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Solution Structures of Bis(glycoaldehyde) Phosphodiester and Mixed Glycoaldehyde-triose Phosphodiesters

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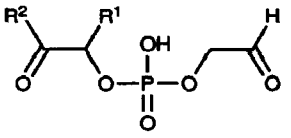
Abstract The solution structures of the potentially prebiotic compounds, bis(glycoaldehyde) phosphodiester **1**, dihydroxyacetonephosphoglycoaldehyde **2** and glyceraldehyde-2-phosphoglycoaldehyde **3** have been elucidated. The aldehyde moieties of **1**, **2**, and **3** are fully hydrated in aqueous solution whereas the ketone moiety of **2** is only approximately 50% hydrated. The hydration behaviour of **2** results in ketone enolisation being kinetically preferred to aldehyde enolisation.

Introduction

In a previous paper[1] we have presented a novel retrosynthetic analysis of the RNA polymer that suggests a potentially prebiotic synthesis involving aldol polymerisation chemistry, intramolecular redox transfer and ring closure *via* a mesomeric heterocyclic betaine intermediate. Our analysis suggests that bis(glycoaldehyde) phosphodiester **1** and simple glycoaldehyde-triose mixed phosphodiesters **2** and **3**, Fig. 1, might be important in the prebiogenesis of RNA in that they might function as intermediates in the production of our proposed RNA polymerisation monomer **4** (B=A, C, G or U).



1 R^1=R^2=H
2 R^1=H, R^2=CH_2OH
3 R^1=CH_2OH, R^2=H
4 R^1=H, R^2=CH_2B

Fig. 1. Potentially Prebiotic Phosphodiesters

Accordingly we have initiated a programme aimed at synthesising such mixed phosphodiesters and studying their behaviour in aqueous solution. In the preceding paper we have described efficient syntheses of bis(glycoaldehyde) phosphodiester **1**, dihydroxyacetonephosphoglycoaldehyde **2** and glyceraldehyde-2-phosphoglycoaldehyde **3** and in this paper we report upon the structures adopted by these compounds in aqueous solution.

Results and discussion

Bis(glycoaldehyde) phosphodiester **1** has previously been prepared by Eschenmoser's group by ozonolysis of diallyl-hydrogenphosphate[2]. They reported ^{1}H NMR data for the trimethylammonium salt and assigned the bis hydrate structure to **1** in aqueous solution. Our data [δ_H (200 MHz, D_2O) 4.89 (2H, t, C<u>H</u>$(OH)_2$, *J* 5), 3.52 (4H, dd, C<u>H</u>$_2$OP, *J* 5, 7); δ_C (50 MHz, D_2O) 85.65 (d, *J* 9), 65.7 (d, *J* 5)] for the parent compound are in full accord with this assignment, in particular the presence of a doublet at δ = 85 ppm in the ^{13}C NMR spectrum and the absence of any signal further downfield are strongly indicative of both the aldehyde groups of **1** being fully hydrated. The situation with dihydroxyacetonephosphoglycoaldehyde **2** is more complicated and is extremely interesting in light of our retrosynthetic analysis of RNA[1]. The proton-decoupled ^{31}P NMR spectrum revealed there to be two phosphodiester species present in solution in an approximately 1:1 ratio [δ_P

D_2O (101.2 MHz) 0.806 (s), 1.306 (s)]. ^1H and ^{13}C NMR spectra indicated that these two species resulted from the ketone moiety of **2** being approximately 50% hydrated: δ_H (500 MHz, D_2O) 5.11 (1H, t, $C\underline{H}(OH)_2$, *J* 5), 5.09 (1H, t, $C\underline{H}(OH)_2$, *J* 5), 4.58 (2H, d, $OC\underline{H}_2C{=}OCH_2OH$, *J* 8), 4.44 (2H, s, $HOC\underline{H}_2C{=}O$), 3.80 (2H, d, $OC\underline{H}_2C(OH)_2CH_2OH$, *J* 5), 3.75 (2H, t, $OC\underline{H}_2CH(OH)_2$, *J* 7), 3.74 (2H, t, $OC\underline{H}_2CH(OH)_2$, *J* 7), 3.52 (2H, s $HOC\underline{H}_2C(OH)_2$); δ_C (125.7 MHz, D_2O) 209.26 (d, C=O, *J* 7), 94.73 (d, $CH_2\underline{C}(OH)_2CH_2$ *J* 8), 88.80 (d, $\underline{C}H(OH)_2$, *J* 8), 88.73 (d, $\underline{C}H(OH)_2$, *J* 8), 68.36, 68.27, 68.23, 67.17, 65.55, 63.94. The negative ion electrospray mass spectrum of **2** gave peaks at *m/z* 210.5 and 228.7 consistent with the dicarbonyl form (*calc.* 211) and a monohydrated form (*calc.* 229) however it is known that, whilst this form of mass spectroscopy can detect hydration of carbonyl groups, the degree of hydration is less than that in solution[3].Similar spectroscopic analysis suggests that glyceraldehyde-2-phosphoglycoaldehyde **3** adopts the bis-hydrate structure in aqueous solution: δ_H (200 MHz, D_2O) 5.14 (1H, t, $CH_2C\underline{H}(OH)_2$, *J* 5), 5.10 (1H, d, $CHC\underline{H}(OH)_2$, *J* 4.5), 4.09-3.97 (1H, m, $C\underline{H}$), 3.86-3.73 (4H, m 2x $C\underline{H}_2$); δ_C (50 MHz, D_2O) 89.57 (d, $\underline{C}H(OH)_2$, *J* 7), 89.13 (d, $\underline{C}H(OH)_2$, *J* 11), 79.24 (d, $\underline{C}HCH(OH)_2$, *J* 9), 68.63 (d, $\underline{C}H_2$, *J* 7), 61.50 (d, $\underline{C}H_2$, *J* 7).

