11}N_2O_8PNa$, 329.0145; found, 329.0145

Nucleotides phosphorylation

5'-nucleoside amidodiphosphate

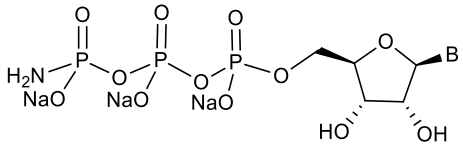


19a B = U
20a B = C
21a B = A
22a B = G

Method A (reaction in solution at pH 5.5): To an Eppendorf tube containing 0.1 M of the 5'-nucleoside monophosphate (1 equiv.) in D_2O, was added DAP (5 equiv.), and zinc chloride (3 equiv.). The pH of the mixture was adjusted to 5.5 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the pH was adjusted to 5.5 twice a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.

Method B (Reactions in solution at pH 7): To an Eppendorf tube containing 0.1 M of the 5'-nucleoside monophosphate (1 equiv.) in D_2O, was added DAP (5 equiv.), imidazole (1 equiv.) and metal chloride (0.5 or 3 equiv. of zinc chloride or magnesium chloride). The pH of the mixture was adjusted to 7 with (4 M) hydrochloric acid (in the case of 3 equiv. of $ZnCl_2$ the pH was raised to 7 with sodium hydroxide (1 M) before the addition of the nucleotide). The reactions were kept at room temperature and the pH was adjusted to 7 once a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.

5'-nucleoside amidotriphosphate



19b B = U
20b B = C
21b B = A
22b B = G

Method A (Reaction in solution at pH 5.5, zinc chloride): To an Eppendorf tube containing 0.1 M of the 5'-nucleoside diphosphate (1 equiv.) in D_2O, was added DAP (5 equiv.) and zinc chloride (3 equiv.). The pH of the reaction mixture was adjusted to 5.5 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the pH was adjusted to 5.5 once a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.

Method B (Reaction in solution at pH 5.5, no zinc chloride): To an Eppendorf tube containing 0.1 M of the 5' nucleoside diphosphate (1 equiv.) in D_2O, was added DAP (5 equiv.). The pH of the reaction mixture was adjusted to 5.5 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the pH was adjusted to 5.5 once a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.

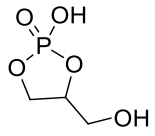
Method C (Reaction in solution at pH 7) To an Eppendorf tube containing 0.1 M of the 5'-nucleoside diphosphate (1 equiv.) in D_2O, was added DAP (5 equiv.), imidazole (1 equiv.) and magnesium chloride (0.5 equiv.). The pH of the reaction mixture was adjusted to 7 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the pH was adjusted to pH 7 once a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.

Oligonucleotides phosphorylation

The commercially available oligonucleotide phosphate was dissolved in 1 mL of RNAse and DNAse free water and the concentration of the oligophosphate was measured using Nanodrop at 260 nm wavelength. From that stock solution, 1 mM oligophosphate was pipetted out in a small Eppendorf tube and dried under vacuum. 20 µL of a solution containing 1 M DAP and 1 M imidazole at pH 7 (adjusted by 1 N HCl) was used to resuspend the dried oligophosphate. The progress of the reaction was monitored by using anion exchange liquid chromatography with different buffer mixture. The starting oligomer signal identity was confirmed by spiking the reaction mixture with the commercial starting oligophosphate and the formation of the phosphorylated oligomer product was confirmed by MALDI.

Vesicles-like structures formation

Formation of Glycerol-1,2-cyclophosphate (**24**)



24

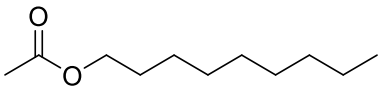
Method A (Reaction in solution at pH 7.5): To an Eppendorf tube containing 0.1 M of the glycerol **23** (0.25 mmol) in D_2O, was added DAP (5 equiv.), imidazole (1 equiv.). The pH of the reaction mixture was adjusted to 7.5 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the pH was adjusted to 7 once a day. The progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.

Method B (Reactions under "paste conditions"): Glycerol **23** (0.25 mmol), DAP (5 equiv.), imidazole (5 equiv.) and about 2 drops of D_2O were added to a watch glass and the resulting mixture was ground using a glass rod to obtain a paste. The paste-reaction was kept at room temperature and ground (for 5 min every day). The progress of the reaction was monitored by taking a small portion of the paste-reaction mixture and dissolving it in 500 µL of D_2O. The pH of the solution was adjusted to ~ 7.5 (from original pH of 8.5 and 9.5 - to ensure consistency in NMR chemical shifts) with hydrochloric acid (4 M) prior to the ^{1}H, ^{13}C and ^{31}P NMR spectroscopy measurements. When all the DAP was consumed (^{31}P NMR) an additional 5 equivalents of DAP were added and reaction was continued.

Method C (Reaction in paste at 50 ºC): Glycerol **23** (0.25 mmol), DAP (5 equiv.), imidazole (5 equiv.) and about 2 drops of D_2O were added to an Eppendorf tube and the resulting mixture was ground using a glass rod to obtain a paste. The paste-reaction was warmed at 50 ºC. The progress of the reaction was monitored by taking a small portion of the paste-reaction mixture and dissolving it in 500 µL of D_2O. The pH of the solution was adjusted to ~ 7.5 (from original pH of 8.5 and 9.5 - to ensure consistency in NMR chemical shifts) with hydrochloric acid (4 M) prior to the ^{1}H, ^{13}C and ^{31}P NMR spectroscopy measurements. When all the DAP was consumed (^{31}P NMR) an additional 5 equivalents of DAP were added and reaction was continued.

Method D (Reaction in microwave): Glycerol **23** (0.25 mmol), DAP (5 equiv.), imidazole (5 equiv.) and D_2O (5 drops) were added to a 5 mL µW vial and was subjected to microwave irradiation. The progress of the reaction was monitored by taking a small portion of the paste-reaction mixture and dissolving it in 500 µL of D_2O. The pH of the solution was adjusted to ~ 7.5 (from original pH of 8.5 and 9.5 - to ensure consistency in NMR chemical shifts) with hydrochloric acid (4 M) prior to the ^{1}H, ^{13}C and ^{31}P NMR spectroscopy measurement. When all the DAP was consumed (^{31}P NMR, every 3-4 hours) an additional 5 equivalents of DAP were added and reaction was continued.

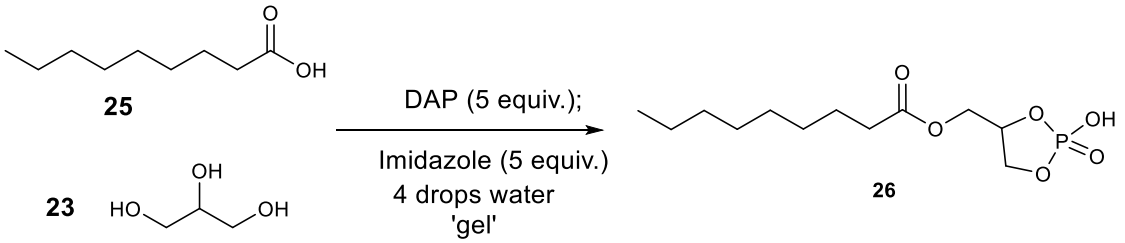
Formation of Nonanoyl acetate



Method (Reactions under "paste-conditions"): 1-Nonanol (0.25 mmol, 1 equiv.), ammonium acetate (0.25 mmol, 1 equiv.), DAP (1.25 mmol, 5 equiv.), imidazole (0.25 mmol, 5 equiv.) and 2 drops of D_2O were added to a watch glass and the resulting mixture was ground using a glass rod to obtain a paste. The paste-reaction was kept at room temperature and ground (for 5 min every day). The progress of the reaction was monitored by taking a small portion of the paste-reaction mixture and dissolving it in 500 µL of D_2O. The pH of the solution was adjusted to ~ 7.5 (from original pH between 8.5 and 9.5 - to ensure consistency in NMR chemical shifts) with hydrochloric acid (4 M) prior to the ^{1}H NMR spectroscopy measurements.

Formation of Phospholipid 26:

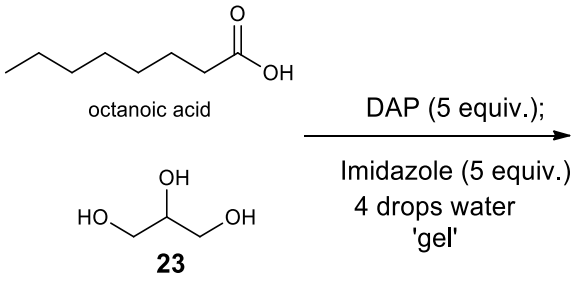
<u>One-pot reaction of nonanoic acid **25**, glycerol **23** in the presence of DAP and imidazole:</u>



Method A1 (Reaction under "paste conditions"): 1-nonanoic acid **25** (0.25 mmol, 1 equiv.), glycerol (0.25 mmol, 1 equiv.), DAP (1.25 mmol, 5 equiv.), imidazole (1.25 mmol, 5 equiv.) and about 4 drops of D_2O were added to an Eppendorf tube and the resulting mixture was ground using a glass rod to obtain a paste. The paste-reaction was kept at room temperature and

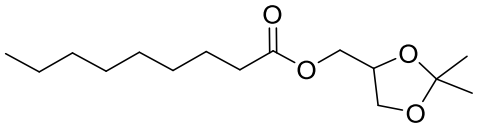
ground (for 5 min every day). The progress of the reaction was monitored by dissolving a small portion of the reaction in 500 µL of D_2O. The pH of the solution was adjusted to ~ 7.5 (from original pH of 8.5 and 9.5 - to ensure consistency in NMR chemical shifts) with hydrochloric acid (4 M) prior to the ^{1}H NMR spectroscopy measurements. The ^{1}HNMR and mass analysis (Fig. S118-S123) suggested the formation of **26** in the crude 'paste-reaction'.

One-pot reaction of octanoic acid, glycerol **23** in the presence of DAP and imidazole.



Method A2 (Reaction under "paste conditions"): 1-octanoic acid (0.25 mmol, 1 equiv.), glycerol (0.25 mmol, 1 equiv.), DAP (1.25 mmol, 5 equiv.), imidazole (1.25 mmol, 5 equiv.) and 4 drops of D_2O were added to an Eppendorf tube and the resulting mixture was ground using a glass rod to obtain a paste. The paste-reaction was kept at room temperature and ground (for 5 min every day).

Three step synthesis of phospholipid **26**: (2-hydroxy-2-oxido-1,3,2-dioxaphospholan-4-yl)methyl nonanoate :

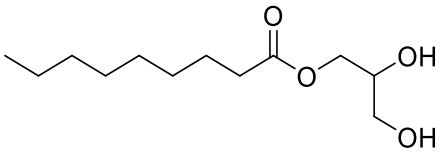


Method B:

Step 1

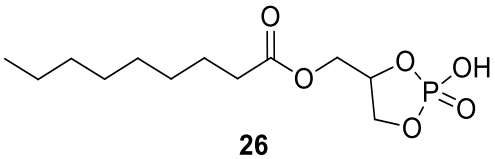
Synthesis of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl nonanoate:[S3] To a solution of nonanoic acid **25** (5 mmol) in dry CH_2Cl_2 was added dimethylaminopyridine (20 mol%) followed by solketal (5 mmol) dissolved in dry CH_2Cl_2. The reaction mixture was cooled to 0 ^oC prior to the addition of dicyclohexylcarbodiimide (5 mmol) and was stirred at room temperature for overnight after which the dicyclohexylurea was filtered. The reaction was taken up in CH_2Cl_2 and washed with $NaHCO_3$ (2X). The organic layer was concentrated *in vacuo* and the crude product was purified using column chromatography to afford (2,2-dimethyl-1,3-dioxolan-4-yl)methyl nonanoate as a colorless liquid (0.32 g, 59 %). ^{1}H NMR (600 MHz, Chloroform-d) δ 4.31 (qd, J = 6.2, 4.7 Hz, 1H), 4.16 (dd, J = 11.5, 4.7 Hz, 1H), 4.13 – 4.05 (m, 2H), 3.74 (dd, J = 8.4, 6.2 Hz, 1H), 2.34 (t, J = 7.6 Hz, 2H), 1.62 (q, J = 7.4 Hz, 2H), 1.43 (s, 3H), 1.37 (s, 3H), 1.34 – 1.20 (m, 10H), 0.87 (t, J = 7.0 Hz, 3H). ^{13}C NMR (150 MHz, Chloroform-d) δ 173.19, 109.36, 76.34, 33.66, 31.33, 28.74, 28.65, 26.23, 24.94, 24.43, 22.17, 13.62.

Step 2



Synthesis of 2,3-dihydroxypropyl nonanoate:[S4] To an ice-cold solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl nonanoate (1.5 mmol) in MeOH (50 mL) was slowly added 1 M HCl (1.04 mL) and the reaction was stirred at room temperature for 7 hours until the disappearance of starting material as observed by TLC after which the reaction was quenched with $NaHCO_3$ (5 mL). The reaction mixture was taken up in CH_2Cl_2 (2X) and the organic layer was separated, dried using sodium sulfate and concentrated in vacuo. The crude mixture was purified by column chromatography to obtain 2,3-dihydroxypropyl nonanoate as a white solid (0.14 g, 49 %). ^{1}H NMR (600 MHz, Chloroform-d) δ 4.18 (qd, J = 11.7, 5.3 Hz, 2H), 3.93 (q, J = 5.2 Hz, 1H), 3.69 (q, J = 5.5, 4.9 Hz, 1H), 3.59 (dd, J = 11.5, 5.8 Hz, 1H), 2.35 (t, J = 7.7 Hz, 2H), 2.04 (q, J = 6.5 Hz, 1H), 1.68 – 1.60 (m, 2H), 1.28 (d, J = 9.8 Hz, 10H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR (150 MHz, Chloroform-d) δ 173.91, 69.81, 64.72, 62.87, 33.70, 31.33, 28.73, 28.64, 24.45, 22.17, 13.62.

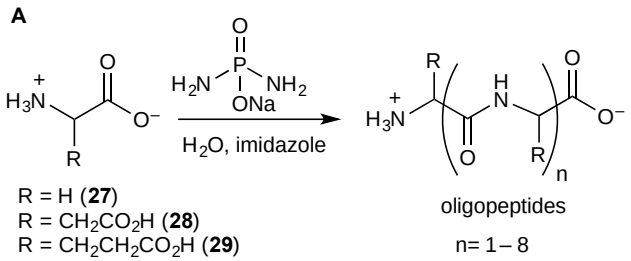
Step 3



26

Synthesis of (2-hydroxy-2-oxido-1,3,2-dioxaphospholan-4-yl)methyl nonanoate **26**: To a solution of $POCl_3$ (10 equiv.) in dry THF (4 mL) at -78 ºC, under an argon atmosphere, was added pyridine (quantity). The reaction mixture was stirred for 5 minutes at -78 ºC, after which a solution of 2,3-dihydroxypropyl nonanoate (0.58 mmol) in dry THF (4 mL) was added to it dropwise over 5 minutes, while the temperature was maintained at -78 ºC for 30 minutes after which the reaction was warmed to room temperature and stirred for 2 hours.[S5] The reaction mixture was poured into ice cold solution of $NaHCO_3$ (2.43 g in 20 mL) and the aqueous layer was washed with ethyl acetate. The aqueous layer was concentrated and the semi-solid white residue was washed with methanol. The mother liquor was concentrated and purified by flash chromatography (80% ethyl acetate and methanol) to yield 26 as a white solid (0.16 g, 94 %). ^{1}H NMR (600 MHz, D_2O) δ 4.37 – 4.25 (m, 2H), 4.18 (dd, J = 12.2, 6.1 Hz, 1H), 4.03 (td, J = 9.5, 6.9 Hz, 1H), 2.41 (t, J = 7.4 Hz, 2H), 1.58 (q, J = 7.3 Hz, 2H), 1.34 – 1.17 (m, 11H), 0.87 – 0.76 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 176.38, 73.59, 65.29, 63.75, 33.24, 30.62, 27.82, 27.75, 23.77, 21.56, 12.99; ^{31}P NMR{H-decoupled} (162 MHz, D2O) δ ppm: 18.6 (s); ^{31}P NMR {H-coupled} (162 MHz, D_2O) δ ppm: 18.6 (dd, J = 17.3, 10.5 Hz); HRMS (ESI) m/z calcd for $[C_{12}H_{23}O_6P + H]^+$ 295.1305, found 295.1307.

DAP mediated oligomerization of amino acids



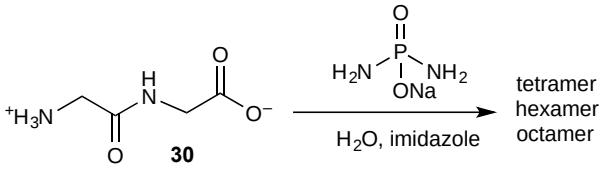
Method A: To an Eppendorf tube containing 0.1 M of the amino acid (0.25 mmol) in D_2O, was added DAP (5 equiv.), imidazole (1 equiv.). The pH of the reaction mixture was adjusted to 7.3-7.8 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the progress of the reaction was monitored by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy and mass analysis.

Method B**:** To an Eppendorf tube containing 0.1 M of the amino acid (0.25 mmol) in H_2O, was added DAP (0.5 equiv.), imidazole (1 equiv.). The pH of the reaction mixture was adjusted to 7.0-7.5 with (4 M) hydrochloric acid or (1 M) sodium hydroxide. The reactions were kept at room temperature and the progress of the reaction was monitored by ESI mass analysis after 7 days.

Control reaction without DAP

To an Eppendorf tube containing 0.1 M of the glycine **27** (0.25 mmol) in D_2O was added imidazole (1 equiv.). The pH of the reaction mixture was adjusted to 7.3-7.8 with (4 M) hydrochloric acid. The reactions were kept at room temperature and the progress of the reaction was monitored by ^{1}H NMR spectroscopy.

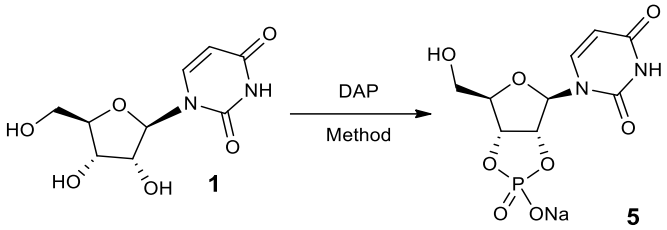
DAP mediated oligomerization of diglycine **30**



Method A: To an Eppendorf tube containing 0.1 M of the diglycine **30** (0.25 mmol) in D_2O, was added DAP (5 equiv.), imidazole (1 equiv.). The pH of the reaction mixture was adjusted to 7.3-7.8 with (4 M) hydrochloric acid. The oligomerization of diglycine was monitored by LCMS analysis (60 days).

Supplementary Table 1:

Formation of cUMP **5** by the phosphorylation of uridine **1** using DAP

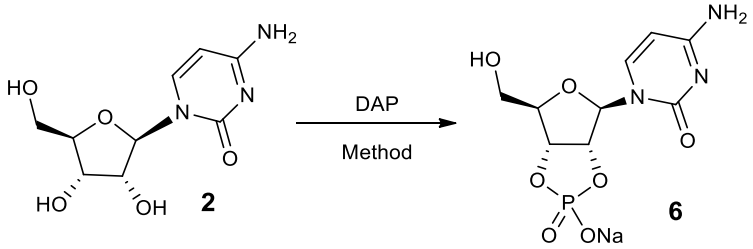


Entry	Method[π]	Amount of **1** (mmol)	Metal Chloride added (equiv.)	Time (days)	Conversion (%)*
			Solution pH 5.5		
1[±¥]	A	0.6	Zn (5)	30	29
2[±]	A	0.1	Zn (3)	9	7
3[±]	A	0.1	Mg (3)	9	7
4	A	0.1	Zn (0.5)	8	17
5	A	0.1	Zn (3)	8	16
				16	27
				30	43
6	A	0.1	Mg (0.5)	8	18
7	A	0.1	Mg (3)	8	16
				17	24
				21	27
			Solution pH 7		
8	B	0.1	Zn (0.5)	28	20
9	B	0.1	Zn (3)	28	24
10	B	0.1	Mg (0.5)	28	22
11	B	0.1	Mg (3)	28	29
				13	25
12[§]	B	0.1	Mg (3)	11	19
13[€]	B	0.1	Mg (3)	31	<2
			Paste Condition		
14[§]	C	0.1	NA	18	89
15	C	0.41	NA	30	~80
16	C	4.1	NA	36	83 (65[※]) (5'-amidophosphorylated c-UMP as well as short oligonucleotides were detected, please see Supplementary Fig 93-95)
			Microwave		
17[†]	C	0.2	NA	19 hours	51
18[‡]	C	0.2	NA	2 hours	37
			pH 7.8 – 10		
19[δ]	NA	0.1	NA	3.5 months	~6.5
20[≠]	NA	0.1	NA	7 months	<2

[π]: See 2',3'-cyclic nucleotide formation for methods; *: Based on the ^{1}H-NMR integral ratio of C_6-*H for* **1** and **5**; [¥]: reaction at 1 M instead of 0.1 M; [±]: no imidazole; [§]: 5 M DAP (0.1 M uridine, 50 equiv. DAP); [€]: 0.05 M DAP (0.01 M uridine, 5 equiv. DAP) [※]: isolated; [†]: microwave, 60°C ; [‡]: microwave 100°C; [δ]: The pH was not adjusted ranging between 7.8 and 8.4. No imidazole was use in this reaction; [≠]: The pH of a set of 4 reactions was adjusted to 8.5, 9, 9.5 and 10 respectively.

Supplementary Table 2:

Formation of cCMP **6** by the phosphorylation of cytosine **2** using DAP

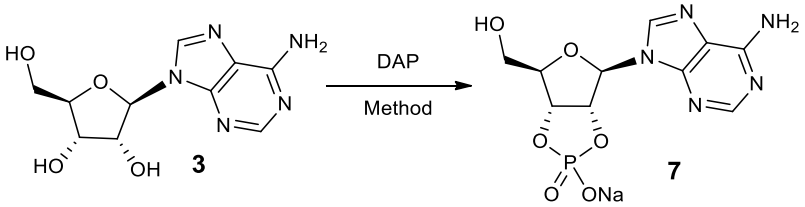


Entry	Method [π]	Amount of **2** (mmol)	Metal Chloride added (equiv.)	Time (days)	Conversion (%)*
			Solution pH 5.5		
1[±]	A	0.1	Zn (3)	14	23
			Solution pH 7		
2	B	0.1	Zn (0.5)	21	18
3	B	0.1	Zn (3)	21	14
4	B	0.1	Mg (0.5)	21	23
5	B	0.1	Mg (3)	21	23
			Paste Condition		
6	C	0.1	NA	10	42
7 [§]	C	0.25	NA	18	83

[π]: See 2',3'-cyclic nucleotide formation for methods; *: Based on the ^{1}H-NMR integral ratio of C_6-*H for* **2** and **6**; [§]: 50 equiv. of DAP; [±] : no imidazole

Supplementary Table 3:

Formation of cAMP **7** by the phosphorylation of adenosine **3** using DAP

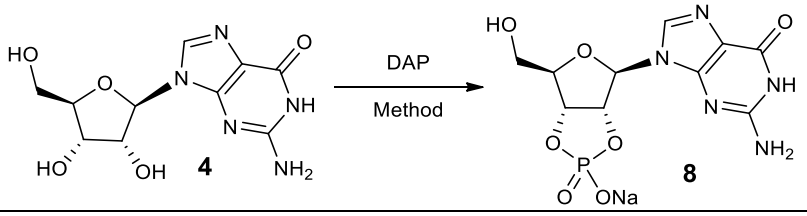


Entry	Method [π]	Amount of **3** (mmol)	Metal Chloride added (equiv.)	Time (days)	Conversion (%)*
			Solution pH 5.5		
1[§±]	A	0.1	Zn (3)	8	12
2	A	0.1	Mg (3)	23	31
			Solution pH 7		
3[§]	B	0.1	Zn (0.5)	17	13
4[§]	B	0.1	Zn (3)	17	11
5[§]	B	0.1	Mg (0.5)	17	13
6	B	0.1	Mg (3)	23	31
			Paste condition		
7	C	0.1	NA	16	<5

[π]: See 2',3'-cyclic nucleotide formation for methods; *: Based on the ^{1}H-NMR integral ratio average between C_1'*-H*, C_2-*H and* C_8-*H of* **3** and **7**; [±] : no imidazole; [§] : Reaction set up assuming 0.1 M of **3** even though its solubility in water is 0.022 mol L^{-1}

Supplementary Table 4:

Formation of cGMP **8** by the phosphorylation of guanosine **4** using DAP

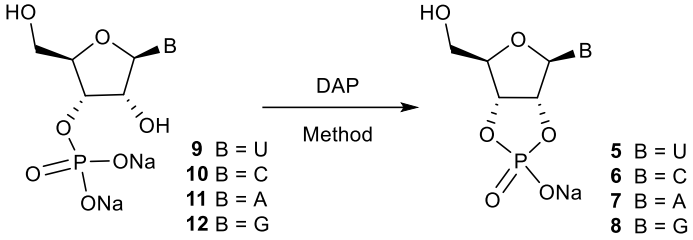


Entry	Method [π]	Amount of **4** (mmol)	Metal Chloride added (equiv.)	Time (days)	Conversion (%)*
			Solution pH 5.5		
1[§±]	A	0.1	Zn (3)	14	16
2[§]	A	0.1	Zn (3)	8	13
3	A	0.1	Mg (3)	12	27
			Solution pH 7		
4[§]	B	0.1	Zn (0.5)	17	10
5[§]	B	0.1	Zn (3)	17	13
6[§]	B	0.1	Mg (0.5)	11	14
7	B	0.1	Mg (3)	12	24
			Paste condition		
8	C	0.1	NA	20	17

[π]: See 2',3'-cyclic nucleotide formation for methods; * : Based on the 1H-NMR integral ratio average of C_1'-*H and* C_8-*H of* **4** and **8**; [±] : no imidazole; [§] : Reaction set up assuming 0.1 M on **4** even though its solubility in water is $2.6 .10^{-3}$ mol L^{-1}

Supplementary Table 5:

Formation of c-NMP by the phosphorylation of 3'-NMP using DAP



9 B = U
10 B = C
11 B = A
12 B = G

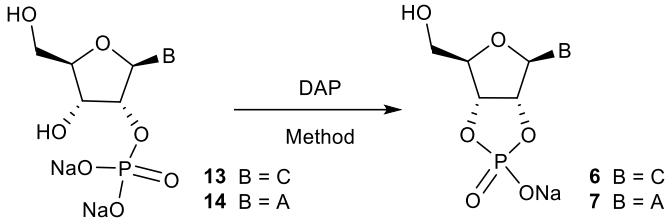
5 B = U
6 B = C
7 B = A
8 B = G

Entry	Method [¤]	Base	Metal Chloride added (equiv.)	Time (days)	Conversion (%)*
			Solution pH 5.5		
1	A	Uracil	Mg (0.5)	5	82
2	A	Uracil	Zn (0.5)	18 hours	87
3	A	Cytosine	Mg (0.5)	5	72
4	A	Adenine	Mg (0.5)	5	81
5[※]	A	Guanine	Mg (0.5)	8	48
			Solution pH 7		
6	B	Uracil	Mg (0.5)	3	58
				4	76
7[†]	B	Uracil	Mg (0.5)	3	56
8	B	Uracil	Zn (0.5)	5	49
9	B	Cytosine	Mg (0.5)	4	76
10[±]	B	Cytosine	Mg (0.5)	5	16
11	B	Adenine	Mg (0.5)	8	84
12[※]	B	Guanine	Mg (0.5)	5	31

[¤]: See 2',3'-cyclic nucleotide formation for methods; *: Based on the ^{1}H-NMR integral ratio average of C^{6}-*H* for the pyrimidines and C_{1}'-*H* and C_{8}-*H* for the purines; [※]: reaction at 0.05 M because of solubility issues; [†]: ^{15}N-labeled imidazole was used ; [±]: no imidazole

Supplementary Table 6:

Formation of c-NMP by the phosphorylation of 2'-NMP using DAP

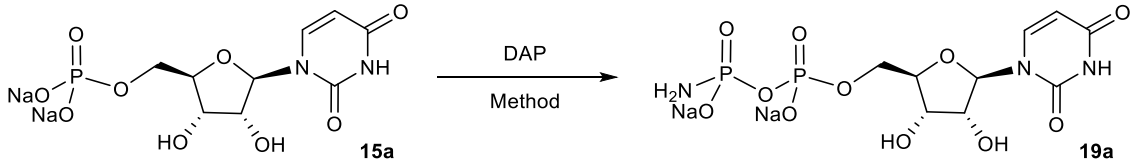


Entry	Method [ɪɪ]	Base	Metal Chloride added (equiv.)	Time (days)	Conversion (%)*
			Solution pH 7		
1	B	Cytosine	Mg (0.5)	5	67
2	B	Adenine	Mg (0.5)	5	47

[ɪɪ]: See formation of 2',3'-cyclic nucleotide for methods; *: Based on the ^{1}H-NMR integral ratio average of C^{6}-*H* for the pyrimidines and C_{1}'-*H* and C_{8}-*H* for the purines

Supplementary Table 7:

Formation of 5'-UDP^{NH2} **19a** by the amidophosphorylation of 5'-UMP **15a** using DAP

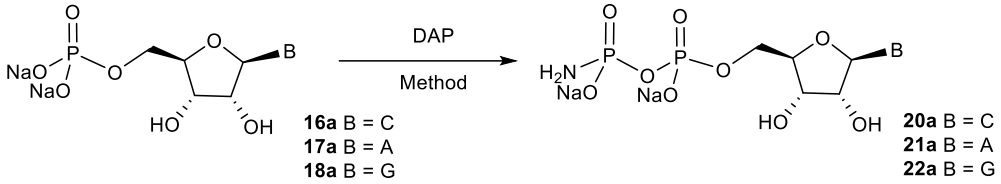


Entry	Method [π]	Amount (mmol)	Metal Chloride added (equiv.)	Time days	Conversion %* (% cUMP)
			Solution pH 5.5		
1[±]	A	0.1	Zn (3)	2	81[‡]
			Solution pH 7		
2	B	0.1	Zn (0.5)	6	55 (3)
3	B	0.1	Zn (3)	6	32 (1)
4	B	0.1	Mg (0.5)	6	82 (6)
5[†]	B	0.1	Mg (0.5)	1	45 (ND)
6	B	0.1	Mg (3)	6	79 (9)
			Paste Condition		
7[※]	C	0.1	NA	5	40(ND)

[π]: See nucleotides phosphorylation (5'-nucleoside amidodiphosphate) for methods; *: Based on the ^{1}H-NMR integral ratio average of C_6-*H* for **15a** and **19a**; [±]: no imidazole; [‡]: A fast amidophosphorylation of **15a** was observed during the first 24 h followed by the hydrolysis of **19a**. Since DAP was still present in the mixture during the first 6 days, a competition between hydrolysis and amidophosphorylation is observed when 3 equiv. of zinc chloride were used. [†]: ^{15}N-labeled imidazole was used; [※]: A substantial amount of side product were observed by NMR. NA: Not applicable; ND: Not determined

Supplementary Table 8:

Formation of 5'-NDP^{NH2} by the amidophosphorylation of 5'-NMP using DAP

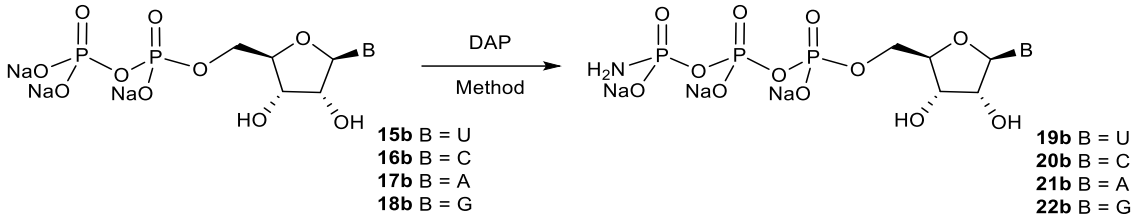


Entry	Method [π]	Base	Metal Chloride added (equiv.)	Time (days)	Conversion (%)* (% cUMP)
			Solution pH 5.5		
1	A	Cytosine	Zn (3)	2	88 [‡]
2	A	Adenine	Zn (3)	2	90 [‡]
3	A	Guanine	Zn (3)	2	87 [‡]
			Solution pH 7		
4	B	Cytosine	Mg (0.5)	5	81 (4)
5	B	Adenine	Mg (0.5)	5	79(ND)
6	B	Guanine	Mg (0.5)	5	77 (6)

[π]: See nucleotides phosphorylation (5'-nucleoside amidodiphosphate) for methods; *: Based on the ^{1}H-NMR integral ratio average of C_6-*H* for the pyrimidines and C_1'-*H* and C_8-*H* for the purines; [‡]: A fast amidophosphorylation of NMPs were observed during the first 24h followed by the slow hydrolysis of the resulting NDP^{NH2}s. Since DAP was still present in the mixture during the first 6 days a competition between hydrolysis and amidophosphorylation is observed when 3 equiv. of zinc chloride was used. ND: Not determined

Supplementary Table 9:

Formation of 5'-NTP^{NH2} by the amidophosphorylation of 5'-NDP using DAP

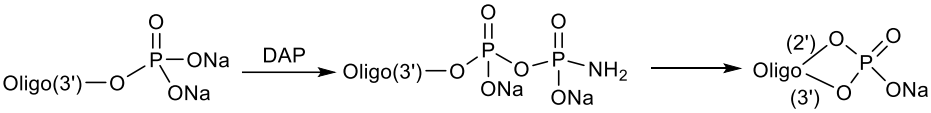


Entry	Method [π]	Base	Metal Chloride added (equiv.)	Time (days)	Conversion (%)*
			Solution pH 5.5, no imidazole		
1	A‡	Uracil	Zn (3)	11	83
2	A‡	Cytosine	Zn (3)	2	ND
3	A‡	Adenine	Zn (3)	2	ND
4	A‡	Guanine	Zn (3)	2	ND
			Solution pH 5.5		
5	B	Uracil	NA	2	42
6	B	Cytosine	NA	2	35
				5	45
7	B	Adenine	NA	2	39
				5	47
8	B	Guanine	NA	2	38
				5	44
			Solution pH 7		
9	C	Uracil	Mg (0.5)	2	85
				7	91
10	C	Cytosine	Mg (0.5)	2	50
				7	73
11	C	Adenine	Mg (0.5)	2	85
12	C	Guanine	Mg (0.5)	2	61
				7	79

[π]: See nucleotides phosphorylation (5'-nucleoside amidotriphosphate) for methods; *: relative conversion based on the ^{31}P-NMR ratio of the "α" phosphate of NDP and NTP^{NH2}; ‡ Addition of $ZnCl_2$ forms a white precipitate. The progress of the formation of **19b** only was monitored. For other nucleotides **16b**-**18b** the precipitate formation interfered with the monitoring by NMR. NA: Not applicable; ND: Not determined

Supplementary Table 10:

Amidophosphorylation of oligonucleotide-3'-(mono)phosphate

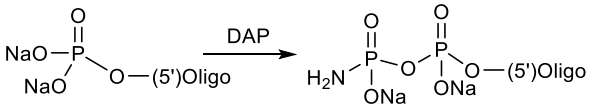


Entry	Oligophosphate	Time (days)	% conversion *
1	5’-A_4U_3A- PO_3^{2-}-3’	3	31#
2	5’-GGUGCCAGUC- PO_3^{2-}-3’	8	29#
3	5’-$d(T)_8$- PO_3^{2-}-3’	28	59

See oligonucleotides phosphorylation for method; * : based on the FPLC integration peak ratio of starting oligomer and phosphorylated oligomer using condition 2; #: 2’,3’-cyclic phosphates.

Supplementary Table 11:

Amidophosphorylation of oligonucleotide-5'-(mono)phosphate

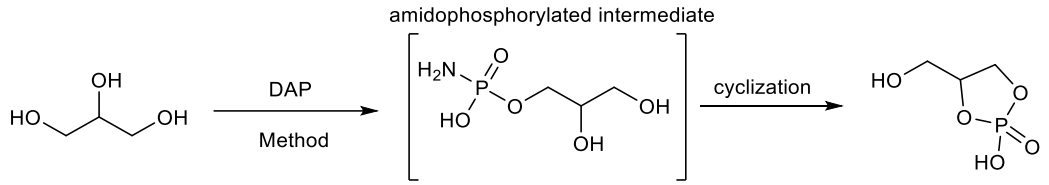


Entry	Oligophosphate	Time (days)	% conversion *
1	5'-PO_3^{2-}-A_{12}-3'	7	32
2	5'-PO_3^{2-}-U_{12}-3'	4	50‡
3	5'-PO_3^{2-}-UAUUAUUA-3'	7	23
4	5'-PO_3^{2-}-GGUUCUC-3'	7	30
5	5'-PO_3^{2-}—$d(T)_{10}$-3'	28	66±
6	5'-PO_3^{2-}—$d(T)_{12}$-3'	28	58±
7	d(5'- PO_3^{2-}-GCTACG- 3')	3	36

See oligonucleotides phosphorylation for method; *: based on the FPLC integration peak ratio of starting oligomer and phosphorylated oligomer using Condition 2. ‡: based on the FPLC integration peak ratio of starting oligomer and phosphorylated oligomer using Condition 1; ±: % conversion of the phosphorylated oligomer product is not exact due to unreacted dT_{10} and dT_{12} present in the commercial sample merging at the same retention time as the product.

Supplementary Table 12:

Formation of glycerol-2,3-cyclic phosphate **24** by the phosphorylation of glycerol **23** using DAP



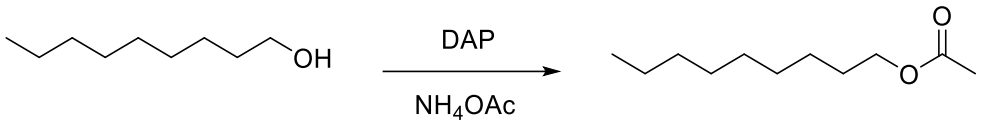
23 **24i** **24**

Entry	Method [π]	Time (days)	Conversion (%)*
		Solution at pH 7.5	
5	A	30	4
6	A	68	12
		Paste	
1	B	40	35
2	B	45	47
3	B	49	58
4	B	60	70
		Paste at 50 °C	
7	C	5	17
8	C	12	30
9	C	15	37
10	C	19	46
		Microwave	
11	D	5 hours	47
12	D	8 hours	52
13	D	11 hours	50
14	D	13 hours	49

[π] : See vesicles-like structures formation (glycerol-1,2-cyclophosphate) for methods; * : Based on the ^{1}H-NMR integral ratio of C_1-H_H for glycerol **23** and C_2-H for glycerol-1,2-cyclophosphate **24**

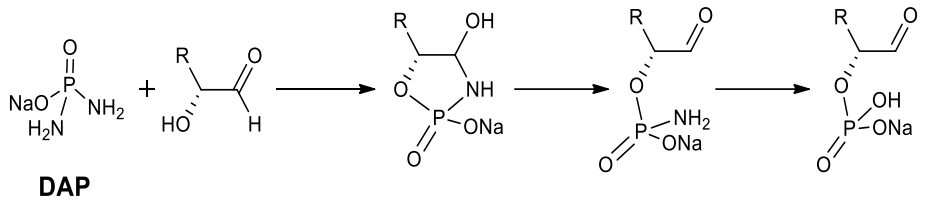
Supplementary Table 13.

DAP/imidazole mediated esterification of 1-nonanol with ammonium acetate

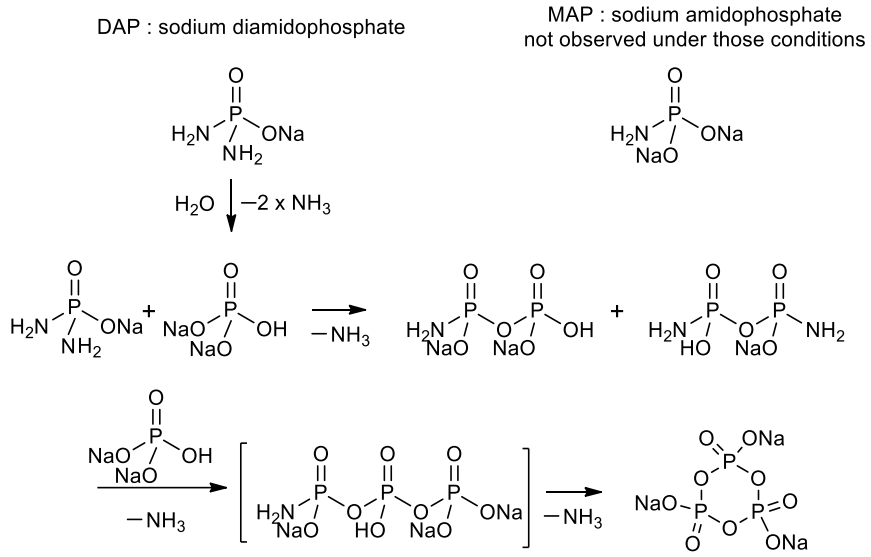


Entry	Time (days)	Conversion (%)*
1	3	9
2	15	16
3	22	20
4‡	22	7

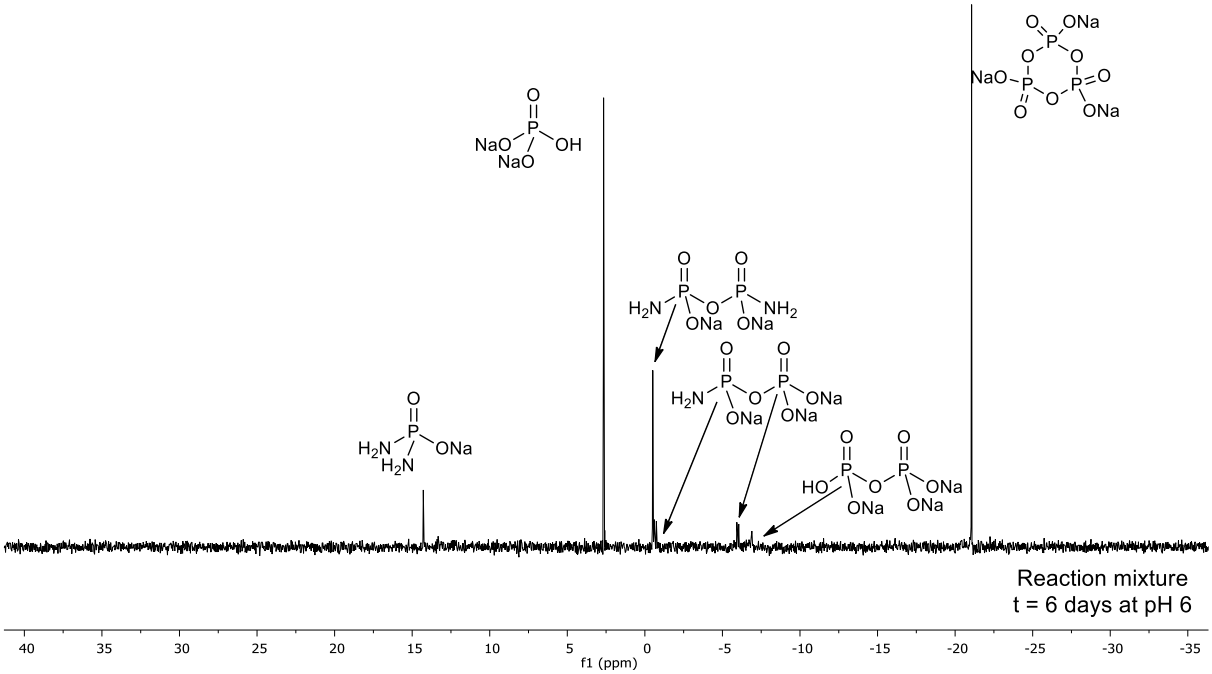
See vesicles-like structures formation (nonanoyl acetate) for methods; *: Based on the ^{1}H NMR integral ratio of C_8-*H* for 1-nonanol and -$COOCH_3$ for methyl nonanoate; ‡: Control experiment without DAP.



Supplementary Figure 1: DAP (sodium diamidophosphate) mediated intramolecular phosphorylation of α-hydroxy aldehyde.[10]

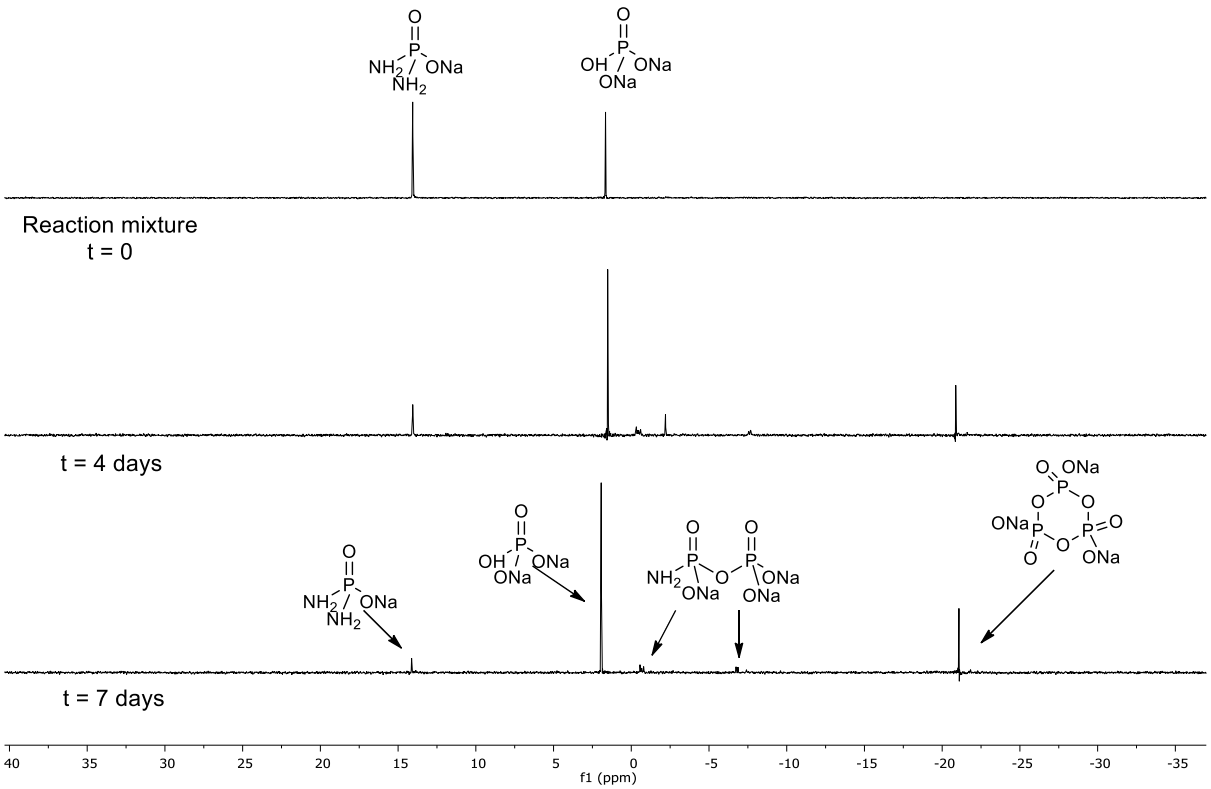


Supplementary Figure 2:
Self-condensation of DAP alone in water at pH 6. ^{31}P NMR (Supplementary Fig. 1) shows the formation of various condensed phosphates.



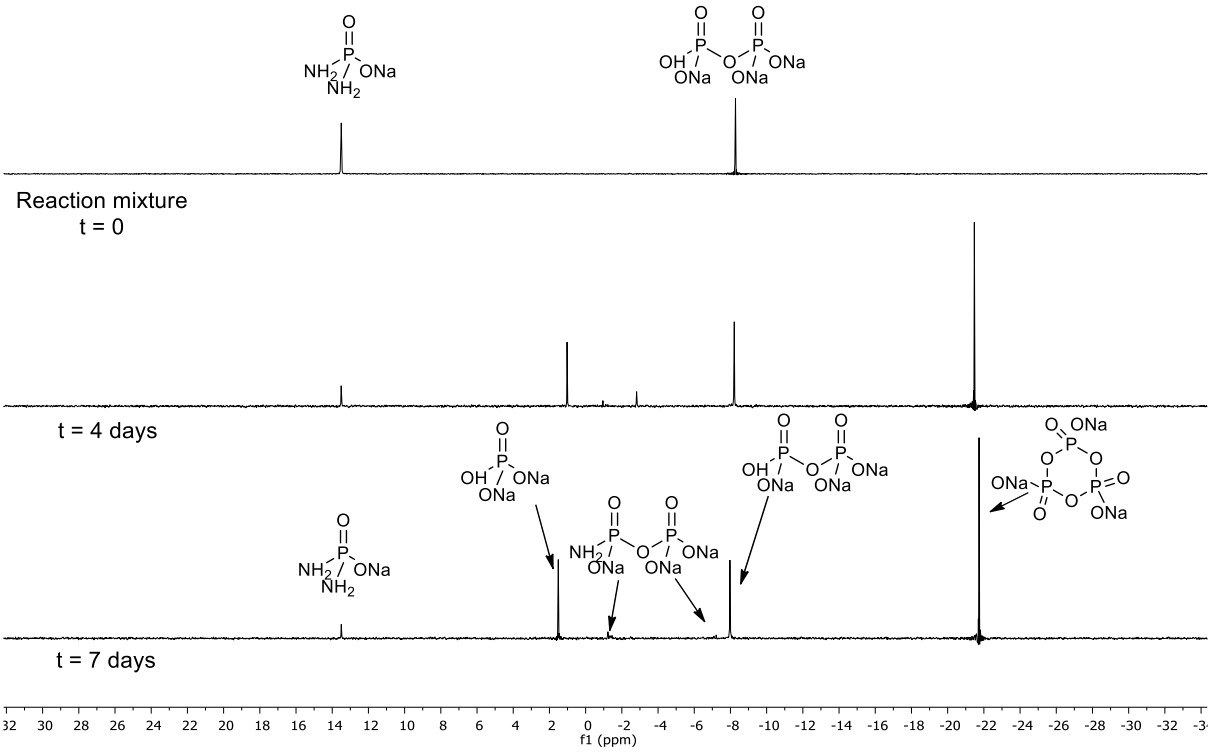
Supplementary Figure 3:

{H-decoupled}^{31}P NMR spectrum of the self-condensation of DAP alone at pH 6 after 6 days in D_2O. DAP (0.08 M) in 1 mL of H_2O (initial pH 8) was adjusted to pH 5 ~ 6 by the addition on IR-120 (H^+) resin. The mixture was maintained at room temperature and the reaction progress was monitored by ^{31}P NMR.



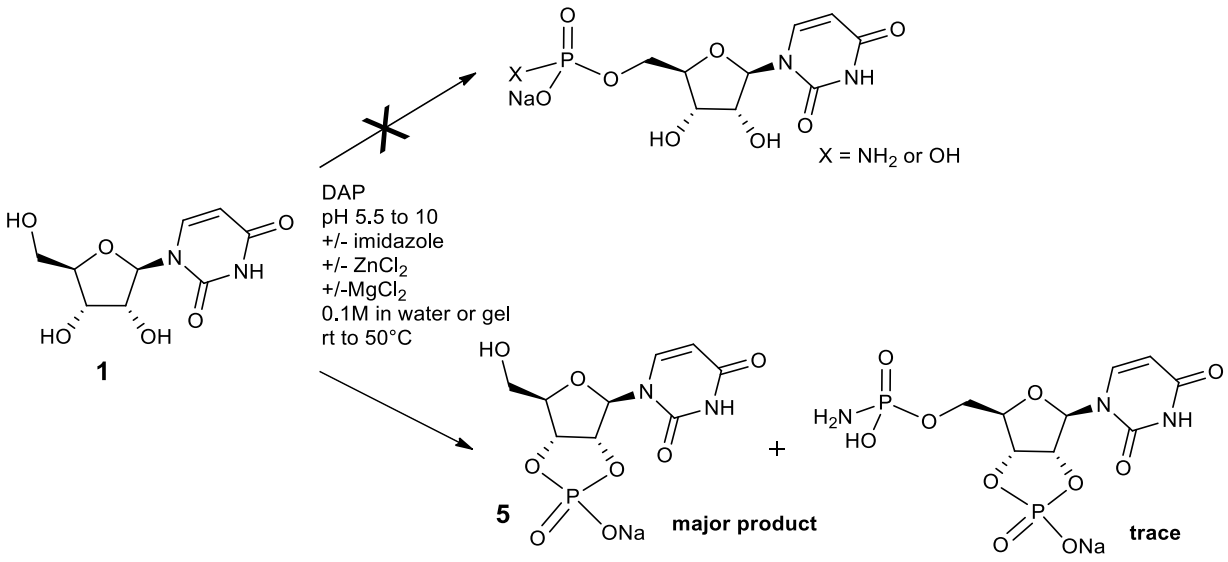
Supplementary Figure 4:

{H-decoupled}^{31}P NMR spectra of the condensation reaction of DAP in the presence of orthophosphate at pH 6 after 4 and 7 days in D_2O. Method: Phosphoric acid (13 µL, 0.25 mmol) was dissolved in 3 mL of H_2O. The pH was adjusted to 7 by the addition of 0.1 M NaOH. Then, H_2O was added to make the total volume 5 mL. DAP was added to make a 0.05M solution and pH was again adjusted to pH 6 by addition of IR-120 (H^+) resin.



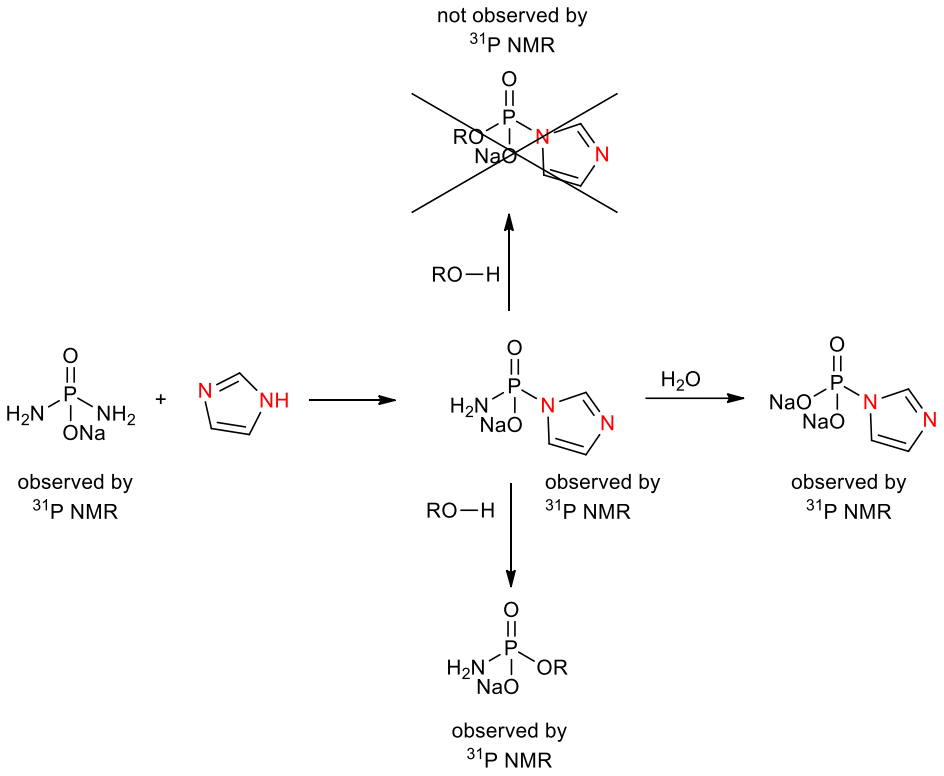
Supplementary Figure 5:

{H-decoupled}^{31}P NMR spectra of the condensation reaction of DAP in the presence of pyrophosphate at pH 6 after 4 and 7 days in D_2O. To DAP (0.05 M) in 5 mL of water was added tetra potassium diphosphate (82.6 mg, 0.25 mmol). The pH was adjusted to 6 by addition of IR-120 (H^+) resin.



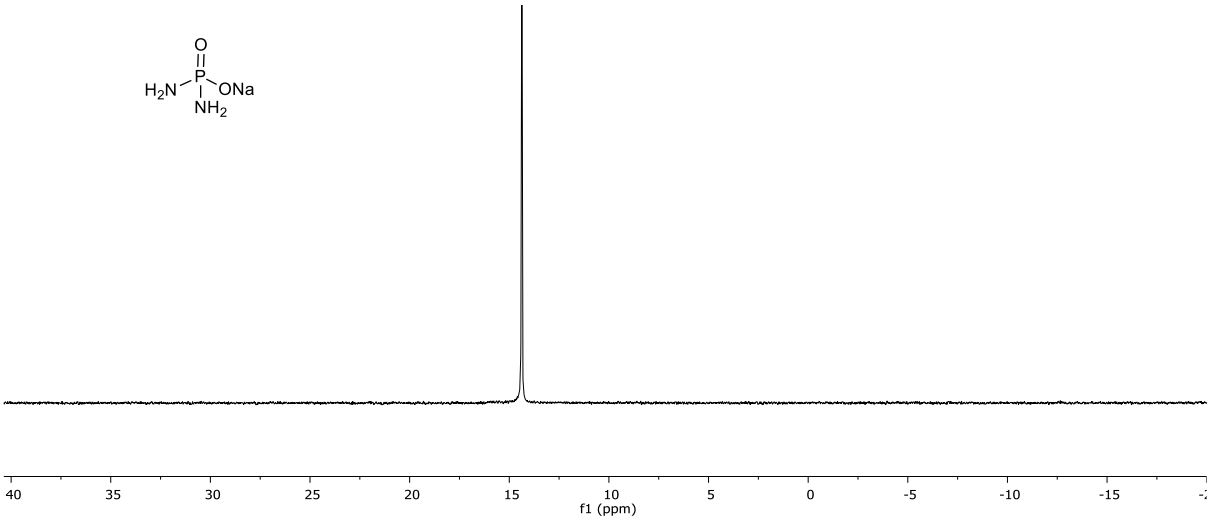
Supplementary Figure 6:

Phosphorylation of **1** afforded 2',3'-cUMP **5** as the major product and traces of the 5'-amidophosphate of 2',3'-cUMP under certain conditions (for 5'-amidophosphorylated c-UMP formation see entry 16 of the Supplementary Table 1 and Supplementary Fig. 93). No trace of the mono phosphate 5'-UMP was observed under the conditions studied.



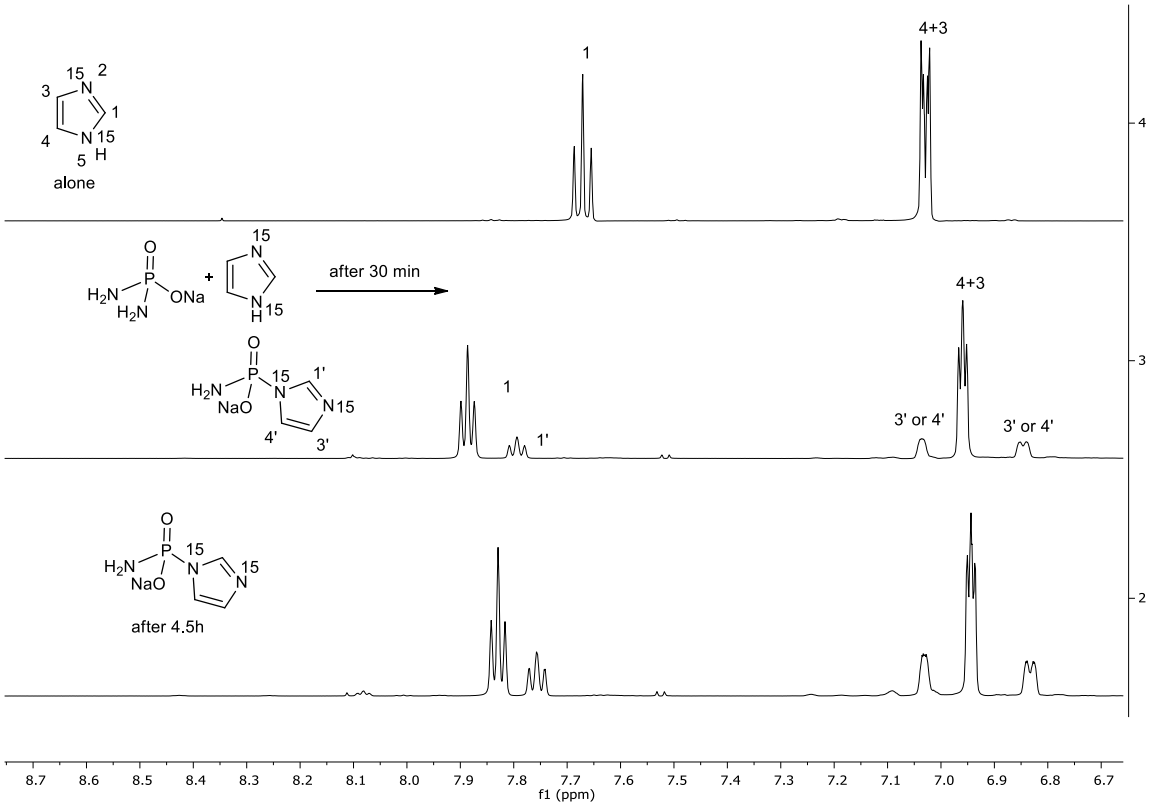
Supplementary Figure 7:

Mechanism of phosphorylation using imidazole. For the ^{15}N-labeled imidazole and ^{15}N-labeled DAP NMR studies please see Supplementary Fig. 9-16.



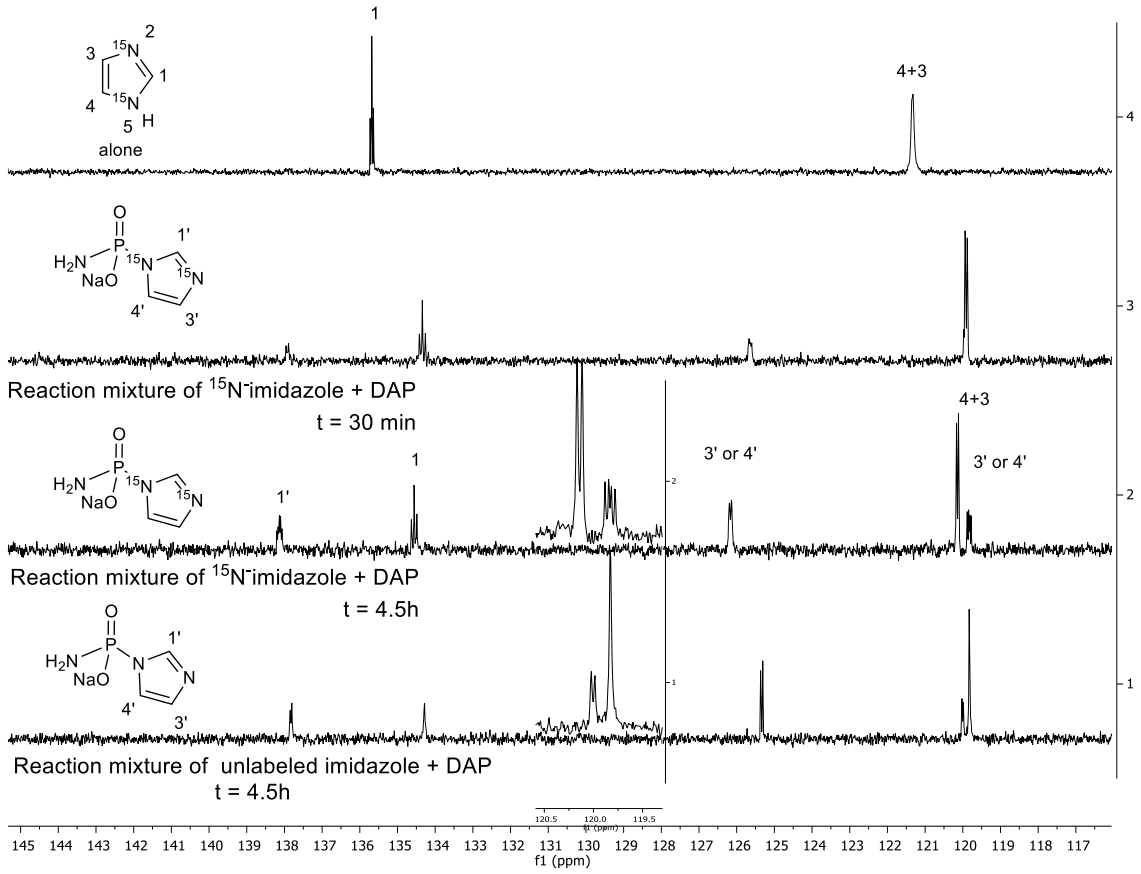
Supplementary Figure 8:

{H-coupled} ^{31}P NMR spectrum of 0.1 M DAP in D_2O. The pH was at 8.1 and was not adjusted.



