Synthesis and Properties of Glycolaldehyde Di- and Triphosphate

Anthony J. Lawrence, John D. Sutherland*

Department of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK
Fax +44(161)2754939; E-mail: john.sutherland@man.ac.uk

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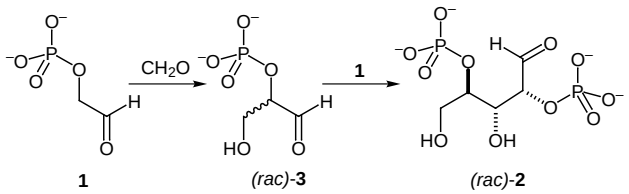
Abstract: Use of photolabile acetal protecting groups enables the synthesis of glycolaldehyde di- and triphosphate **4** and **5**, which undergo enolisation at significantly lower pH values than glycolaldehyde phosphate **1**. At pH > 10, **5** is converted to **1** and inorganic pyrophosphate.

Key words: aldehydes, bioorganic chemistry, ozonolysis, phosphorylations, photochemistry

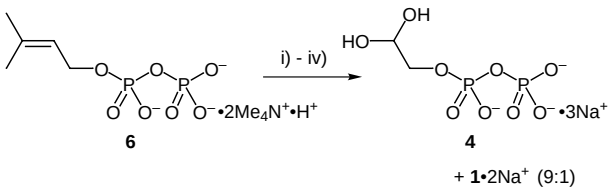
The aldolisation of glycolaldehyde phosphate **1** in the presence of formaldehyde has been shown by Eschenmoser et al. to constitute an efficient route to pentose-2,4-diphosphates with ribose-2,4-diphosphate **2** predominating.[1] The sequence involves reaction of glycolaldehyde phosphate with formaldehyde to give glyceraldehyde-2-phosphate **3** which acts as an aldol acceptor to a second molecule of **1** (Scheme 1).



Scheme 1

An aldolisation route to nucleic acid backbones would gain credence as a potentially prebiotic pathway if it could be shown to operate under conditions at which nucleic acids (in particular RNA) are stable. The phosphorylation of glycolaldehyde using amidotriphosphate[2] and the aldol reaction of **3** with **1** in the presence of double layer metal hydroxide minerals[3] both fulfil this criterion, taking place in aqueous solutions at near neutral pH. The aldol reaction of **1** with formaldehyde however only proceeds in a strongly alkaline medium,[1] conditions under which RNA is hydrolytically labile. In connection with related work we had cause to prepare glycolaldehyde diphosphate **4** and glycolaldehyde triphosphate **5** the properties of which suggest a possible solution to this problem.

Alkylation of *tris*(tetra-*n*-butylammonium) hydrogen pyrophosphate with dimethylallyl bromide in dry acetonitrile as described by Dixit et al.[4] furnished dimethylallyl diphosphate **6** which was purified by ion-exchange chromatography (Dowex® 1X8-400 eluting with an aqueous ammonium bicarbonate gradient) and then converted to the tetramethylammonium salt (treatment with H-Dowex® followed by titration to pH 7 with tetramethylammonium hydroxide solution). Ozonolysis in methanol at –78 °C followed by reductive work-up then provided **4** contaminated with ca. 10% **1** as judged by ^{1}H and ^{31}P NMR (Scheme 2).[1,5] No resonances for non-hydrated aldehydic protons were observed in the ^{1}H NMR spectrum indicating that both species were > 95% hydrated. It is not clear how **1** is formed during this procedure although it is possible that a carbonyl oxide or α-methoxy-hydroperoxide attacks the α-phosphorus nucleophilically resulting in pyrophosphate cleavage.[6] Fortuitously the monophosphate **1** served as a convenient internal standard in subsequent experiments. Although dimethylallyl triphosphate was successfully prepared by alkylation of *tetrakis*(tetra-*n*-butylammonium) hydrogen triphosphate, subsequent ozonolysis with reductive work-up gave at least four glycolaldehyde derivatives by ^{1}H NMR.



Scheme 2 *Reagents and conditions:* i) O_3, MeOH, –78 °C; ii) Me_2S; iii) repeated evaporation from H_2O; iv) Na-Dowex®

Investigation of the sample of **4** containing **1** by ^{1}H NMR (in D_2O) revealed that the α-protons of **4** fully exchange at 20 °C, pD 8.0 after 4 days ($t_{1/2}$ ca. 20 h) whereas those of **1** do not (Figure 1). The greater electron-withdrawing capacity of the diphosphate group compared to the monophosphate group is probably responsible for this phenomenon. The increase in electron-withdrawal presumably biases the hydration equilibrium of **4** in favour of hydrate further than that of **1** but the residual aldehyde of **4** is rendered intrinsically more prone to enolisation than the residual aldehyde of **1**. Evidently the effect on intrinsic enolisation rate outweighs the effect on the hydration equilibrium. This extremely subtle electron-demand/hydration controlled enolisation chemistry prompted us to establish a secure synthetic route to **5** and a cleaner route to **4**.

