

Supporting Information

for

**Prebiotic Synthesis of Aminooxazoline-5'-Phosphates in
Water by Oxidative Phosphorylation**

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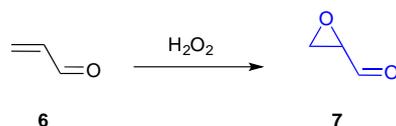
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General Experimental

Reagents and anhydrous organic solvents were obtained and used without further purification, unless specified. Sodium methanethiolate, hydrogen peroxide, potassium cyanide, glyceraldehyde-3-phosphate (**2**), methylglyoxal, potassium thioacetate, cyanamide (**19**), anhydrous copper (II) sulphate and sodium hydrogen phosphate were purchased from *Sigma Aldrich*. 2-Aminooxazole (**11**) and D-xylose was purchased from *Alfa Aesar*. Sodium dihydrogen phosphate, D-lyxose, diphenyl chlorophosphate and pyridine were purchased from *Acros Organics*. Glyceraldehyde (**5**), was purchased from *Carbosynth*. Acrolein (**6**) was purchased from *Manchester Organics*. Ammonium chloride was purchased from *Fluorochem*. Methanethiol (**23**) was generated by dropping sodium methanethiolate (21% aqueous solution) onto mono-basic sodium phosphate. Dowex® 50W×8 resin was purchased from *Acros Organics* and regenerated with HCl solution. Deionised water was obtained from an Elga Option 3 purification system. Automated flash column chromatography was carried out using a Biotage Isolera Four purification system and Biotage KP-C18-HS Snap Cartridge. Solution pH values were measured using a Mettler Toledo Seven Compact pH meter with a Mettler Toledo InLab semi-micro pH probe. The readings for D₂O solutions are reported as pD, and corrected according to Covington et al.¹ The readings for H₂O and H₂O/D₂O solutions are reported uncorrected. NMR Spectra were recorded on a Bruker Avance III (400) equipped with a gradient probe or Bruker Avance III (600) spectrometer, equipped with a cryoprobe. Chemical shifts (δ) are reported in parts per million (ppm) relative to the residual solvent peak, and ¹H chemical shifts relative to TMS were calibrated using the residual solvent peak: HOD (δ_{H} 4.75 ppm) or CDCl₃ (δ_{H} 7.26 ppm). Coupling constants (J) are reported in Hertz (Hz). Spin multiplicities are indicated by symbols: s (singlet); d (doublet); t (triplet); q (quartet); ABX (geminal (AB) spin system coupled to one other nuclei (X)); ABXY (geminal (AB) spin system coupled to two other nuclei (X, Y)); obs. (obscured/coincidental signals), or a combination of these. NMR data are reported as follows: chemical shift (multiplicity, coupling constants (J), number of protons, nuclear assignment). Spectra were recorded at 298 K. Signal assignments are made by correlation of COSY, HMBC, HSQC and DEPT experiments. ¹H NMR spectra (H₂O/D₂O) are solvent suppressed (noesygprr1d) with presaturation and spoil gradients. Stacked spectra are offset, $x=0.25$ ppm. Infrared (IR) spectra were recorded with a *Shimadzu* 100 FTIR spectrometer. Absorption maxima are reported in wavenumbers (cm⁻¹). Mass spectra were determined by the University College London mass spectrometry service by electrospray ionisation (ESI) using a Waters LCT Premier XE or Thermo Finnigan MAT 900XP instrument.

Incubation of acrolein (**6**) and hydrogen peroxide



Acrolein (**6**; 9.3 μL , 0.14 mmol) was added to a stirred solution of hydrogen peroxide (13.2 μL , 10.6M, 0.14 mmol) in $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1, 1 mL) at room temperature and pH 8.0. The solution was adjusted to pH 8.5 with 1M NaOH and ^1H NMR spectra were periodically acquired. Glycidaldehyde (**7**; 90% yield by ^1H NMR integration) was observed after 2 h.

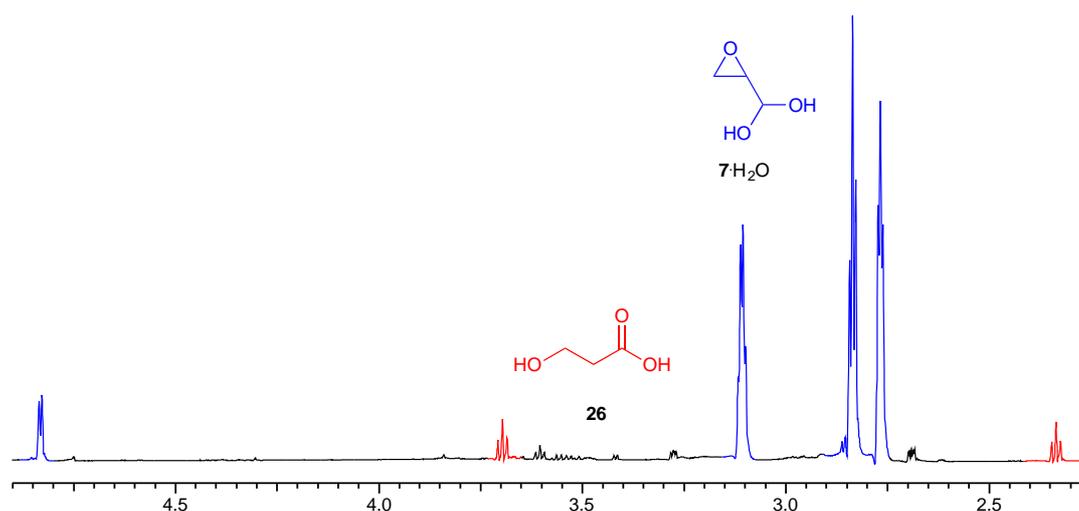


Figure S1: ^1H NMR spectra (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1); 1.0–9.0 ppm) of glycidaldehyde (**7**) furnished by incubation of acrolein (**6**; 140mM) and hydrogen peroxide (1 eq.) at pH 8.5 and room temperature after 2 h. 3-Hydroxypropanoic acid (**26**; 8%) was observed.

Preparative synthesis of glycidaldehyde (**7**)

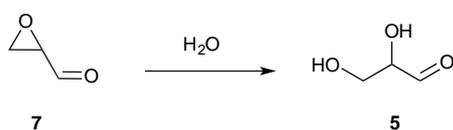


Glycidaldehyde (**7**) was prepared by adaption of Payne's synthesis² and Golding's continuous batch extraction.³ To a solution of hydrogen peroxide (13.2 mL, 10.6M, 0.14 mol) in water (95 mL) adjusted to pH 8.0, acrolein (**6**, 9.3 mL, 0.14 mol) was added dropwise over 10 min. The pH was maintained between 8.0 and 8.5 (with 1M HCl/NaOH) and the solution was stirred for 2 h. The solution was then continuously extracted with CH_2Cl_2 for 48 h. The CH_2Cl_2 fraction was subsequently dried with MgSO_4 and filtered, giving a 34% yield (by ^1H NMR analysis) of extracted glycidaldehyde (**7**). The CH_2Cl_2 fraction containing the product was distilled at 120

Torr, glycidaldehyde (**7**, 2.74 g, 27%) was collected as a colourless liquid and stored at -20 °C under an argon atmosphere.

B.p. 62–64 °C, 120 Torr (lit³. 57–58 °C, 100 Torr). ¹H NMR (600 MHz, CDCl₃) δ 8.93 (d, *J* = 6.5 Hz, 1H, H-(C1)); 3.34 (ddd, *J* = 6.5, 4.5, 2.5 Hz, 1H, H-(C2)); 3.13 (dd, *J* = 5.3, 4.5 Hz, 1H, H-(C3)); 3.03 (dd, *J* = 5.3, 2.5 Hz, 1H, H-(C3')). ¹H NMR (600 MHz, D₂O) δ 4.83 (d, *J* = 4.2 Hz, 1H, H-(C1)); 3.11 (tdd, *J* = 4.2, 3.0, 0.7 Hz, 1H, H-(C2)); 2.84 (td, *J* = 4.2, 0.7 Hz, 1H, H-(C3)); 2.77 (dd, *J* = 4.2, 3.0 Hz, 1H, H-(C3')). ¹³C NMR (150 MHz, D₂O) δ_c 89.7 (C1); 54.4 (C2); 45.3 (C3). HRMS (*m/z*): [M+H⁺]⁺ calcd for formula C₃H₅O₂⁺, 73.02895; found, 73.028791.

Hydrolysis of glycidaldehyde (**7**)



Glycidaldehyde (**7**, 400mM) and potassium hydrogen phthalate (KHP; 26.2mM, internal standard) was dissolved in H₂O/D₂O (9:1). The solution was adjusted to specified pH (pH 3.0–9.0). The solution pH was then continuously monitored, adjusting as required with 1M NaOH/HCl, whilst ¹H NMR spectra were periodically acquired. Glyceraldehyde (**5**) synthesis was confirmed by sample spiking with a commercial standard.

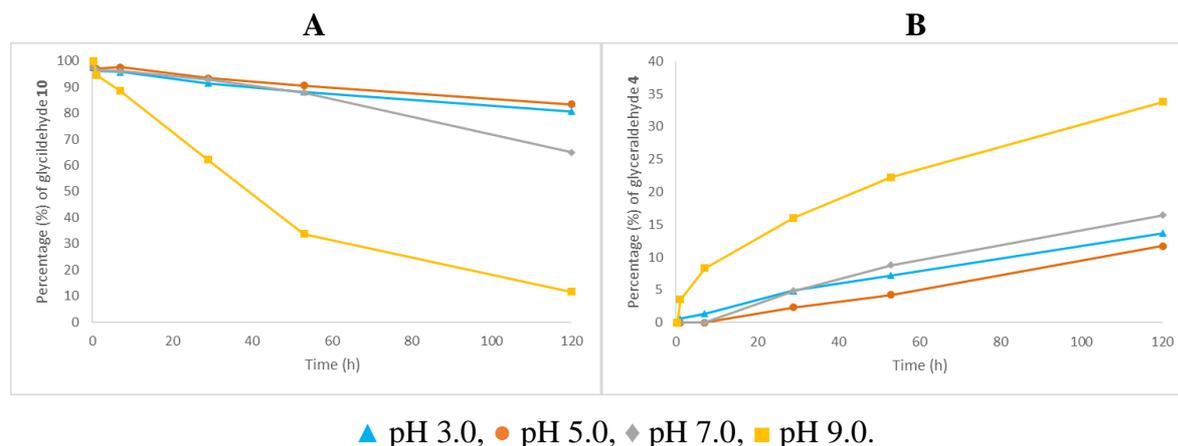
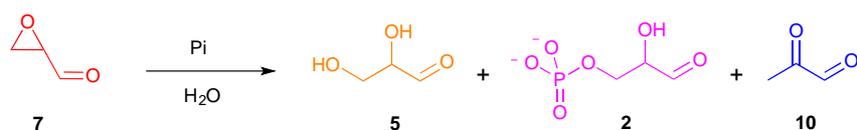


Figure S2: Graph to show: **A**) the degradation of glycidaldehyde (**7**, 400mM); **B**) the formation of glyceraldehyde (**5**) during the incubation of glycidaldehyde (**7**, 400mM) in water at specified pH. The yield (%) glyceraldehyde (**5**) and residual (%) glycidaldehyde (**7**) are measured by comparative ¹H NMR integration with internal standard.

Phosphorylation of glyceraldehyde (7)



Glyceraldehyde (7, 250mM) was dissolved in phosphate solution (0.25–0.5M, 1 mL, pH 5.0–8.0) and incubated at room temperature. ¹H and ³¹P NMR spectra were periodically acquired and production of glyceraldehyde-3-phosphate (2) monitored. Glyceraldehyde-3-phosphate (2), glyceraldehyde (5) and methylglyoxal (10) synthesis was confirmed by sample spiking with a commercial standard.

Glyceraldehyde 3-phosphate (2): ¹H NMR (600 MHz, H₂O/D₂O (9:1)) δ 4.86 (d, *J* = 5.8 Hz, 1H, H-(C1)); 3.77 (ddd, *J* = 11.3, 6.5, 3.3 Hz, 1H, H-(C3)); 3.68 (dt, *J* = 11.3, 6.5 Hz, 1H, H-(C3')); 3.52 (td, *J* = 5.8, 3.3 Hz, 1H, H-(C2)). ³¹P (121 MHz, H₂O/D₂O (9:1)) δ 6.41 (t, *J* = 6.5 Hz).

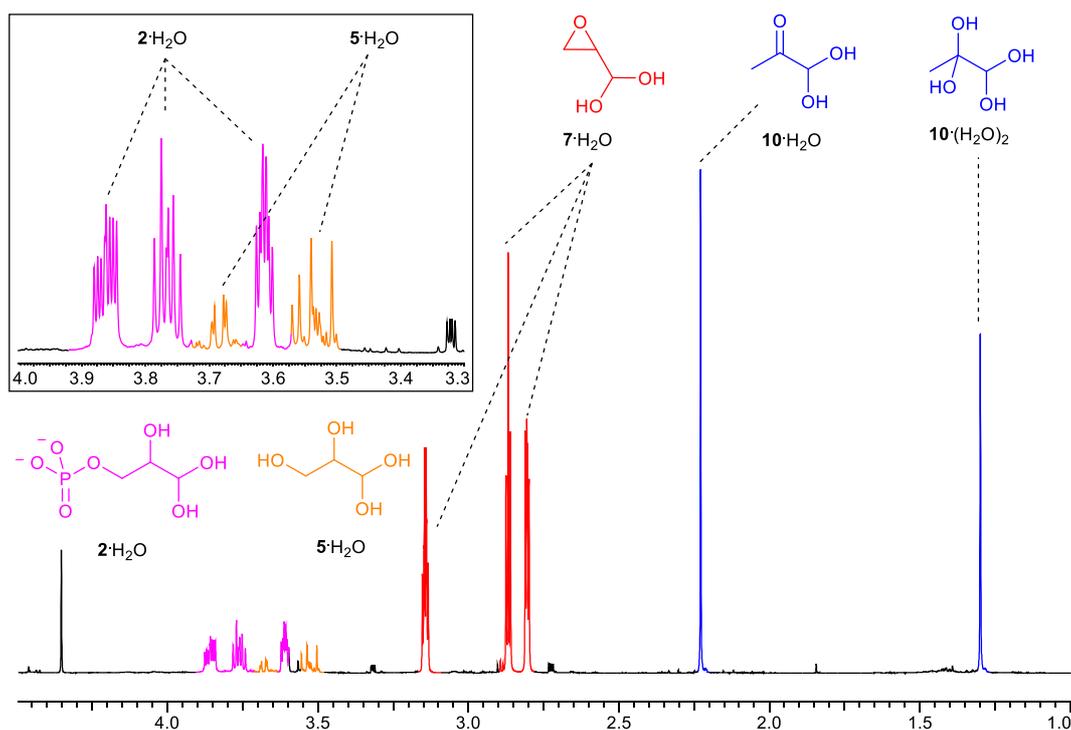
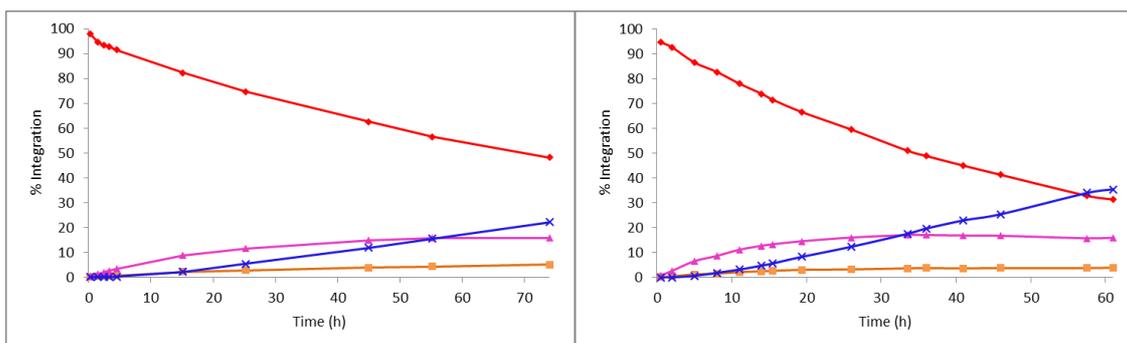


Figure S3: ¹H NMR spectra (600 MHz, H₂O/D₂O (9:1), 1.0–4.5 ppm) of the reaction of glyceraldehyde (7; 250mM) in phosphate solution (1M; pH 7.0) after incubation at room temperature for 36 h. Two methyl resonances corresponding to methylglyoxal mono- and dihydrate (10·H₂O/10·(H₂O)₂) are observed. Inset: ¹H NMR spectra (600 MHz, H₂O/D₂O (9:1), 3.3–4.0 ppm) showing the peaks corresponding to 2·H₂O and 5·H₂O.

A

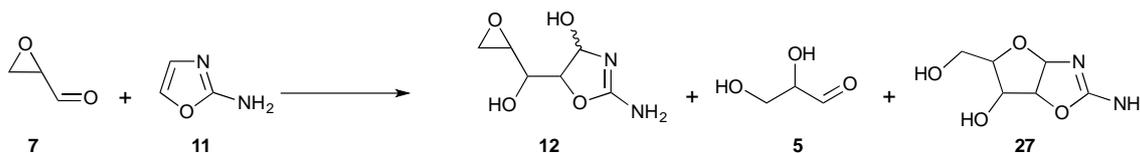
B



▲ Glyceraldehyde 3-phosphate **2**, ◆ Glycidaldehyde **7**, ■ Glyceraldehyde **5**,
 × Methylglyoxal **10**.

Figure S4: Graph to show the yield (%) of glyceraldehyde 3-phosphate (**2**) observed from the reaction of glycidaldehyde (**7**; 250mM) at pH 7.0 in phosphate solution: **A**) 2 eq. of phosphate; **B**) 4 eq. of phosphate. Amount of each species was determined by integration of ^1H NMR spectra.