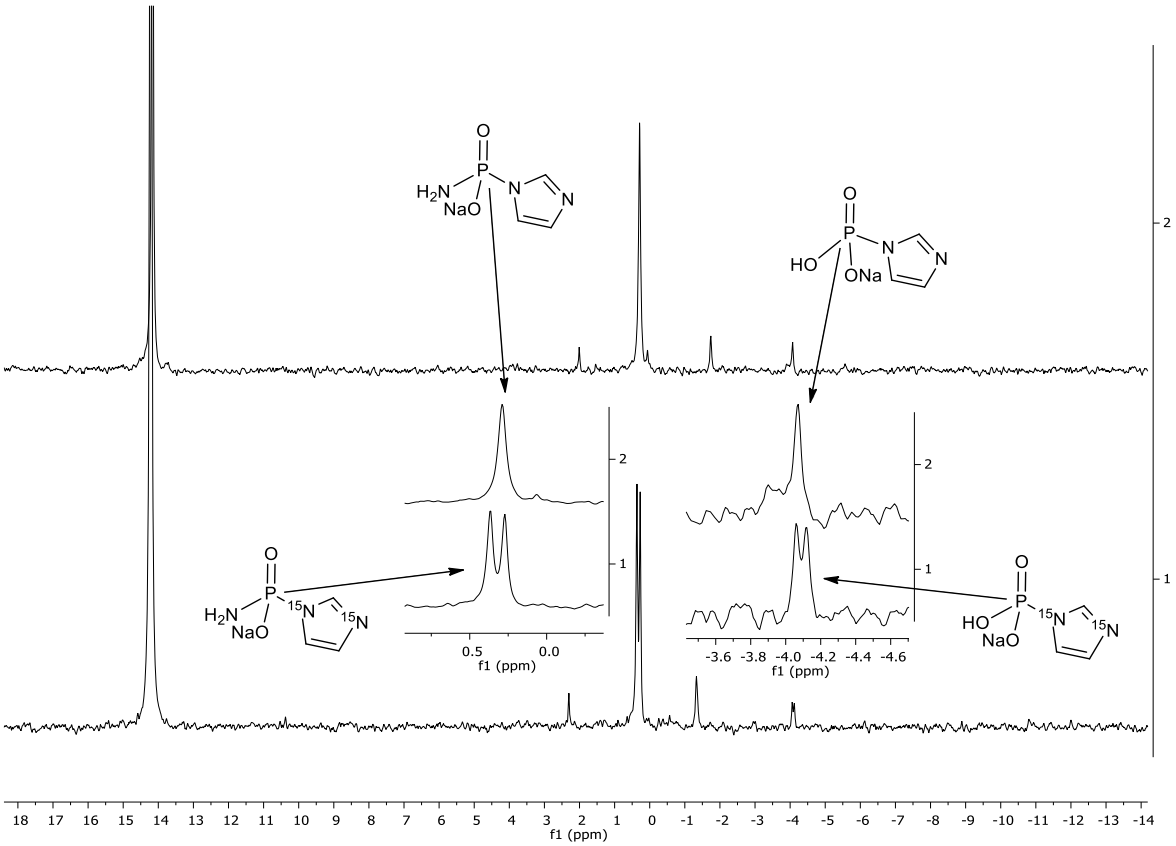
Supplementary Figure 9:

^{1}H NMR spectra showing the reaction with 1 equiv. of DAP and 1 equiv. of ^{15}N-labeled imidazole at pH 7 (pH adjusted by amberlite-H^{+} resin) at 0.1 M in D_2O. The top spectrum shows ^{15}N-labeled imidazole alone. Then from the top to the bottom are shown the time progress of the reaction of DAP and ^{15}N-labeled imidazole after 30 min and 4.5 h respectively. After addition of DAP, three new signals appear indicating that imidazole binds to the phosphorus center by replacing one of the -NH_2 of DAP



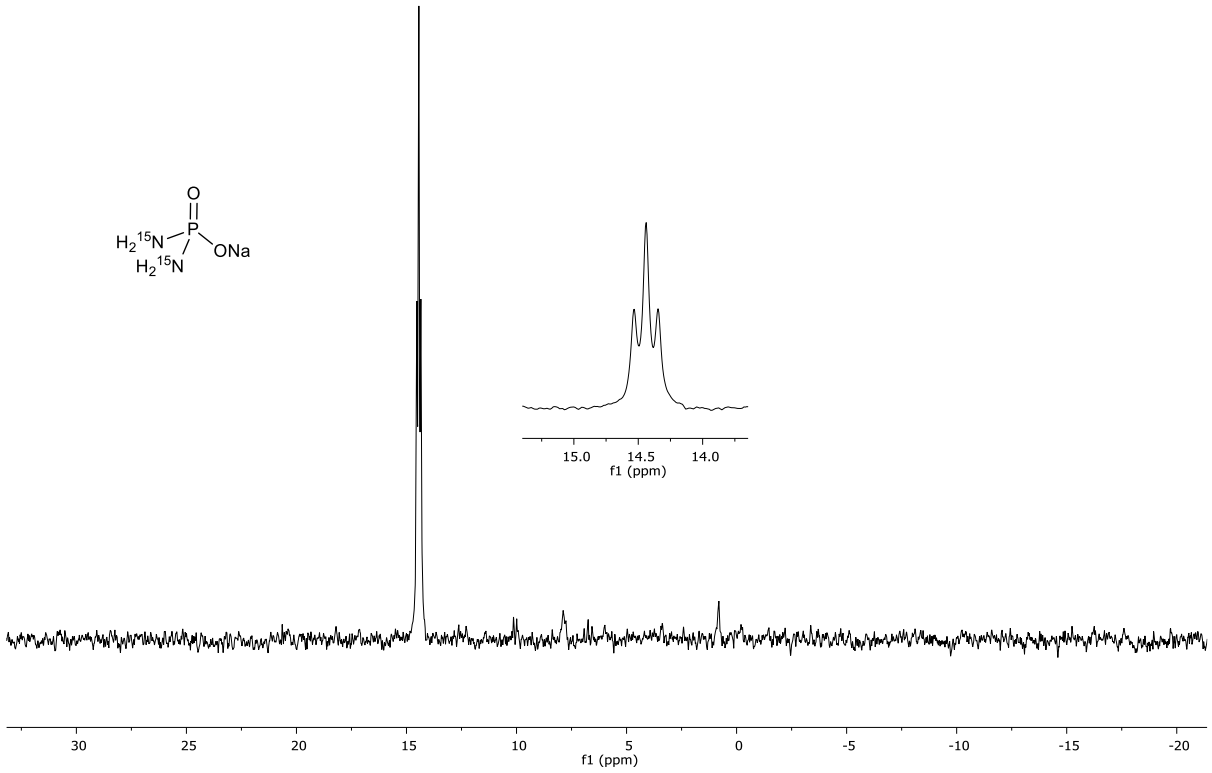
Supplementary Figure 10:

^{13}C NMR spectra of the reaction of 1 equiv. of DAP and 1 equiv. of ^{15}N-labeled imidazole at pH 7 (pH adjusted by amberlite-H^+ resin) at 0.1 M in D_2O. The top spectrum shows ^{15}N-labeled imidazole alone. Then from the spectrum 2 (from top) to spectrum 3 show the time progress of the reaction of DAP and ^{15}N-labeled imidazole after 30 min and 4.5 h respectively. The bottom spectrum shows the reaction of DAP with unlabeled imidazole after 4.5 h for comparison.

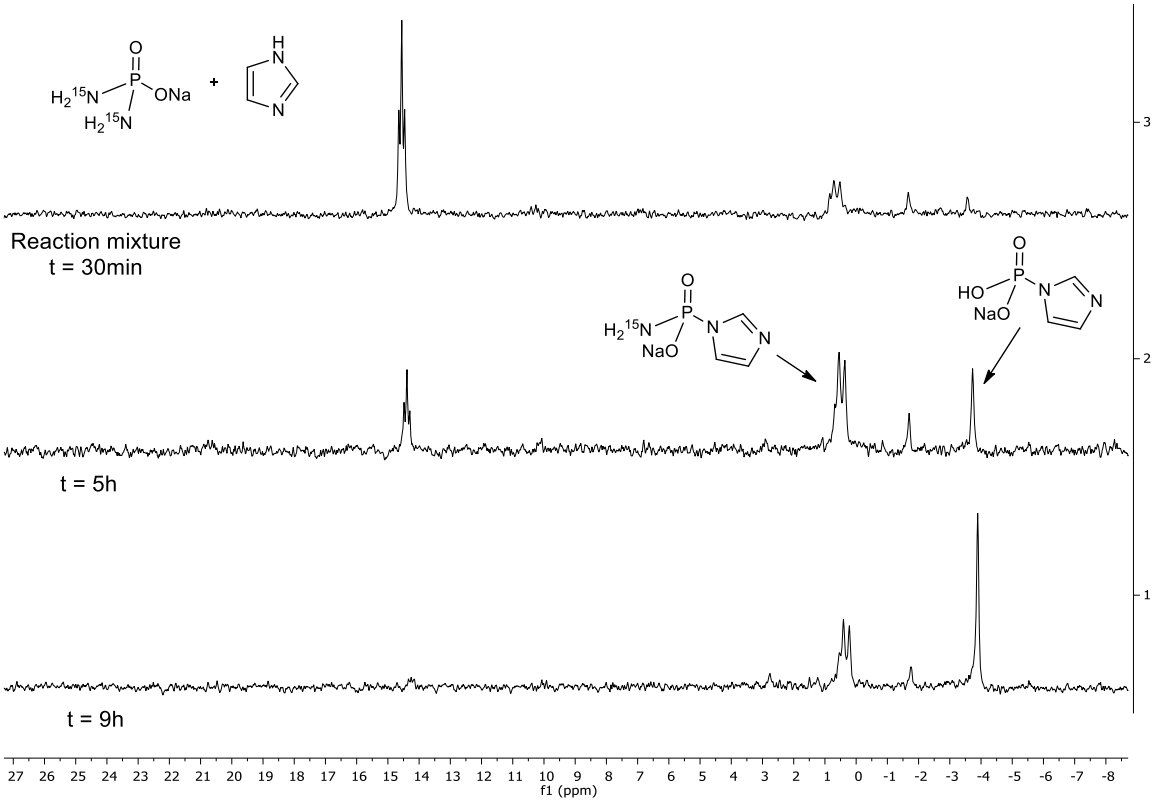


Supplementary Figure 11:

{H-decoupled}^{31}P NMR spectra comparison of the reaction of DAP with unlabeled imidazole (top spectrum) and with ^{15}N-labeled imidazole (bottom spectrum) after 4.5 h at pH 7 (pH adjusted by amberlite-H^+ resin) at 0.1 M in D_2O.

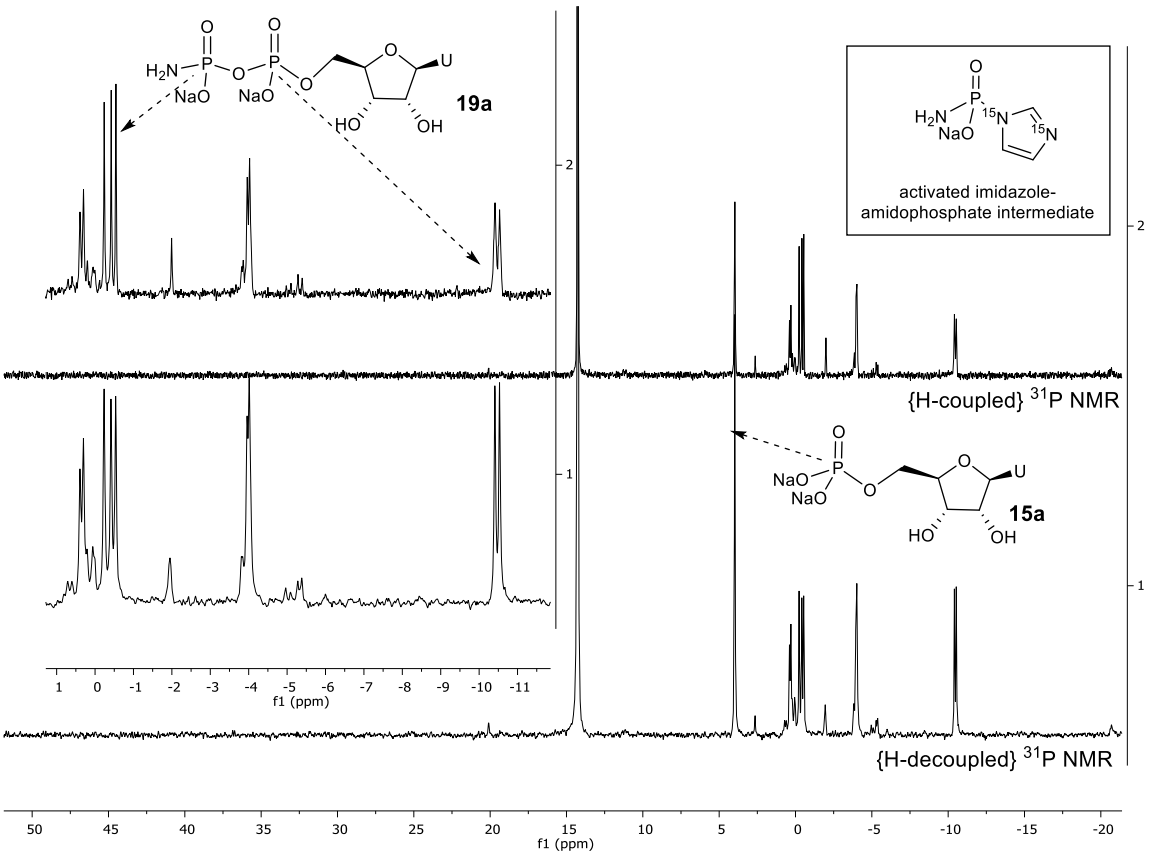


Supplementary Figure 12:
{H-decoupled} ^{31}P NMR spectrum of ^{15}N-labeled DAP alone in D_2O at pH 9.5



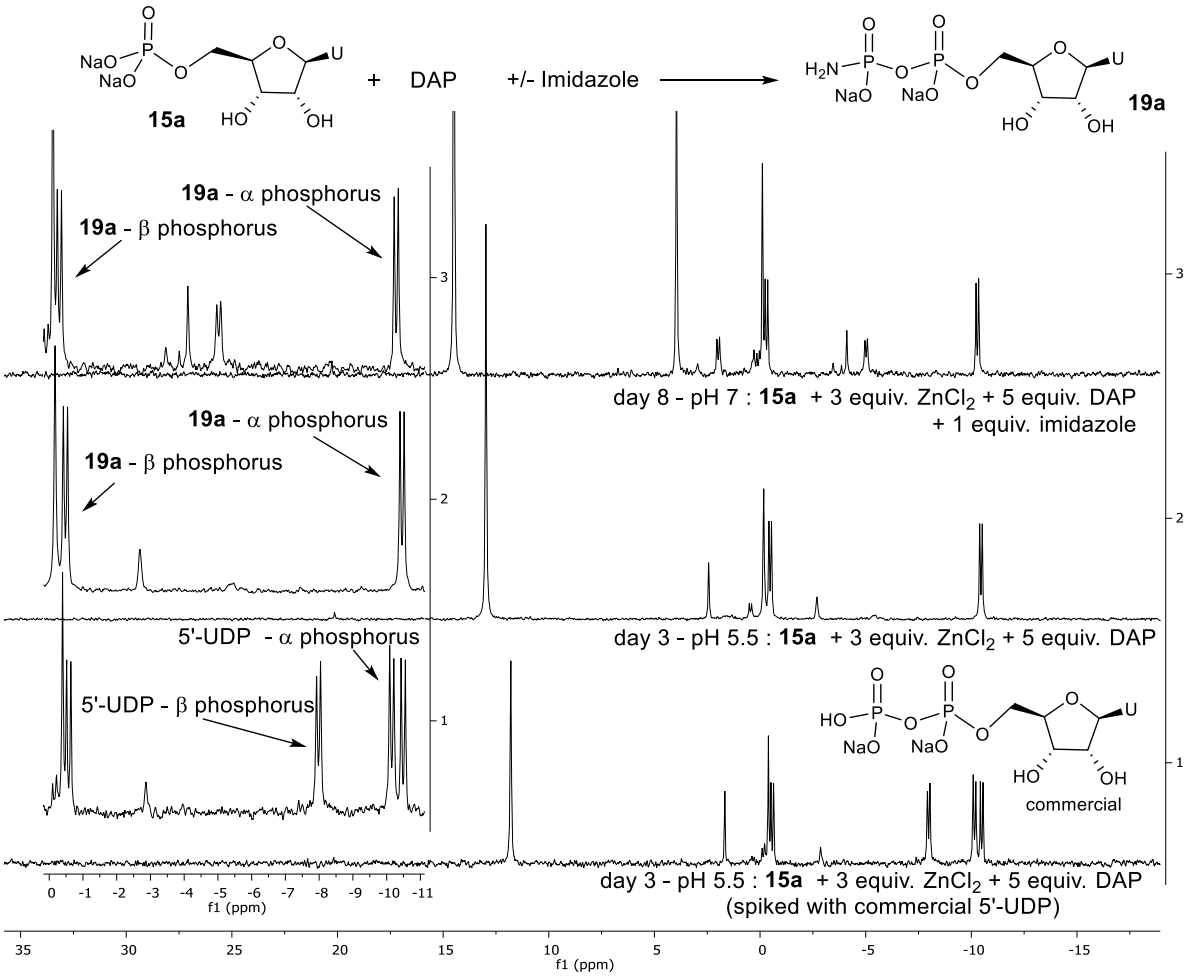
Supplementary Figure 13:

{H-decoupled} ^{31}P NMR spectra of the reaction of 1 equiv. of ^{15}N-labeled DAP and 1 equiv. of imidazole at pH 7 (pH adjusted with 4 M HCl) at 0.1 M in D_2O. This study further confirms the assignment of the constitution of the species. The peak at ~0.5 ppm is attributed to the compound containing both $–NH_2$ (doublet) and imidazole ligand (Supplementary Figure. 7).



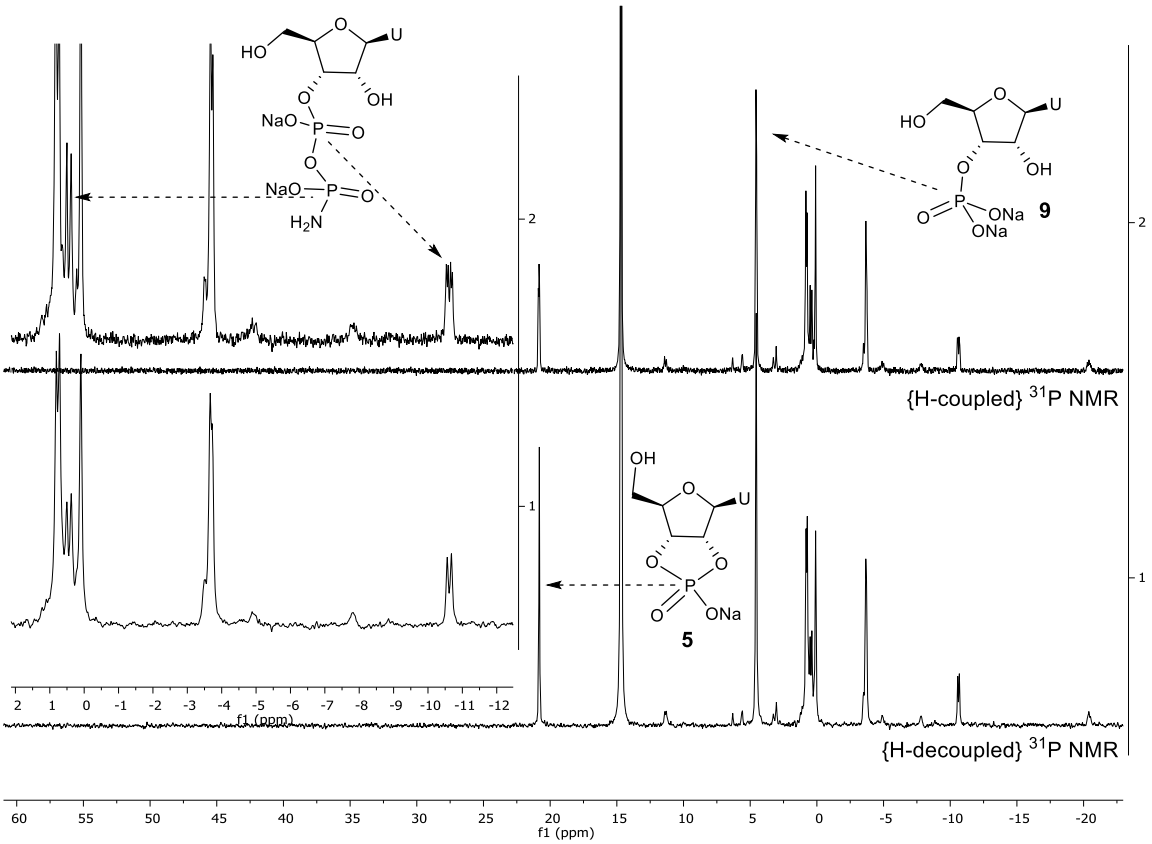
Supplementary Figure 14:

{H-coupled} ^{31}P NMR (top) and {H-decoupled} ^{31}P NMR (bottom) spectra of the reaction of 15a (1 equiv.), ^{15}N-labeled imidazole (1 equiv.) and DAP (5 equiv.) in D_2O (0.1 M) at pH 7 after 24 h of reaction. The results confirm that the imidazole, not -NH_2 of the activated imidazole-amidophosphate intermediate acts as the leaving group. If the NH_2 would have been the leaving group, imidazole would bind to the phosphorus in product **19a** and the phosphorus (at -0.5 ppm) would be a quadruplet because it would be coupled both to the phosphorus and to the ^{15}N-imidazole. Also, the chemical shifts are in accordance with a NH_2 bound to the phosphorous of **19a**.



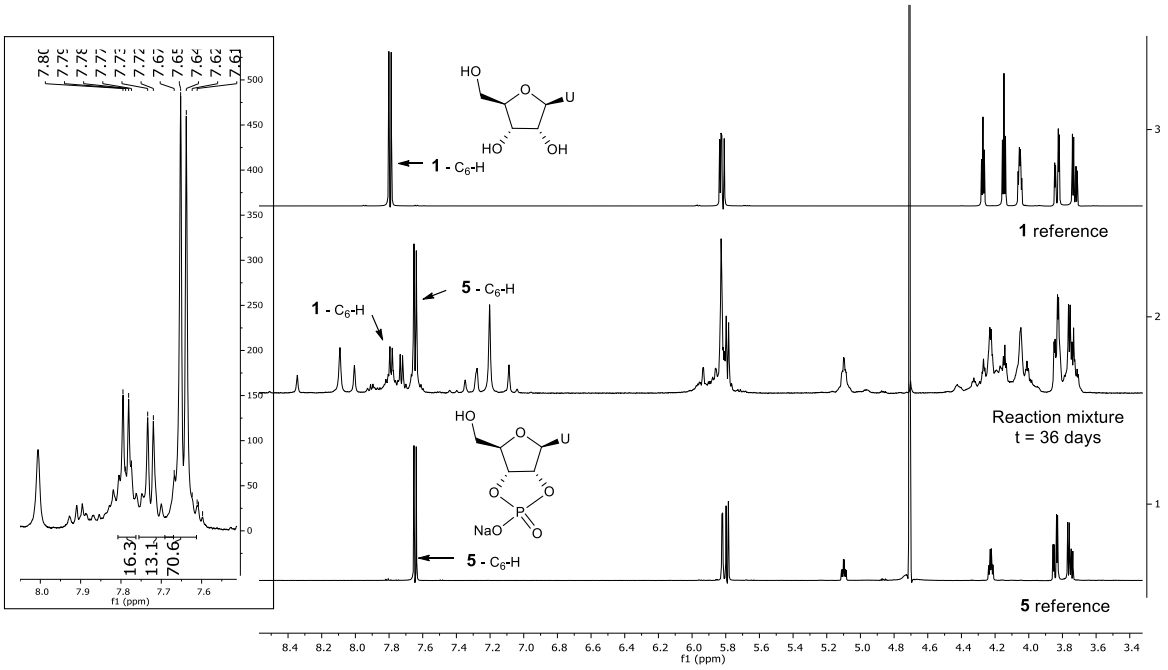
Supplementary Figure 15:

{H-decoupled} ^{31}P NMR spectra of the reaction of **15a** with imidazole (top spectrum), without imidazole (middle spectrum) and spiking of reaction without imidazole with commercial 5'UDP (bottom spectrum). The fact that the phosphorus signal of **19a** are similar with and without imidazole and that those signals do not superimpose with the phosphorus signals of the commercial 5'-UDP further confirms that NH_2 is bound to the terminus phosphate.



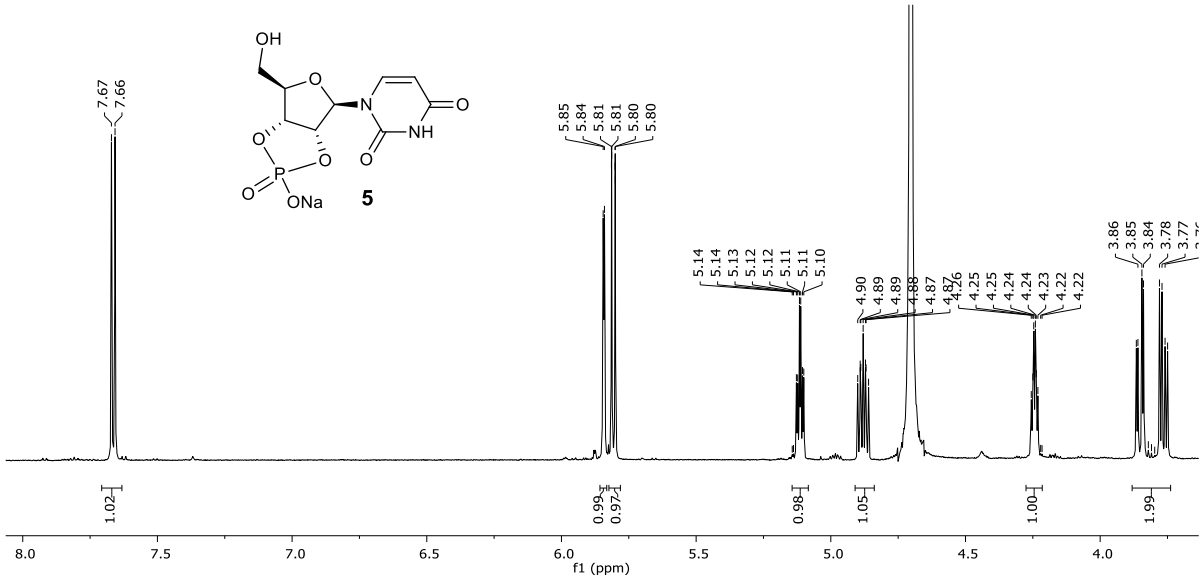
Supplementary Figure 16:

{H-coupled} ^{31}P NMR (top) and {H-decoupled} ^{31}P NMR (bottom) spectra of the reaction of 9 (1 equiv.), ^{15}N-labeled imidazole (1 equiv.) and DAP (5 equiv.) in D_2O (0.1M) at pH 7 after 24 h of reaction. If the imidazole was bound to the phosphorus of the 3'-diphosphate intermediate the phosphorus (at -0.5 ppm) of the intermediate would be a quadruplet due to coupling with both the phosphorus and the ^{15}N -labeled imidazole.



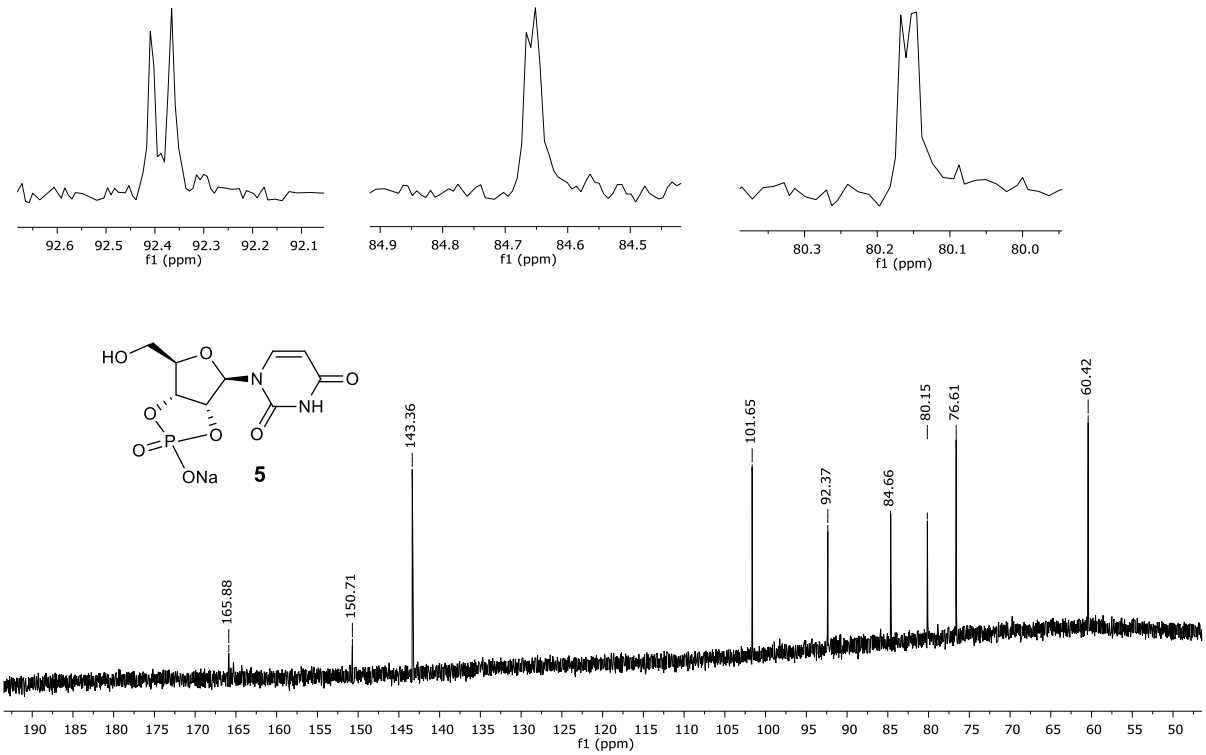
Supplementary Figure 17:

{Water suppression} ^{1}H NMR spectra of gram scale phosphorylation of **1** leading to the formation of **5** after 36 days in paste-reaction following the method C (Supplementary Table 1, entry 16, middle spectrum). The spectra of **1** (uridine, top spectrum) and **5** (cUMP, bottom spectrum) are shown for comparison.



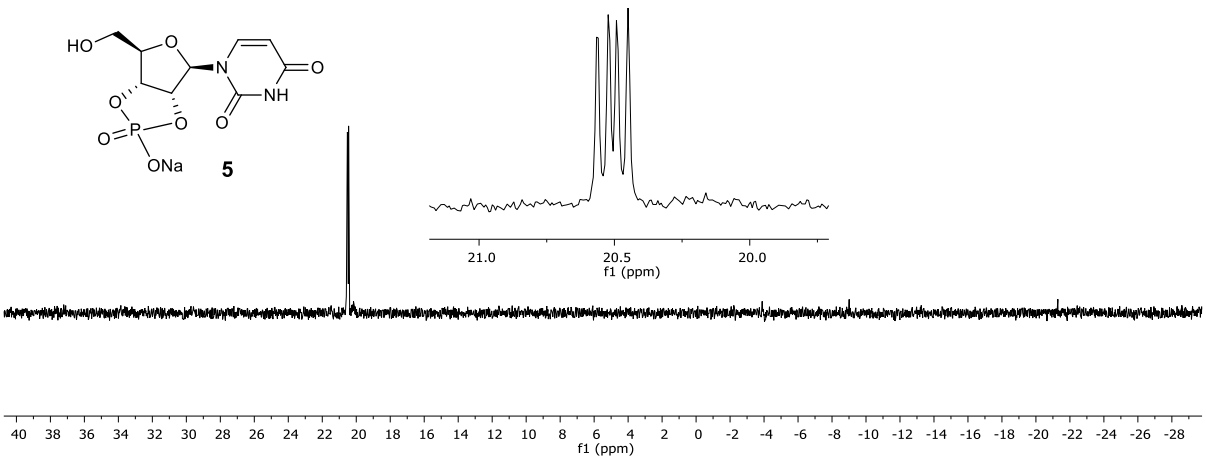
Supplementary Figure 18:

^{1}H NMR spectrum of 5 in D_2O at pH 7 after purification. The product was obtained pure in the fraction eluted with 0.50 M of TEAB buffer after the SEPHADEX column.



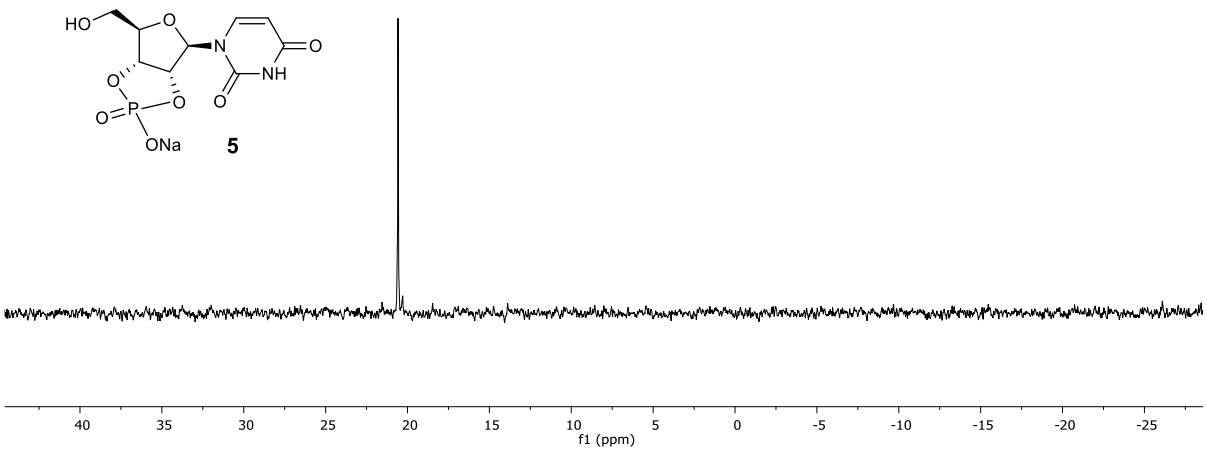
Supplementary Figure 19:

^{13}C NMR spectrum of **5** in D_2O at pH 7 after purification. Expansion of the 92, 84 and 80 ppm area shows the ^{31}P-^{13}C-coupling for C_1', C_4' and C_2' respectively. The product was obtained pure in the fraction eluted with 0.50 M of TEAB buffer after the SEPHADEX column.



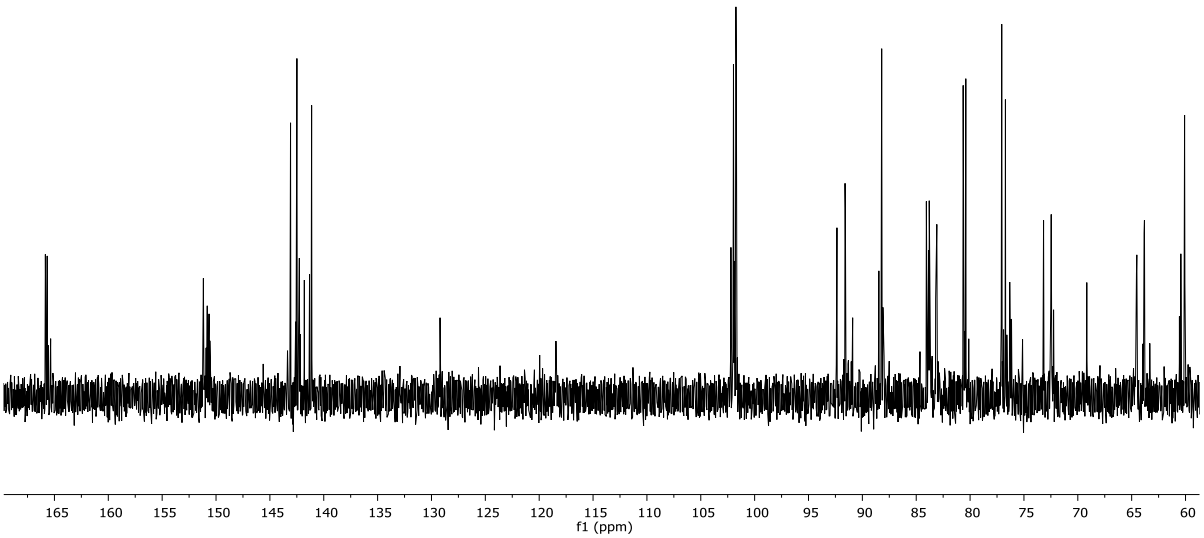
Supplementary Figure 20:

{H-coupled} ^{31}P NMR spectrum of **5** in D_2O at pH 7 after purification. The zoom of the signal at 20.5 ppm shows a doublet of doublet because of the coupling between the phosphate and the C_2'-H and C_3'-H protons. The product was obtained pure in the fraction eluted with 0.50 M of TEAB buffer after the SEPHADEX column.



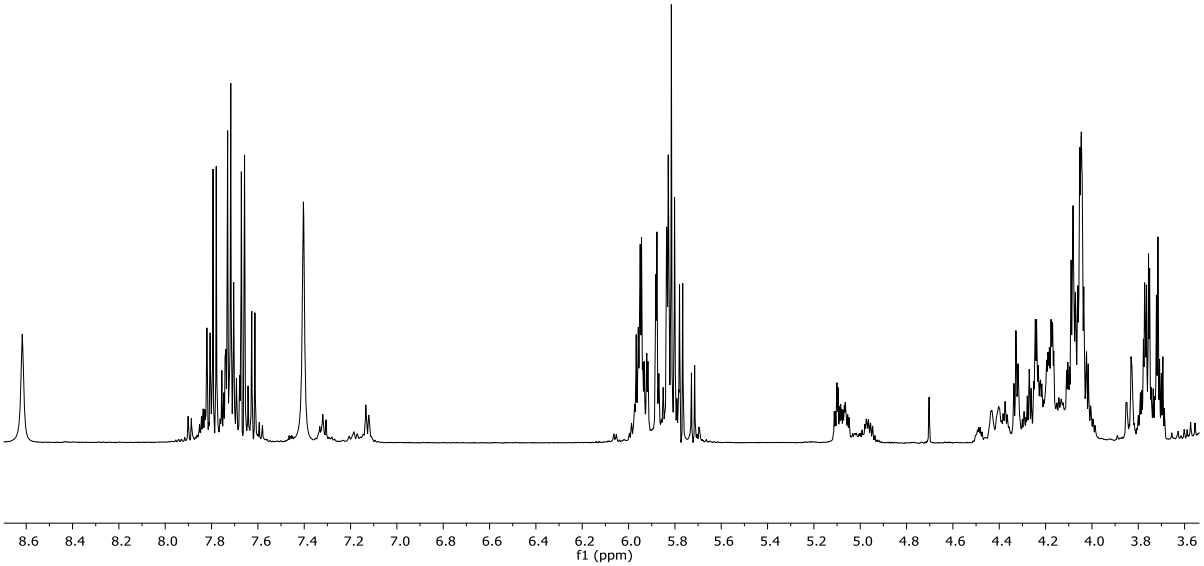
Supplementary Figure 21:

{H-decoupled} ^{31}P NMR spectrum of **5** in D_2O at pH 7 after purification. The product was obtained pure in the second fraction eluted with 0.50 M of TEAB buffer after the SEPHADEX column.



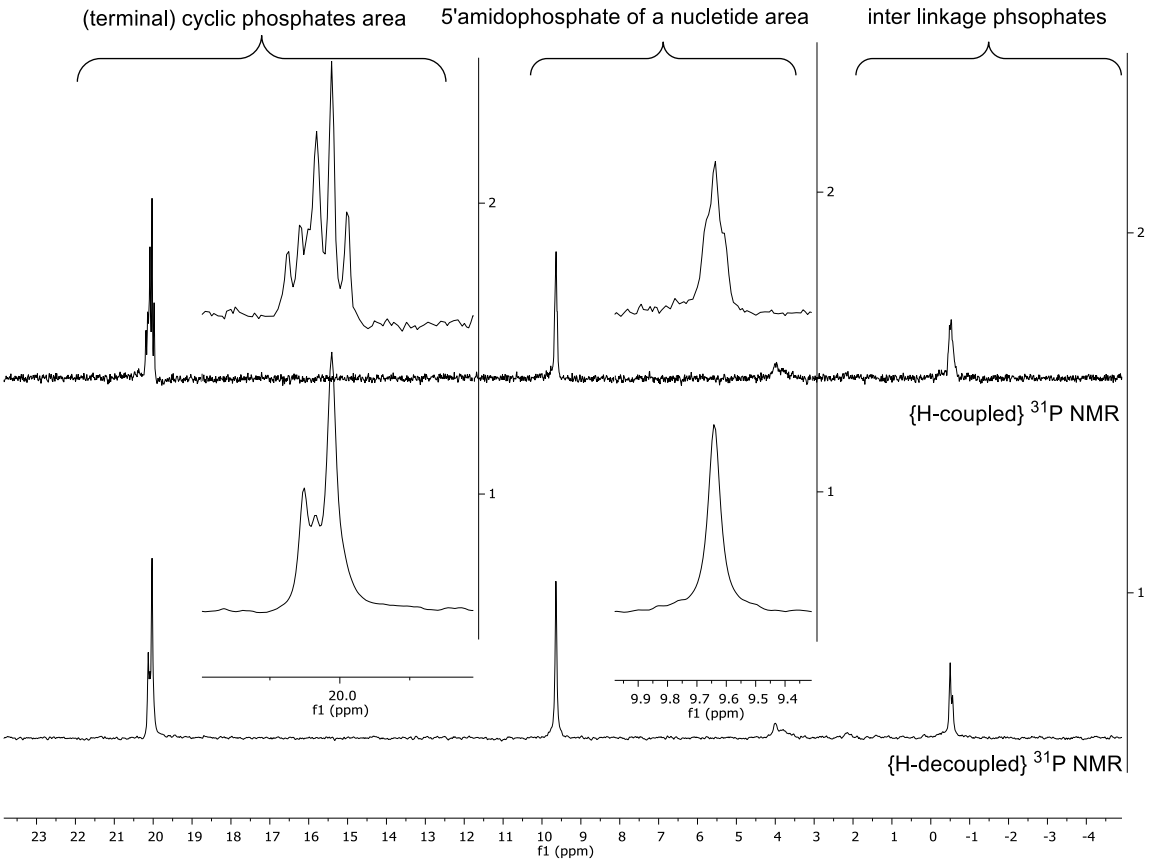
Supplementary Figure 22:

^{13}C NMR spectrum of the second fraction eluted with 0.75M of TEAB buffer, last fraction of the 1 gram paste-reaction purification containing UV active products. This mixture of products was analyzed by mass spectroscopy that showed the presence of 5' amidophosphate of **5** together with dimer, trimer, tetramer of uridine.



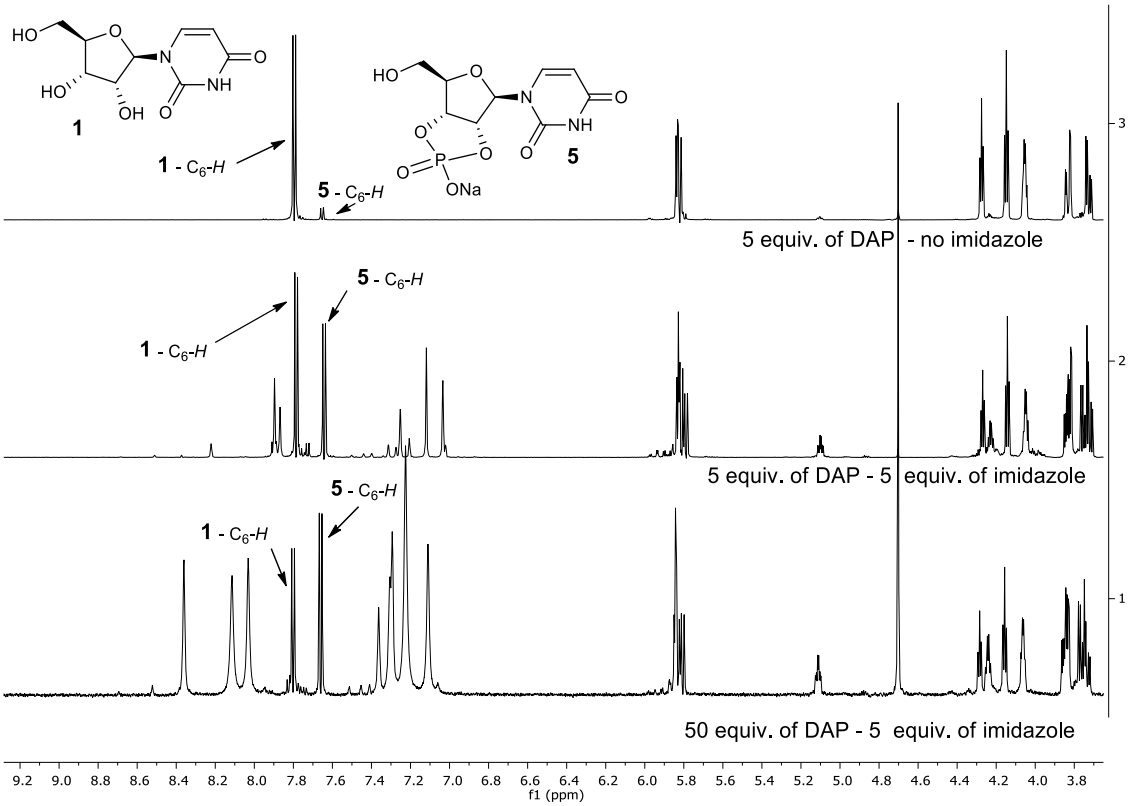
Supplementary Figure 23:

^{1}H NMR spectrum of the second fraction eluted with 0.75 M of TEAB buffer, last fraction of the 1 gram paste-reaction purification containing UV active products. This mixture of product was analyzed by mass spectroscopy that showed the presence of 5' amidophosphate of **5** together with dimer, trimer, tetramer of uridine.



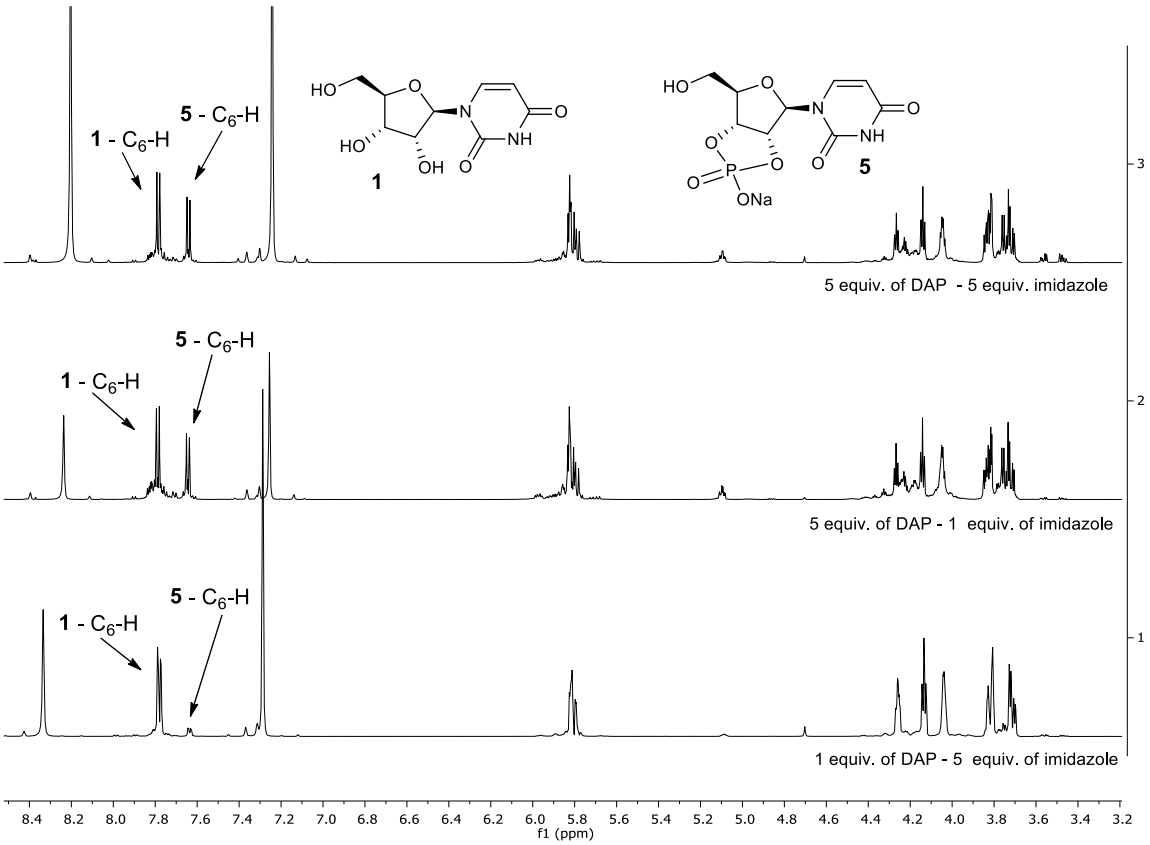
Supplementary Figure 24:

{H-coupled} ^{31}P NMR spectra of the fraction eluted with 0.75-2 M of TEAB buffer, last fraction of the 1 gram paste-reaction purification containing UV active products. This mixture of products was analyzed by mass spectroscopy that showed the presence of 5' amidophosphate of **5** together with dimer, trimer, tetramer of uridine.



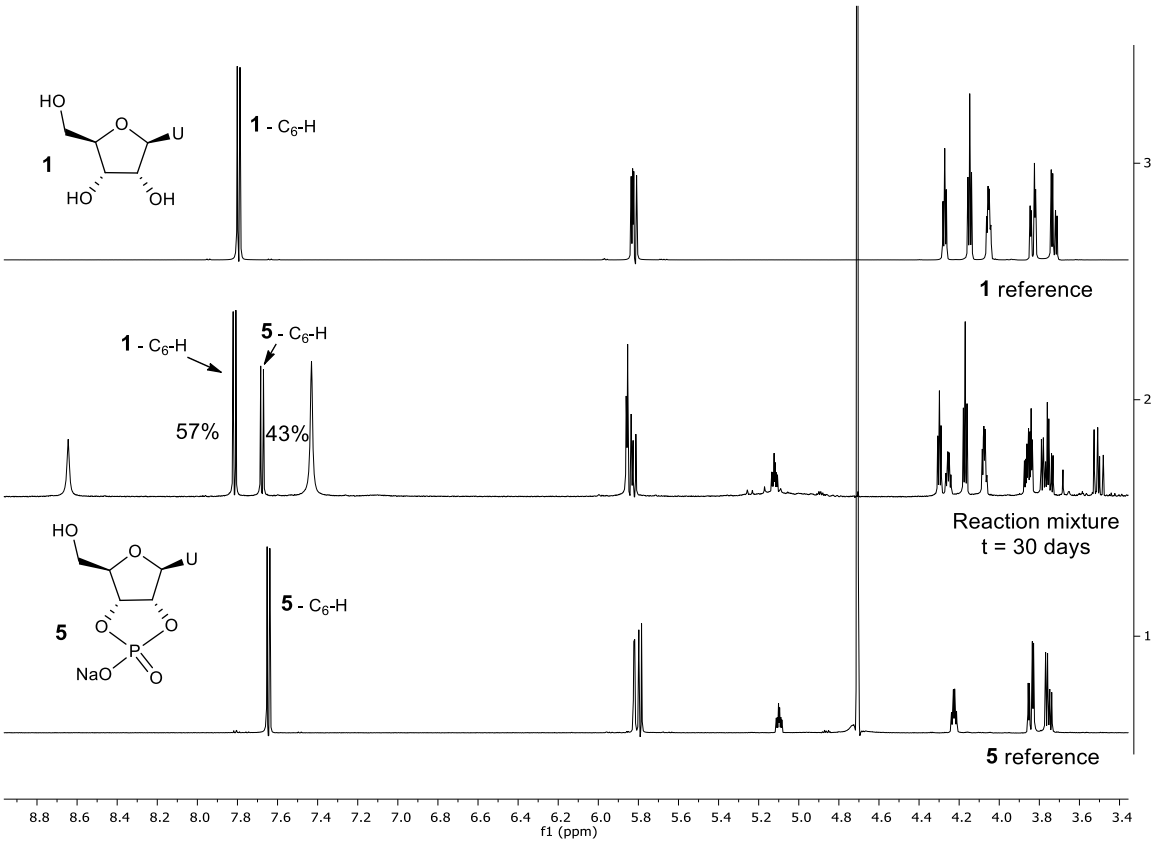
Supplementary Figure 25:

{Water suppression} ^{1}H NMR spectra of the phosphorylation of **1** in paste-reaction conditions at room temperature. Comparison between 5 equiv. of DAP and no imidazole (top), 5 equiv. of DAP and 5 equiv. of imidazole (middle) and 50 equiv. of DAP and 5 equiv. of imidazole (bottom). The spectra were recorded after 7 days of reaction by dissolving an aliquot of the crude paste-reaction in 500 µL of D_2O followed by adjusting the pH to 7 with 4 M HCl.



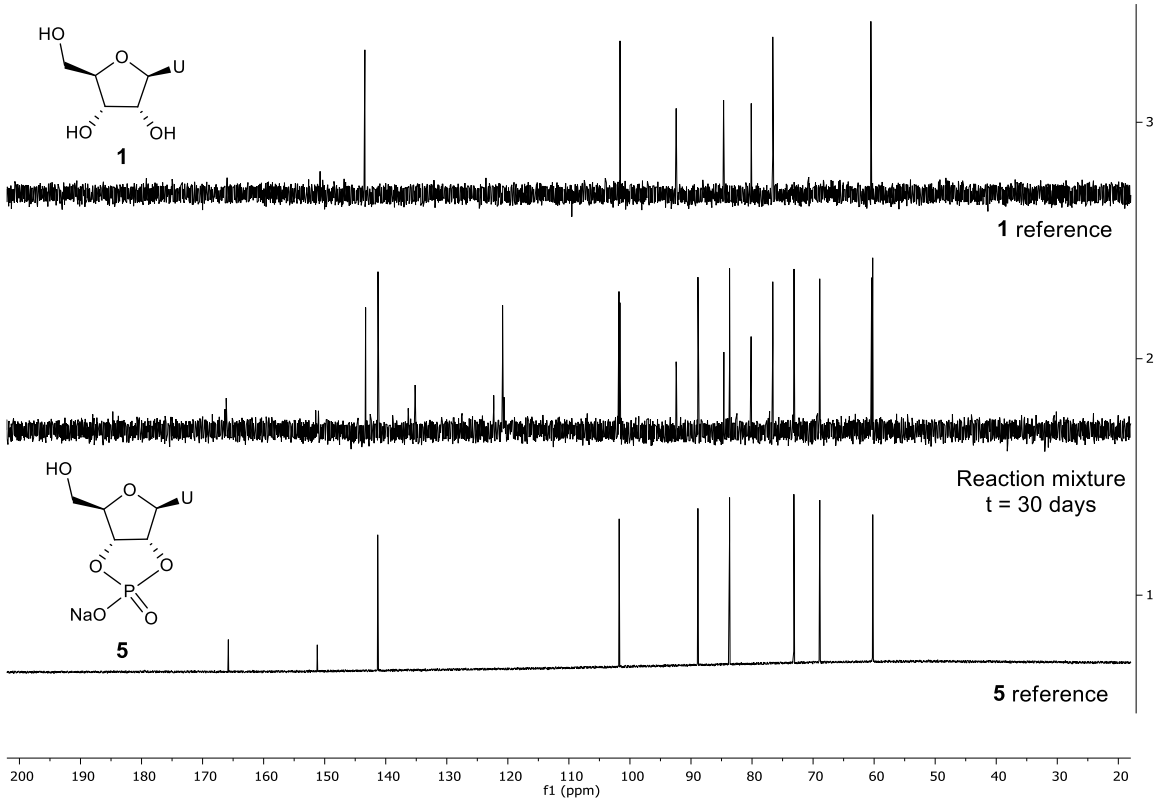
Supplementary Figure 26:

{Water suppression} ^{1}H NMR spectra of the phosphorylation of **1** in paste-reaction conditions at 40°C. Comparison between 5 equiv. DAP and 5 equiv. imidazole (top), 5 equiv. DAP and 1 equiv. imidazole (middle) and 1 equiv. DAP and 5 equiv. imidazole (bottom). The spectra were recorded after 4 days of reaction by dissolving an aliquot of the crude paste-reaction in 500 µL of D_2O followed by adjusting the pH to 7 with 4 M HCl.



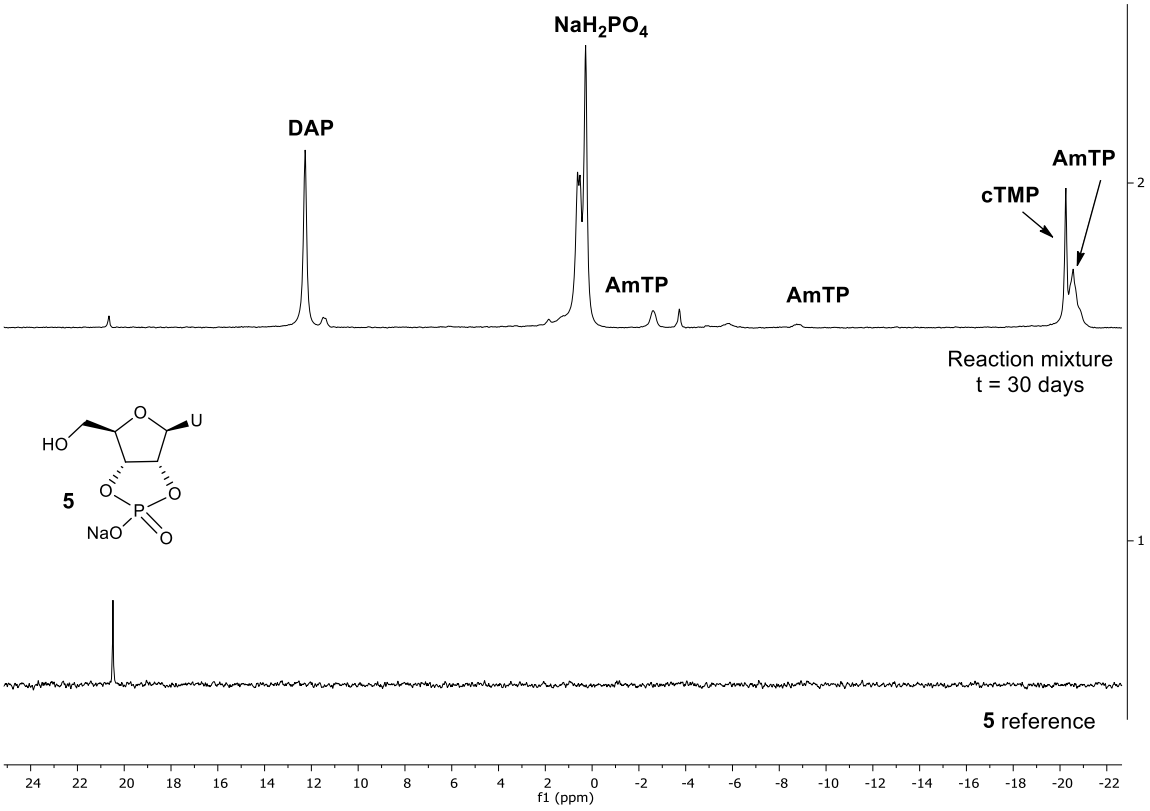
Supplementary Figure 27:

{Water suppression} ^{1}H NMR spectra of phosphorylation of **1** in solution at pH 5.5 in D_2O following the Method A (Supplementary Table 1, entry 5, day 30, middle spectrum). The spectra of starting material **1** (top spectrum) and reference product **5** (cUMP, bottom spectrum) are shown for comparison.



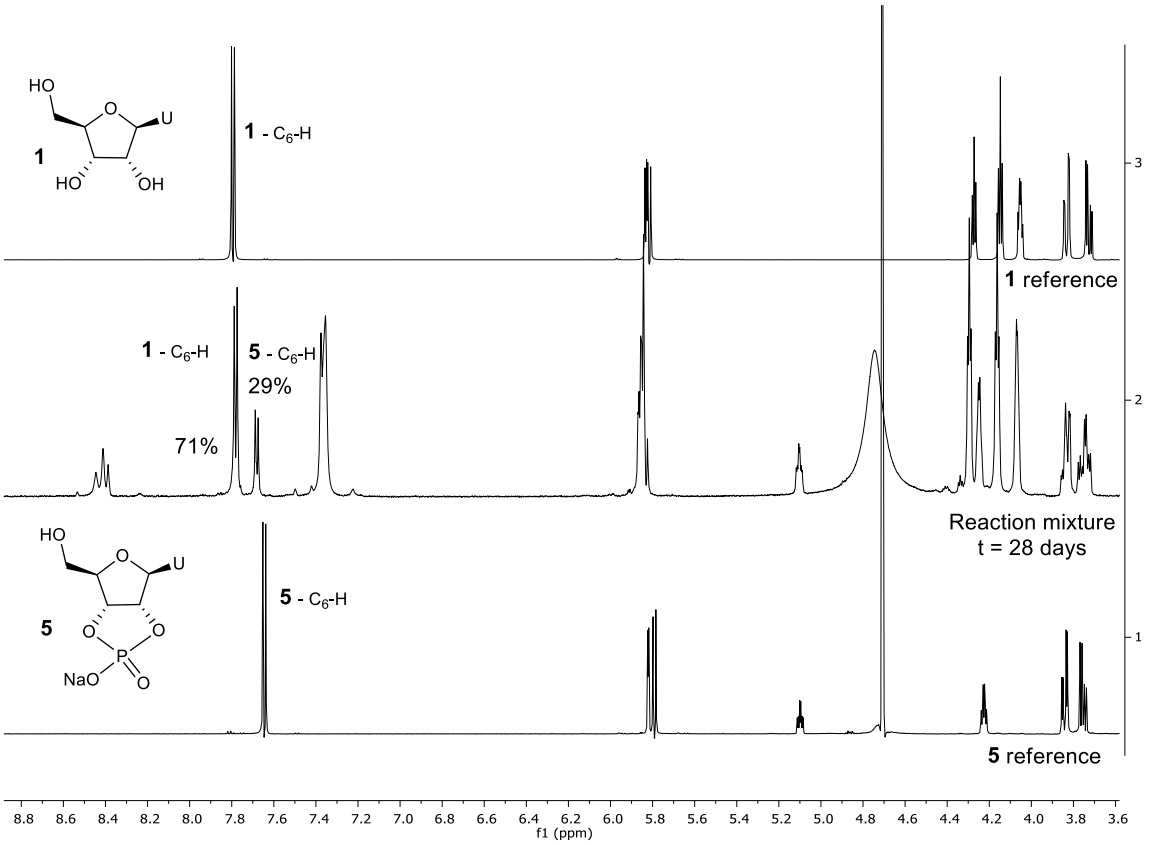
Supplementary Figure 28:

^{13}C NMR spectra of phosphorylation of **1** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 1, entry 5, day 30, middle spectrum). The spectra of starting material **1** (uridine, top spectrum) and reference product **5** (cUMP, bottom spectrum) are shown for comparison.



