Pergamon

Tetrahedron, Vol. 53, No. 34, pp. 11595-11626, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00466-3

Studies on a Potentially Prebiotic Synthesis of RNA

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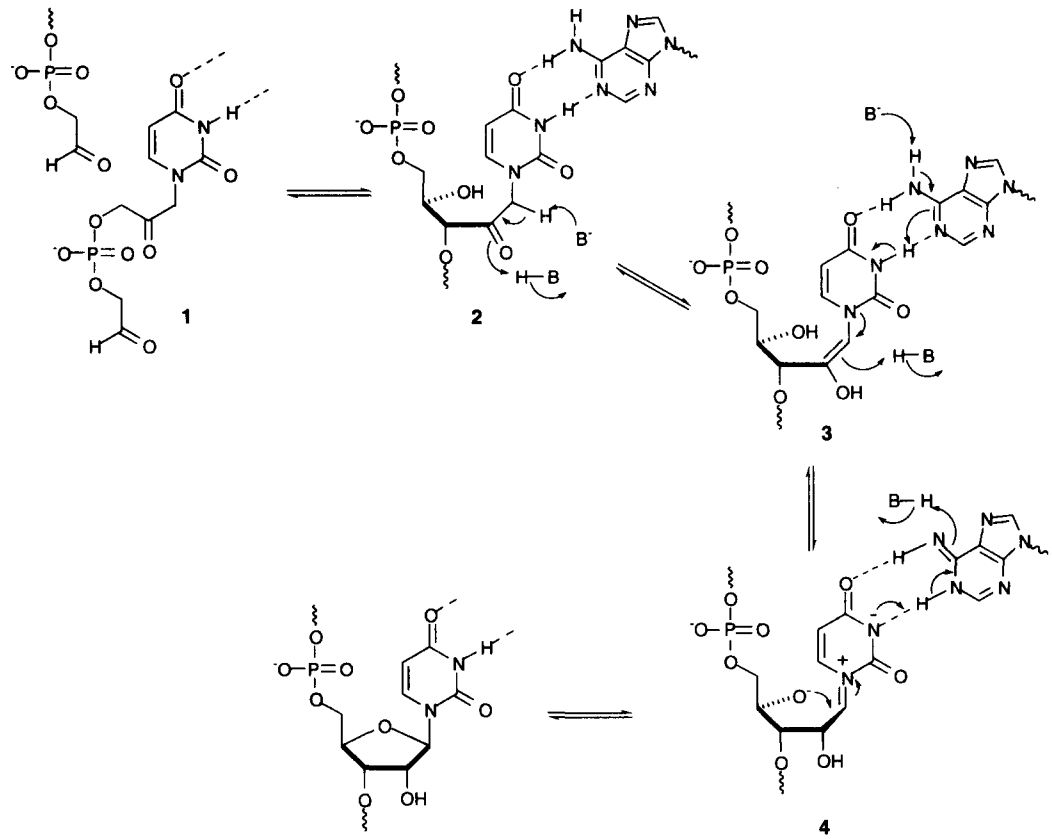
Abstract Current thinking supports an early phase of evolution in which information transfer and catalysis were mediated by evolving RNA. A novel potentially prebiotic synthesis of RNA is proposed involving polymerisation through aldol condensation followed by a retro-Amadori rearrangement and ring closure *via* a base-paired mesomeric heterocyclic betaine intermediate. The proposed monomer **1** is an achiral mixed phosphodiester and herein we report synthetic routes to **1** containing each of the four RNA bases. The solution phase behaviour of **1** containing adenine and uracil has been investigated and preliminary results of polymerisation experiments are also presented. © 1997 Elsevier Science Ltd.

Introduction

The origin of life is one of the most significant questions of biology to remain unanswered. The most widely accepted theory suggests that there was a time before the origin of protein synthesis when life was comprised of replicating RNA species, a scenario which has been termed the 'RNA world'.[1] This theory has gained credence with the recent discoveries that RNA has both catalytic potential[2,3] and the capacity to evolve *in vitro*,[4, 5, 6, 7] properties which potentially enable it to catalyse its own replication in addition to acting as a carrier of genetic information. A plausible potentially prebiotic synthesis of RNA is a central requirement of the 'RNA world' hypothesis but remains to be demonstrated. As discussed in the preceding report, we recently proposed a novel, potentially prebiotic route to RNA involving aldol polymerisation of monomers **1**, **Scheme 1**. Following the initial aldol condensation it is proposed that a retro-Amadori rearrangement of **2** could occur involving participation of the nitrogen lone-pair of the component base. The Amadori rearrangement[8] involves azaenolisation followed by ketonisation and is seen in the contemporary biosynthesis of tryptophan, histidine and several coenzymes.[9, 10] Subsequent 5-exo-trig ring-closure of **4** to form the ribose moiety completes the synthesis, driven by the neutralisation of positive charge in the component base.

In order to test the foregoing hypothesis a chemical synthesis of the monomers **1** was required. In this paper we describe efficient synthetic routes to monomers **1** containing the RNA bases uracil, cytosine, adenine and guanine. Preliminary results of the solution behaviour and aldolisation properties of these monomers are also presented and methods for analysis of the results are described. Whilst direct proof of the mechanisms involved in the origin of life can never be obtained, the early evidence suggests that a modified version of this theory of prebiotic RNA formation should be considered a realistic possibility.

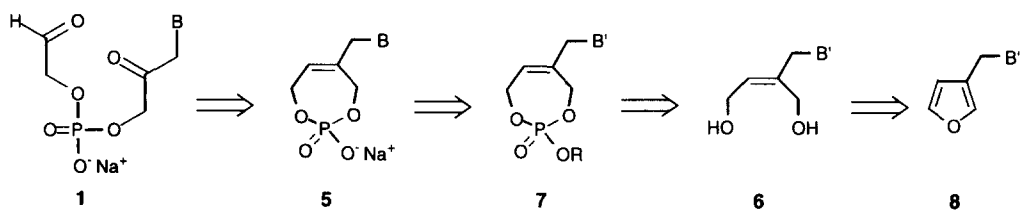


Scheme 1 Proposed polymerisation mechanism

Results and Discussion

Retrosynthetic Analysis

Due to the inherent reactivity of the two carbonyl groups in the target monomers, retrosynthetic analysis was directed at cyclic phosphate **5**, **Scheme 2**. This, it was envisaged, could undergo oxidative cleavage in a mild final step to produce monomer **1**.



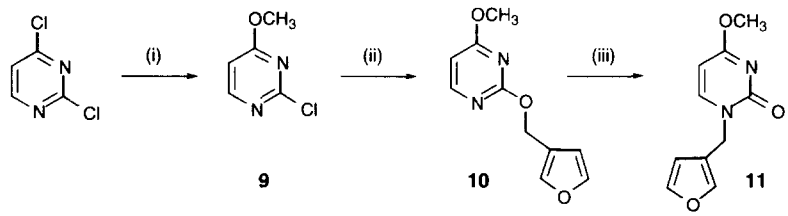
Scheme 2

In turn, disconnection of cyclic phosphate **5** led to the base-protected 2-substituted butene-1,4-diol derivative **6**, through phosphotriester **7**. Previous work[11] had shown that a synthesis beginning with butene-1,4-diol itself was not a viable option and an alternative disconnection to 3-substituted furan **8** was suggested. Preliminary research had shown that such furans undergo a Diels-Alder reaction with singlet oxygen, and borohydride reduction of the resultant ozonide equivalent was expected to produce the required diol **6**.[12]

Based on this analysis we have accomplished efficient syntheses of all four potentially prebiotic RNA precursors.

Uracil Monomer Synthesis

Synthesis of a suitable base-protected 3-methylfuran derivative **8** was the first target. 2,4-Dichloropyrimidine was chosen as a masked uracil template, since sequential displacement of chloride by oxygen nucleophiles was expected to allow facile introduction of a protected carbonyl equivalent at C4. 2-Chloro-4-methoxypyrimidine **9** was prepared according to the method of Kenner *et al.*[13] This was treated with the anion of 3-furanmethanol, prepared from the parent alcohol and sodium hydride in THF, to yield *bis*-alkoxypyrimidine **10** in excellent yield, **Scheme 3**.



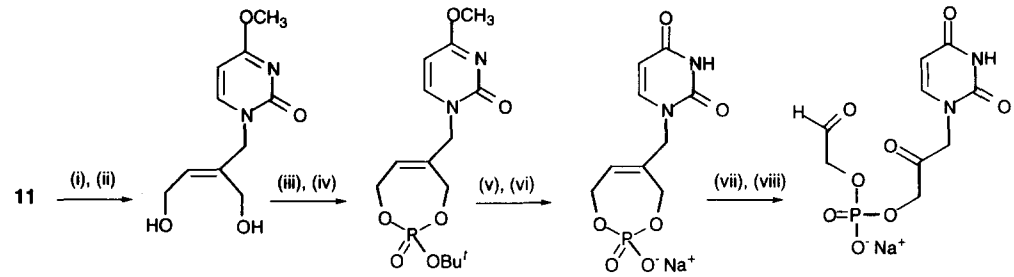
(i) NaOMe, MeOH (74%); (ii) 3-furanmethanol, NaH, THF (97%); (iii) tr. MeI (93%)

Scheme 3

Conversion to *N*-alkylfuran derivative **11** was achieved in excellent yield by rearrangement of **10** induced by the addition of a trace of methyl iodide to the neat oil. This reaction was developed following the observation that 2,4-dibenzyloxypyrimidine contaminated with traces of benzyl or 3-furanmethyl bromide **23** undergoes a clean rearrangement such that the O2-benzyl substituent migrates to N1. The position of alkylation was confirmed in both cases by heteronuclear multiple bond correlation (HMBC) nmr spectrometry.[14]

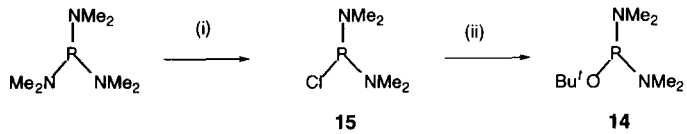
Furan oxidative cleavage *via* singlet oxygen cycloaddition then sodium borohydride reduction, proceeded smoothly and diol **12** was furnished in 62% purified yield, **Scheme 4**. It was found that column chromatography on alumina (Brockmann grade I) was an effective way to remove the pink colouration due to the sensitiser, Rose Bengal which was not eluted from the column.

Preparation of cyclophosphate **13** was conveniently achieved by reaction with the novel reagent *t*-butoxy-*N,N,N',N'*-tetramethylphosphorodiamidite **14**. This was synthesised by modification of the general procedure of Hargis and Alley,[15] involving initial formation of the pyrophoric chloro-*N,N,N',N'*-tetramethylphosphorodiamidite **15** by the exothermic disproportionation reaction of HMPT and PCl_3, **Scheme 5**.[16] The intermediate was distilled with great care under reduced pressure and immediately treated with *t*-butanol and triethylamine to afford the required phosphorodiamidite **14**.



(i) $^1O_2$, MeOH, EtOH, DCM, -78°C; (ii) $NaBH_4$, EtOH, -78°C (62%); (iii) $^tBuOP(NMe_2)_2$ (**14**), 1*H*-tetrazole, MeCN; (iv) ^tBuOOH (70% aq. solution), MeCN (41%); (v) HCl (conc., aq), MeOH; (vi) sodium Dowex, H_2O (quant.); (vii) O_3 (1 eq.), MeOH, -78°C; (viii) Me_2S, MeOH (67%)

Scheme 4



(i) PCl_3, 100°C; (ii) ^tBuOH, Et_3N, Et_2O, 0°C (38%)

Scheme 5

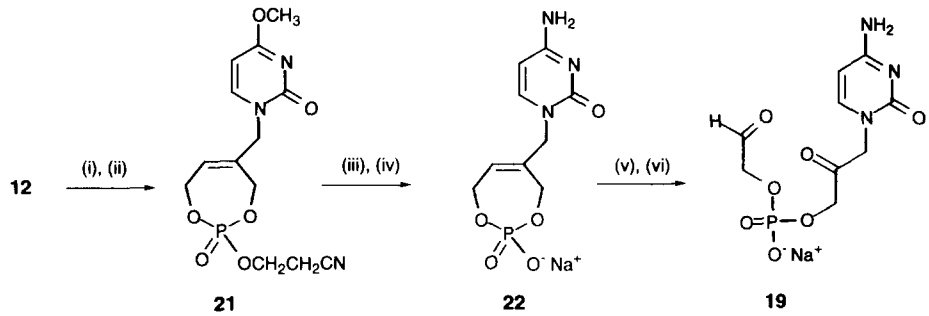
Reaction of **12** with phosphorodiamidite **14** and 1*H*-tetrazole in acetonitrile furnished cyclophosphite **16** which was not isolated but was oxidised *in situ* by *t*-butyl hydroperoxide to afford **13** in 41% purified yield.

Deprotection of **13** under mild conditions, using a trace of concentrated aqueous hydrochloric acid in methanol, was achieved over 24 h, successfully removing both the *t*-butyl group and the lactim methyl ether. The first formed free acid was converted to sodium salt **17** by treatment with sodium Dowex resin and this salt was purified by reverse phase HPLC.

A method for selective double bond cleavage had to be developed due to the lability of the pyrimidine C5-C6 double bond towards cleavage methods such as standard ozonolysis. Initial work had shown the cyclophosphate double bond to be the more reactive and the required selectivity was achieved using one equivalent of ozone, quantified using ozonisible dyes.[17] After reduction with dimethyl sulphide and reverse phase HPLC purification, **18** was obtained in 67% yield. HMBC and HMQC techniques were used to facilitate nmr spectral assignment.