*Reaction of glycidaldehyde (**7**) and 2-aminooxazole (**11**)*

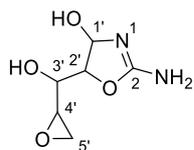


Glycidaldehyde (**7**; 128mM) and 2-aminooxazole (**11**; 1.1 eq.) were dissolved in $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1). The solution was adjusted to the desired pH (3.0–12.0), the pH was then continuously monitored and adjusted as required with 1M NaOH/HCl. ^1H NMR spectra were periodically acquired. Aminooxazolines **27** and epoxide **12a-d** were identified by their characteristic H-(C1') peaks.

pH	Time (h)	Product yield (%)			
		12	27	7	5
3	30	17	0	52	29
5	59	72	0	18	10
7	59	66	22	0	0
9	34	53	26	0	0
12	6	6	50	0	0

Table S1: Product yields (%) observed in the reaction of glycidaldehyde (**7**, 128mM) and 2-aminooxazole (**11**; 1.1 eq.) at specified pH and time. Amount of each species was determined by ^1H NMR integration, and yields (%) are reported at the time after which no further significant change was observed.

Preparative synthesis of epoxide **12**



2-Aminooxazole (**11**; 87 mg, 1.03 mmol), glycidaldehyde (**7**; 62 mg, 0.86 mmol) and KHP (17mM, internal standard) were dissolved in H₂O/D₂O (9:1, 10 mL). The solution was adjusted to pH 5.0 with 1M HCl, monitored and re-adjusted periodically as required (pH fluctuations ± 0.08 were observed). ¹H NMR spectra were periodically acquired. After 18 h at room temperature a maximum yield of epoxide (**12**, 78%) was observed (the yield (%) was calculated by integrating the H-C1' proton resonances relative to internal potassium hydrogen phthalate standard).

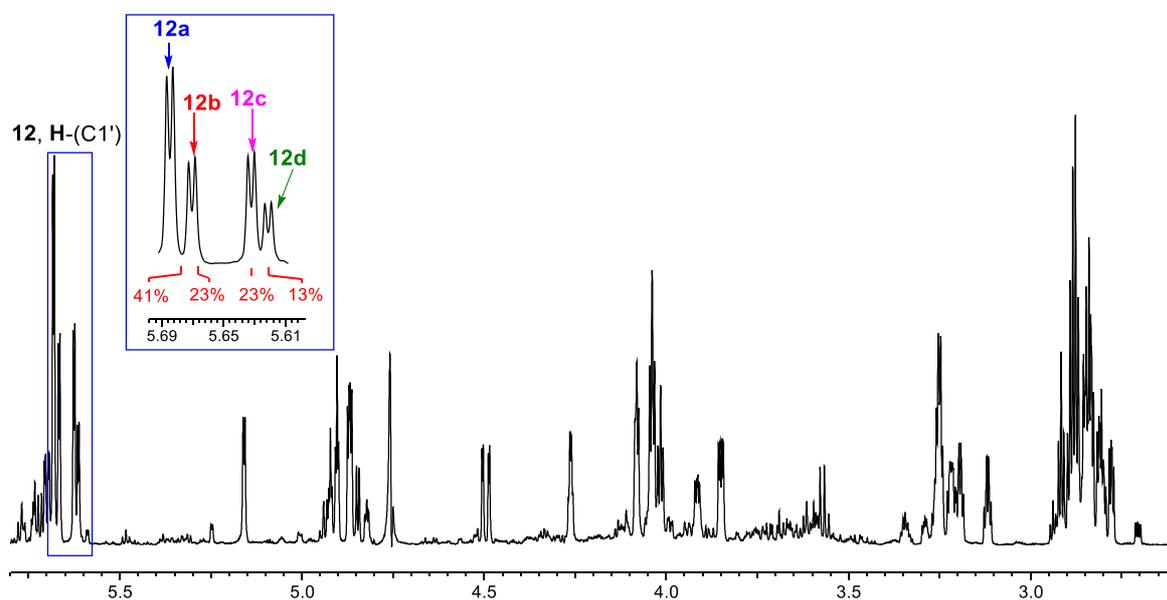


Figure S5: ¹H NMR spectra (600 MHz, H₂O/D₂O (9:1), 2.6–5.8 ppm) to show the reaction of glycidaldehyde (**7**; 86mM) and 2-aminooxazole (**11**; 103mM) at pH 5.0 and room temperature after 18 h. Inset: ¹H NMR spectra (600 MHz, H₂O/D₂O (9:1), 5.59–5.70 ppm) showing the integration for the H-(C1') of epoxides **12**.

Epoxide **12a**

¹H NMR (600 MHz, H₂O/D₂O (9:1), partial data) δ 5.69 (d, $J = 2.6$ Hz, 1H, H-(C1')); 4.88 (dd, $J = 4.4, 2.6$ Hz, 1H, H-(C2')); 4.05 (dd, $J = 4.4, 3.4$ Hz, 1H, H-(C3')); 3.26 (dd, $J = 4.2, 3.4,$

3.1 Hz, 1H, H-(C4')); 2.95–2.75 (obs., 2H, H-(C5')). ¹³C NMR (151 MHz, H₂O/D₂O (9:1)) δ_C 163.1 (C2); 91.0 (C2'); 80.5 (C1'); 68.0 (C3'); 52.3 (C4').¹

Epoxide **12b**

¹H NMR (600 MHz, H₂O/D₂O (9:1), partial data) δ 5.67 ppm (d, *J* = 2.8 Hz, 1 H, H-(C1')); 4.83 (dd, *J* = 3.9, 2.8 Hz, 1 H, H-(C2')); 4.02 (dd, *J* = 4.3, 3.9 Hz, 1 H, H-(C3')); 3.20 (td, *J* = 4.3, 4.3, 2.9 Hz, 1 H, H-(C4')); 2.95–2.75 (obs., 2 H, H-(C5')). ¹³C NMR (151 MHz, H₂O/D₂O (9:1)) δ_C 163.1 ppm (C2); 90.8 (C2'); 80.6 (C1'); 68.8 (C3'); 51.6 (C4').

Epoxide **12c**

¹H NMR (600 MHz, H₂O/D₂O (9:1), partial data) δ 5.63 (d, *J* = 2.7 Hz, 1 H, H-(C1')); 4.91 (t, *J* = 2.7 Hz, 1 H, H-(C2')); 3.86 (dd, *J* = 5.5, 2.7 Hz, 1 H, H-(C3')); 3.22 (m, 1 H, H-(C4')); 2.95–2.75 (obs., 2 H, H-(C5')). ¹³C NMR (151 MHz, H₂O/D₂O (9:1)) δ_C 163.4 ppm (C2); 90.7 (C2'); 81.6 (C1'); 69.8 (C3'); 51.8 (C4').

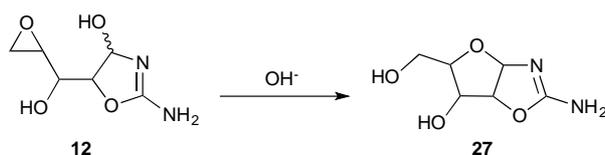
Epoxide **12d**

¹H NMR (600 MHz, H₂O/D₂O (9:1), partial data) δ 5.62 (d, *J* = 2.7 Hz, 1 H, H-(C1')); 4.93 (t, *J* = 2.7 Hz, 1 H, H-(C2')); 3.92 (dd, *J* = 4.6, 2.7 Hz, 1 H, H-(C3')); 3.28–3.26 (obs., 1 H, H-(C4')); 2.95–2.75 (obs., 2 H, H-(C5')). ¹³C NMR (151 MHz, H₂O/D₂O (9:1)) δ_C 163.4 (C2); 91.3 (C1'); 81.8 (C2'); 69.8 (C3'); 53.0 (C4').

Glyceraldehyde (**5**): 3.61 (m, 1H, H-(C3)).

Glycidaldehyde (**7**): 3.12 (m, 1H, H-(C2)).

Hydrolysis of epoxide **12** at high pH



Glycidaldehyde (**7**; 128mM), 2-aminooxazole (**11**; 1.3 eq.) and KHP (24.2mM, internal standard) were dissolved in H₂O/D₂O (9:1). The solution was adjusted to pH 5.0, the pH was then continuously monitored and adjusted as required with 1M HCl. ¹H NMR spectra were periodically acquired, and conversion to epoxide **12** (68%) was observed after 13 h at room temperature. Epoxide **12** was then adjusted specified pH (7.0–12.0) and incubated at room

¹ Shifts are pH dependent, report at pH 5.0. All H-(C5') overlap at 2.95-2.75 ppm; ¹³C was assigned by ¹³C NMR and HSQC; however, it was not possible to assign peaks for C5' for every stereoisomer as all the peaks appear in the same spectral region 46.4-44.5 ppm.

temperature. ^1H NMR spectra were again periodically acquired to monitor the conversion of epoxide **12** to aminooxazoline **27** (50 % yield of aminooxazoline **27** after 6 h at pH 12.0).

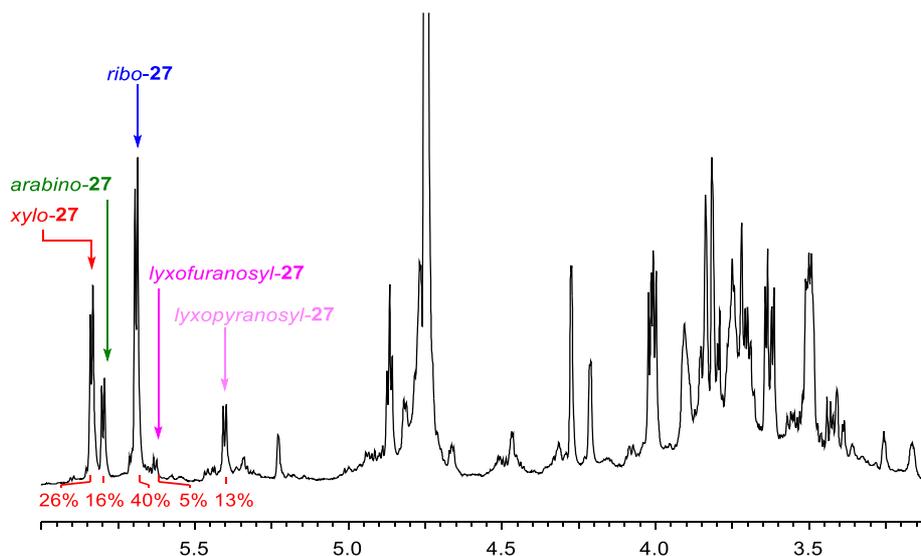
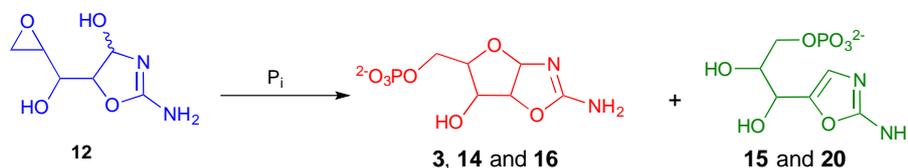


Figure S6: ^1H NMR spectra (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1), 3.2–6.0 ppm) to show the hydrolysis of epoxide **12** (87mM) at pH 12.0 and room temperature after 22 h. The yield of each aminooxazoline **27** was calculated by integration of the anomeric peaks H-(C1').

*Phosphorylation in water of epoxide **12** by addition of phosphate*



Glycidaldehyde (**7**; 86mM), 2-aminooxazole (**11**; 1.2 eq.) and KHP (17mM, internal standard) were dissolved in $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1). The solution was adjusted to pH 5.0, continuously monitored and adjusted as required with 1M HCl. ^1H NMR spectra were periodically acquired, showing conversion to epoxide **12** after 18 h at room temperature (78% yield based on internal standard). A mixture of sodium dihydrogen phosphate and sodium hydrogen phosphate was added to the reaction in one portion (to give 0.14–1.12M phosphate, at the specified pH). The solution pH was monitored, no adjustments were required to maintain the desired pH (pH 5.0–9.0). ^1H and ^{31}P NMR spectra were periodically acquired. When epoxide **12** was fully consumed by the reaction a mixture of aminooxazoline **3** (14 %), **14** (5 %) and **16** (17 %) and oxazole **15** (7 %) and **20** (7 %) were observed. The solution was adjusted to pH 10.0, and purified by ion exchange chromatography (Dowex® 1×8, 1 mL, hydroxide-form, prewashed) elution as follow: water (3×5 mL), 0.5M aqueous HCO_2H (5 mL), and 1M aqueous HCO_2H (3×5 mL). The fractions containing aminooxazolines **3**, **14** and **16** were combined, diluted two-fold and

lyophilised. The lyophilite was then dissolved in D₂O (0.5 mL) and ¹H and ³¹P NMR spectra were acquired. Aminooxazolines **3**, **14** and **16** and oxazole **15** and **20** were identified by their characteristic H-(C1') peaks. Aminooxazolines **3**, **14** and **16** were confirmed by spiking with authentic samples.

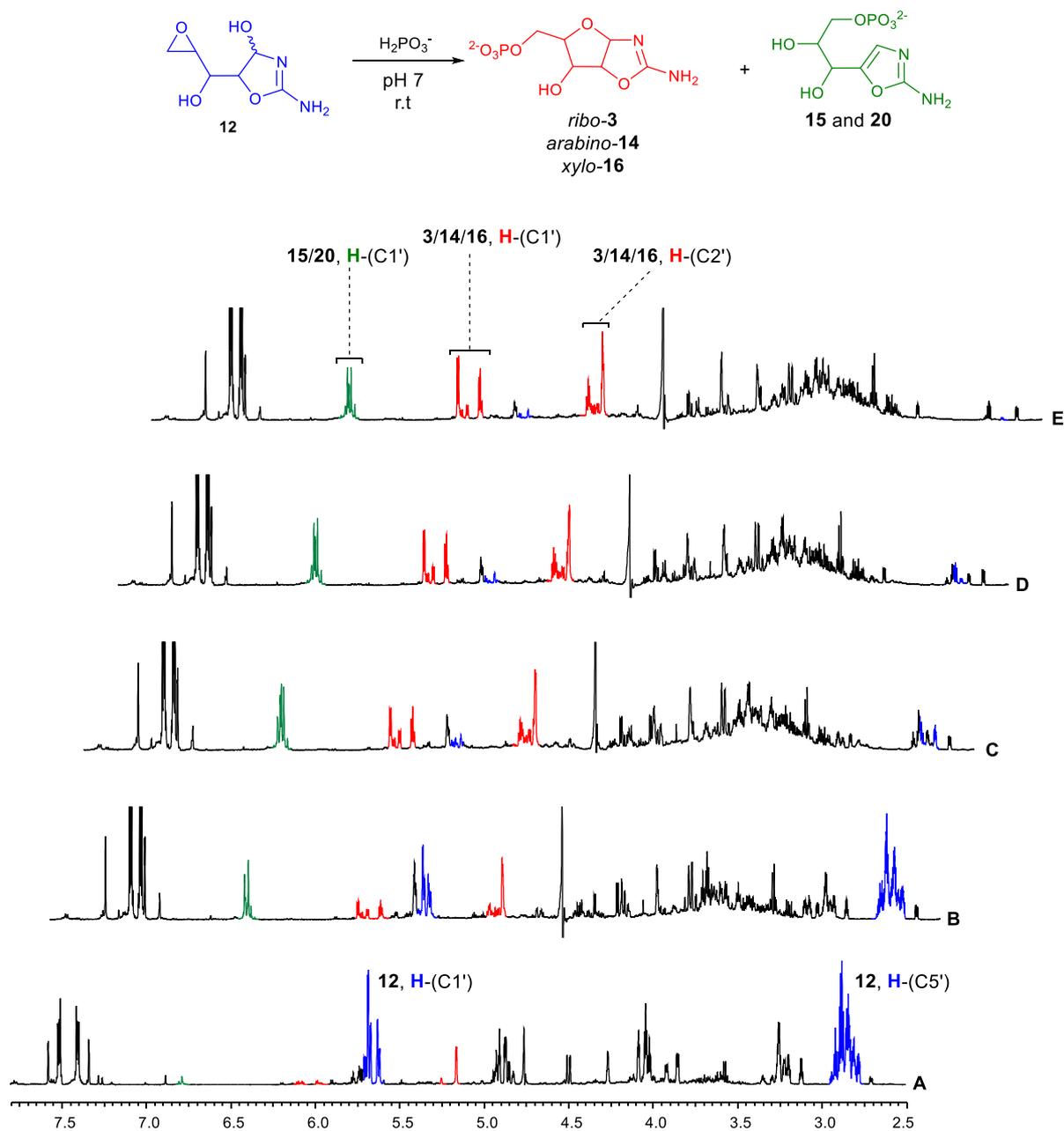


Figure S7: ¹H NMR spectra (600 MHz, H₂O/D₂O (9:1), 2.5–7.8 ppm) for the phosphorylation of epoxide **12** (67mM) with inorganic phosphate (13 eq.) at pH 7.0 after: **A**) 30 minutes; **B**) 1 d; **C**) 2 d; **D**) 3 d; **E**) 5 d.

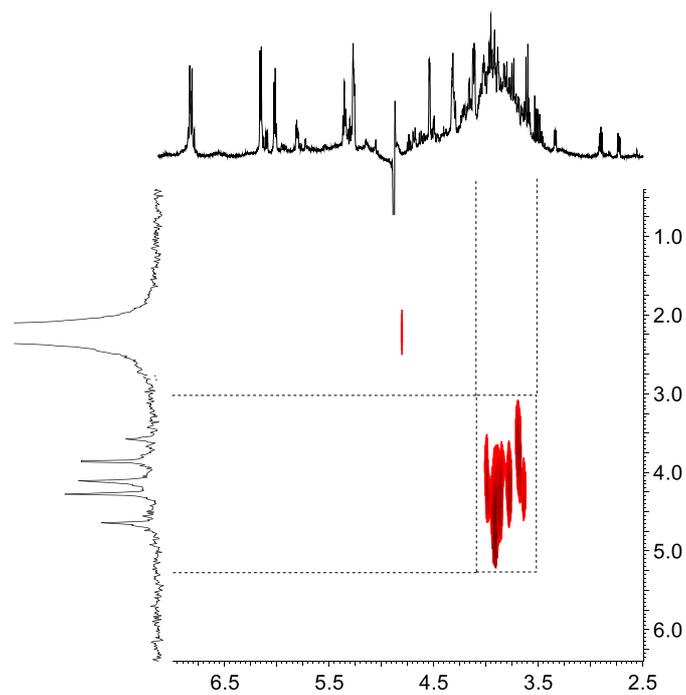


Figure S8: ^1H - ^{31}P HMBC NMR (400 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1)) to show the phosphorylation reaction of epoxide **12** (67mM) with inorganic phosphate (13 eq., pH 7.0) after 5 d the characteristic H-(C5')-phosphorus coupling are highlighted in box.