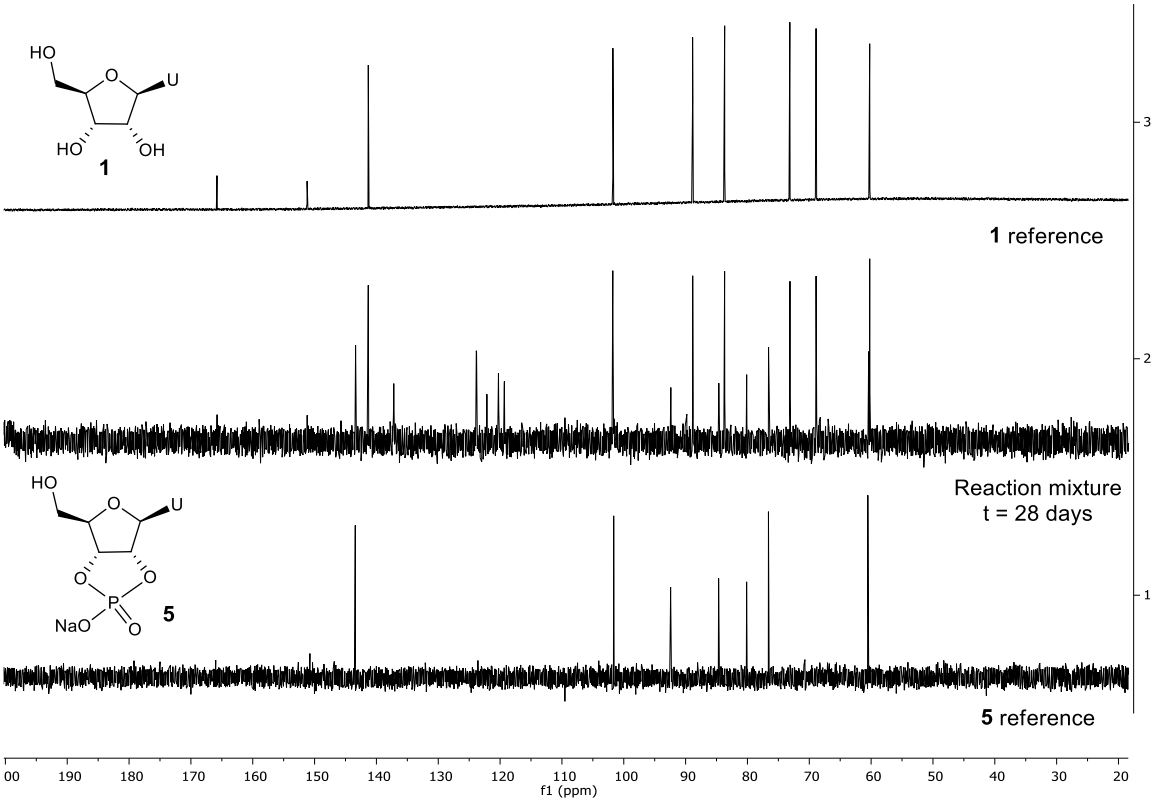
Supplementary Figure 29:

{H-decoupled} ^{31}P NMR spectra of phosphorylation of **1** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 1, entry 5, day 30, middle spectrum). The spectrum of the reference product **5** (cUMP, bottom spectrum) is shown for comparison. The signals are relatively broad because there is a high concentration of inorganic phosphates in the crude sample.



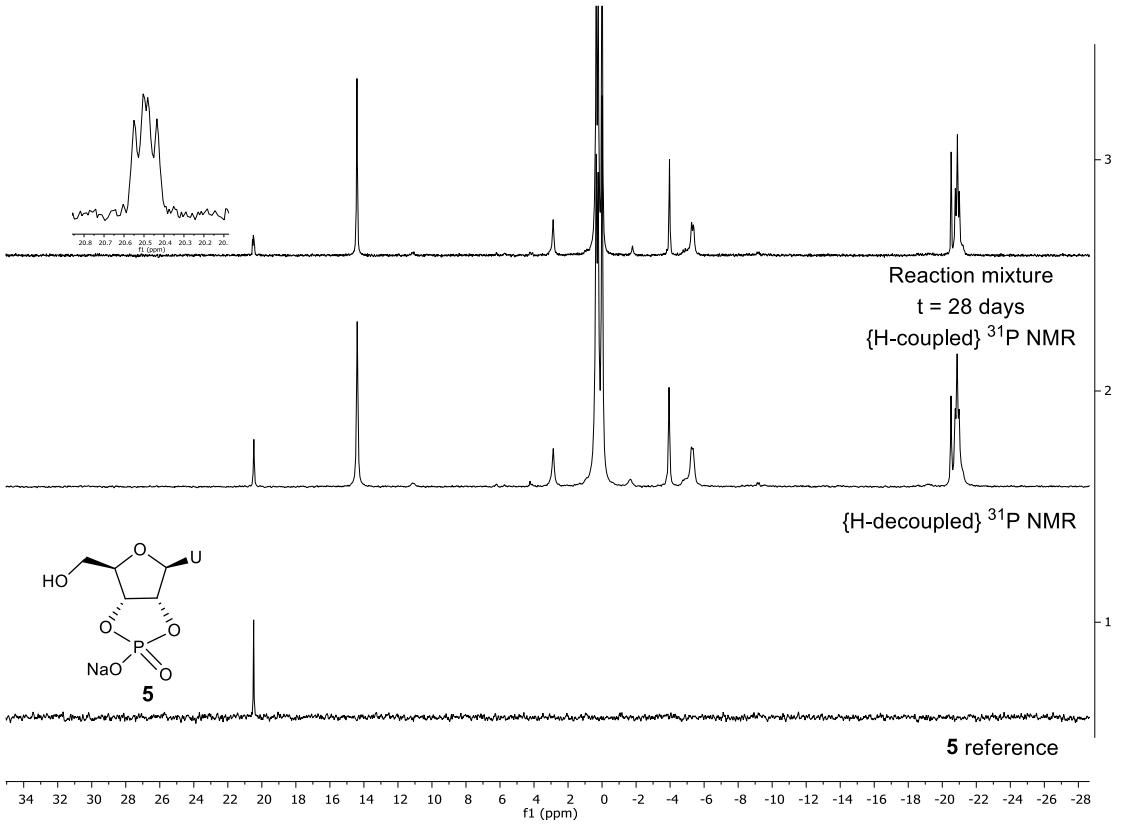
Supplementary Figure 30:

{Water suppression} ^{1}H NMR spectra of phosphorylation of **1** in solution at pH 7 in D_2O following the method B (Supplementary Table 1, entry 11, day 28, middle spectra). The spectra of starting material **1** (top spectrum) and reference product **5** (bottom spectrum) are shown for comparison.



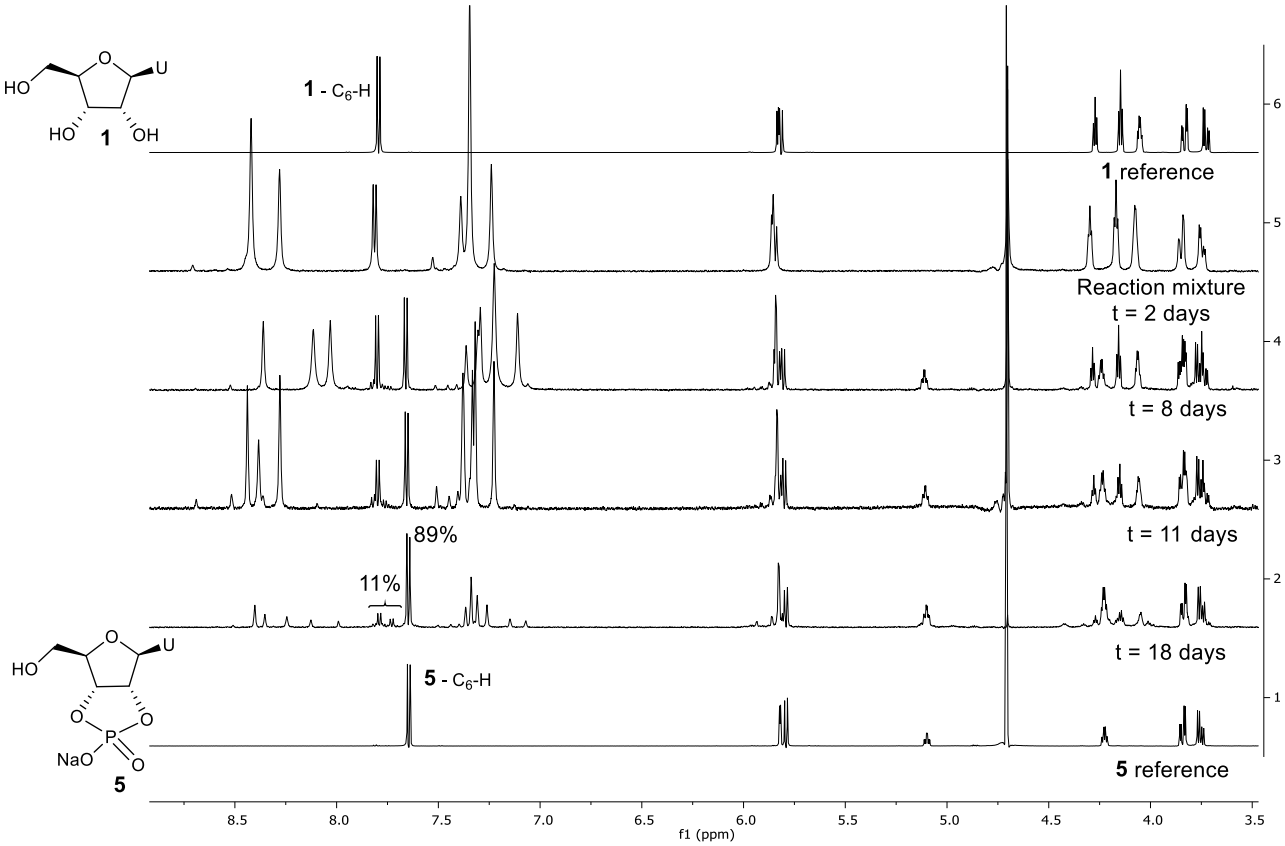
Supplementary Figure 31:

^{13}C NMR spectra of phosphorylation of **1** in solution at pH 7 in D_2O following the method B (Supplementary Table 1, entry 11, day 28, middle spectrum). The spectra of starting material **1** (top spectrum) and reference product **5** (bottom spectrum) are shown for comparison.



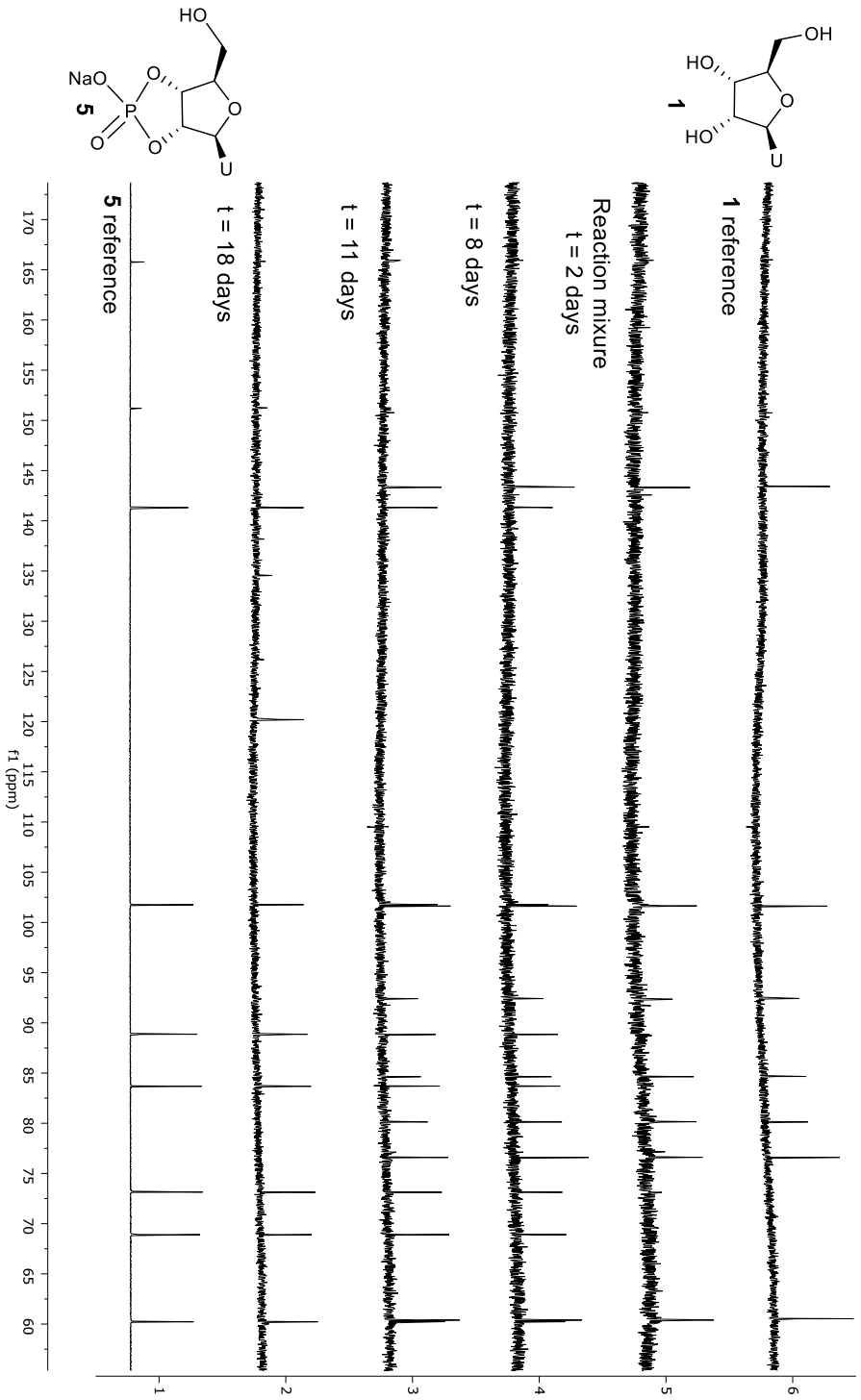
Supplementary Figure 32:

{H-coupled} ^{31}P NMR and {H-decoupled} ^{31}P NMR spectra of phosphorylation of **1** in solution at pH 7 in D_2O following the method B (Supplementary Table 1, entry 11, day 28, top and middle spectra). The spectrum of the reference product **5** (bottom spectrum) is shown for comparison.



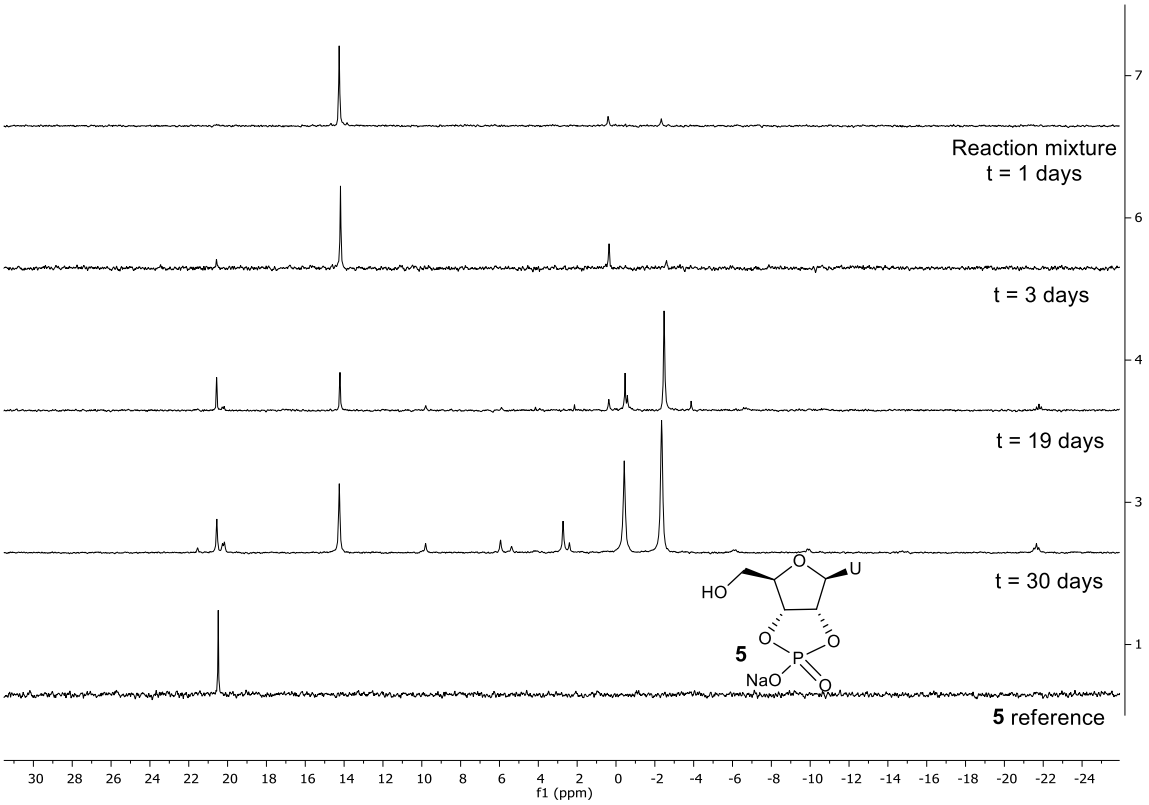
Supplementary Figure 33:

{Water suppression} ^{1}H NMR spectra of phosphorylation of **1** in paste-reaction following the method C (Supplementary Table 1, entry 14, 18 days, spectrum 2 from bottom). The spectra of starting material **1** (uridine, top spectrum) and reference product **5** (cUMP, bottom spectrum) are shown for comparison.



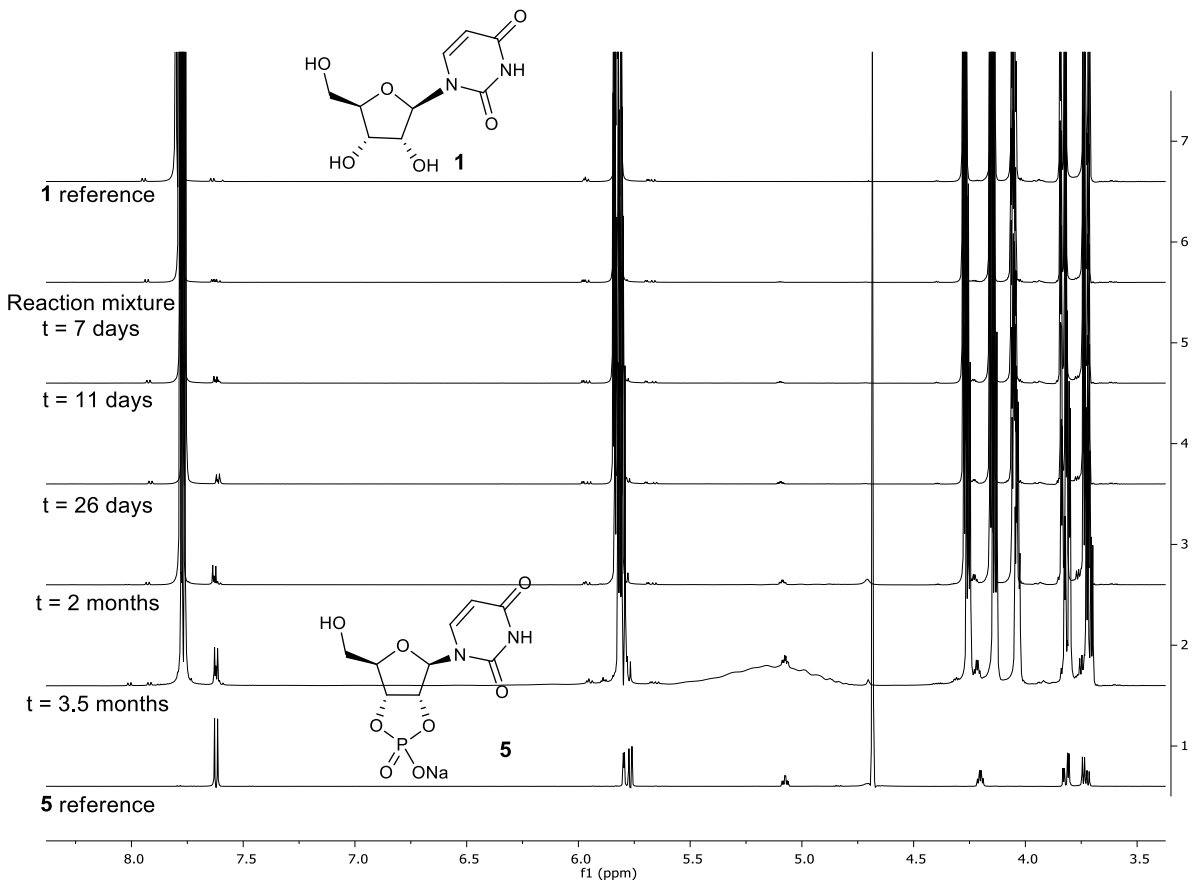
Supplementary Figure 34:

^{13}C NMR spectra of phosphorylation of **1** in paste-reaction following the method C (Supplementary Table 1, entry 14, 18 days, spectrum 2 from bottom). The spectra of starting material **1** (top spectrum) and reference product **5** (bottom spectrum) are shown for comparison



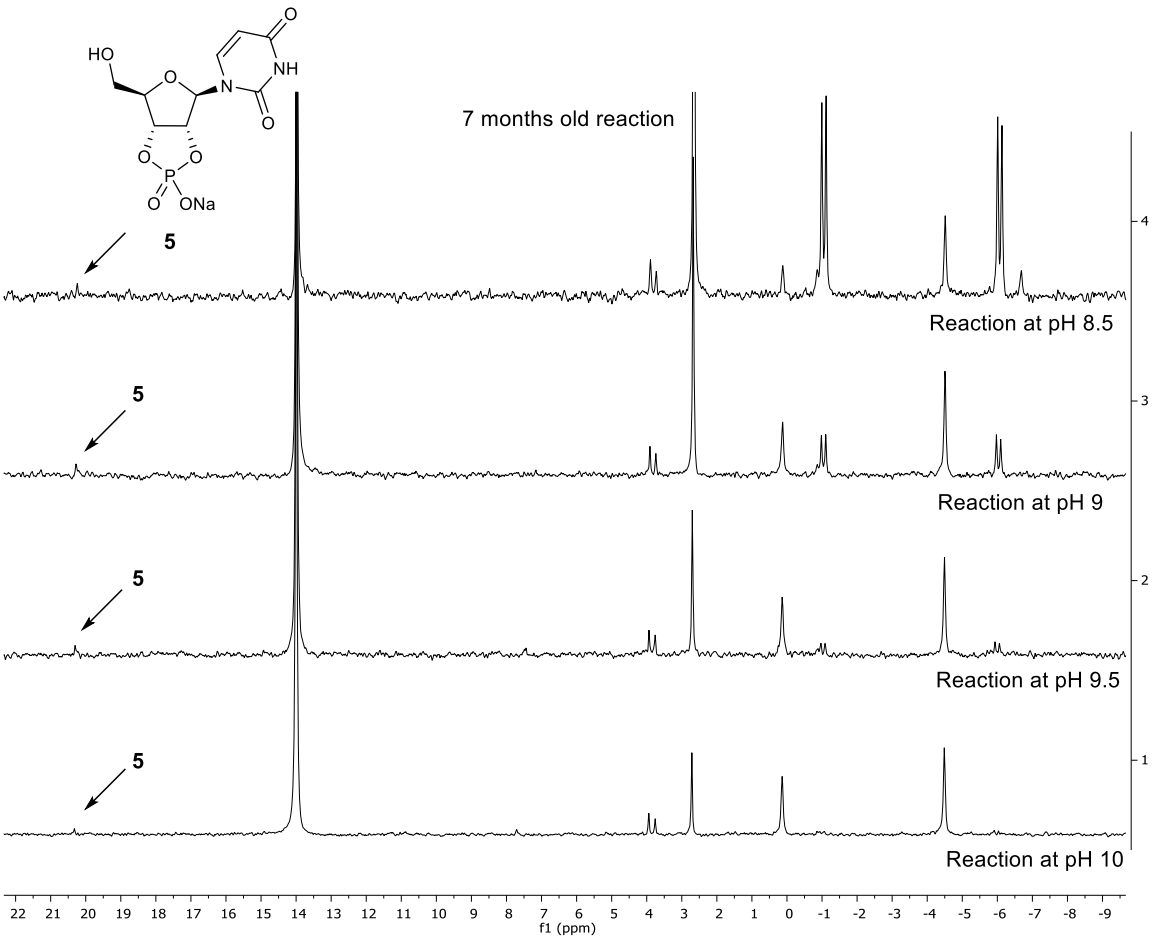
Supplementary Figure 35:

{H-decoupled} ^{31}P NMR spectra of phosphorylation of **1** in paste-following the method C (Supplementary Table 1, entry 15, 30 days, spectrum 2 from bottom). The spectrum the reference product spectrum of **5** (bottom spectrum) is shown for comparison.



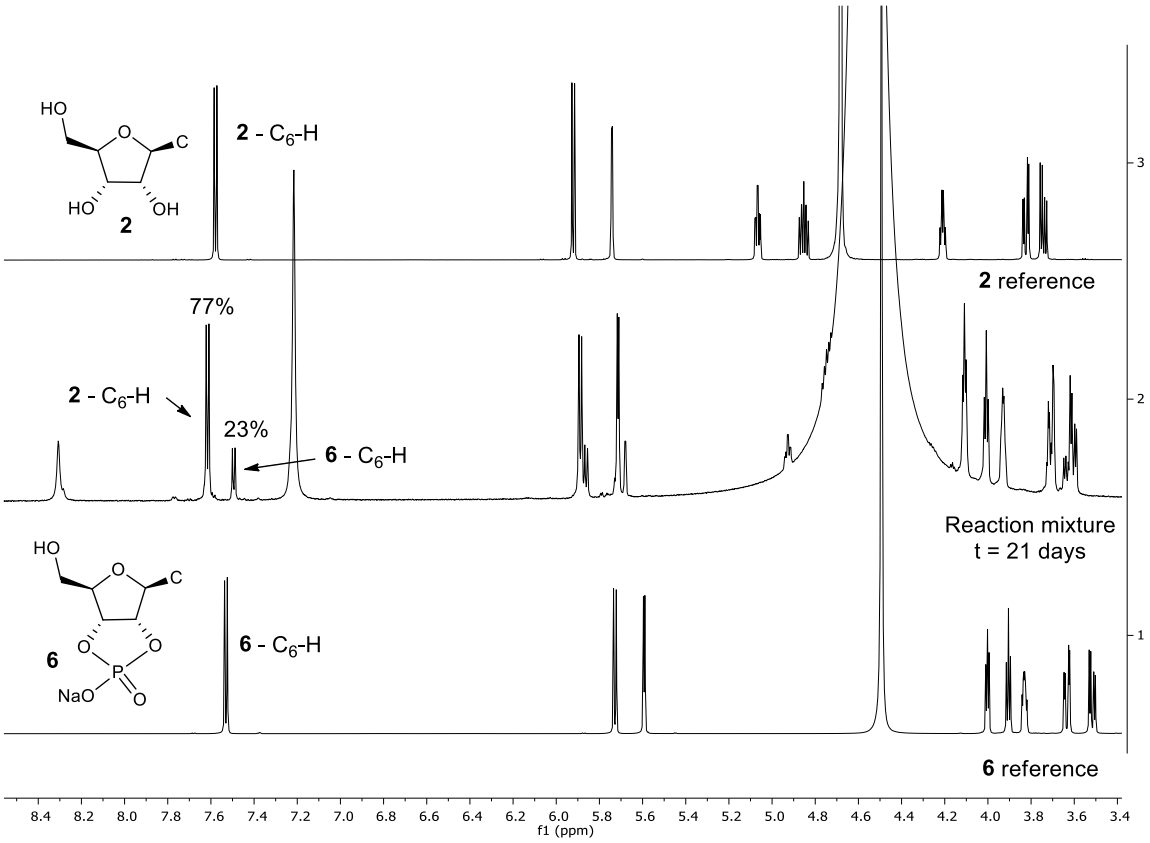
Supplementary Figure 36:

{Water suppression} ^{1}H NMR spectra showing the phosphorylation of **1** (uridine) with 5 equiv. of DAP at 0.1 M in D_2O. The pH was 8.3 (the pH has not been adjusted). The formation of **5** (cUMP) is slow (~6 % in 3.5 months) suggesting that the reaction is accelerated by the presence of imidazole and/or by pH adjustment.



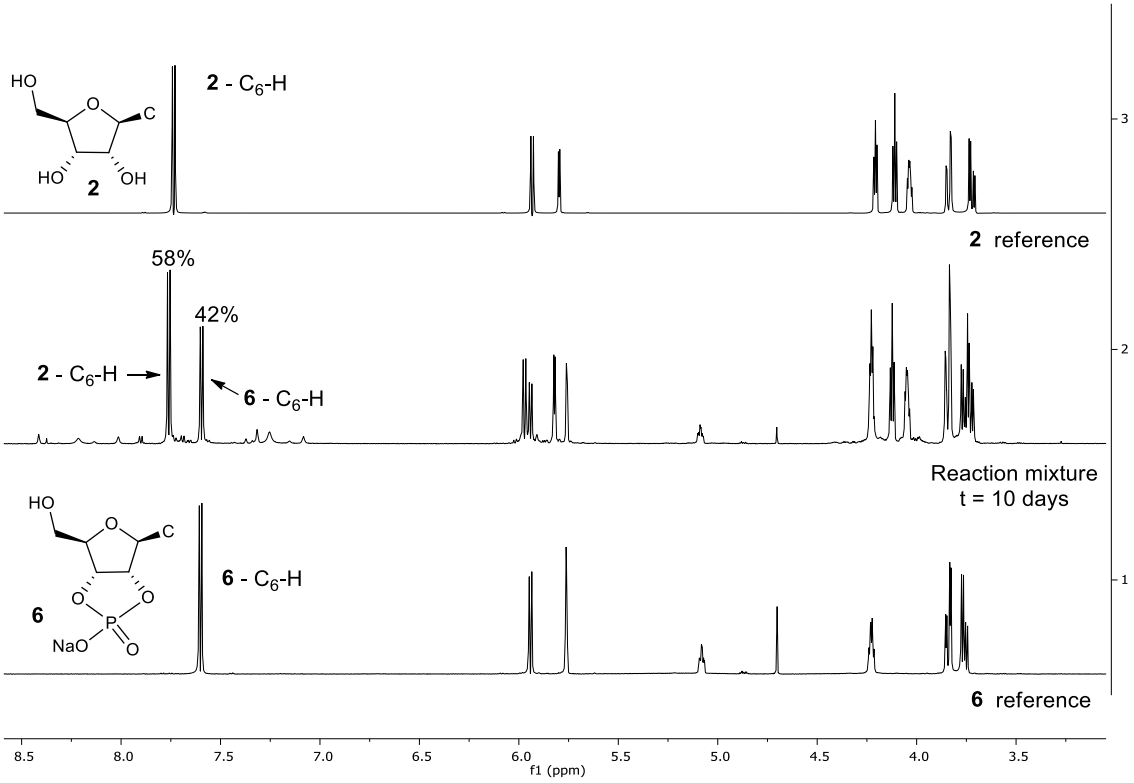
Supplementary Figure 37:

{H-decoupled} ^{31}P NMR spectra (7-month-old reactions) of the phosphorylation of **1** with 5 equiv. of DAP and 1 equiv. of imidazole leading to the formation of **5** in D_2O at different pH. The pH of the reactions was adjusted using 0.1 M NaOH to 10, 9.5, 9 and 8.5 (spectra 1, 2, 3 and 4 respectively) while setting up the reactions. No further DAP was added. Formation of **5** is observed even at basic pH, though slowly.



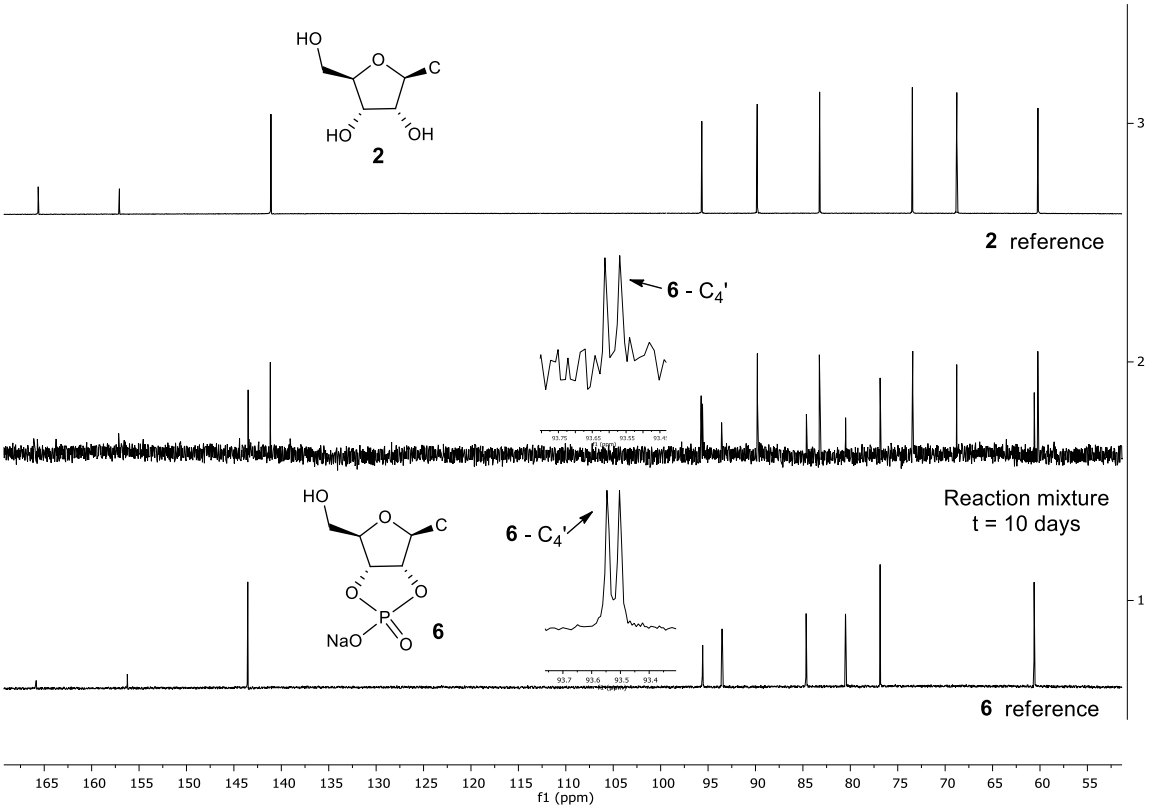
Supplementary Figure 38:

^{1}H NMR spectra of phosphorylation of **2** in solution at pH 7 in D_2O following the method B (Supplementary Table 2, entry 5, 21 days, middle spectrum). The spectra of starting material **2** (top spectrum) and reference product **6** (bottom spectrum) are shown for comparison.



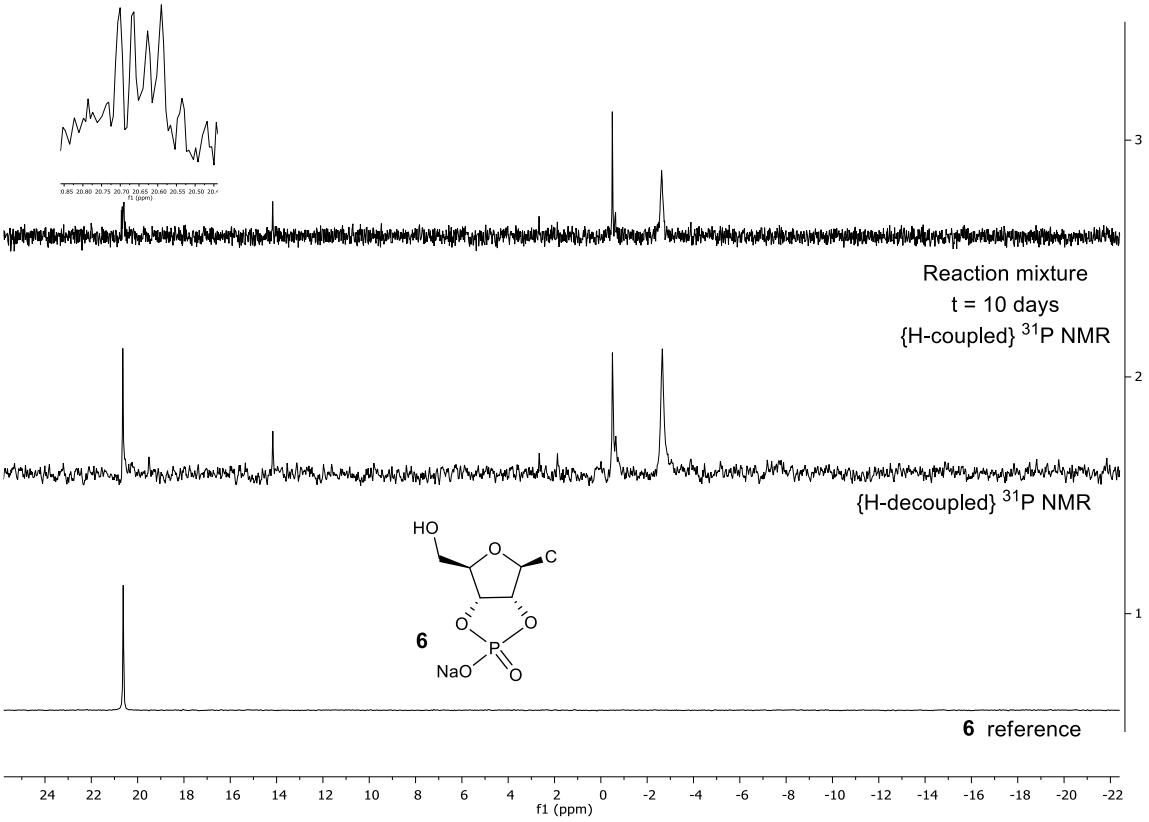
Supplementary Figure 39:

{Water suppression} ^{1}H NMR spectra of phosphorylation of **2** in paste-reaction following the method C (Supplementary Table 2, entry 6, 10 days, middle spectrum). The spectra of starting material **2** (top spectrum) and reference product **6** (bottom spectrum) are shown for comparison.



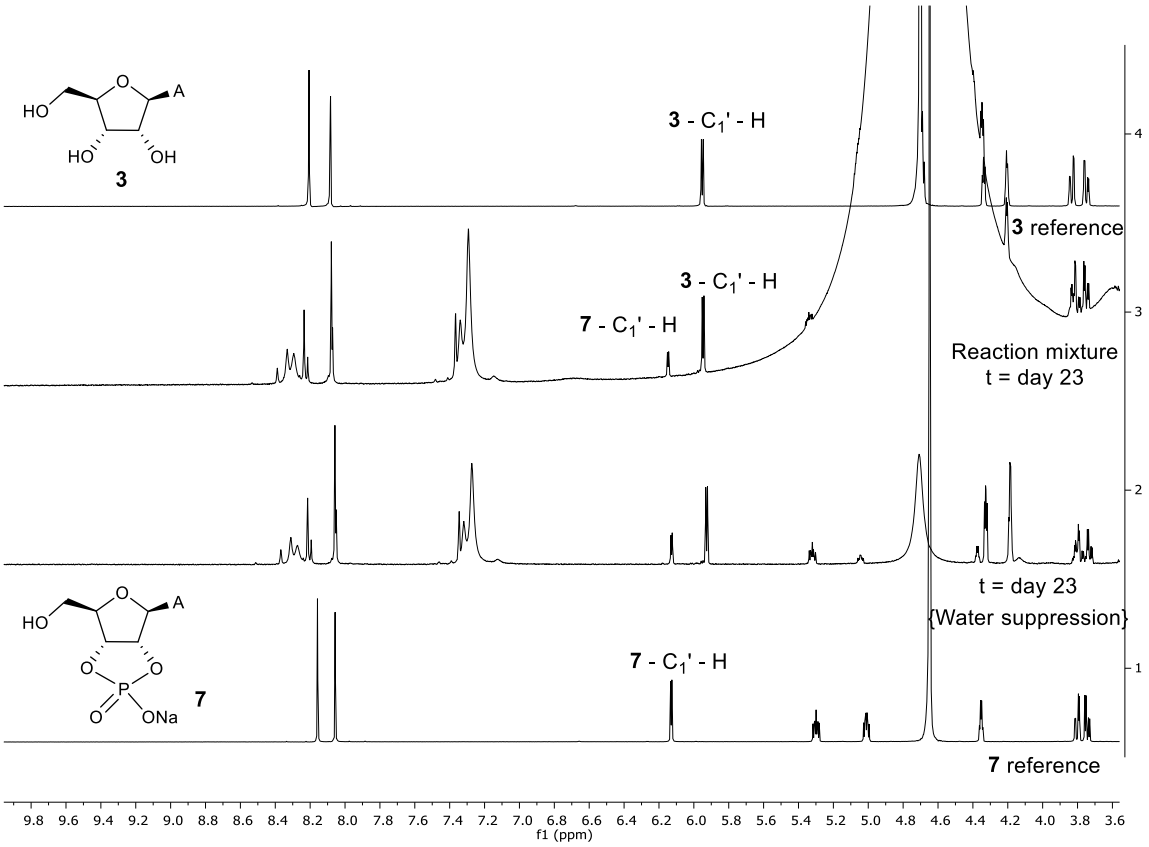
Supplementary Figure 40:

^{13}C NMR spectra of phosphorylation of **2** in paste-reaction following the method C (Supplementary Table 2, entry 6, 10 days, middle spectrum). The spectra of starting material **2** (top spectrum) and reference product **6** (bottom spectrum) are shown for comparison.



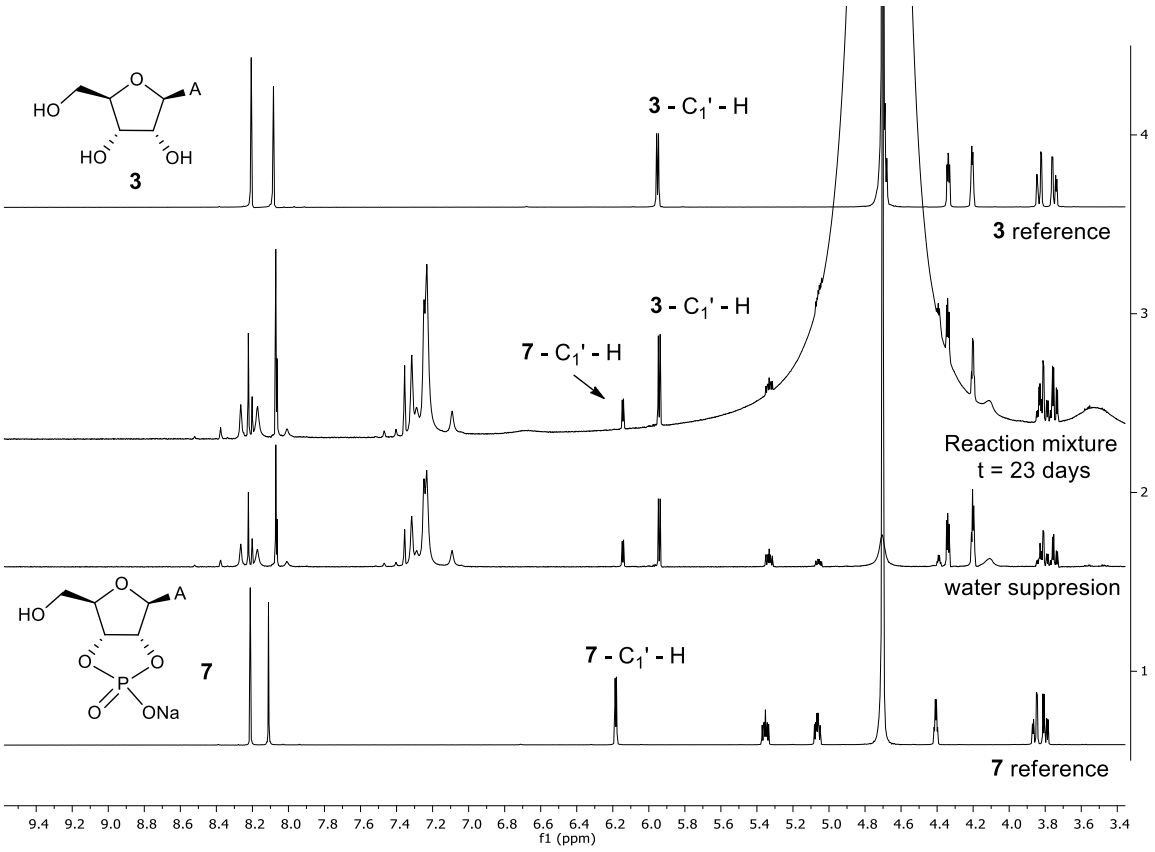
Supplementary Figure 41:

{H-coupled} ^{31}P NMR (top spectrum) and {H-decoupled} ^{31}P NMR (middle spectrum) spectra of phosphorylation of **2** in paste-reaction following the method C (Supplementary Table 2, entry 6, 10 days, top and middle spectra respectively). The spectrum of the reference product **6** (bottom spectrum) is shown for comparison.



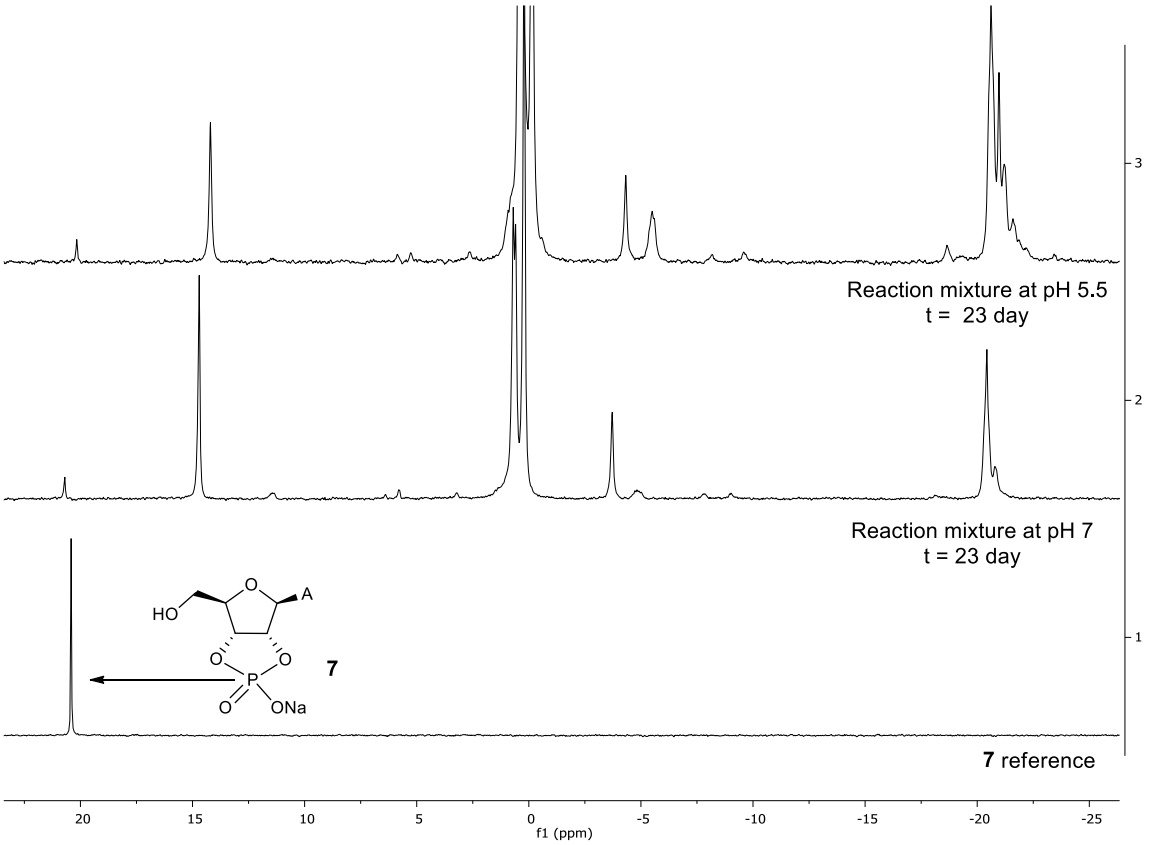
Supplementary Figure 42:

^{1}H NMR and {water suppression} ^{1}H NMR spectra of phosphorylation of **3** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 3, entry 2, 23 days, middle two spectra). The spectra of starting material **3** (top spectrum) and reference product **7** (bottom spectrum) are shown for comparison.



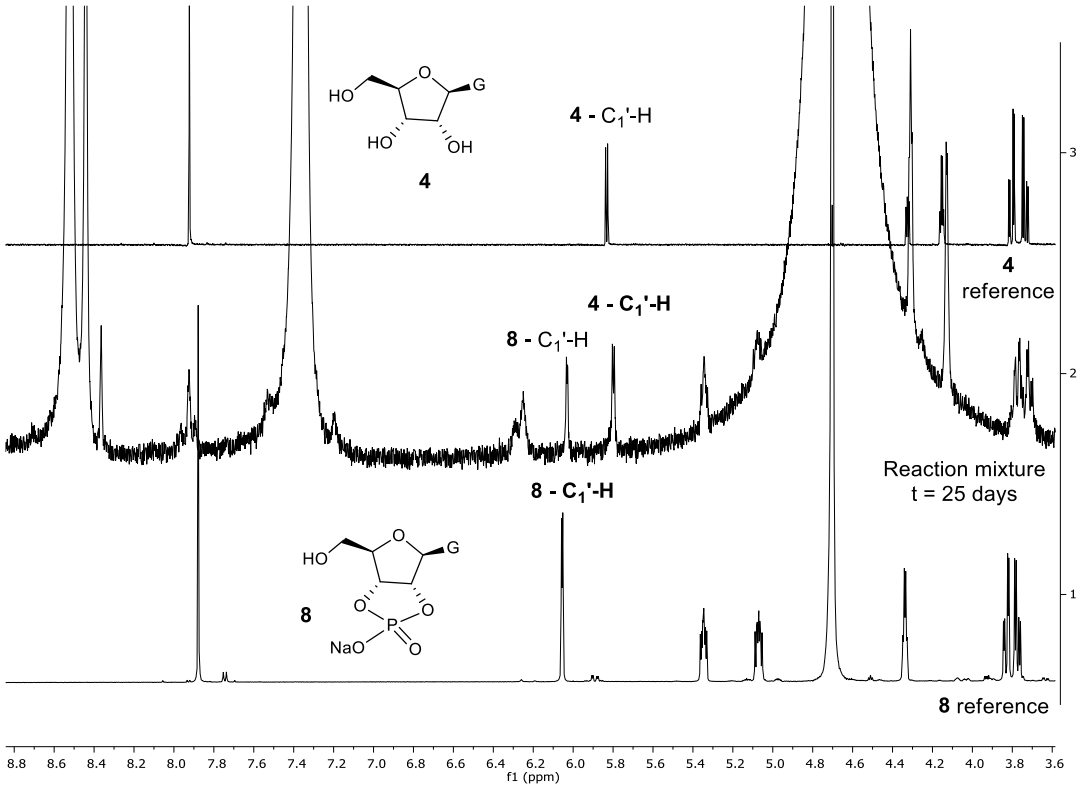
Supplementary Figure 43:

^{1}H NMR and {water suppression} ^{1}H NMR spectra of phosphorylation of **3** in solution at pH 7 in D_2O following the method B (Supplementary Table 3, entry 6, 23 days middle two spectra). The spectra of starting material **3** (top spectrum) and **7** reference product (bottom spectrum) are shown for comparison.



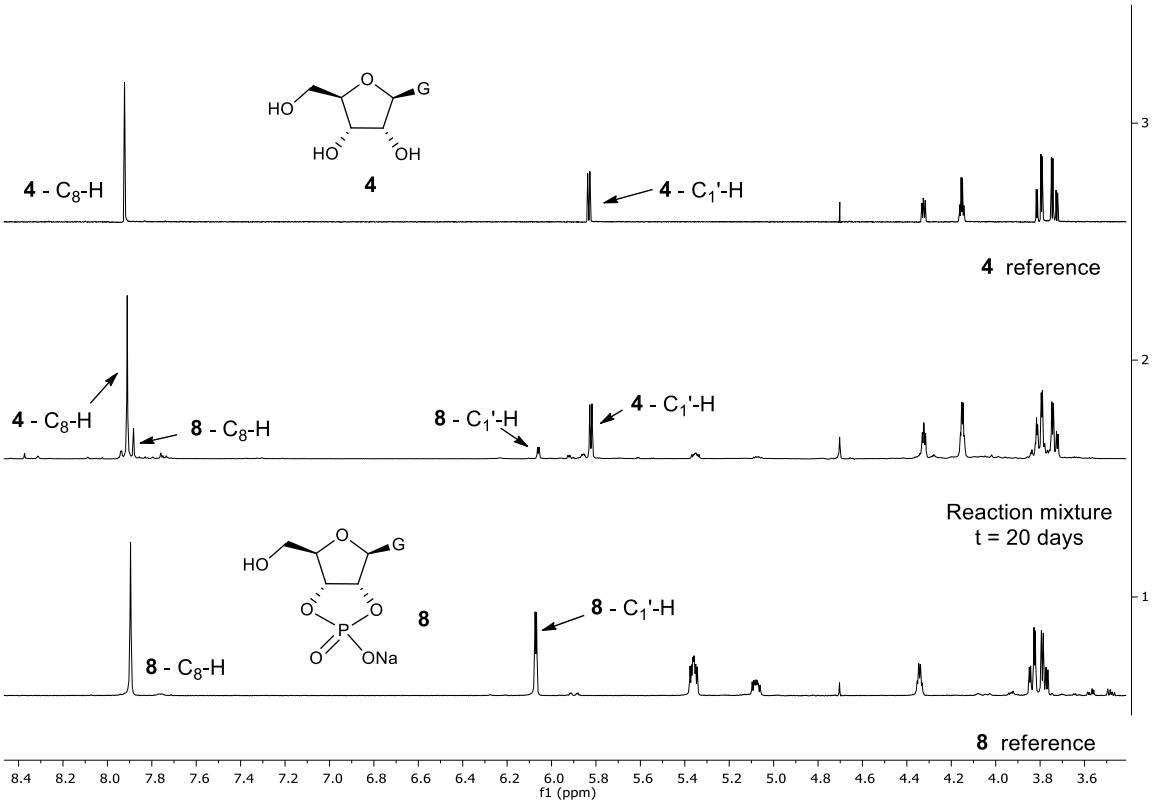
Supplementary Figure 44:

{H-decoupled} ^{31}P NMR spectra of phosphorylation of **3** in solution at pH 5.5 and at pH 7 in D_2O following the method A and B respectively (Supplementary Table 3, entry 2 and 6 respectively, 23 days, top and middle respectively). The spectrum of the reference product **7** (bottom spectrum) is shown for comparison.



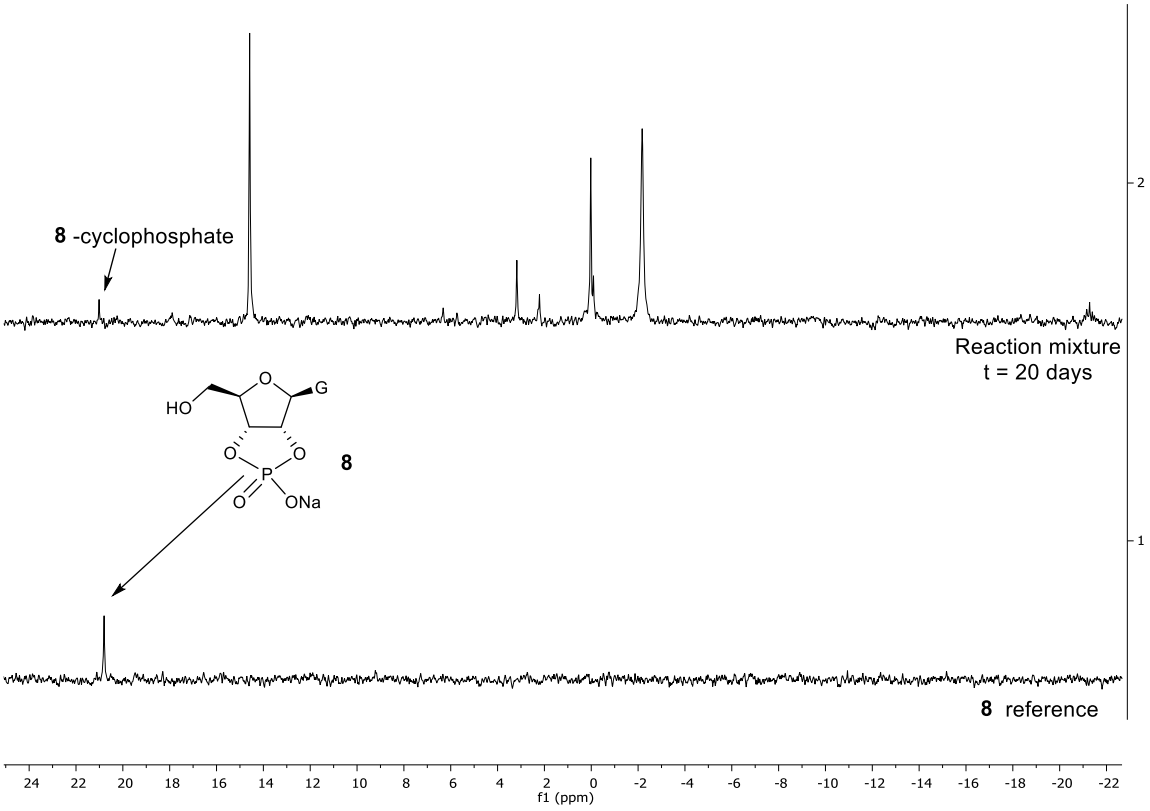
Supplementary Figure 45:

^{1}H NMR spectra of phosphorylation of **4** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 4, the middle spectrum represents the progress of the reaction using Method A on day 25th; the % conversion was determined using day 12th spectrum, entry 3). The spectra of starting material 4 (top spectrum) and reference product **8** (bottom spectrum) are shown for comparison.



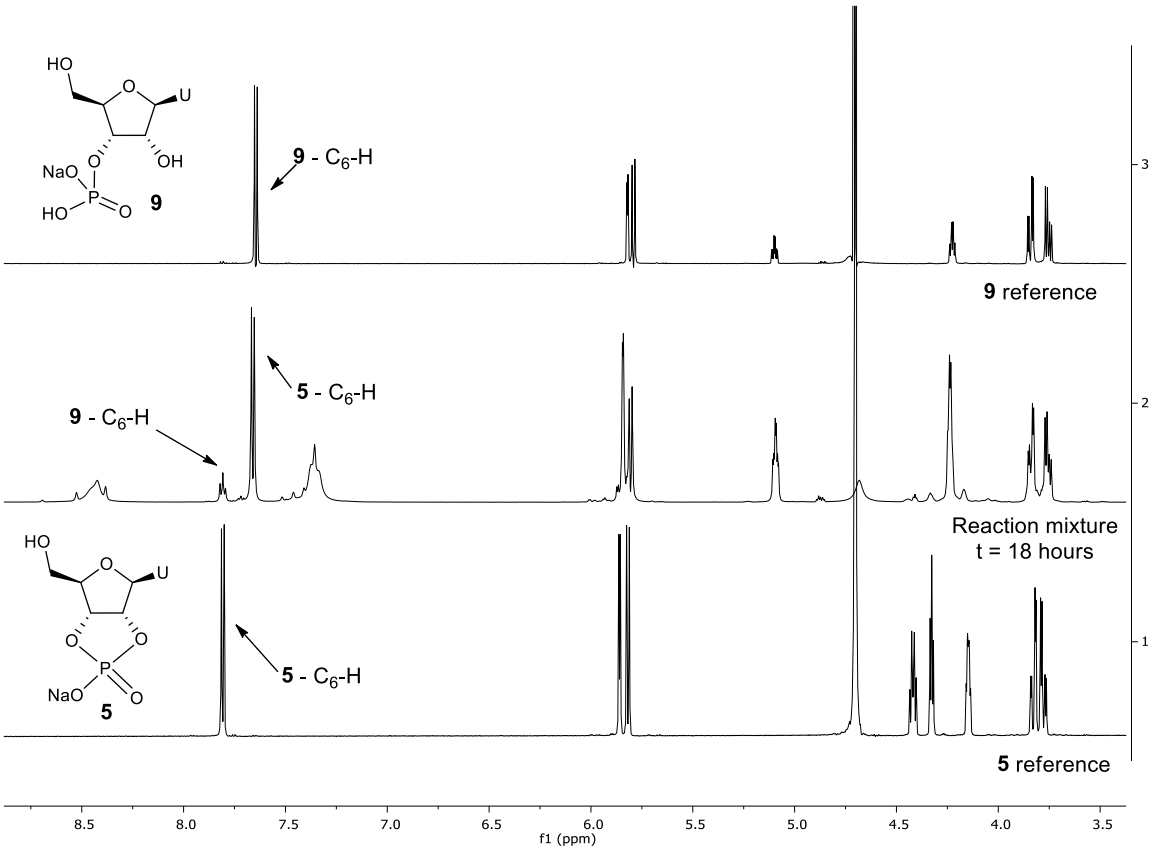
Supplementary Figure 46:

{Water suppression} ^{1}H NMR spectrum of phosphorylation of **4** in paste-reaction following the method C (Supplementary Table 4, entry 8, 20 days, middle spectrum). The spectra of starting material **4** (top spectrum) and reference product **8** (bottom spectrum) are shown for comparison.



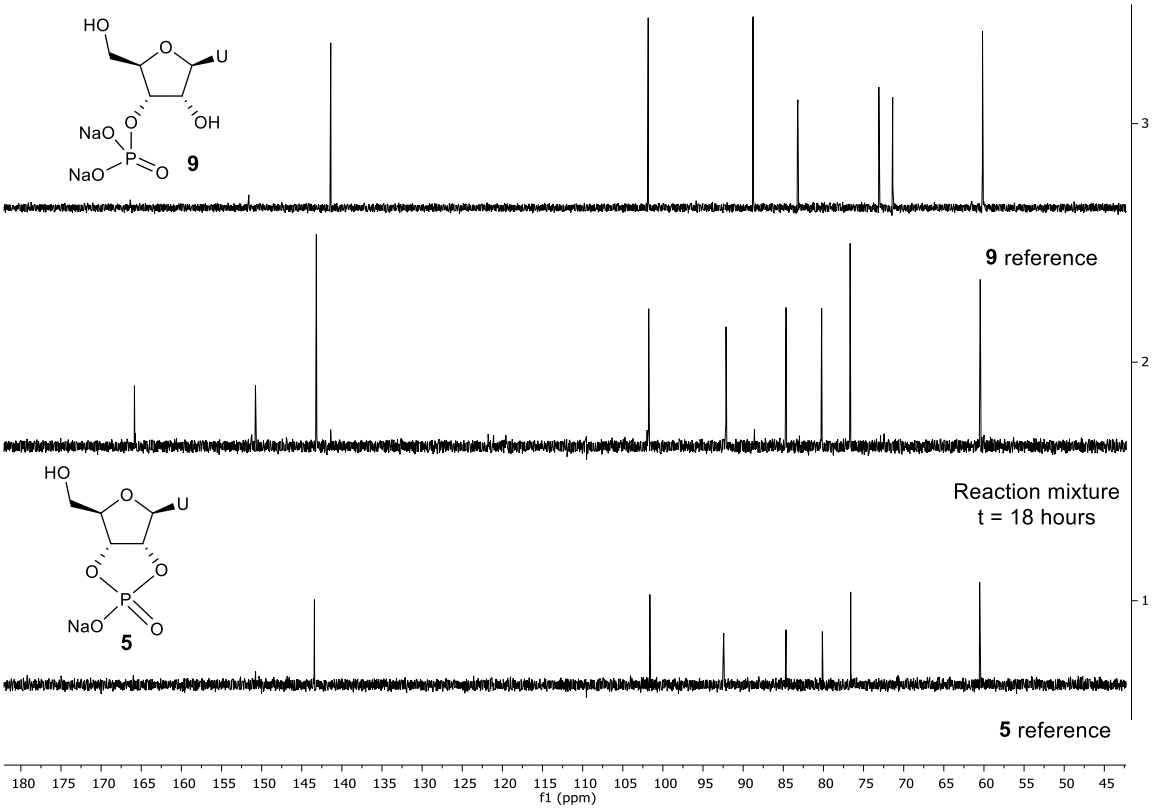
Supplementary Figure 47:

{H-decoupled} ^{31}P NMR spectrum of phosphorylation of **4** in paste-reaction following the method C (Supplementary Table 4, entry 8, 20 days, top spectrum). The spectrum of reference product **8** (bottom spectrum) is shown for comparison.