Cytosine Monomer Synthesis

Base-protected diol **12** provided an ideal starting point for synthesis of cytosine monomer **19**; derivatives containing the free cytosine base are accessible by displacement of the 4-methoxy substituent by ammonia, as described for 2,4-dimethoxypyrimidine by Shen *et al.*[18] In order to maintain protecting group compatibility, the β-cyanoethyl group was chosen as a base labile phosphate protecting group. A synthesis of the required phosphitylating reagent β-cyanoethoxy-*N,N,N',N'*-phosphorodiamidite **20** was readily achieved following the general procedure used in the synthesis of **14**. Phosphitylation and oxidation proceeded smoothly to afford cyclic phosphotriester **21** in 74% purified yield, **Scheme 6**.



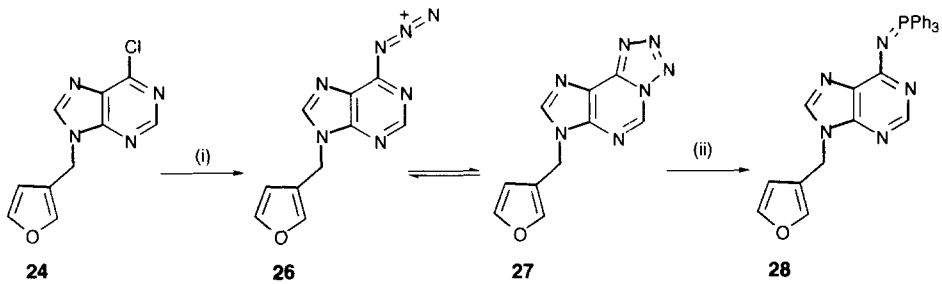
(i) $NCCH_2CH_2OP(NMe_2)_2$, 1*H*-tetrazole, MeCN; (ii) ^tBuOOH (70% aq. solution), MeCN (74%); (iii) NH_4OH, 75°C; (iv) sodium Dowex, H_2O (88%); (v) O_3 (1 eq.), MeOH, -78°C; (vi) Me_2S, MeOH (65%)

Scheme 6

Deprotection of automatically synthesised oligonucleotides containing the β-cyanoethyl phosphate group, is commonly accomplished by heating with concentrated aqueous ammonia.[19] Under these conditions at 75°C **21** was fully deprotected to cytosine cyclophosphate **22** in excellent yield after HPLC purification. Selective ozonolysis of **22** carried out as before resulted in successful formation of cytosine monomer **19** in good yield after purification by reverse phase HPLC. Nmr spectral assignment of **19** and later monomers was facilitated by comparison with data from uracil monomer **18**.

Adenine Monomer Synthesis

The sodium salt of 6-chloropurine was treated with a solution of 3-furanmethyl bromide **23** in DMF, freshly prepared following the procedure described by Lohmar and Steglich for synthesis of the 2-isomer.[20] A 5:1 mixture of N9 : N7 regioisomers was observed and, after column chromatography on silica, the required 9-isomer **24** was isolated in 51% yield, an 11% yield of the 7-isomer **25** was also obtained. The position of alkylation was again confirmed by HMBC nmr techniques. Introduction of a protected nitrogen substituent at C6 of the purine was next required since the 6-amino group of adenine conventionally requires protection during phosphorylation chemistry. Sodium azide effected clean nucleophilic aromatic substitution of the 6-chloro substituent to furnish 6-azidopurine derivative **26** in 81% purified yield, **Scheme 7**.

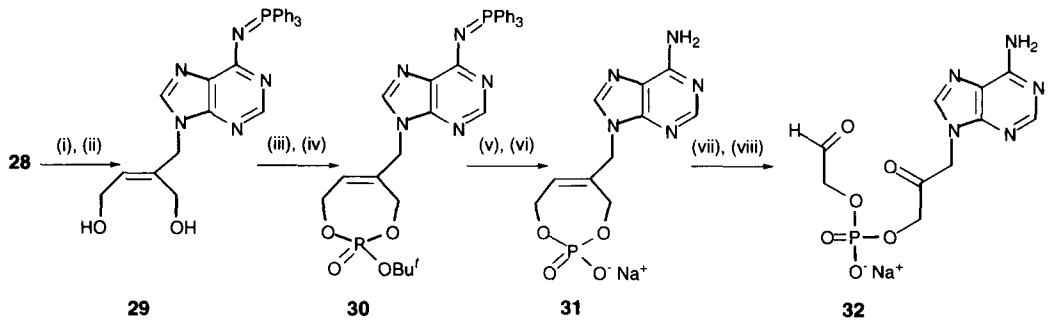


(i) NaN_3 (10 eq.), DMF (81%); (ii) PPh_3, 50°C, THF (80%)

Scheme 7

This compound exhibited a solvent dependent equilibrium with cyclic tetrazole derivative **27** which in part explains its higher than expected stability. In $CDCl_3$ a 2:3 mixture of azido:tetrazolo species was present but in DMSO-d_6 only the tricyclic material was observed. An IR spectrum run as a solution in chloroform showed a weak azide stretch at ν_{max} 2359 cm^{-1} whilst a spectrum with the sample as a nujol mull showed no absorption in this region.[21]

Staudinger-type reduction of **26** was carried out by heating overnight with triphenylphosphine in THF and iminophosphorane **28** was isolated in 80% recrystallised yield. The unusual stability of this product towards water, mild acid, alcoholic solvents and redox transfer reagents is presumed to derive from extensive delocalisation of electron density at N6 into the purine ring system and allowed use of the iminophosphorane as an amino protecting group. Single crystal X-ray analysis of **28** confirmed the structure, **Fig. 1**. The remaining synthetic steps were carried out in an analogous manner to the syntheses of the two pyrimidine monomers and proved straightforward. Oxidative cleavage of the furan moiety of **28** proceeded smoothly to afford diol **29** in 87% yield. Predicting removal of the iminophosphorane group under acidic conditions, *t*-butylphosphorodiamidite **14** was the chosen phosphitylation reagent. Cyclic phosphotriester **30** was obtained in excellent yield after oxidation of the intermediate phosphite triester with *t*-butylhydroperoxide, **Scheme 8**. Treatment of **30** with hydrochloric acid in methanol for 48h was followed by organic extraction of triphenylphosphine oxide and cation exchange using sodium Dowex, to afford cyclophosphate **31** in 83% yield after purification by reverse phase HPLC. Final stage cyclophosphate cleavage was achieved without complication by bubbling a stream of ozone/oxygen through a solution of **31** in methanol, until a blue colouration was observed indicating an excess of ozone. Dimethyl sulphide reduction afforded adenine monomer **32** in 62% yield after HPLC purification.



(i) $^1O_2$, MeOH, EtOH, DCM, -78°C; (ii) $NaBH_4$, EtOH, -78°C (87%); (iii) $^tBuOP(NMe_2)_2$, 1*H*-tetrazole, MeCN; (iv) ^tBuOOH (70% aqueous solution), MeCN (89%); (v) HCl (conc., aq.), MeOH; (vi) sodium Dowex, H_2O (83%); (vii) O_3, MeOH; (viii) Me_2S, MeOH (62%)

Scheme 8

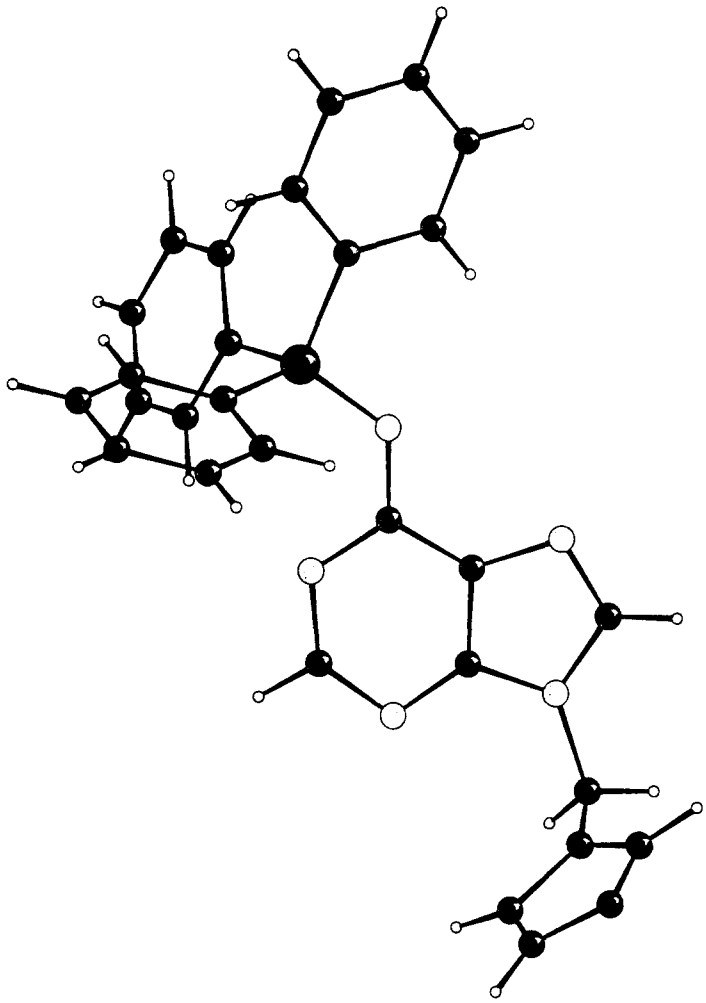
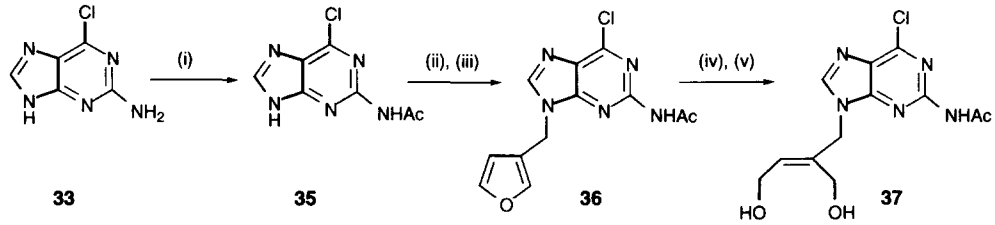


Fig. 1 X-ray crystal structure of 3-(6-Triphenyliminophosphoranopurin-9-ylmethyl)furan, **28**

Guanine Monomer Synthesis

In a similar manner to the adenine series, 2-amino-6-chloropurine **33** was used as a template for the synthesis of guanine monomer **34**. Selective acetylation of the 2-amino group of **33** was achieved in moderate yield by heating with acetic anhydride in *N,N*-dimethylacetamide at 150°C, in accordance with the procedure of Bowles *et al.*, **Scheme 9**.[22]

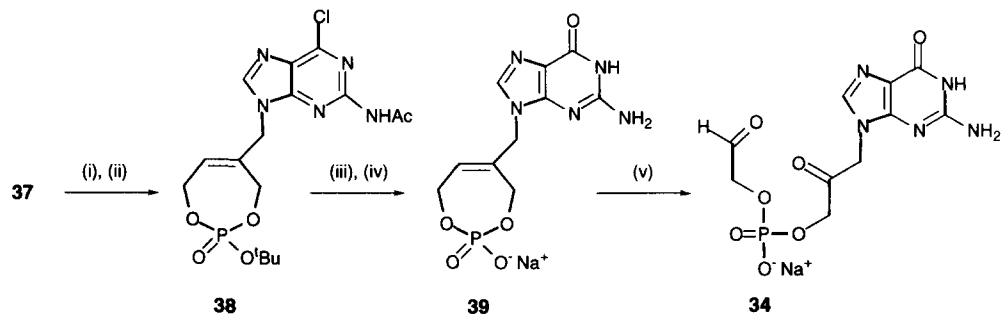


(i) Ac_2O, DMA, 150°C (58%); (ii) Bu_4NOH, DCM; (iii) 3-furanmethyl bromide, DMF (58%); (iv) $^1O_2$, MeOH, EtOH, DCM, -78°C; (v) $NaBH_4$, EtOH, -78°C (85%)

Scheme 9

Alkylation of **35** with 3-furanmethyl bromide in DMF made use of tetra-*n*-butylammonium hydroxide as the preferred base, following the observation by Bisacchi *et al.* that this counterion enhances N9 regioselectivity and improves the solubility of the resultant purinide in such alkylations.[23] 3-(2-Acetamido-6-chloropurin-9-ylmethyl)furan **36** was isolated in 58% yield.

Furan oxidative cleavage by singlet oxygen addition followed by sodium borohydride reduction afforded diol **37** which was phosphitylated utilising *t*-butyl-*N,N,N',N'*-phosphorodiamidite and 1*H*-tetrazole. An excess of the acidic/nucleophilic catalyst was required to ensure protonation of liberated dimethylamine in order to prevent substitution of the 6-chloro substituent. Subsequent *in situ* oxidation of the first-formed phosphite using *t*-butylhydroperoxide furnished cyclic phosphodiester **38**, **Scheme 10**.



(i) ^{t}BuOP(NMe_2)$_2$, 1*H*-tetrazole (6 eq.), MeCN; (ii) ^{t}BuOOH (70% aqueous solution), MeCN (83%); (iii) 2M HCl, dioxane, 50°C; (iv) sodium Dowex, H_2O (61%); (v) OsO_4, $NaIO_4$, H_2O (73%)

Scheme 10

Deprotection using aqueous hydrochloric acid resulted in loss of the 2-amino and phosphate protecting groups but effected only partial displacement of the 6-chloro substituent. Full deprotection of **38** to give guanine

cyclophosphate salt **39** was achieved by the action of aqueous hydrochloric acid in dioxane at 50°C, followed by treatment with sodium Dowex and reverse phase HPLC purification.