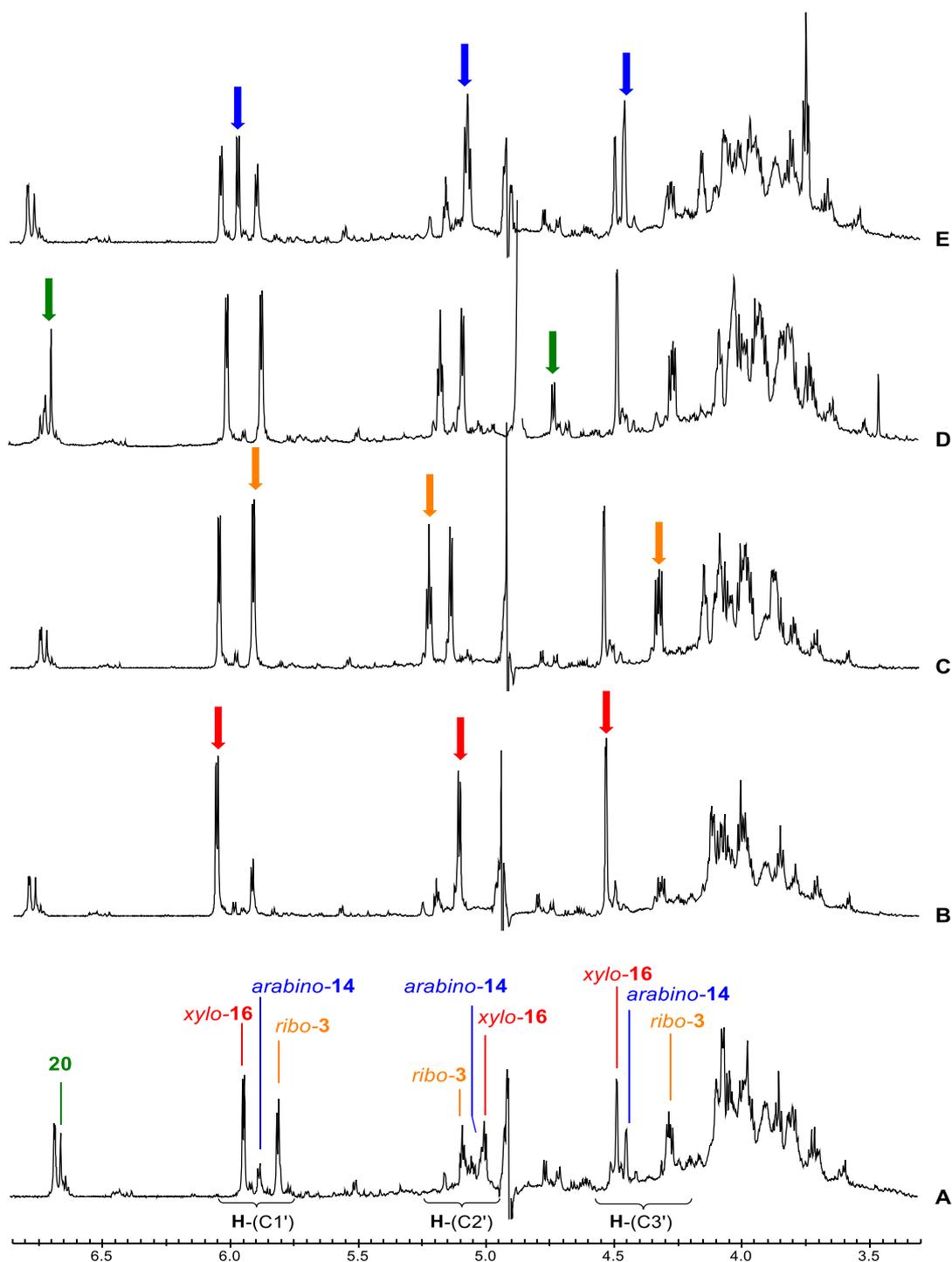
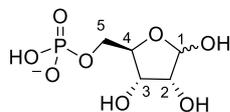


Figure S9: ^1H NMR spectra (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1), 3.30–6.85 ppm) to show: **A**) the phosphorylation of epoxide **12** (90mM) with inorganic phosphate (1.1M, pH 7.0, 12 eq.) for 11 d then the solution was adjusted to pH 9.0 to eliminate hydrates **28**. The reaction mixture observed in spectrum **A** was then spiked sequentially with: **B**) xylose aminooxazoline-5'-phosphate (**16**); **C**) ribose aminooxazoline-5'-phosphate (**3**); **D**) oxazole **20**. **E**) A second aliquot of the reaction mixture observed in spectrum **A** was spiked with an authentic standard of arabinose aminooxazoline-5'-phosphate (**14**).

Preparative synthesis of aminooxazolines-5'-phosphate **3**, **14** and **16**

D-Ribose 5-phosphate (**1**)



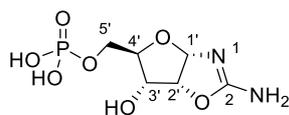
β -D-Adenosine-5'-phosphate **29** (1.50 g, 4.33 mmol) was added to a suspension of Dowex[®] 50W \times 8 resin (10 g, H⁺-form, prewashed) in water (20 mL). The suspension was refluxed for 30 min, then allowed to cool to room temperature. The resin was then removed by filtration and washed with water (2 \times 50 mL). The combined filtrates were adjusted to pH 7.5 at 0 °C with 4M NaOH and lyophilised to afford ribose 5-phosphate **1** (0.88 g, 74 %) as a white solid. β -anomer: ¹H NMR (600 MHz, D₂O) δ 5.31 ppm (d, J = 2.0 Hz, 1H, H-(C1)); 4.37 (dd, J = 5.2, 4.9 Hz, 1H, H-(C3)); 4.16 (ddd, J = 5.2, 4.6, 4.4 Hz, 1H, H-(C4)); 4.12 (dd, J = 4.9, 2.0 Hz, 1H, H-(C2)); 4.06 (ABXY, J = 11.2, 4.6, 4.4 Hz, 1H, H-(C5)); 3.93–4.01 (m, 1H, H-(C5')). ¹³C NMR (151 MHz, D₂O) δ 101.8 (C1); 82.4 (C4); 76.0 (C2); 71.3 (C3); 65.8 (C5).

α -anomer: ¹H NMR (600 MHz, D₂O) δ 5.36 (d, J = 3.2 Hz, 1H, H-(C1)) 4.17–4.23 (obs., 1 H, H-(C4)) 4.11–4.17 (obs., 2H, H-(C2), H-(C3)) 3.80–3.91 (obs., 2H, H-(C5)). ¹³C NMR (151 MHz, D₂O) δ 97.0 (C1); 83.0 (d, C4); 71.4, 70.9 (C2, C3); 65.0 (d, C5).

HRMS (ESI) (m/z): [M-H]⁻ calcd for formula C₅H₁₀O₈P⁻, 229.0119; found, 229.0112.

*The spectroscopic properties of this compound were consistent with the data reported in the literature.*⁴

D-Ribose aminooxazoline-5'-phosphate (**3**)

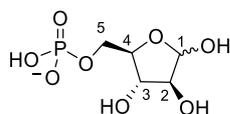


Cyanamide **19** (1.25 g, 29.7 mmol) was added to a solution of ribose 5-phosphate **1** (3.58 g, 13.1 mmol) in aqueous NH₄OH (3.5%, 40 mL), stirred at 60 °C for 3 h and then lyophilised. The product was purified by ion exchange chromatography with Dowex[®] 50W \times 8 resin (40 g, H⁺-form, prewashed, eluting with water (100 mL), HCl 0.125M (100 mL), 0.25M (100 mL), 0.5M (100 mL), 1M (100 mL) and 2M (100 mL). Aminooxazoline **3** was found in fractions 0.125–0.25M HCl, and confirmed by ¹H NMR; these fractions were lyophilised. Lyophilised fractions afforded D-ribose aminooxazoline-5'-phosphate (**3**, 2.33 g, 70% isolated yield) as a yellowish solid.

^1H NMR (600 MHz, D_2O) δ 5.97 (d, $J = 5.2$ Hz, 1H, H-(C1')); 5.37 (t, $J = 5.2$ Hz, 1H, H-(C2')); 4.28 (dd, $J = 9.3, 5.2$ Hz, 1H, H-(C3')); 4.14 (ABXY, $J = 12.0, 5.6, 2.0$ Hz, 1H, H-(C5')); 3.98 (ABXY, $J = 12.0, 7.0, 4.5$ Hz, 1H, H-(C5'')); 3.91 (ddd, $J = 9.3, 7.0, 2.0$ Hz, 1H, H-(C4')). ^{13}C NMR (151 MHz, D_2O) δ 164.0 (C2); 88.5 (C1'); 86.0 (C2'); 78.3 (d, C4'); 70.0 (C3'); 63.7 (d, C5'). ^{31}P NMR (162 MHz, D_2O , ^1H -decoupled) δ 0.04.

*The spectroscopic properties of this compound were consistent with the data reported in the literature.*⁵

D-Arabinose-5-phosphate (**30**)



Adenine 9- β -D-arabinofuranoside-5'-phosphate **31** (0.52 g, 1.5 mmol) was added to a suspension of Dowex® 50W \times 8 resin (5 g, H^+ -form, prewashed) in water (10 mL). The solution was refluxed for 30 min, then cool to room temperature and the resin removed by filtration and washed with water (2×50 mL). The combined filtrates were adjusted to pH 7.5 with 4M NaOH, concentrated *in vacuo* (to 10 mL) and then lyophilised to afford compound arabinose-5-phosphate **30** (378 mg, quant. yield) as a white solid.

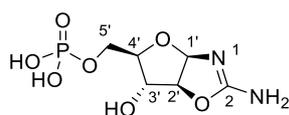
β anomer: ^1H NMR (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1)) δ 5.19 (d, $J = 2.8$ Hz, 1H, H-(C1)); 4.12 (dd, $J = 5.4, 3.7$ Hz, 1H, H-(C4)); 3.98–4.02 (obs., 1H, H-(C3)); 3.93–3.97 (obs., 1H, H-(C2)); 3.84–3.93 (obs., 2H, H-(C5)). ^{13}C NMR (151 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1)) δ_{C} 101.8 (C1); 82.5 (C4); 82.0 (C2); 76.2 (C3); 66.0 (C5). ^{31}P NMR (162 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1), ^1H -decoupled): δ 1.8. HRMS (ESI⁺) (m/z): $[\text{M}+\text{Na}^+]^+$ calcd for formula $\text{C}_5\text{H}_{11}\text{NaO}_8\text{P}^+$, 253.0084; found, 253.0087.

α -anomer: ^1H NMR (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1)) δ 5.21 (d, $J = 4.5$ Hz, 1H, H-(C1)); 4.04–4.08 (obs., 1H, H-(C3)); 4.01–4.04 (obs., 1 H, H-(C2)); 3.84–3.93 (obs., 2 H, H-(C5)); 3.81–3.84 (obs., 1H, H-(C4)). ^{13}C NMR (151 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ 9:1) δ 95.9 (C1); 80.8 (C4); 76.8 (C2); 74.8 (C3); 64.8 (C5).

Open chain (partial data): ^1H NMR (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1)) δ 4.97 (d, $J = 7.0$ Hz, 1H, H-(C1)).

*The spectroscopic properties of this compound were consistent with the data reported in the literature.*⁴

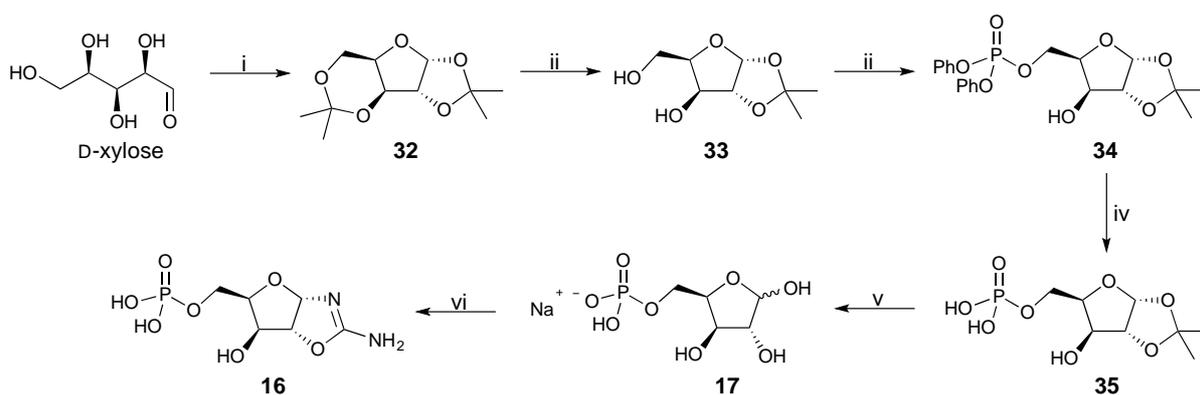
D-Arabinose aminooxazoline-5'-phosphate (**14**)



Cyanamide (**19**; 378 mg, 9 mmol) was added to a solution of arabinose-5-phosphate **30** (1.13 g, 4.5 mmol) in water (5 mL). The resulting solution was stirred at 60 °C for 4 h then lyophilised. The product was purified by ion exchange chromatography with Dowex® 50W×8 resin (40 g, H⁺-form, prewashed, eluting with water (100 mL), HCl 0.125M (100 mL), 0.25M (100 mL), 0.5M (100 mL), 1M (100 mL) and 2M (100 mL). *D*-Arabinose aminooxazoline-5'-phosphate (**14**) was found in fractions 0.25M–0.5M HCl, and confirmed by ¹H NMR. The fractions containing *D*-arabinose aminooxazoline-5'-phosphate (**14**) were combined and lyophilised to yield **14** (827 mg, 72% isolated yield) a yellowish solid.

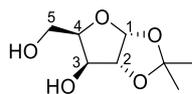
¹H NMR (600 MHz, D₂O) δ 6.11 (d, *J* = 5.7 Hz, 1H, H-(C1')); 5.37 (d, *J* = 5.7 Hz, 1H, H-(C2')); 4.61 (d, *J* = 2.3 Hz, 1H, H-(C3')); 4.31 (dt, *J* = 4.1, 2.3 Hz, 1H, H-(C4')); 3.82–3.86 (m, 2H, H-(C5')). ¹³C NMR (151 MHz, D₂O) δ 163.2 (C2); 92.8 (C2'); 90.3 (C1'); 86.8 (C4'); 75.0 (C3'); 65.4 (C5'). ³¹P NMR (162 MHz, D₂O, ¹H-decoupled): δ 0.2. HRMS (ESI⁺) (*m/z*): [M+H⁺]⁺ calcd for formula C₆H₁₂N₂O₇P⁺, 255.0377; found, 255.0375.

Synthesis of *D*-xylofuranosyl aminooxazoline-5'-phosphate (**16**)



Scheme 1: Reagents and conditions: (i) CuSO₄, H₂SO₄, (CH₃)₂CO, 4 days, r.t.; (ii) 0.1M HCl_{aq}, 3 h, r.t., 44% over two steps; (iii) (PhO)₂POCl, CH₂Cl₂, pyridine, 7 h, 0 °C, 90%; (iv) H₂, PtO₂, MeOH, 16 h, r.t., 97%; (v) H₂O, 16 h, 50 °C, quant.; (vi) H₂NCN, NH₄OH, H₂O, 64%.

1,2-*O*-Isopropylidene- α -D-xylofuranose (**33**)

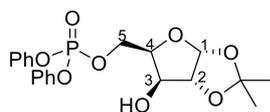


D-Xylose (4.98 g, 33.20 mmol) was suspended in acetone (40 mL). Anhydrous copper (II) sulphate (5.01 g, 31.94 mmol) and concentrated sulphuric acid (0.4 mL) were added. The reaction mixture was stirred for 4 d at room temperature then filtered. The filtrate was neutralised with concentrated NH_4OH solution and the resultant precipitate separated by filtration. The filtrate was concentrated *in vacuo* to give diisopropylidene **32** (3.73 g) as a yellow oil. Crude diisopropylidene **32** was dissolved in 0.1M HCl (30 mL) and incubated for 3 h at room temperature. Solid NaHCO_3 was added until the solution was neutralised, and the solution was extracted with Et_2O (40 mL). The aqueous solution was then concentrated *in vacuo* to yield a white solid. This solid was triturated with CH_2Cl_2 (40 mL), and the CH_2Cl_2 filtrate was then dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column (Hexane/ EtOAc 9:1–0:1) to afford 1,2-*O*-isopropylidene- α -D-xylofuranose **33** (2.775 g, 44% over two steps) as a colourless oil.

^1H NMR (600 MHz, CDCl_3) δ 6.00 (d, $J = 3.7$ Hz, 1H, H-(C1)); 4.53 (d, $J = 3.7$ Hz, 1H, H-(C2)); 4.34 (t, $J = 3.0$ Hz, 1H, H-(C3)); 4.12–4.22 (m, 2H, H-(C4), H-(C5)); 4.06 (ABXY, $J = 12.0, 7.6, 2.0$ Hz, 1H, H-(C5')); 3.99 (d, $J = 3.0$ Hz, 1H, C3-OH); 2.64 (dd, $J = 7.6, 4.6$ Hz, 1H, C5-OH); 1.49 (s, 3H, $\text{C}(\text{CH}_3)_3$); 1.33 (s, 3H, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (151 MHz, CDCl_3) δ 111.8 ($\underline{\text{C}}(\text{CH}_3)_3$); 104.9 (C1); 85.7 (C2); 78.5 (C4); 77.1 (C3); 61.3 (C5); 26.8 ($\text{C}(\underline{\text{C}}\text{H}_3)_3$); 26.1 ($\text{C}(\underline{\text{C}}\text{H}_3)_3$).

*The spectroscopic properties of this compound were consistent with the data reported in the literature.*⁶

1,2-*O*-Isopropylidene- α -D-5-diphenylphosphoxyxylofuranose (**34**)



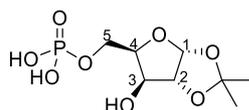
1,2-*O*-Isopropylidene- α -D-xylofuranose **33** (794 mg, 4.18 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 (200 mL) and anhydrous pyridine (30 mL). The solution was cooled to 0 °C and diphenyl chlorophosphate (1.1 mL, 5.31 mmol) was added, and the solution was stirred at 0 °C for 7 h. The reaction was then quenched by addition of water (30 mL) and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane/ EtOAc 4:1 to 2:3)

to afford 1,2-*O*-isopropylidene- α -D-5-diphenylphosphoxylo-furanose **34** (1.586 g, 90%) as a white solid.