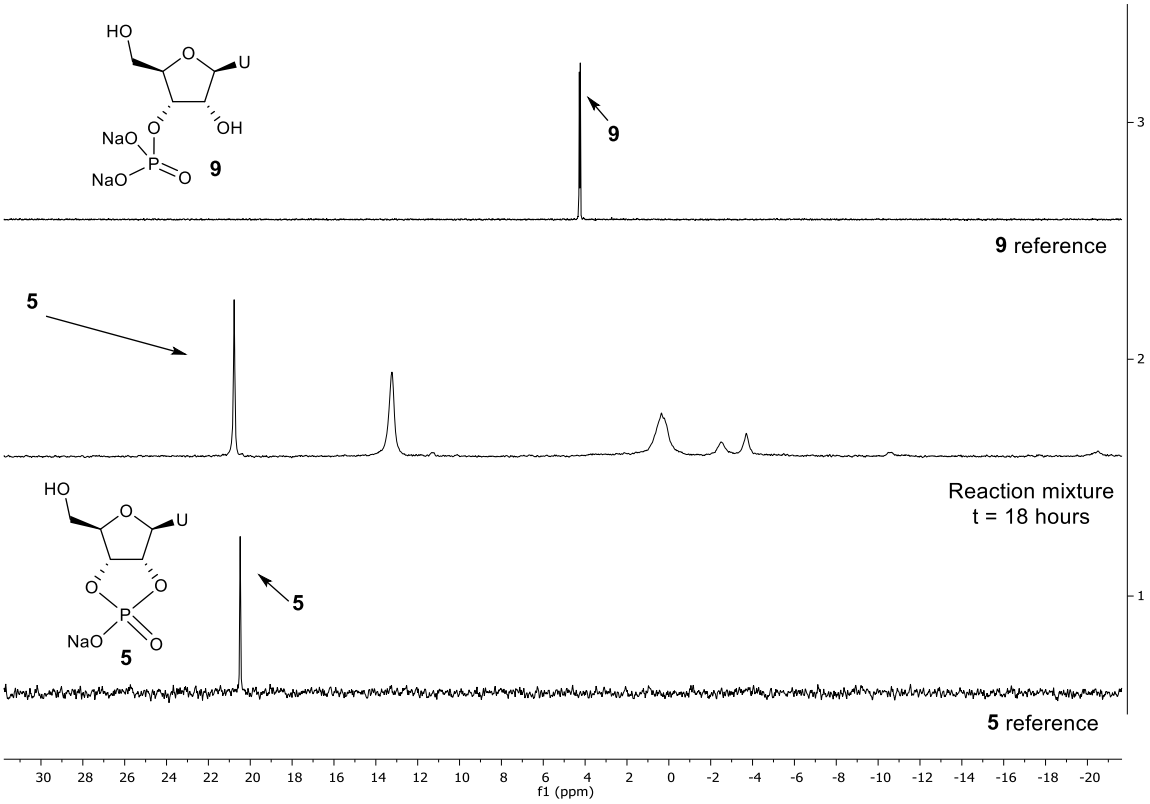
Supplementary Figure 48:

^{1}H NMR spectra of phosphorylation of **9** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 2, 18 hours, middle spectrum). The spectra of starting material **9** (top spectrum) and reference product **5** (bottom spectrum) are shown for comparison.



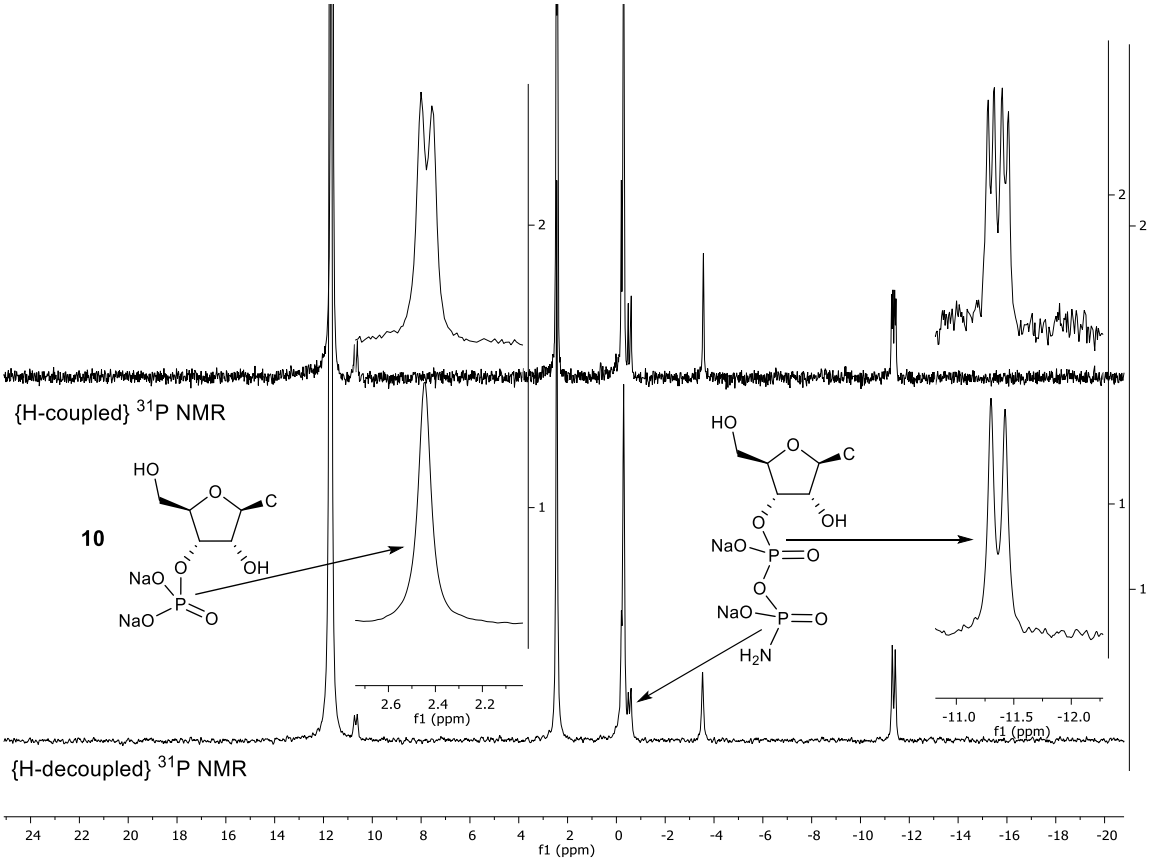
Supplementary Figure 49:

^{13}C NMR spectra of phosphorylation of **9** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 2, 18 hours, middle spectrum). The spectra of starting material **9** (top spectrum) and reference product **5** (bottom spectrum) are shown for comparison.



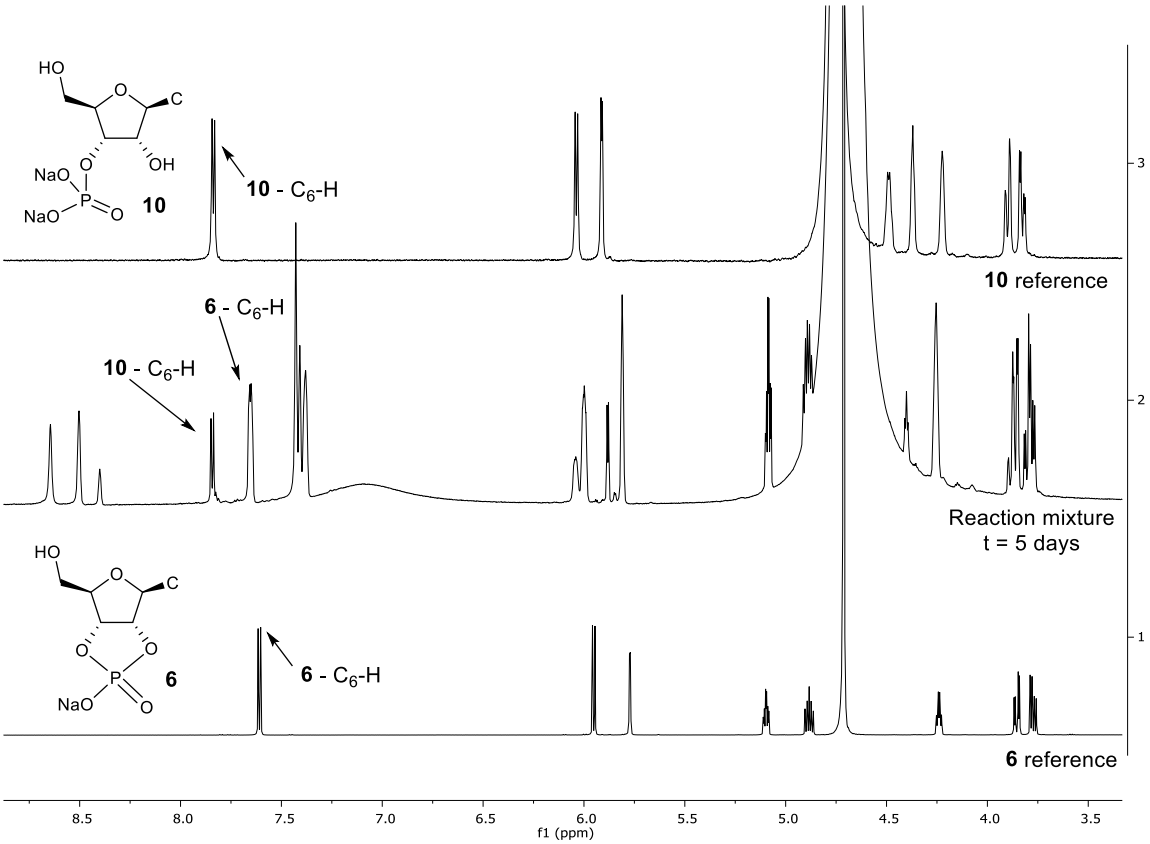
Supplementary Figure 50:

{H-decoupled} ^{31}P NMR spectra of phosphorylation of **9** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 2, 18 hours, middle spectrum). The spectra of starting material **9** (top spectrum) and reference product **5** (bottom spectrum) are shown for comparison.



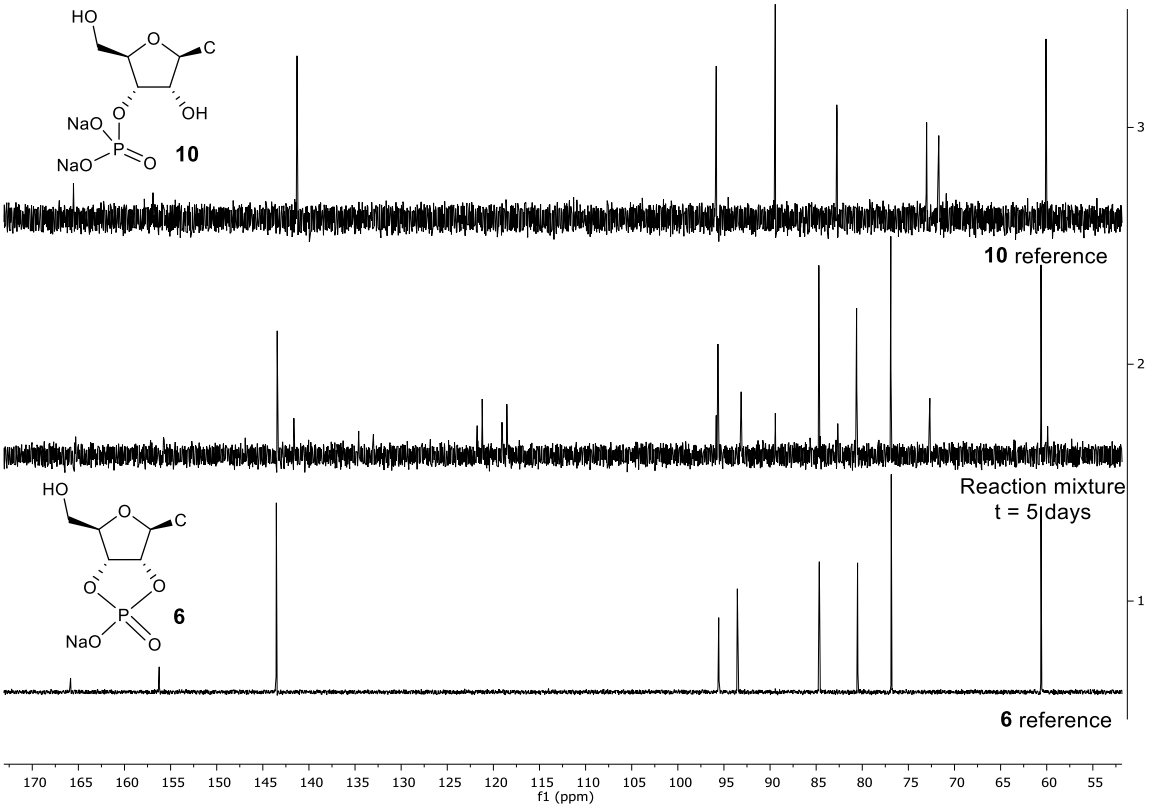
Supplementary Figure 51:

{H-coupled}^{31}P NMR (top spectrum) and {H-decoupled} ^{31}P NMR (bottom spectrum) spectra of phosphorylation of **10** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 3, 5 days). The top {H-coupled} ^{31}P NMR spectrum shows the coupling of the phosphate of **10** and of the intermediate with the C_3'-H.



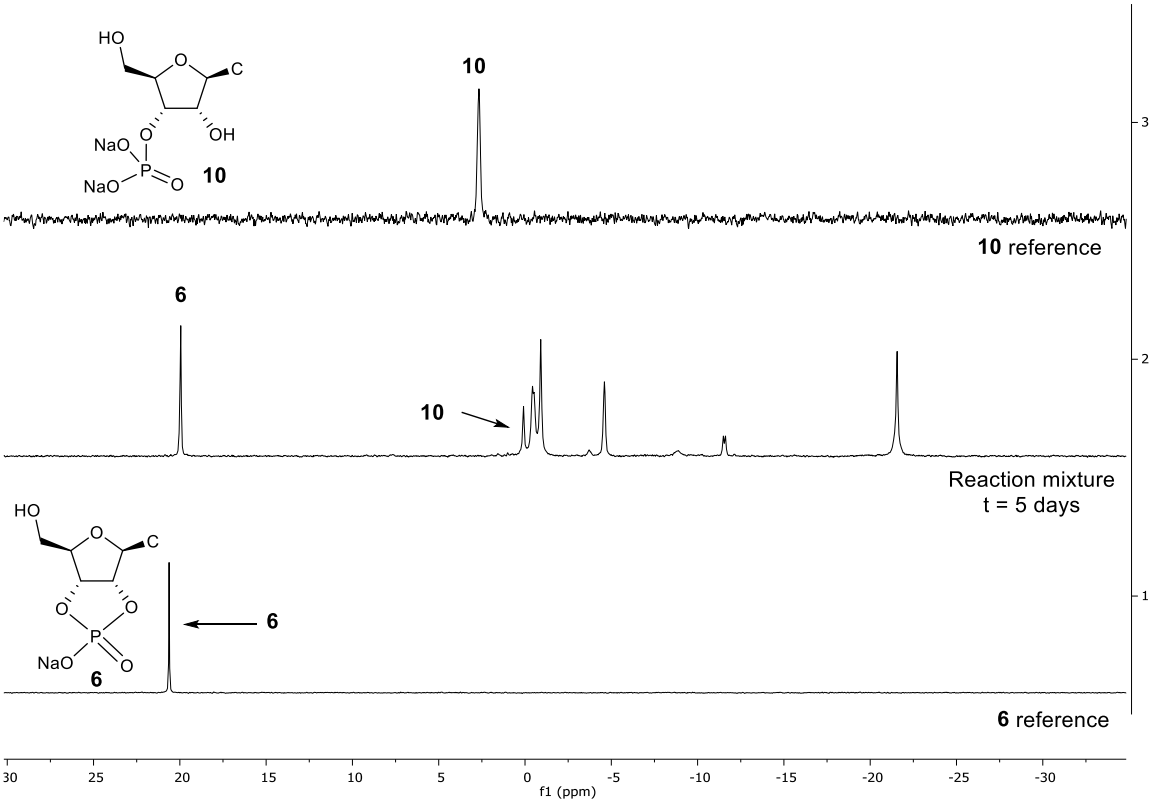
Supplementary Figure 52:

^{1}H NMR spectra of phosphorylation of **10** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 3, 5 days, middle spectrum). The spectra of starting material **10** (top spectrum) and reference product **6** (bottom spectrum) are shown for comparison.



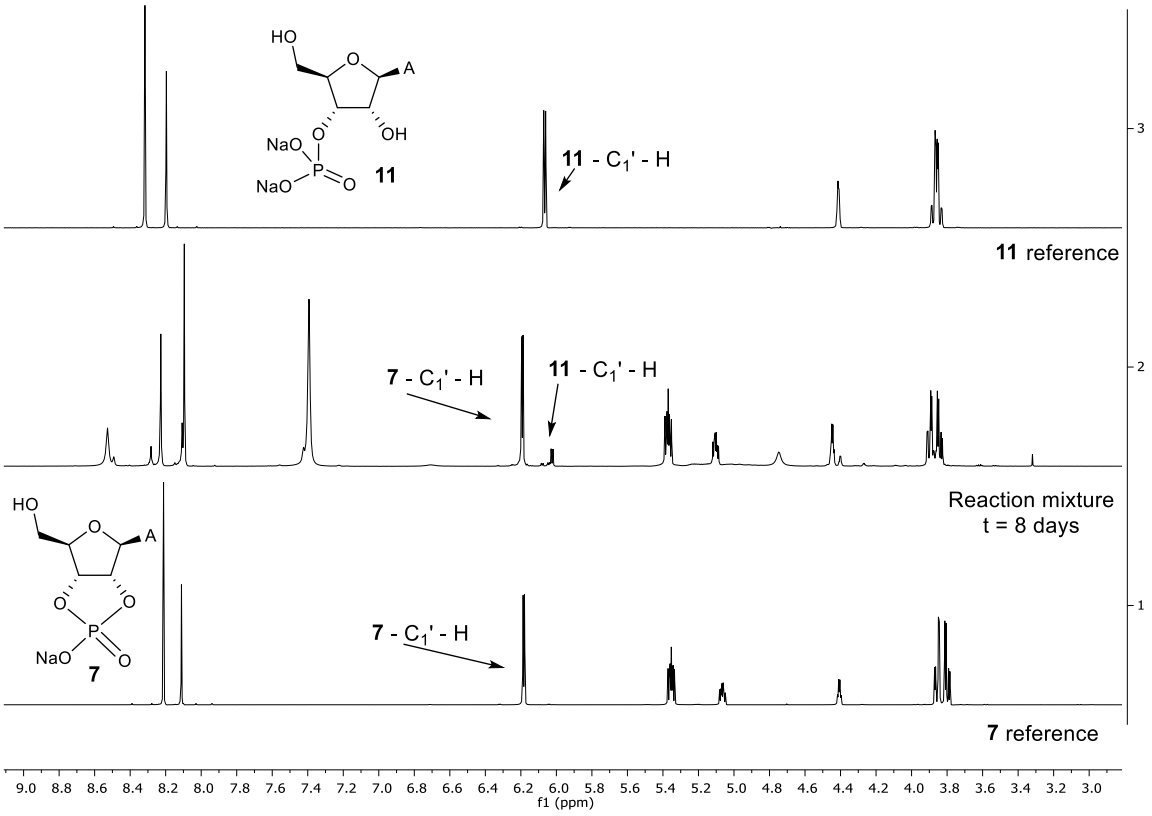
Supplementary Figure 53:

^{13}C NMR spectra of phosphorylation of **10** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 3, 5 days, middle spectrum). The spectra of starting material **10** (top spectrum) and reference product **6** (bottom spectrum) are shown for comparison.



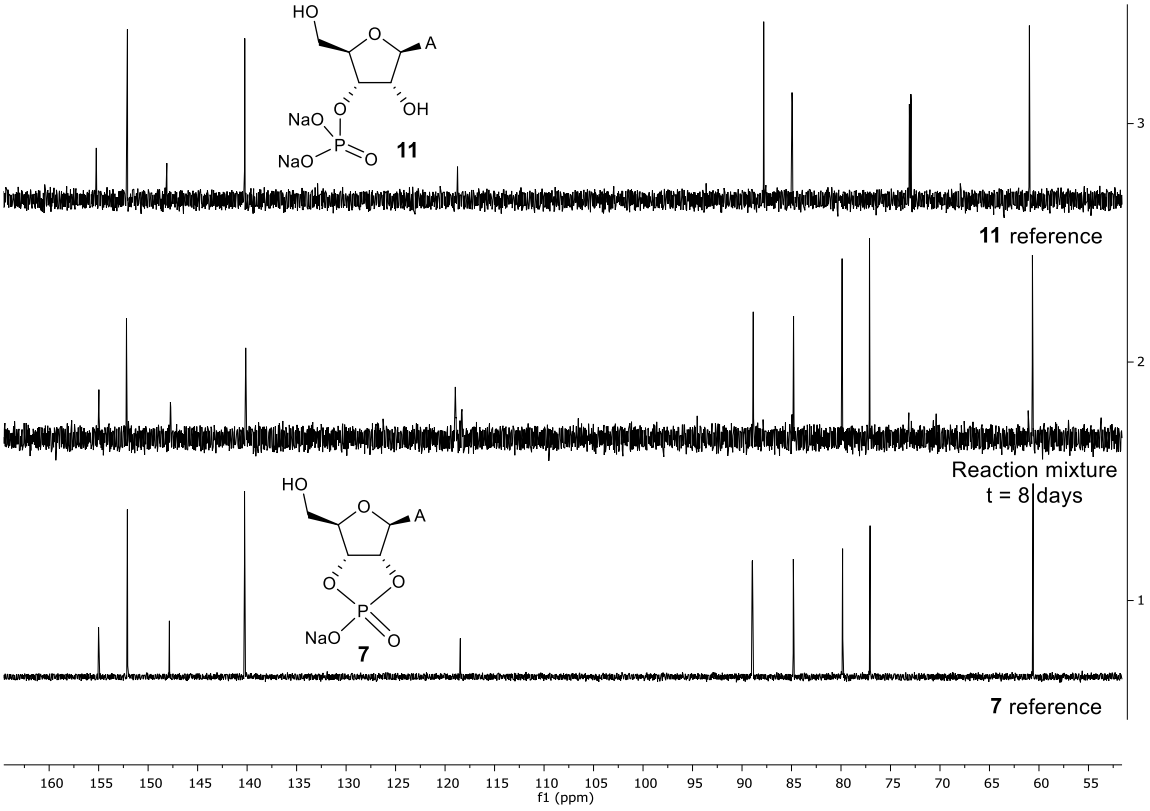
Supplementary Figure 54:

{H-decoupled} ^{31}P NMR spectra of phosphorylation of **10** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 3, 5 days, middle spectrum). The spectra of starting material **10** (top spectrum) and reference product **6** (bottom spectrum) are shown for comparison.



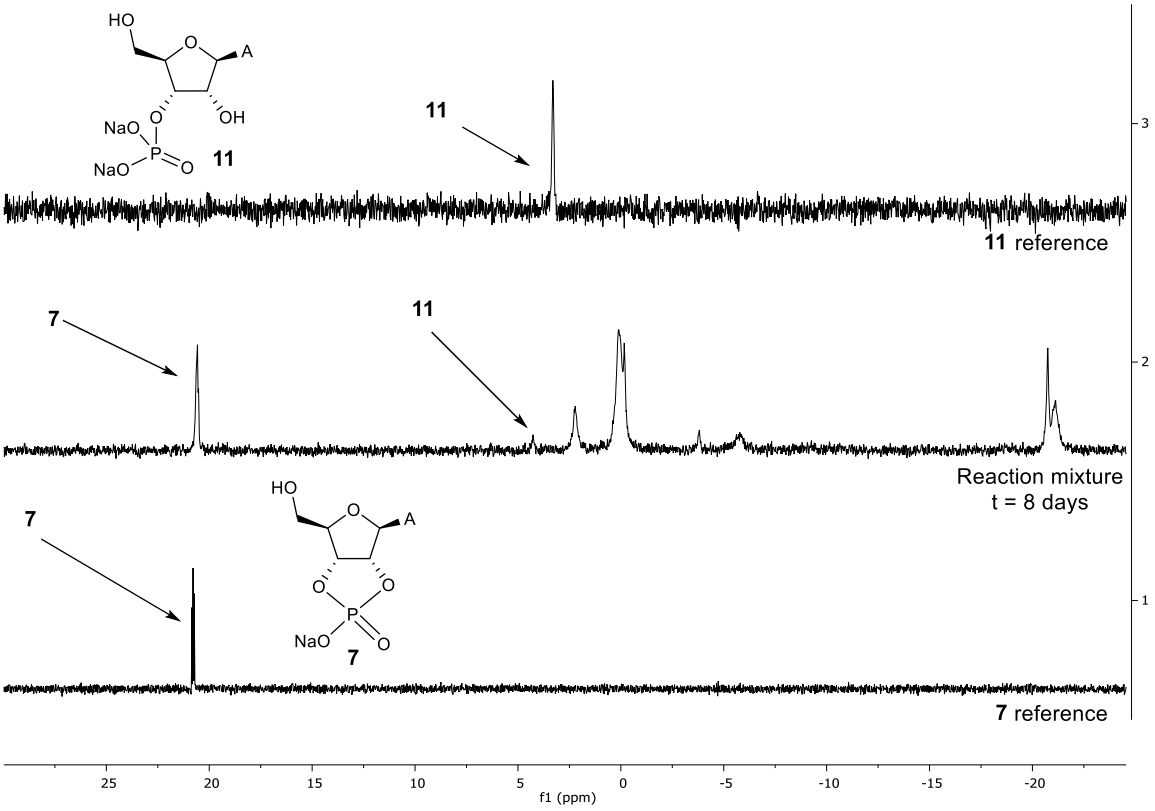
Supplementary Figure 55:

{Water suppression} ^{1}H NMR spectra of phosphorylation of **11** in solution at pH 7 in D_2O following the method B (Supplementary Table 5, entry 11, 8 days, middle spectrum). The spectra of starting material **11** (top spectrum) and reference product **7** (bottom spectrum) are shown for comparison.



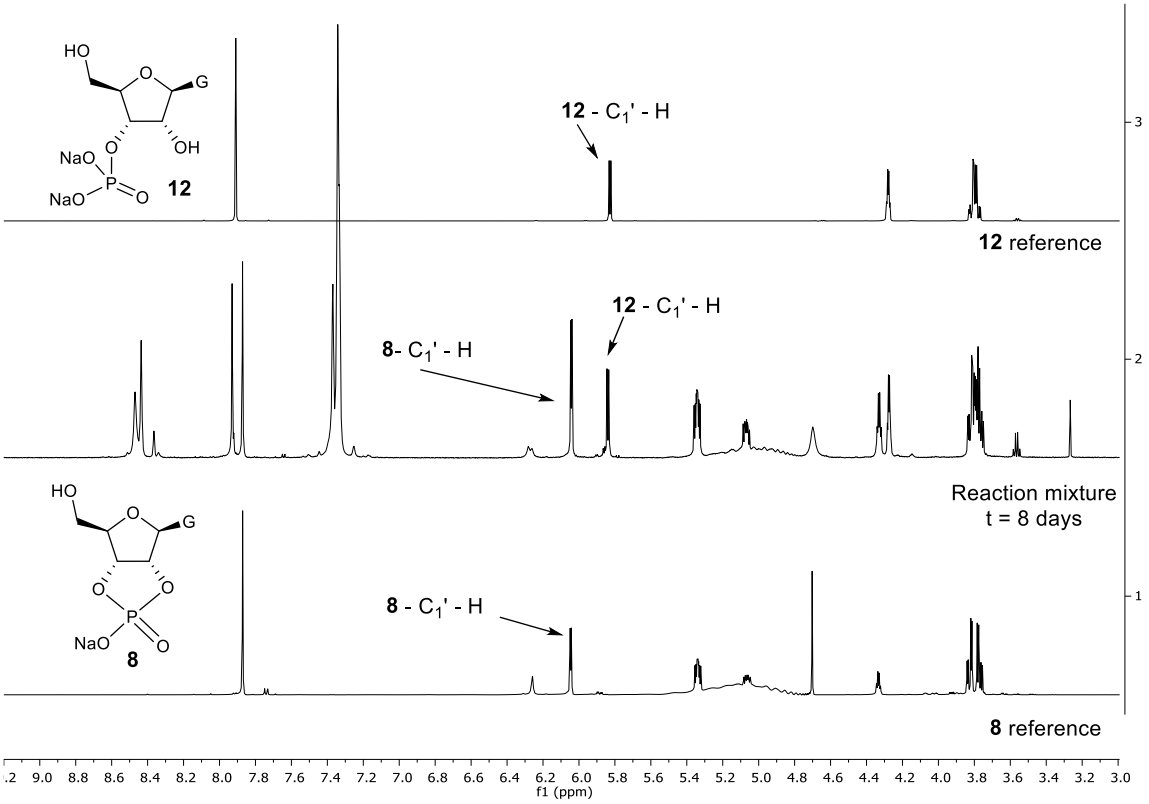
Supplementary Figure 56:

^{13}C NMR spectra of phosphorylation of **11** in solution at pH 7 in D_2O following the method B (Supplementary Table 5, entry 11, 8 days, middle spectrum). The spectra of starting material **11** (top spectrum) and reference product **7** (bottom spectrum) are shown for comparison.



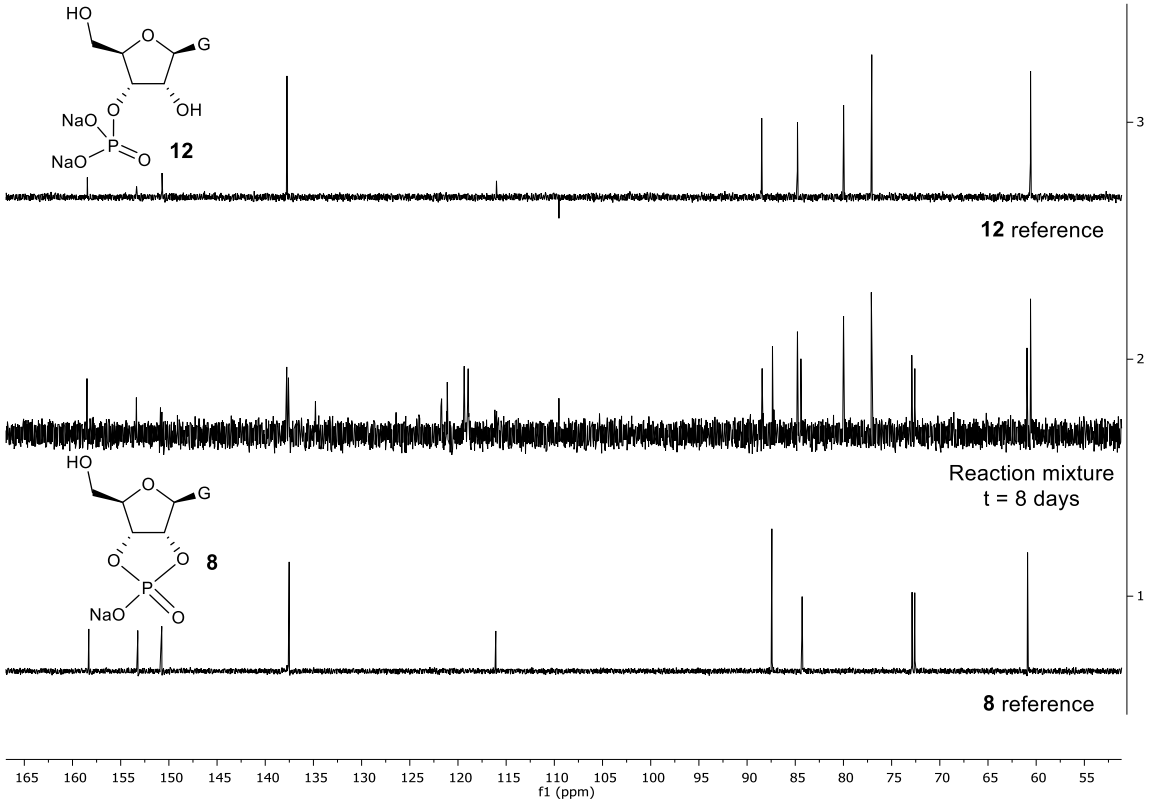
Supplementary Figure 57:

{H-coupled} ^{31}P NMR spectra of phosphorylation of **11** in solution at pH 7 in D_2O following the method B (Supplementary Table 5, entry 11, 8 days, middle spectrum). The spectra of starting material **11** (top spectrum) and reference product **7** (bottom spectrum) are shown for comparison.



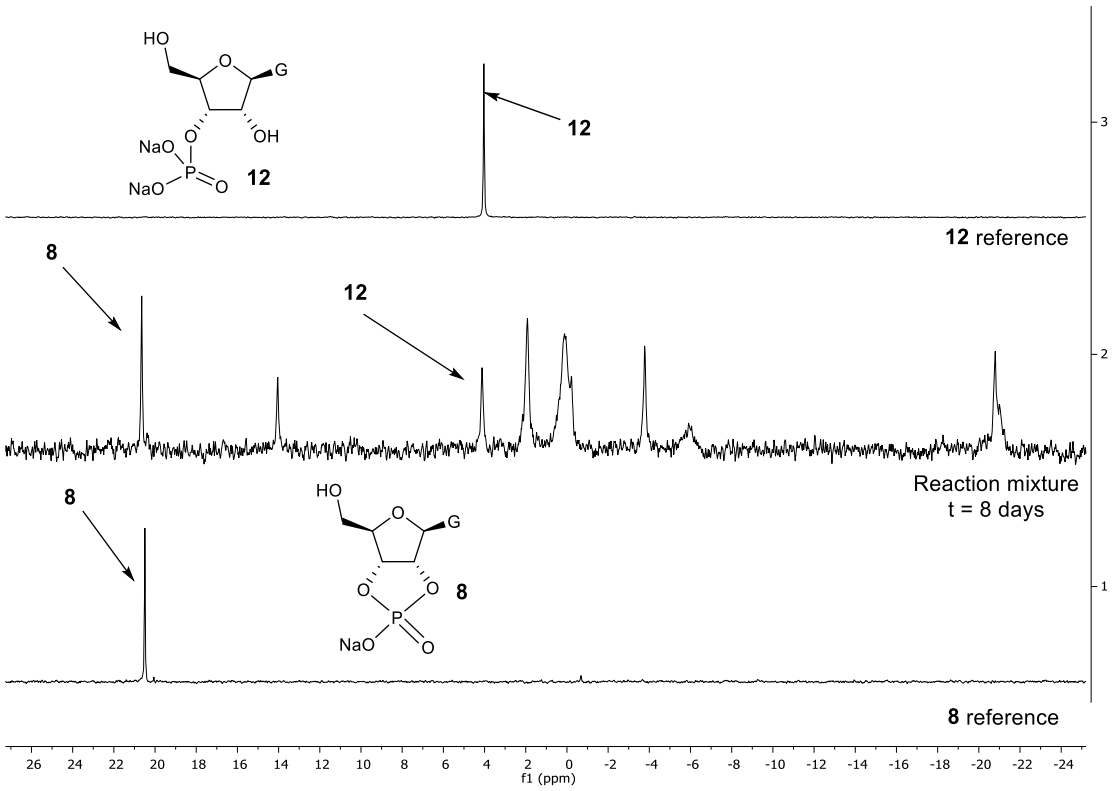
Supplementary Figure 58:

{H-coupled} ^{31}P NMR spectra of phosphorylation of **12** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 5, 8 days, middle spectrum). The spectra of starting material **12** (top spectrum) and reference product **8** (bottom spectrum) are shown for comparison.



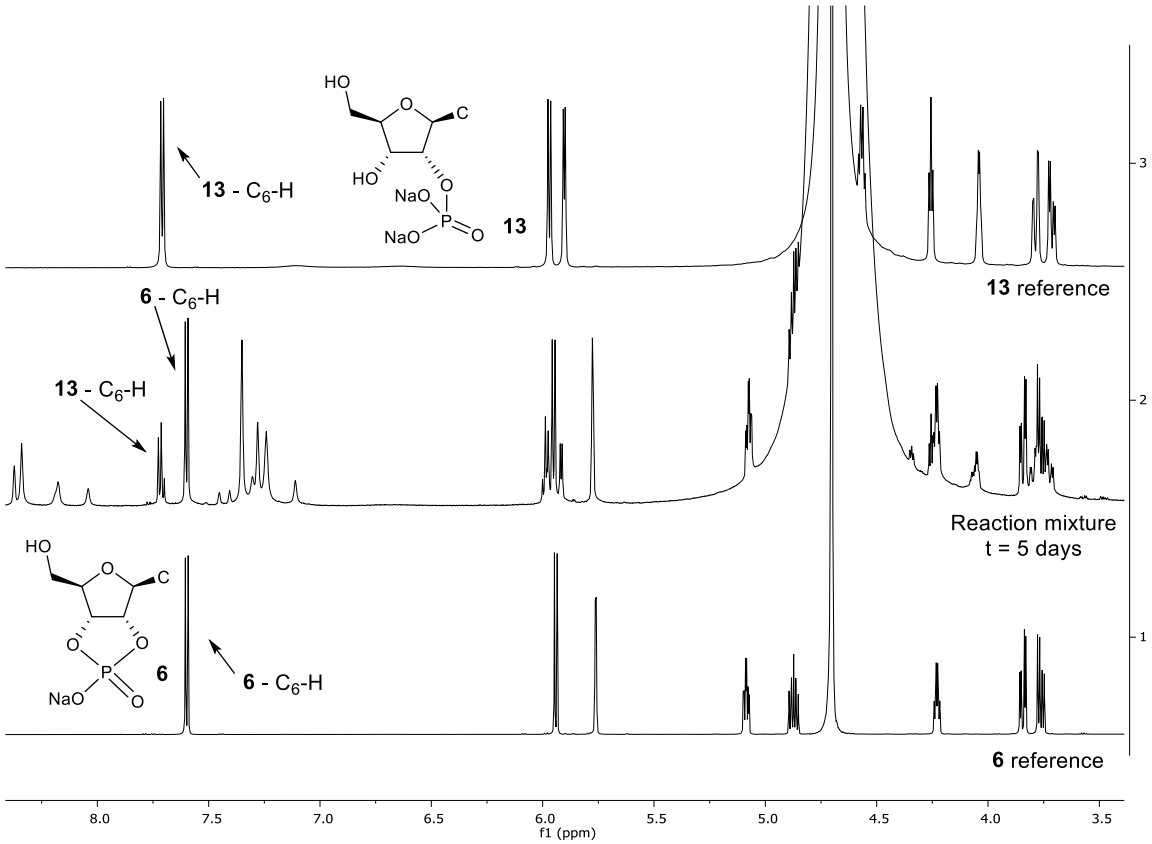
Supplementary Figure 59:

^{13}C NMR spectra of phosphorylation of **12** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 5, 8 days, middle spectrum). The spectra of starting material **12** (top spectrum) and reference product **8** (bottom spectrum) are shown for comparison.



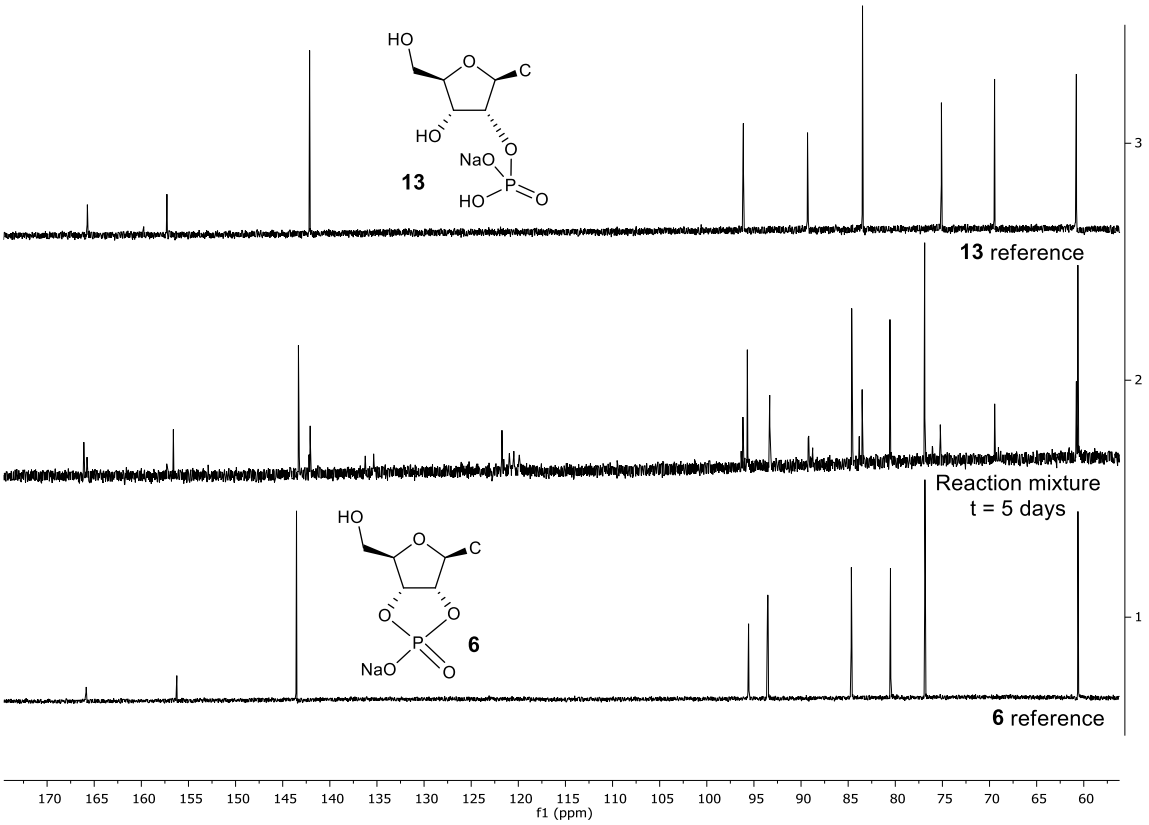
Supplementary Figure 60:

{H-decoupled} ^{31}P NMR spectra of phosphorylation of **12** in solution at pH 5.5 in D_2O following the method A (Supplementary Table 5, entry 5, 8 days, middle spectrum). The spectra of starting material **12** (top spectrum) and reference product **8** (bottom spectrum) are shown for comparison.



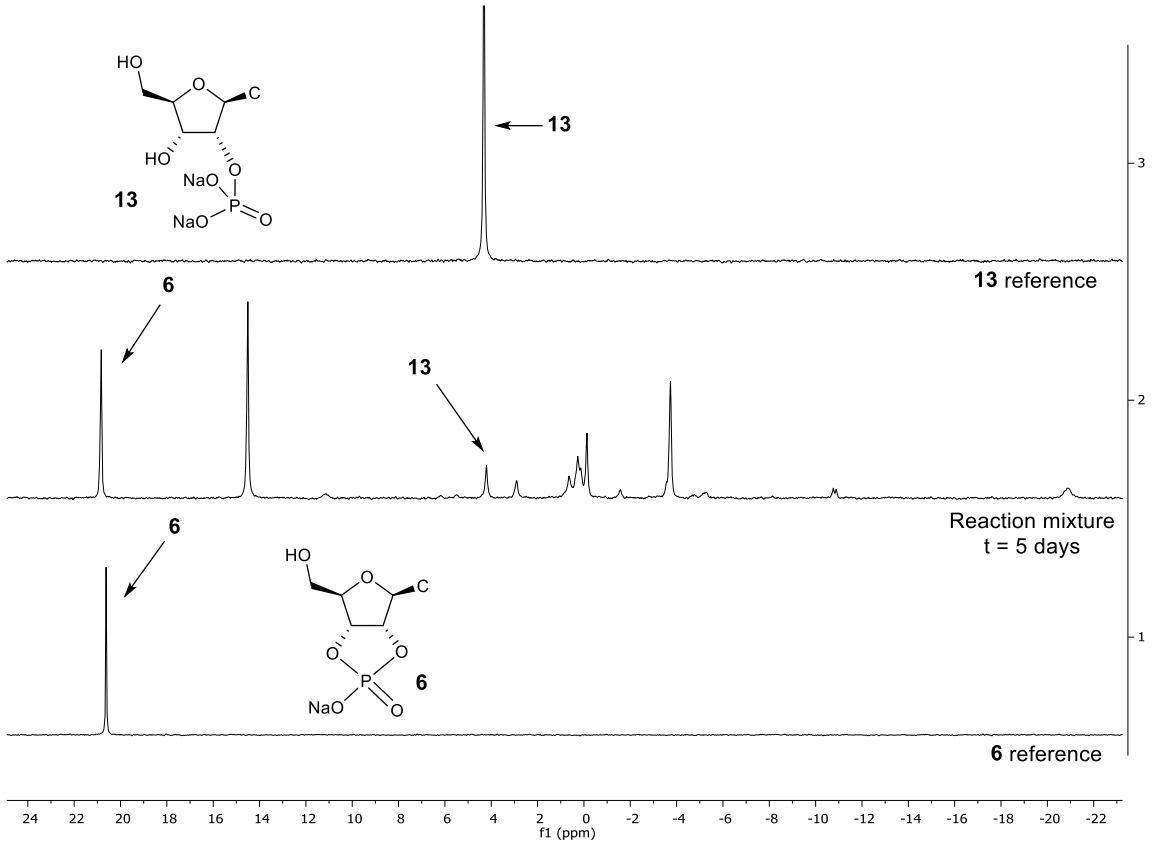
Supplementary Figure 61:

^{1}H NMR spectra of phosphorylation of **13** in solution at pH 7 in D_2O following the method B (Supplementary Table 6, entry 1, 5 days, middle spectrum). The spectra of starting material **13** (top spectrum) and reference product **6** (bottom spectrum) are shown for comparison.



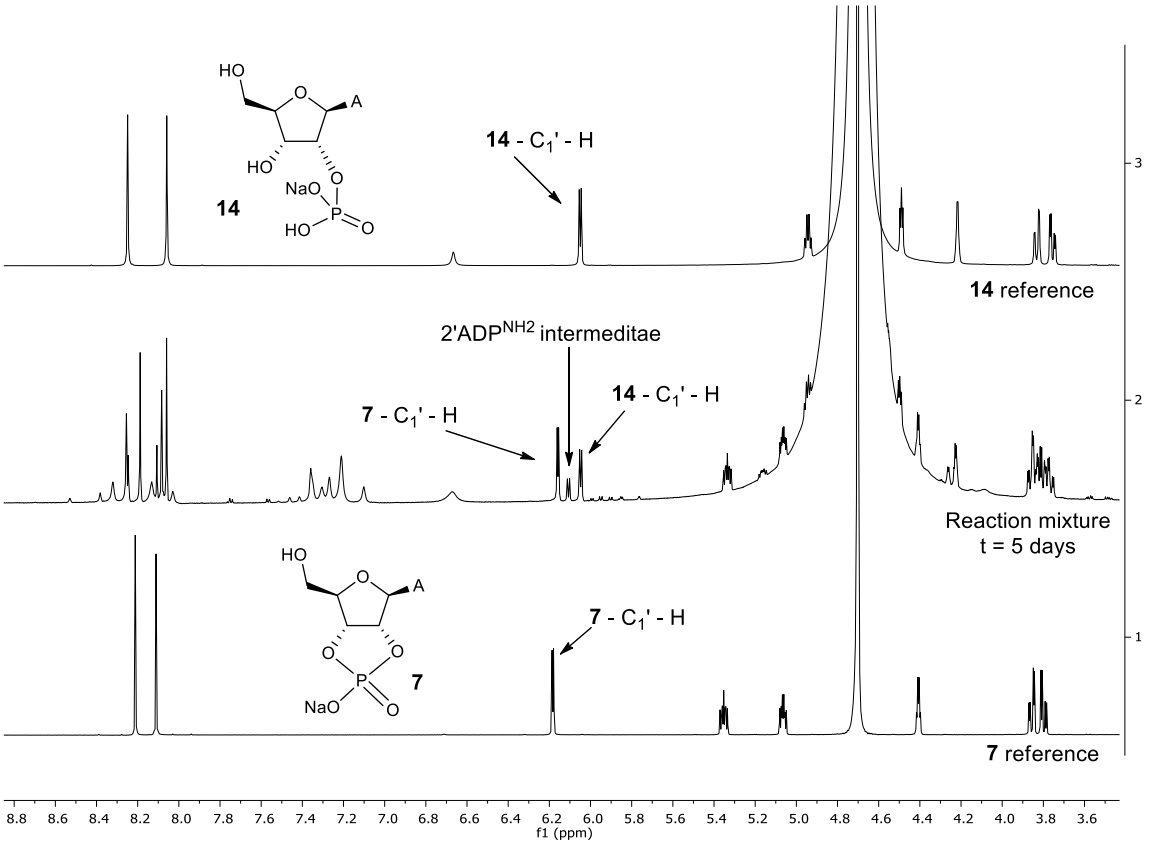
Supplementary Figure 62:

^{13}C NMR spectra of phosphorylation of **13** in solution at pH 7 in D_2O following the method B (Supplementary Table 6, entry 1, 5 days, middle spectrum). The spectra of starting material **13** (top spectrum) and reference product **6** (bottom spectrum) are shown for comparison.



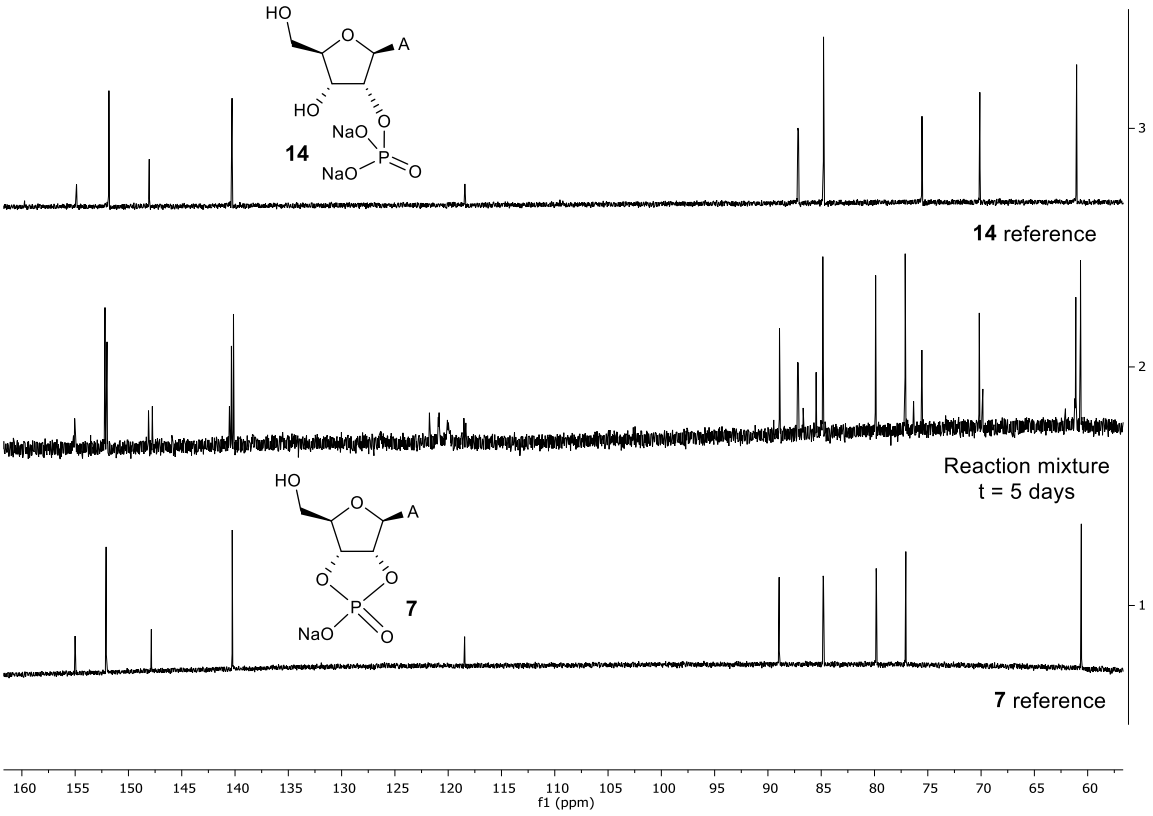
Supplementary Figure 63:

{H-decoupled} ^{31}P NMR spectra of phosphorylation of **13** in solution at pH 7 in D_2O following the method B (Supplementary Table 6, entry 1, 5 days, middle spectrum). The spectra of starting material **13** (top spectrum) and reference product **6** (bottom spectrum) are shown for comparison.



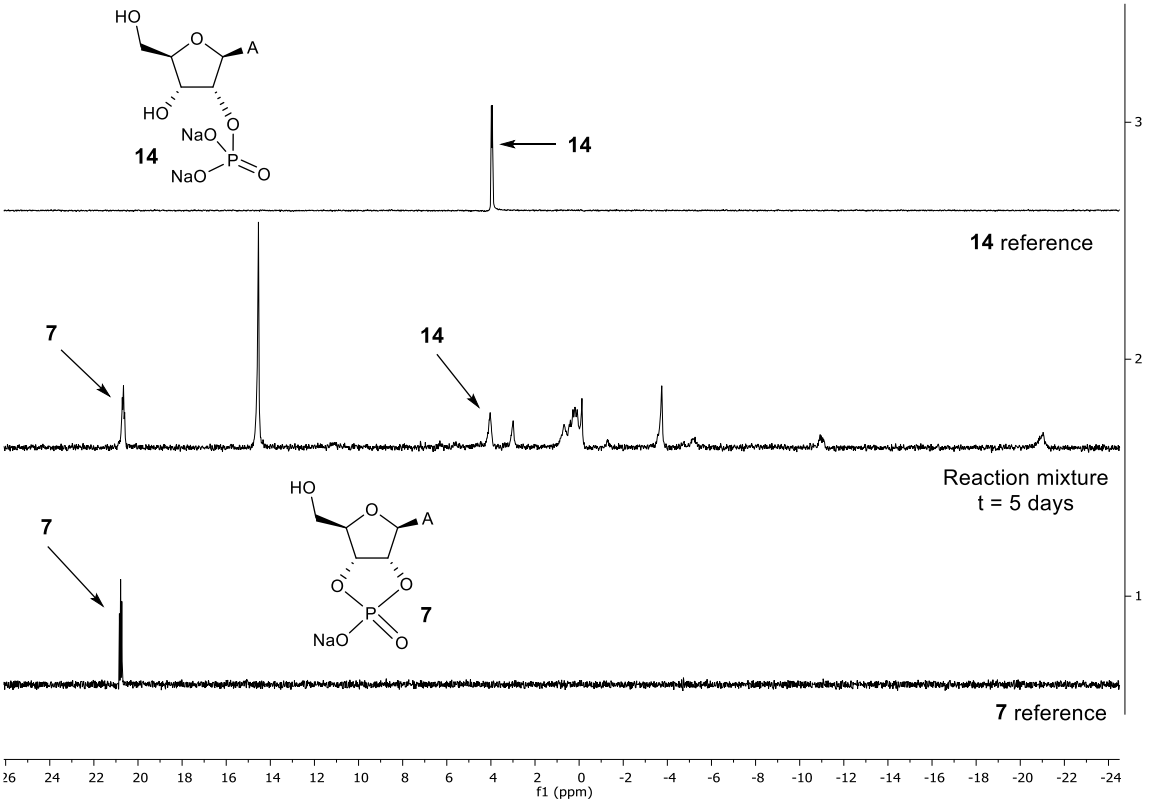
Supplementary Figure 64:

^{1}H NMR spectra of phosphorylation of **14** in solution at pH 7 in D_2O following the method B (Supplementary Table 6, entry 2, 5 days, middle spectrum). The spectra of starting material **14** (top spectrum) and reference product **7** (bottom spectrum) are shown for comparison.



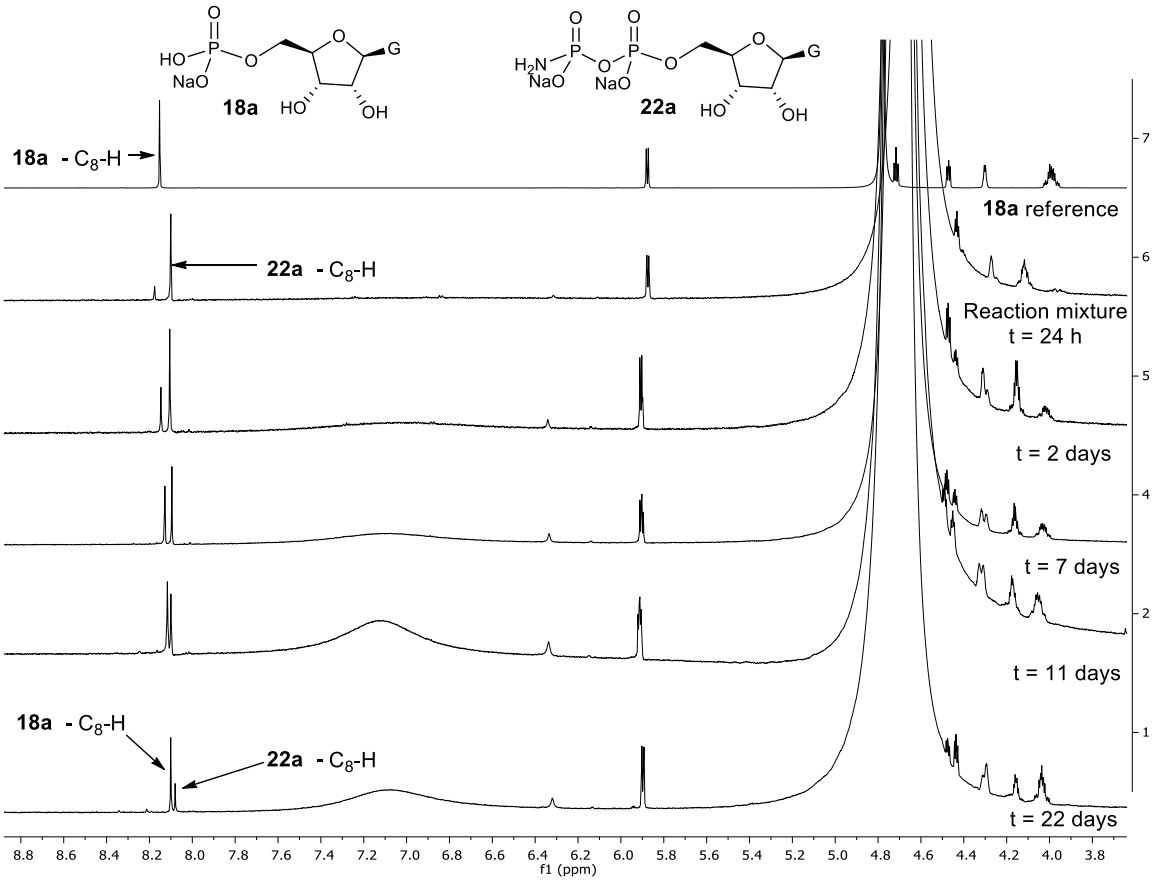
Supplementary Figure 65:

^{13}C NMR spectra of phosphorylation of **14** in solution at pH 7 in D_2O following the method B (Supplementary Table 6, entry 2, 5 days, middle spectrum). The spectra of starting material **14** (top spectrum) and reference product **7** (bottom spectrum) are shown for comparison.



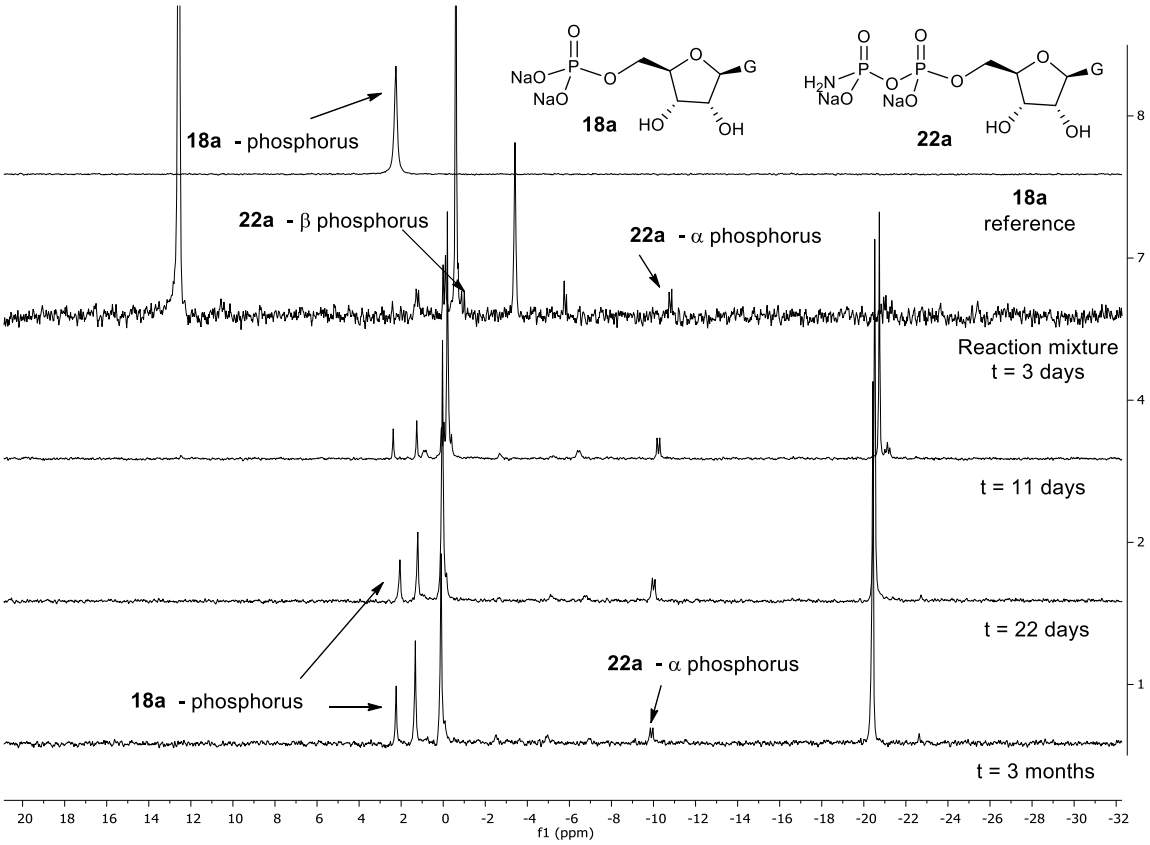
Supplementary Figure 66:

{H-coupled} ^{31}P NMR spectra of phosphorylation of **14** in solution at pH 7 in D_2O following the method B (Supplementary Table 6, entry 2, 5 days, middle spectrum). The spectra of starting material **14** (top spectrum) and reference product **7** (bottom spectrum) are shown for comparison.



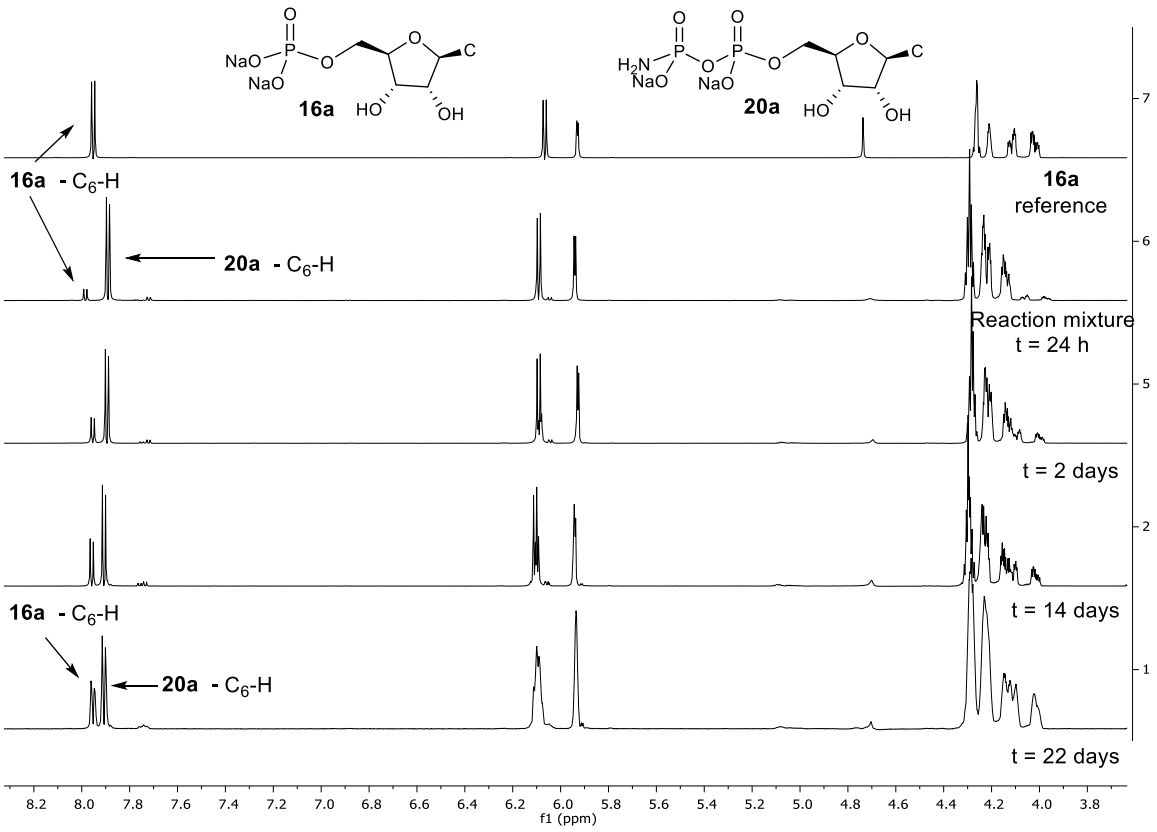
Supplementary Figure 67:

^{1}H NMR spectra showing the formation of **22a** by amidophosphorylation of **18a** and its subsequent hydrolysis in solution at pH 5.5 in D_2O with 3 equiv. of $ZnCl_2$ following the method A (Supplementary Table 8). The spectrum of starting material **18a** (top spectrum) is shown for comparison.