The importance of this hydration behaviour in aqueous solution *vis à vis* the potential prebiotic synthesis of RNA we have proposed[1] can be appreciated by considering the potential intermolecular aldol behaviour of **2**. Eschenmoser's group have clearly demonstrated that the aldol chemistry leading from glycoaldehyde phosphate and formaldehyde to ribose-2,4-diphosphate is kinetically controlled during the first week of reaction[4]. It thus appears reasonable to consider the potential aldol chemistry of **2** from a kinetic point of view[5]. Depending on concentration, the kinetic control must be exerted either in the formation of the enolate or in the subsequent aldol step. We consider first the potential effect of the hydration equilibrium on the kinetics of enolate formation. The effect of a highly displaced hydration equilibrium on the rate of enolate formation has not been given wide coverage in the literature. It has been noted, however, that whilst hexafluoroacetylacetone is a strong acid in aqueous solution, titration with sodium hydroxide takes several hours[6]. This data has subsequently been interpreted as indicating that the stable form of the anion is the enolate which is formed slowly from the trace of unhydrated ketone present[7]. In other words, formation of the enolate from a carbonyl group electronically and sterically predisposed towards enolate formation can be substantially slowed in water because the same factors favour displacement of the hydration equilibrium to delayingly high levels of the unreactive hydrate. Both inductive and steric effects are involved in determining the position of a hydration equilibrium (assessed by the value of K_d where $K_d = [R_1R_2C{=}O]/[R_1R_2C(OH)_2]$) but the predicted *sc sc* conformation of **2** has the two alkyl groups disposed away from each other in space so any steric effect should be minimum leaving the inductive effect predominant. The dilithium salt of dihydroxyacetonephosphate has $K_d = 1.2$ at pH 7 but $K_d = 0.82$ at pH 1 reflecting the difference in magnitude of the inductive effect of a phosphate group as it is protonated. Our data for **2** ($K_d \sim 1$ for the ketone moiety at pH 7) suggest that the inductive effect of a phosphomonoester substituent is qualitatively similar to that of a protonated phosphate group. An accurate value for the hydrate dissociation equilibrium constant, K_d is unavailable for glycoaldehyde monophosphate but the NMR spectrum of this monoester at pH 7 in which an aldehyde signal is barely discernible suggests that K_d is most likely less than 0.05 (by way of reference; K_d for chloroacetaldehyde = 0.027[8]). The glycoaldehyde moiety in **2** would be expected to be even more hydrated than glycoaldehyde monophosphate since **2** is a phosphodiester. The data and arguments presented here indicate that the value $[C{=}O]_{ket}/[C{=}O]_{ald}$ for **2** is greater than 10.