It was found that the guanine moiety of **39** was unstable to ozonolysis, initially selected as a method for cleavage of the cyclophosphate double bond. The use of osmium tetroxide with sodium periodate as cooxidant proved more selective and enabled isolation of target compound **34** in good yield after purification by reverse phase HPLC.

Solution Behaviour and Polymerisation Studies

Polymerisation studies are currently at an early stage. In order to simplify what would otherwise present an enormous analytical task, the monomers were first studied individually and then as base complementary pairs to enable the development of an elementary understanding of their reactivity and to allow optimisation of the required conditions.

The initial study of uracil- and adenine-containing monomers **18** and **32** involved an investigation of their behaviour in aqueous solution over the pH range 7.0 - 9.5. The negative ion electrospray mass spectra of all four monomers each show three peaks corresponding to the four possible hydration states of the carbonyl functionalities; $M\text{-}H^+$, 2 x $((M\text{-}H^+) + H_2O)$ and $((M\text{-}H^+) + 2H_2O)$. ^1H, ^{13}C and ^{31}P nmr spectrometry clearly show that the monomers exist as equilibrating mixtures of ketone **40** and ketone-hydrate **41** in neutral aqueous solution, **Scheme 11**. Both components contain a fully hydrated aldehyde functionality as evidenced by a characteristic triplet *ca.* δ 5.0 - 5.2 ppm in the ^1H nmr spectrum due to the glycoaldehyde proton.

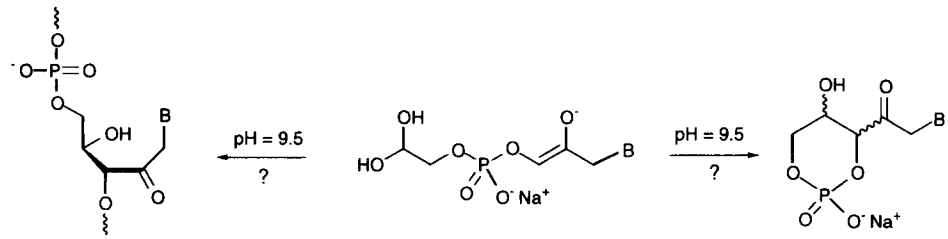
40 **41**

Scheme 11

This observation has extremely important consequences in the context of polymerisation for which first considerations present numerous possible courses of reaction. There are three enolisable positions in monomer **1** (H_b, H_c, H_d) and two potential aldol acceptors (ketone and aldehyde), giving rise to a possible three *intra-* and six *intermolecular* reaction pathways (neglecting stereochemistry). However, several of these options are intrinsically less favourable than others. Hydration of the aldehyde moiety in aqueous solution significantly reduces the potential for enolisation of H_b, since the rate of enolisation is proportional to the effective concentration of the carbonyl group, necessarily low if the group is hydrated. This has been previously reported in an earlier publication from this laboratory[24] following a study of structurally related dihydroxyacetonephosphoglycoaldehyde **42**. In addition, for ketone enolate formation the protons adjacent to the phosphate group (H_c) are more acidic than those adjacent to the base (H_d) and should therefore be the most readily enolised. This reduces the many possible courses of reaction to the kinetically preferred formation of a single ketone enolate **43** and its subsequent intra- or intermolecular reaction with an aldehyde, **Scheme 12**.

The model reactions previously carried out in this laboratory on *bis*-glycoaldehydephosphodiester **44** and glyceraldehyde-2-phosphoglycoaldehyde[25, 26] have indicated that intramolecular cyclisation to form **45** is

unlikely to be a favourable process due to the steric hindrance provided by the base. This leaves the most favourable pathway (again neglecting the issue of stereochemistry) being that leading to **46**, the required mode of polymerisation.



46 **43** **45**

Scheme 12

The monomers are stable in neutral aqueous solution for several weeks at 4°C but, on standing in D_2O slow deuterium exchange is observed. Two deuterium atoms are incorporated into the molecule after two weeks, as confirmed by negative ion electrospray ionisation mass spectrometry, displaying peaks at $(M - H^+) + 1$ and $(M - H^+) + 2$, **Fig. 2**.

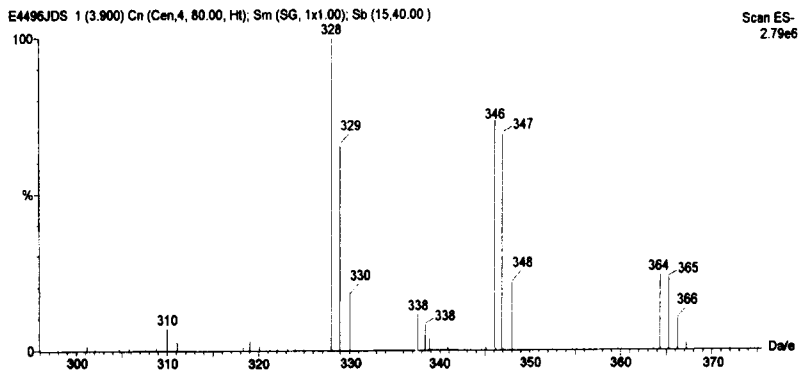


Fig. 2 Mass spectrum of adenine monomer **32** after standing in D_2O for 2 weeks

The ^1H nmr spectrum of uracil monomer **18** after prolonged standing in D_2O, indicates a decrease in intensity of the signals corresponding to the CH_2 protons between the ketone and phosphate groups (H_c), suggesting exchange of these protons. This is an encouraging result in the context of the proposed theory of polymerisation, indicating a preference for formation of the required enolate.

Polymerisation Experiments

Adenine monomer **32** was dissolved in water in an Eppendorf tube, at a concentration of 60 mg/ml. The pH was adjusted to 9.5 by the addition of 0.1 M sodium hydroxide but was observed to drop to *ca.* pH 8 during the experiment and was readjusted to pH 9.5 periodically over the course of the reaction. Samples for analysis were removed at intervals and the reaction quenched after by acidification to pH 5 using 0.1 M HCl. Product analysis was carried out by a variety of methods.

The reaction mixture was evaporated to dryness and dissolved in D_2O, for ^1H and ^{31}P nmr spectrometry. The 500 MHz ^1H nmr spectrum showed that the triplets at δ 5.10 and 5.14 ppm, corresponding to the glycoaldehyde protons had almost disappeared after just 1 h suggesting rapid consumption of the starting material. A broad band of peaks was observed in the aromatic (adenine) region, resolved as discernible pairs of singlets spanning from δ 7.6 - 8.4 ppm. A complex multiplet, presumably corresponding to the CH_2 protons and showing discrete spin systems was observed at δ 3.3 - 4.6 ppm, **Fig. 3**.

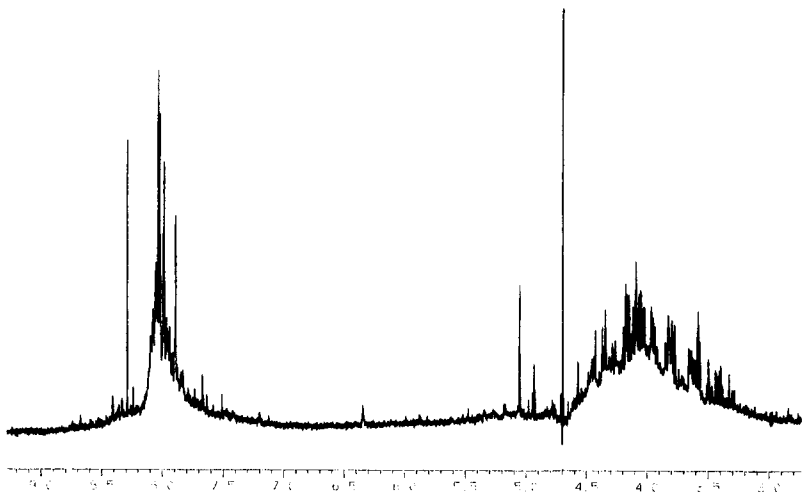


Fig. 3(a) 500 MHz ^1H nmr spectrum of products obtained after standing **103** in H_2O at pH 9.5 for 63 h

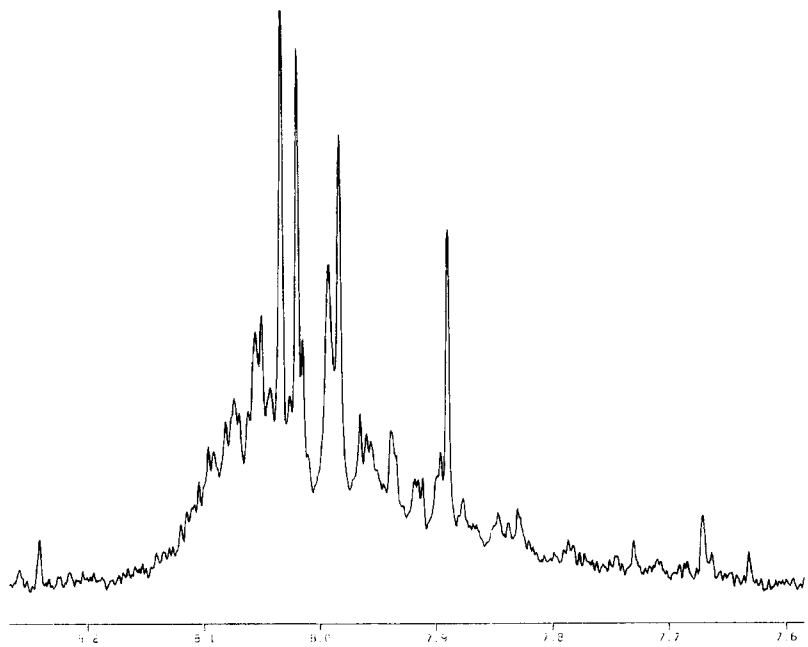


Fig. 3(b) Expansion of adenine region

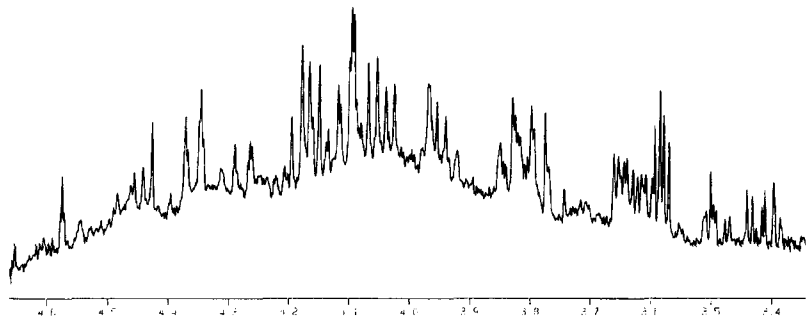


Fig. 3(c) Expansion of CH_2 region

The complex, yet structured nature of the spectra are consistent with polymer formation and the broad span of heterocyclic signals suggests a flexible, unconstrained structure in which there are many different proton environments. In contrast, the 200 MHz ^1H nmr spectra of commercial samples of polyadenylic acid (poly A) and polyuridylic acid (poly U) show remarkably discrete chemical shifts in the regions of glycosidic, heterocyclic and ring protons, presumably a consequence of the regularity of the polymer. There is no evidence for the presence of glycosidic protons (expected to resonate *ca.* 5.0 ppm) in the spectra of the products of the polymerisation experiment.

^{31}P nmr showed two major peaks at -3.76 ppm and 5.75 ppm; between these peaks a complex, broad band of signals was observed. The ^{31}P nmr spectrum of poly A shows a single sharp peak at δ_P -0.13 ppm while that of the more irregular poly [A,U] shows a significantly broader peak centred at δ_P -0.12 ppm. Again the results are consistent with the formation of an acyclic polymeric material containing many phosphorus environments.

The combined data therefore support the theory that aldol condensation has occurred but do not provide evidence for cyclisation to form the ribose ring. If this is the case the system would have many potential degrees of freedom creating many different adenine proton environments along the chain.

Uracil monomer **18** exhibited similar behaviour. After standing for 3 h in D_2O at pD 9.5, a complex but structured multiplet was observed in the 500 MHz ^1H nmr spectrum at δ 3.3 - 4.6 ppm. The uracil signals were represented by two broad patterns of distinct doublets centred around δ 5.9 and δ 7.6 ppm which indicated the presence of several different heterocyclic environments.

Electrospray ionisation mass spectrometry (ESI MS) is an ideal technique for the identification of polymeric species and has been widely used to characterise oligonucleotides.[27, 28] Ionisation by this method is known to be a low energy process which rarely causes excessive fragmentation, thus greatly facilitating spectral interpretation.

The negative ion electrospray mass spectrum of the reaction mixture provided convincing evidence for polymerisation in the adenine series and is shown in **Fig. 4**. The major peak in all the spectra measured over the course of the reaction was seen at 328 which corresponds to M - H^+ for monomer **32**. The ^1H nmr spectrum showed no monomer remaining after 1 h however, suggesting either oligomer hydrolysis (which would afford a mixture of 3'-, 5'- and 3',5'-cyclic monophosphates) or the presence of cyclised monomer **45**.