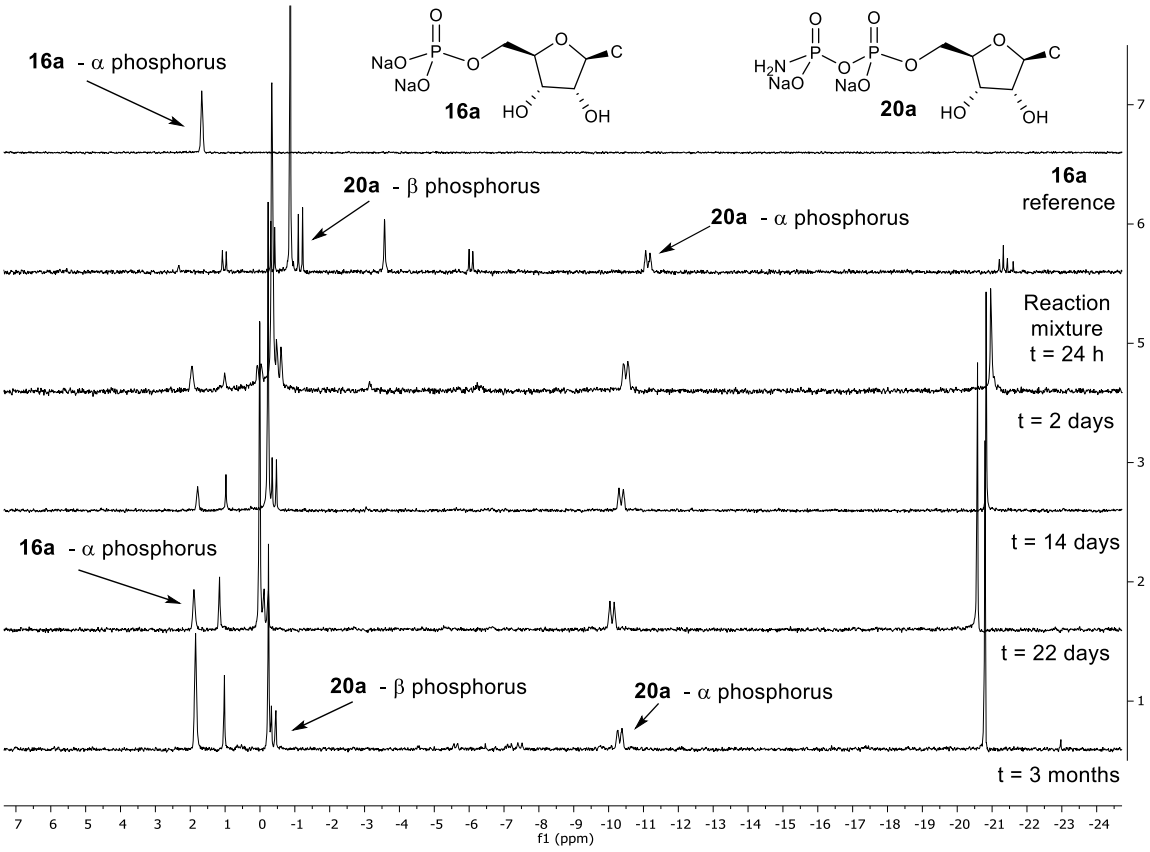
Supplementary Figure 68:

{H-decoupled} ^{31}P NMR spectra showing the formation of **22a** by amidophosphorylation of **18a** and its subsequent hydrolysis in solution at pH 5.5 in D_2O with 3 equiv. of $ZnCl_2$ following the method A (Supplementary Table 8). The spectrum of starting material **18a** (top spectrum) is shown for comparison.



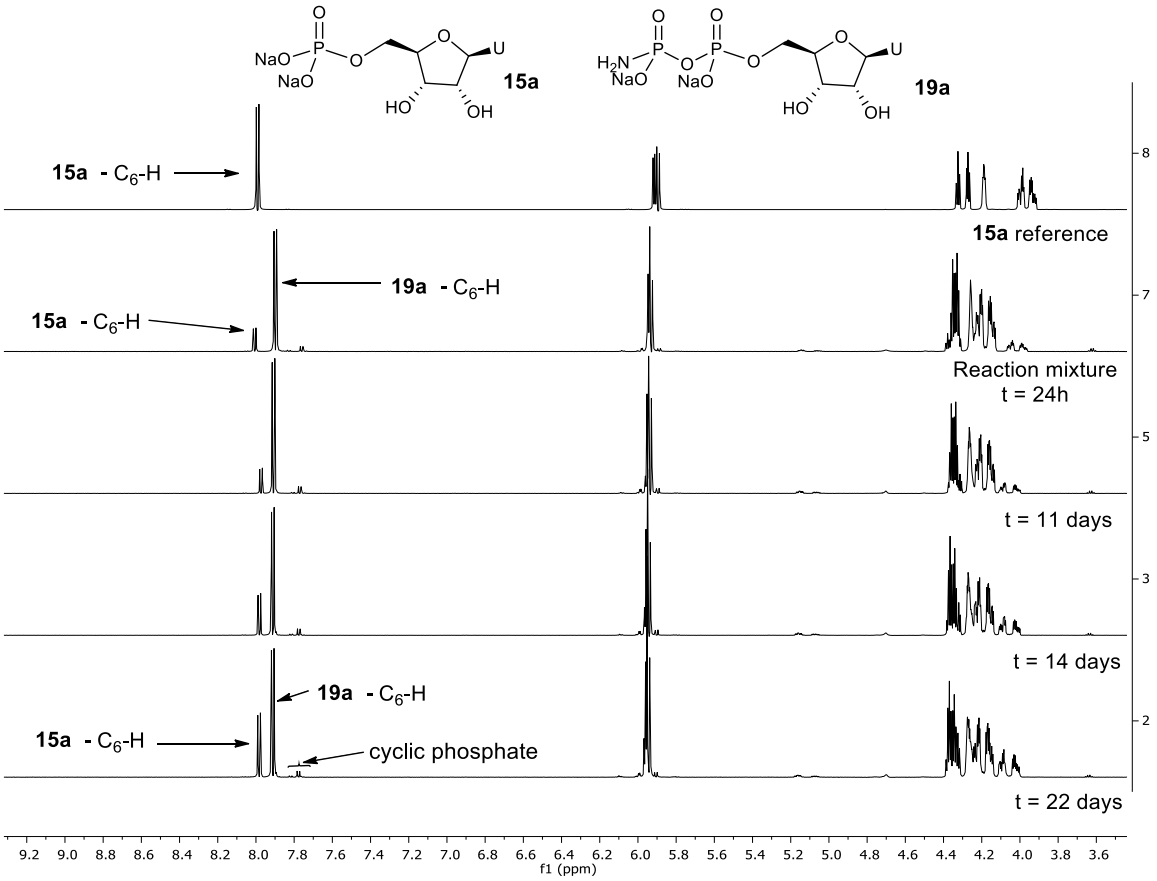
Supplementary Figure 69:

{Water suppression} ^{1}H NMR spectra showing the formation of **20a** by amidophosphorylation of **16a** and its subsequent hydrolysis in solution at pH 5.5 in D_2O with 3 equiv. of $ZnCl_2$ following the method A (Supplementary Table 8). The spectrum of starting material **16a** (top spectrum) is shown for comparison.



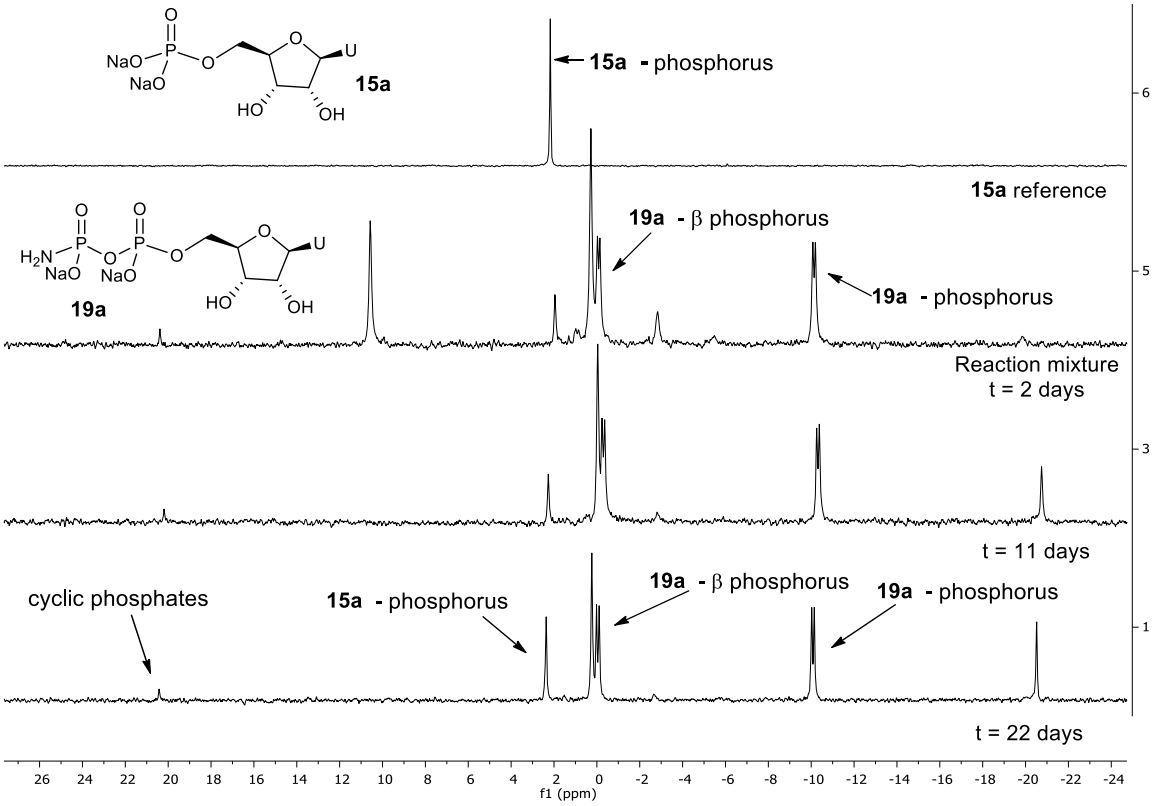
Supplementary Figure 70:

{H-coupled} ^{31}P NMR spectra showing the formation of **20a** by amidophosphorylation of **16a** and its subsequent hydrolysis in solution at pH 5.5 in D_2O with 3 equiv. of $ZnCl_2$ following the method A (Supplementary Table 8). The spectrum of starting material **16a** (top spectrum) is shown for comparison.



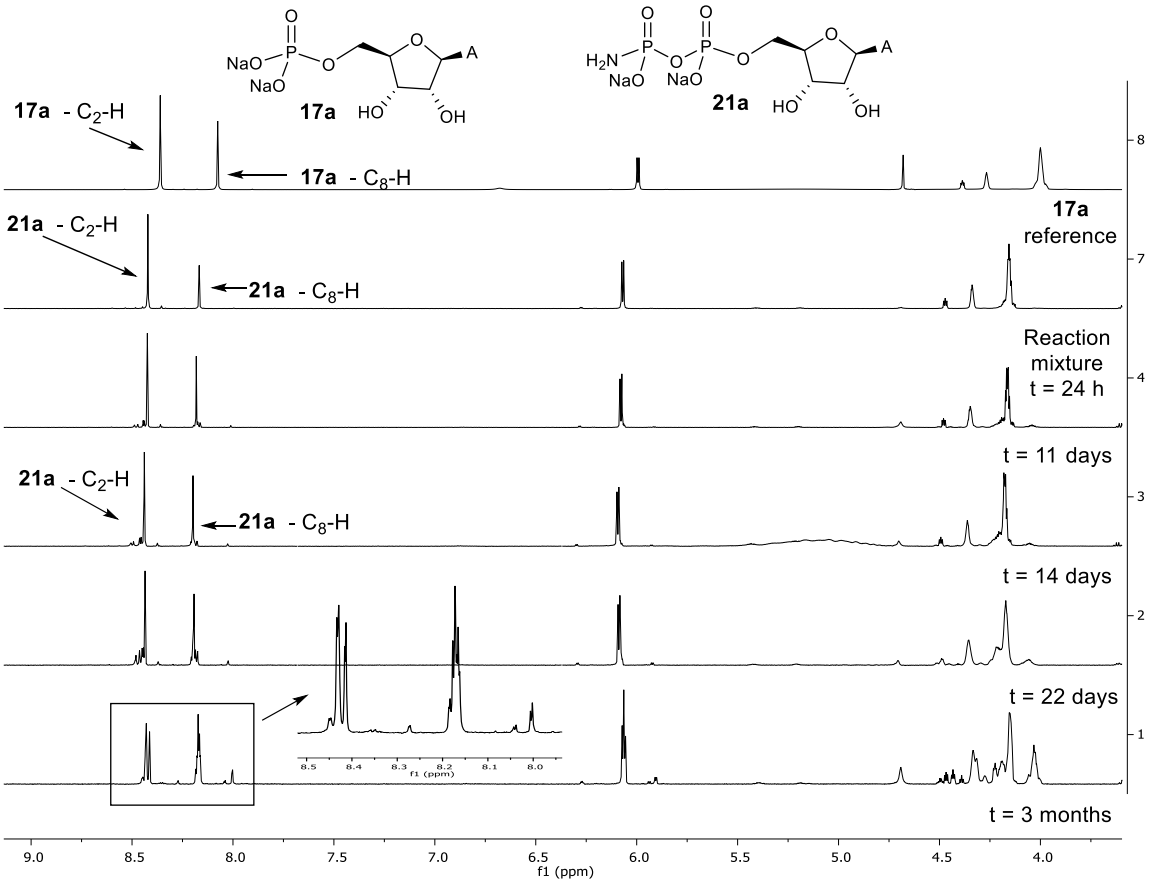
Supplementary Figure 71:

{Water suppression} ^{1}H NMR spectra showing the formation of **19a** by amidophosphorylation of **15a** and its subsequent hydrolysis in solution at pH 5.5 in D_2O with 3 equiv. of $ZnCl_2$ following the method A (Supplementary Table 7). The spectrum of starting material **15a** (top spectrum) is shown for comparison.



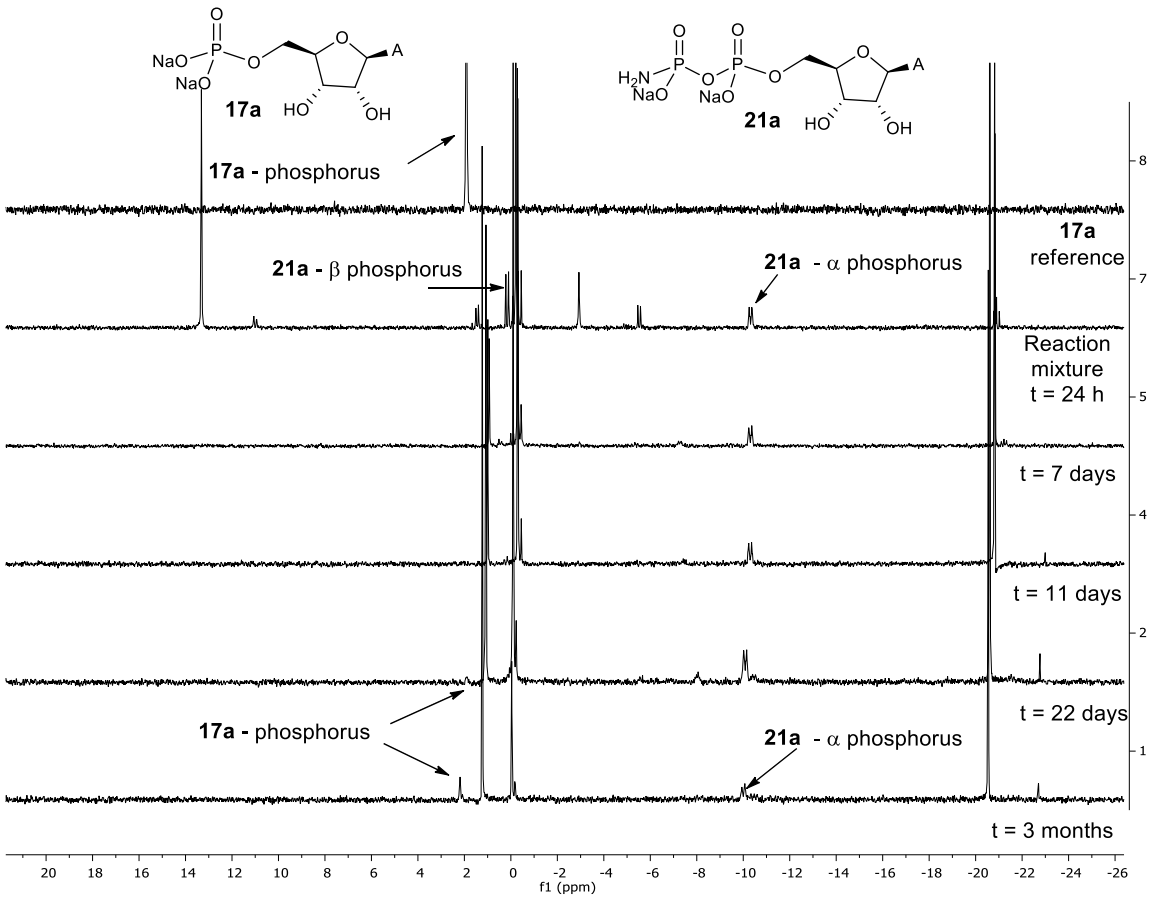
Supplementary Figure 72:

{H-decoupled} ^{31}P NMR spectra showing the formation of **19a** by amidophosphorylation of **15a** and its subsequent hydrolysis in solution at pH 5.5 in D_2O with 3 equiv. of $ZnCl_2$ following the method A (Supplementary Table 7). The spectrum of starting material **15a** (top spectrum) is shown for comparison.



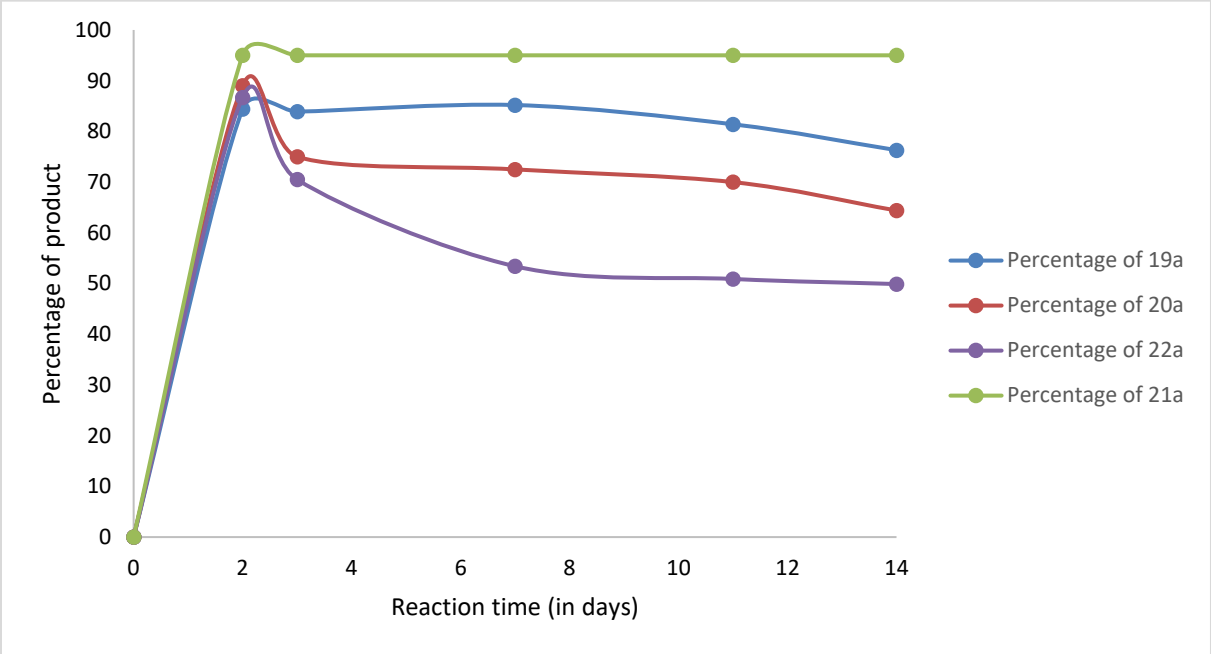
Supplementary Figure 73:

{Water suppression} ^{1}H NMR spectra showing the formation of **21a** by amidophosphorylation of **17a** and its subsequent hydrolysis in solution at pH 5.5 in D_2O with 3 equiv. of $ZnCl_2$ following the method A (Supplementary Table 8). The spectrum of starting material **17a** (top spectrum) is shown for comparison.



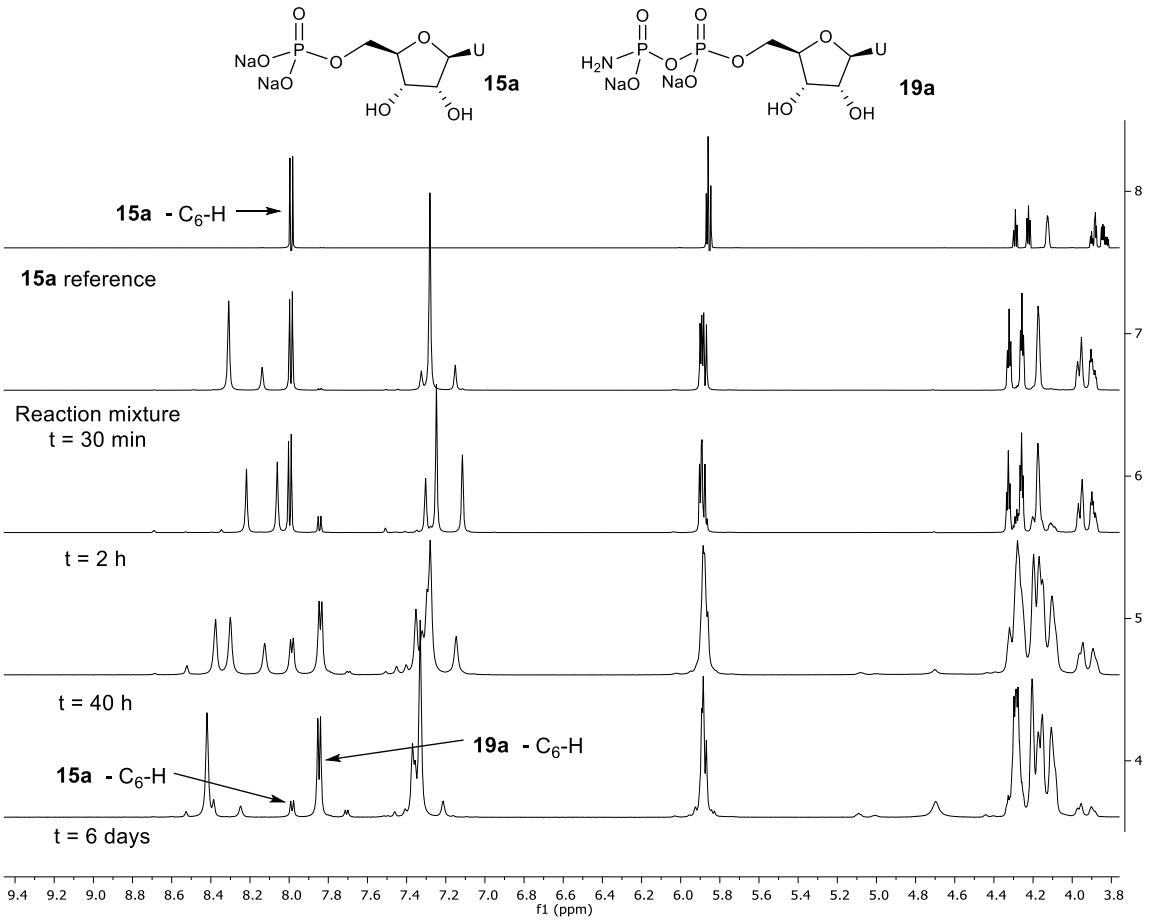
Supplementary Figure 74:

{H-coupled} ^{31}P NMR spectra showing the formation of **21a** by amidophosphorylation of **17a** and its subsequent hydrolysis in solution at pH 5.5 in D_2O with 3 equiv. of $ZnCl_2$ following the method A (Supplementary Table 8). The spectrum of starting material **17a** (top spectrum) is shown for comparison.



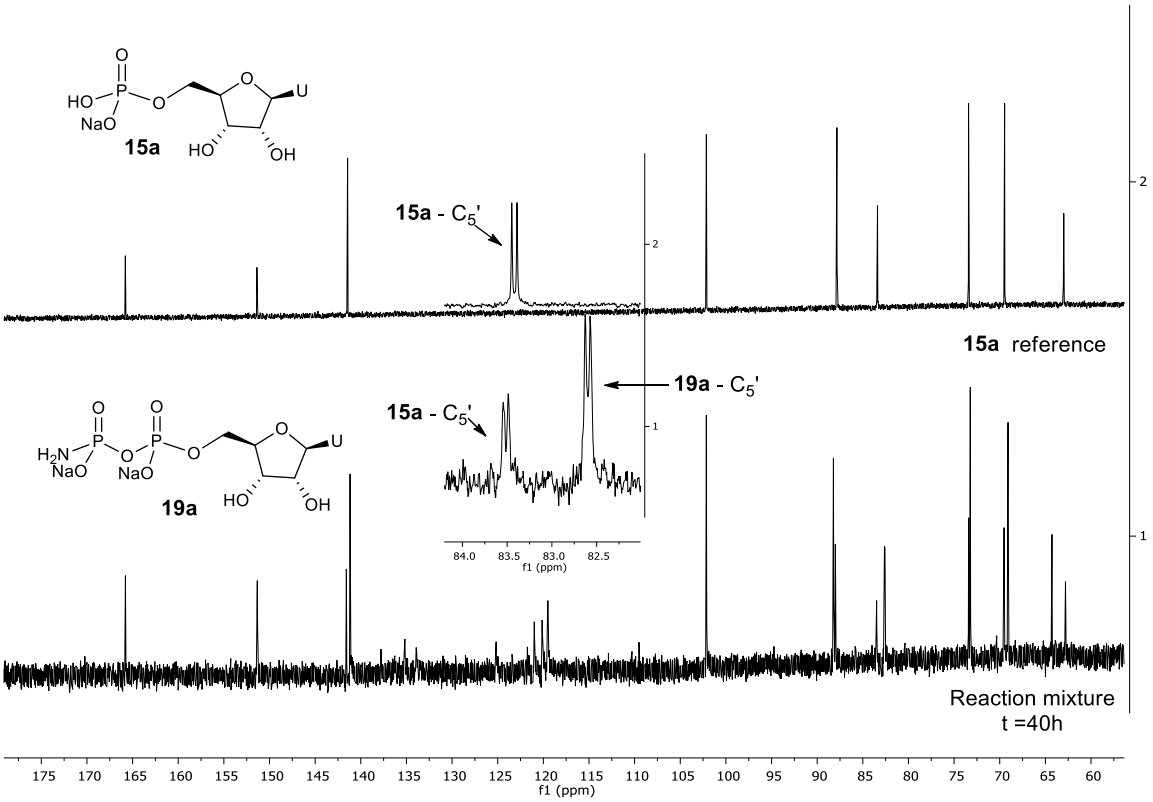
Supplementary Figure 75:

Reaction profile, as monitored by ^{1}H-NMR, showing the formation of **19a**, **20a**, **21a** and **22a** by amidophosphorylation of the NMPs and their hydrolysis back to **15a**, **16a**, **17a** and **18a**: reaction in solution at pH 5.5 in D_2O in presence of 3 equiv. of zinc chloride. The hydrolysis rate follows this order GDP^{NH2} >> CDP^{NH2} > UDP^{NH2} >>ADP^{NH2}. The spectra given in Supplementary Fig. 67 to 74 show these results.



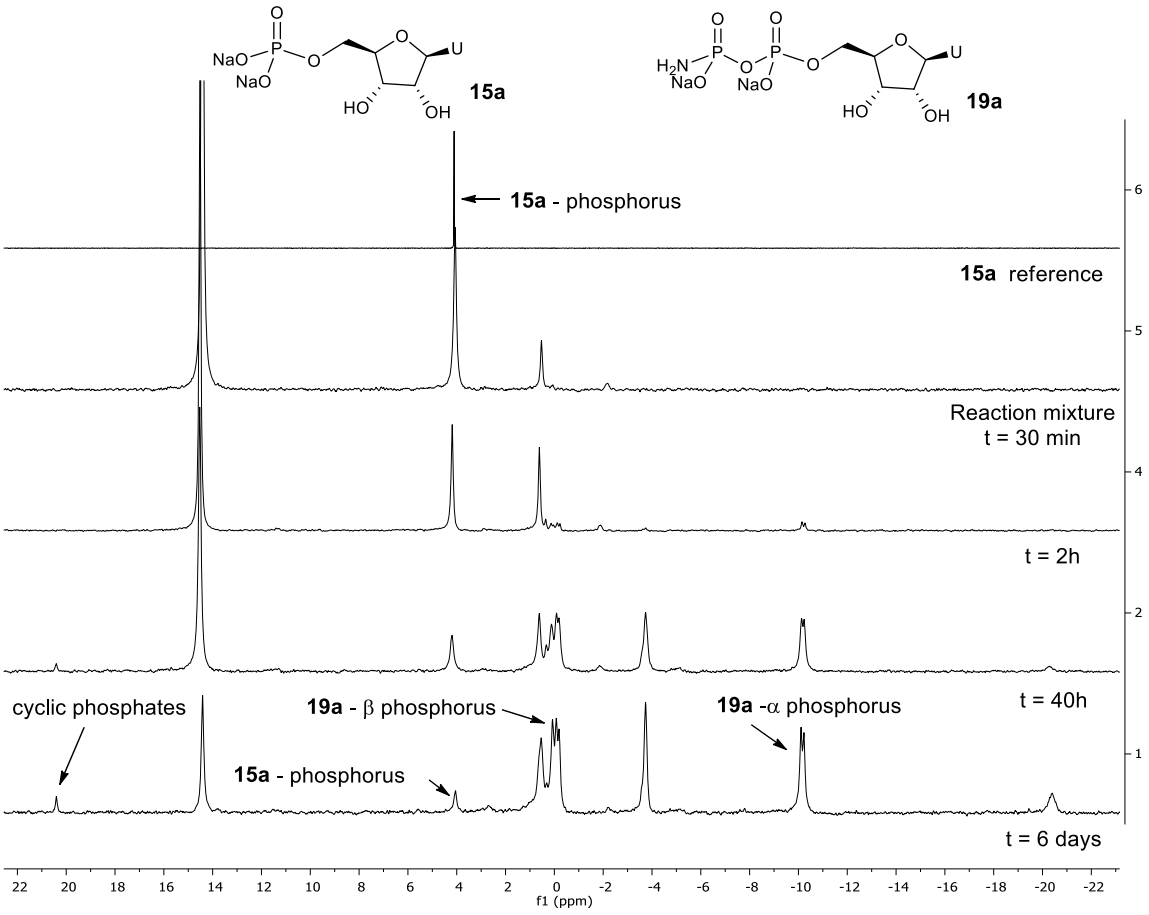
Supplementary Figure 76:

{Water suppression} ^{1}H NMR spectra of the formation of **19a** by amidophosphorylation of **15a** in solution at pH 7 in D_2O following the method B (Supplementary Table 7, entry 4). The spectrum of **15a** (top spectrum) is shown for comparison.



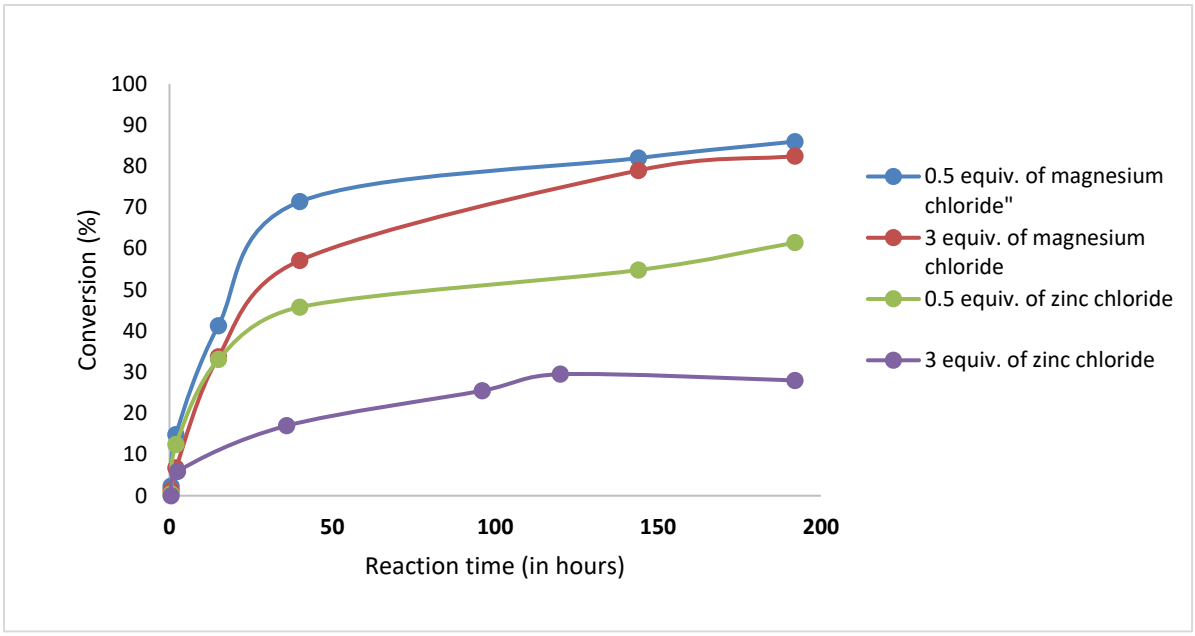
Supplementary Figure 77:

^{13}C NMR spectra of the formation of **19a** by amidophosphorylation **15a** in solution at pH 7 in D_2O following the method B after 40 h of reaction (bottom spectrum). The spectrum of **15a** (top spectrum) is shown for comparison.



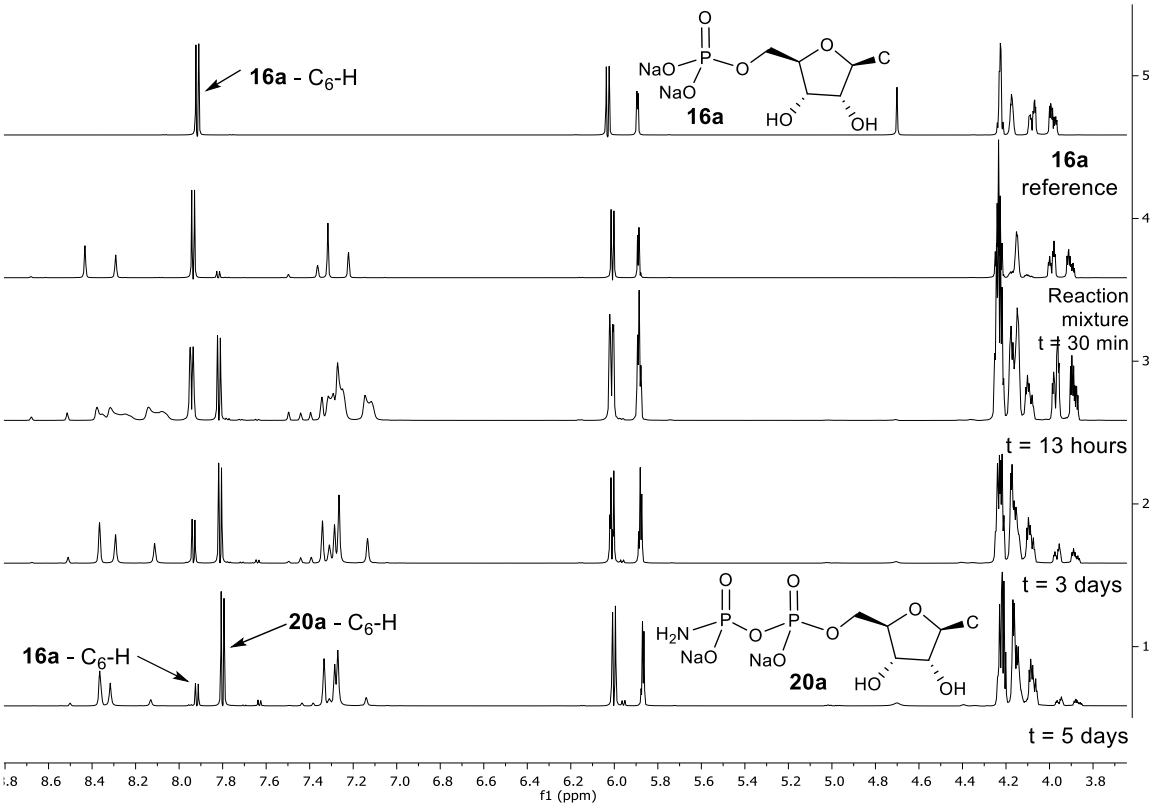
Supplementary Figure 78:

{H-decoupled} ^{31}P NMR spectra of the formation of **19a** by amidophosphorylation **15a** in solution at pH 7 in D_2O following the method B (Supplementary Table 7). The spectrum of **15a** (top spectrum) is shown for comparison.



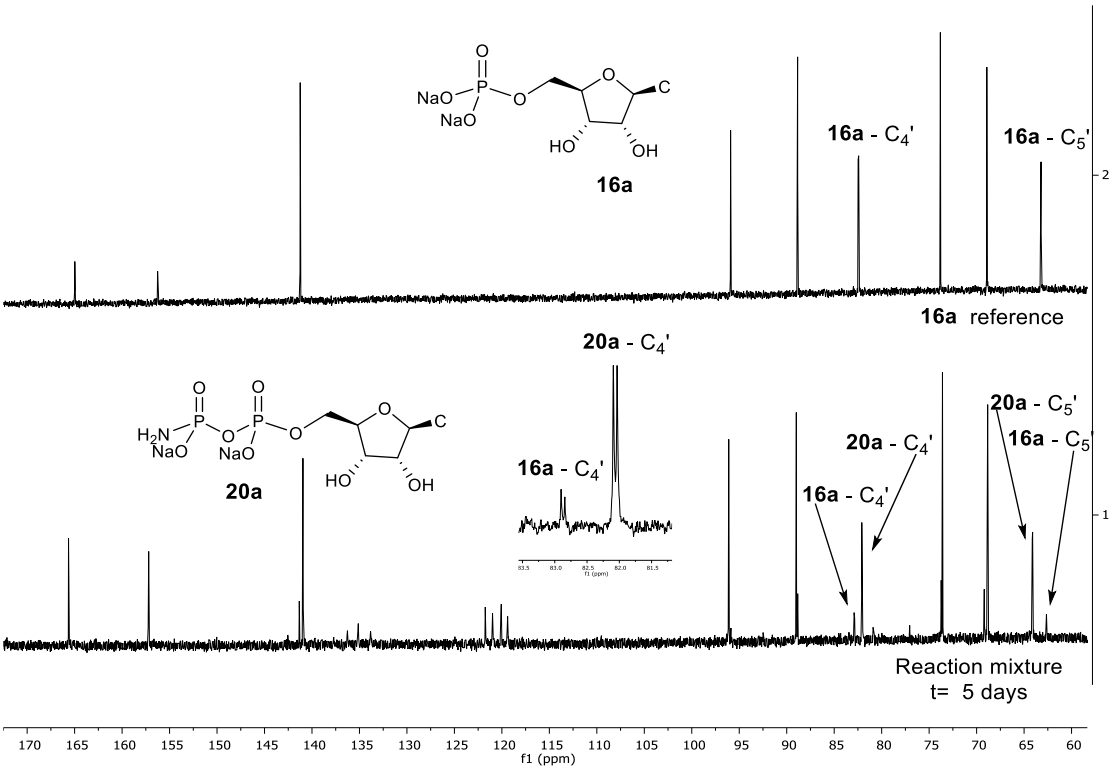
Supplementary Figure 79:

Comparison of the effect of 0.5 and 3 equiv. of zinc chloride and magnesium chloride at pH 7 for the amidophosphorylation of **15a**. The conversion percentage has been determined by the ^{1}H-NMR integral ratio of C_6-*H* for **15a** and **19a**. The results from Supplementary Fig. 76-78 is represented by the blue line.



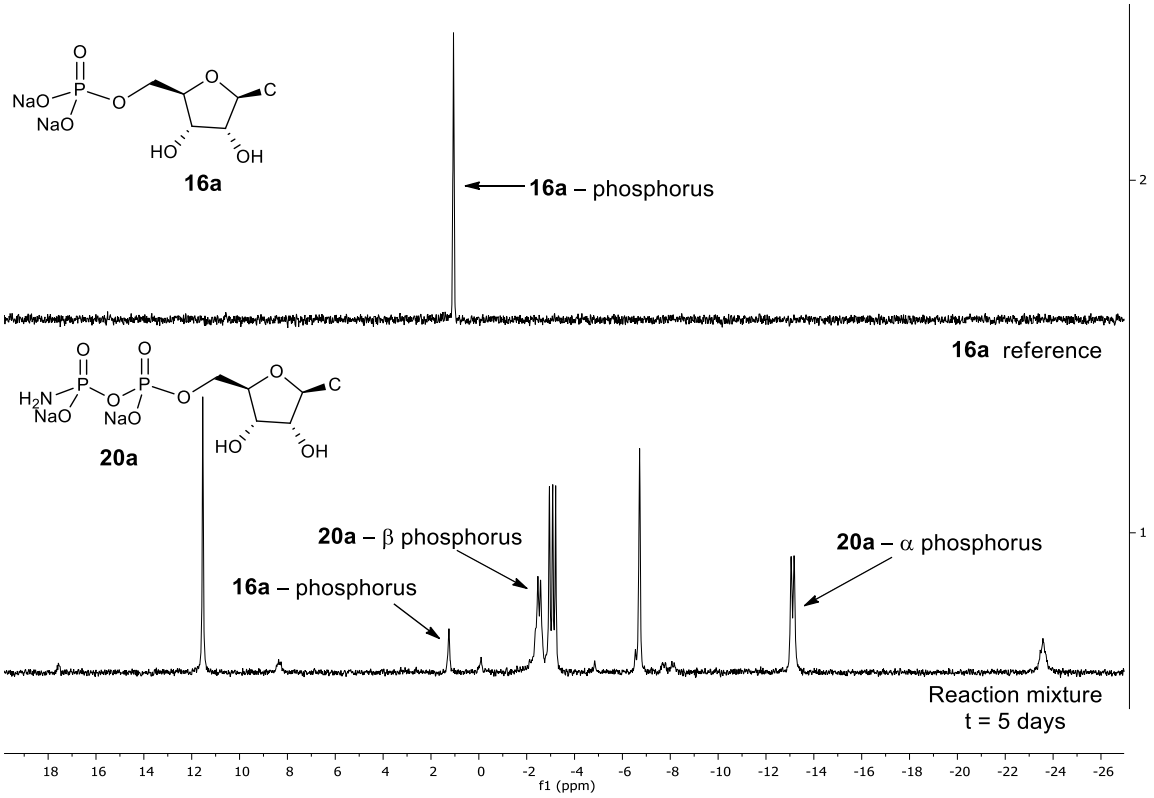
Supplementary Figure 80:

{Water suppression} ^{1}H NMR spectra of the formation of **20a** by amidophosphorylation **16a** in solution at pH 7 in D_2O following the method B (Supplementary Table 8, entry 4). The spectrum of **16a** (top spectrum) is shown for comparison.



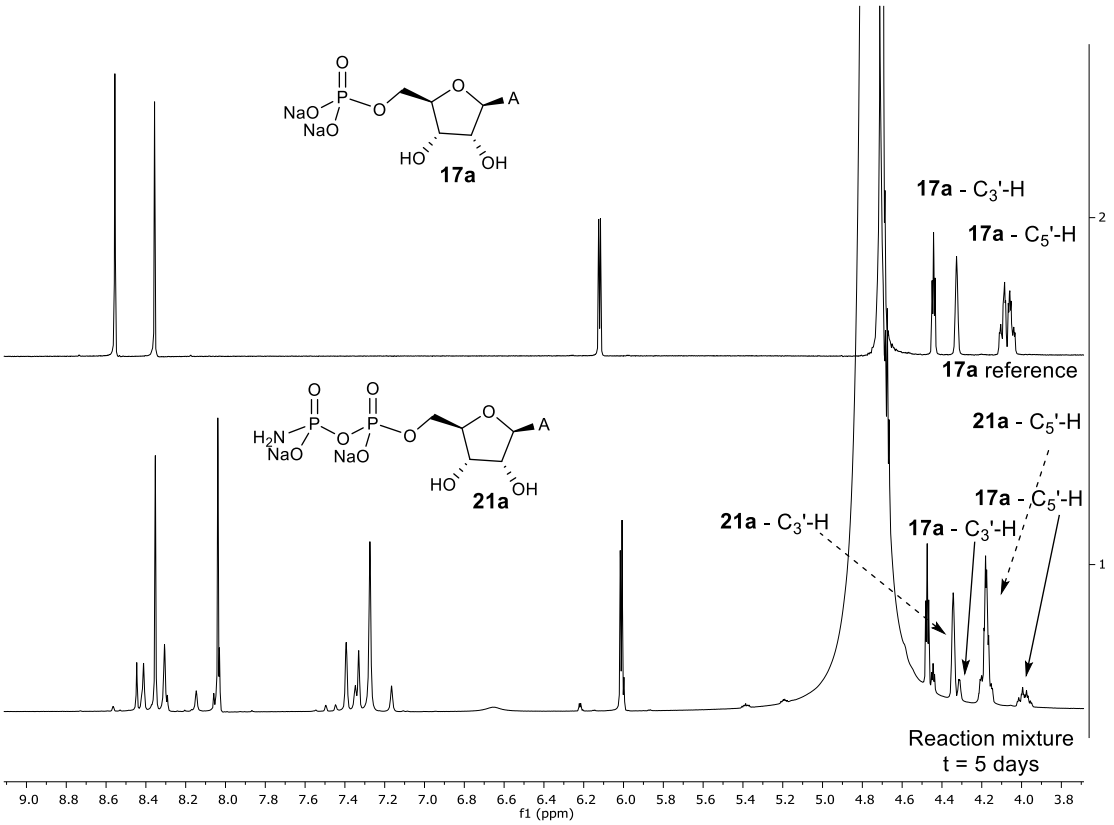
Supplementary Figure 81:

^{13}C NMR spectra of the formation of **20a** by amidophosphorylation of **16a** in solution at pH 7 in D_2O following the method B (Supplementary Table 8, entry 4, bottom spectrum). The spectrum of **16a** (top spectrum) is shown for comparison.



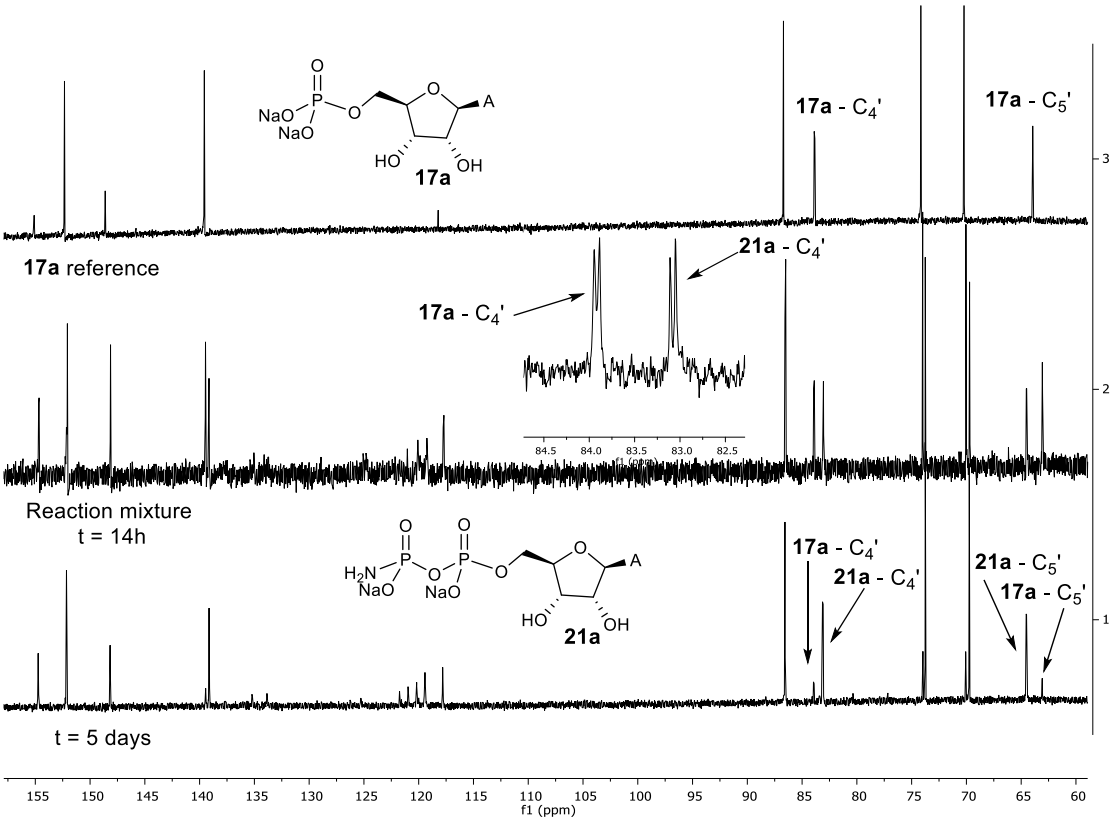
Supplementary Figure 82:

{H-decoupled} ^{31}P NMR spectra of the formation of **20a** by amidophosphorylation of **16a** in solution at pH 7 in D_2O following the method B (Supplementary Table 8, entry 4, bottom spectrum). The spectrum of **16a** (top spectrum) is shown for comparison.



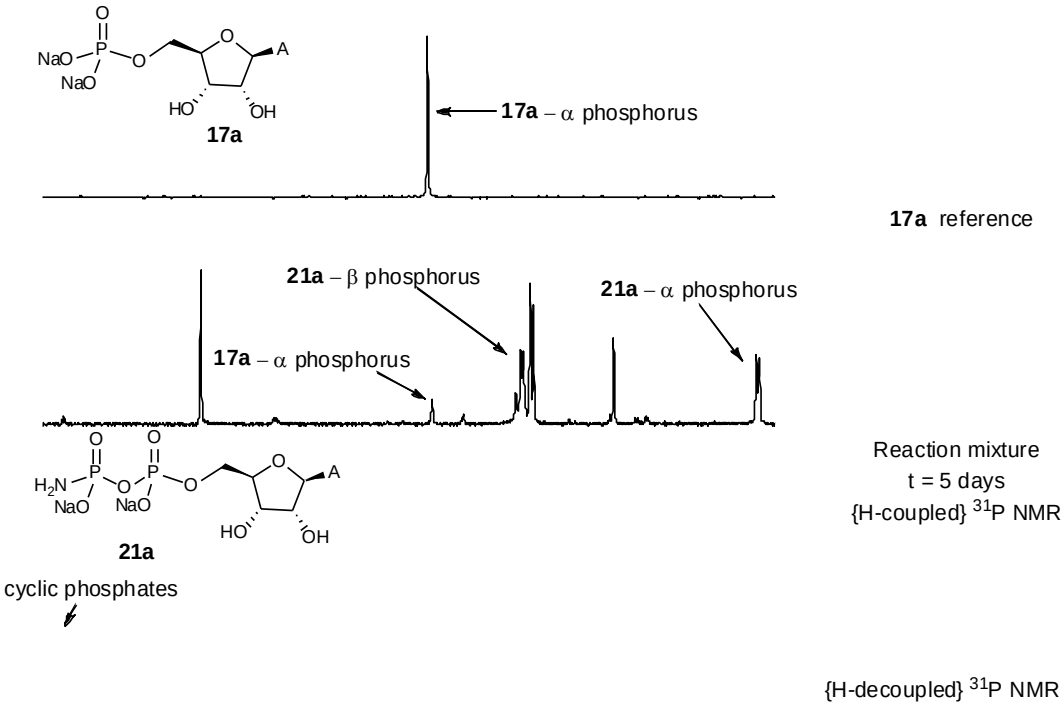
Supplementary Figure 83:

^{1}H NMR spectra of the formation of **21a** by amidophosphorylation of **17a** in solution at pH 7 in D_2O following the method B (Supplementary Table 8, entry 5). The spectrum of **17a** (top spectrum) is shown for comparison.



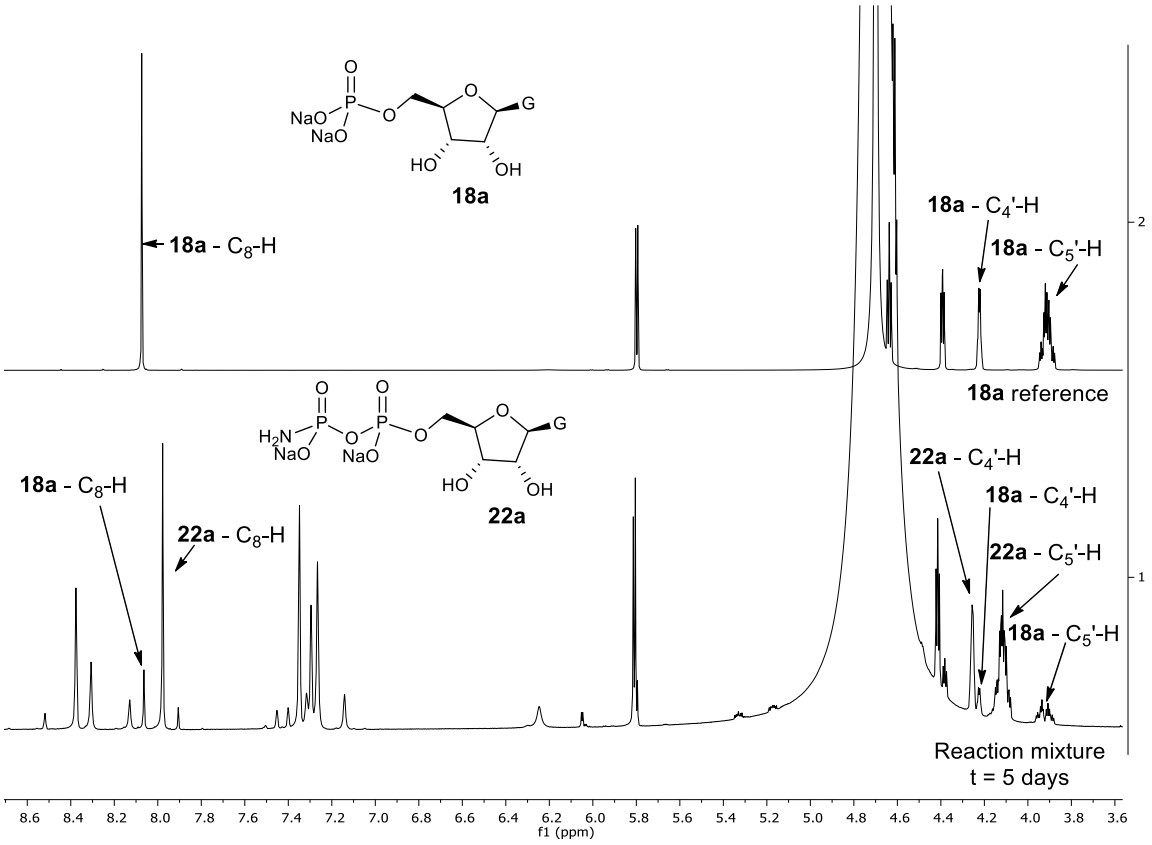
Supplementary Figure 84:

^{13}C NMR spectra of the formation of **21a** by amidophosphorylation of **17a** in solution at pH 7 in D_2O following the method B (Supplementary Table 8, entry 5, bottom spectrum). The spectrum of **17a** (top spectrum) is shown for comparison.



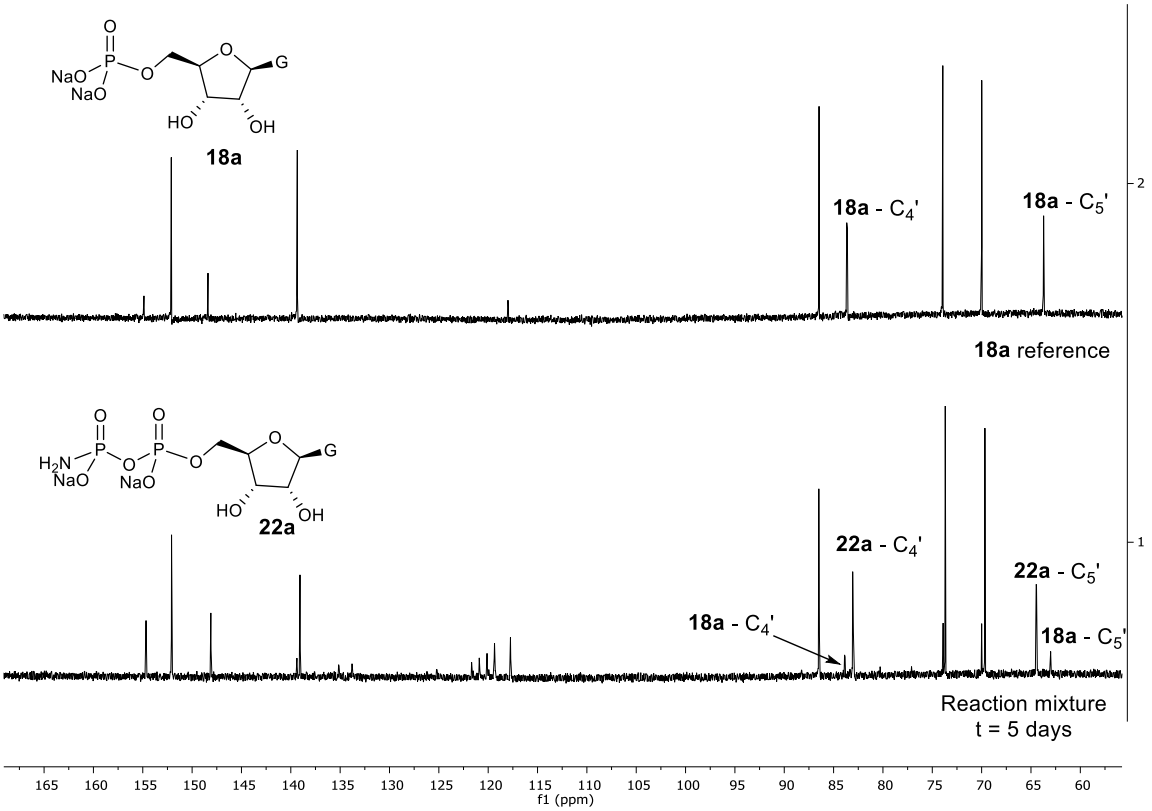
Supplementary Figure 85:

{H-coupled} ^{31}P NMR (middle spectrum) and {H-decoupled} ^{31}P NMR (bottom spectrum) spectra of the formation of **21a** by amidophosphorylation of **17a** in solution at pH 7 in D_2O following the method B (Supplementary Table 8, entry 5). The spectrum of **17a** (top spectrum) is shown for comparison.



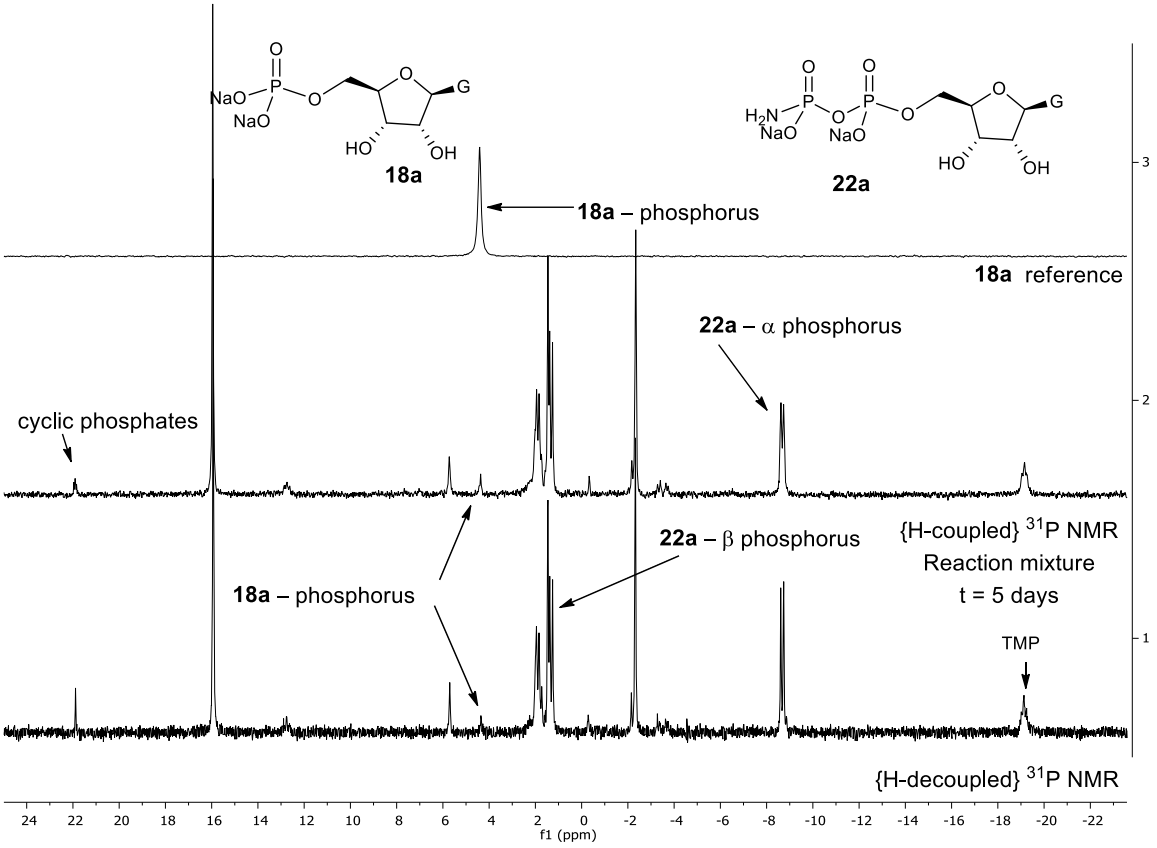
Supplementary Figure 86:

^{1}H NMR spectra of the formation of **22a** by amidophosphorylation of **18a** in solution at pH 7 in D_2O following the method B (Supplementary Table 8, entry 6). The spectrum of **18a** (top spectrum) is shown for comparison.



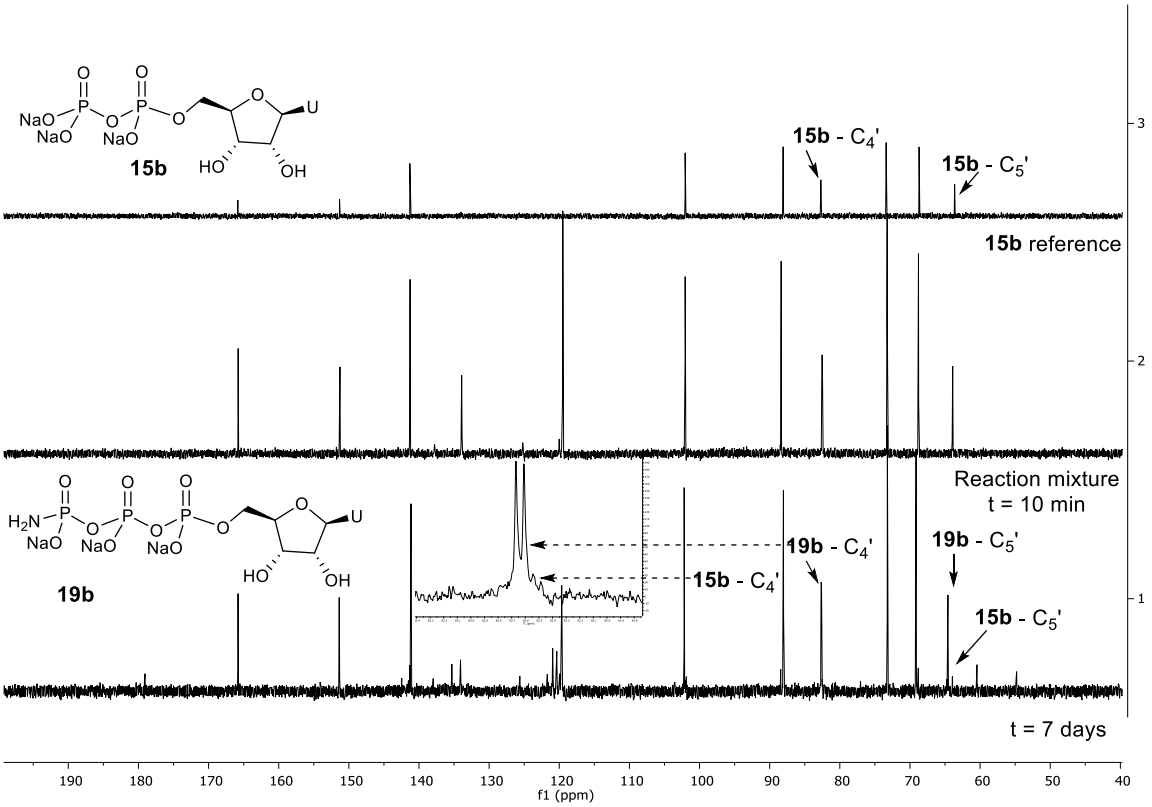
Supplementary Figure 87:

^{13}C NMR spectra of the formation of **22a** by amidophosphorylation of **18a** in solution at pH 7 in D_2O following the method B (Supplementary Table 8, entry 6). The spectrum of **18a** (top spectrum) is shown for comparison.