Unable to use late stage ozonolysis to reveal the sensitive aldehyde groups of **4** and **5**, we developed a strategy based

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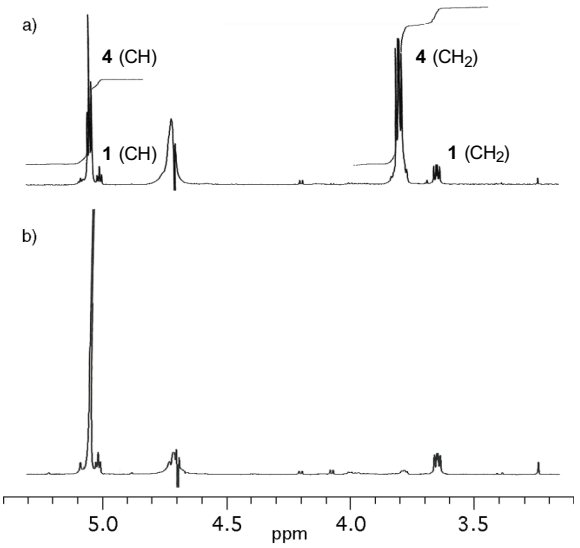
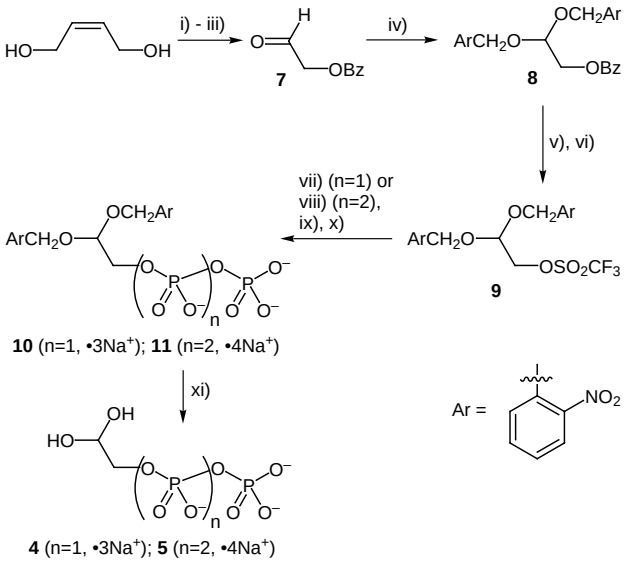


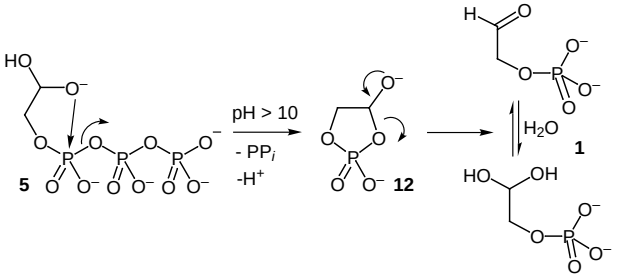
Figure 1 ^{1}H NMR spectra (500 MHz, D_2O, pD 8.0 with HOD suppression) of a mixed sample containing **1** and **4** (1:9) maintained at 20 °C; a) t = 0, b) t = 4 days



Scheme 3 *Reagents and conditions:* i) BzCl, pyridine; ii) O_3, CH_2Cl_2, –78 °C; iii) Me_2S; iv) *o*-NO_2-$C_6H_4CH_2OSiMe_3$, $Me_3SiOSO_2CF_3$, –78 °C to r.t. (over 3 h); v) LiOH (1 M), H_2O/1,4-dioxan (1:1); vi) $(CF_3SO_2)_2O$, pyridine/CH_2Cl_2, 0 °C, 2 min ($HCl_{(aq.)}$ (1 M) quench); vii) $(Bu^n{}_4N)_3HP_2O_7$, CH_3CN; viii) $(Bu^n{}_4N)_4HP_3O_{10}$, CH_3CN; ix) HPLC (C-18: $Et_3NH{\cdot}HCO_3$ (50 mM), CH_3CN gradient); x) Na-Dowex®; xi) hν, EtOAc:dil. $HCl_{(aq.)}$ (pH 4.5)