^1H NMR (600 MHz, CDCl_3) δ 7.34–7.40 (m, 4H, Ph); 7.19–7.26 (m, 6H, Ph); 5.89 (d, $J = 3.6$ Hz, 1H, H-(C1)); 4.48–4.56 (m, 2H, H-(C2), H-(C5)); 4.28–4.36 (m, 2H, H-(C4), H-(C5')); 4.10 (br. s., 1H, H-(C3)); 3.85 (d, $J = 3.5$ Hz, 1H, OH); 1.49 (s, 3H, $\text{C}(\text{CH}_3)_2$); 1.31 (s, 3H, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (151 MHz, CDCl_3) δ 150.3, 150.1, 130.0, 125.9, 125.8, 120.2, 120.0 (Ph); 111.9 ($\underline{\text{C}}(\text{CH}_3)_2$); 105.0 (C1); 85.0 (C2); 78.7 (d, C4); 73.6 (C3); 64.5 (d, C5); 26.9 ($\underline{\text{C}}(\text{CH}_3)_2$); 26.2 ($\text{C}(\underline{\text{C}}\text{H}_3)_2$). ^{31}P NMR (162 MHz, CDCl_3 , ^1H -decoupled) δ -9.78. HRMS (ESI) (m/z): $[\text{M}+\text{H}^+]$ calcd for formula $\text{C}_{20}\text{H}_{24}\text{O}_8\text{P}$, 423.1203; found, 423.1202.

*The spectroscopic properties of this compound were consistent with the data reported in the literature.*⁶

1,2-*O*-Isopropylidene- α -D-5-phosphoxylofuranose (**35**)

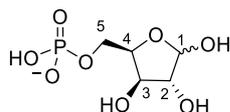


1,2-*O*-Isopropylidene- α -D-5-diphenylphosphoxylofuranose **34** (3.71 g, 8.78 mmol) was dissolved in methanol (60 mL) and PtO_2 (20 mg, catalytic) was added under an atmosphere of Ar. The Ar atmosphere was removed *in vacuo* and then replaced by an atmosphere of hydrogen. The heterogeneous mixture was stirred under the atmosphere of hydrogen for 16 h, then filtered through Celite[®]. The filtrate was concentrated *in vacuo* to obtain 1,2-*O*-isopropylidene- α -D-5-phosphoxylofuranose **35** (2.2915 g, 97%) as a white solid.

^1H NMR (600 MHz, D_2O) δ 6.01 (d, $J = 3.7$ Hz, 1H, H-(C1)); 4.66 (d, $J = 3.7$ Hz, 1H, H-(C2) partially obscured by HOD signal); 4.38 (ddd, $J = 7.1, 5.3, 2.6$ Hz, 1H, H-(C4)); 4.29 (d, $J = 2.6$ Hz, 1H, H-(C3)); 4.05 (ABXY, $J = 11.2, 6.9, 5.3$ Hz, 1H, H-(C5)); 3.95 (ABXY, $J = 11.2, 7.1, 6.9$ Hz, 1H, H-(C5')); 1.50 (s, 3H, $\text{C}(\text{CH}_3)_2$); 1.33 (s, 3H, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (151 MHz, D_2O) δ 113.40 ($\underline{\text{C}}(\text{CH}_3)_2$); 105.23 (C1); 85.17 (C2); 80.7 (d, C4); 74.37 (C3); 63.0 (d, C5); 26.2 ($\text{C}(\underline{\text{C}}\text{H}_3)_2$); 25.8 ($\text{C}(\underline{\text{C}}\text{H}_3)_2$). ^{31}P NMR (162 MHz, CDCl_3 , ^1H -decoupled) δ 1.71. HRMS (ESI) (m/z): $[\text{M}+\text{H}^+]$ calcd for formula $\text{C}_8\text{H}_{16}\text{O}_8\text{P}$, 271.0577; found, 271.0579.

*The spectroscopic properties of this compound were consistent with the data reported in the literature.*⁶

D-Xylose 5-phosphate (**17**)



1,2-*O*-Isopropylidene- α -D-5-phosphoxylofuranose **35** (700 mg, 2.59 mmol) was dissolved in water (100 mL) and stirred at 50 °C for 16 h. The solution was then adjusted from pH 1.5 to pH 6.5 by addition of 4M NaOH and concentrated *in vacuo* to give D-xylose 5-phosphate **17** (653 mg, quant. α/β 1:1) as a white solid. The crude product was used immediately in the next step without further purification.

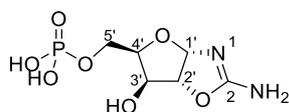
β -anomer: ^1H NMR (600 MHz, D_2O) δ 5.36 (d, $J = 4.5$ Hz, 1H, H-(C1)); 4.28 (q, $J = 5.3$ Hz, 1H, H-(C4)); 4.23 (dd, $J = 5.3, 4.5$ Hz, 2H, H-(C3)); 4.09 (t, $J = 4.5$ Hz, 1H, H-(C2)); 3.83–3.91 (m, 1H, H-(C5)); 3.76–3.83 (m, 1H, H-(C5')). ^{13}C NMR (151 MHz, D_2O) δ 96.4 (C1); 78.3 (d, C4); 76.1 (C2); 75.7 (C3); 63.3 (d, C5).

α -anomer: ^1H NMR (600 MHz, D_2O) δ 5.10 (d, $J = 1.5$ Hz, 1H, H-(C1)); 4.25 (q, $J = 5.3$ Hz, 1H, H-(C4)); 4.16 (dd, $J = 5.3, 3.0$ Hz, 1H, H-(C3)); 4.04 (dd, $J = 3.0, 1.5$ Hz, 1H, H-(C2)); 3.95–4.00 (m, 1H, H-(C5)); 3.83–3.91 (m, 1H, H-(C5')). ^{13}C NMR (151 MHz, D_2O) δ 102.4 (C1); 81.4 (d, C4); 80.8 (C2); 75.4 (C3); 63.6 (d, C5).

^{31}P NMR (162 MHz, D_2O , ^1H -decoupled) δ 3.55 (α -anomer), 3.45 (β -anomer). HRMS (ESI) (m/z): $[\text{M}-\text{H}]^-$ calcd for formula $\text{C}_5\text{H}_{10}\text{O}_7\text{P}$, 229.0119; found, 229.0125.

The spectroscopic properties of this compound were consistent with the data reported in the literature.^{4,6}

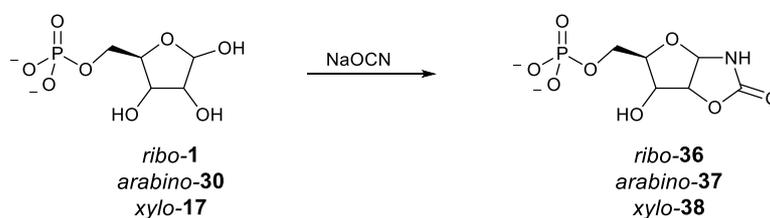
D-Xylose aminooxazoline-5'-phosphate (**16**)



Cyanamide **19** (212 mg, 5.04 mmol) was added to a solution of D-xylose 5-phosphate **17** (580 mg, 2.3 mmol) in aqueous NH_4OH (3.5%, 8 mL). The solution was stirred at 60 °C for 4 h and then lyophilised. The product was purified by ion exchange chromatography with Dowex® 50W \times 8 resin (40 g, H^+ -form, prewashed), eluting with water (100 mL), HCl 0.125M (100 mL), 0.25M (100 mL), 0.5M (100 mL), 1M (100 mL), and 2M (100 mL). D-Xylose aminooxazoline-5'-phosphate (*xylo-5-5P*) was found in fractions 0.125–0.25M HCl, and confirmed by ^1H NMR. The combined fraction containing D-xylose aminooxazoline-5'-phosphate (**16**) were lyophilised to yield **16** (375 mg, 64% isolated yield) as a colourless solid.

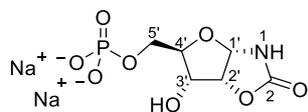
^1H NMR (600 MHz, D_2O) δ 6.11 (d, $J = 5.2$ Hz, 1H, H-(C1')); 5.30 (d, $J = 5.2$ Hz, 1H, H-(C2')); 4.52 (d, $J = 2.9$ Hz, 1H, H-(C3')); 4.21 (ddd, $J = 7.1, 4.5, 2.9$ Hz, 1H, H-(C4')); 4.11 (ABXY, $J = 11.3, 7.1, 4.5$ Hz, 1H, H-(C5')); 3.99 (ABXY, $J = 11.3, 7.1, 5.4$ Hz, 1H, H-(C5')). ^{13}C NMR (151 MHz, D_2O) δ 163.4 (C2); 90.8 (C2'); 89.1 (C1'); 79.8 (d, C4'); 72.8 (C3'); 63.1 (d, C5'). ^{31}P NMR (162 MHz, D_2O , ^1H -decoupled) δ 0.61. HRMS (ESI) (m/z): $[\text{M}+\text{H}^+]^+$ calcd for formula $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_7\text{P}^+$, 255.0377; found, 255.0375.

Preparative synthesis of ribose aminooxazolidinone-5'-phosphate (ribo-36), arabinose aminooxazolidinone-5'-phosphate (arabino-37) and xylose aminooxazolidinone-5'-phosphate (xylo-38)



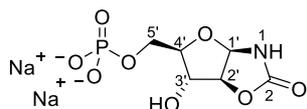
Pentose 5'-phosphate (**1**, **30** or **17**, 0.15M) and sodium cyanate (2 eq.) were dissolved in aqueous ammonium chloride (0.4M) then stirred at 60 °C for 7 h. The solution was cooled to room temperature and lyophilised. The white lyophilite was used directly without further purification.

Ribose oxazolidinone-5'-phosphate (**36**)



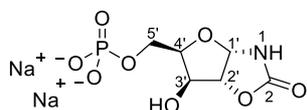
Starting from 176 mg D-ribose-5-phosphate **1**, yield = 182 mg (95% crude). ^1H NMR (600 MHz, D_2O) δ 5.72 (d, $J = 5.2$ Hz, 1H, H-(C1')); 5.05 (dd, $J = 5.5, 5.2$ Hz, 1H, H-(C2')); 4.18 (dd, $J = 8.6, 5.5$ Hz, 1H, H-(C3')); 3.97 (ABXY, $J = 12.1, 4.0, 2.1$ Hz, 1H, H-(C5')); 3.79–3.91 (m, 2H, H-(C4'), H-(C5')). ^{13}C NMR (151 MHz, D_2O) δ 161.1 (C2); 86.0 (C1'); 80.5 (C2'); 78.2 (d, C4'); 70.7 (C3'); 62.5 (d, C5'). ^{31}P NMR (162 MHz, D_2O , ^1H -decoupled) δ 3.86. HRMS (ESI) (m/z): $[\text{M}-\text{H}]^-$ calcd for formula $\text{C}_6\text{H}_9\text{NO}_8\text{P}$, 254.0071; found, 254.0074.

Arabinose oxazolidinone-5'-phosphate (**37**)



Starting from 91 mg D-arabinose-5-phosphate **30**, yield = 95 mg (96% crude). ^1H NMR (600 MHz, D_2O) δ 5.79 (d, $J = 5.8$ Hz, 1H, H-(C1')); 4.98 (dd, $J = 5.8, 1.2$ Hz, 1H, H-(C2')); 4.40 (dd, $J = 3.4, 1.2$ Hz, 1H, H-(C3')); 4.12 (td, $J = 6.3, 3.4$ Hz, 1H, H-(C4')); 3.66–3.75 (m, 2H, H-(C5')). ^{13}C NMR (151 MHz, D_2O) δ 160.3 (C2); 87.2 (C2'); 87.1 (C1'); 85.0 (d, C4'); 75.6 (C3'); 64.1 (d, C5'). ^{31}P NMR (162 MHz, D_2O , ^1H -decoupled) δ 4.02. HRMS (ESI) (m/z): [$\text{M}-\text{H}^-$] calcd for formula $\text{C}_6\text{H}_9\text{NO}_8\text{P}$, 254.0071; found, 254.0079.

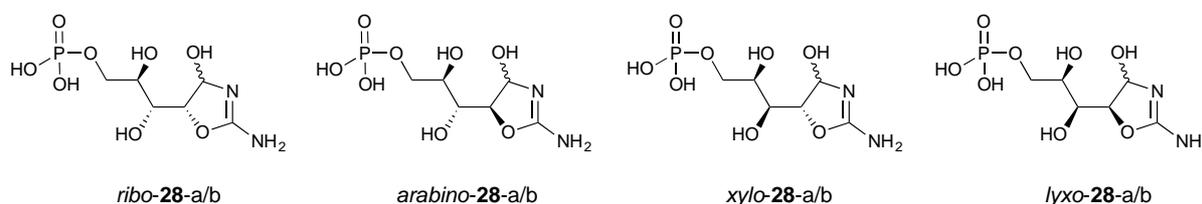
Xylose oxazolidinone-5'-phosphate (**38**)



Starting from 96 mg D-xylose-5-phosphate **17**, yield = 96 mg (91% crude). ^1H NMR (600 MHz, D_2O) δ 5.83 (d, $J = 5.5$ Hz, 1H, H-(C1')); 4.96 (d, $J = 5.5$ Hz, 1H, H-(C2')); 4.41 (d, $J = 2.6$ Hz, 1H, H-(C3')); 4.16 (td, $J = 6.3, 2.6$ Hz, 1H, H-(C4')); 3.95 (ABXY, $J = 11.0, 7.0, 6.3$ Hz, 1H, H-(C5')); 3.87 (ABXY, $J = 11.0, 7.0, 6.3$ Hz, 1H, H-(C5')). ^{13}C NMR (151 MHz, D_2O) δ 160.7 (C2); 86.8 (C1'); 85.7 (C2'); 79.3 (d, C4'); 73.6 (C3'); 61.3 (d, C5'). ^{31}P NMR (162 MHz, D_2O , ^1H -decoupled) δ 4.73. HRMS (ESI) (m/z): [$\text{M}+\text{Na}^+$] calcd for formula $\text{C}_6\text{H}_{10}\text{NNaO}_8\text{P}$, 278.0036; found, 278.0034.

Studies of C2'-stereochemical inversion of ribo- (3), arabino- (14) and xylo- (16) aminooxazoline-5'-phosphate

Pentose aminooxazoline-5'-phosphate (**3**, **14** or **16**; 100mM) was dissolved in H₂O, 1M phosphate solution, a mixture of H₂O/D₂O (1:1) or 1M phosphate solution (D₂O/H₂O 1:1) at specified pH. The solution was then incubated at the specified temperature and ¹H NMR spectra were periodically acquired. The solution pH was periodically monitored, no significant fluctuation in pH was observed. The reaction was monitored by acquisition of ¹H NMR spectra for 7 to 20 days, then spiked with authentic standards of pentose oxazolidone 5'-phosphates (**36**, **37** or **38**) and ¹H NMR spectra acquired. Oxazole **15** and **20** were identified by characteristic H-(C1') signal (6.70 ppm, s and 6.62 ppm, s respectively). Minor open chain hydrates of oxazoline **3**, **14** or **16** were identified by the characteristic signals:



ribo-28-a: 5.46 ppm, d, $J = 4.2$ Hz, H-(C1').

ribo-28-b: 5.56 ppm, d, $J = 2.8$ Hz, H-(C1').

arabino-28-a: 5.47 ppm, d, $J = 4.2$ Hz, H-(C1').

arabino-28-b: 5.62 ppm, d, $J = 2.8$ Hz, H-(C1').

xylo-28-a: 5.61 ppm, d, $J = 2.4$ Hz, H-(C1').

xylo-28-b: 5.87 ppm, d, $J = 4.2$ Hz, H-(C1').

lyxo-28-a: 5.72 ppm, d, $J = 2.4$ Hz, H-(C1').

lyxo-28-b: not observed.

(by comparison to hydrate of aminooxazoline *ribo-27*, *arabino-27* and *xylo-27*).⁷

Incubation of ribose aminooxazoline-5'-phosphate (3)

Ribose aminooxazoline-5'-phosphate (**3**) was observed to be stable to C2'-epimerisation (in both deionised water and 1M phosphate solution) at room temperature for 7 d, oxazole **15** was observed but not the formation of arabinose aminooxazoline-5'-phosphate (**14**) (Fig. S12). By contrast extensive C2'-epimerisation of arabinose aminooxazoline-5'-phosphate (**14**) was observed (in both deionised water and 1M phosphate solution) at room temperature; C2'-epimerisation of arabinose aminooxazoline-5'-phosphate (**14**) to 25 and 50% conversion to

ribose aminooxazoline-5'-phosphate (**3**) was observed after 7 and 20 days respectively. At 40 °C C2'-epimerisation of ribose aminooxazoline-5'-phosphate (**3**) to arabinose aminooxazoline-5'-phosphate (**14**; 5–11%) was observed after 7 d with or without phosphate solution (Fig. S13), however the extent of C2'-epimerisation of arabinose aminooxazoline-5'-phosphate (**14**) to ribose aminooxazoline-5'-phosphate (**3**; 50–60%) over 7 d was significantly greater. It was also of note that maximum rate of conversion of arabinose aminooxazoline-5'-phosphate (**14**) to ribose aminooxazoline-5'-phosphate **3** occurred at pH 6.0, closely reflecting the pK_a of the pentose aminooxazolines (pK_a=6.5).

