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Studies on a Potentially Prebiotic Synthesis of RNA

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Abstract A novel, potentially prebiotic synthesis of RNA is proposed in which the key steps are an aldol mode of polymerisation, intramolecular redox transfer and ring closure *via* a mesomeric heterocyclic betaine intermediate. The key monomers in this scheme are derivable, in theory, from the presumed prebiotic compound, bis(glycoaldehyde) phosphodiester 1 *via* its mono-formaldehyde aldol adduct 2.

Introduction

The recent discoveries that RNA has both catalytic potential[1] and the capacity to evolve *in vitro*[2] have added experimental weight to the theory of an 'RNA world'[3]. In this theory RNA is both the purveyor of genetic information and the catalyst of its own replication. In attempts to explain how the first RNA molecules might have arisen on the primitive Earth, most workers in this area have analysed RNA in a subjective retrosynthetic sense[4] and disconnected the polymer by an oxygen-phosphorus bond cleavage to activated nucleotide monomers. This *superficially* simple phosphodiester disconnection could, in principle, lead to activated 2', 3' or 5'-phosphonucleosides as RNA polymerisation precursors although most favour the latter due to the propensity of the former to undergo cyclisation rather than polymerisation[4] and also, one presumes, because RNA is currently biosynthesised in this way. Much work has been done in this area and it has been shown that, under carefully optimised conditions, oligomerisations can be contrived to take place in the absence of any enzymic catalysis[5]. There are however many problems with this approach[6]. The activated precursors are prone to hydrolysis[7], 2'-5'-linkages are almost always formed along with the desired 3'-5'-linkages[8], 5'-5'-pyrophosphate linkages are also formed[9], there exists the phenomenon of enantiomeric cross-inhibition[10] and perhaps most importantly no potentially prebiotic route to the activated precursors has been demonstrated[11]. Many have invoked alternative polymers[12] (and even minerals[13]) as RNA precursors because of the problems seemingly associated with RNA synthesis. We believe however that a potentially prebiotic synthesis can be found, but only by considering an alternative retrosynthetic disconnection of the RNA polymer in which carbon-carbon bond forming reactions are involved in the polymerisation. This disconnection involves cleaving the ribose moiety of the RNA polymer rather than a phosphodiester linkage and we were prompted to investigate aldol disconnections as being the most likely in this context[14]. For aldol disconnections to be possible however some kind of intramolecular redox transfer is essential so that a carbon atom other than C-1' is at the carbonyl oxidation level. Two ways in which C-1' could be retrosynthetically converted into a reducible centre were considered. In the first, Fig. 1, the base is lost at the behest of the antiperiplanar ring oxygen lone-pair to give an oxonium ion.

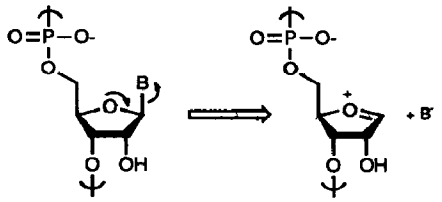

Fig. 1. Oxonium Ion Glycosyl-Disconnection

This option was deemed unlikely in the synthetic sense as conditions for oxonium ion formation (Lewis acid catalysis on an appropriate precursor) would appear not be compatible with the bases being in a nucleophilic (deprotonated) form. In addition we had it in mind that, while most electrophiles react with the four component bases of RNA in a non-selective manner, Michael acceptors are known to alkylate pyrimidines cleanly at N-1[15] and adenine at N-9[16]. We thus wished to introduce the bases by Michael addition at an earlier stage in the synthesis. Further to these considerations is a belief that the bases might be important in the assembly and reactions of the polymerisation monomer by some form of tautomeric catalysis[17]. The second option for glycosyl disconnection involved endocyclic cleavage driven by the (albeit weakly available) glycosyl nitrogen lone-pair, Fig. 2.

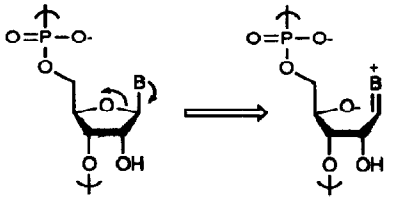

Fig. 2. Iminium Ion Glycosyl-Disconnection

This option initially looked discouraging because of the anticipated instability of such iminium ions attached to the component bases. At this point however we realised that such iminium ions could be stabilised by deprotonation of the base to produce mesomeric heterocyclic betaines. Literature analysis failed to indicate any stable betaines of this exact form but did reveal, *inter alia*, the natural product, Herbipolin[18], Fig. 3, which is a stable mesomeric heterocyclic betaine[19].