upon the use of photolabile protecting groups. Benzoyl glycolaldehyde **7** was prepared conveniently by quantitative dibenzoylation of *Z*-but-2-ene-1,4-diol followed by ozonolysis with reductive work-up in 86% yield. The *bis*(*o*-nitrobenzyl) acetal **8** was obtained in 70% yield using Noyori's procedure;[7,8] other methods were unsuccessful (Scheme 3). Saponification of the ester function of **8** in 75% yield followed by careful triflylation (quantitative) gave the key intermediate **9**. Displacement of the triflate with *tris*(tetra-*n*-butylammonium) hydrogen pyrophosphate followed by reverse-phase HPLC gave the acetal **10** in 16% yield (compromised for purity). Deprotection at pH 4.5 by irradiation with a standard slide projector in a biphasic solvent system[9] proceeded smoothly to give **4** in quantitative yield and a high state of purity as judged by ^{1}H and ^{31}P NMR. All data were consistent with those obtained from the route outlined in Scheme 2.[5] Displacement of the triflate with *tetrakis*(tetra-*n*-butylammonium) hydrogen triphosphate was successful and, after similar HPLC purification, **11** was obtained in 33% yield (again compromised for purity) from **9**. Photolytic deprotection of **11** proceeded smoothly and the triphosphate **5** was obtained in quantitative yield, in a high state of purity as judged by ^{1}H and ^{31}P NMR.[10]

With pure samples of **4** and **5** in hand, an investigation of their solution behaviour was undertaken. The two compounds were found to undergo comparable H/D exchange ($t_{1/2}$ ca. 20 h) in D_2O at 20 °C, pD 8.0; conditions under which **1** remains unchanged. Further studies were carried out with **5** at higher pH values. At pH 10 and above, **5** was observed to hydrolyse to **1** and inorganic pyrophosphate (^{1}H, ^{31}P NMR) with a half-life of ca. 1 hour at pH 10.5, 20 °C. Since the hydrolysis is not seen below pH 10 and the *gem*-diol of **5** is likely to have a pK_a near 10 it is probable that the hydrolysis mechanism involves attack of the hydrate alkoxide on the α-phosphorus atom resulting in the displacement of inorganic pyrophosphate and the formation of the cyclic intermediate **12** which can then ring-open to **1** (Scheme 4).[11]



Scheme 4 Proposed mechanism for the conversion of **5** to **1**

The ease with which **5** enolises coupled with its propensity for hydrolysis suggests that it might be possible at lower pH values, where RNA is stable, for **5** to aldolise with formaldehyde giving glyceraldehyde-2-triphosphate which could then hydrolyse to **3**. Current work is aimed at addressing this possibility and at investigating possible prebiotic routes to **5**.

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(5) **Compound 4**: ^{1}H NMR (500 MHz, D_2O, pD 8.0): δ = 3.79 (2 H, dd, *J* = 4.5, *J* = 6.5, CH_2), 5.04 (1 H, t, *J* = 4.5, CH); ^{13}C NMR (125 MHz, D_2O, pD 8.0): δ = 67.9 (d, *J* = 5.0), 88.5 (d, *J* = 9.0); ^{31}P NMR (83 MHz, 10 mM EDTA in D_2O, pD 8.0): δ = –9.25 (d, *J* = 22.0), –5.06 (d, *J* = 22.0); MS (ES-, CV -15 V): *m/z* (%) = 237(100), $[M^{3-} + 2\ H^{+}]^{-}$; MS (ES-, CV -25 V): *m/z* (%) = 237(100), $[M^{3-} + 2\ H^{+}]^{-}$), 219(100), $[M^{3-} + 2\ H^{+} - H_2O]^{-}$); MS (ES-, CV -35 V): *m/z* (%) = 219(100), $[M^{3-} + 2\ H^{+} - H_2O]^{-}$).

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(10) **Compound 5**: ^{1}H NMR (300 MHz, D_2O, pD 8.5): δ = 3.78 (2 H, dd, *J* = 4.5, *J* = 7.5, CH_2), 5.04 (1 H, t, *J* = 4.5, CH); ^{13}C NMR (75 MHz, D_2O, pD 8.5): δ = 68.1 (d, *J* = 5.5), 88.3(brs); ^{31}P NMR (83 MHz, 1 mM EDTA in D_2O, pD 9.0): δ = –20.85 (t, *J* = 19.5), –9.70 (d, *J* = 19.5), –4.95 (d, *J* = 19.5); MS (ES-, CV -35V): *m/z* (%) = 321(20), $[M^{4-} + 2\ H^{+} + Na^{+} - H_2O]^{-}$), 299(5) $[M^{4-} + 3\ H^{+} - H_2O]^{-}$).

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