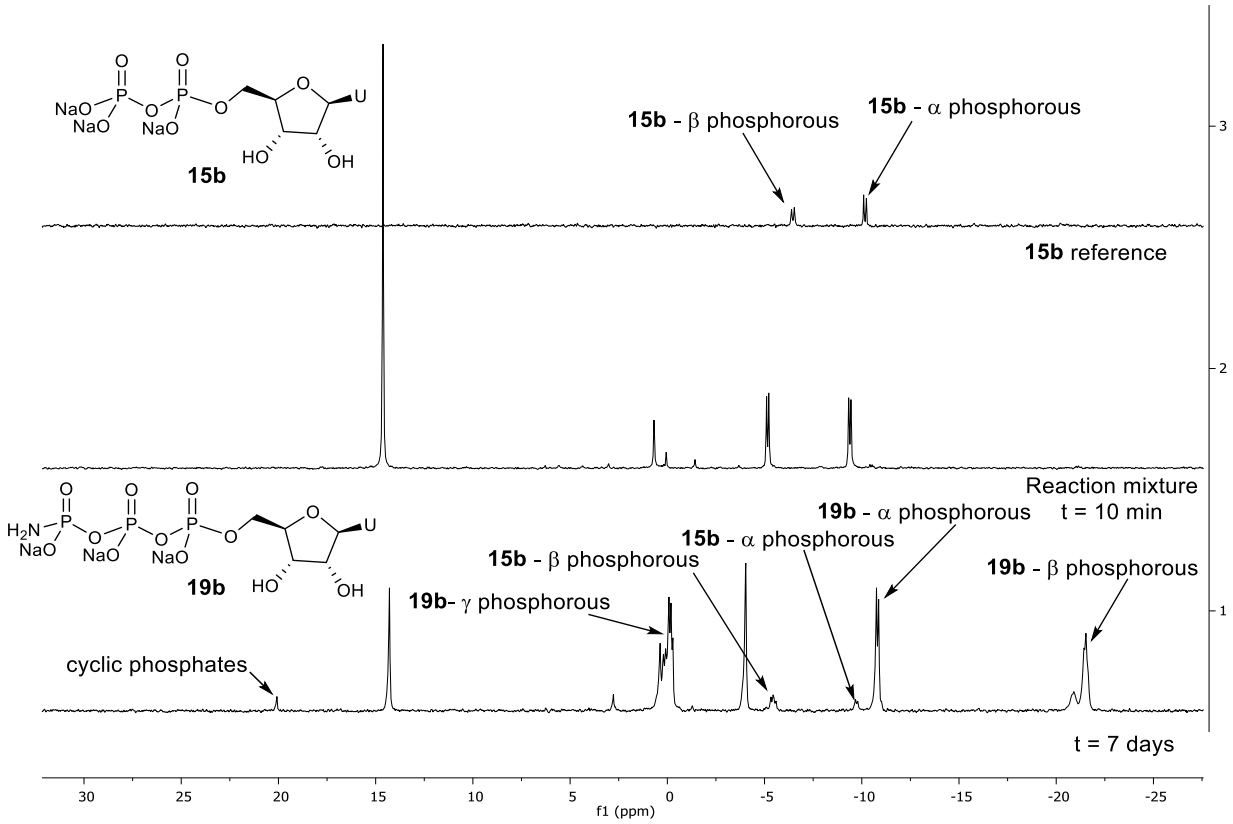
Supplementary Figure 88:

{H-coupled} ^{31}P NMR (middle spectrum) and {H-decoupled} ^{31}P NMR (bottom spectrum) spectra of the formation of **22a** by amidophosphorylation of **18a** in solution at pH 7 in D_2O following the method B (Supplementary Table 8, entry 6). The spectrum of the starting material **18a** (top spectrum) is shown for comparison.



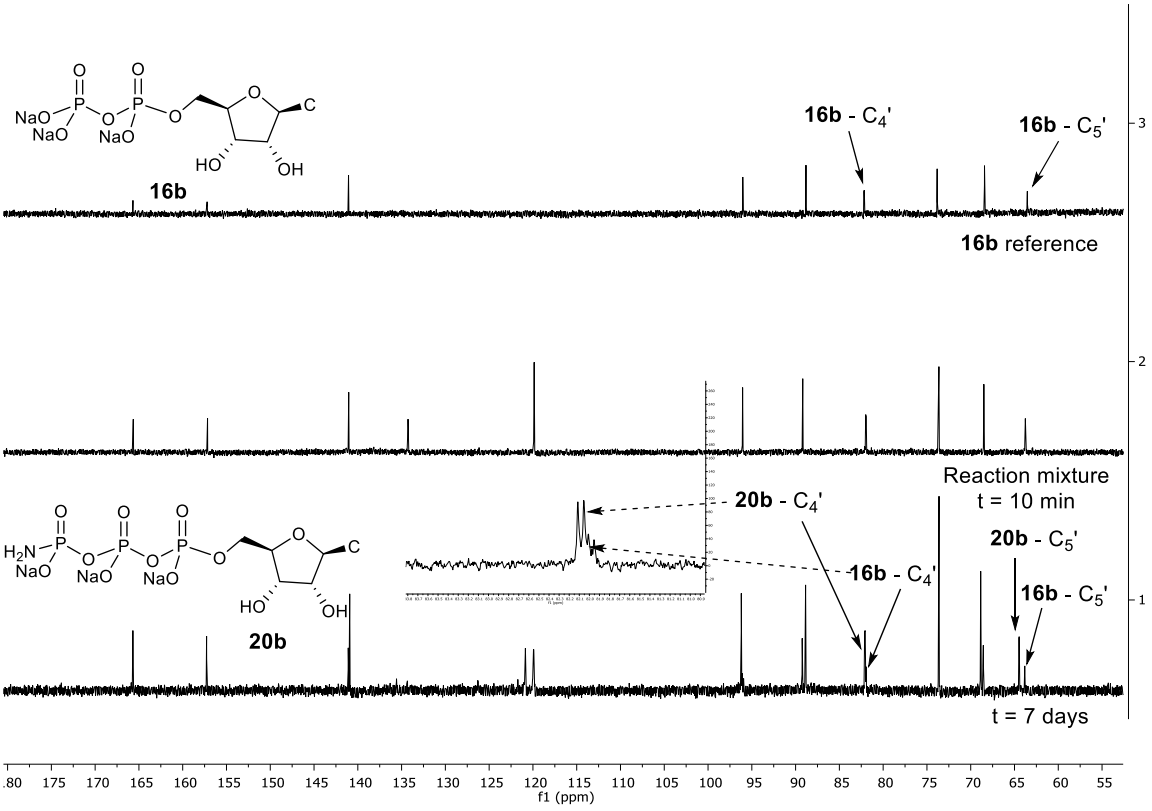
Supplementary Figure 89:

^{13}C NMR spectra of the formation of **19b** by amidophosphorylation of **15b** in solution at pH 7 in D_2O following the method C (Supplementary Table 9, entry 9). The spectrum of the starting material **15b** (top spectrum) is shown for comparison.



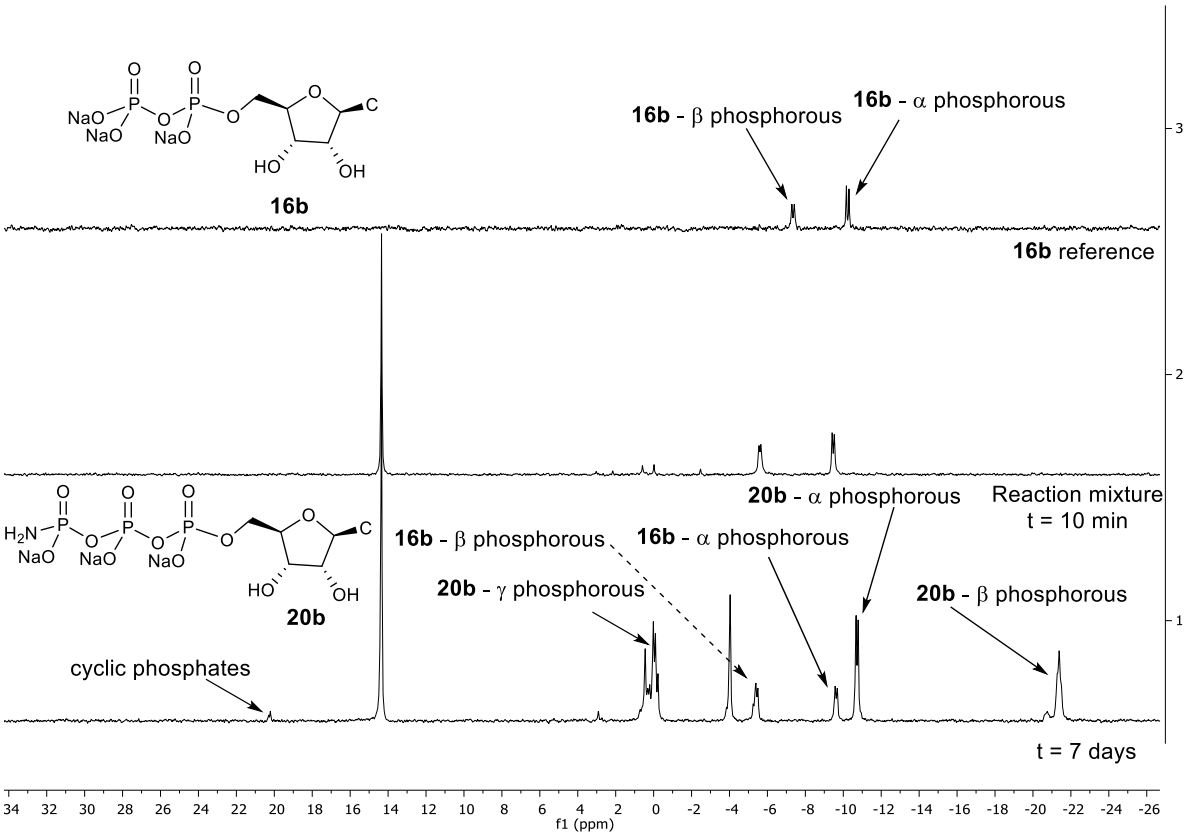
Supplementary Figure 90:

{H-decoupled} ^{31}P NMR spectra of the formation of **19b** by amidophosphorylation of **15b** in solution at pH 7 in D_2O following the method C (Supplementary Table 9, entry 9). The spectrum of the starting material **15b** (top spectrum) is shown for comparison.



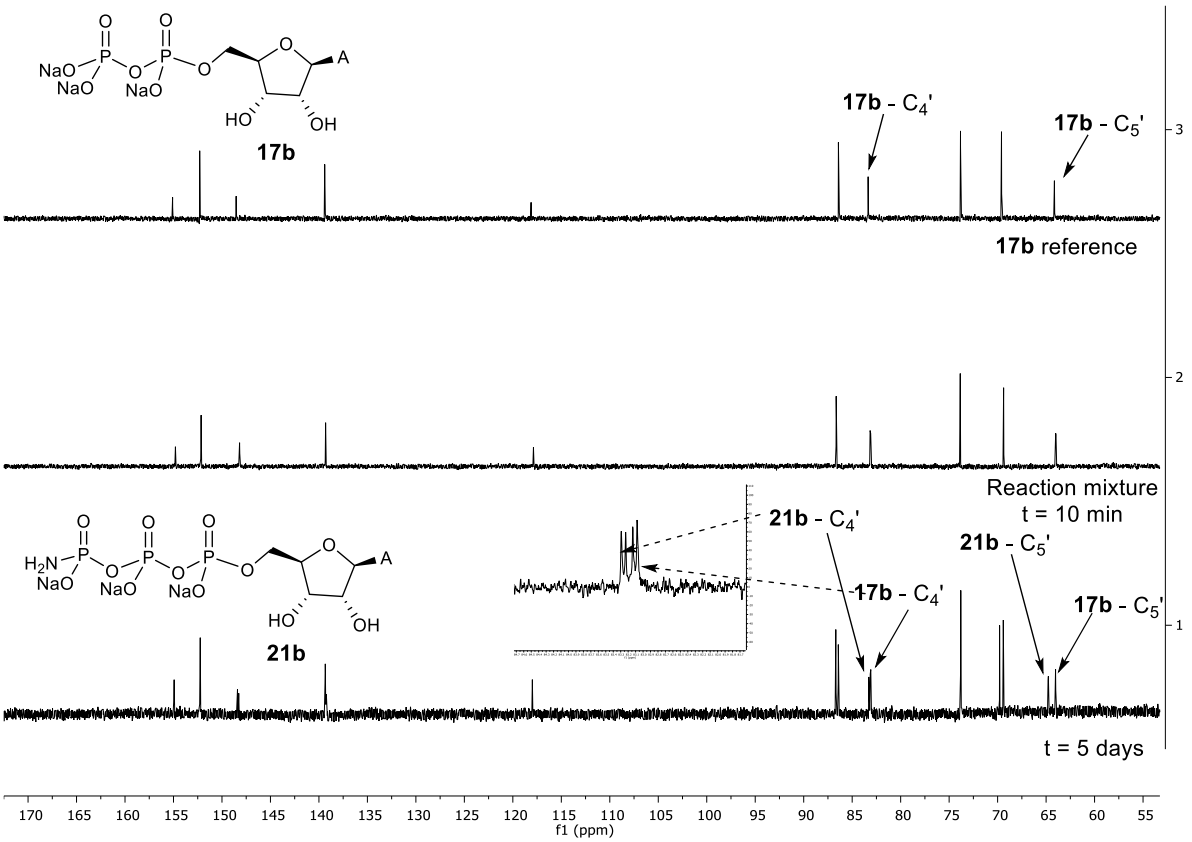
Supplementary Figure 91:

^{13}C NMR spectra of the formation of **20b** by amidophosphorylation of **16b** in solution at pH 7 in D_2O following the method C (Supplementary Table 9, entry 10). The spectrum of the starting material **16b** (top spectrum) is shown for comparison.



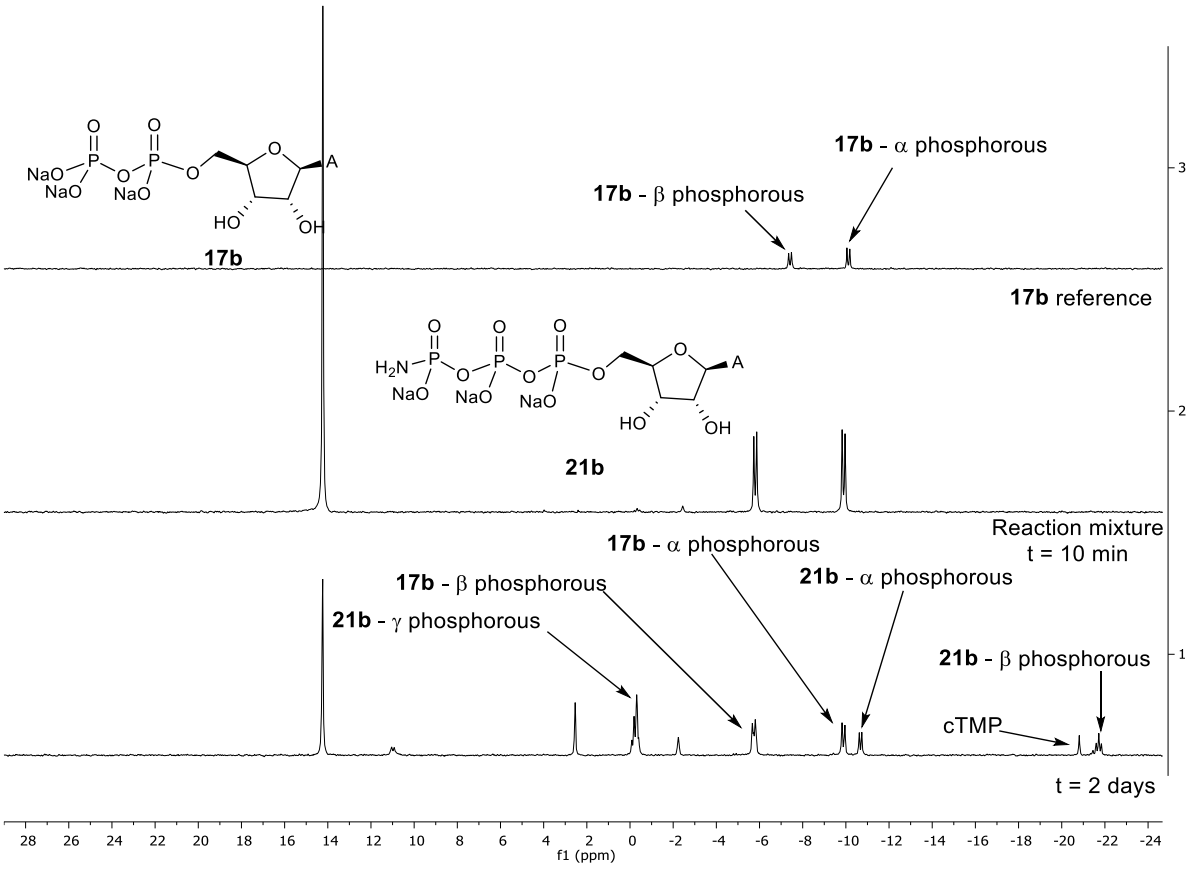
Supplementary Figure 92:

{H-decoupled} ^{31}P NMR spectra of the formation of **20b** by amidophosphorylation of **16b** in solution at pH 7 in D_2O following the method C (Supplementary Table 9, entry 10). The spectrum of the starting material **16b** (top spectrum) is shown for comparison.



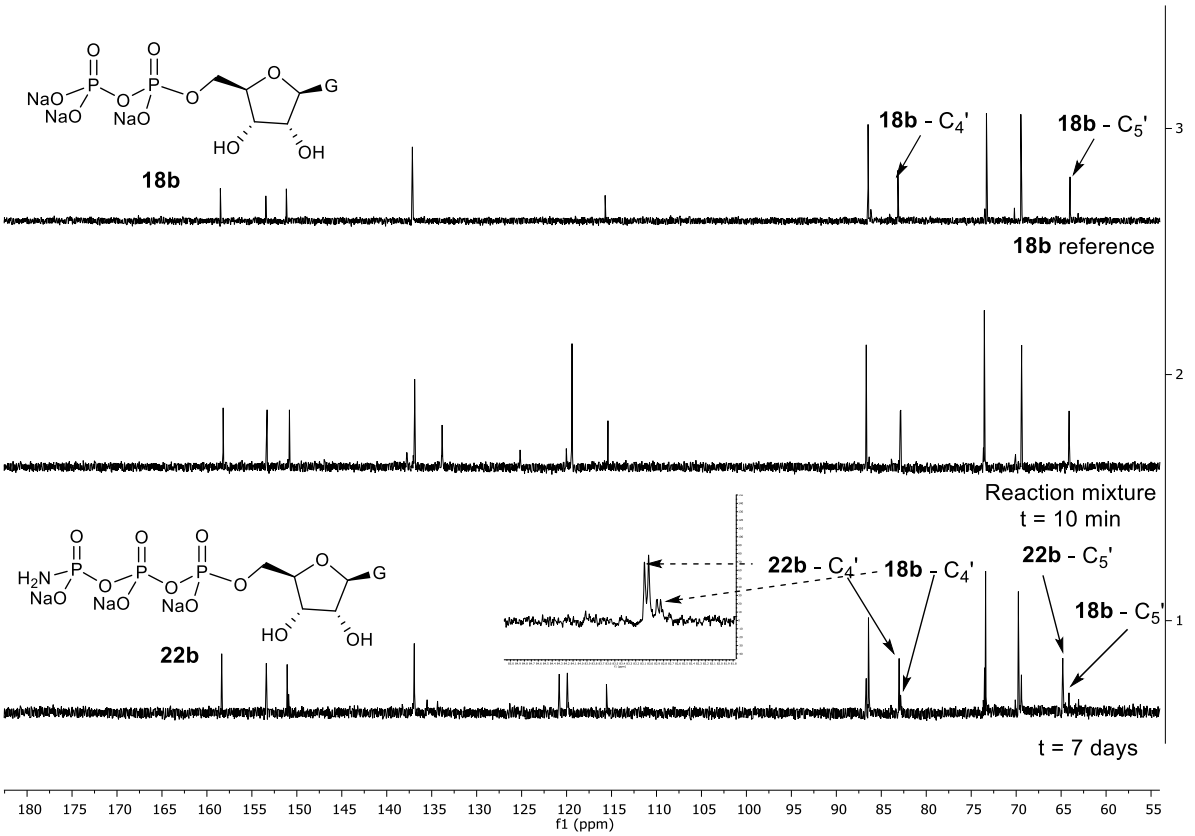
Supplementary Figure 93:

^{13}C NMR spectra of the formation of **21b** by amidophosphorylation of **17b** in solution at pH 5.5 in D_2O following the method B (Supplementary Table 9, entry 7). The spectrum of the starting material **17b** (top spectrum) is shown for comparison.



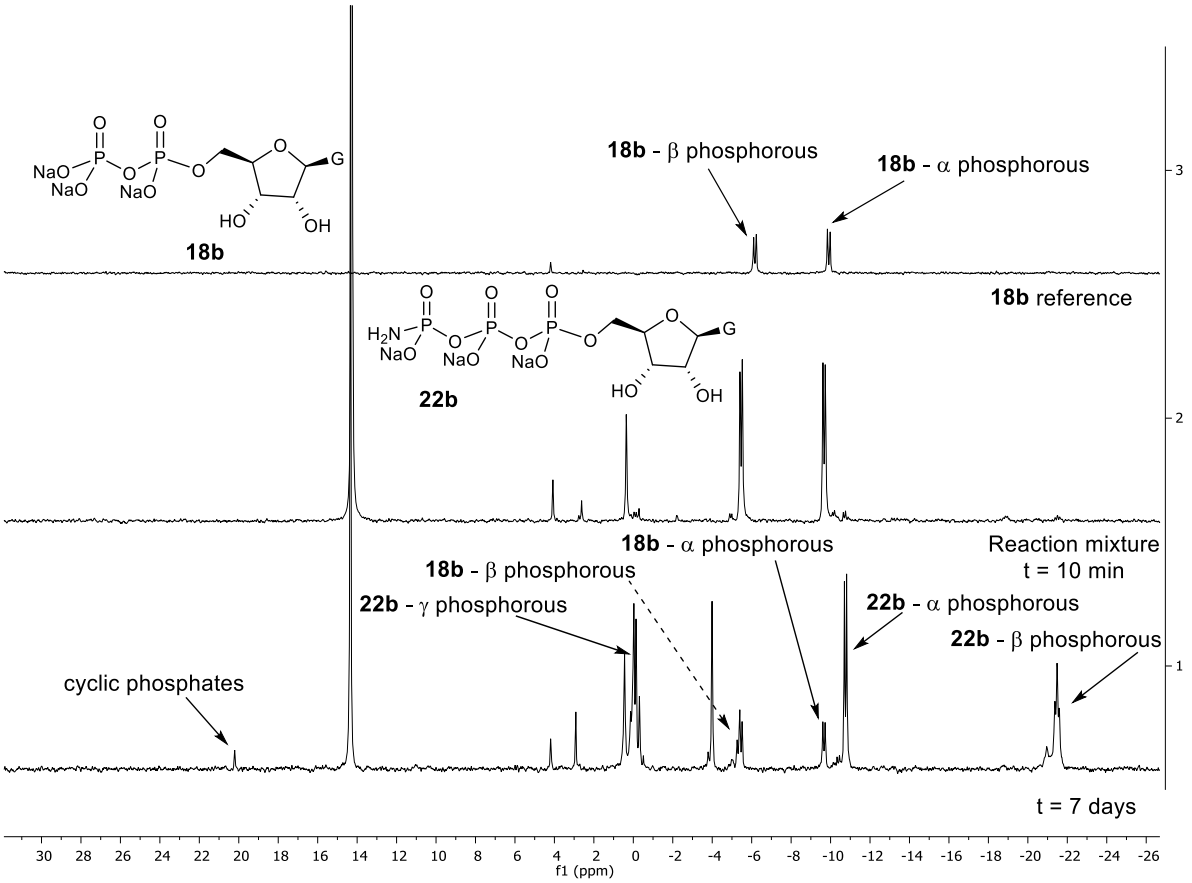
Supplementary Figure 94:

{H-decoupled} ^{31}P NMR spectra of the formation of **21b** by amidophosphorylation of **17b** in solution at pH 5.5 in D_2O following the method B (Supplementary Table 9, entry 7). The spectrum of the starting material **17b** (top spectrum) is shown for comparison.



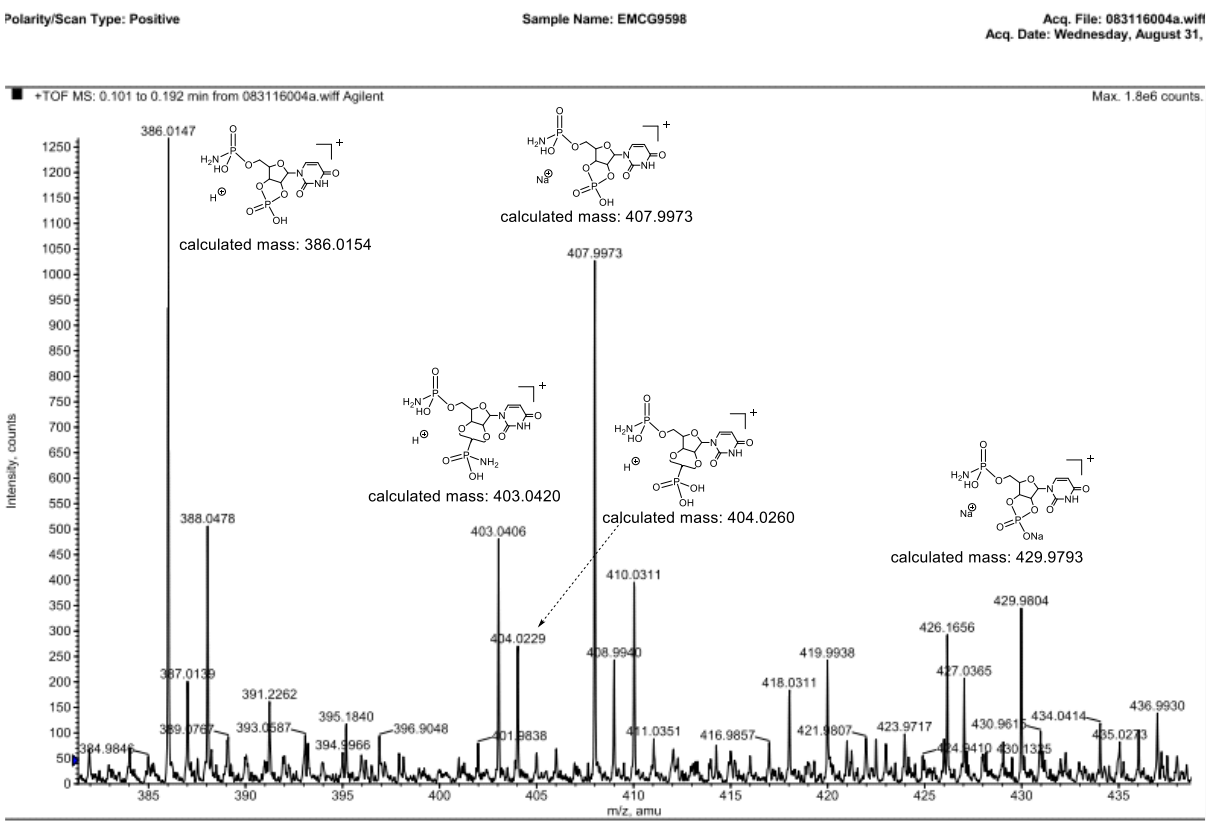
Supplementary Figure 95:

^{13}C NMR spectra of the formation of **22b** by amidophosphorylation of **18b** in solution at pH 7 in D_2O following the method C (Supplementary Table 9, entry 12). The spectrum of the starting material **18b** (top spectrum) is shown for comparison.



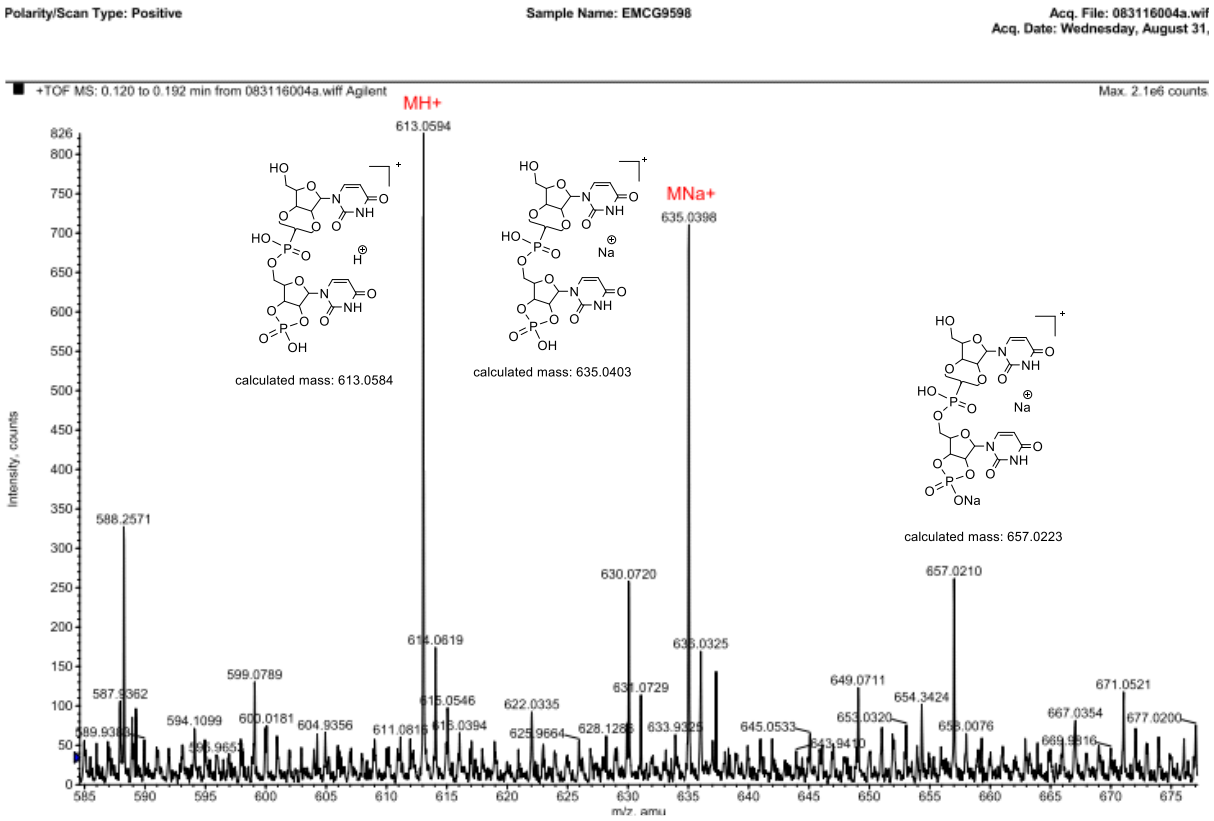
Supplementary Figure 96:

{H-decoupled} ^{31}P NMR spectra of the formation of **22b** by amidophosphorylation of **18b** in solution at pH 7 in D_2O following the method C (Supplementary Table 9, entry 12). The spectrum of the starting material **18b** (top spectrum) is shown for comparison.



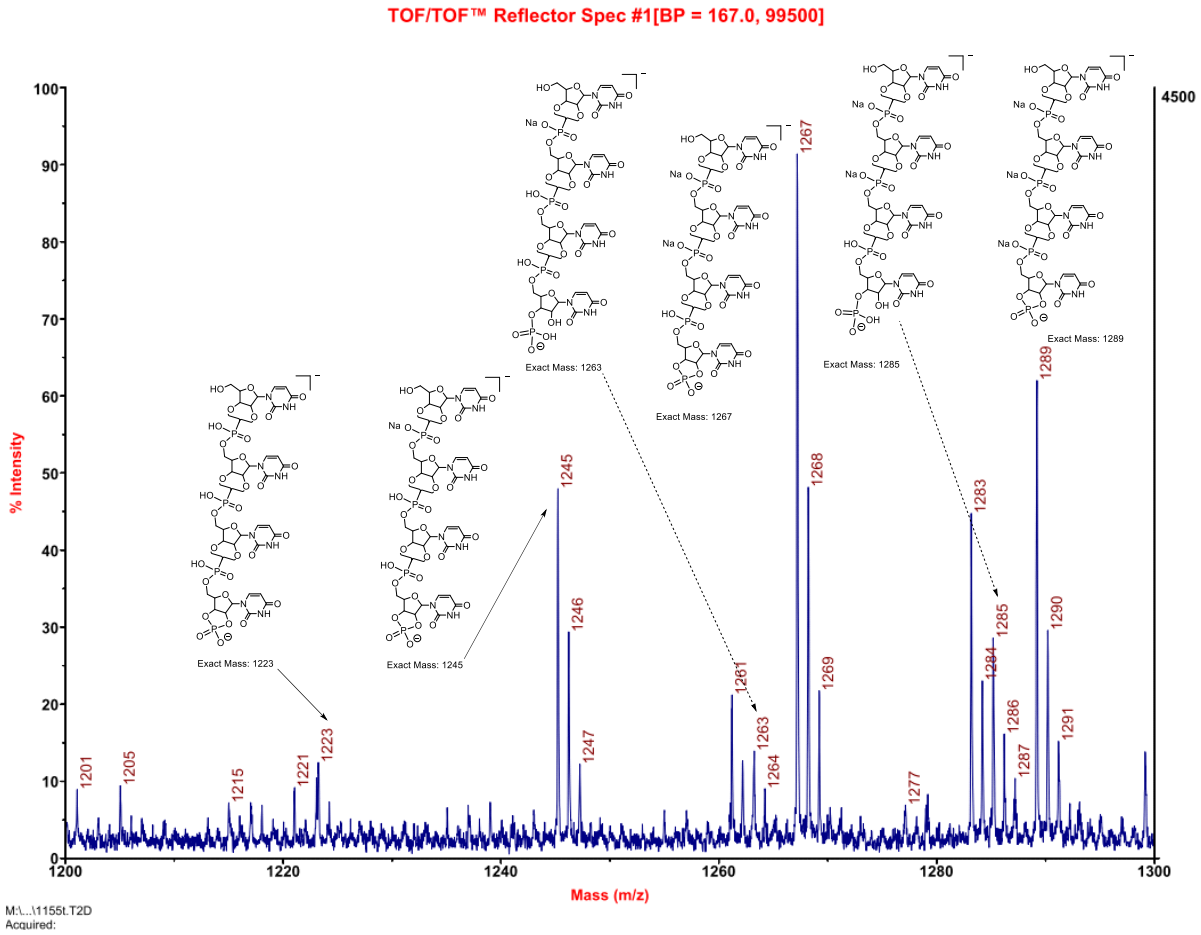
Supplementary Figure 97:

Positive mode of the ESI-TOF high-accuracy performed on the fraction eluted with 0.75 M of TEAB for the purification of a gram scale phosphorylation of **1** (see Supplementary Table 1, entry 16) showing the exact mass of the 5'-amidophosphorylated c-UMP.



Supplementary Figure 98:

Positive mode of the ESI-TOF high-accuracy performed on the fraction eluted with 0.75 M TEAB for the purification of a gram scale phosphorylation of **1** (see Supplementary Table 1, entry 16) showing the exact mass of the uridine dimer bearing a cyclic phosphate at the terminal 2',3'-OH. The nature of the linkage 5'-2' or 5'-3' has not been determined yet.

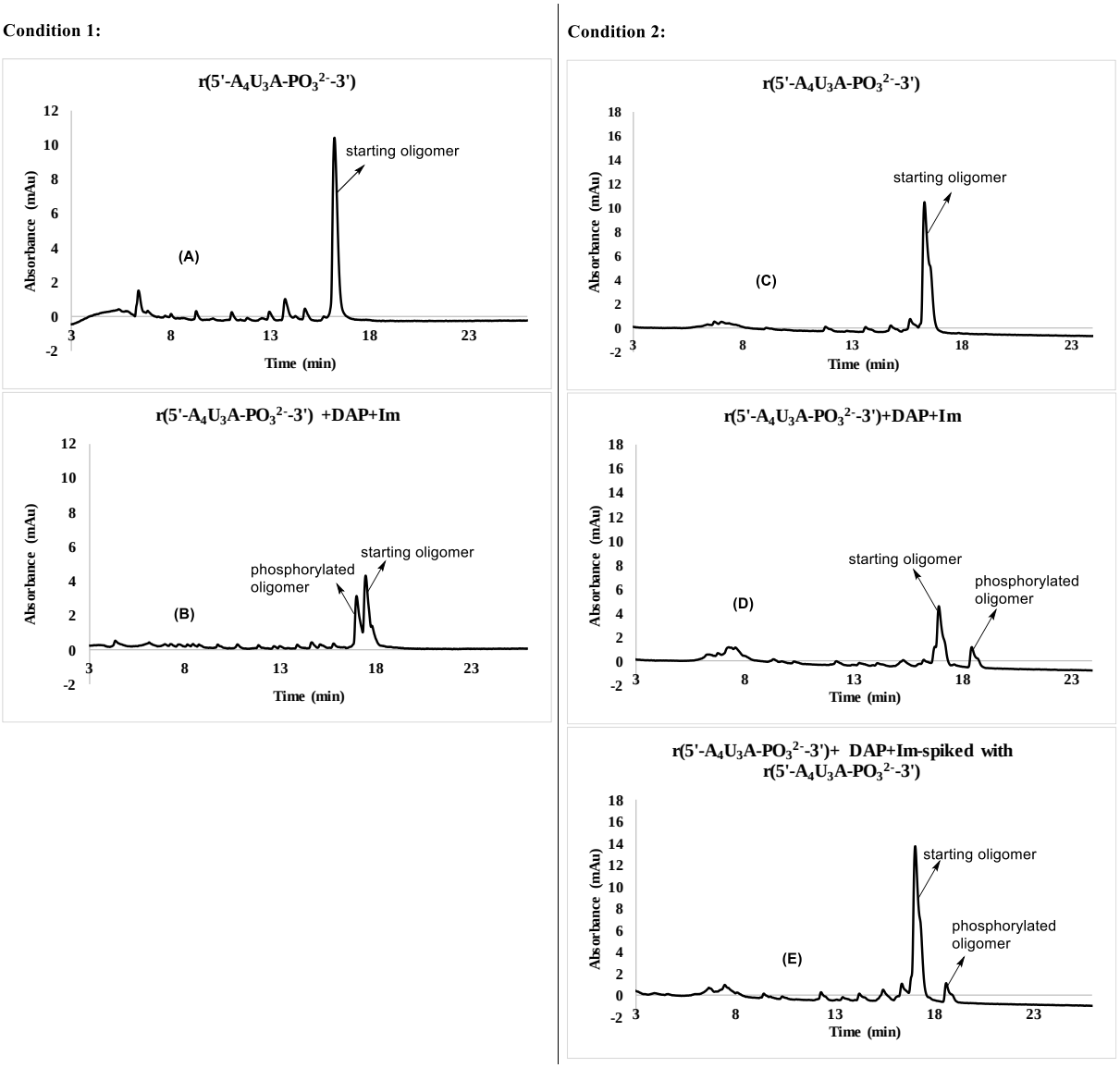


Supplementary Figure 99:

Negative mode of the MALDI-TOF performed on the second fraction eluted with 0.75 M TEAB for the purification of a gram scale phosphorylation of **1** (see Supplementary Table 1, entry 16) showing the uridine tetramer bearing a cyclic phosphate at the terminal 2',3'-hydroxyls or an open phosphate at 2' or 3' hydroxyl. The nature of the linkage 5'-2' or 5'-3' has not been determined yet.

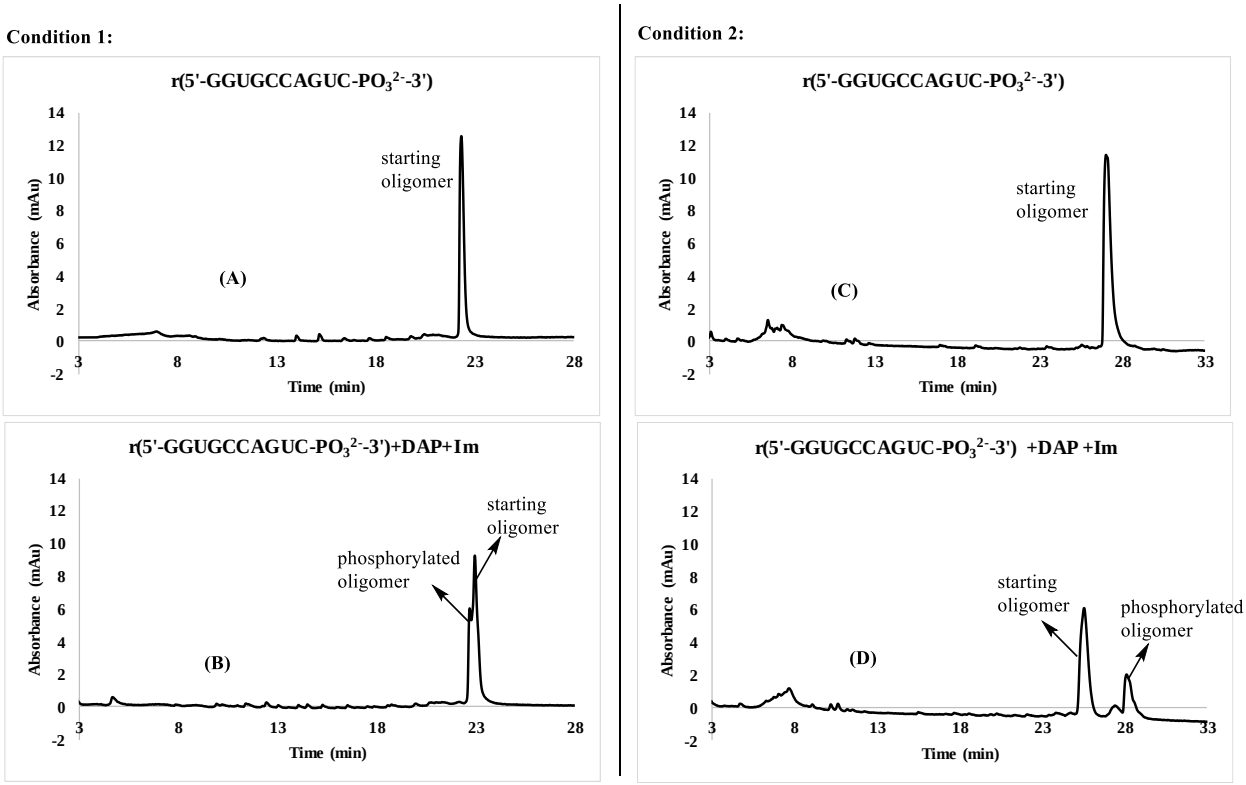
Oligonucleotides

Buffers for FPLC: Condition 1: Buffer A: 5 mM Tris base buffer, pH 8.2. Buffer B: 5 mM Tris base, 330 mM $NaClO_4$ buffer, pH 8.2. Condition 2: Buffer A: 10 mM Na_2HPO_4, pH 10.5. Buffer B: 10 mM Na_2HPO_4, 1 M NaCl, pH 10.5. The gradient is specified under each profile.



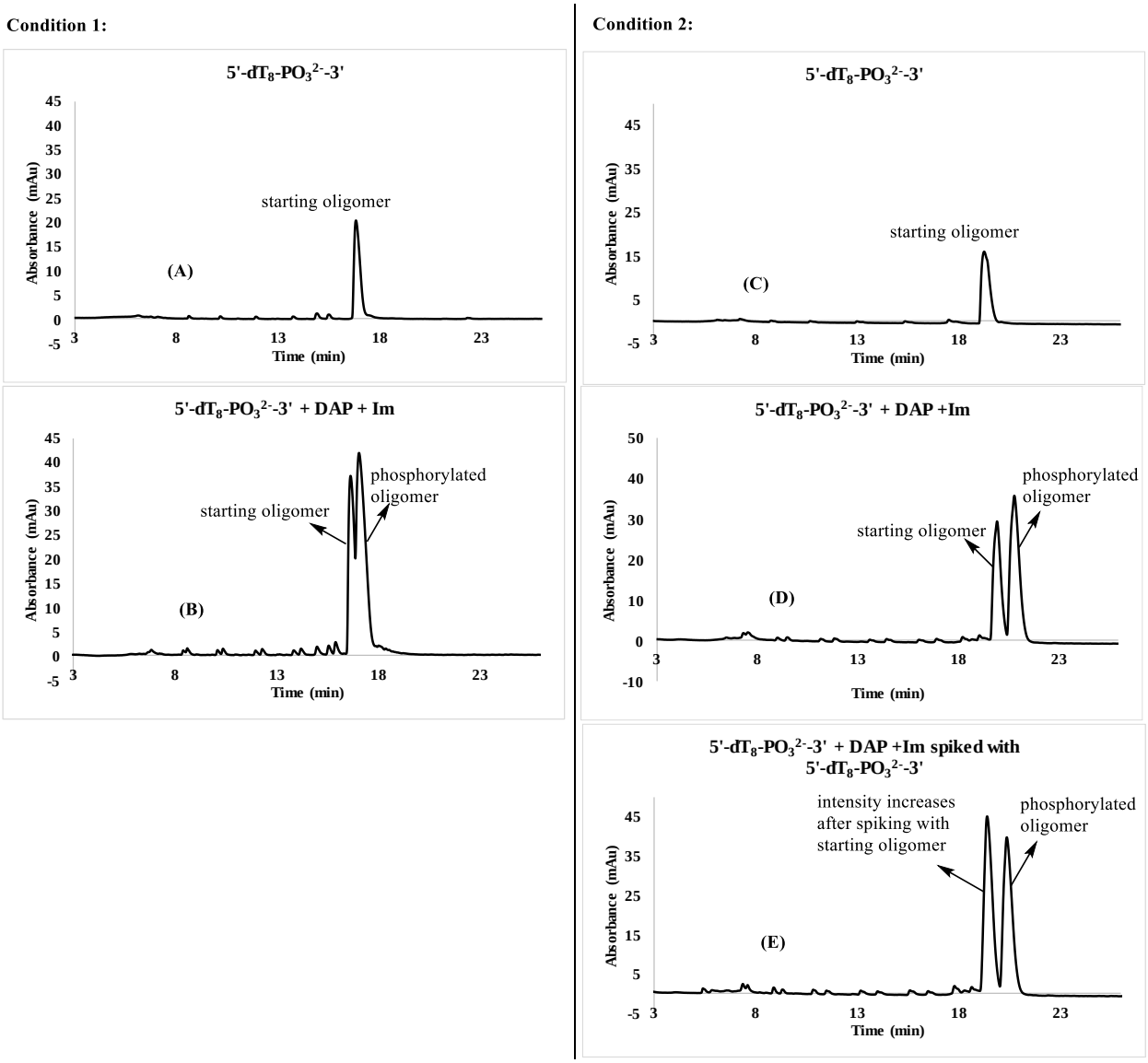
Supplementary Figure 100:

(A) FPLC trace of r(5'-A_4U_3A-PO_3^{2-}-3') in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of r(5'-A_4U_3A-PO_3^{2-}-3') after 3 days in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (C) FPLC trace of r(5'-A_4U_3A-PO_3^{2-}-3') in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient; (D) FPLC trace of the phosphorylation reaction of r(5'-A_4U_3A-PO_3^{2-}-3') after 6 days in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient (Table S10, entry 1); (E) FPLC trace of the phosphorylation reaction of r(5'-A_4U_3A-PO_3^{2-}-3') after 6 days in condition 2 spiked with starting oligo r(5'-A_4U_3A-PO_3^{2-}-3') using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient.



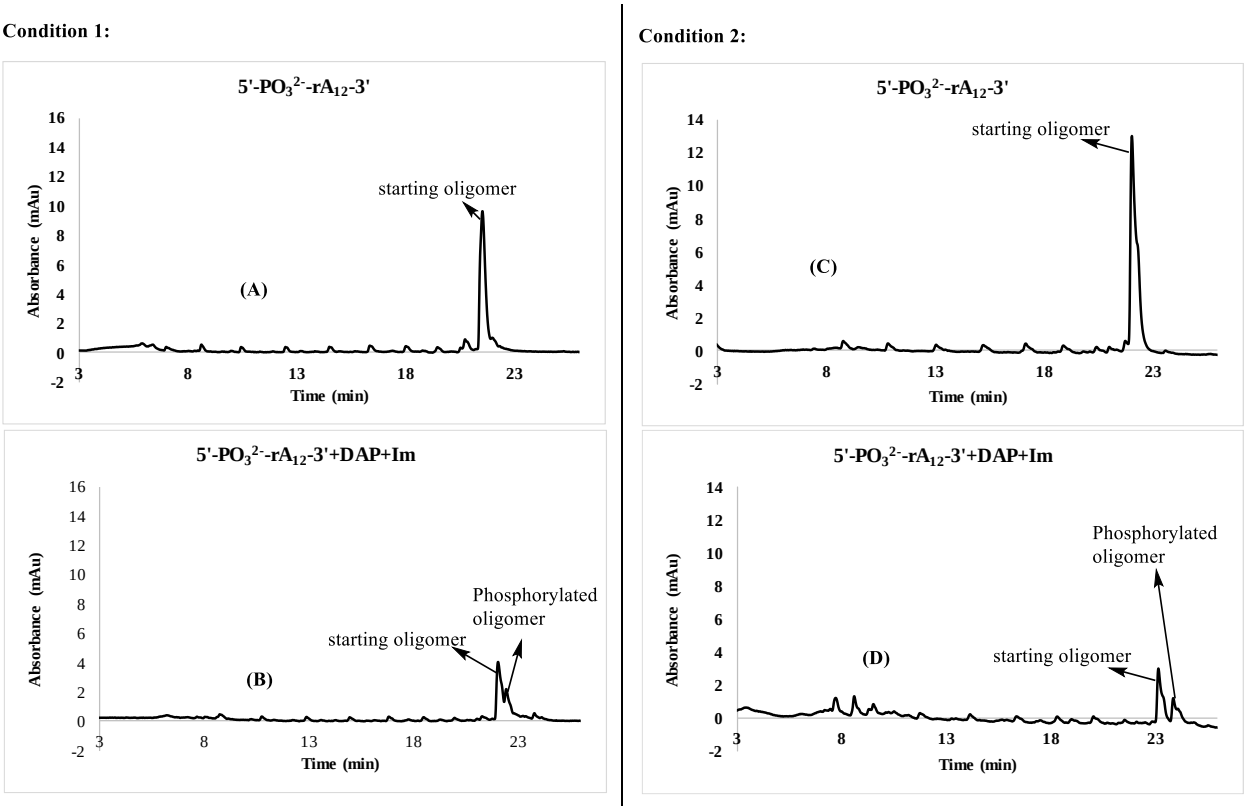
Supplementary Figure 101:

(A) FPLC trace of r(5'-GGUGCCAGUC- PO_3^{2-}-3') in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of r(5'-GGUGCCAGUC- PO_3^{2-}-3') after 4 days in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (C) FPLC trace of r(5'-GGUGCCAGUC- PO_3^{2-}-3') in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient; (D) FPLC trace of the phosphorylation reaction of r(5'-GGUGCCAGUC- PO_3^{2-}-3') after 6 days in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient (Supplementary Table 10, entry 2).



Supplementary Figure 102:

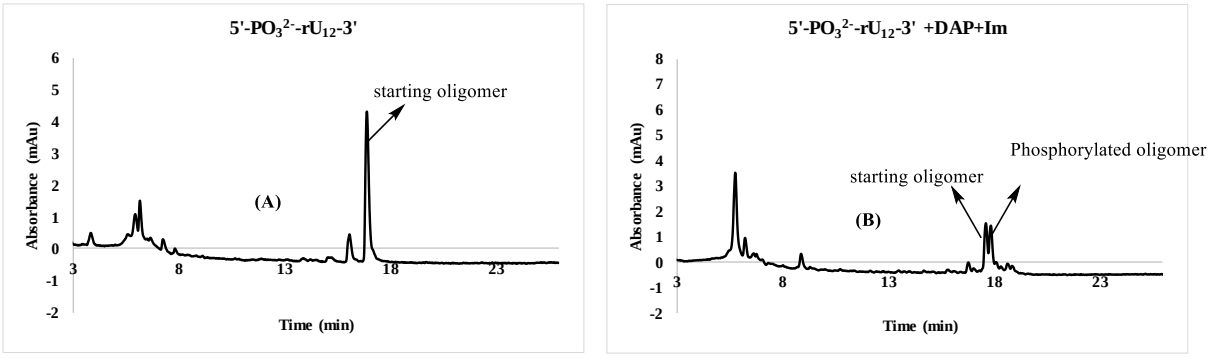
(A) FPLC trace of 5'-dT_8-PO_3^{2-} in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of 5'-dT_8-PO_3^{2-} after 25 days in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (C) FPLC trace of 5'-dT_8-PO_3^{2-} in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient; (D) FPLC trace of the phosphorylation reaction of 5'-dT_8-PO_3^{2-} after 28 days in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient (Table S10, entry 3); (E) FPLC trace of the phosphorylation reaction of 5'-dT_8-PO_3^{2-} after 28 days spiked with starting 5'-dT_8-PO_3^{2-} -3' in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient.



Supplementary Figure 103:

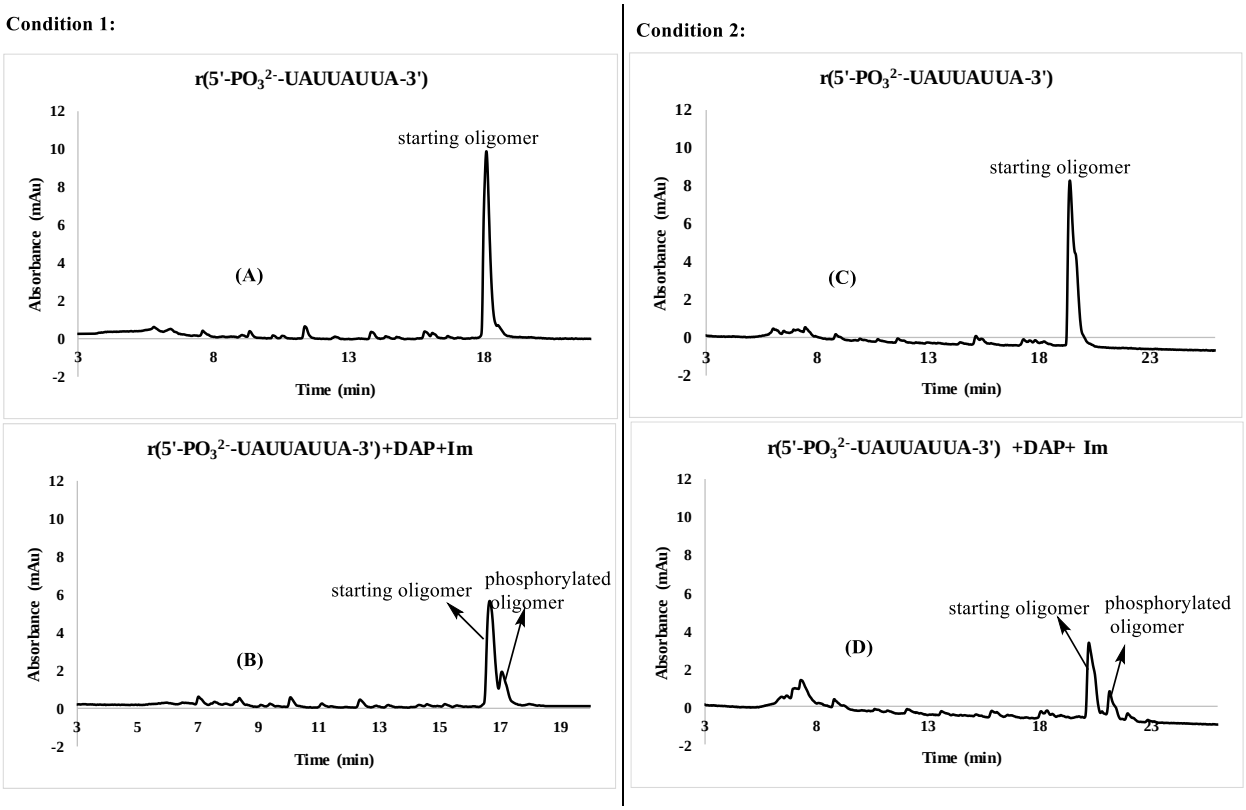
(A) FPLC trace of 5'-PO_3^{2-}-rA_{12}-3' in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of 5'-PO_3^{2-}-rA_{12}-3' after 4 days in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (C) FPLC trace of 5'-PO_3^{2-}-rA_{12}-3' in condition 2 using 0 to 15 % buffer B in 1 min then 15 to 60 % buffer B in 30 min gradient; (D) FPLC trace of the phosphorylation reaction of 5'-PO_3^{2-}-rA_{12}-3' after 6 days in condition 2 using 0 to 15 % buffer B in 1 min then 15 to 60 % buffer B in 30 min gradient (Supplementary Table 11, entry 1).

Condition 1:



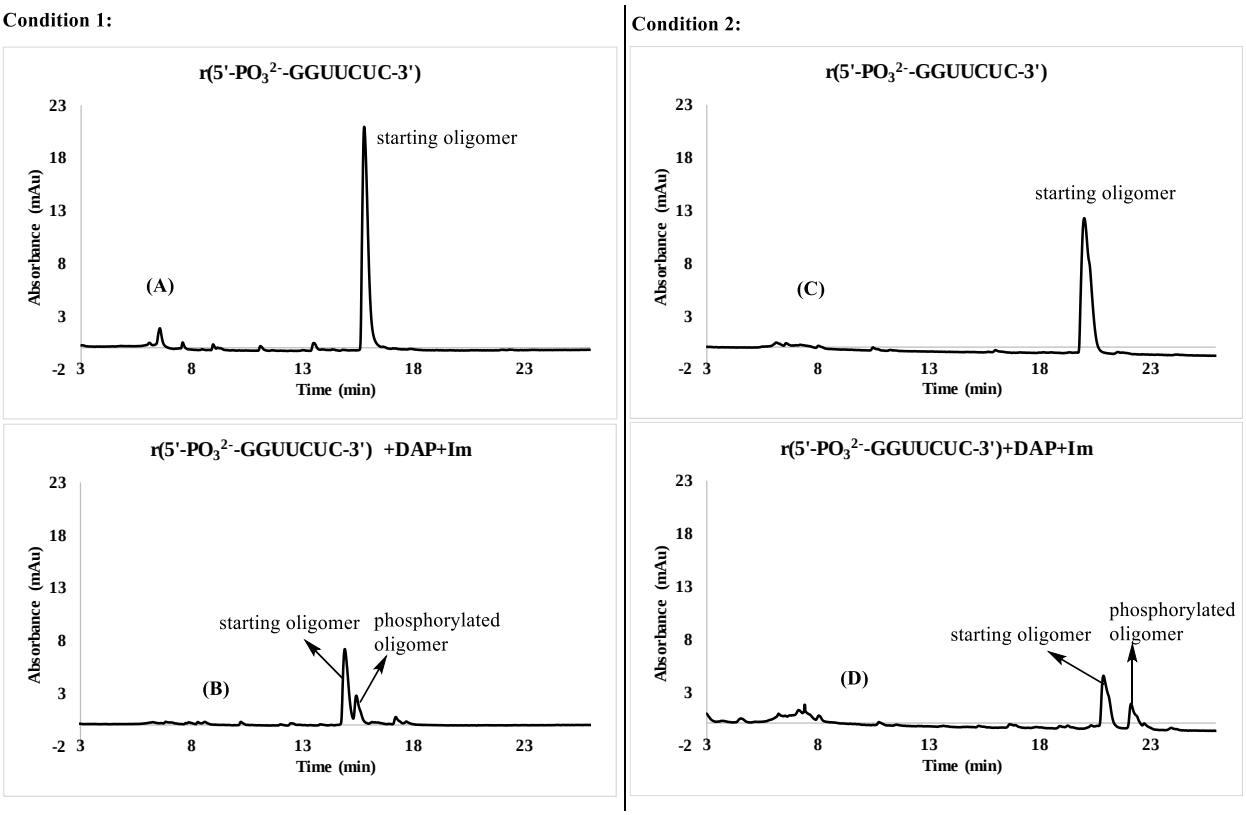
Supplementary Figure 104:

(A) FPLC trace of r(5'- PO_3^{2-}-U_{12}-3') in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 50 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of r(5'- PO_3^{2-}-U_{12}-3') after 4 days in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 50 % buffer B in 30 min gradient (Supplementary Table 11, entry 2).



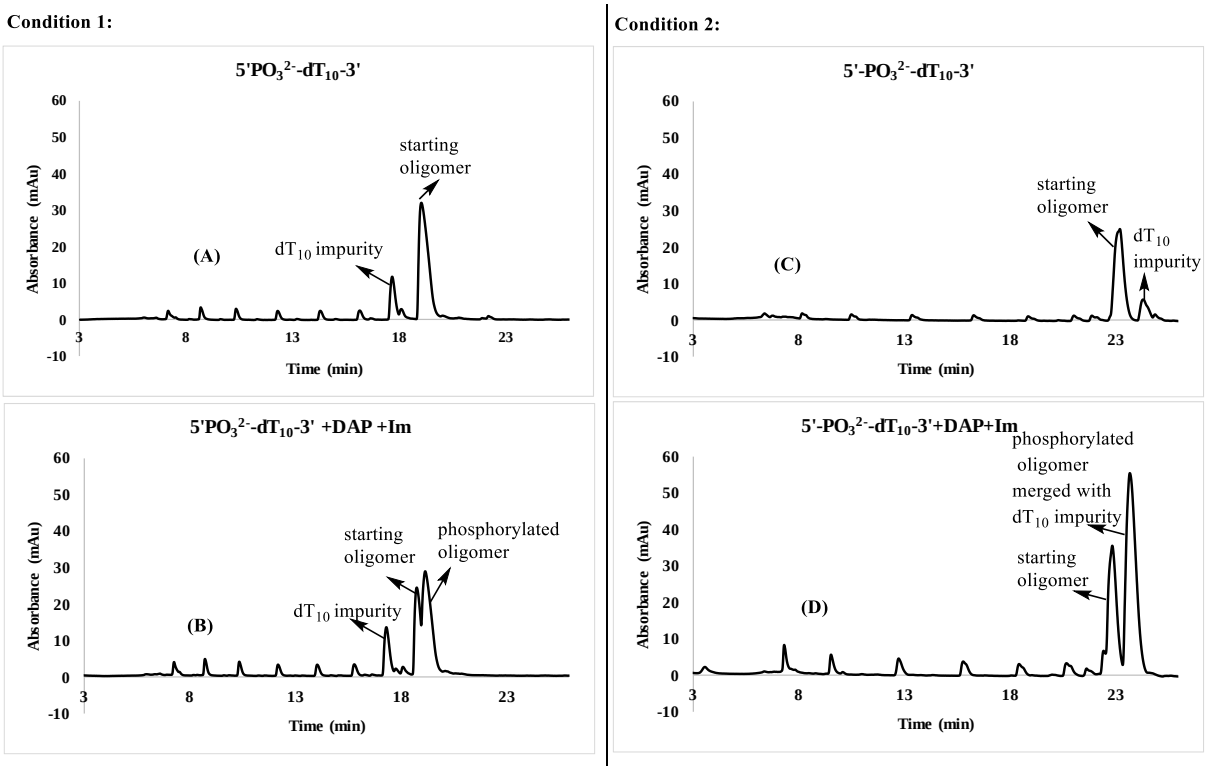
Supplementary Figure 105:

(A) FPLC trace of r(5'-PO_3^{2-}-UAUUAUUA-3') in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of r(5'-PO_3^{2-}-UAUUAUUA-3') after 4 days in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (C) FPLC trace of r(5'-PO_3^{2-}-UAUUAUUA-3') in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient; (D) FPLC trace of the phosphorylation reaction of r(5'-PO_3^{2-}-UAUUAUUA-3') after 6 days in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient (Supplementary Table 11, entry 3).