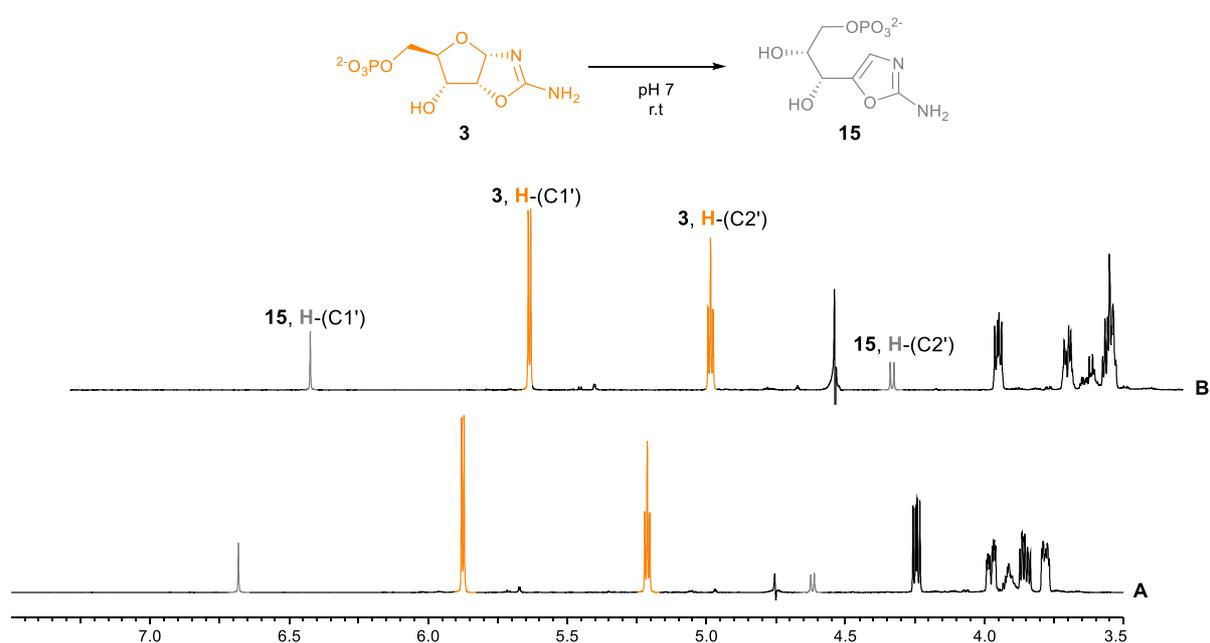


Figure S10: ¹H NMR spectra (600 MHz, D₂O, 3.5–7.5 ppm) for the incubation of ribose aminooxazoline-5'-phosphate (**3**, 100mM) for 7 d at room temperature and pH 7.0: **A**) in H₂O; **B**) in 1M phosphate solution. Oxazole **15** is observed to accumulate.

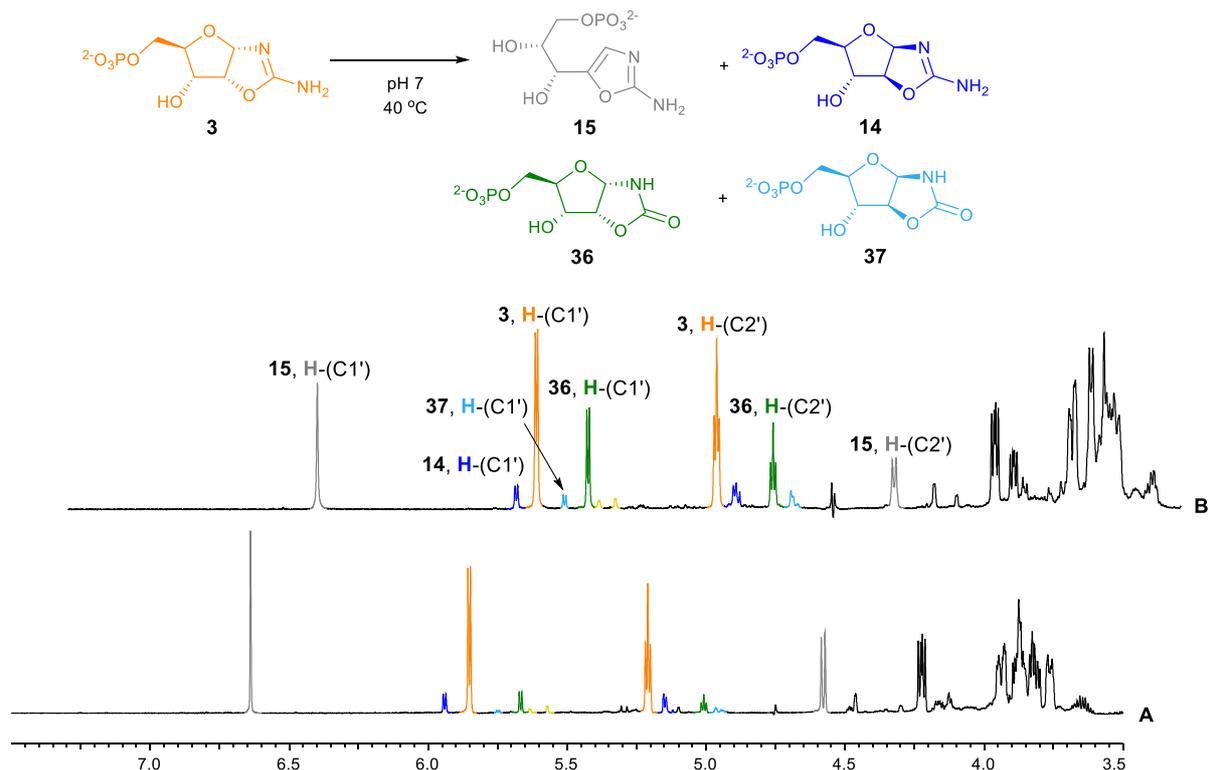


Figure S11: ¹H NMR spectra (600 MHz, D₂O, 3.5–7.5 ppm) for the incubation of ribose aminooxazoline-5'-phosphate (**3**, 100mM) for 7 d at 40 °C and pH 7.0: **A**) in H₂O; **B**) in 1M phosphate solution. Oxazole **15** and ribose oxazolidinone-5'-phosphate (**36**) are the major products; small amounts of arabinose aminooxazoline-5'-phosphate (**14**) and arabinose oxazolidinone-5'-phosphate (**37**) are observed.

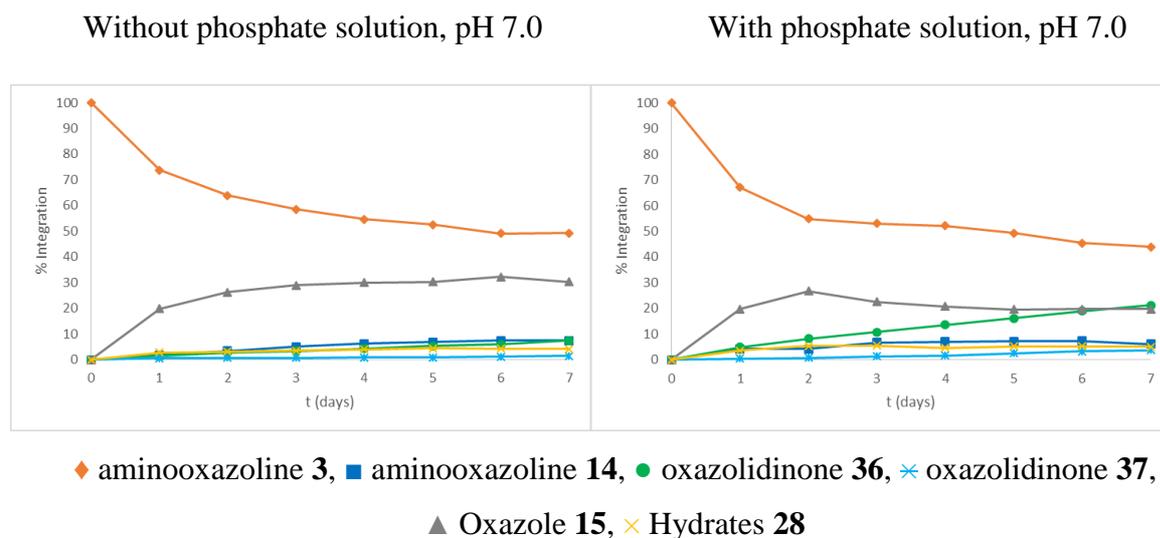


Figure S12: Graphs to show the reaction of ribose aminooxazoline-5'-phosphate (**3**, 100mM) with and without phosphate solution at pH 7.0 at 40 °C. Amount of each species was determined by integration of ¹H NMR spectra.

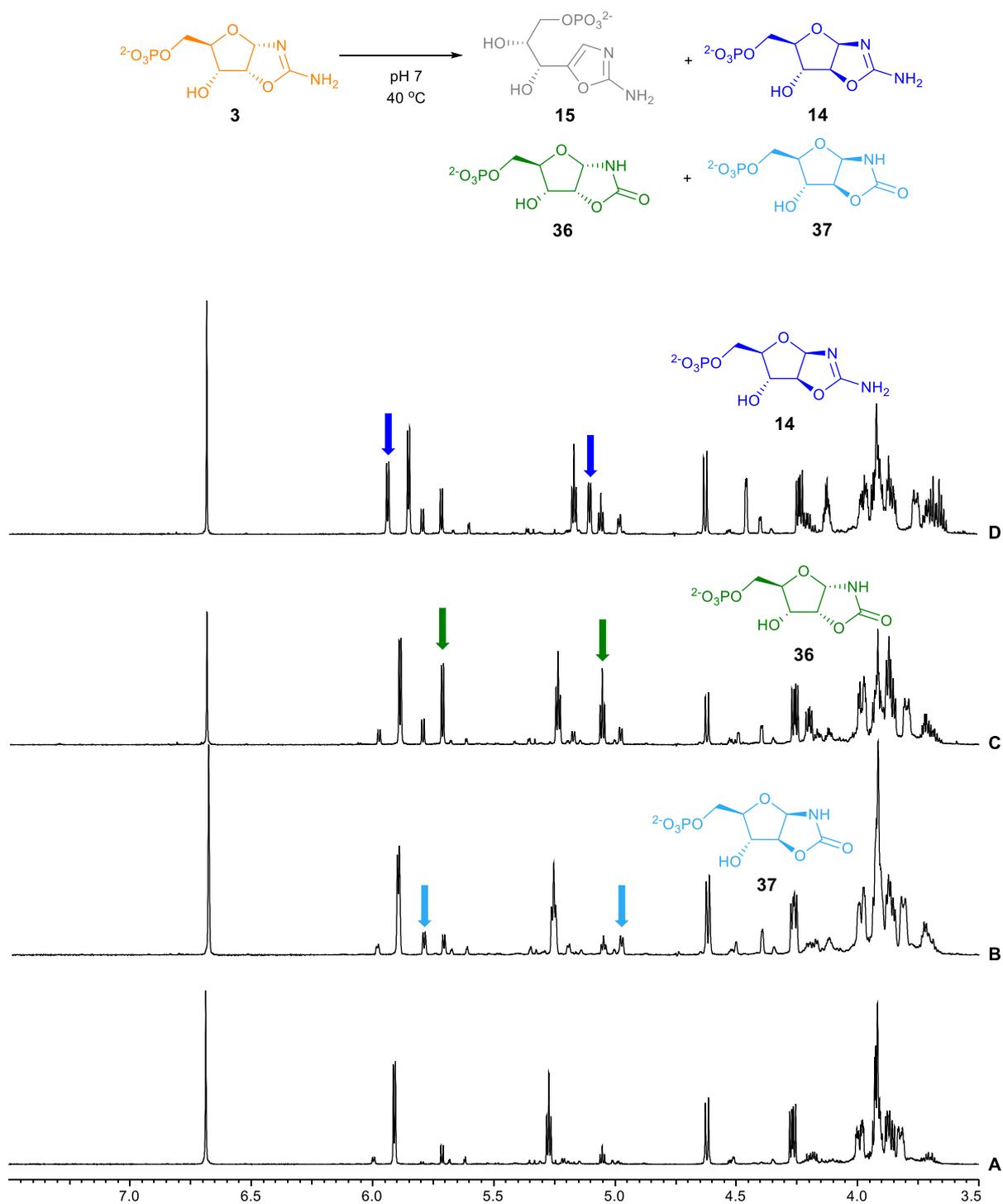


Figure S13: ¹H NMR spectra (600 MHz, D₂O, 3.5–7.5 ppm) for **A**) the residue after lyophilisation of the incubation of ribose aminooxazoline-5'-phosphate (**3**, 100mM) at pH 7.0 at 40 °C for 6 d in H₂O; **B**) spiked with arabinose oxazolidinone-5'-phosphate (**37**); **C**) spiked with ribose oxazolidinone-5'-phosphate (**36**); **D**) spiked with arabinose aminooxazoline-5'-phosphate (**14**).

Incubation of arabinose aminooxazoline-5'-phosphate (**14**)

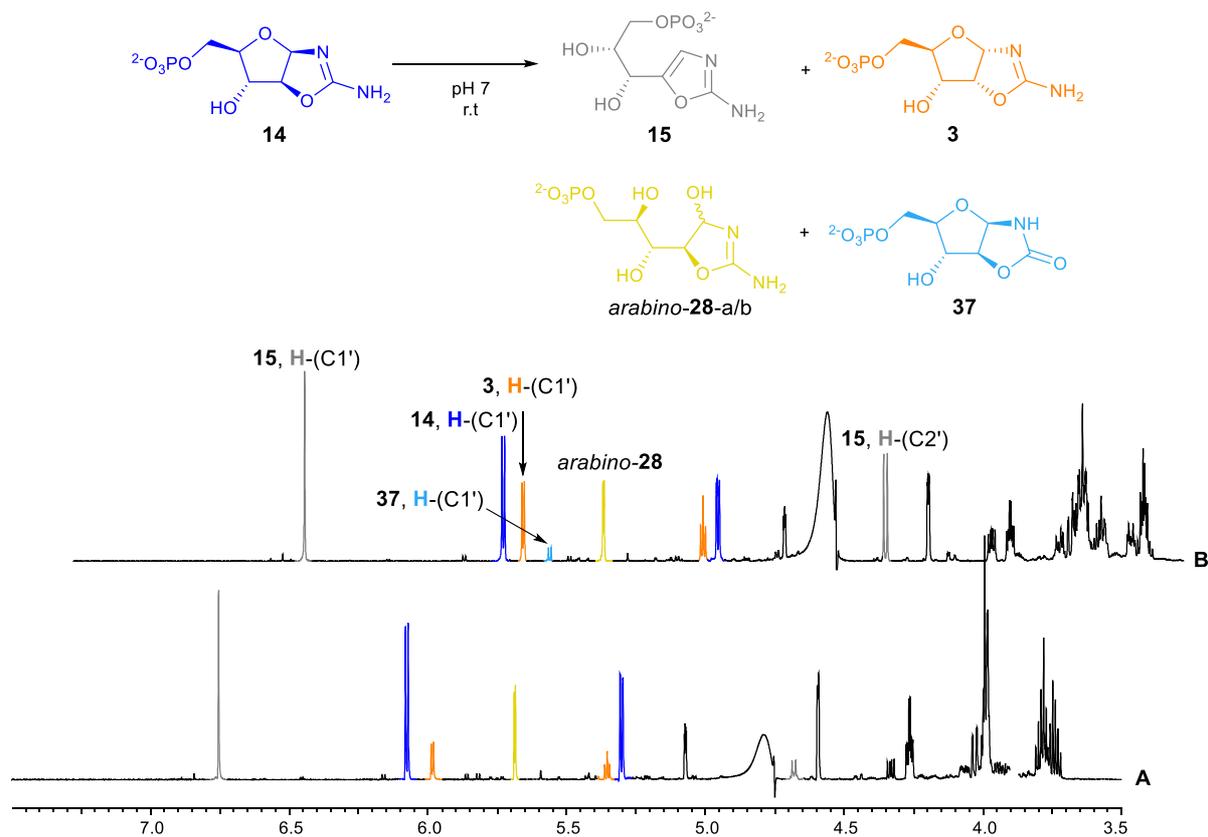
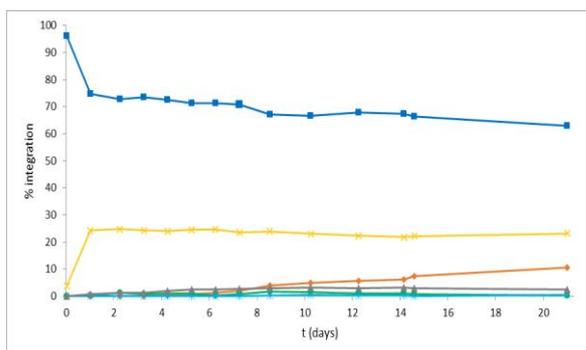
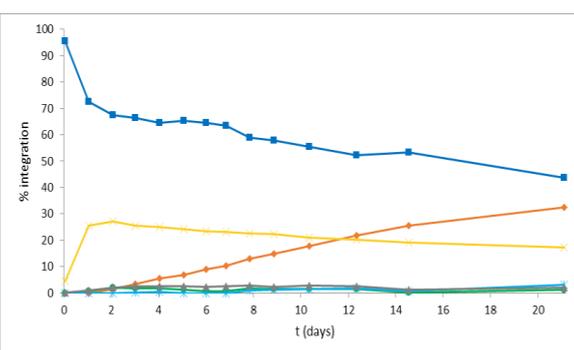


Figure S14: ¹H NMR spectra (600 MHz, D₂O/H₂O (1:1), 3.5–7.5 ppm) for the incubation of arabinose aminooxazoline-5'-phosphate (**14**) for 7 d at room temperature and pH 7.0: **A**) in H₂O; **B**) in 1M phosphate solution. Oxazole **15**, ribose aminooxazoline-5'-phosphate (**3**) and hydrate *arabino-28* are the major products; a small amount of arabinose oxazolidinone-5'-phosphate (**37**) is observed.