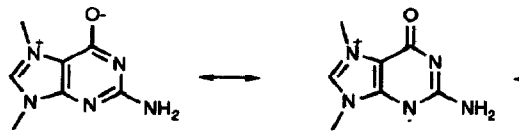

Fig. 3. Herbipolin

Whilst the guanine and uracil bases of unbase-paired RNA can be significantly deprotonated (at N-1 and N-3 respectively) at pH values at which RNA is resistant to base catalysed hydrolysis, the adenine and cytosine bases cannot. Deprotonation of A or C in base-paired RNA however is conceivable because base-pairing can be maintained if minor base tautomers[20] are invoked. If this deprotonation occurs prior to or at the same time as endocyclic ribose cleavage then stable, base-paired mesomeric heterocyclic betaines would result, this phenomenon is illustrated in Fig. 4.

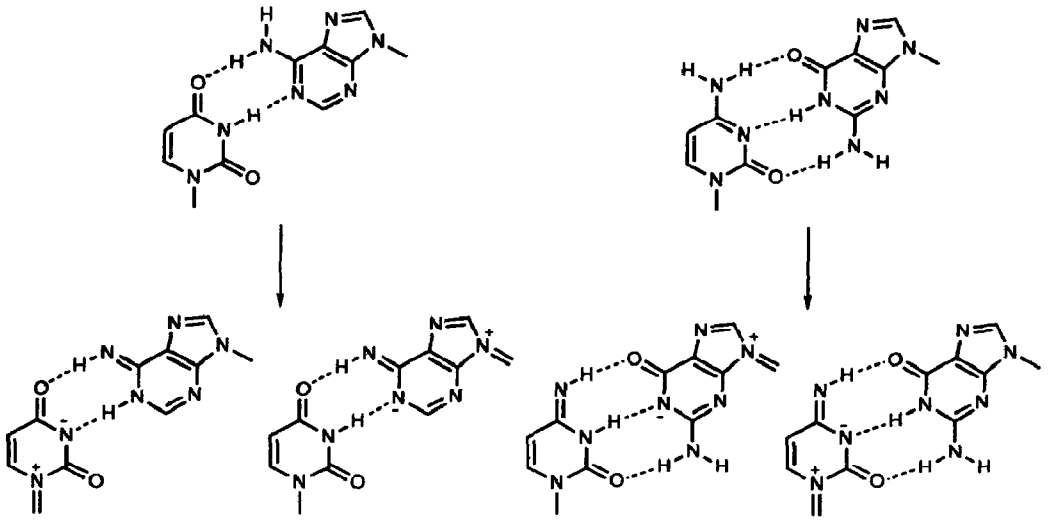

Fig. 4. Base-Paired Mesomeric Heterocyclic Betaines

To reach a point where the aldol disconnection can be applied redox transfer between C-1' and C-2' is necessary. Formally this can be achieved by an Amadori process (involving azaenolisation followed by ketonisation) but, for a variety of reasons, we prefer a 1,2 hydride shift (reminiscent of a base catalysed α-ketol rearrangement) assisted, in the retrosynthetic sense, by intramolecular general base catalysis from the C-4' alkoxide, Fig. 5.

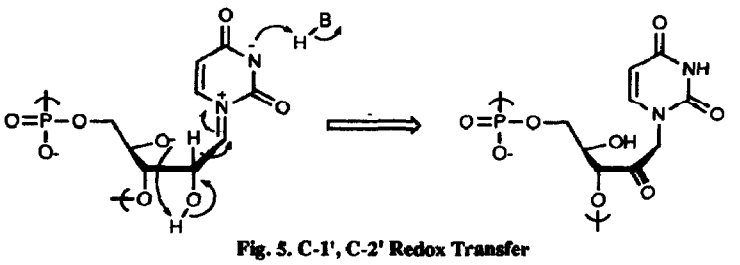

Fig. 5. C-1', C-2' Redox Transfer

Associated with the redox transfer the base pair would be expected to be reprotonated. With this disconnection the target was now in a form where the aldol disconnection could be applied, Fig. 6.