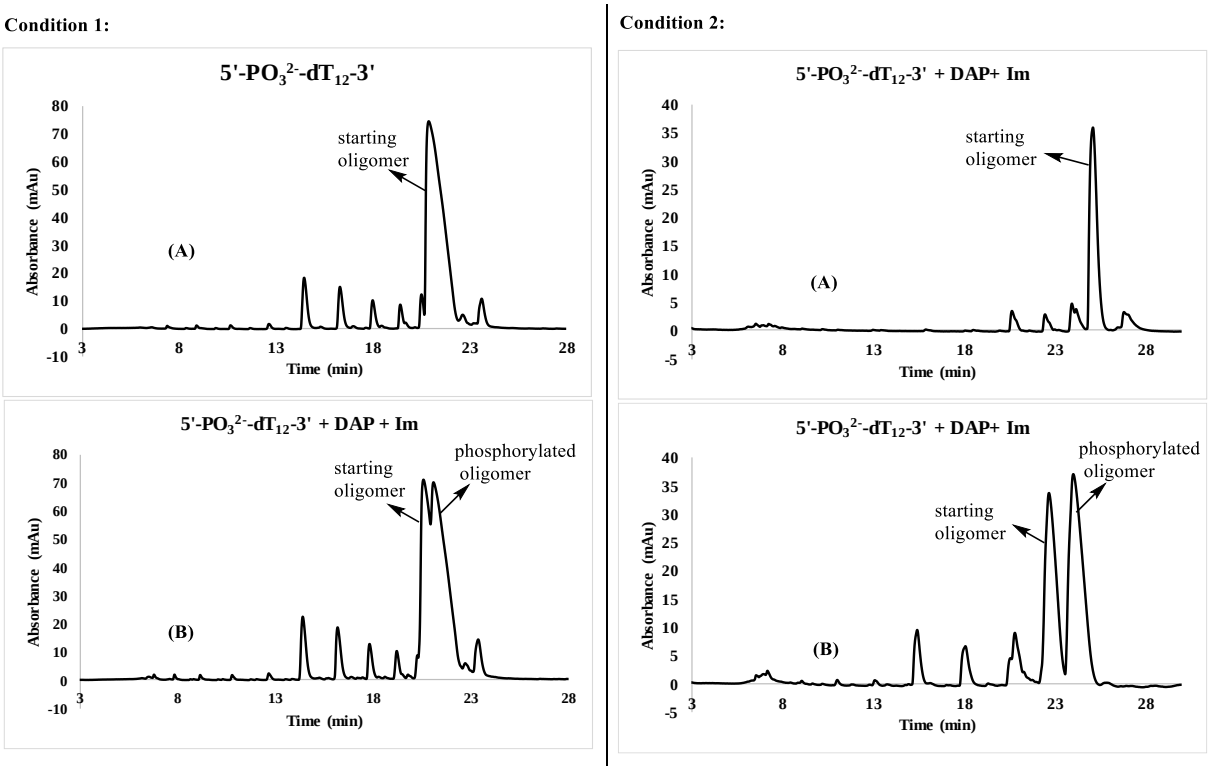
Supplementary Figure 106:

(A) FPLC trace of r(5'-PO_3^{2-}-UAUUAUUA-3') in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of r(5'-PO_3^{2-}-UAUUAUUA-3') after 4 days in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (C) FPLC trace of r(5'-PO_3^{2-}-UAUUAUUA-3') in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient; (D) FPLC trace of the phosphorylation reaction of r(5'-PO_3^{2-}-UAUUAUUA-3') after 6 days in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient (Supplementary Table 11, entry 4).



Supplementary Figure 107:

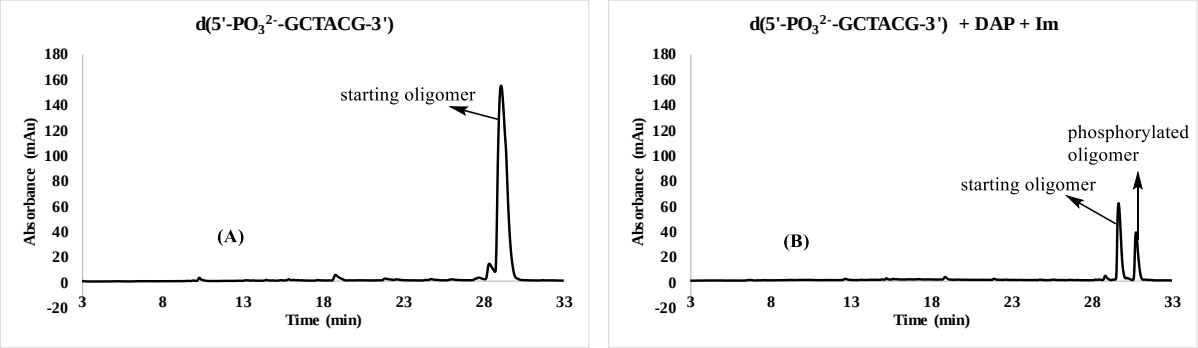
(A) FPLC trace of 5'-PO_3^{2-}-d(T)$_{10}$-3' in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of 5'-PO_3^{2-}-d(T)$_{10}$-3' after 25 days in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (C) FPLC trace of 5'-PO_3^{2-}-d(T)$_{10}$-3' in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient; (D) FPLC trace of the phosphorylation reaction of 5'-PO_3^{2-}-d(T)$_{10}$-3' after 28 days in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient (Supplementary Table 11, entry 5).



Supplementary Figure 108:

(A) FPLC trace of 5'-PO_3^{2-}-dT_{10}-3' in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of 5'-PO_3^{2-}-dT_{10}-3' after 25 days in condition 1 using 0 to 10 % buffer B in 1 min then 10 to 40 % buffer B in 30 min gradient; (C) FPLC trace of 5'-PO_3^{2-}-dT_{10}-3' in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient; (D) FPLC trace of the phosphorylation reaction of 5'-PO_3^{2-}-dT_{10}-3' after 28 days in condition 2 using 0 to 30 % buffer B in 1 min then 30 to 80 % buffer B in 30 min gradient (Supplementary Table 11, entry 6).

Condition 2:



Supplementary Figure 109:

(A) FPLC trace of r(5'-A_4U_3A-PO_3^{2-}-3') in condition 2 using 0 to 50 % buffer B in 30 min gradient; (B) FPLC trace of the phosphorylation reaction of d(5'-GCTACG-PO_3^{2-}-3') with 1 M DAP and1 M imidazole after 4 days in condition 2 using 0 to 50 % buffer B in 30 min gradient (Supplementary Table 11, entry 7).

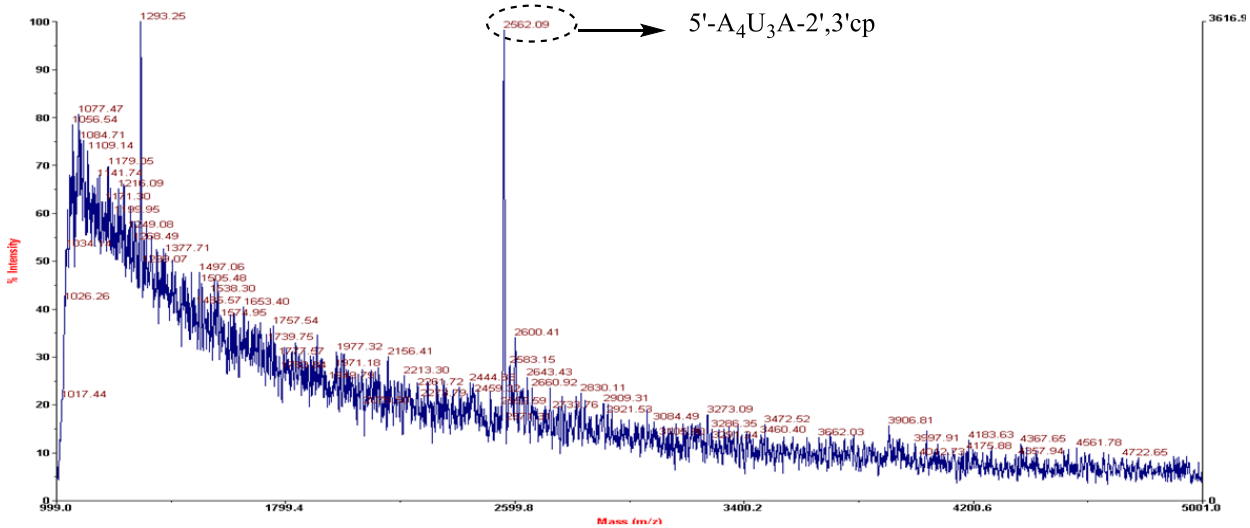
MALDI results of oligonucleotide-5'-phosphate phosphorylation with DAP and Im

Entry	Oligonucleotide-5'-phosphate	Expected average mass	Observed mass
1	5'-PO_3^{2-}-rA_{12}-3'	4047.5	4048.5
2	5'-PO_3^{2-}-rU_{12}-3'	3771.6	3769.6
3	r(5'-PO_3^{2-}-UAUUAUUA-3')	2615.4	2613.8
4	r(5'-PO_3^{2-}-GGUUCUC-3')	2316.3	2315.7
5	5'-PO_3^{2-}—$d(T)_{10}$-3'	3138.9	3135.3
6	5'-PO_3^{2-}—$d(T)_{12}$-3'	3748.4	3743.7
7	d(5'-GCTACG- PO_3^{2-}-3')	1951.2	1950.2

MALDI results of oligonucleotide-3'-phosphate phosphorylation with DAP and Im

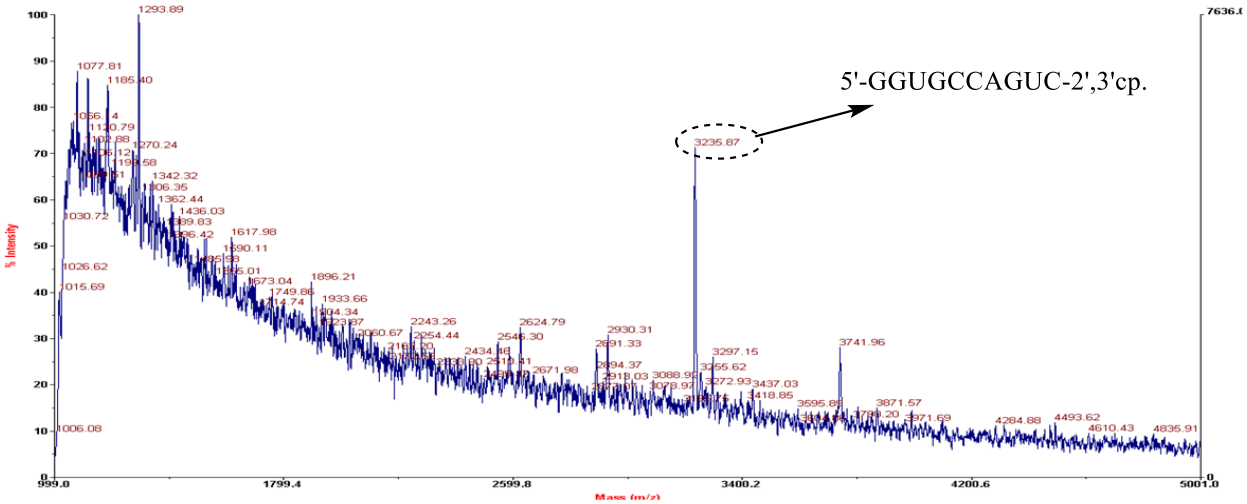
Entry	Oligonucleotide-3'-phosphate	Expected average mass	Observed mass
1	r(5'-A_4U_3A- PO_3^{2-}-3')	2564.5*	2562.1*
2	r(5'-GGUGCCAGUC- PO_3^{2-}-3')	3237.9*	3235.9*
3	5'-$d(T)_8$- PO_3^{2-}-3'	2530.6	2527.3

* : mass of 2',3'- cyclic phosphate



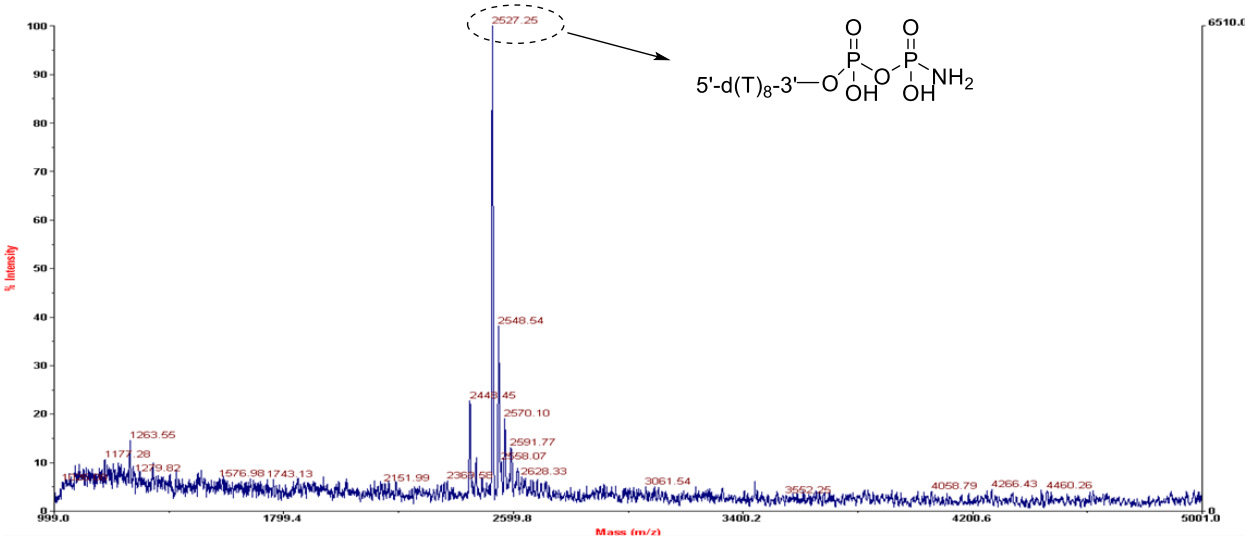
Supplementary Figure 110:

Negative mode MALDI-TOF spectrum of 5'-A_4U_3A- PO_3^{2-}-3' phosphorylation reaction using THAP matrix showing the formation of 5'-A_4U_3A-2',3'cp.



Supplementary Figure 111:

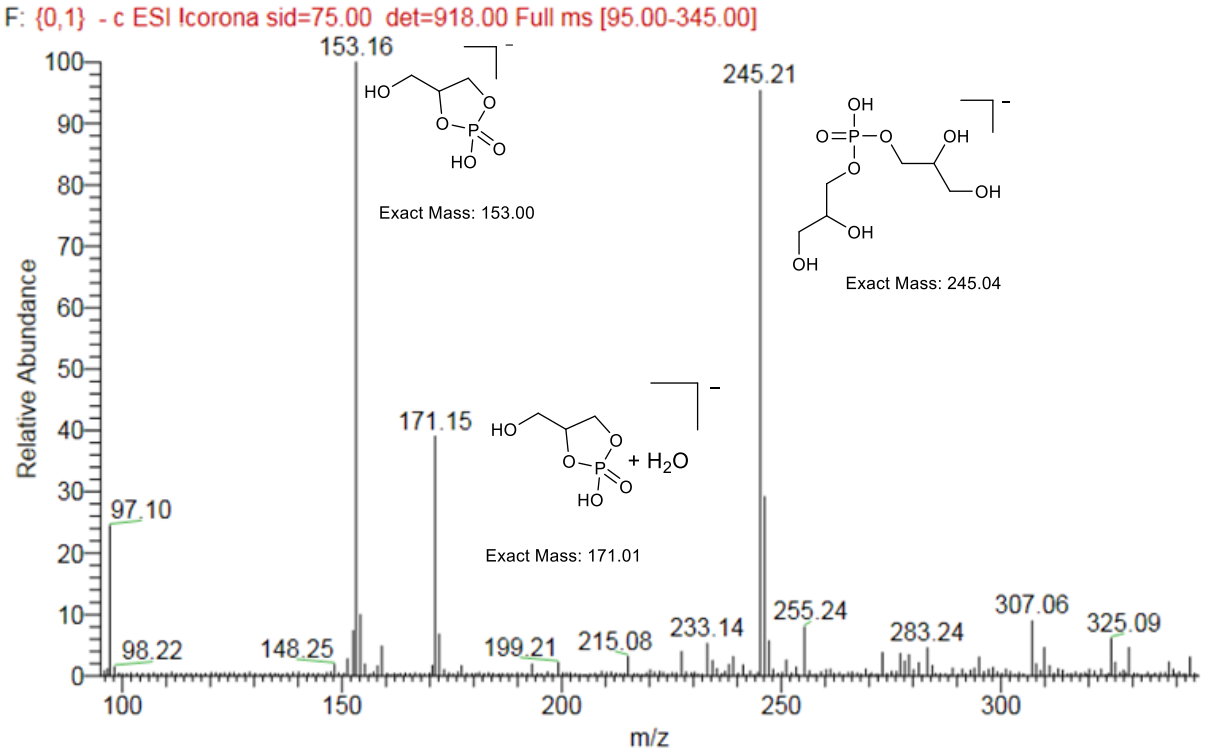
Negative mode MALDI-TOF spectrum of 5’-GGUGCCAGUC- PO_3^{2-}-3’ phosphorylation reaction using THAP matrix showing the formation of r(5’-GGUGCCAGUC-2’,3’cp).



Supplementary Figure 112:

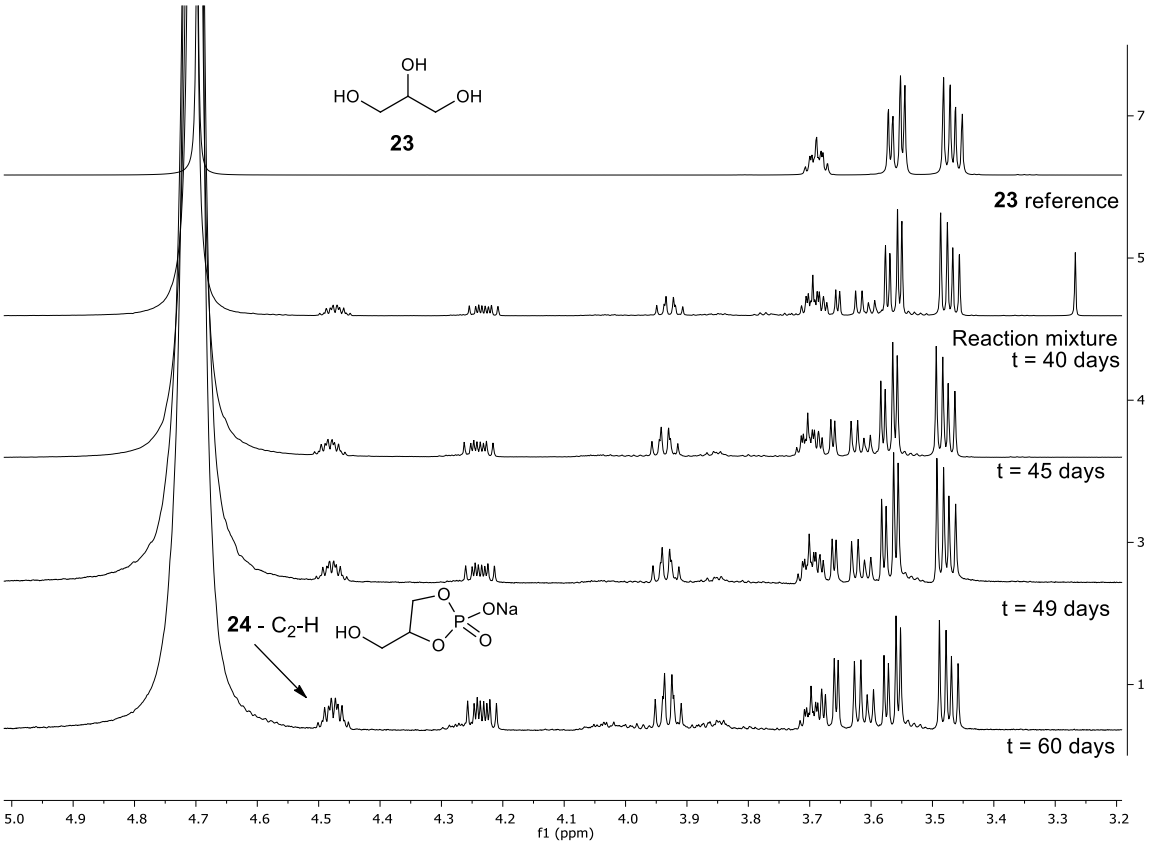
Negative mode MALDI-TOF spectrum of 5'-d(T)$_8$- PO_3^{2-}-3' phosphorylation reaction using THAP matrix showing the formation of 5'-d(T)$_8$- $DP^{NH}{}_2$-3'.

Vesicle-like structure formation study



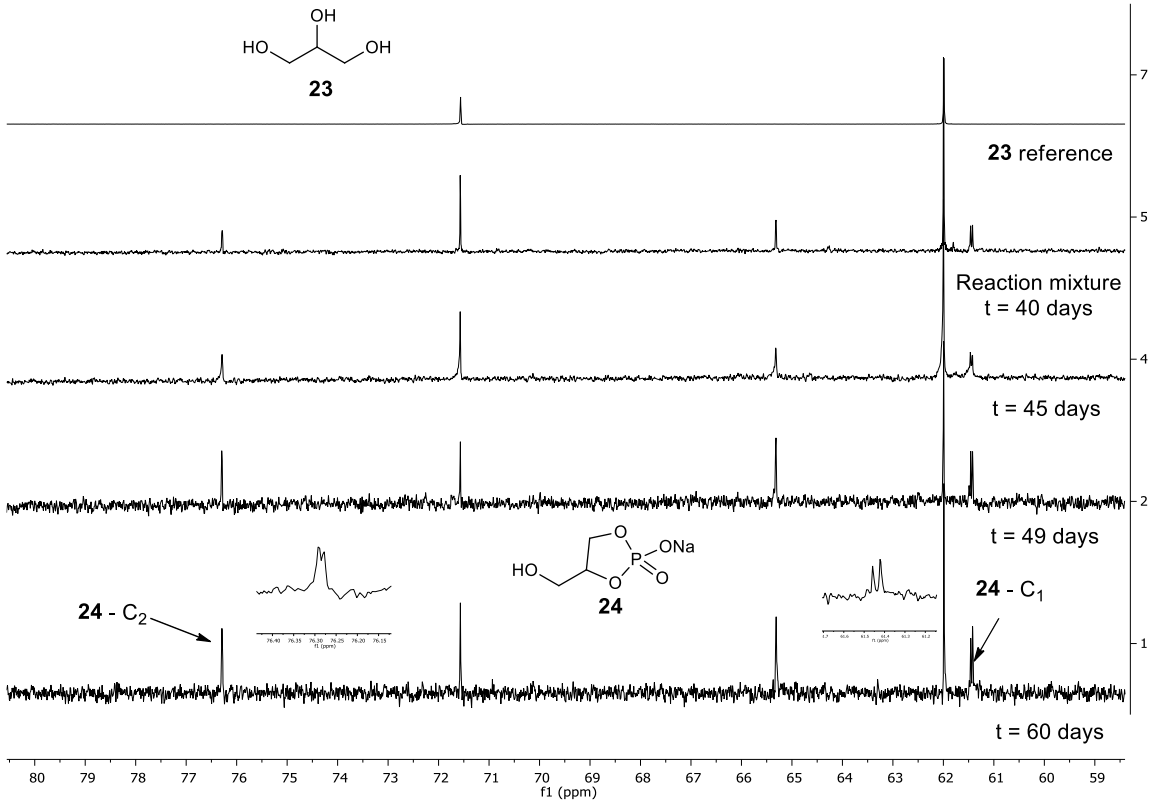
Supplementary Figure 113:

Negative mode direct inject ESI-MS of the reaction of glycerol **23** with DAP and imidazole at 50 °C showing the formation of diglycerol-phosphodiester (m/z = 245.04) and glycerol-1,2-cyclophosphate (m/z = 153.00).



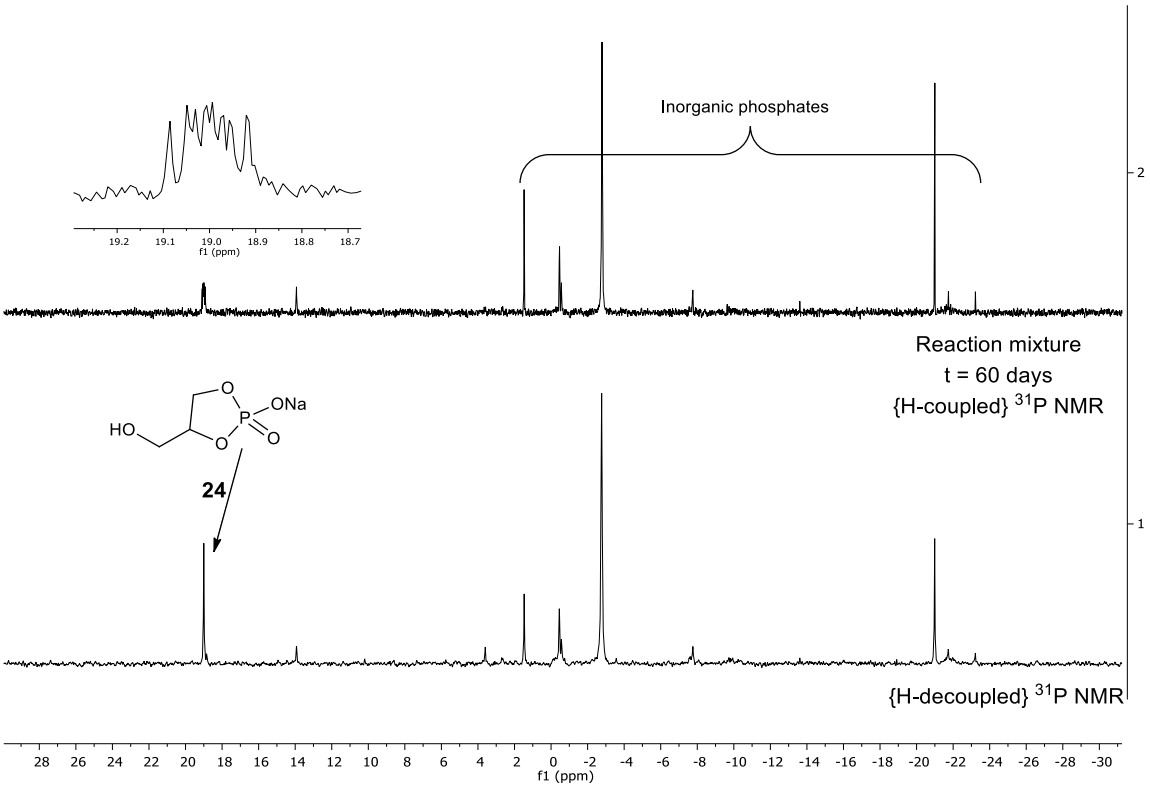
Supplementary Figure 114:

^{1}H NMR spectra of phosphorylation of **23** in paste following the method B (Supplementary Table 12 entries 3 - 6). The spectrum of starting material **23** (top spectrum) is shown for comparison.

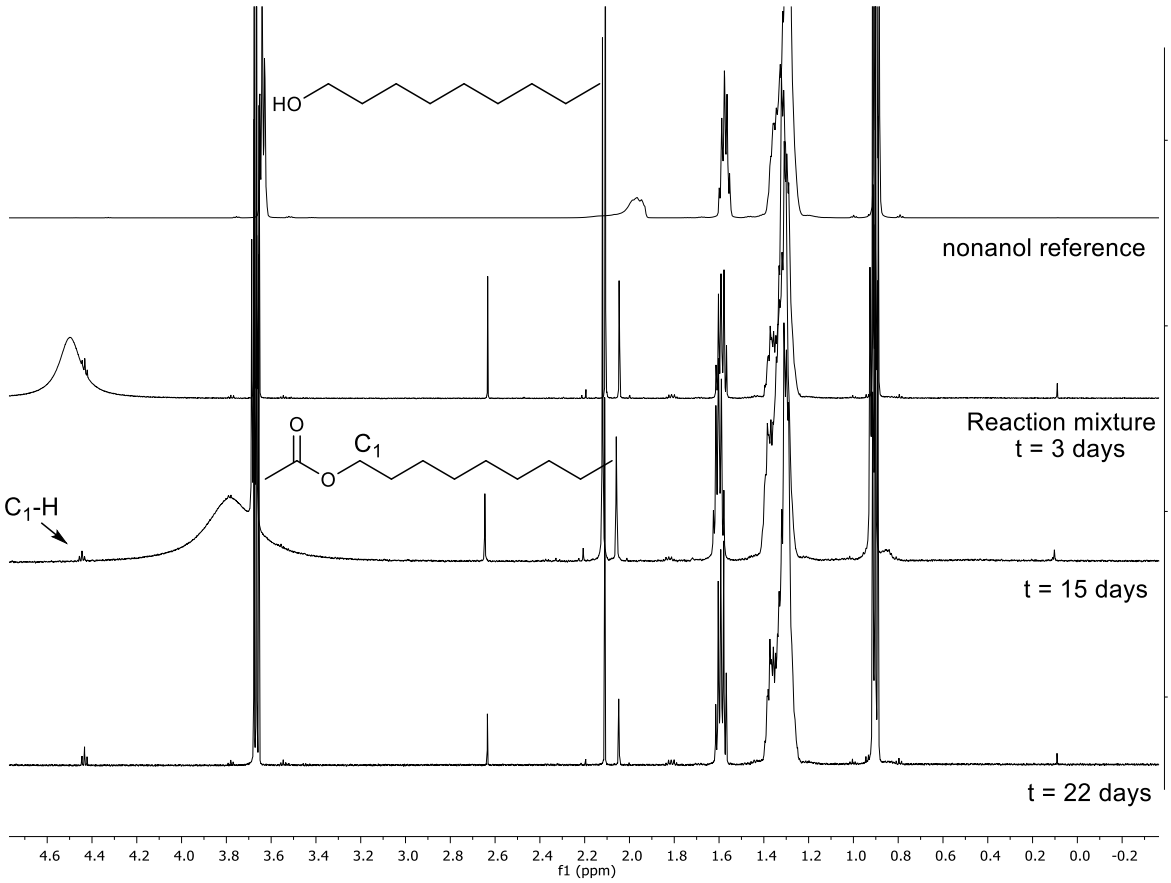


Supplementary Figure 115:

^{13}C NMR spectra of phosphorylation of **23** in paste following the method B (Supplementary Table 12 entries 3 - 6). The spectrum of starting material **23** (top spectrum) is shown for comparison.

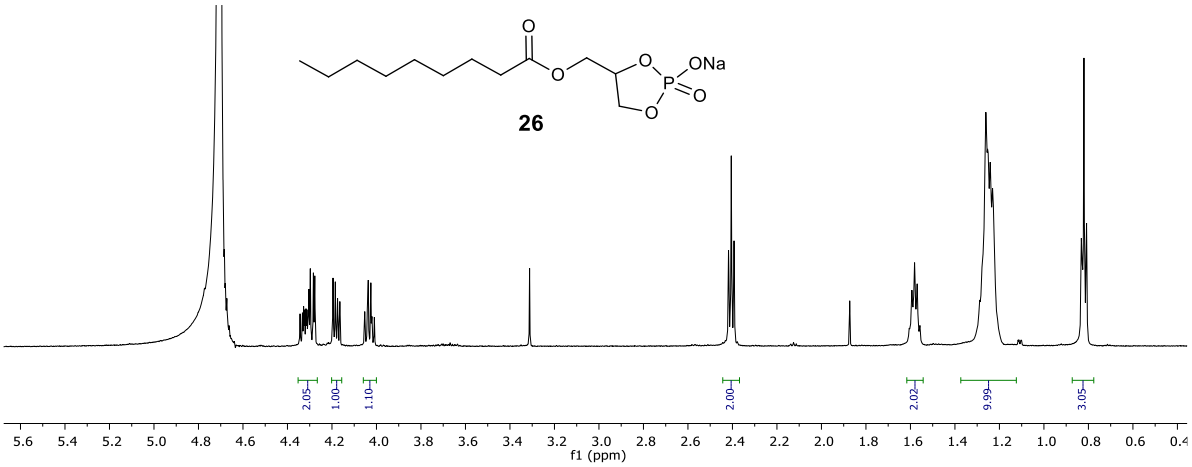


Supplementary Figure 116:
{H-coupled} ^{31}P NMR (top spectrum) and {H-decoupled} ^{31}P NMR (bottom spectrum) spectra of phosphorylation of **23** in paste following the method B (Supplementary Table 12 entry 6).

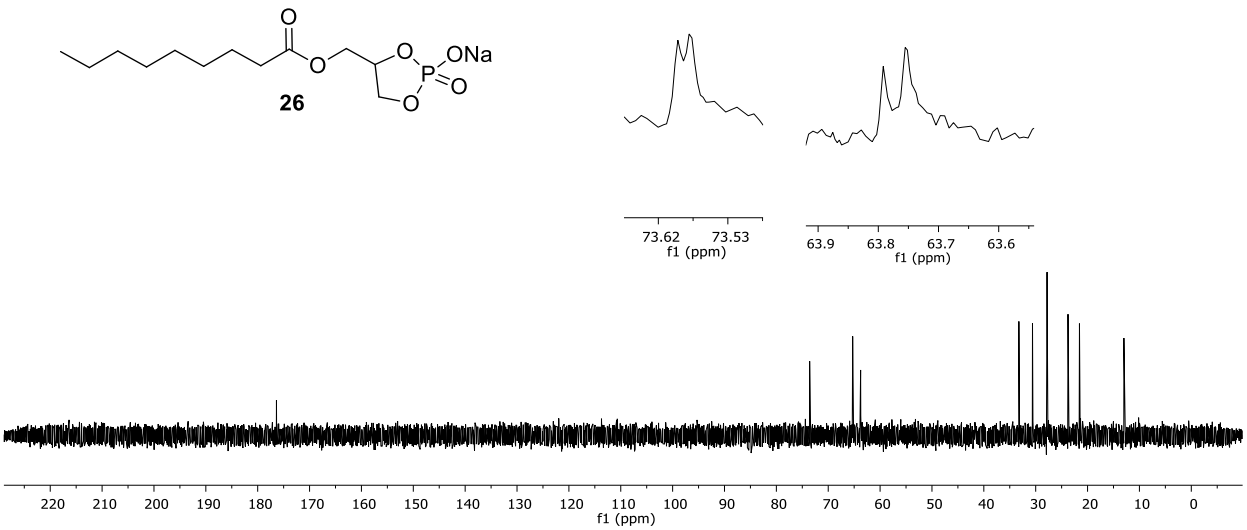


Supplementary Figure 117:

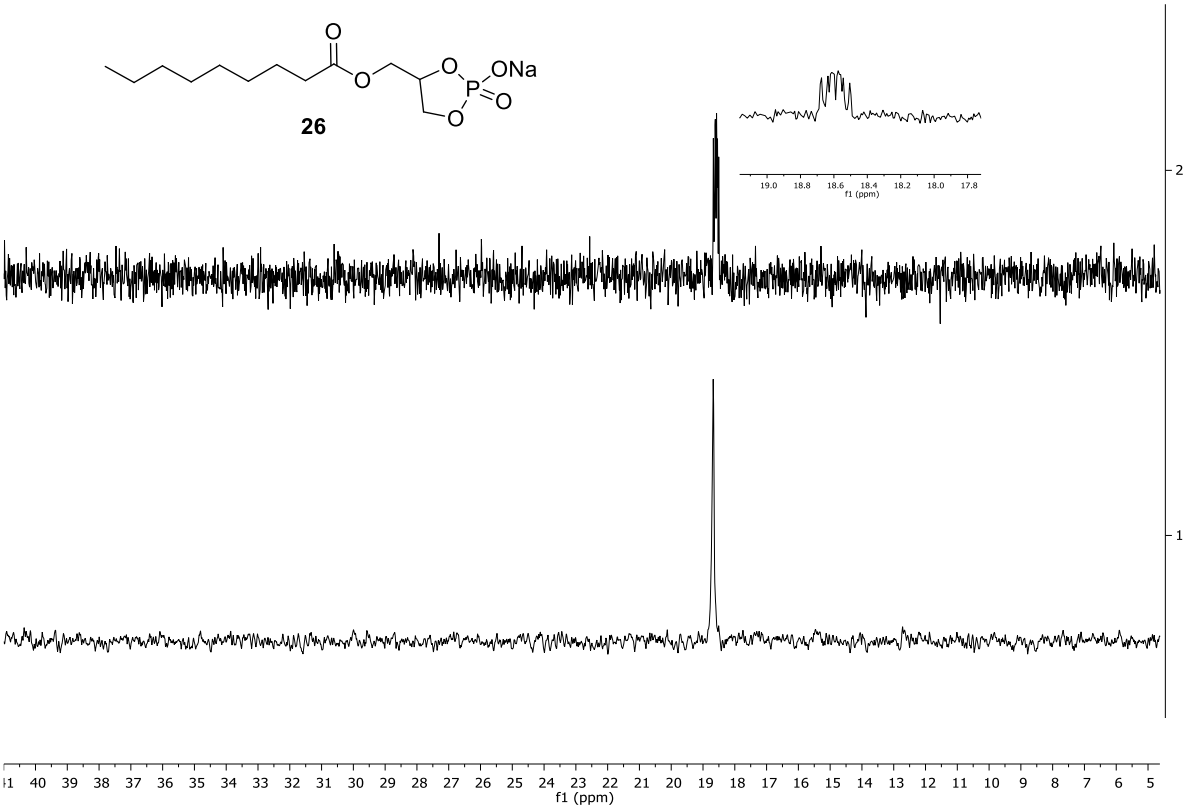
^{1}H NMR spectra ($CDCl_3$) of esterification of 1-nonanol and ammonium acetate with DAP under gel. (Supplementary Table 13, entries 1 - 3). The spectrum of starting material 1-nonanol (in $CDCl_3$, top spectrum) is shown for comparison.



Supplementary Figure 118:
^{1}H NMR spectrum of synthesized phospholipid **26** in D_2O.

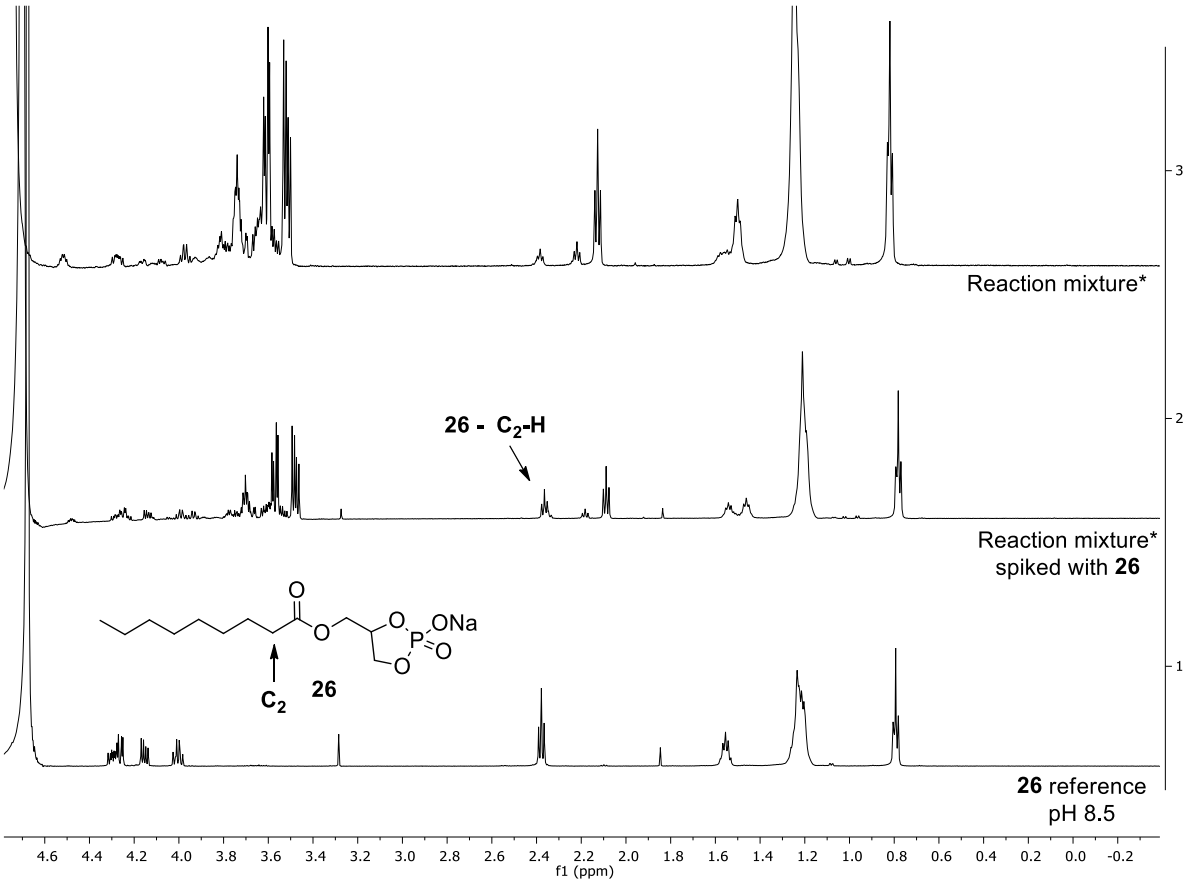


Supplementary Figure 119:
^{13}C NMR spectrum of synthesized phospholipid **26** in D_2O.



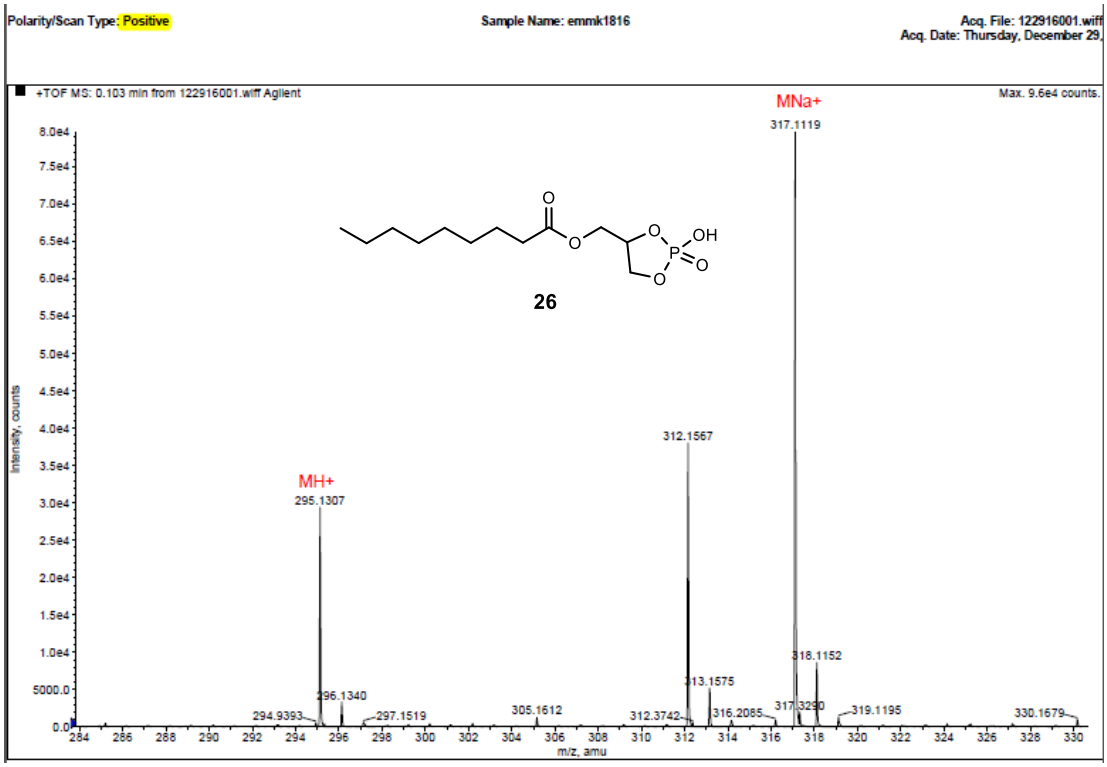
Supplementary Figure 120:

{H-coupled} ^{31}P (top spectrum) and {H-decoupled} ^{31}P (bottom spectrum) spectra of synthesized phospholipid **26** in D_2O.

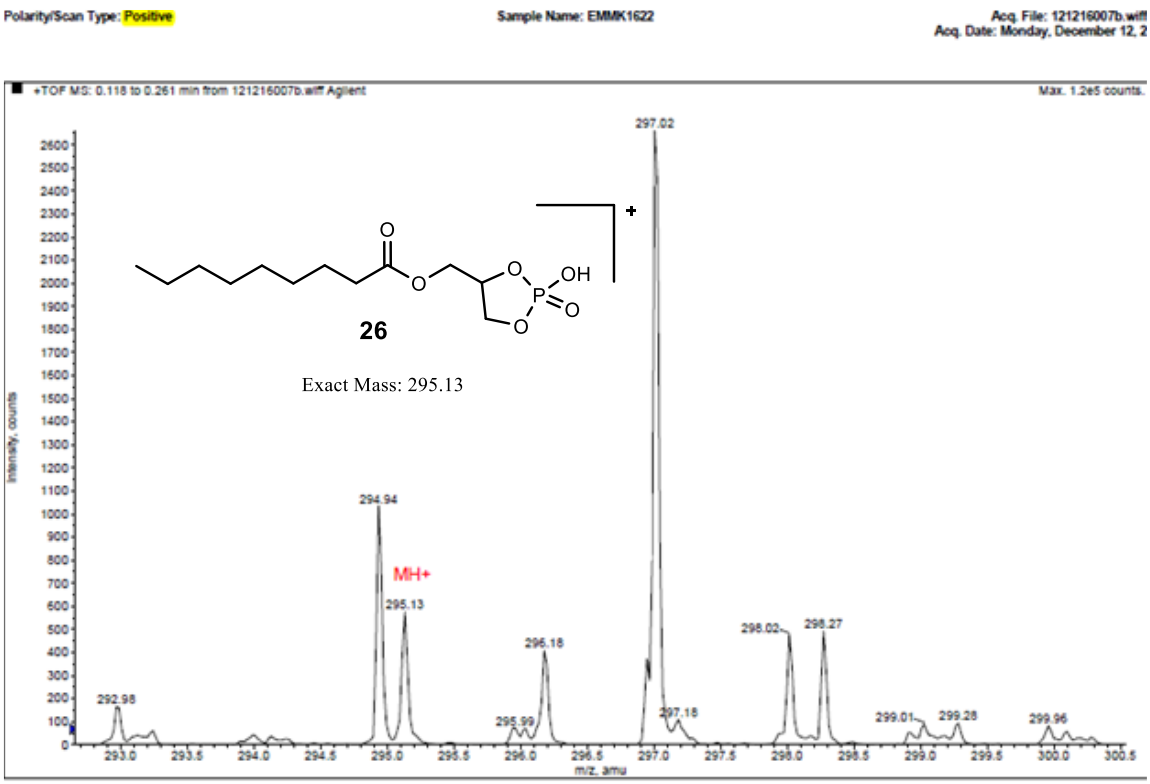


Supplementary Figure 121:

^{1}H NMR spectra (in D_2O) comparing the synthesized phospholipid **26** (bottom spectrum) with crude reaction mixture (following Method A1, top spectrum) and crude reaction mixture spiked with synthesized phospholipid **26** (middle spectrum).



Supplementary Figure 122:
HRMS (ESI-TOF; positive mode) of the synthesized phospholipid **26**.



Supplementary Figure 123:

ESI-MS (positive mode) of crude reaction mixture following Method A1 showing the presence of phospholipid **26** as MH^+ = 295.13 (calculated = 295.13).

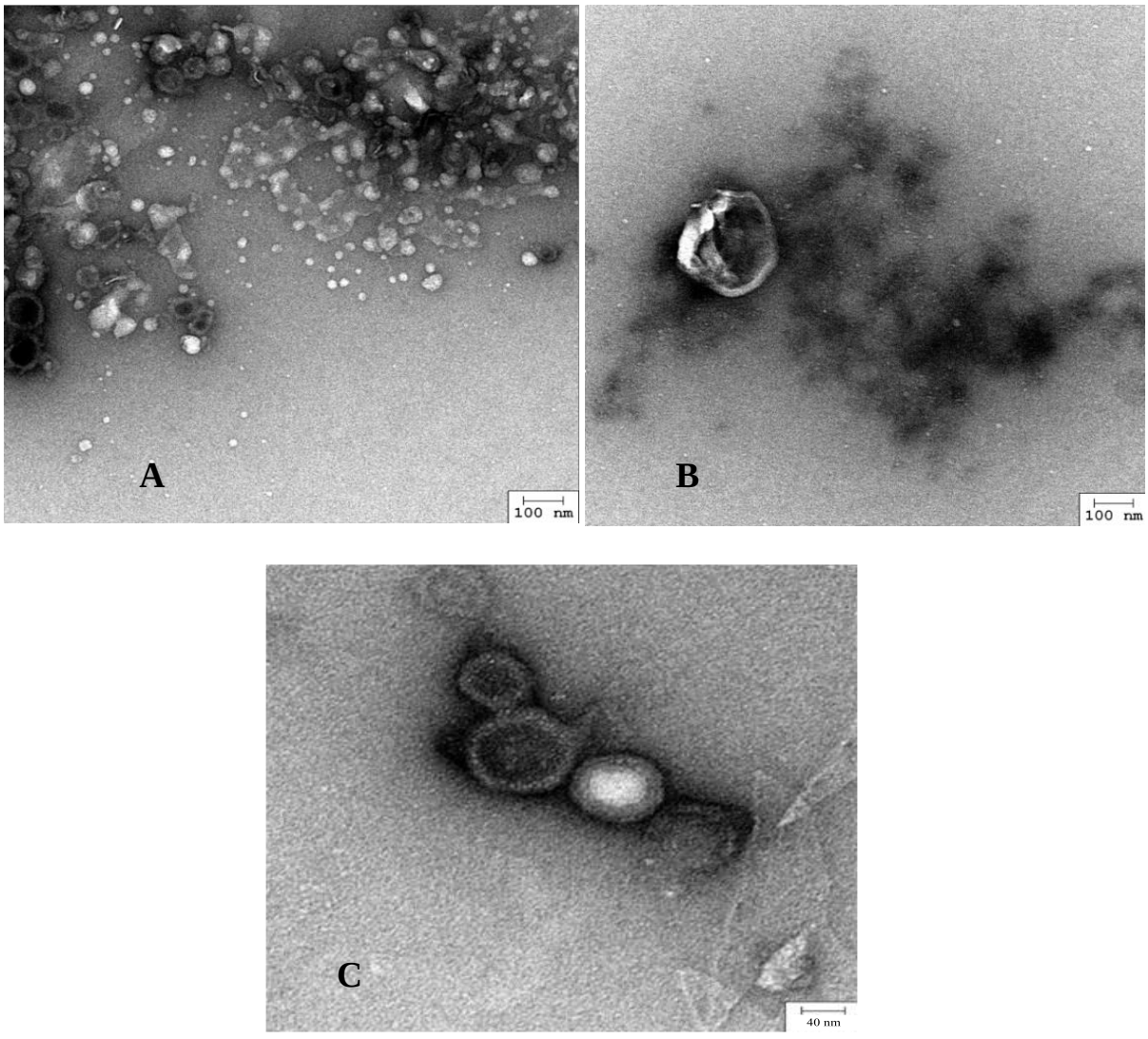
Generation of micelle and/or vesicle-like structures and sample preparation for study by negative stain transmission electron microscopy (TEM).

Procedure A: *Sample preparation (with sonication)* -The crude reaction mixture (obtained following Method A1, page S9) (0.015 g) was dissolved in water (1 mL) and the pH of the solution was adjusted to ~ 8.5 with 1 M NaOH. The solution was vortexed. Upon sonication, the solution becomes clear and was filtered through 0.22 μm PVDF filter unit. The resulting solution was taken for analysis by negative stain transmission electron microscopy (TEM).

Procedure B: *Sample preparation (without sonication)* -The crude reaction mixture (obtained following Method A1, page S9) (0.015 g) was dissolved in water (1 mL) and the pH of the solution was adjusted to ~ 8.5 with 1 M NaOH. The solution was vortexed which appeared slightly opaque and was taken for analysis by negative stain TEM.

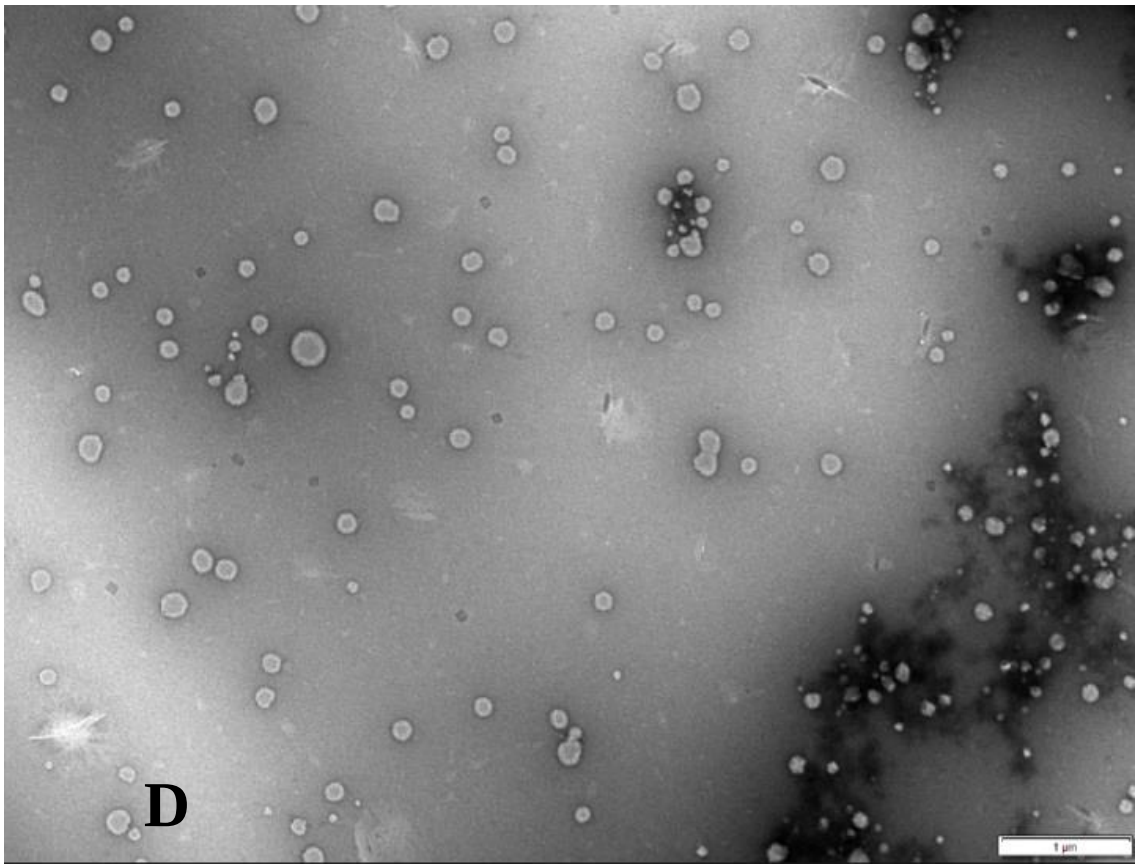
*Procedure C: Sample preparation for phospholipid **26** (without sonication)* - 1 mg of the synthetic phospholipid **26** (obtained following Method B, Page S9) was diluted with 1 mL water, pH adjusted to ~ 8.5 with 1 M NaOH. The solution was vortexed and was taken for analysis by negative stain TEM.

Procedure D: Sample preparation (without sonication) -The crude reaction mixture (obtained following Method A2, page S9) (0.015 g) was dissolved in water (1 mL) and the pH of the solution was adjusted to ~ 8.5 with 1 M NaOH. The solution was vortexed which appeared slightly opaque and was taken for analysis by negative stain TEM.



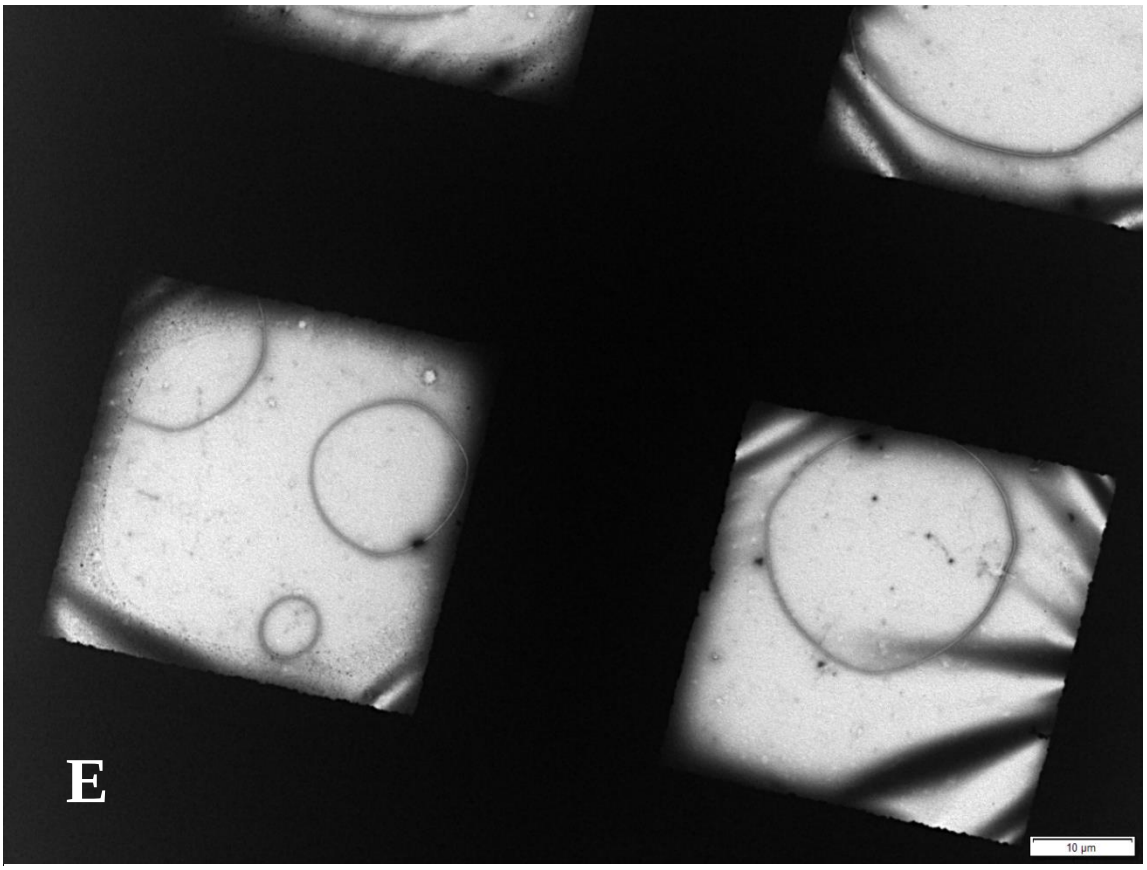
Supplementary Figure 124:

(Image A, B and C) Representative electron micrographs of micelle/vesicle-like structures obtained by the procedure A (Method A1, 45 days old reaction mixture). The pH was ~8.5 and stained with uranyl formate. Vesicle-like structures appear to have a diameter in the range of 30 - 110 nm.



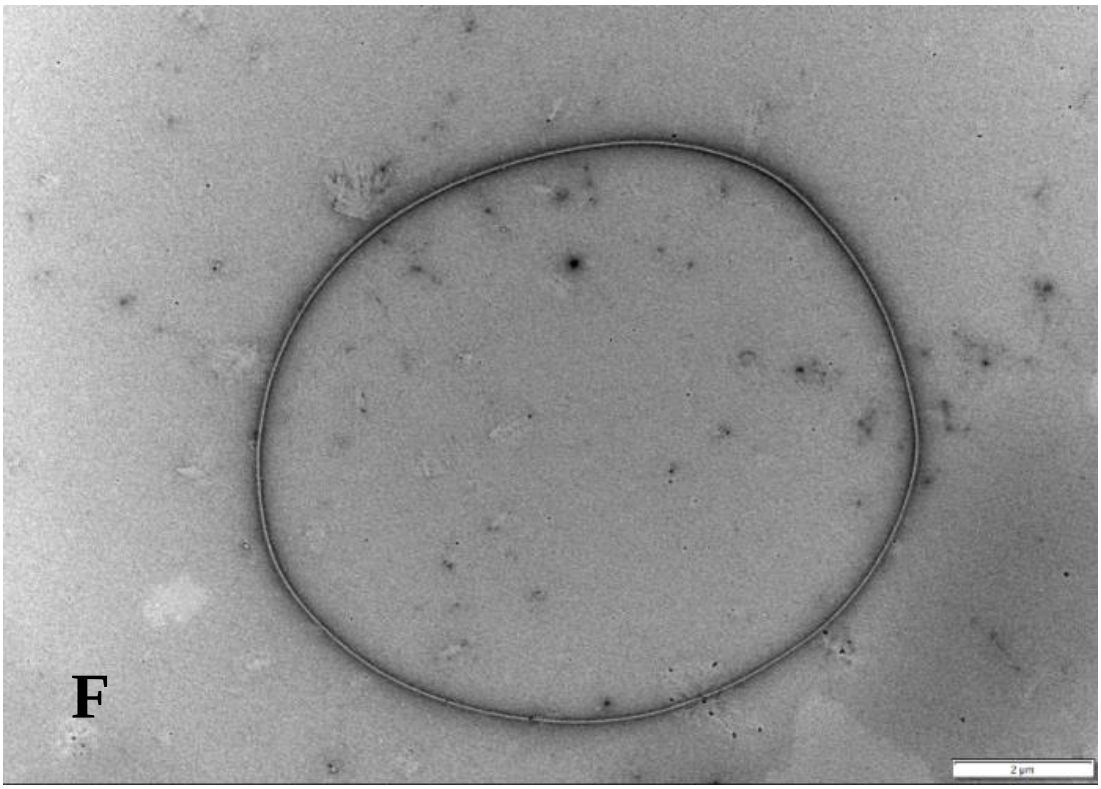
Supplementary Figure 125:

(Image D) Representative electron micrograph of micelle/vesicle-like structures obtained by the Procedure B (Method A1, 45 days old reaction mixture; one cross-sectional area of the grid). The pH was ~8.5 and stained with uranyl acetate. Vesicle-like structures appear to have diameter up to ~ 280 nm. The scale bar represents 1 µm.



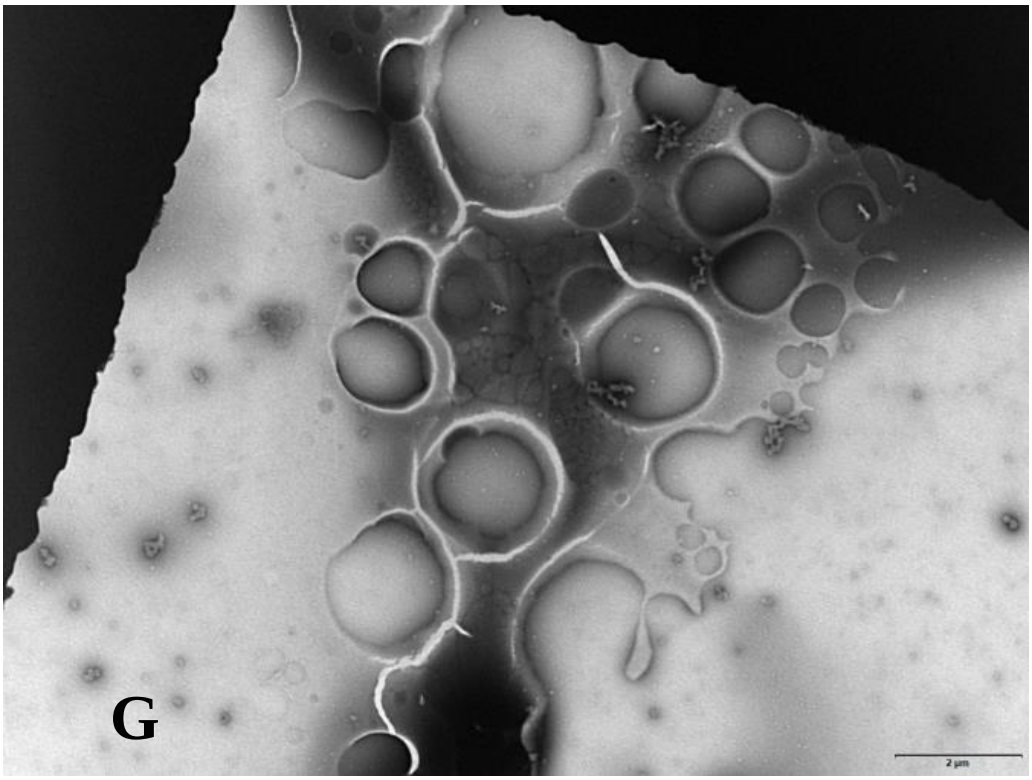
Supplementary Figure 126:

(Image E) Representative electron micrograph of vesicle-like structures obtained by the Procedure B (Method A1, 45 days old reaction mixture; another cross-sectional area of the above grid). The pH was ~8.5 and stained with uranyl acetate. The diameter measurements on these vesicle-like structures are in the range of 5.7 to 24.7 μm. The scale bar represents 10 μm.