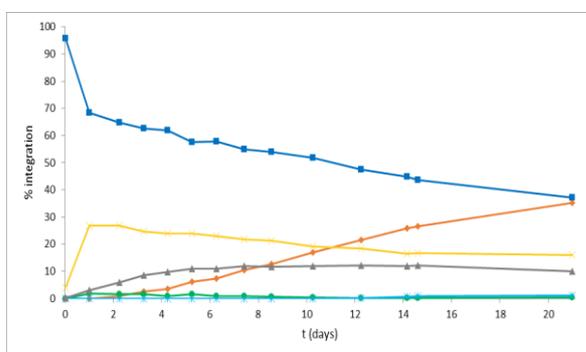
Without phosphate solution, pH 5.0



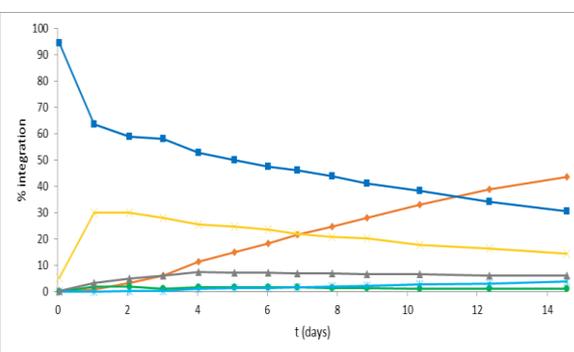
With phosphate solution, pH 5.0



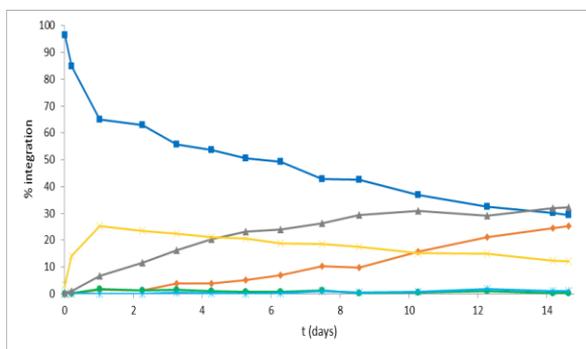
Without phosphate solution, pH 6.0



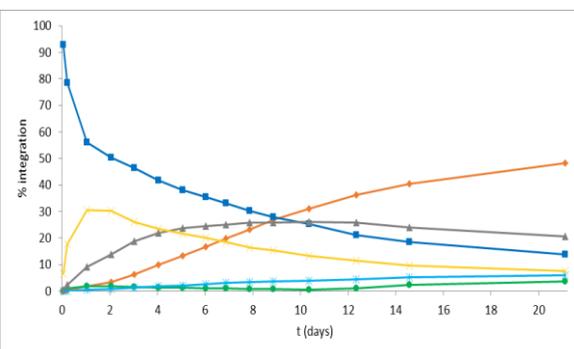
With phosphate solution, pH 6.0



Without phosphate solution, pH 7.0



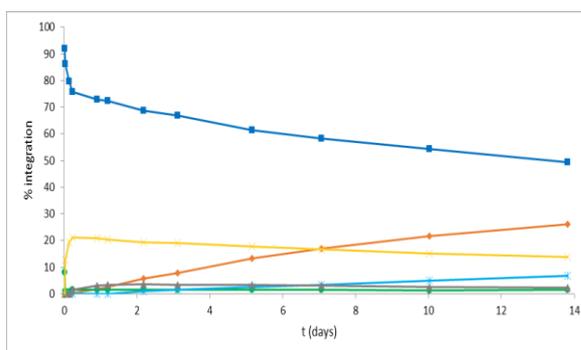
With phosphate solution, pH 7.0



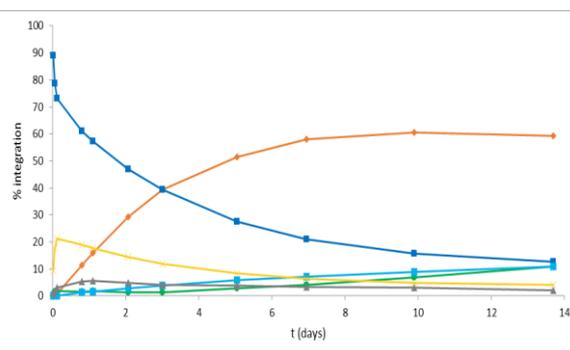
♦ aminooxazoline **3**, ■ aminooxazoline **14**, ● oxazolidinone **36**, ✧ oxazolidinone **37**,
▲ Oxazole **15**, × Hydrates **28**

Figure S15: Graphs to show the reaction of arabinose aminooxazoline-5'-phosphate (**14**) with and without phosphate solution at pH 5.0, 6.0 and 7.0 at room temperature. Amount of each species was determined by integration of ^1H NMR spectra.

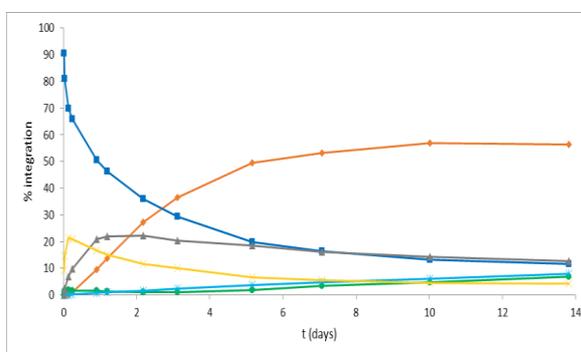
Without phosphate solution, pH 5.0



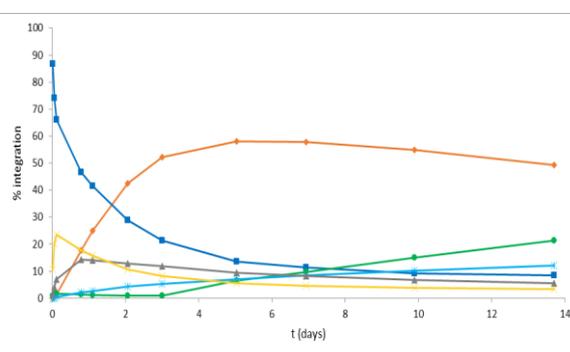
With phosphate solution, pH 5.0



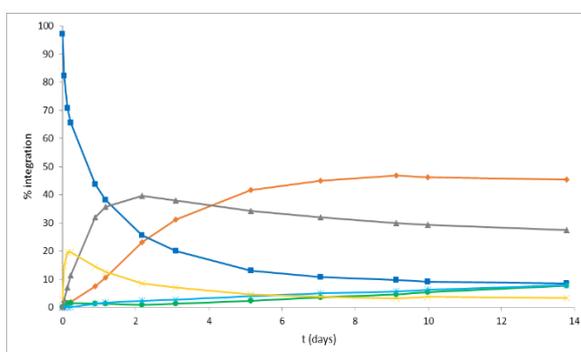
Without phosphate solution, pH 6.0



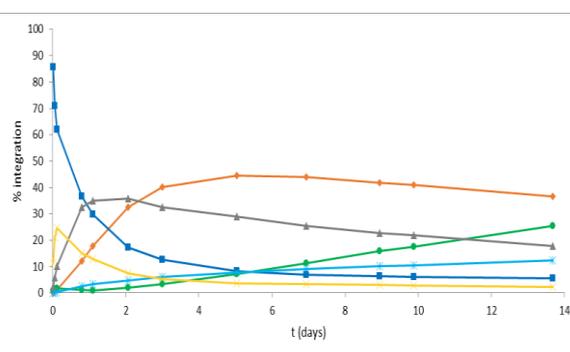
With phosphate solution, pH 6.0



Without phosphate solution, pH 7.0



With phosphate solution, pH 7.0



◆ aminooxazoline **3**, ■ aminooxazoline **14**, ● oxazolidinone **36**, ✖ oxazolidinone **37**,
▲ Oxazole **15**, × Hydrates **28**

Figure S16: Graphs to show the reaction of arabinose aminooxazoline-5'-phosphate (**14**, 100mM) with and without phosphate solution at pH 5.0, 6.0 and 7.0 at 40 °C. Amount of each species was determined by integration of ¹H NMR spectra.

Incubation of xylose aminooxazoline-5'-phosphate (**16**)

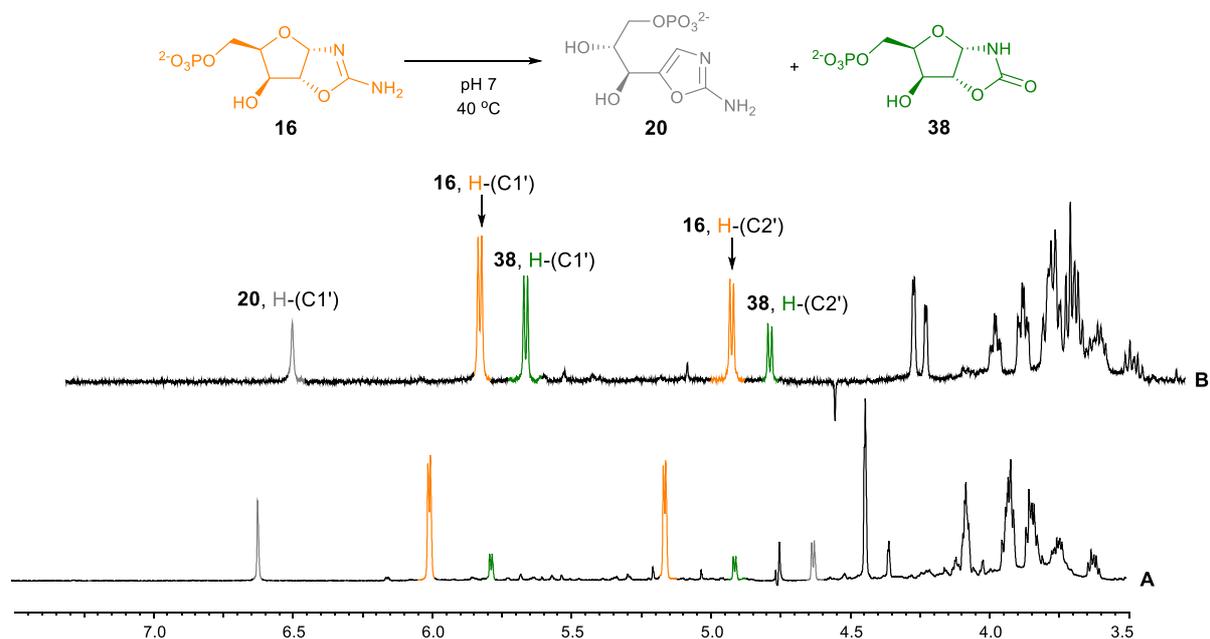


Figure S17: ¹H NMR spectra (600 MHz, D₂O, 3.5-7.5 ppm) for the incubation of xylose aminooxazoline-5'-phosphate (**16**, 100mM) for 7 d at 40 °C and pH 7.0: **A**) in H₂O; **B**) in 1M phosphate solution. Oxazole **20** and xylose oxazolidinone-5'-phosphate (**38**) are the only products.

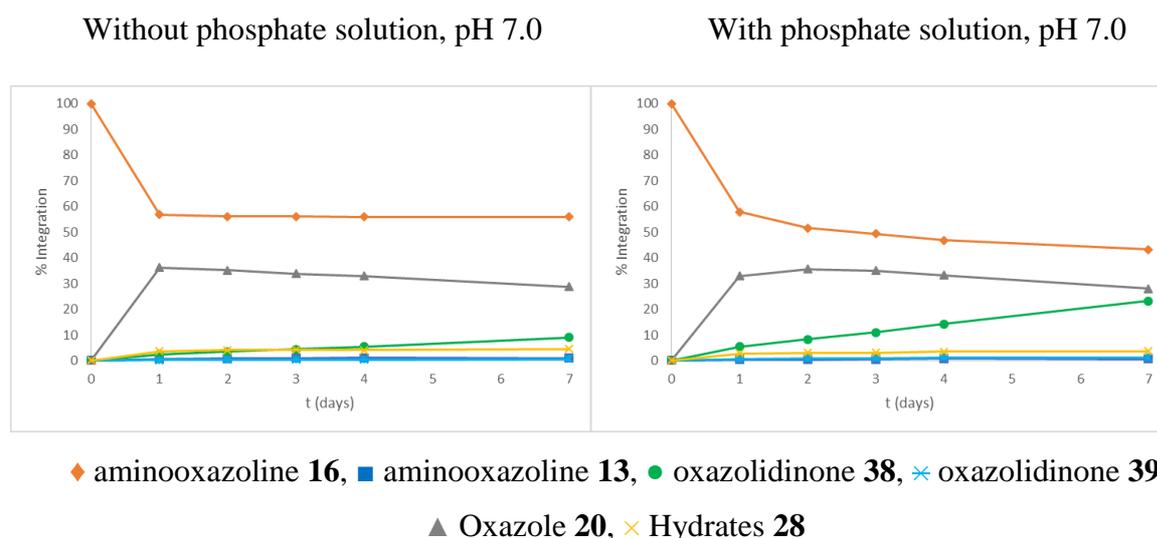


Figure S18: Graphs to show the reaction of xylose aminooxazoline-5'-phosphate (**16**, 100mM) with and without phosphate solution at pH 7.0 at 40 °C. Amount of each species was determined by integration of ¹H NMR spectra. Lyxose aminooxazoline-5'-phosphate **13** and lyxose oxazolidinone-5'-phosphate **39** were not observed.

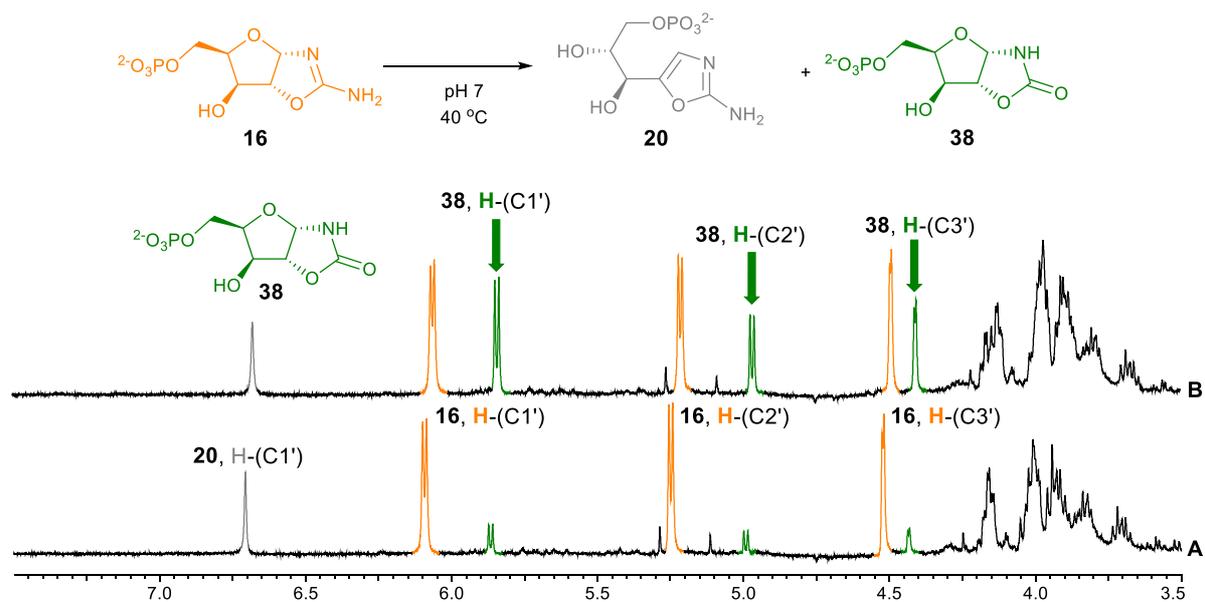
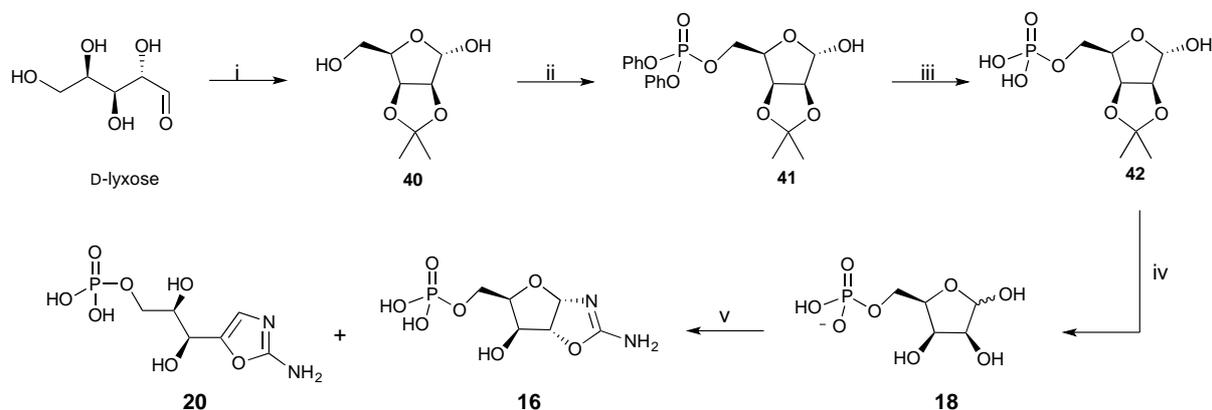


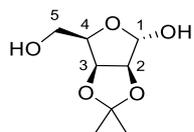
Figure S19: ¹H NMR spectra (600 MHz, D₂O, 3.5–7.5 ppm) for **A**) the residue after lyophilisation of the incubation of xylose aminooxazoline-5'-phosphate (**16**, 100mM) at pH 7.0 at 40 °C for 7 d in H₂O; **B**) spiked with xylose oxazolidinone-5'-phosphate (**38**). Oxazole **20** was assigned by its characteristic peak at 6.62 ppm.

Preparative synthesis of D-lyxose 5-phosphate (**18**)



Scheme 2: Reagents and conditions: (i) H_2SO_4 , $(\text{CH}_3)_2\text{CO}$, 2 h, r.t., 93%; (ii) $(\text{PhO})_2\text{POCl}$, CH_2Cl_2 , pyridine, 6 h, 0 °C, 92%; (iii) H_2 , PtO_2 , MeOH, 18 h, r.t., quant.; (iv) H_2O , 16 h, 50 °C, quant.; (v) H_2NCN , NH_4OH , H_2O .

2,3-*O*-Isopropylidene-*D*-lyxofuranose (**40**)

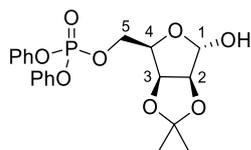


D-Lyxose (5.00 g, 33.3 mmol) was suspended in dry acetone (170 mL), cooled to 0 °C and concentrated sulfuric acid (220 μL , 3.9 mmol) was added dropwise. The solution was stirred at 0 °C for 10 min, then warmed to room temperature and stirred for a further 2 h. Aqueous NH_4OH solution (3.5 %) was added to neutralise the solution, which was then filtered and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/MeOH 9:1) to yield to yield α -2,3-*O*-isopropylidene-*D*-lyxofuranose (**40**; 5.923 g, 93%) as a colourless oil.

$[\alpha]_{\text{D}}^{25} = +21.25$ ($c=0.16$, Acetone). IR (cm^{-1}) 3630–3120 (O-H), 3060–2810 (C-H). ^1H NMR (600 MHz, CDCl_3) δ 5.44 (s, 1H, H-(C1)); 4.82 (dd, $J = 5.8, 3.9$ Hz, 1H, H-(C3)); 4.63 (d, $J = 5.8$ Hz, 1H, H-(C2)); 4.26–4.30 (m, 1H, H-(C4)); 3.92–3.96 (m, 2 H, H-(C5)); 1.47 (s, 1H, $\text{C}(\text{CH}_3)_2$); 1.32 (s, 1H, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (150 MHz, CDCl_3) δ 112.7 ($\underline{\text{C}}(\text{CH}_3)_2$); 100.9 (C1); 85.6 (C2); 80.4 (C3); 79.7 (C4); 61.1 (C5); 26.0 ($\underline{\text{C}}(\text{CH}_3)_2$); 24.5 ($\underline{\text{C}}(\text{CH}_3)_2$). HRMS (ES^+) (m/z): $[\text{M}+\text{NH}_4^+]^+$ calcd for formula $\text{C}_8\text{H}_{18}\text{O}_5\text{N}^+$, 208.1179; found, 208.1180.