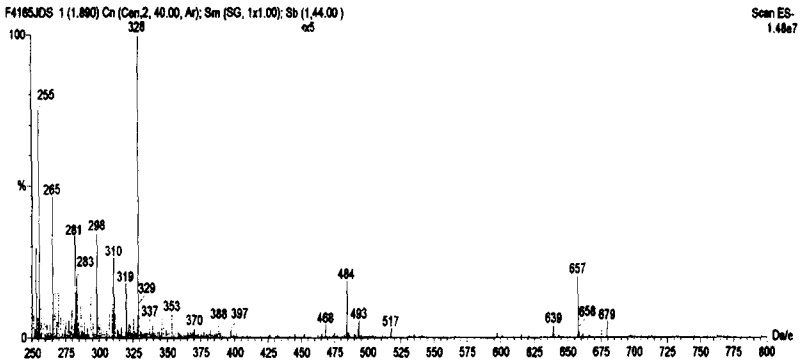
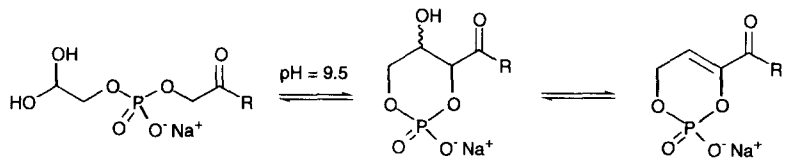


Fig. 4 Mass spectrum of products obtained after standing **103** in H_2O at pH 9.5 for 41 h

In support of the presence of **45**, a peak at 310 could be tentatively assigned as corresponding to α,β-unsaturated ketone **47**, **Scheme 13** by analogy with the similarly observed peak at (M - H^+) -18 corresponding to **48**, seen as a consequence of the cyclisation of *bis*-glycoaldehyde phosphodiester **44** under these conditions.[26]



32 R = CH_2B
44 R = H

45 R = CH_2B

47 R = CH_2B
48 R = H

Scheme 13

Deuteration studies showed that the intramolecular aldol condensation process is reversible for **44** and in the case of monomer **32** this would almost certainly be the case, due to the increased steric bulk of the heterocyclic base which disfavours cyclisation. In such an equilibrating mixture, monomer cyclisation should not therefore preclude oligomerisation, which could potentially be the sterically preferred course of reaction. The gradual emergence of peaks at higher mass indicated the presence of dimers and trimers of **32**, **Fig. 5** The peak at 657 corresponds to the monoanion of a dimer and the peak at 493 to the dianion of a trimer; both were clearly visible after 41 h. It should be noted that the observed charge does not increase uniformly with chain length; for example a trimer is ionised as a mixture of dianion and monoanion. This leads to a cluster of peaks around just a few *m/z* values. Under the particular mass spectrometry conditions employed higher oligomers might not be detectable. An additional complicating feature of these spectra is the presence of different hydration states. The extent of hydration or dehydration observed by ESI MS is dependent on the applied cone voltage, high cone voltages causing dehydration. The presence of such species can however be diagnostic of oligomerisation, for example the peak at 657 corresponding to dimer shows a dehydration peak at 639, the difference of 18 confirming it is a monoanion. The peak at 493 corresponding to trimer shows two dehydration peaks at 484 and 475, the incremental difference of 9 consistent with a dianionic species and the potential for loss of two water molecules requiring the presence of three units of **32**. The identification of small amounts of higher oligomers becomes extremely complex when these dehydration states are taken into account.

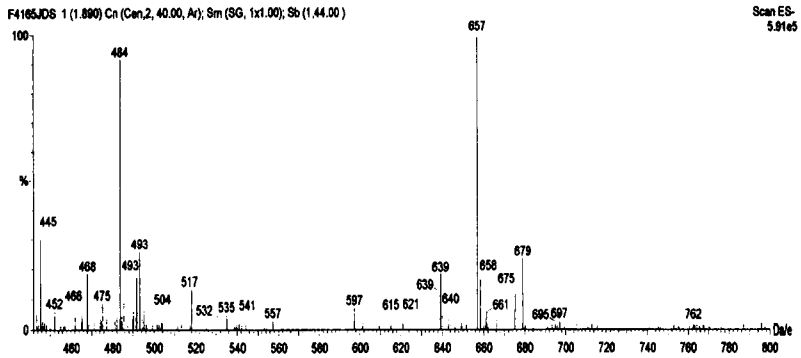


Fig. 5 Expansion of the mass spectrum given in **Fig. 5**, showing the presence of dimers and trimers

These data corroborate the tentative evidence from nmr spectrometry that cyclisation to ribose has not taken place in the resultant polymer.

The reverse phase HPLC trace for the reaction mixture showed a single peak at a retention time of 3.2 min, similar to the monomer (3.7 min) and perhaps corresponding to cyclised monomer **45** or hydrolysed polymer. More interesting was a larger and more complex peak at an earlier retention time of 1.7 min, suggesting the presence of multiple charges and potentially corresponding to oligomeric material.

Anion-exchange HPLC was carried out under conditions adapted from those of Baldwin *et al.*.[29] After an initial pre-wash to remove unbound material, a large peak was seen at 10.3 min followed by a significant broad band of lower intensity peaks between 12.5 and 18 min, **Fig. 6**. This was an earlier retention time than that seen for two short length DNA oligomers (22 and 37 nucleotides) run under identical conditions but later than monomer **32** which eluted at around 10.3 min, again suggesting the presence of multiply charged polymeric material.

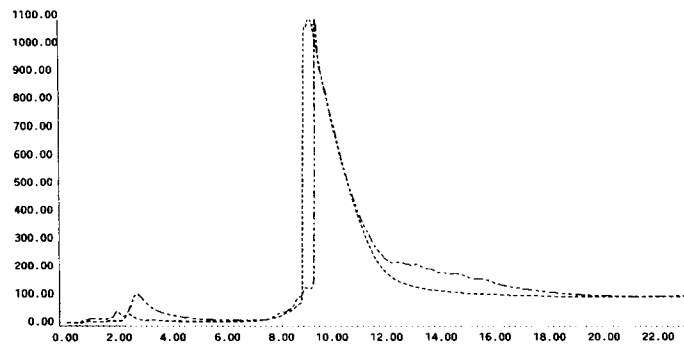


Fig. 6 Anion exchange HPLC trace of **103** (····) and after standing in H_2O at pH 9.5 for 63 h (–..–..)

Analytical methods based on enzymatic digestion procedures are now being developed.

A preliminary investigation of the effect of base pairing on the polymerisation procedure was carried out with the uracil and adenine-containing monomers together at pD 9.5 in D_2O, for direct analysis by ^1H nmr spectrometry. After 2.5 h the intensities of all the glycoaldehyde signals were again significantly reduced and the CH_2 signals had been transformed into a complex multiplet at δ 3.45 - 4.60 ppm. After a prolonged reaction time the spectrum appeared to be simply a combination of those from the individual monomer experiments; the effect of base pairing in facilitating ring closure to ribose seems negligible from these preliminary data.

Conclusions

The chemical synthesis of the four monomers **18**, **19**, **32** and **34** has formed an essential part of the study of the proposed prebiotic synthesis of RNA. Efficient synthetic routes to all four monomers and conditions for their purification by HPLC have been established. A preliminary study of the solution behaviour of the adenine and uracil containing monomers (**32** and **18** respectively) demonstrated their potential for polymerisation by aldol condensation. On the basis of the experiments carried out to date the following conclusions can be drawn. In under 48 h at pH 9.5, polymerisation of monomers **32** and **18** occurs, as evidenced by ^1H and ^{31}P nmr spectrometry, anion-exchange HPLC and mass spectrometry. Agarose gel electrophoresis of polymerisation mixtures (with ethidium bromide staining) repeatedly showed only a blue fluorescence at the gel front which could not be explained. The results were potentially complicated by polymer hydrolysis and/or monomer cyclisation. The structure of the resultant polymer is not known but from the complexity of the nmr spectroscopic data it appears to be acyclic and the absence of signals that may be attributed to the glycosyl protons in the ^1H nmr spectrum suggests it does not contain a ring-closed sugar unit. This is further supported by the presence of dehydrated species in the mass spectrum. The data obtained is consistent with formation and accumulation of the required aldol polymer **46** which has failed to undergo the required ring closure to afford the ribose unit of RNA. No definite proof of the actual structure of the polymeric material has yet been obtained however and this hypothesis remains to be confirmed. These results represent the first potentially prebiotic route to naturally occurring nucleic acid polymers *via* an aldol condensation mechanism from an acyclic monomeric precursor. It must be stressed that the results of polymerisation experiments outlined above are preliminary findings. It remains to establish the exact structure of the polymeric material derived from the adenine and uracil monomers and to confirm the presence of longer oligomers, hydrolysis products or cyclised material after prolonged reaction times. The inclusion of oligonucleotide templates and Mg^{2+} ions may be beneficial in this context.

The lack of redox-transfer and ring-closure after aldolisation suggests that the heterocyclic lone pair in the monomers **1** is not sufficiently available to allow these reactions. These preliminary experiments thus indicate that the theory of a potentially prebiotic nucleic acid synthesis we proposed should be modified. The consequences of the modified theory are indicated in the preceding report.

Acknowledgements

We thank the EPSRC for financial support of this work and Dr. A. Edwards for performing the crystal structures of **28**, Dr. T. Claridge and Mrs E. McGuinness for HMBC and high field nmr experiments, Dr. R. T. Aplin and colleagues for mass spectrometry and Dr G. Weaver (Dyson Perrins) and Dr. S. Pitsch (ETH, Zürich) for helpful discussions.

Experimental

Experimental Techniques

Melting points were determined on a Phillip Harris melting point apparatus model C49547/8. Values are quoted to the nearest degree centigrade and are uncorrected. *Fourier transform infrared spectra* were recorded on a Perkin Elmer 1750 Fourier Transform spectrometer and only selected absorptions are reported. *Ultraviolet spectra* were recorded using a Perkin Elmer Lambda 2 UV/VIS spectrometer. *Proton nmr spectra* were recorded on Varian Gemini 200 (200 MHz), Bruker AC200 (200 MHz), Bruker AM500 (500 MHz) and Bruker AMX500 (500 MHz) spectrometers. Chemical shifts (δ_H) are quoted in ppm relative to TMS and are internally referenced to the residual solvent peak. Coupling constants (J) are given in Hz to the nearest 0.5 Hz. *Carbon nmr spectra* were recorded on Varian Gemini 200 (50.3 MHz), Bruker AC200 (50.3 MHz), Bruker AM500 (125.8 MHz) and Bruker AMX500 (125.8 MHz) spectrometers using DEPT editing to aid peak assignment where necessary. Chemical shifts (δ_C) are quoted in ppm and were internally referenced to the residual solvent peak. Samples in D_2O were referenced externally to 1,4-dioxane. *Phosphorus nmr spectra* were recorded on Bruker AM250 (101.2 MHz) and Bruker AM500 (202.4 MHz) spectrometers and were referenced externally to 85% phosphoric acid at $\delta_P = 0$ ppm. *Mass spectra* were recorded on a VG Micromass ZAB 1F (CI, DCI, FAB) spectrometer or a VG BIO-Q triple quadrupole atmospheric pressure mass spectrometer equipped with an electrospray interface. *Analytical thin layer chromatography* was carried out on aluminium sheets using Merck Kieselgel 60 F_{254} 0.2 mm precoated plates which were visualised by the quenching of u.v. fluorescence (λ_{max} = 254 nm) or by the application of heat after staining with 10% w/v ammonium molybdate in 2M H_2SO_4. Retention factors (R_f) are quoted to the nearest 0.1. *Flash column chromatography* was accomplished using Sorbisil® C60 (mpd 60Å) silica gel (30-60 μm) or Laporte Actal UG alumina (Brockmann grade I) following the method of Still *et al.*[30] *High performance liquid chromatography* (HPLC) was carried out on a Gilson HPLC system equipped with Gilson 305 and 306 pumps, a variable wavelength detector, an 806 manometric module and a model 231 Biosample injector. HPLC instrument control, data collection and analysis were performed on an IBM PS1 model 486DX-33 computer equipped with Gilson 715 v.1.2 software. Retention times are quoted to the nearest 0.1 min. *Reverse phase HPLC* was carried out using a C_{18} Hypersil® column, at a flow rate of 5 ml/min, eluting with water under isocratic conditions and with u.v. detection at 220 nm. *Anion-exchange HPLC* was carried out using a Mono-Q® HR/55 column eluting with a gradient profile of 0% B in A for 5 min then 0-80% B in A over 20 min; (Buffer A: 20 mM Tris.HCl, 1mM EDTA, pH 8.3; Buffer B: 20 mM Tris.HCl, 1mM EDTA, 1M NaCl, pH 8.3). A flow rate of 1 ml/min was used with u.v. detection at 254 nm. All samples for HPLC purification were prepared by dissolution in water followed by centrifugation using a Jouan A-14 centrifuge at 14,000 rpm. *Accurate pH* was measured using a Hanna Piccolo 2 ATC pH meter or Hydrion Microfine pH test paper.

Tetrahydrofuran and diethyl ether were dried using sodium benzophenoneketyl under argon. Anhydrous pyridine was obtained by standing over potassium hydroxide for 12 h followed by distillation from calcium hydride under argon. Anhydrous DMF and anhydrous acetonitrile were obtained by standing over calcium hydride for 24 h followed by distillation under reduced pressure. Methanol was dried by distillation from magnesium methoxide.

Experimental Procedures

Sodium Dowex cation exchange resin .

Dowex-50WX8-200 cation exchange resin (2.0 g) was washed with Milli-Q water (50 ml), then with aqueous sodium hydroxide (1 M, 20 ml) and finally with Milli-Q water until the pH of the washings was neutral.

3-Furanmethyl bromide (**23**).