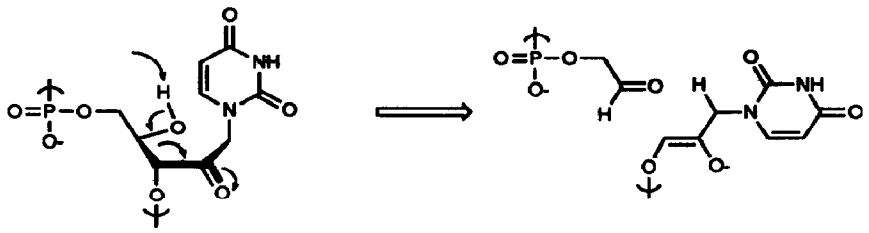

Fig. 6. Aldol Disconnection

The retrosynthetic analysis presented above thus reduces the RNA polymer to the achiral precursor monomer **5**[21], Fig 7. A chemically plausible route whereby this monomer might be produced from the presumed prebiotic compound, bis(glycoaldehyde) phosphodiester **1**[22], formaldehyde and the component bases is shown in Fig. 7. The starting point is bis(glycoaldehyde) phosphodiester **1** which Eschenmoser's group have shown to be produced by reaction of inorganic phosphate and either aziridinecarbonitrile or oxiranecarbonitrile. It is proposed that this compound undergoes base catalysed aldol condensation with formaldehyde to give the adduct **2**[23] which can dehydrate to give the phosphoenolpyruvaldehyde derivative **3**. Michael addition of a component base to **3** then gives the enolate **4** which is set up to rearrange to the enolate form of the proposed monomer **5**.

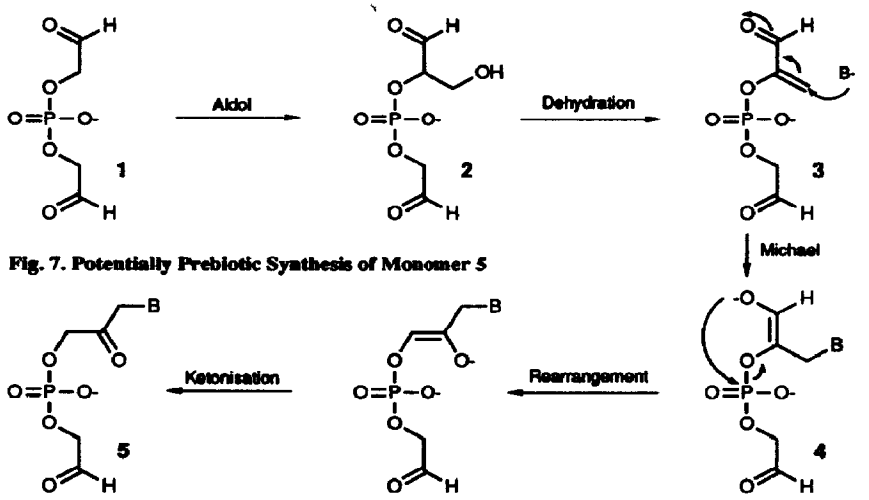

Fig. 7. Potentially Prebiotic Synthesis of Monomer *5*

This proposed enolate rearrangement has some literature precedent in the work of Ramirez *et al.*[24] who showed that acetoin enediolcyclophosphate **6** reacts with alcohols to give mixed phosphodiesters **7** of the alcohol and acetoin, Fig. 8. This reaction is proposed to proceed *via* nucleophilic addition of the alcohol to **6** to generate a trigonal bipyramidal pentacoordinate oxyphosphorane intermediate **8** which exists in rapidly interconverting forms by pseudorotational isomerism[25], Fig. 8. Loss of the enol leaving group and tautomerisation then furnishes the product **7**.

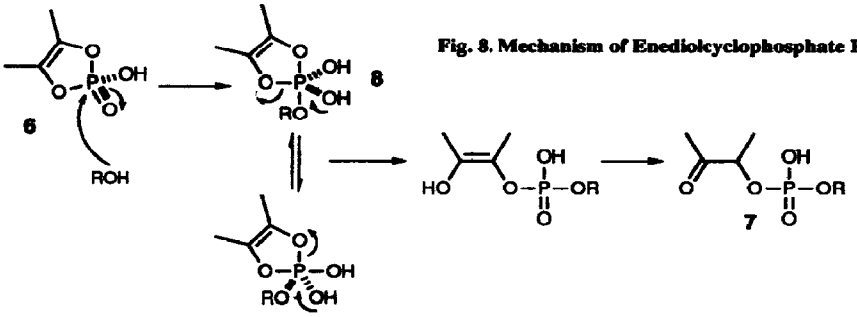

Fig. 8. Mechanism of Enediolcyclophosphate Ring Opening

By the principle of microscopic reversibility the ring opened enol intermediate can revert to the pentacoordinate oxyphosphorane **8** and this can pseudorotate before ring opening again. In principle therefore, a phosphodiester of an 'unsymmetrical' α-hydroxycarbonyl compound can isomerise *via* this mechanism.

The retrosynthetic analysis outlined in this paper is novel and requires experimental evaluation. In order to provide such evaluation we have initiated a programme aimed at synthesising compounds **1**, **2**, **3**, **4** (the aldehyde form) and **5** and examining their behaviour with respect to the foregoing proposals. Synthetic work directed at some of these targets and preliminary results of potentially prebiotic reactions are described in the following papers in this issue.

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