The spectroscopic properties of this compound were consistent with the data reported in the literature.⁸

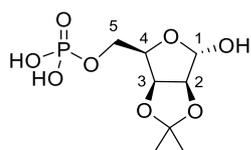
2,3-*O*-Isopropylidene-D-5-diphenylphospholyxofuranose (**41**)



2,3-*O*-Isopropylidene-D-lyxofuranose (**40**; 1.105 g, 5.8 mmol) was dissolved in a mixture of anhydrous CH₂Cl₂ (270 mL) and anhydrous pyridine (40 mL), cooled to 0 °C and diphenyl chlorophosphate (1.49 mL, 7.15 mmol) added dropwise and stirred at 0 °C. After 6 h the solution warmed to room temperature, water (40 mL) was added and the solution was concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/hexane 1:4 to 1:0) to yield α -2,3-*O*-isopropylidene-D-5-diphenylphospholyxofuranose (**41**; 2.27 g, 92%) as a colourless oil.

$[\alpha]_D^{25} = +16.90$ ($c=0.073$, CHCl₃). IR (cm⁻¹) 3560–3250 (O-H), 3120–2810 (C-H) 1175 (P=O). ¹H NMR (600 MHz, CDCl₃) δ 7.31–7.36 (m, 4 H, Ph); 7.22–7.25 (m, 4H, Ph); 7.17–7.21 (m, 2H, Ph); 5.39 (s, 1H, H-(C1)); 4.74 (dd, $J = 5.8, 3.4$ Hz, 1H, H-(C3)); 4.60 (d, $J = 5.8$ Hz, 1H, H-(C2)); 4.53–4.57 (m, 1H, H-(C5)); 4.40–4.45 (m, 2H, H-(C4) and H-(C5')); 1.43 (s, 1H, C(CH₃)₂); 1.28 (s, 1H, C(CH₃)₂). ¹³C NMR (150 MHz, CDCl₃) δ 150.4 (Ph); 129.7 (Ph); 125.4 (Ph); 120.1 (Ph); 112.8 (C(CH₃)₂); 101.2 (C1); 85.4 (C2); 79.5 (C3); 78.3 (C4); 67.1 (C5); 25.9 (C(CH₃)₂); 24.6 (C(CH₃)₂). ³¹P NMR (121 MHz, CDCl₃, 1H-decoupled): δ -12.1. HRMS (ESI) (m/z): [M+H]⁺ calcd for formula C₂₀H₂₄O₈P⁺, 423.1203; found, 423.1204.

2,3-*O*-Isopropylidene-D-5-phospholyxofuranose (**42**)

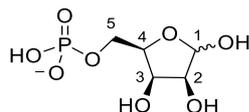


2,3-*O*-Isopropylidene-D-5-diphenylphospholyxofuranose (**41**, 280 mg, 0.66 mmol) was dissolved in methanol (14 mL) under a N₂ atmosphere, and PtO₂ (25 mg, catalytic) was added. The suspension was stirred vigorously under a H₂ atmosphere for 16 h. The reaction was then flushed with N₂ and filtered through Celite[®], which was washed with methanol (3 \times 5 mL). The combined filtrates were concentrated *in vacuo* to yield α -2,3-*O*-isopropylidene-D-5-phospholyxofuranose (**42**, 170 mg, quant. yield) as a colourless oil.

$[\alpha]_D^{25} = +1.55$ ($c=0.344$, CH₃OH). IR (cm⁻¹) 3500–3130 (O-H), 3000–2810 (C-H), 1195 (P=O). ¹H NMR (600 MHz, CD₃OD) δ 5.24 (s, 1H, H-(C1)); 4.81 (dd, $J = 5.8, 3.8$ Hz, 1H, H-(C3)); 4.55 (d, $J = 5.8$ Hz, 1H, H-(C2)); 4.31–4.35 (m, 1H, H-(C4)); 4.23 (ABXY, $J = 10.7, 6.8, 4.8$

Hz, 1H, H-(C5)); 4.08 (ABXY, $J = 10.7, 6.8, 6.8$ Hz, 1H, H-(C5')); 1.40 (s, 1H, C(CH₃)₂); 1.29 (s, 1H, C(CH₃)₂). ¹³C NMR (150 MHz, CD₃OD) δ 113.8 ppm (C(CH₃)₂); 102.4 (C1); 87.3 (C3); 81.2 (C2); 80.0 (C4); 65.9 (C5); 26.5 (C(CH₃)₂); 25.0 (C(CH₃)₂). ³¹P NMR (162 MHz, CD₃OD, ¹H-decoupled): δ 0.1 ppm. HRMS (ES⁻) (m/z): [M]⁻ calcd for formula C₈H₁₄O₈P⁻, 269.0432; found, 269.0435.

D-Lyxose 5-phosphate (**18**)



2,3-*O*-Isopropylidene-*D*-5-phosphoxyfuranose (**42**, 170 mg, 0.66 mmol) was dissolved in water (25 mL) and heated at 50 °C for 16 h. The solution was then adjusted to pH 6.5 and concentrated *in vacuo* to yield *D*-lyxose 5-phosphate (**18**, 145 mg, quant.) as a colourless oil.

β -anomer: ¹H NMR (600 MHz, H₂O/D₂O (9:1)) δ 5.22 (d, $J = 4.8$ Hz, 1H, H-(C1)); 4.31–4.37 (m, 1H, H-(C4)); 4.26 (t, $J = 3.9$ Hz, 1H, H-(C3)); 4.03 (dd, $J = 4.8, 3.9$ Hz, 1H, H-(C2)); 3.96 (ABXY, $J = 10.9, 6.7, 5.6$ Hz, 1H, H-(C5)); 3.83 (ABXY, $J = 10.9, 6.7, 6.6$ Hz, 1H, H-(C5')). ¹³C NMR (150 MHz, H₂O/D₂O (9:1)) δ 101.4 (C1); 79.8 (C4); 77.7 (C2); 71.7 (C3); 63.9 (C5). ³¹P NMR (162 MHz, H₂O/D₂O (9:1), ¹H-decoupled): δ 2.1 ppm.

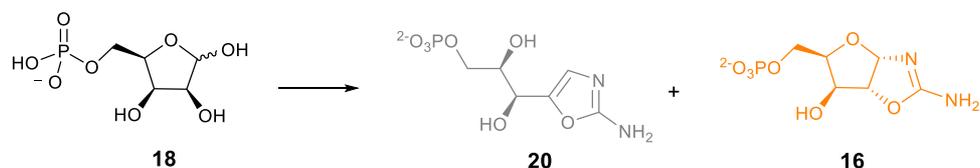
α -anomer: ¹H NMR (600 MHz, H₂O/D₂O (9:1)) δ 5.18 (d, $J = 4.9$ Hz, 1H, H-(C1)); 4.23 (t, $J = 4.1$ Hz, 1H, H-(C3)); 4.11 (dd, $J = 4.8, 3.9$ Hz, 1H, H-(C2)); 4.08–4.10 (m, 1H, H-(C4)); 4.01–4.05 (obs., 1H, H-(C5), *peak overlaps with β anomer H-(C2)*); 3.89 (ABXY, $J = 10.9, 6.9, 6.6$ Hz, 1H, H-(C5')). ¹³C NMR (150 MHz, H₂O/D₂O (9:1)) δ 96.2 (C1); 80.0 (C4); 72.0 (C2); 70.4 (C3); 64.6 (C5). ³¹P NMR (162 MHz, H₂O/D₂O (9:1), ¹H-decoupled): δ 2.1 ppm.

Open chain isomer (partial data): ¹H NMR (600 MHz, H₂O/D₂O (9:1)) δ 4.91 ppm (d, $J = 4.1$ Hz, 1 H, H-(C1)); 4.04–4.08 (m, 1 H, H-(C2)). ¹³C NMR (150 MHz, H₂O/D₂O (9:1)) δ 76.7 ppm (C2); 71.6 (C1).

HRMS (ES⁻) (m/z): [M]⁻ calcd for formula C₅H₁₀O₈P⁻, 229.0119; found, 229.0118.

The spectroscopic properties of this compound were consistent with the data reported in the literature.^{4,6}

Reaction of D-lyxose 5-phosphate (**18**) and cyanamide **19**



Lyxose aminooxazoline (*lyxo-27*) is the only pentose aminooxazoline (**27**) that exists predominately as its pyranosyl-form (*p-lyxo-27*).⁹ Therefore, it was expected that lyxose aminooxazoline 5'-phosphate (**13**), which cannot adopt a pyranosyl ring, would preferentially adopt open-chain forms. To test this hypothesis, D-lyxose-5-phosphate (**18**; 11.6 mg, 0.05 mmol) and cyanamide (**19**; 4.2 mg, 0.1 mmol) were dissolved in aqueous NH₄OH (3%, 0.5 mL, H₂O/D₂O (9:1)) and incubated at 60 °C. The reaction was analysed periodically by ¹H NMR. After 4.5 h at 60 °C xylose aminooxazoline 5'-phosphate (**16**; 30%) was observed, alongside oxazole (**20**; 70%). Lyxose aminooxazoline 5'-phosphate (**13**) was not observed under these conditions and further investigation of the synthesis of lyxose aminooxazoline 5'-phosphate (**13**) was not undertaken.

Xylose aminooxazoline-5'-phosphate (**16**): 5.94 ppm, d, *J* = 5.0 Hz, H-(C1'); 5.02 ppm, d, *J* = 5.0 Hz, H-(C2').

Oxazole **20**: 6.62 ppm, s, H-(C1').

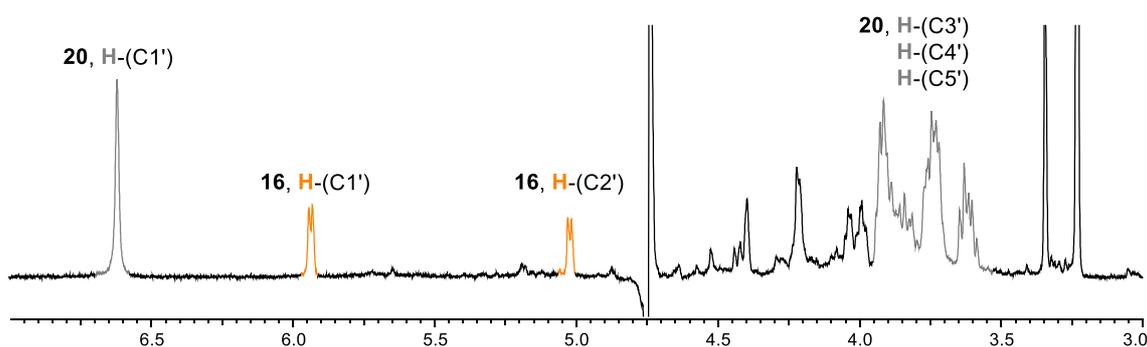
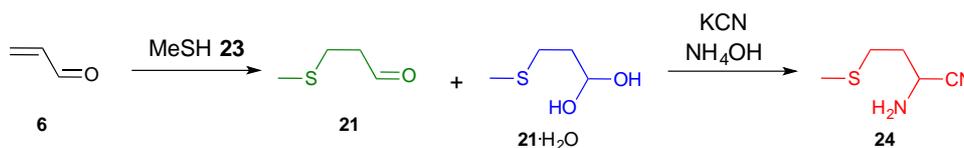


Figure S21: ¹H NMR spectrum (400 MHz, H₂O/D₂O (1:1), 3.0–7.0 ppm) to show oxazole **20** (blue) and xylose aminooxazoline-5'-phosphate (**16**; orange) formed during the reaction of lyxose-5-phosphate (**18**, 22mM) with cyanamide (**19**; 3 eq.) in water at pH 7.0 after 4.5 h at 60 °C.

Incubation of acrolein (6) and methanethiol (9) followed by Strecker synthesis



A flow of methanethiol (**23**), generated by dropping sodium methanethiolate (21% aqueous solution) onto mono-basic sodium phosphate, was bubbled continuously through a solution of phosphate solution (0.5 mL, 1M, pH 7.0) whilst a solution of acrolein (**6**, 9.3 μ L, 0.14 mmol) in H₂O/D₂O (9:1, 0.5 mL) was added dropwise over 5 min. After 15 min NMR spectra were acquired and 3-(methylthio)propanal (**21**, >94% by ¹H NMR integration) was observed as a mixture of aldehyde **21** and hydrate **21**·H₂O (1:2.5). The synthesis of 3-(methylthio)propanal (**21**) was confirmed by spiking with an authentic standard and used without further purification. Potassium cyanide (1.5 eq.) and then ammonium hydroxide solution (5 eq.) were added to an aliquot of the reaction mixture (0.5 mL, 0.14mM, 70 μ mol) and the solution was adjusted to pH 10.0 with 1M NaOH. ¹H NMR spectra were periodically acquired and after 20 h at room temperature synthesis of 2-amino-4-(methylthio)butanenitrile (**24**) was observed.

3-(Methylthio)propanal hydrate (**21**·H₂O): ¹H NMR (600 MHz, H₂O/D₂O (9:1)) 5.07 (t, *J* = 5.5 Hz, 1H, H-(C1)); 2.51 (t, *J* = 7.6 Hz, 2H, H-(C3)); 2.04 (s, 3H, SCH₃); 1.81 (dt, *J* = 7.6, 5.5 Hz 2H, H-(C2)). ¹³C NMR (151 MHz, H₂O/D₂O (9:1)) δ _C 90.6 (C1); 37.2 (C2); 29.2 (C3); 15.0 (SCH₃).

3-(Methylthio)propanal (**21**): ¹H NMR (600 MHz, H₂O/D₂O (9:1)) 9.60 (s, 1 H, H-(C1)); 2.80 (t, *J* = 6.6 Hz, 2H, H-(C3)); 2.73 (t, *J* = 6.6 Hz, 2H, H-(C2)); 2.04 (s, 3H, SCH₃). ¹³C NMR (151 MHz, H₂O/D₂O (9:1)) δ 207.4 (C1); 43.0 (C3); 26.2 (C2); 14.9 (SCH₃).

2-Amino-4-(methylthio)butanenitrile (**24**): ¹H NMR (600 MHz, H₂O/D₂O (9:1)) 3.96 (t, *J* = 7.2 Hz, 1H, H-(C1)); 2.54–2.69 (m, 2H, H-(C3)); 2.06 (s, 3H, SCH₃); 1.99 (m, 2H, H-(C2)). ¹³C NMR (151 MHz, H₂O/D₂O (9:1)) δ 123.1 (CN); 42.4 (C1); 33.8 (C2); 29.6 (C3); 14.8 (SCH₃).

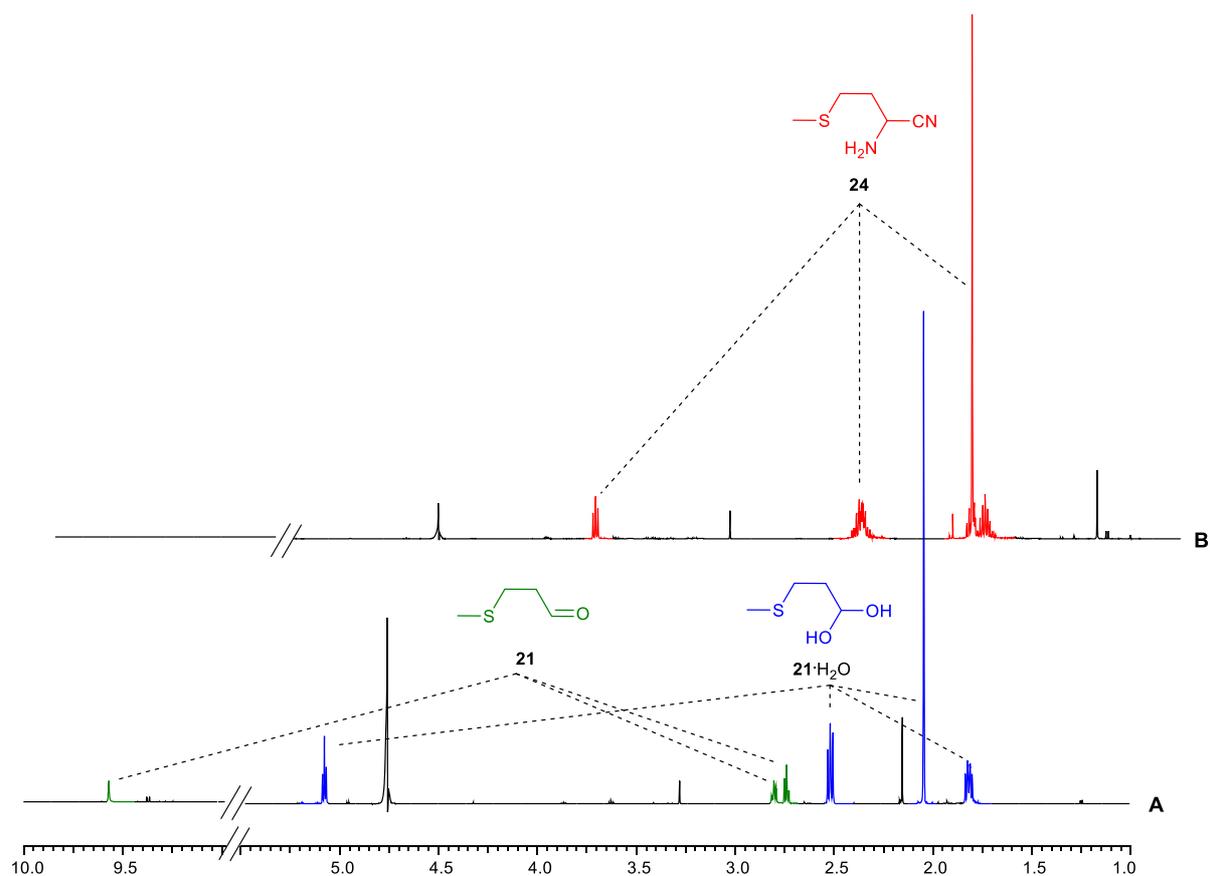
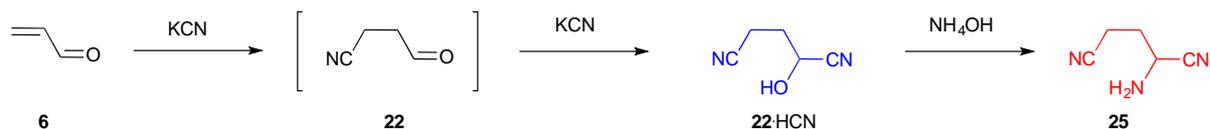


Figure S22: ^1H NMR spectra (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1); 1.0–10.0 ppm) of: **A**) 3-(Methylthio)propanal (**21**) and its hydrate (**21**· H_2O) furnished by incubation of acrolein (**6**) (140mM) and methanethiol (**23**, sat. sol.) in phosphate solution (1M, pH 7.0) at room temperature for 30 minutes. **B**) 2-Amino-4-(methylthio)butanenitrile (**24**) synthesized upon addition of potassium cyanide (1.5 eq.) and ammonium hydroxide (5 eq.) to solution shown in spectrum **A**, after 20 h at pH 10.0 and room temperature.