A solution of 3-furanmethanol (3.43 g, 35 mmol) and dry pyridine (0.94 ml, 12 mmol) in anhydrous diethyl ether (30 ml) was stirred at -10°C (ice/salt) and treated dropwise with a solution of phosphorus tribromide (0.98 ml, 12 mmol) in anhydrous diethyl ether (10 ml). An off-white solid was formed almost immediately and after stirring for 3 h the supernatant of the resultant mixture was decanted into a separating funnel containing 30 ml water. The solid remaining was washed with diethyl ether (2 x 20 ml) and the washings added to the funnel. The ethereal layer was washed with $NaHCO_3$ (aq., sat.) (10 ml) and brine (5 ml), then dried ($MgSO_4$). Dilution with DMF (25 ml) followed by removal of diethyl ether under reduced pressure, afforded a clear yellow solution of the title compound in DMF. The title compound was used in all further experiments without isolation as a solution in DMF, its hazardous nature preventing characterisation.[31]

Chloro-N,N,N',N'-tetramethylphosphorodiamidite (**15**).

The following method was adapted from that of Hargis and Alley.[15] Hexamethylphosphorustriamide (4.55 ml, 25 mmol) was treated dropwise with phosphorus trichloride (1.09 ml, 12.5 mmol) and the mixture heated at 100°C for 20 min with stirring. During the addition the mixture became hot and white fumes were evolved. The mixture, containing an orange precipitate, was cooled and then distilled under reduced pressure (b.p. 45-50°C, 3 mbar). Extreme care was needed during the distillation due to the pyrophoric nature of the material, which was afforded as a colourless liquid and presumed to be the expected intermediate, chloro-*N,N,N',N'*-tetramethylphosphorodiamidite. This material was used immediately, without characterisation.

Alkoxy-N,N,N',N'-tetramethylphosphorodiamidite (**14**, **20**).

Following dissolution of freshly distilled chloro-*N,N,N',N'*-tetramethylphosphorodiamidite (**15**) in anhydrous diethyl ether (30 ml) the slightly cloudy solution was added dropwise to a stirred solution of the appropriate alcohol (21 mmol) and triethylamine (2.90 ml, 21 mmol) in dry diethyl ether (20 ml) at 0°C. A white solid, assumed to be triethylammonium hydrochloride was formed immediately and after stirring for 15 min was removed by filtration and washed with diethyl ether. The ethereal filtrates were washed with water (2 x 5 ml) and brine (3 ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure.

t-butoxy-N,N,N',N'-tetramethylphosphorodiamidite (**14**) was afforded as a colourless liquid (3.21 g, 80% crude yield). Distillation under reduced pressure (b.p. 27-30 °C, 3 mbar) yielded the title compound as a colourless liquid (1.54 g, 38%). δ_H (200 MHz, $CDCl_3$) 1.31 (9 H, s, $C(CH_3)_3$), 2.44 (6 H, s, NCH_3) and 2.49 (6 H, s, NCH_3); δ_C (50.3 MHz, $CDCl_3$) 30.5 (d, $J_{P\text{-}C}$ 9, $C(CH_3)$), 36.5 (d, $J_{P\text{-}C}$ 19, $N(CH_3)_2$) and 36.8 (d, $J_{P\text{-}C}$ 20, $N(CH_3)_2$); *m/z* (CI) 193 (MH^+, 100%).

β-Cyanoethoxy-N,N,N',N'-tetramethylphosphorodiamidite (**20**) was afforded as a colourless liquid (3.59 g, 91% crude yield). Distillation under reduced pressure (b.p. 72-75 °C, 3 mbar) yielded the title compound as a colourless liquid (2.78 g, 70%). ν_{max}/cm^{-1} 2253s (CN); δ_H (200 MHz, $CDCl_3$) 2.56 (6 H, s, $N(CH_3)_2$), 2.61 (6 H, s, $N(CH_3)_2$), 2.56 - 2.69 (2 H, m, CH_2CN) and 3.82 (2 H, dt, J_{H-H} 6.5, J_{P-H} 8, OCH_2); δ_C (50.3 MHz, $CDCl_3$) 20.2 (d, J_{P-C} 7,CH_2CN), 34.4 (d, J_{P-C} 6, OCH_2), 36.1 (d, J_{P-C} 18, $N(CH_3)_2$) and 36.6 (d, J_{P-C} 18, $N(CH_3)_2$); *m/z* (CI) 190 (MH^+, 100%).

2-Chloro-4-methoxypyrimidine (**9**).

A solution prepared by adding sodium (0.23 g, 10 mmol) to methanol (7 ml) was added slowly to a stirred solution of 2,4-dichloropyrimidine (1.5 g, 10 mmol) in methanol (10 ml). After 40 min, diethyl ether (30 ml) was added to complete precipitation of a white solid, presumed to be sodium chloride which was then removed by filtration through Celite®. Concentration of the filtrate *in vacuo* afforded the title compound as a white solid (1.34 g, 93%). Recrystallisation from pet. ether afforded 2-chloro-4-methoxypyrimidine as colourless needles (1.07 g, 74%). R_f = 0.5 [Et_2O]; m.p. 53 - 55°C (from pet. ether) (lit., 55°C)[13]; δ_H (200 MHz, $CDCl_3$) 4.01 (3 H, s, OCH_3), 6.68 (1 H, d, *J* 5.5, C(5)H) and 8.29 (1 H, d, *J* 5.5, C(6)H); δ_C (50.3 MHz, $CDCl_3$) 54.5 (OCH_3), 107.2 (C(5)), 158.9 (C(6)), 160.6 (C(2)) and 171.0 (C(4)); *m/z* (CI, NH_3) 145 (MH^+, 100%).

2-(3-Furanmethoxy)-4-methoxypyrimidine (**10**).

Sodium hydride (0.31 g, 7.6 mmol, 60% dispersion) was freed from mineral oil by successive washing with pet. ether (3 x 5 ml). To this was then added anhydrous THF (10 ml) and 3-furanmethanol (0.60 ml, 7.0 mmol) at 0°C and stirring continued until effervescence ceased. The resultant suspension was then added to a solution of **9** (1.0 g, 7.0 mmol) in dry THF (15 ml) and the mixture stirred for 2.5 h. THF was removed *in vacuo* and the orange residue mixed with ethyl acetate (50 ml). Filtration through Celite® followed by concentration *in vacuo* afforded a yellow oil shown to be the title compound (1.43 g, 97%). The compound was further purified for analysis by flash column chromatography on silica gel [pet. ether:diethyl ether, (3:1) as eluent] to yield the title compound as a yellow oil (1.16 g, 81%). R_f = 0.3 [pet. ether:Et_2O, (1:1)]; λ_{max} ($CHCl_3$)/nm 276.5 ($\varepsilon/M^{-1}cm^{-1}$ 6700); δ_H (200 MHz, $CDCl_3$) 3.99 (3 H, s, OCH_3), 5.32 (2 H, s, OCH_2), 6.40 (1 H, d, *J* 5.5, pyrimidine C(5)H), 6.56 (1 H, brs, furan C(4)H), 7.42 (1 H, d, *J* 1.5, furan C(5)H), 7.57 (1 H, s, furan C(2)H) and 8.21 (1 H, d, *J* 5.5, pyrimidine C(6)H); δ_C (50.3 MHz, $CDCl_3$) 53.7 (OCH_3), 60.6 (OCH_2), 102.3 (pyrimidine C(5)), 110.9 (furan C(4)), 120.9 (furan C(3)), 141.8 (furan C(2)), 143.4 (furan C(5)), 158.5 (pyrimidine C(6)), 165.0 ($COCH_2$) and 171.8 ($COCH_3$); *m/z* (CI, NH_3) 207 (MH^+, 100%) and 81 (3-furanmethyl cation, 6); (Found: MH^+ 207.0770. $C_{10}H_{10}N_2O_3$ requires MH^+ 207.0691).

3-(4-Methoxypyrimidin-2-on-1-ylmethyl)furan (**11**).

To the neat oil **10** (0.50 g, 2.5 mmol) was added a catalytic amount of methyl iodide (*ca.* 20 μl). The reaction vessel was sealed and left at room temperature for 3 days after which time the mixture had solidified. The resultant pale yellow solid was purified by column chromatography on silica gel [ethyl acetate as eluent] to afford the title compound as a white solid (0.46 g, 93%). Further purification by recrystallisation from ethyl acetate provided a sample for elemental analysis as fine white crystals (0.11 g, 21%). The position of alkylation

was determined by HMBC nmr spectrometry. R_f = 0.3 [EtOAc]; m.p. 124 - 126°C (from EtOAc); (Found: C, 58.36; H, 4.54; N, 13.84; $C_{10}H_{10}N_2O_3$ requires C, 58.25; H, 4.89; N, 13.59%); λ_{max} ($CHCl_3$)/nm 258.7 ($\varepsilon/M^{-1}cm^{-1}$ 5900); ν_{max}/cm^{-1} (nujol) 1664s (C=O); δ_H (200 MHz, $CDCl_3$) 3.97 (3 H, s, OCH_3), 4.90 (2 H, s, CH_2N), 5.87 (1 H, d, *J* 7, pyrimidine C(5)H), 6.42 (1 H, brs, furan C(4)H), 7.41 (1 H, d, *J* 7, pyrimidine C(6)H), 7.42 (1 H, d, *J* 1.5, furan C(5)H) and 7.52 (1 H, s, furan C(2)H); δ_C (50.3 MHz, $CDCl_3$) 43.7 (CH_2N), 54.4 (OCH_3), 96.0 (pyrimidine C(5)), 110.5 (furan C(4)), 120.0 (furan C(3)), 141.7 (furan C(2)), 144.3 (furan C(5)), 146.1 (pyrimidine C(6)), 157.0 (C=O) and 172.5 (*C*OCH_3); *m/z* (CI, NH_3) 207 (MH^+, 100%) and 81 (3-furanmethyl cation, 7).

2-(4-Methoxypyrimidin-2-on-1-ylmethyl)-Z-but-2-ene-1,4-diol (**12**).

A solution of **11** (0.50 g, 2.4 mmol) and Rose Bengal (*ca.* 5 mg) in ethanol (5 ml), methanol (5 ml) and dichloromethane (5 ml) was stirred at -78°C (acetone/CO_2) and irradiated with a 150W tungsten filament lamp whilst a stream of oxygen was bubbled through the solution. After 3 h the mixture was treated with a solution of sodium borohydride (0.10 g, 2.7 mmol) in ethanol (5 ml) and maintained at -78°C for a further 2.5 h with stirring. Equilibration to room temperature was followed by addition of 5 ml of water to quench the reaction. The mixture was concentrated *in vacuo* and the resultant pink residue was pre-absorbed onto alumina then purified by flash column chromatography on alumina [methanol:ethyl acetate (1: 9) as eluent] to afford the title compound as a colourless gum (0.33 g, 62%). R_f = 0.3 [MeOH:EtOAc, (1:4)]; (Found: C, 52.96; H, 6.27; N, 12.36; $C_{10}H_{14}N_2O_4$ requires C, 53.09; H, 6.24; N, 12.38%); λ_{max} ($CHCl_3$)/nm 276.7 ($\varepsilon/M^{-1}cm^{-1}$ 5900); ν_{max}/cm^{-1} ($CHCl_3$) 3340br (OH), 1640br (C=C and C=O); δ_H (200 MHz, CD_3OD) 3.91 (3 H, s, OCH_3), 4.11 (2 H, s, C(1)H_2OH), 4.16 (2 H, d, *J* 6.5, C(4)H_2OH), 4.56 (2 H, s, CH_2N), 5.51 (1 H, t, *J* 6.5, C=CH), 6.06 (1 H, d, *J* 7.5, pyrimidine C(5)H) and 7.81 (1 H, d, *J* 7, pyrimidine C(6)H); δ_C (50.3 MHz, CD_3OD) 52.1 (CH_2N), 53.5 (OCH_3), 57.2 and 57.3 (2 x CH_2OH), 95.9 (pyrimidine C(5)), 130.0 (H*C*=C), 135.7 (HC=*C*), 148.8 (pyrimidine C(6)), 158.0 (C=O) and 172.6 ($COCH_3$); *m/z* (CI, NH_3) 227 (MH^+, 100%) and 453 ($(M)_2H^+$, 6).

t-Butyl 2-(4-methoxypyrimidin-2-on-1-ylmethyl)-Z-but-2-ene-1,4-diylcyclophosphate (**13**).