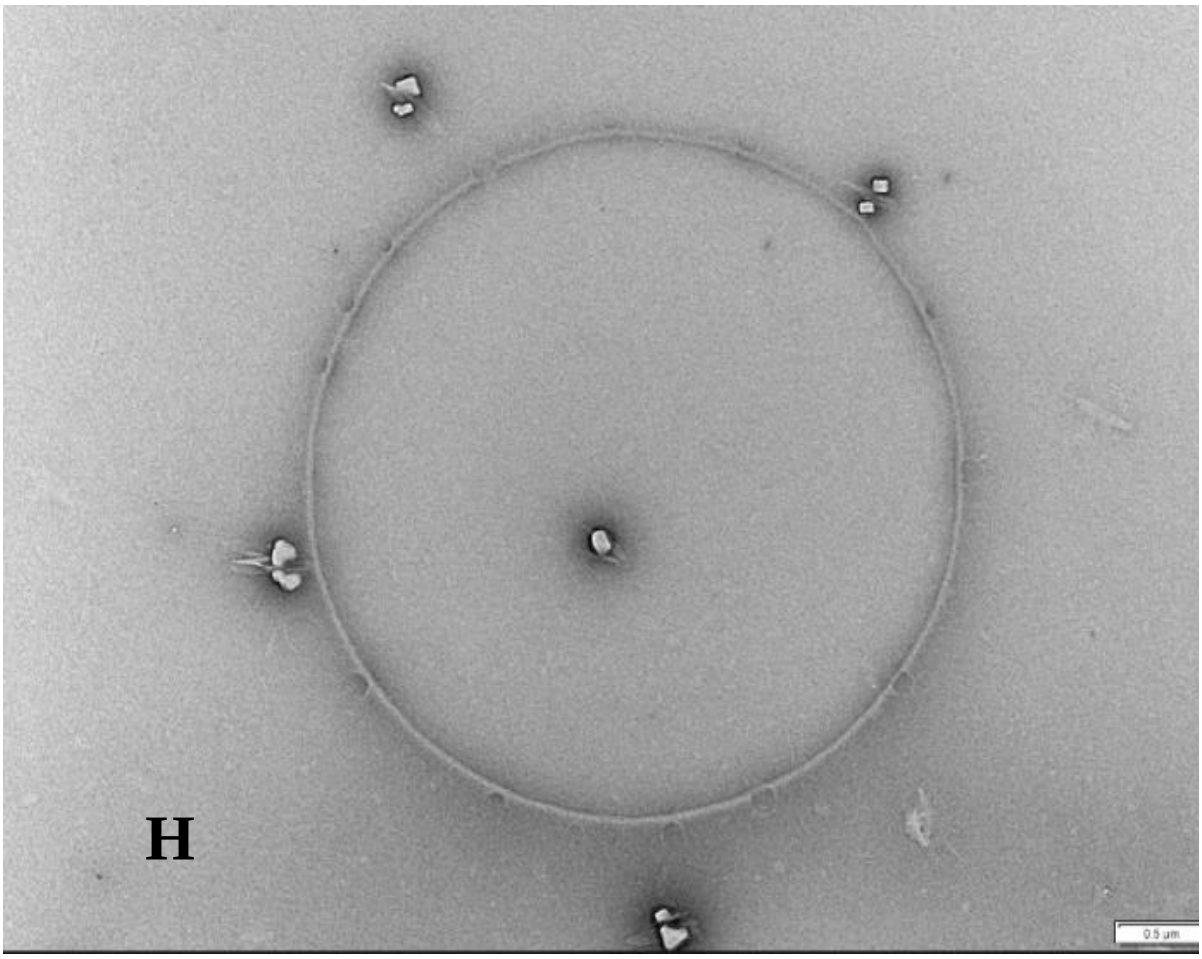
Supplementary Figure 127:

(Image F) Representative electron micrograph of vesicle-like structure obtained by the Procedure B (Method A1, 45 days old reaction mixture; magnified version of image E). The pH was 8.5 and stained with uranyl acetate. The scale bar represents 2 µm.



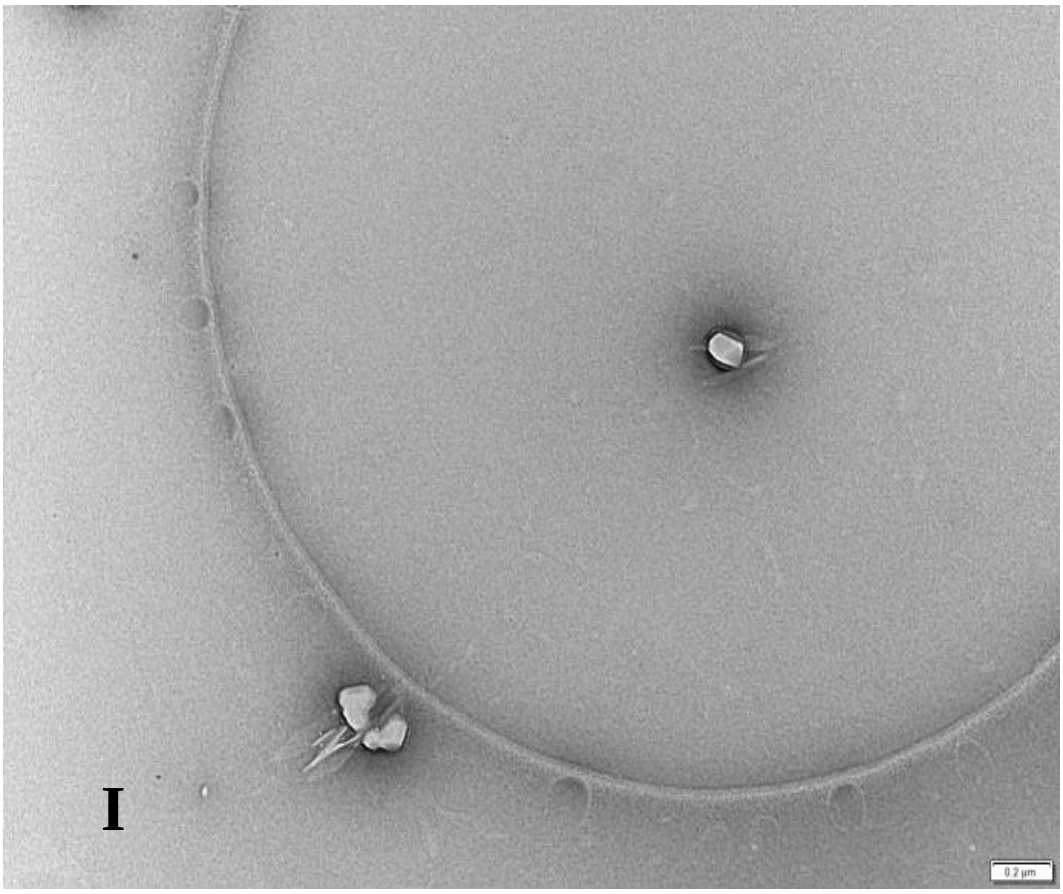
Supplementary Figure 128:

(Image G) Representative electron micrograph of structures obtained by the Procedure C (synthesized phospholipid **26**). The pH was 8.5 and stained with uranyl acetate. The scale bar represents 2 µm

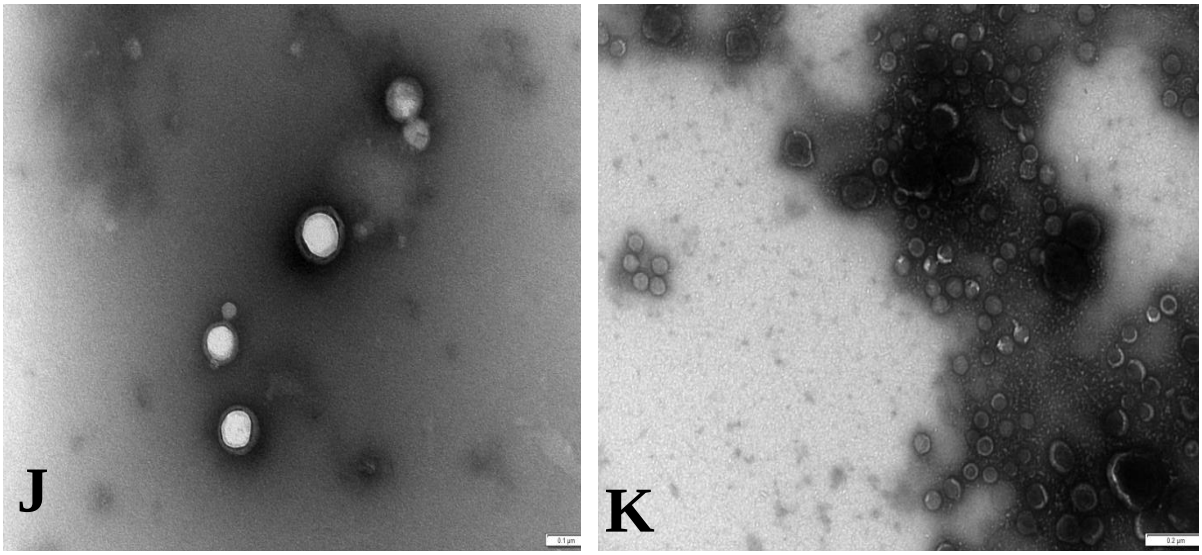


Supplementary Figure 129:

(Image H) Representative electron micrograph of vesicle-like structures obtained by the Procedure C (synthesized phospholipid **26**). The pH was 8.5 and stained with uranyl acetate. Vesicle-like appears to have a diameter of 3.5 µm. The scale bar represents 0.5 µm.



Supplementary Figure 130:
(Image I) Representative electron micrograph of vesicle-like structures obtained by the Procedure C (synthesized phospholipid **26**; magnified version of image H). The pH was 8.5 and stained with uranyl acetate. Small adherent vesicle-like structures are visible in this image. The scale bar represents 0.2 µm.

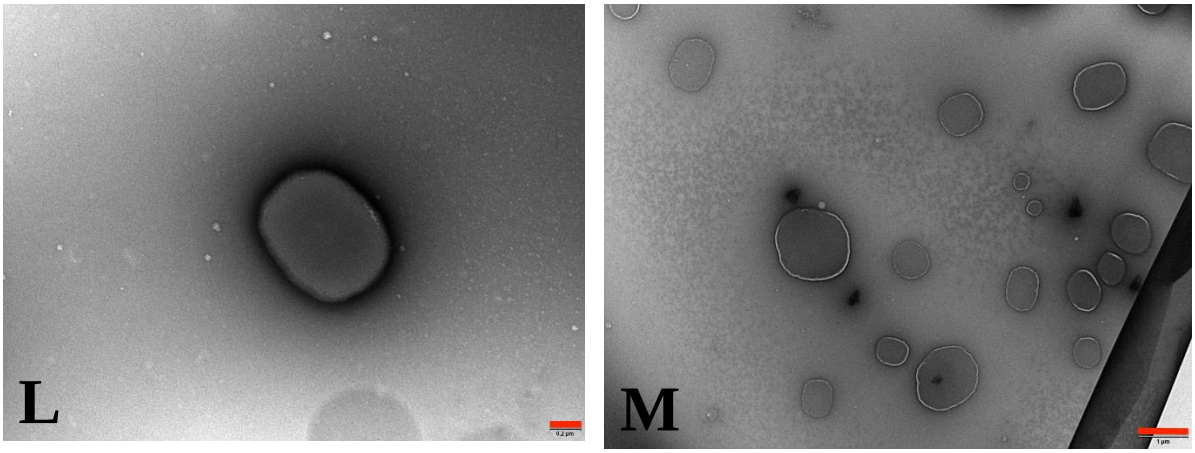


Supplementary Figure 131:

(Image J; left and Image K; right) Representative electron micrograph of vesicle-like structures obtained by the Procedure D (octanoic acid; Method A2). The pH was 8.5 and stained with uranyl acetate. The scale bar represents 0.1 μm (image J) and 0.2 μm (image K).

Control experiments for the crude one-pot reaction of nonanoic acid, glycerol, DAP and imidazole under paste conditions (Procedure B).

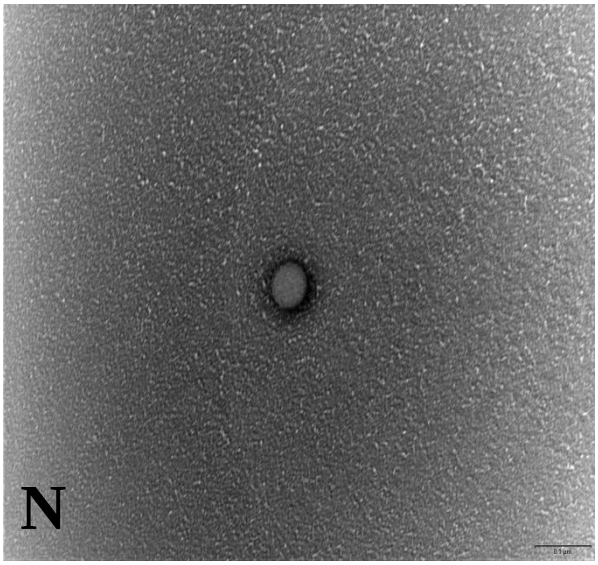
Nonanoic acid	Glycerol	DAP	Imidazole
Yes	*Yes*	*Yes*	*No*



Supplementary Figure 132:

(Images L; left and Image M; right) Representative electron micrographs of vesicle-like structures obtained by the Procedure B (Method A1; 30 days old reaction mixture). The pH was 8.5 and stained with uranyl acetate. Vesicle-like structures appear to have diameter size in the range of 200 nm – 400 nm. The scale bars represent 0.2 μm (image L) and 1 μm (image M).

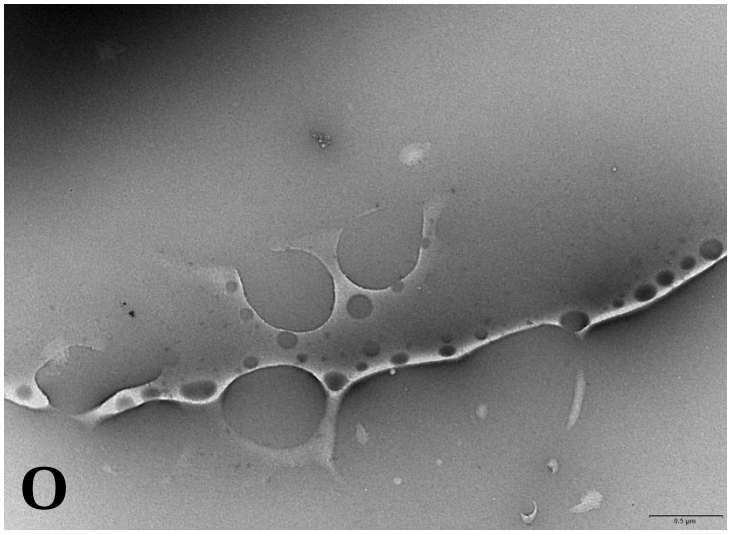
Nonanoic acid	Glycerol	DAP	Imidazole
Yes	*Yes*	*No*	*Yes*



Supplementary Figure 133:

(Image N) Representative electron micrograph obtained by the Procedure B (Method A1; 30 days old reaction mixture). The pH was 8.5 and stained with uranyl acetate. The scale bar represents 0.1 μm.

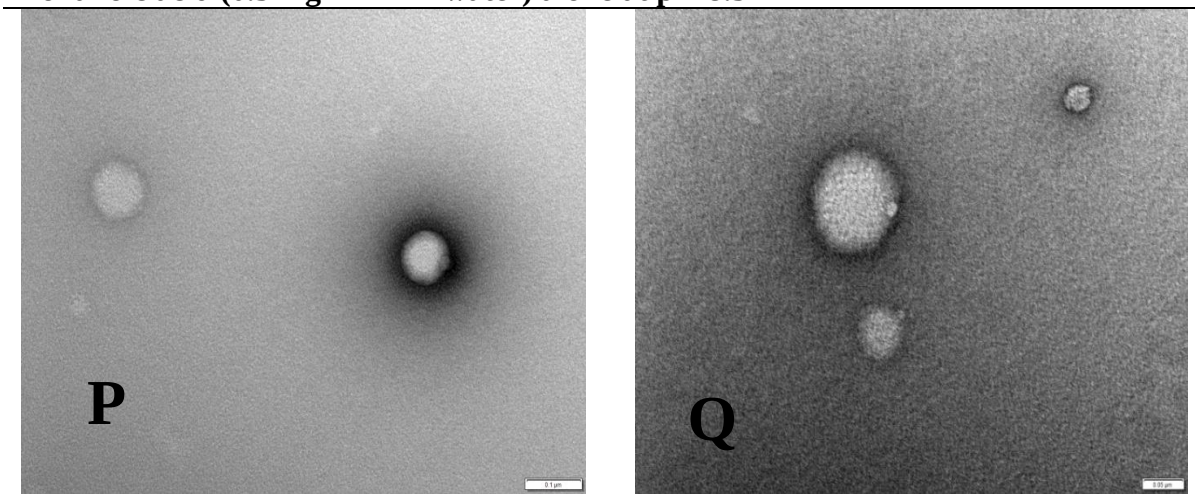
Nonanoic acid	Glycerol	DAP	Imidazole
Yes	*No*	*Yes*	*Yes*



Supplementary Figure 134:

(Image O) Representative electron micrograph obtained by the Procedure B (Method A1; 30 days old reaction mixture). The pH was 8.5 and stained with uranyl acetate. The scale bar represents 0.5 μm.

Nonanoic acid (0.5 mg in 1 mL water) alone at pH 8.5



Supplementary Figure 135:

(Image P, left and image Q, right) Representative electron micrographs of nonanoic acid alone at pH 8.5. Stained with Uranyl Acetate. The micelles appear to be in the range of diameter 34 nm – 102 nm. The scale bar represents 0.1 μm and 0.05 μm for images P and Q respectively.

Confocal Microscopy Study

Quantities of materials used:

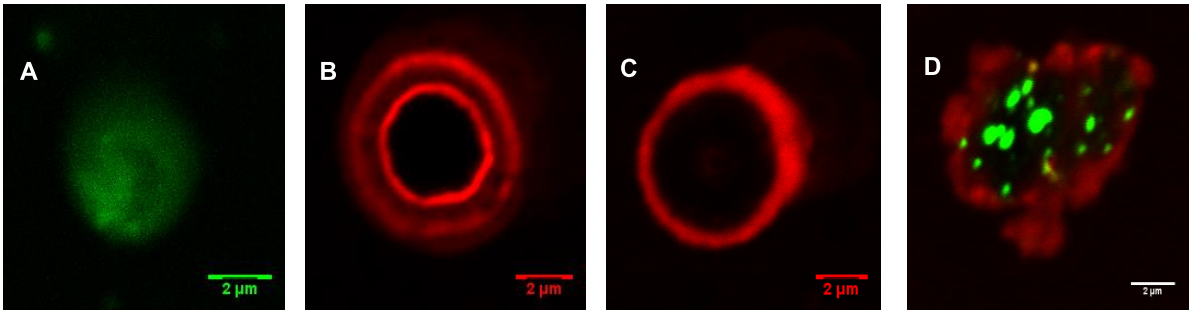
Synthetic Phospholipid **26**: 2 mg in 0.1 mL water, pH 8.5
Rhodamine B: 1 µL of 1 mM
Pyranine: 1 µL of 5 mM

Vesicle preparation: 2 mg of phospholipid **26** was dissolved in 0.1 mL water and the pH was adjusted to 8.5. The solution was vortexed. The three samples which were visualized by confocal microscopy were prepared as following:

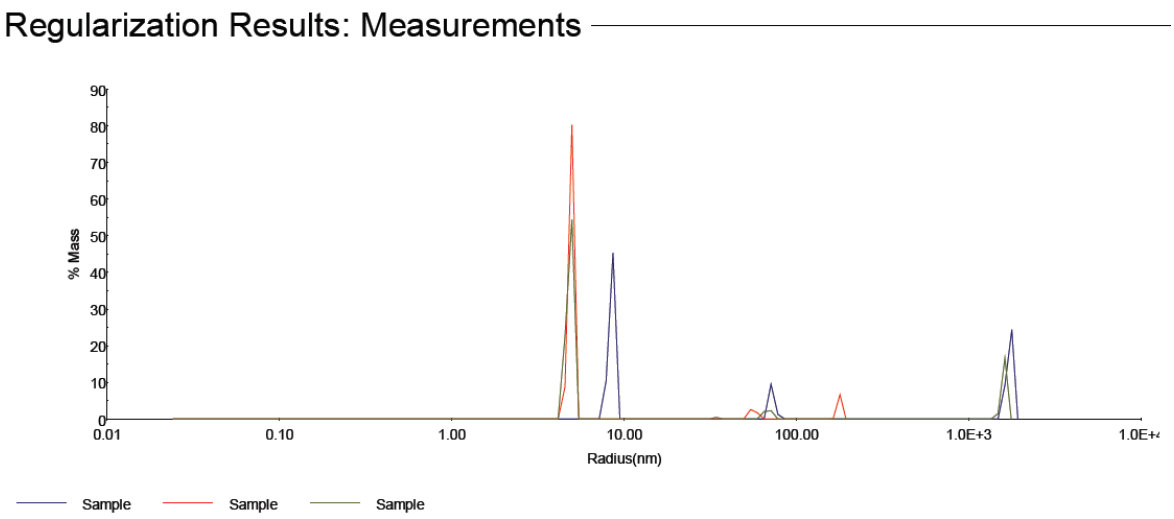
Sample A (dye encapsulation): Phospholipid **26** in water was mixed with pyranine dye (5 mM, 1 µL) and tumbled for 30 minutes. It was passed through sephadex G-50 size exclusion spin column to get rid of excess of dye. 10 µL was placed on a cover slip and visualized by confocal microscopy.

Sample B: Phospholipid **26** in water was mixed with rhodamine b dye (1 mM, 1 µL) and tumbled for 30 minutes. 10 µL was placed on a cover slip and visualized by confocal microscopy.

Sample C: Phospholipid **26** in water was mixed with pyranine dye (5 mM, 1 µL) and tumbled for 30 minutes. It was passed through sephadex G-50 size exclusion spin column to get rid of excess of dye followed by addition of rhodamine b dye. 10 µL of the sample containing dyes was placed on a cover slip and visualized by confocal microscopy.

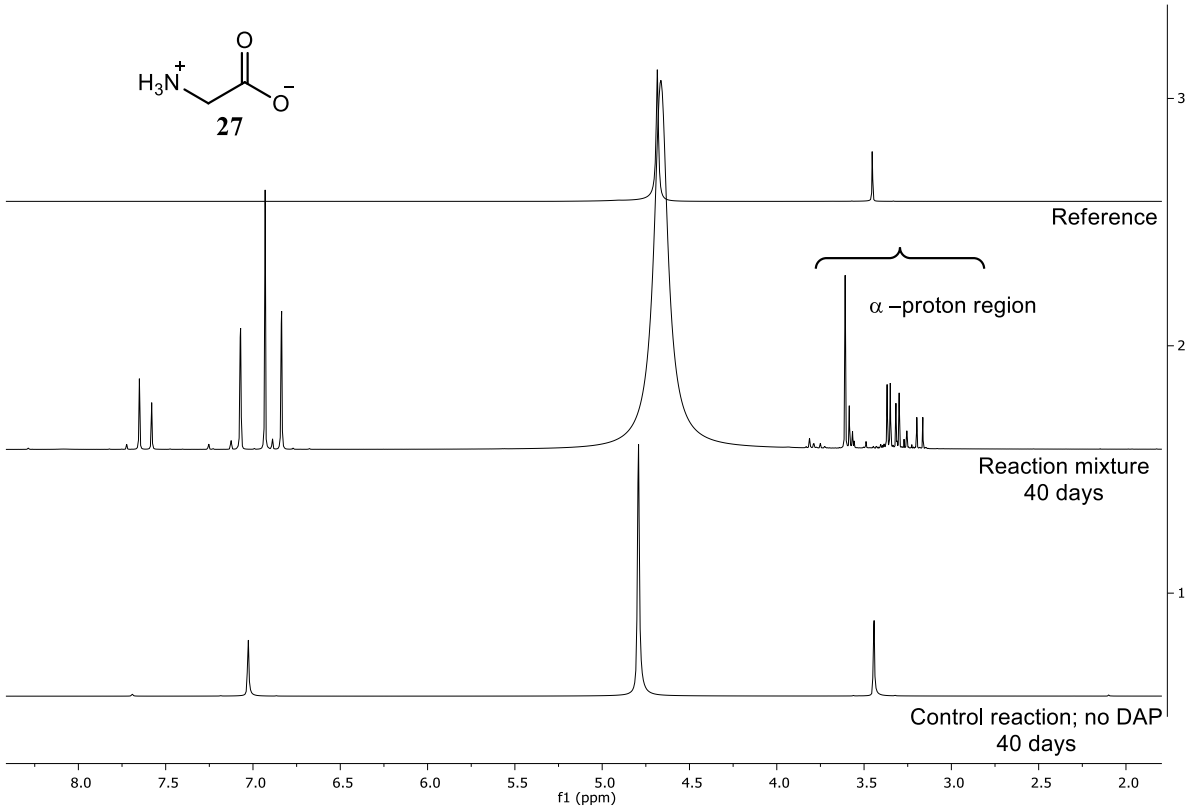


Supplementary Figure 136:
Confocal laser scanning microscopy fluorescence images of vesicle prepared with phospholipid **26** (2 mg in 0.1 mL water). (A) Green fluorescence indicates hydrophilic pyranine dye encapsulated in the cavity. (B and C) Red fluorescence indicates rhodamine b dye labelling the bilayer membrane of the phospholipid (D) Fluorescence merged image of phospholipid vesicle prepared with both rhodamine b dye and pyranine dye. The scale bar represents 2 µm.



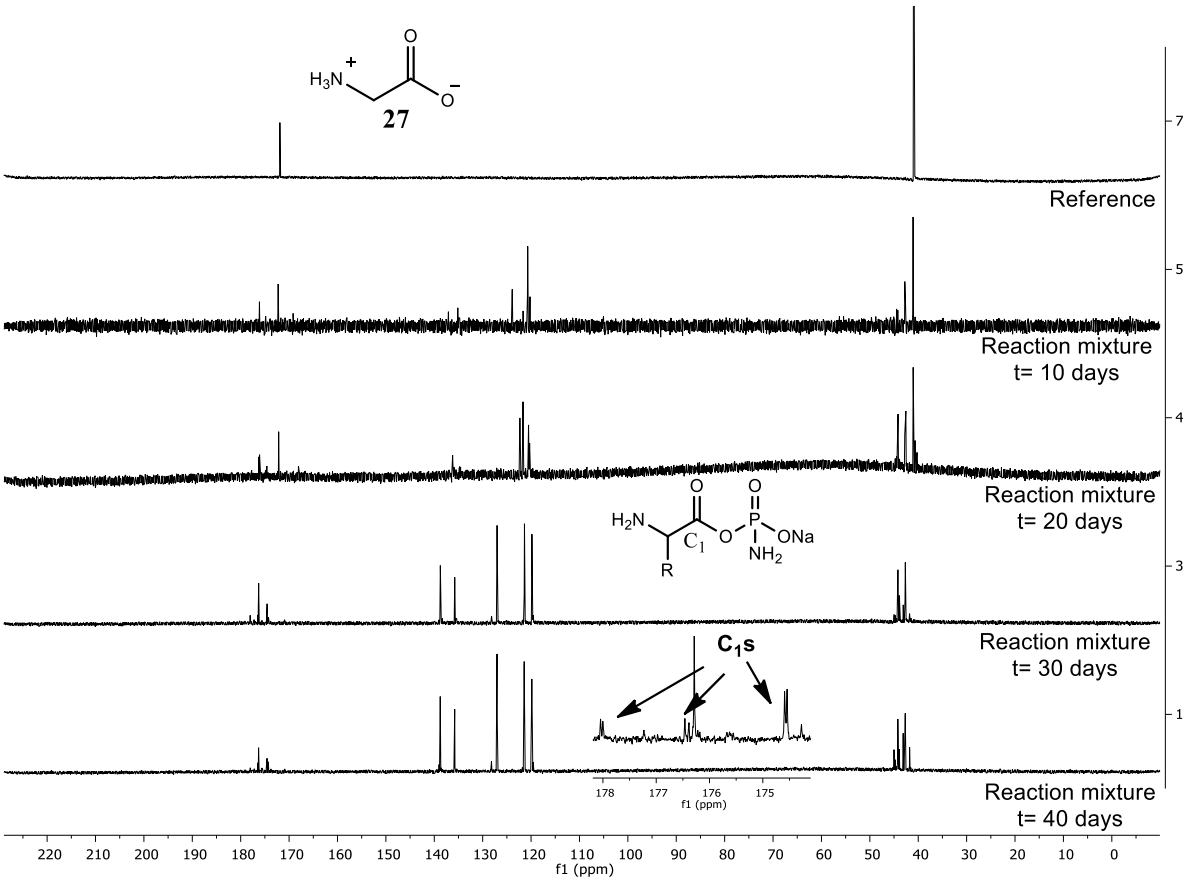
Supplementary Figure 137 :

Dynamic light scattering (DLS) distribution function for sample prepared by procedure A (Method A1, 45 days old reaction mixture).



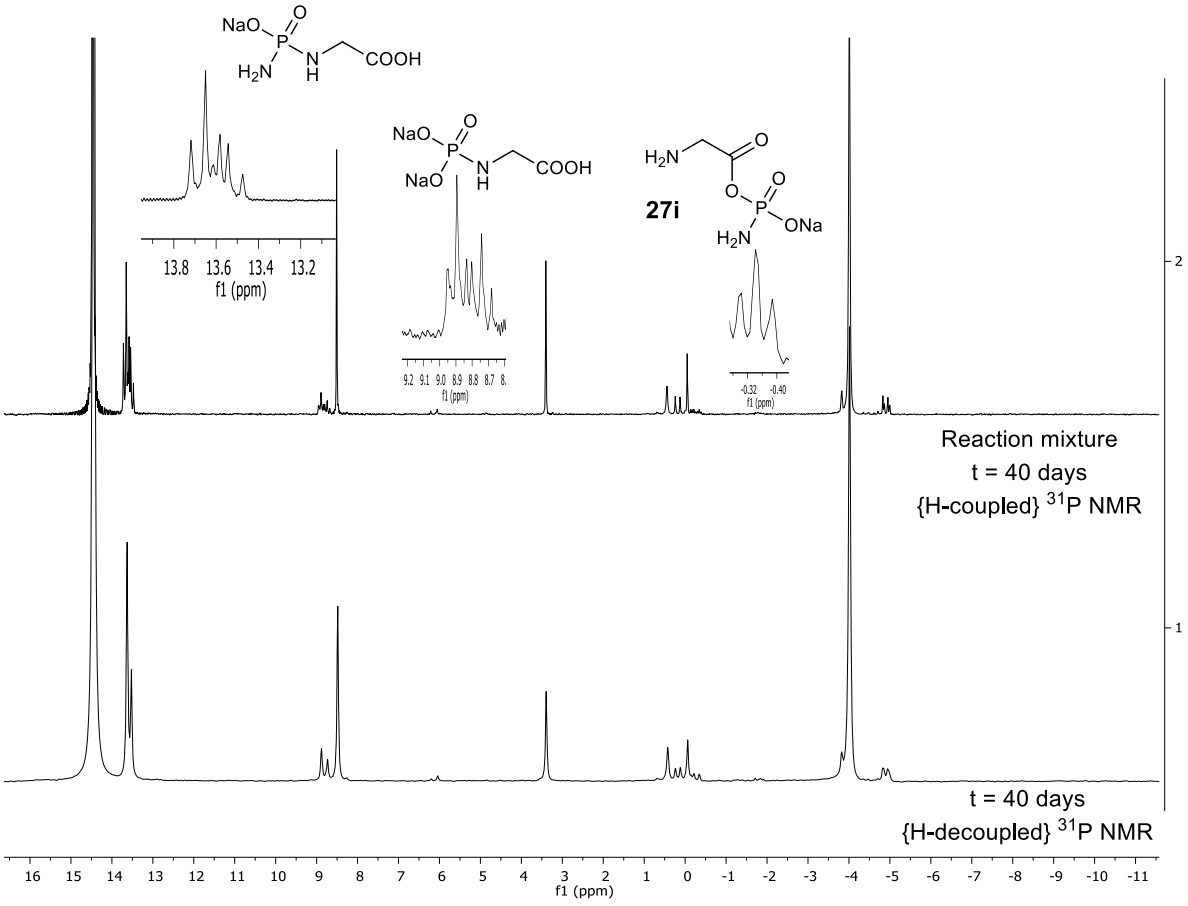
Supplementary Figure 138:

^{1}H NMR spectra of the crude reaction of **27** with DAP and imidazole in D_2O following the Method A (middle spectrum, 40 days) showing complete conversion of **27** to higher peptides. The bottom spectrum is the reaction of **27** with imidazole in D_2O in the absence of DAP (control) showing no formation of higher order peptides in 40 days. The spectrum of starting material **27** (top spectrum) is shown for comparison.



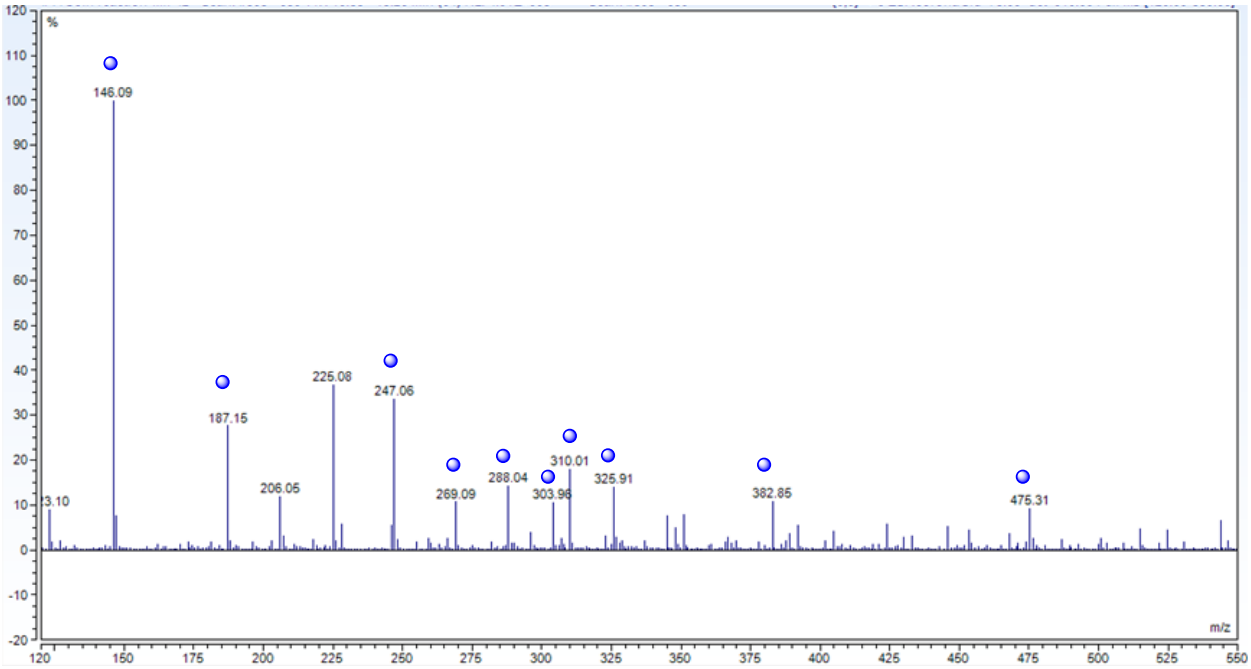
Supplementary Figure 139:

^{13}C NMR spectra of the crude reaction of **27** with DAP and imidazole in D_2O following the Method A (bottom spectrum, 40 days) showing the formation of acyl phosphates of glycine and its homologues in the region between 174 -180 ppm. The spectrum of starting material **27** top spectrum) is shown for comparison.



Supplementary Figure 140:

{H-decoupled} ^{31}P NMR (bottom spectrum) and {H-coupled} ^{31}P NMR (top spectrum) spectra of the crude reaction of **27** with DAP and imidazole in D_2O following the Method A (top spectrum, 40 days). The signals at 13.6, 8.9 and -0.3 ppm shows the phosphorylation of α-amino and the α-carboxyl positions.[S6, S7]

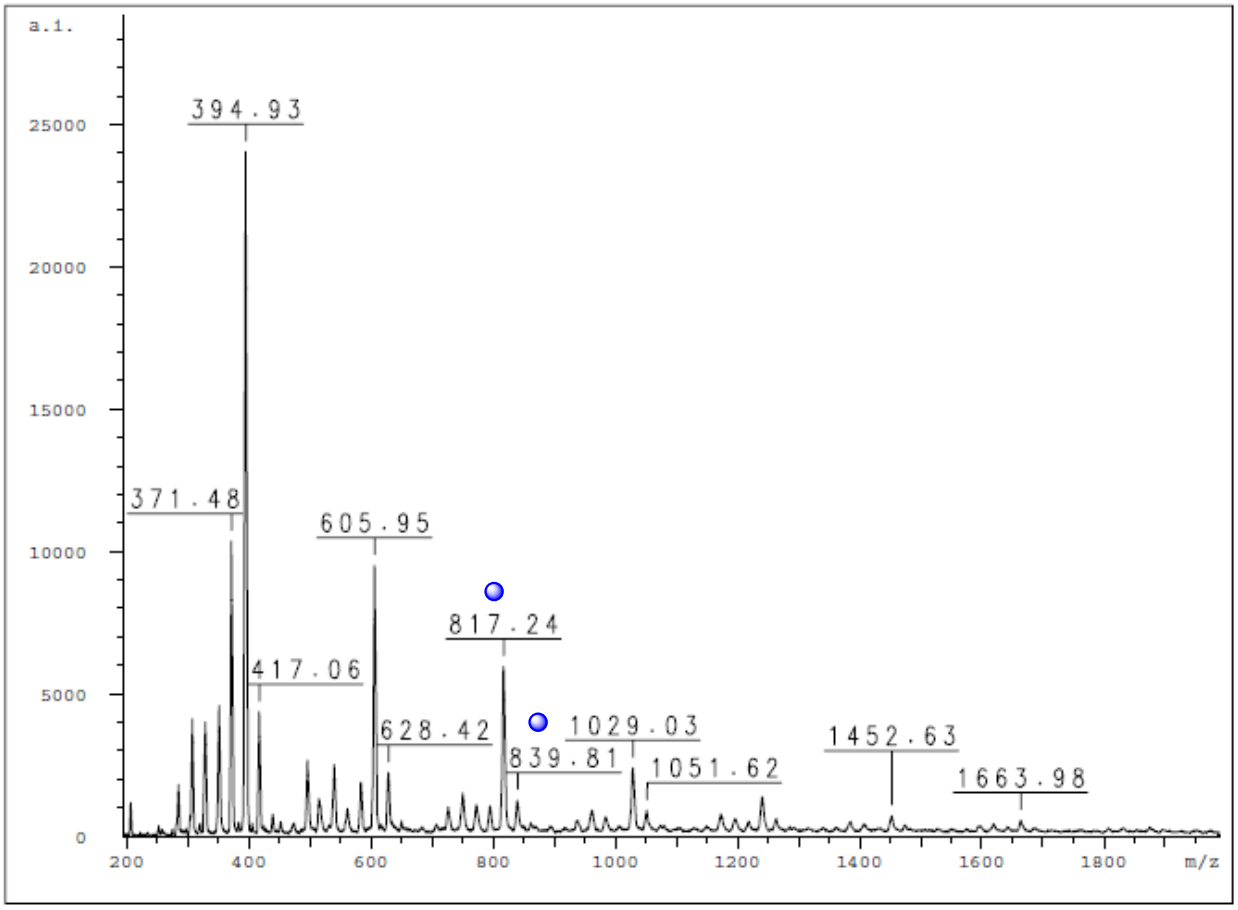


Entry	Species	Calculated m/z	Observed m/z
1.	$(Gly_4+2Na)/2$	146.04	146.09
2.	$(Gly_4+2Na + 2\ ACN)/2$	187.06	187.15
3.	Gly_4+H	247.09	247.06
4.	Gly_4+Na	269.09	269.10
5.	$Gly_4+ ACN + Na$	310.11	310.02
6.	$Gly_5+ H$	304.13	303.96
7.	Gly_5+Na	325.91	326.10
8.	Gly_6+Na	383.12	382.85
9.	$Gly_8+ H$	475.18	475.31

Supplementary Figure 141:

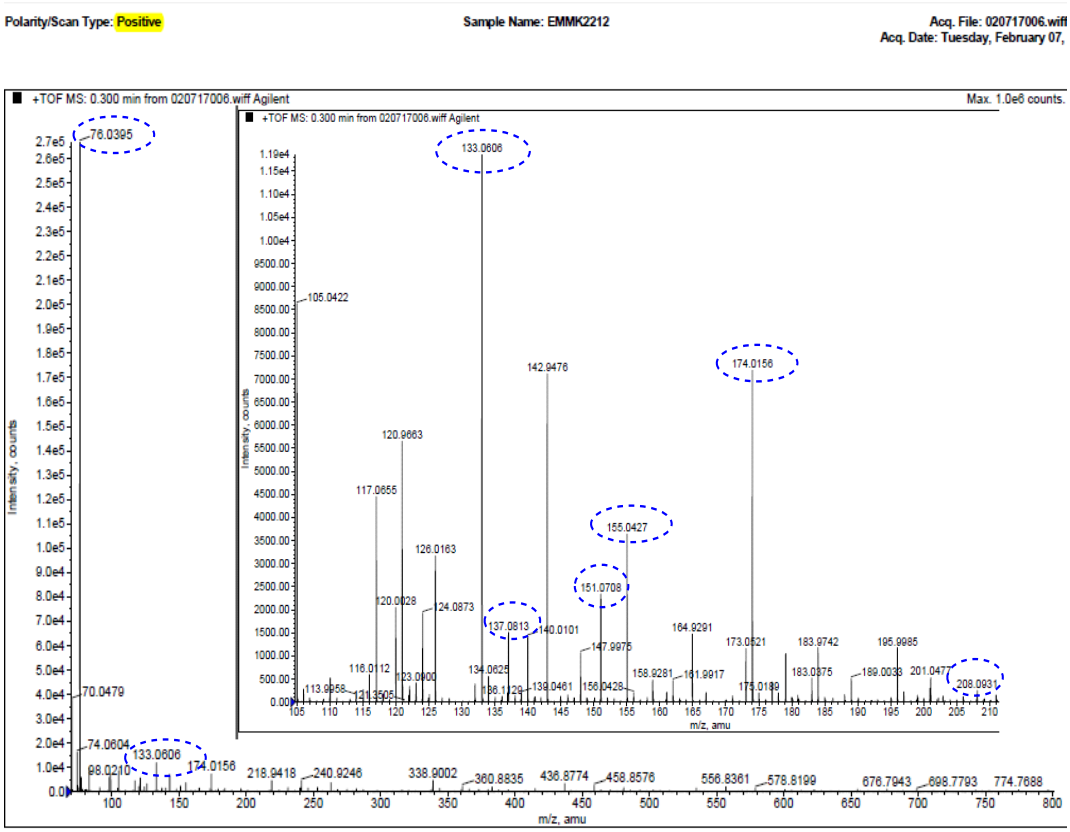
LCMS-spectrum of the crude reaction (Method A) showing the glycine oligomerization products after 80 days. Glycine oligomeric peaks up to 8-mer can be identified as H^+ adducts (along with a series of Na^+ and (Na^+ + ACN) adducts). Characterization of the peaks in the above mass spectrum is depicted in the table (color dot refers to the assigned peak). LCMS sample preparation: An aliquot of crude reaction mixture (30 µL) was mixed with HPLC grade acetonitrile (30 µL) and 10 % HCOOH.

(For more information on mass spectrometry adduct calculation-seehttp://fiehnlab.ucdavis.edu/staff/kind/Metabolomics/MS-Adduct-Calculator)



Supplementary Figure 142:

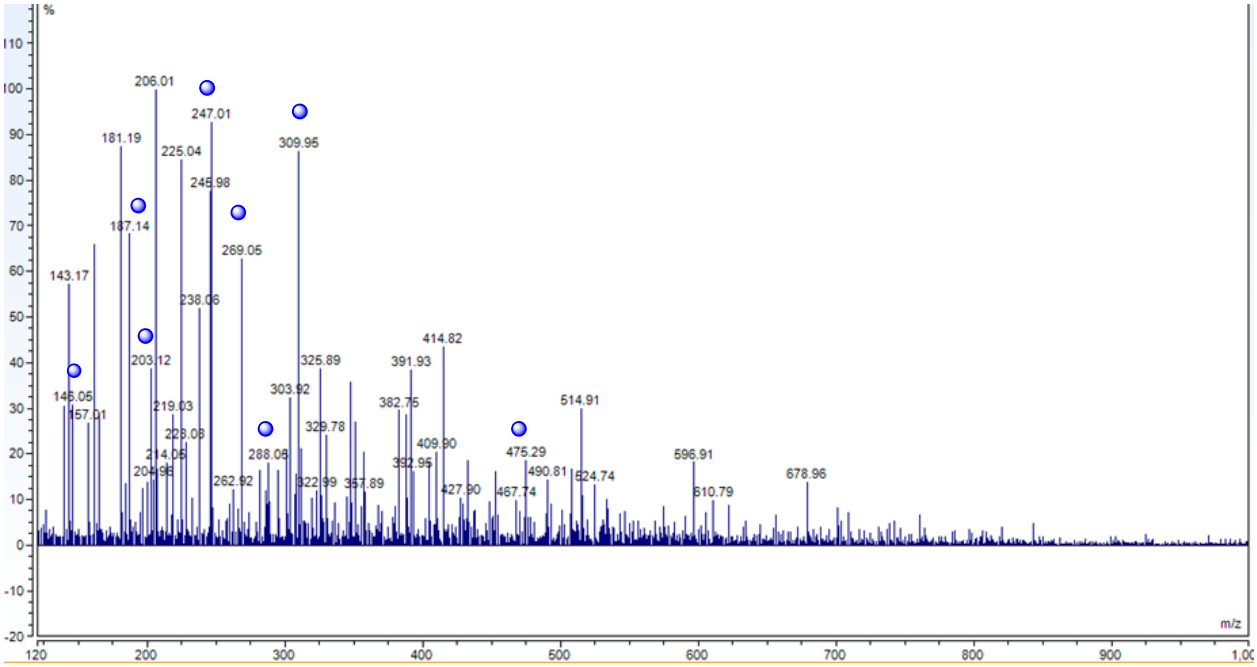
MALDI-TOF analysis of the crude reaction of **27** with DAP and imidazole (Method A). Note peak (assigned with blue dot) at 817.24, corresponding to $[C_{28}H_{44}N_{14}O_{15}H]^+$ (theoretical value: 817.31), and peak at 839.81 corresponding to its sodium adduct, the composition of $(Gly)_{14}$. The other peaks could be attributed to inorganic phosphates present in the reaction.



Entry	Species	Calculated m/z	Observed m/z
1.	Gly +H	75.0320	75.0395
2.	2Gly+H	151.0641	151.0708
3.	DKP+ Na	137.0327	137.0813
4.	Gly_2+H	133.0535	133.0606
5.	Gly_2+Na	155.0423	155.0427
6.	Gly_2+ H +ACN	174.0879	174.0156
7.	Gly_3+H+ H_2O	208.0931	208.0933

Supplementary Figure 143:

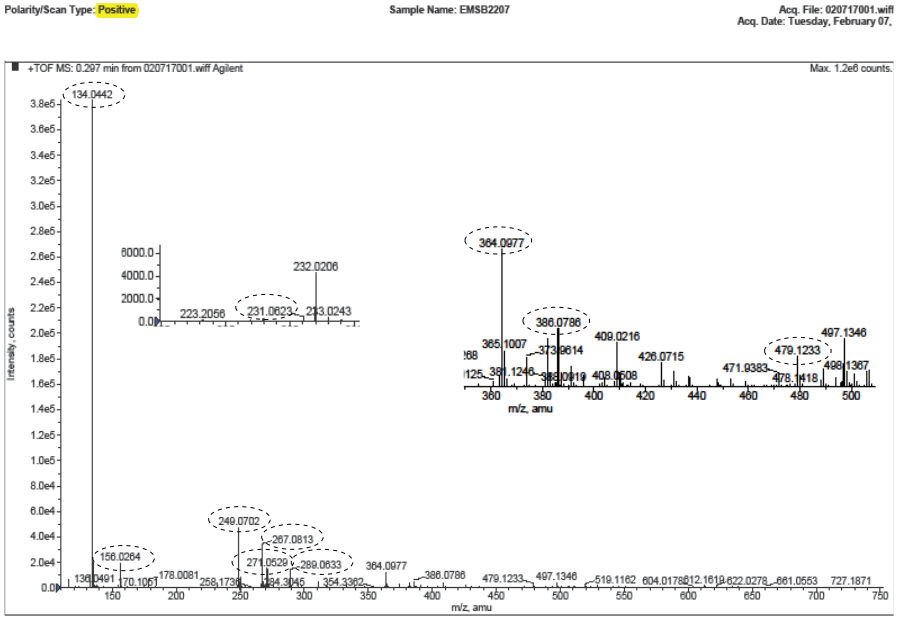
Positive mode ESI-MS of the crude reaction of **27** with DAP and imidazole following method B. Characterization of the peaks in the above mass spectrum is depicted in the table (dotted circled peaks).



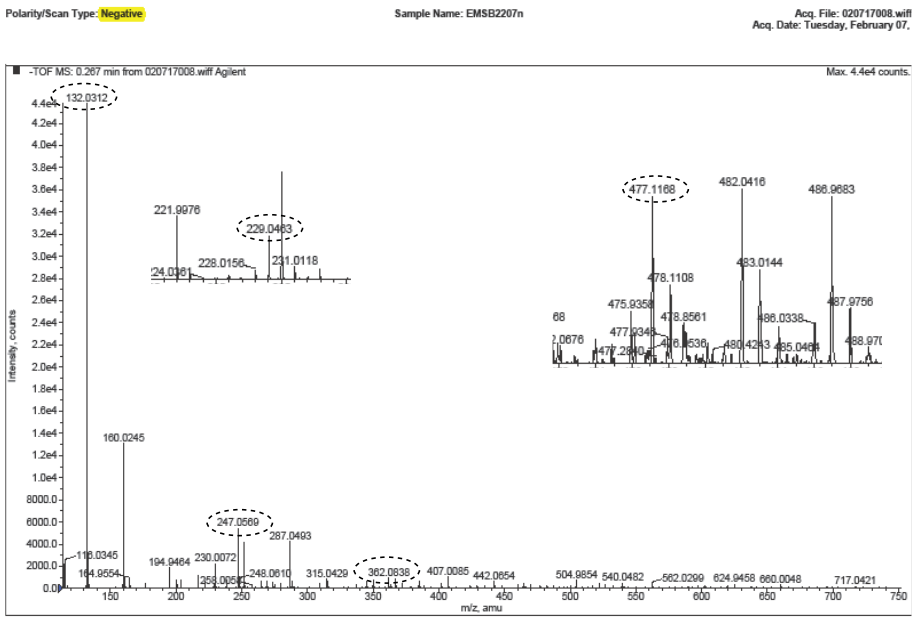
Entry	Species	Calculated m/z	Observed m/z
1.	$(Gly_4+2Na)/2$	146.04	146.09
2.	$(Gly_4+2Na + 2\ ACN)/2$	187.06	187.15
3.	Gly_4+H	247.09	247.01
4.	Gly_4+Na	269.09	269.05
5.	$Gly_4+ACN+H$	288.13	288.05
6.	$Gly_4+ ACN + Na$	310.11	309.95
7.	$(Gly_6 + 2Na)/2$	203.06	203.12
8.	$Gly_8 + H$	475.18	475.29

Supplementary Figure 144:

LCMS-spectrum (positive mode) of the crude reaction of diglycine **30** with DAP and imidazole (Method A) showing the oligomerization products after 60 days. Diglycine oligomeric peaks up to 8-mer can be identified as H^+ adducts (along with a series of Na^+ and (Na^+ + ACN) adducts). Characterization of the peaks in the above mass spectrum is depicted in the table (Color dot refers to the assigned peak). LCMS sample preparation: An aliquot of crude reaction mixture (30 µL) was mixed with HPLC grade acetonitrile (30 µL) and 10 % HCOOH.



(A)

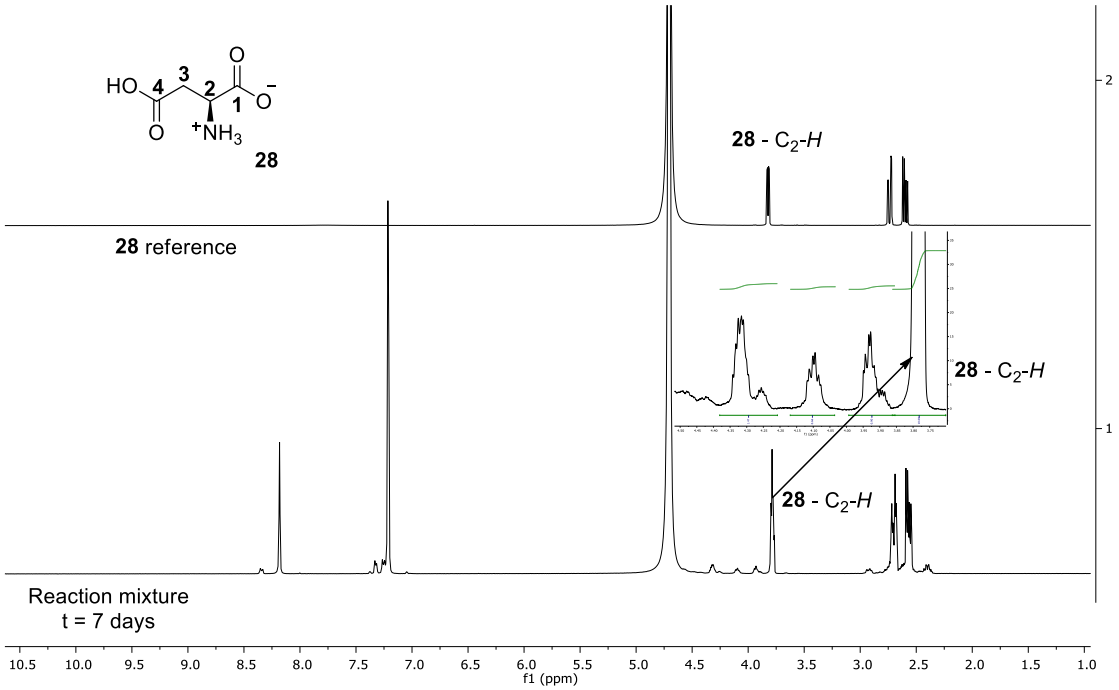


(B)

Entry	Species		Positive mode		Negative mode	
			Calculated mass	Observed mass	Calculated mass	Observed mass
1	Asp	M+H	134.0453	134.0442	132.0297	132.0312
		M+Na	156.0273	156.0264		
2	DKP	M+H	231.0617	231.0623	229.0461	229.0463
3	Asp-Asp	M+H	249.0723	249.0702	247.0566	
		M+Na	271.0542	271.0529		
4	2Asp	M+H	267.0828	267.0813	265.0672	265.0682
		M+Na	289.0648	289.0633		
5	Asp-Asp-Asp	M+H	364.0992	364.0977	362.0836	362.0838
		M+Na	386.0812	386.0786		
6	Asp-Asp-Asp-Asp	M+H	479.1262	479.1233	477.1105	477.1168
7	Asp-Asp-Asp-Asp-Asp	M+H	-	-	-	-

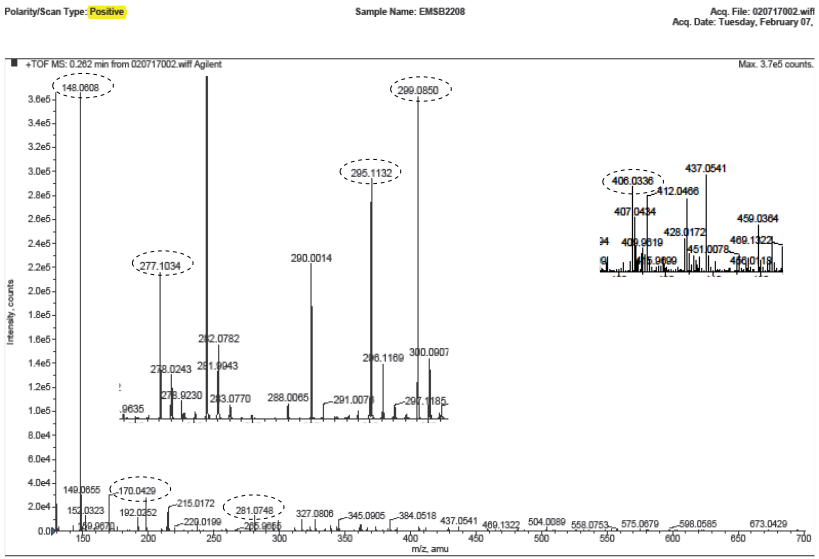
Supplementary Figure 145:

(A) Positive mode and (B) negative mode ESI-MS of crude reaction of aspartic acid 28 reaction with DAP and imidazole following method B. The above circled peaks correspond to the product peptides formed in that reaction. The peaks are listed in the following table (C).

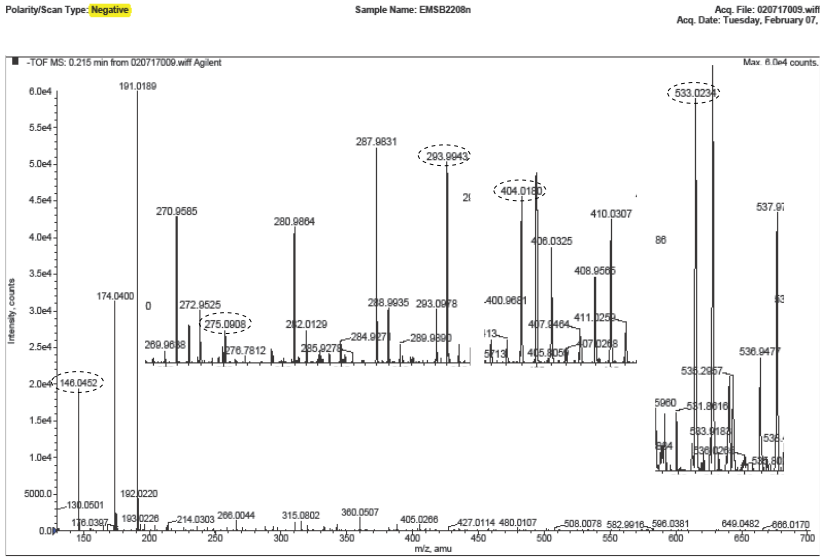


Supplementary Figure 146:

^{1}H NMR spectra of crude reaction of **28** with DAP and imidazole following method B. The zoomed area shows the formation of higher order peptides of aspartic acid. The integration of newly formed peak with the starting peak shows about 23% conversion of starting material **28** to the higher order products.



(A)

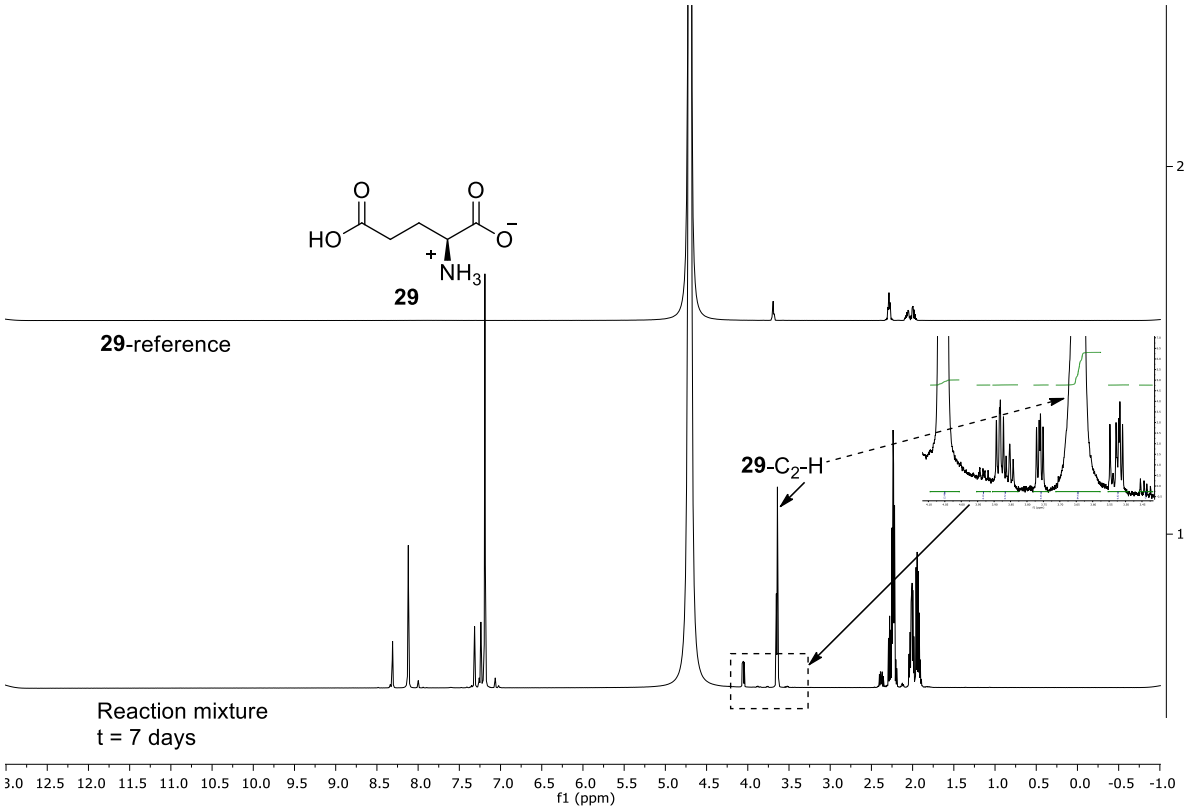


(B)

Entry	Species		Positive mode		Negative mode	
			Calculated mass	Observed mass	Calculated mass	Observed mass
1	Glu	M+H	148.0610	148.0608	146.0453	146.0452
		M+Na	170.0429	170.0429		
2	DKP	M+H	259.0930	-	257.0774	-
		M+Na	281.0750	281.0748		
3	2Glu	2M+H	295.1141	295.1132	293.0985	293.0978
		2M+Na	317.0961	317.0958		
4	Glu-Glu	M+H	277.1036	277.1034	275.0879	275.0908
		M+Na	299.0855	299.0850		
5	Glu-Glu-Glu	M+H	406.1462	406.0336	404.1305	404.0180
		M+Na	428.1281	428.0172		
6	Glu-Glu-Glu-Glu	M+H	535.1888	535.0454	533.1731	533.0234
		M+Na	557.1707	557.0245		
7	Glu-Glu-Glu-Glu-Glu	M+H	-	-	-	-

Supplementary Figure 147:

(A) Positive mode and (B) negative mode ESI-MS of crude reaction of glutamic acid **29** reaction with DAP and imidazole following method B. The above circled peaks correspond to the product peptides formed in that reaction. The peaks are listed in table (C).



Supplementary Figure 148:

^{1}H NMR spectra of the crude reaction of **29** with DAP and imidazole following method B. The zoomed area shows the formation of higher order peptides of glutamic acid. The integration of newly formed peak with the starting peak shows about 15% conversion of starting material **29** to the higher order products.

Supplementary References:

S1. Watanabe, M. & Sato, S. The synthesis and thermal behaviour of sodium phosphorodiamidate. *J. Mat. Sci*. **21**, 2623-2627 (1986).

S2. Perur, N., Yahara, M., Kamei, T. & Tamaoki, N. A non-nucleoside triphosphate for powering kinesin-microtubule motility with photo-tunable velocity. *Chem. Commun*. **49**, 9935-9937 (2013).

S3. Neises, B. & Steglich, W. Einfaches Verfahren zur veresterung von carbonsäuren. *Angew. Chem*. **90**, 556-557 (1978).

S4. Coleman, B. E. *et al*. Modular approach to the synthesis of unsaturated 1-monoacyl glycerols. *Synlett* **8**, 1339-1342 (2004).

S5. Timmer, M. S. *et al*. Discovery of lipids from B. longum subsp. infantis using whole cell MALDI analysis. *J. Org. Chem*. **79**, 7332-7341 (2014).

S6. Wu, L. Y. & Berkman, C. E. Synthesis of *N*-phosphoryl amino acids using bis(9-fluorenylmethyl) phosphite. *Tetrahedron Lett*. **46**, 5301-5303 (2005).

S7. Leman, L. J., Orgel, L. E., Ghadiri, M. R. Amino acid dependent formation of phosphate anhydrides in water mediated by carbonyl sulfide. *J. Am. Chem. Soc*. **128**, 20-21 (2006).