Incubation of acrolein (6) and potassium cyanide followed by Strecker synthesis



Potassium cyanide (45.6 mg, 0.7 mmol) was dissolved in $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1, 1 mL) and the solution was adjusted to pH 9.2 using 1M HCl. Acrolein (**6**; 9.3 μL , 0.14 mmol) was added, the solution

was adjusted to pH 9.2 with 1M HCl. The reaction was then incubated at room temperature and ^1H NMR spectra were periodically acquired. Conversion to 2-hydroxypentanedinitrile (**22**-HCN, 65% yield by ^1H NMR integration) was observed after 4 h, which was used without further purification. Then ammonium hydroxide solution (5 eq.) was added to an aliquot of the reaction mixture (0.5 mL, 0.14mM, 70 μmol) and the solution was adjusted to pH 9.2 with 1M HCl. ^1H NMR spectra were periodically acquired, and after 8 h at room temperature synthesis of 2-aminopentanedinitrile (**25**) was observed (*Figure S23, Spectrum B*).

2-Hydroxypentanedinitrile (**22**-HCN): ^1H NMR (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1)) 4.75 (obscured by HOD signal, H-(C1)); 2.68 (t, $J = 7.1$ Hz, 2H, H-(C2)); 2.08–2.27 (m, 2H, H-(C3)). ^{13}C NMR (151 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1)) δ 120.8 (CN); 60.0 (C1); 30.6 (C3); 13.3 (C2).

*The spectroscopic properties of this compound were consistent with the data reported in the literature.*¹⁰

2-Aminopentanedinitrile (**25**): ^1H NMR (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1)) 4.11 (t, $J = 7.2$ Hz, 1H, H-(C1)); 2.80 (td, $J = 7.3, 1.9$ Hz, 2H, H-(C3)); 2.17–2.31 (m, 2 H, H-(C2)).

*The spectroscopic properties of this compound were consistent with the data reported in the literature.*¹⁰

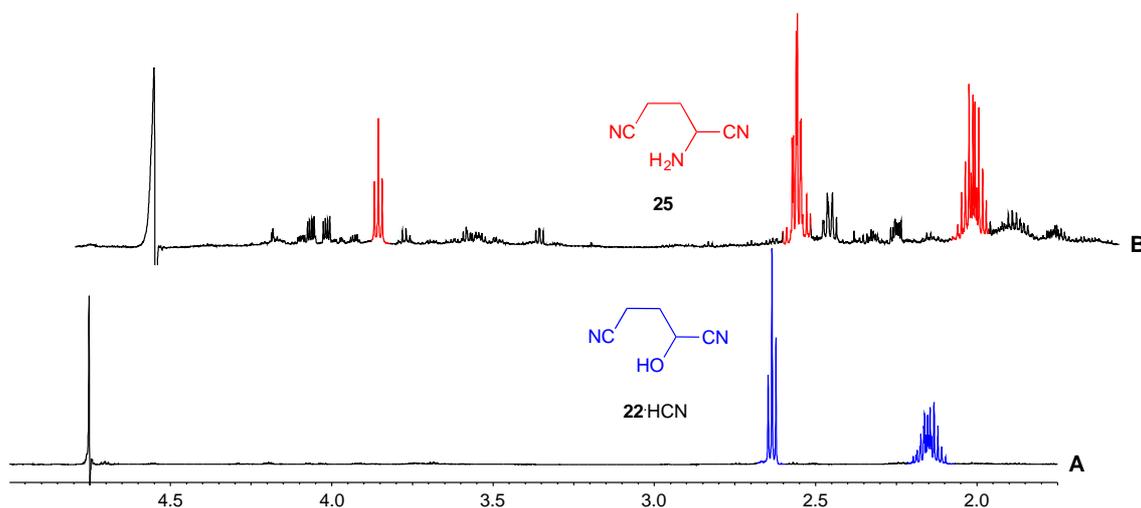


Figure S23: ^1H NMR spectra (600 MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1), 1.75–5.00 ppm) of: **A**) 2-Hydroxypentanedinitrile **7**-HCN furnished by incubation of acrolein **6** (140mM) and potassium cyanide (5 eq.) at pH 9.2 and room temperature for 4 h. **B**) 2-Aminopentanedinitrile **25** synthesized upon addition of ammonium hydroxide (5 eq.) to solution shown in spectrum **A** after 8 h at pH 9.2 and room temperature.

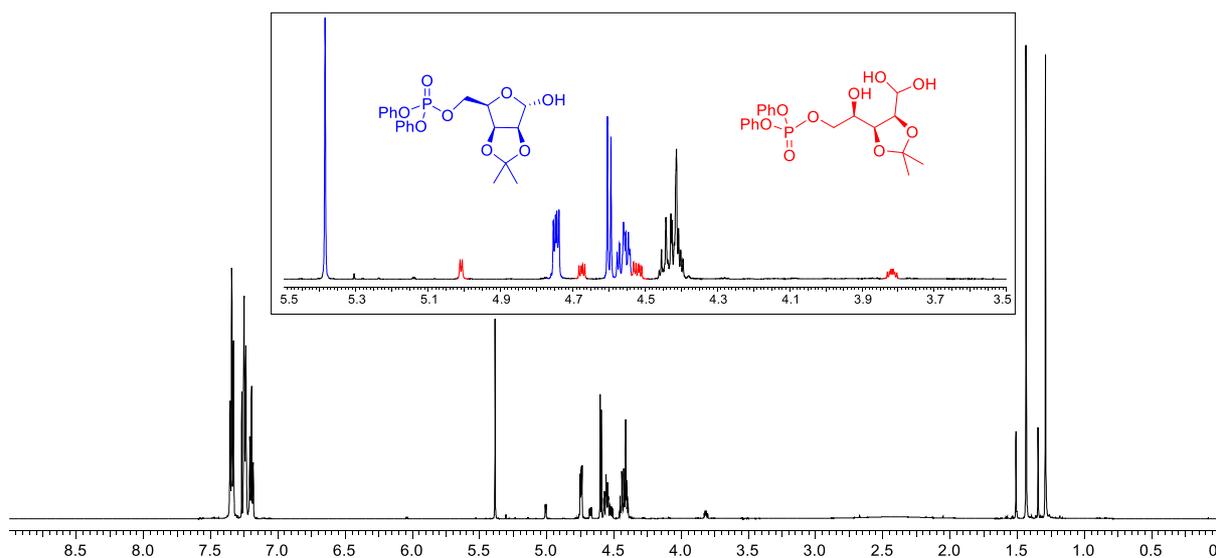


Figure S24: ^1H NMR spectra (600 MHz, CDCl_3 , 0 – 9.0 ppm) for 2,3-*O*-isopropylidene-D-5-diphenylphosphoxyfuranose (**41**). Inset: ^1H NMR spectrum (600 MHz, CDCl_3 , 3.5 – 5.5 ppm) for 2,3-*O*-isopropylidene-D-5-diphenylphosphoxyfuranose (**41**).

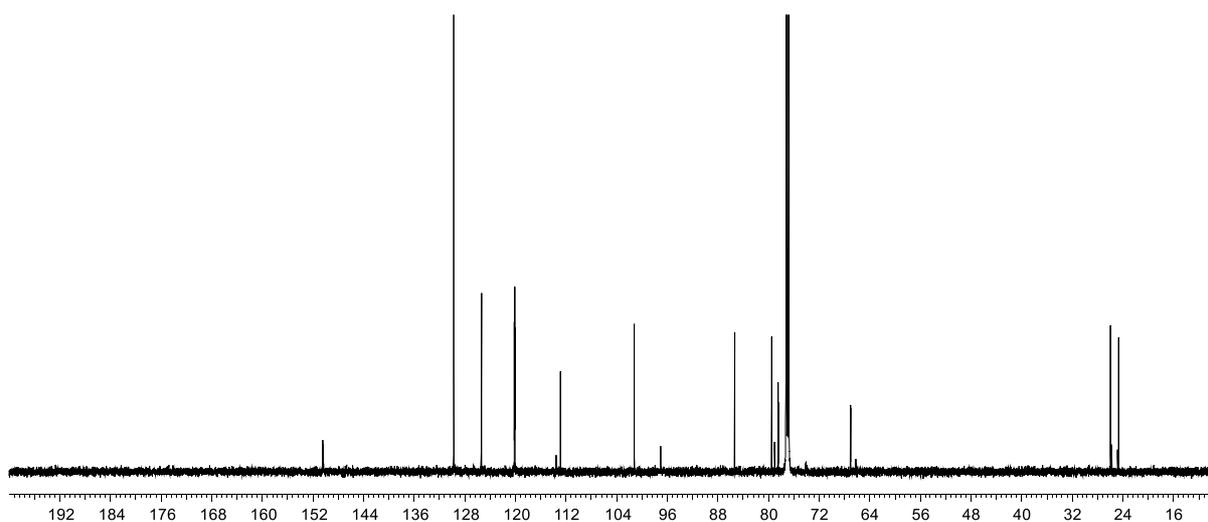


Figure S25: ^{13}C NMR spectra (150 MHz, CDCl_3 , 0 – 200 ppm) for 2,3-*O*-isopropylidene-D-5-diphenylphosphoxyfuranose (**41**).

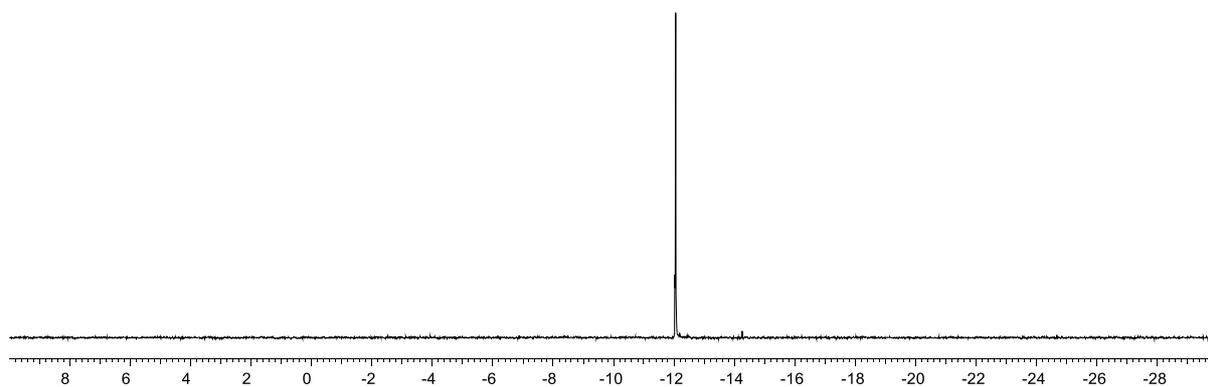


Figure S26: ^{31}P NMR spectra (162 MHz, CDCl_3 , -30 – 10 ppm) for 2,3-*O*-isopropylidene-D-5-diphenylphospholyxofuranose (**41**).

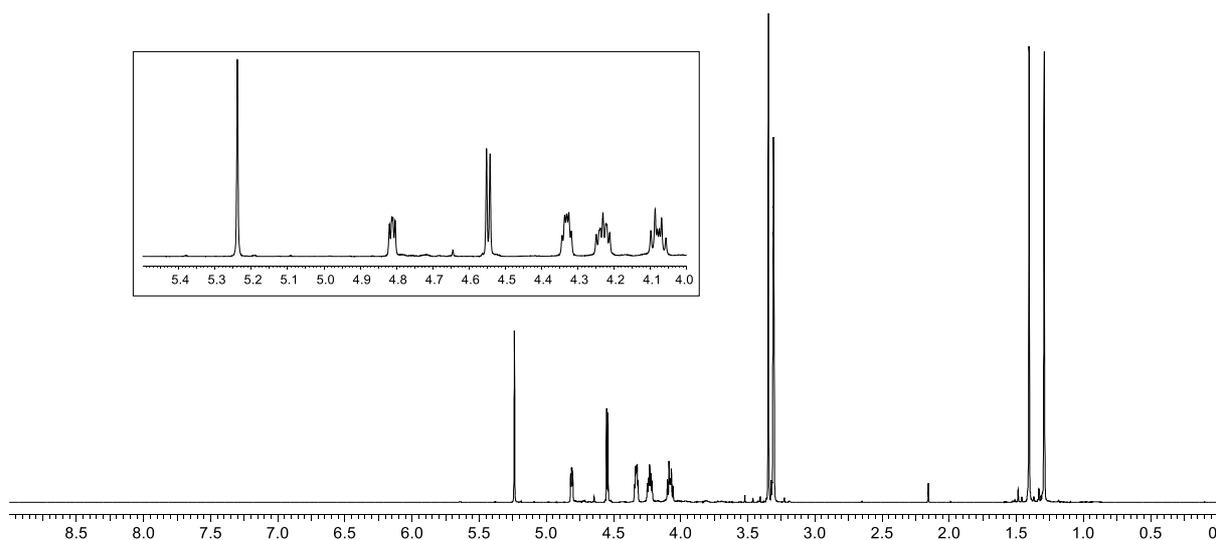


Figure S27: ¹H NMR spectra (600 MHz, CDCl₃, 0 – 9.0 ppm) for 2,3-*O*-isopropylidene-D-5-phosphoxyfuranose (**42**). Inset: ¹H NMR spectrum (600 MHz, CDCl₃, 4.0 – 5.5 ppm) for 2,3-*O*-isopropylidene-D-5-phosphoxyfuranose (**42**).

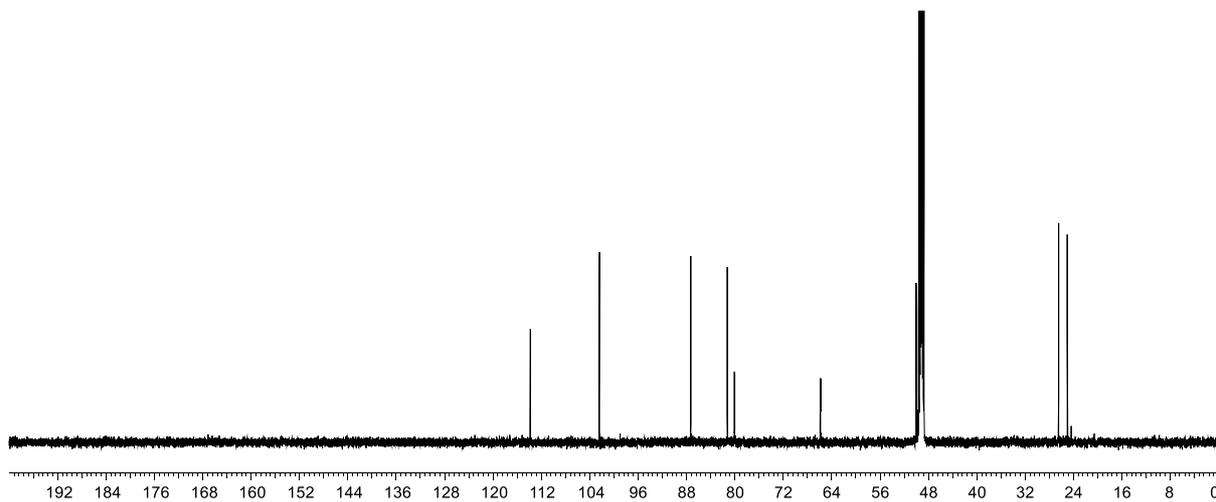


Figure S28: ¹³C NMR spectra (150 MHz, CDCl₃, 0 – 200 ppm) for 2,3-*O*-isopropylidene-D-5-phosphoxyfuranose (**42**).

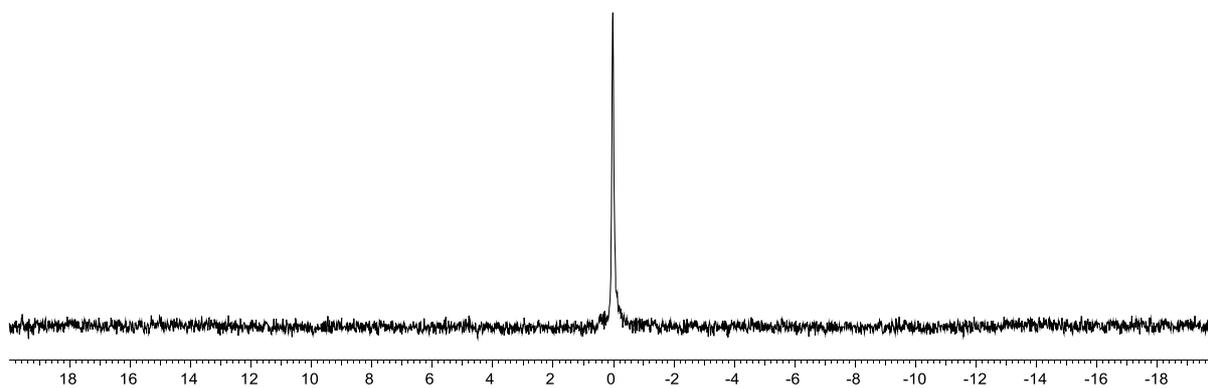


Figure S29: ^{31}P NMR spectra (162 MHz, CDCl_3 , -20 – 20 ppm) for 2,3-*O*-isopropylidene-D-5-phosphoxyfuranose (**42**).

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