12 (0.28 g, 1.2 mmol) was first dissolved in acetonitrile (2 x 5 ml) and the solution evaporated to remove residual water, then dried under vacuum over P_2O_5 overnight. The sample was dissolved in oligonucleotide synthesis grade acetonitrile (10 ml) and to the stirred solution under argon was added *t*-butoxy-*N,N,N',N'*-tetramethylphosphorodiamidite (0.36 g, 1.9 mmol) as a solution in acetonitrile (2 ml), followed by 1*H*-tetrazole (0.26 g, 1.9 mmol). The homogeneous mixture was stirred under argon for 1.5 h (monitored by t.l.c.), then *t*-butyl hydroperoxide (0.08 ml, 0.61 mmol, 70% aqueous solution) was added as a solution in acetonitrile (2 ml) and stirring continued for a further 1 h. The mixture was concentrated *in vacuo* and separated by column chromatography on silica gel [5% isopropanol in chloroform as eluent] to afford a colourless oil which solidified on standing to give the title compound as a white solid (0.18 g, 41%). Recrystallisation from dichloromethane/pet. ether afforded fine white crystals for analytical purposes. R_f = 0.3 [EtOAc:MeOH, (4:1)]; m.p. 125 - 130°C (dec.) (DCM/pet. ether); (Found: C, 48.61; H, 5.85; N, 8.52. $C_{14}H_{21}N_2O_6P$ requires C, 48.84; H, 6.18; N, 8.14%); λ_{max} ($CHCl_3$)/nm 274.5 ($\varepsilon/M^{-1}cm^{-1}$ 6100); ν_{max}/cm^{-1} 1280m (P=O) and 1631m (C=O); δ_H (500 MHz, $CDCl_3$) 1.53 (9 H, s, $C(CH_3)_3$), 3.97 (3 H, s, OCH_3), 4.34 - 4.51 (2 H, ABq, CH_2N),

4.52 - 4.77 (4 H, m, 2 x CH_2OP), 5.69 (1 H, t, *J* 3, HC=C), 5.93 (1 H, d, *J* 7, pyrimidine C(5)H) and 7.38 (1 H, d, *J* 7, pyrimidine C(6)H); δ_C (50.3 MHz, $CDCl_3$) 29.7 ($C(CH_3)_3$), 52.1 ($C(CH_3)_3$), 54.6 (CH_2N and OCH_3), 63.2 (d, J_{P-C} 6, CH_2OP), 64.3 (d, J_{P-C} 6, CH_2OP), 97.0 (pyrimidine C(5)), 126.4 (*HC*=C), 134.7 (HC=*C*), 146.2 (pyrimidine C(6)), 157.1 (C=O) and 172.2 ($COCH_3$); δ_P (101.2 MHz, $CDCl_3$) -0.68 (m); *m/z* (DCI, NH_3) 345 (MH^+, 2%), 209 (39), 127 (pyrimidineH^+, 100) and 58 ($C(CH_3)$, 52).

Sodium 2-(pyrimidin-2,4-dion-1-ylmethyl)-Z-but-2-ene-1,4-diylcyclophosphate (**17**).

To a solution of **13** (0.15 g, 0.45 mmol) in methanol (5 ml) was added concentrated hydrochloric acid (30 µl, 12M) and the mixture left to stand for 24 h. The solvents and volatile side products were removed by concentration *in vacuo* to yield the conjugate acid of the title compound as a white solid (0.12 g, quant.). Sodium Dowex resin was freshly prepared and added gradually to an aqueous solution of the product until the pH was neutral. After stirring for 5 min the resin was removed by filtration and the filtrate freeze-dried to afford the title compound as a white solid (0.13 g, quant.). The compound was further purified by reverse phase HPLC [water as eluent], retention time = 4.0 min. R_f = 0.3 [EtOAc:EtOH:H_2O, (3:2:1)]; m.p. 175°C (dec.); λ_{max} (MeOH)/nm 264.8 ($\varepsilon/M^{-1}cm^{-1}$ 9700); δ_H (500 MHz, D_2O) 4.31 (2 H, s, CH_2N), 4.40 (2 H, d, J_{P-H} 16, C(1)H_2OP), 4.50 (2 H, dd, J_{P-H} 16, *J* 2, C(4)H_2OP), 5.68 (1 H, brs, HC=C), 5.83 (1 H, d, *J* 8, pyrimidine C(5)H) and 7.57 (1 H, d, *J* 8, pyrimidine C(6)H); δ_C (50.3 MHz, D_2O) 50.7 (CH_2N), 62.2 (d, J_{P-C} 6, CH_2OP), 63.1 (d, J_{P-C} 5, CH_2OP), 102.1 (pyrimidine C(5)), 126.3 (*HC*=C), 133.6 (HC=*C*), 146.6 (pyrimidine C(6)), 152.3 (pyrimidine C(2)=O) and 166.8 (pyrimidine C(4)=O); δ_P (101.2 MHz, D_2O) 6.08; *m/z* (Electrospray, -ve) 273 ($M - H^+$, 100%).

Sodium 3-(pyrimidin-2,4-dion-1-yl)-hydroxyacetone-1-phosphoglycoaldehyde (**18**).

Ozonisible dye, Solvent Red 19 (0.03 g, 0.08 mmol) was dissolved in methanol (5 ml) and the solution cooled to -78°C. A stream of ozone enriched oxygen was passed through the solution and the time taken to the initial diffusion of the red colouration was accurately noted. The oxygen flow was maintained and the gas stream transferred to a solution of **17** (0.02 g, 0.07 mmol) in methanol (5 ml) at -78°C. A stream of ozone-enriched oxygen was passed through the solution for the precise time earlier recorded, then the reaction vessel was purged for several minutes with argon. An excess of dimethyl sulphide (1.0 ml) was added and the mixture allowed to warm up to room temperature. After 2 h the solution was again purged with argon for several minutes to remove excess dimethyl sulphide then concentrated *in vacuo* and twice evaporated from D_2O (2 x 1 ml). The resultant glass was purified by reverse phase HPLC [water as eluent], retention time = 2.9 min, to afford the title compound as a semi-crystalline solid (0.015 g, 67%). The product was seen by ^1H nmr to exist in D_2O as a 2:1 mixture of ketone and ketone-hydrate both with hydrated aldehyde. λ_{max} (MeOH)/nm 262.1 ($\varepsilon/M^{-1}cm^{-1}$ 7600); δ_H (500 MHz, D_2O) 3.72 - 3.84 (6 H, m, $OCH_2CH(OH)_2$ (ketone and hydrate) and $OCH_2C(OH)_2CH_2N$), 3.94 - 3.98 (4 H, m, $OCH_2C(OH)_2CH_2N$ and OCH_2COCH_2N), 4.66 (2 H, d, *J* 8, OCH_2COCH_2N), 5.12 - 5.16 (2 H, m, $CH(OH)_2$ (ketone and hydrate)), 5.81 (1 H, d, *J* 8, pyrimidine C(5)H (hydrate)), 5.84 (1 H, d, *J* 8, pyrimidine C(5)H (ketone)), 7.49 (1 H, d, *J* 8, pyrimidine C(6)H (ketone)) and 7.64 (1 H, d, *J* 8, pyrimidine C(6)H (hydrate)); δ_C (125.8 MHz, D_2O) 49.0 (s, $OCH_2C(OH)_2CH_2N$ and OCH_2COCH_2N), 67.3 - 68.4 (m, $OCH_2C(OH)_2CH_2N$, OCH_2COCH_2N and $OCH_2CH(OH)_2$ (ketone and hydrate)), 82.7 ($CH_2C(OH)_2CH_2N$), 88.2 ($OCH_2CH(OH)_2$ (ketone)), 89.2 ($OCH_2CH(OH)_2$ (hydrate)),

101.1 (pyrimidine C(5) (hydrate)), 101.6 (pyrimidine C(5) (ketone)), 147.0 (pyrimidine C(6) (ketone)), 148.0 (pyrimidine C(6) (hydrate)), 151.9 (pyrimidine C(2) (ketone)), 152.6 (pyrimidine C(2) (hydrate)), 166.6 (pyrimidine C(4) (ketone and hydrate)) and 203.9 (OCH_2COCH_2N); δ_P (101.2 MHz, D_2O) 1.29 (ketone) and 1.71 (hydrate); *m/z* (Electrospray, -ve) 341 ((M - H^+) + $2H_2O$, 12%). 323 ((M - H^+) + H_2O, 100) and 305 ((M - H^+), 39).

2-Cyanoethyl-2-(4-methoxypyrimidin-2-on-1-ylmethyl)-Z-but-2-ene-1,4-diylcyclophosphate (**21**).

12 (0.40 g, 1.8 mmol) was first dissolved in acetonitrile (2 x 10 ml) and evaporated to remove residual water, then dried under vacuum over P_2O_5 overnight. The dry sample was then dissolved in oligonucleotide synthesis grade acetonitrile (10 ml) and to the stirred solution was added freshly distilled β-cyanoethyl-*N,N,N',N'*-tetramethylphosphorodiamidite (0.37 g, 1.9 mmol), as a solution in acetonitrile (5 ml), followed by 1*H*-tetrazole (0.27 g, 1.9 mmol). The resultant solution was stirred under argon for 2 h (monitored by t.l.c.), then *t*-butyl hydroperoxide (0.27 ml, 1.9 mmol, 70% aqueous solution) added and stirring continued for a further 2 h. The mixture was concentrated *in vacuo* and purified by column chromatography on silica gel [5% isopropanol in chloroform as eluent] to afford the title compound as a colourless gum (0.44 g, 74%). R_f = 0.3 [EtOAc:MeOH, (4:1)]; λ_{max} ($CHCl_3$)/nm 276.3 (ε/$M^{-1}cm^{-1}$ 4600); ν_{max}/cm^{-1} ($CHCl_3$) 3425brs (NH), 2254s (CN) and 1680brs (C=O and C=C); δ_H (200 MHz, CD_3OD) 2.91 (2 H, t, *J* 5.5, CH_2CH_2CN), 3.90 (3 H, s, OCH_3), 4.33 (2 H, dt, *J* 6, 7.5, CH_2CH_2CN), 4.49 (2 H, s, CH_2N), 4.71 - 4.80 (4 H, m, CH_2OP), 5.77 (1 H, t, *J* 1.5, C=CH), 6.08 (1 H, d, *J* 7, pyrimidine C(5)H) and 7.85 (1 H, d, *J* 7, pyrimidine C(6)H); δ_C (50.3 MHz, CD_3OD) 18.8 (CH_2CH_2CN, J_{P-C} 7.5), 51.8 (CH_2N), 53.7 (OCH_3), 63.1 (d, J_{P-C} 4, CH_2OP), 64.1 (d, J_{P-C} 5.5, CH_2OP), 65.4 (d, J_{P-C} 6, CH_2OP), 96.5 (pyrimidine C(5)), 117.4 (CH_2CN), 125.5 (HC=C), 134.9 (HC=*C*), 148.2 (pyrimidine C(6)), 157.8 (pyrimidine C(2)) and 172.7 (pyrimidine C(4)); δ_P (101.2 MHz. D_2O) 4.18; *m/z* (CI, NH_3) 342 (MH^+, 4%), 243 (41), 209 (100) and 127 (methoxypyrimidine, 30).

Sodium 2-(4-aminopyrimidin-2-on-1-ylmethyl)-Z-but-2-ene-1,4-diylcyclophosphate (**22**).

21 (0.10 g, 0.04 mmol) was dissolved in concentrated aqueous ammonia (1.0 ml, 18.8 mmol), transferred to a screw cap Eppendorf tube and heated in a water bath at 75°C for 24 h. The sample was then cooled in ice and the screw cap removed. Concentration of the resultant solution *in vacuo* afforded a gum. Freshly prepared sodium Dowex resin was added to a solution of the above gum in Milli Q water (10 ml) until the pH was neutral. After stirring for 5 min the resin was removed by filtration and the filtrate freeze-dried to afford the title compound as a white solid (0.08 g, 88%). The product was purified by reverse phase HPLC [water as eluent], retention time = 3.2 min. R_f = 0.1 [EtOAc:EtOH:H_2O, (3:2:1)]; m.p. 161 - 163°C (dec.); λ_{max} (MeOH)/nm 274.3 (ε/$M^{-1}cm^{-1}$ 8900); δ_H (200 MHz, D_2O) 4.07 (2 H, s, CH_2N), 4.11 - 4.29 (4 H, m, 2 x CH_2OP), 5.28 (1 H, brs, HC=C), 5.75 (1 H, brs, pyrimidine C(5)H) and 7.28 (1 H, d, *J* 7.5, pyrimidine C(6)H); δ_C (50.3 MHz, D_2O) 52.3 (CH_2N), 63.0 (d, J_{P-C} 5.5, CH_2OP), 63.8 (d, J_{P-C} 5.5, CH_2OP), 96.7 (pyrimidine C(5)H), 125.7 (HC=C), 135.0 (HC=*C*), 147.1 (pyrimidine C(6)H), 158.6 (pyrimidine C(2)) and 166.9 (pyrimidine C(4)); δ_P (101.2 MHz. D_2O) 6.10; *m/z* (Electrospray, -ve) 272 (M - H^+, 100%).

Sodium 3-(4-aminopyrimidin-2-on-1-yl)-hydroxyacetone-1-phosphoglycoaldehyde (**19**).

Ozonisible dye Solvent Red 19 (0.03 g, 0.08 mmol) was dissolved in methanol (5 ml) and the solution cooled to -78°C. A stream of ozone enriched oxygen was passed through the solution and the time taken to initial diffusion of the red colouration was accurately noted. The gas stream was maintained and transferred to a solution of **22** (0.02 g, 0.07 mmol) in methanol (5 ml) at -78°C. A stream of ozone enriched oxygen was passed through the solution for the precise time earlier recorded then the reaction vessel was purged for several minutes with argon. An excess of dimethyl sulphide (0.3 ml) was then added and the mixture allowed to warm up to room temperature. After 2 h the solution was again purged with argon for several minutes to remove excess dimethyl sulphide and concentrated *in vacuo* to afford a glassy solid. Evaporation from D_2O (2 x 1 ml) followed, to hydrolyse the first formed hemiacetal and afforded a colourless gum which was purified by reverse phase HPLC [water as eluent], retention time = 3.3 min, to afford the title compound as a colourless glass (0.014 g, 65 %). The product was seen by ^1H nmr spectrometry to exist in D_2O as a 2:1 mixture of ketone and ketone-hydrate both with hydrated aldehyde. λ_{max} (MeOH)/nm 271.1 ($\varepsilon/M^{-1}cm^{-1}$ 11700); δ_H (500 MHz, D_2O) 3.64 - 3.76 (6 H, m, $OCH_2CH(OH)_2$ (ketone and hydrate) and $OCH_2C(OH)_2CH_2N$), 3.87 (2 H, d, *J* 5, $OCH_2C(OH)_2CH_2N$), 3.96 (2 H, s, OCH_2COCH_2N), 4.60 (2 H, d, *J* 8, OCH_2COCH_2N), 5.05 - 5.07 (2 H, m, $CH(OH)_2$ (ketone and hydrate)), 5.92 (2 H, d, *J* 7.5, pyrimidine C(5)H (ketone and hydrate)), 7.38 (1 H, d, *J* 7.5, pyrimidine C(6)H (ketone)) and 7.52 (1 H, d, *J* 7.5, pyrimidine C(6)H (hydrate)); δ_C (125.8 MHz, D_2O) 53.8 ($OCH_2C(OH)_2CH_2N$), 61.4 (OCH_2COCH_2N), 73.6 - 74.4 (m, $OCH_2CH(OH)_2$ (ketone and hydrate), $OCH_2C(OH)_2CH_2N$ and OCH_2COCH_2N), 85.6 ($CH_2C(OH)_2CH_2N$), 94.0 ($OCH_2CH(OH)_2$ (hydrate)), 94.1 ($OCH_2CH(OH)_2$ (ketone)), 101.3 (pyrimidine C(5) (hydrate)), 101.6 (pyrimidine C(5) (ketone)), 152.9 (pyrimidine C(6) (ketone)), 154.0 (pyrimidine C(6) (hydrate)), 163.5 (pyrimidine C(2) (ketone and hydrate)), 167.7 (pyrimidine C(4) (ketone and hydrate)) and 209.3 (OCH_2COCH_2N); δ_P (101.2 MHz, D_2O) 1.14 (ketone) and 1.43 (hydrate); *m/z* (Electrospray, -ve) 340 ((M - H^+) + $2H_2O$, 30%), 322 ((M - H^+) + H_2O, 100) and 304 ((M - H^+), 64).