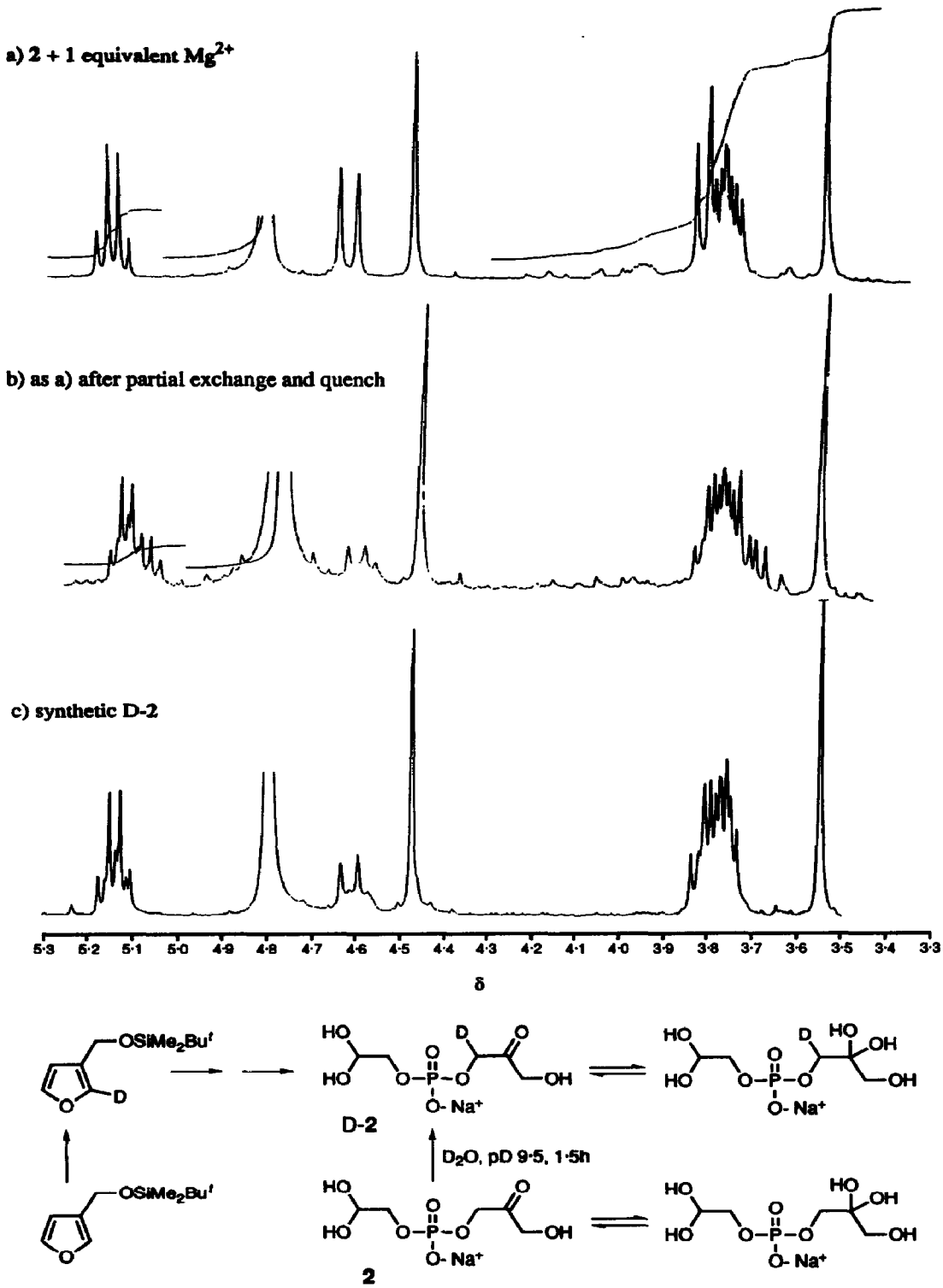


Fig. 2. Enolisation Behaviour of 2 Above: ^1H NMR (200MHz, D_2O)

The rate of enolisation of a carbonyl group depends on the product of the intrinsic rate constant and the concentration of the carbonyl group (rate – k[C=O]). To investigate whether the hydration behaviour of **2** will result in faster ketone enolisation (ie. whether $k_{ket}[C=O]_{ket} > k_{ald}[C=O]_{ald}$ because $[C=O]_{ket} >> [C=O]_{ald}$ despite the fact that k_{ald} would be expected to be greater than k_{ket}) we allowed a solution of **2** in D_2O at an apparent pD of 9.5 to exchange partially. To our delight the ketone was observed to enolise faster and after 1·5h the sample was quenched to pD 7 and appeared to be predominantly monodeuterated, Fig. 2, this was confirmed by comparison with an authentic sample of monodeuterated material synthesised from 2-deutero-3-*tert*-butyldimethylsilyloxymethyl-furan using the procedure described in the preceding paper. Given that the ketone enolate is formed kinetically then it can undergo intermolecular aldol reaction either with another ketone or with an aldehyde group. Similar kinetic arguments apply again but for this reaction the intrinsic rate constant for attack of a ketone is much smaller than that for attack of an aldehyde for steric reasons. It is thus possible that the hydration and hence enolisation behaviour of **2** will predispose aldol chemistry to produce a pentose-3,5-diphosphate linked polymer.

The hydration behaviour of **4** (B = A or U) is very similar to that of **2** (unpublished results) augurring well for the outcome of aldol polymerisation experiments with **4**. Such experiments are now being carried out and will be reported in due course. Finally, it is worth noting that the proposal outlined in the first paper out of three in this issue lead us to synthesise **2**, and that the hydration behaviour of **2** subsequently uncovered offers a means whereby the aldol reaction suggested for the potentially prebiotic synthesis of RNA appears less naive. It remains to be seen whether our proposal has any additional substance particularly in light of Eschenmoser's recent suggestion that RNA might be produced by isomerisation of pyranosyl-RNA[9].

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References and footnotes

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5 Preliminary experiments on the aldol behaviour of **1** with formaldehyde indicate that it is substantially more reactive than glycoaldehyde monophosphate, this behaviour is likely to be true throughout the diester series (Sutherland, J. D.; Weaver, G. W. unpublished work). Our aldol experiments are conducted in the presence of Mg^{2+} since this ion is crucial for the function of most catalytic RNA species and because of its presumed prebiotic presence.

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