3-(6-Chloropurin-9-ylmethyl)furan (**24**).

Sodium hydride (0.86 g, 21 mmol, 60% dispersion) was freed from mineral oil by successive washing with pet. ether (3 x 5 ml) then covered with DMF (10 ml). To the resultant suspension was added 6-chloropurine (3.0 g, 19 mmol) and the mixture stirred until effervescence ceased. A freshly prepared solution of 3-furanmethyl bromide (1.5 eq.) in DMF was then added and after stirring for a further 2.5 h, the mixture was concentrated *in vacuo*. The yellow oil remaining was mixed with ethyl acetate (60 ml) and water (15 ml). The aqueous layer of the yellow two phase filtrate was separated and further extracted with ethyl acetate (3 x 15 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo* to afford a brown oil which crystallised on standing. A ^1H nmr spectrum of the crude mixture showed the presence of two isomers in approximately 5:1 ratio which were separated and purified by flash column chromatography on silica gel [diethyl ether followed by diethyl ether:ethyl acetate, (2:1) as eluents]. The position of alkylation was determined by HMBC nmr spectrometry.

3-(6-chloropurin-9-ylmethyl)furan was afforded as a white solid (2.27 g, 51%); R_f = 0.3 [Et_2O:EtOAc, (2:1)], m.p. 111 - 112°C (from EtOH/pet. ether); (Found: C, 51.18; H, 2.65; N, 23.90. $C_{10}H_7ClN_4O$ requires C, 51.18; H, 3.01; N, 23.87%); λ_{max} ($CHCl_3$)/nm 266 ($\varepsilon/M^{-1}cm^{-1}$ 9600); δ_H (200 MHz, $CDCl_3$) 5.34 (2 H, s,

CH_2N), 6.40 (1 H, brs, furan C(4)H), 7.45 (1 H, brs, furan C(5)H), 7.58 (1 H, s, furan C(2)H), 8.12 (1 H, s, purine C(8)H) and 8.81 (1 H, s, purine C(2)H); δ_C (125.8 MHz, $CDCl_3$) 38.9 (CH_2N), 110.1 (furan C(4)), 119.3 (furan C(3)), 131.6 (purine C(5)), 141.5 (furan C(2)), 144.7 (furan C(5)), 145.0 (purine C(8)H), 151.2 (purine C(6)), 151.7 (purine C(4)) and 152.3 (purine C(2)H); *m/z* (CI, NH_3) 235 (MH^+, 100%) and 81 (furanmethyl cation, 9).

3-(6-chloropurin-7-ylmethyl)furan was afforded as a white solid (0.49 g, 11%) R_f = 0.3 [EtOAc]; m.p. 95 - 97°C; λ_{max} ($CHCl_3$)/nm 270.5 ($\varepsilon/M^{-1}cm^{-1}$ 7100); δ_H (200 MHz, $CDCl_3$) 5.57 (2 H, s, CH_2N), 6.38 (1 H, brs, furan C(4)H), 7.49 (1 H, brs, furan C(5)H), 7.52 (1 H, s, furan C(2)H), 8.26 (1 H, s, purine C(8)H) and 8.91 (1 H, s, purine C(2)H); δ_C (125.8 MHz, $CDCl_3$) 42.2 (CH_2N), 109.4 (furan C(4)), 119.3 (furan C(3)), 122.3 (purine C(5)), 141.0 (furan C(2)), 143.0 (purine C(6)), 144.6 (furan C(5)), 148.6 (purine C(8)H), 152.4 (purine C(2)H) and 161.9 (purine C(4)); *m/z* (CI, NH_3) 235 (MH^+, 100%) 155 (MH^+ - furanmethyl cation, 23), 121 (11) and 81 (furanmethyl cation, 9).

3-(6-Azidopurin-9-ylmethyl)furan (**26**).

To a solution of **24** (1.00 g, 4.26 mmol) in DMF (10 ml) was added sodium azide (2.77 g, 42.6 mmol) and the mixture stirred at room temperature for 3 days. The mixture was concentrated *in vacuo* to afford an orange solid which was mixed with ethyl acetate (20 ml) and washed with water (2 x 5 ml). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to yield the title compound as a yellow solid (1.00 g, 98%). Further purification by flash column chromatography on silica gel [diethyl ether then diethyl ether:ethyl acetate, (2:1) as eluents] afforded the title compound as a pale yellow solid (0.83 g, 81%). For analytical purposes the product was recrystallised from ethyl acetate / dichloromethane. The free azide exhibited a solvent dependent equilibrium with the cyclic tetrazole derivative 3-(tetrazolo[5.1-i]purin-9-ylmethyl)furan **27** seen as a 2:3 mixture in the ^1H nmr spectrum in $CDCl_3$ and solely as the tri-cyclic material in the ^1H nmr spectrum in DMSO-d_6 and by X-ray crystallography[21]. R_f = 0.2 [Et_2O:EtOAc, (2:1)]; m.p. 136 - 138°C (from EtOAc / DCM); (Found: C, 49.93; H, 2.62; N, 40.48. $C_{10}H_7N_7O$ requires C, 49.79; H, 2.92; N, 40.65%); λ_{max} ($CHCl_3$)/nm 283.2 ($\varepsilon/M^{-1}cm^{-1}$ 10900); ν_{max}/cm^{-1} ($CHCl_3$) 2359w (N_3); δ_H (200 MHz, $CDCl_3$) open chain form: 5.30 (2 H, s, CH_2N), 6.39 (1 H, d, *J* 1, furan C(4)H), 7.44 (1 H, d, *J* 1.5, furan C(5)H), 7.55 (1 H, s, furan C(2)H), 7.99 (1 H, s, purine C(8)H) and 8.72 (1 H, s, purine C(2)H), cyclised form : 5.46 (2 H, s, CH_2N), 6.42 (1 H, brs, furan C(4)H), 7.47 (1 H, d, *J* 1, furan C(5)H), 7.61 (1 H, s, furan C(2)H), 8.19 (1 H, s, purine C(8)H) and 9.54 (1 H, s, purine C(2)H); δ_H (200 MHz, DMSO-d_6) 5.45 (2 H, s, CH_2N), 6.54 (1 H, brs, furan C(4)H), 7.60 (1 H, brs, furan C(5)H), 7.74 (1 H, s, furan C(2)H), 8.70 (1 H, s, purine C(8)H) and 10.10 (1 H, s, purine C(2)H); δ_C (50.3 MHz, DMSO-d_6) 39.1 (CH_2N), 110.3 (furan C(4)), 119.8 (purine C(5)), 120.7 (furan C(3)), 135.9 (purine C(8)H) 141.3 (furan C(2)), 142.1 (purine C(6)), 144.1 (furan C(5) and purine C(2)H) and 145.5 (purine C(4)); *m/z* (DCI, NH_3) 242 (MH^+, 100%), 216 (MH_3^+ - N_2, 37) and 81 (furanmethyl cation, 20).

3-(6-Triphenyliminophosphoranopurin-9-ylmethyl)furan (**28**).

To a stirred solution of **26** (2.20 g, 8.9 mmol) in THF (50 ml) was added triphenylphosphine (2.34 g, 8.9 mmol) and the mixture heated at 50°C for 5 h. Concentration *in vacuo* afforded a yellow oily solid which was dissolved in a small amount of hot ethanol (5 ml). On standing overnight the solution crystallised and after

filtration and washing of the crystals with diethyl ether the title compound was afforded as a white solid (3.00 g, 70%). Further crystallisation from the mother liquors yielded more of the required product (overall 3.40 g, 80%). R_f = 0.3 [EtOAc]; m.p. 179 - 181°C (from EtOAc / DCM); (Found: C, 70.93; H, 4.56; N, 14.47. $C_{28}H_{22}N_5OP$ requires C, 70.73; H, 4.66; N, 14.73%); ν_{max}/cm^{-1} (nujol) 1463s P-Ph; λ_{max} ($CHCl_3$)/nm 296.7 ($\varepsilon/M^{-1}cm^{-1}$ 20900); δ_H (200 MHz, $CDCl_3$) 5.19 (2 H, s, CH_2N), 6.35 (1 H, brs, furan C(4)H), 7.37 - 7.55 (11 H, m, furan C(5)H and Ph), 7.79 (1 H, s, furan C(2)H), 7.87 - 7.98 (6 H, m, purine C(8)H and Ph), 8.13 (1 H, s, purine C(2)H); δ_C (50.3 MHz, $CDCl_3$) 38.1 (CH_2N), 110.1 (furan C(4)), 120.9 (furan C(3)), 128.1 (d, J_{P-C} 32, *ipso*-Ph), 128.6 (d, J_{P-C} 15, *o*-Ph), 130.1 (purine C(5)), 132.1 (*p*-Ph), 133.6 (d, J_{P-C} 10, *m*-Ph), 139.2 (furan C(2)), 140.7 (furan C(5)), 144.1 (purine C(8)H) and 152.4 (purine C(2)H); δ_P (101.2 MHz, $CDCl_3$) 18.21; *m/z* (FAB +/-) 476 (MH^+, 100%), 183 (28), 262 (PPh_3, 17), and 81 (furanmethyl cation, 17). A list of crystallographic data has been sent to the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ.

2-(6-Triphenyliminophosphoranopurin-9-ylmethyl)-Z-but-2-ene-1,4-diol (**29**).

A solution of **28** (1.00 g, 2.10 mmol) and Rose Bengal (*ca.* 5 mg) in ethanol (7 ml), methanol (7 ml) and dichloromethane (7 ml) was stirred at -78°C (acetone/CO_2) and irradiated with a 150W tungsten filament lamp whilst a stream of oxygen was bubbled through the solution. After 3 h the mixture was treated with a solution of sodium borohydride (0.09 g, 2.30 mmol) in ethanol (7 ml) and maintained at -78°C for a further 3 h with stirring. Equilibration to room temperature was followed by addition of water (5 ml) and concentration *in vacuo*. The resultant pink residue was purified by dry flash column chromatography on alumina [dichloromethane (2 x 100 ml), then dichloromethane : ethyl acetate (1:1) (200 ml) then 10% ethanol in dichloromethane:ethyl acetate (1:1) (100 ml) as eluents]. The appropriate fractions, identified by t.l.c., were combined and concentrated *in vacuo* to afford the title compound as a white solid (0.91 g, 87%). R_f = 0.4 [EtOAc:MeOH, (4:1)]; m.p. 110 - 112°C; λ_{max} (MeOH)/nm 295.2 ($\varepsilon/M^{-1}cm^{-1}$ 24700); ν_{max}/cm^{-1} (nujol) 3205br (OH) and 1456s (P-Ph); δ_H (200 MHz, CD_3OD) 4.10 (2 H, s, C(1)H_2OH), 4.12 (2 H, d, *J* 8, C(4)H_2OH), 4.86 (2 H, s, CH_2N), 5.41 (1 H, t, *J* 6.5, HC=C), 7.45 - 7.64 (6 H, m, PPh_3), 7.86 - 7.97 (9 H, m, PPh_3), 7.97 (1 H, s, purine CH) and 8.06 (1 H, s, purine CH); δ_C (50.3 MHz, CD_3OD) 46.7 (CH_2N), 57.0 and 57.1 (2 x CH_2OH), 127.7 (purine C(5)), 128.5 (d, J_{P-C} 13, *o*-Ph), 129.5 (d, J_{P-C} 30, *ipso*-Ph), 129.7 (C=*C*H), 132.3 (*p*-Ph), 133.2 (d, J_{P-C} 9.5, *m*-Ph), 136.2 (*C*=CH), 141.0 (purine C(8)H), 149.5 (purine C(6)), 151.7 (purine C(2)H) and 161.2 (purine C(4)); δ_P (101.2 MHz, DMSO-d_6) 16.53 (PPh_3); *m/z* (FAB) 496 (MH^+, 100%), 396 (purine$PPh_3H_2^+$, 36), 262 (PPh_3, 28), 183 (34), 119 (60), 103 (2-methyl butenediol cation, 39) and 85 (62); (Found: MH^+, 496.1939. $C_{28}H_{26}N_5O_2P$ requires MH^+, 496.1902.).

t-Butyl-2-(6-triphenyliminophosphoranopurin-9-ylmethyl)-Z-but-2-ene-1,4-diyl cyclophosphate (**30**).

29 (0.30 g, 0.6 mmol) was first dissolved in acetonitrile (2 x 10 ml) and evaporated to remove residual water, then dried under vacuum over P_2O_5 overnight. The dry sample was then dissolved in oligonucleotide synthesis grade acetonitrile/DCM, (2:1) (10 ml) and to the stirred solution was added freshly distilled *t*-butoxy-*N,N,N',N'*-tetramethylphosphorodiamidite (0.13 g, 0.7 mmol) as a solution in acetonitrile (2 ml), followed by 1*H*-tetrazole (0.09 g, 1.3 mmol). The resultant solution was stirred under argon for 2 h (monitored by t.l.c.), then *t*-butyl hydroperoxide (0.09 ml, 0.7 mmol, 70% aqueous solution) was added as a solution in acetonitrile

(2 ml) and stirring continued for a further 2 h. The mixture was concentrated *in vacuo* and purified by flash column chromatography on silica gel [5% isopropanol in chloroform as eluent] to afford a colourless oil which solidified on standing to give the title compound as white crystals (0.33 g, 89%). R_f = 0.5 [EtOAc:MeOH, (4:1)]; m.p. 84 - 86°C; λ_{max} (MeOH)/nm 294.8 ($\varepsilon/M^{-1}cm^{-1}$ 25900); δ_H (200 MHz, $CDCl_3$) 1.50 (9 H, s, $C(CH_3)_3$), 4.54 - 4.65 (4 H, m, 2 x CH_2OP), 4.70 (2 H, s, CH_2N), 5.56 (1 H, t, *J* 1.5, C=CH), 7.41 - 7.59 (9 H, m, Ph), 7.77 (1 H, s, purine CH), 7.86 - 7.96 (6 H, m, Ph) and 8.09 (1 H, s, purine CH); δ_C (50.3 MHz, $CDCl_3$) 29.7 ($C(CH_3)_3$), 30.2 ($C(CH_3)_3$), 46.3 (CH_2N), 63.1 (d, J_{P-C} 5.5, CH_2OP), 64.1 (d, J_{P-C} 5.5, CH_2OP), 125.1 (C=*C*H), 128.0 (d, J_{P-C} 30, *ipso*-Ph), 128.6 (d, J_{P-C} 12.5, *o*-Ph), 130.0 (purine C(5)), 132.1 (*p*-Ph), 133.5 (d, J_{P-C} 9.5, *m*-Ph), 134.9 (H*C*=C), 139.2 (purine C(8)H), 150.2 (purine C(6)), 152.6 (purine C(2)H) and 161.6 (purine C(4)); δ_P (101.2 MHz, $CDCl_3$) -0.57 (P=O) and 18.48 (PPh_3); *m/z* (FAB+) 614 (MH^+, 21%), 558 (MH^+ - $C(CH_3)_3$, 100), 396 ((6-triphenyliminophosphoranopurine)H^+, 21) and 262 (PPh_3, 27).

Sodium 2-(6-Aminopurin-9-ylmethyl)-Z-but-2-ene-1,4-diyl cyclophosphate (**31**).

To a solution of **30** (0.15 g, 0.24 mmol) in methanol (5 ml) was added concentrated hydrochloric acid (50 µl, 12M) then water (2 ml) and the mixture left to stand for 48 h. The solvents and volatile side products were removed by concentration *in vacuo* and the residue partitioned between dichloromethane (5 ml) and water (15 ml). The washing procedure was repeated three times. Concentration of the combined aqueous layers *in vacuo* was followed by treatment with freshly prepared sodium Dowex resin which was added gradually to an aqueous solution of the above formed free acid until the pH was neutral. After stirring for 5 min the resin was removed by filtration and the filtrate freeze-dried to afford the title compound as a white solid (0.07 g, 83%). The product was purified by reverse phase HPLC [water as eluent], retention time = 6.3 min. R_f = 0.2 [EtOAc:EtOH:H_2O, (3:2:1)]; m.p. 130°C (dec.); λ_{max} (MeOH)/nm 259.8 ($\varepsilon/M^{-1}cm^{-1}$ 12900); δ_H (200 MHz, D_2O) 4.27 - 4.37 (4 H, m, 2 x CH_2OP), 4.70 (2 H, s, CH_2N), 5.52 (1 H, brs, C=CH), 8.16 (1 H, s, purine C(8)H) and 8.22 (1 H, s, purine C(2)H); δ_C (50.3 MHz, D_2O) 47.3 (CH_2N), 62.2 (d, J_{P-C} 5.5, CH_2OP), 63.1 (d, J_{P-C} 5.5, CH_2OP), 118.2 (purine C(5)), 127.2 (C=*C*H), 144.6 (purine C(8)H), 144.9 (purine C(2)H), 149.1 (purine C(6)) and 150.0 (purine C(4)); δ_P (101.2 MHz, D_2O) 6.05; *m/z* (Electrospray, -ve) 296 (M - H^+, 100%).

Sodium 3-(6-Aminopurin-9-yl)-hydroxyacetone-1-phosphoglycoaldehyde (**32**).

A stream of ozone enriched oxygen was bubbled through a solution of **31** (0.07 g, 0.2 mmol) in methanol (10 ml) until a blue colouration was observed in the mixture indicating an excess of ozone. A stream of oxygen was passed over the surface of the mixture to remove excess ozone, then dimethyl sulphide (0.3 ml, excess) added and the reaction vessel allowed to warm to room temperature overnight. Concentration *in vacuo* afforded a glassy solid which was then twice evaporated from D_2O (2 x 1 ml). Purification by reverse phase HPLC [water as eluent], retention time = 3.7 min, afforded the title compound as a semi-crystalline solid (0.05 g, 62%). The product was seen by ^1H nmr spectrometry to exist in D_2O as a 2:1 mixture of ketone and ketone-hydrate both with hydrated aldehyde. λ_{max} (MeOH)/nm 260.0 ($\varepsilon/M^{-1}cm^{-1}$ 15400); δ_H (500 MHz, D_2O) 3.75 (4 H, dd, *J* 5 and 6.5, $OCH_2CH(OH)_2$ (ketone and hydrate)), 3.82 - 3.84 (2 H, m, $CH_2C(OH)_2CH_2N$), 3.82 (2 H, s, $CH_2C(OH)_2CH_2N$), 3.83 (2 H, s, OCH_2COCH_2N), 4.78 (2 H, d, *J* 8, OCH_2COCH_2N), 5.10 (1 H, t, *J* 5, $CH(OH)_2$ (ketone)), 5.14 (1 H, t, *J* 5, $CH(OH)_2$ (hydrate)), 8.07 (1 H, brs, purine C(8)H (hydrate), 8.20 (1

H, brs, purine C(8)H (ketone) and 8.24 (2 H, brs, purine C(2)H (ketone and hydrate)); δ_C (125.8 MHz, D_2O) 49.7 ($OCH_2CO\underline{C}H_2N$), 51.1 ($CH_2C(OH)_2\underline{C}H_2N$), 68.4 - 69.7 (m, $O\underline{C}H_2CH(OH)_2$ (ketone and hydrate), $O\underline{C}H_2C(OH)_2CH_2N$ and $O\underline{C}H_2COCH_2N$), 89.3 ($OCH_2\underline{C}H(OH)_2$ (ketone)), 89.6 ($OCH_2\underline{C}H(OH)_2$ (hydrate)), 94.4 ($CH_2\underline{C}(OH)_2CH_2N$), 118.4 (purine C(5) (hydrate)), 118.8 (purine C(5) (ketone)), 143.8 (purine C(8)H (hydrate)), 144.4 (purine C(8)H (ketone)), 152.6 (purine C(2)H (ketone)), 153.1 (purine C(2)H (hydrate)), 150.0 (purine C(6) (hydrate)), 150.5 (purine C(6) (ketone)), 155.8 (purine C(4) (ketone)), 156.1 (purine C(4) (hydrate)) and 203.6 ($OCH_2\underline{C}OCH_2N$); δ_P (101.2 MHz, D_2O) 0.72 (hydrate) and 0.93 (ketone); *m/z* (Electrospray, -ve) 364 ((M - H^+) + $2H_2O$, 23%), 346 ((M - H^+) + H_2O, 73) and 328 ((M - H^+), 100).

2-Acetamido-6-chloro-9H-purine (**35**).[22]

A mixture of *N, N*-dimethylacetamide (60 ml) and acetic anhydride (8 ml) was stirred and heated to 150°C. To this hot solution was added 2-amino-6-chloro-9*H* purine (4.0 g, 24 mmol) slowly with stirring. The solid dissolved to give a yellow solution. After 25 min at 150°C the flask was allowed to cool to *ca.* 90°C, then a mixture of ethanol and water (4:1, 60 ml) was added and cooling allowed to continue overnight. The off-white precipitate which formed was filtered, washed with a small amount of acetone then air-dried to yield an off-white solid, (2.90 g, 58%). A small amount of material was recrystallised from hot *N,N*-dimethylacetamide and water for analytical purposes. R_f = 0.5 [EtOAc:MeOH, (4:1)]; m.p. >250°C (from DMA); (Found: C, 39.69; H, 2.54; N, 33.21. $C_7H_6ClN_5O$ requires C, 39.72; H, 2.86; N, 33.09%); λ_{max} (MeOH)/nm 289.0 and 256.0 ($\varepsilon/M^{-1}cm^{-1}$ 6800 and 8000); ν_{max}/cm^{-1} 1722s (C=O); δ_H (200 MHz, DMSO-d_6) 2.14 (3 H, s, CH_3), 8.50 (1 H, s, purine C(8)H) and 10.79 (1 H, brs, $N\underline{H}COCH_3$); δ_C (125.8 MHz, DMSO-d_6) 24.5 (CH_3), 145.8 (purine C(6)), 148.2 (purine C(4)), 152.1 (purine C(8)H), 154.8 (purine C(2)) and 168.5 (C=O); *m/z* (DCI, NH_3) 212 (MH^+, 100%), 169 (MH^+ - Ac, 50) and 134 (MH^+ - Ac - Cl, 11).

3-(2-Acetamido-6-chloropurin-9-ylmethyl)furan (**36**).

To a suspension of **35** (1.60 g, 7.60 mmol) in dichloromethane (50 ml) was added tetra-*n*-butylammonium hydroxide (4.95 ml, 7.60 mmol, 40% aqueous solution). The insoluble starting material was consumed as the mixture was stirred at room temperature for 1 h and the resultant solution was then concentrated *in vacuo*. The residue was co-evaporated with toluene (3 x 10 ml) and the white solid remaining dried over P_2O_5 overnight. To a suspension of the dried purinide in DMF (30 ml) was added a freshly prepared solution of 3-furanmethyl bromide (1.5 eq.) also in DMF. After stirring at room temperature for 18 h, DMF was removed *in vacuo* and the orange residue mixed with ethyl acetate (50 ml). An insoluble solid, presumed to be tetra-*n*-butylammonium bromide was removed by filtration through Celite® and ethyl acetate removed by concentration *in vacuo* to afford a yellow oil which appeared by ^1H nmr spectrometry to be a mixture of two isomers of the title compound. The isomers were separable by flash column chromatography on silica gel [diethyl ether then diethyl ether:ethyl acetate, (1:1) as eluents]. The title compound was afforded as a white solid (1.27 g, 58%). R_f = 0.4 [EtOAc]; m.p. 169 - 170°C (from EtOH); λ_{max} (MeOH)/nm 288.3 and 259.4 ($\varepsilon/M^{-1}cm^{-1}$ 11300 and 9100); ν_{max}/cm^{-1} (MeOH) 1708s (C=O); δ_H (200 MHz, CD_3OD), 2.30 (3 H, s, CH_3), 5.36 (2 H, s, CH_2N), 6.57 (1 H, d, *J* 1, furan C(4)H), 7.46 (1 H, d, *J* 1, furan C(5)H), 7.72 (1 H, s, furan C(2)H) and 8.40 (1 H, s, purine C(8)H); δ_C (50.3 MHz, CD_3OD) 23.1 (CH_3), 38.1 (CH_2N), 110.1 (furan C(4)), 120.2 (furan C(3)), 141.9 (furan C(α)), 144.0 (purine C(8)), 145.9 (furan C(α)), 152.5 (br, quaternary purines) and 170.4 (C=O); *m/z*

(Electrospray, +ve) 292 (MH^+, 100%), 314 (MNa^+, 18); (Found: MH^+ 292.0601. $C_{12}H_{10}ClN_5O_2$ requires MH^+ 292.0601).

2-(2-Acetamido-6-chloropurin-9-ylmethyl)-Z-but-2-ene-1,4-diol (**37**).

A solution of **36** (0.70 g, 2.4 mmol) and Rose Bengal (*ca.* 5 mg) in ethanol (10 ml), methanol (10 ml) and dichloromethane (10 ml) was stirred at -78°C (acetone/CO_2) and irradiated with a 150W tungsten filament lamp whilst a stream of oxygen was bubbled through the solution. After 2.5 h the mixture was treated with a solution of sodium borohydride (0.11 g, 2.9 mmol) in ethanol (5 ml) and maintained at -78°C for a further 3 h. Equilibration to room temperature was followed by neutralisation using a 10% aqueous solution of citric acid and the combined solvents were then removed by concentration *in vacuo*. The resultant pink residue was purified by flash column chromatography on alumina [ethyl acetate:methanol, (4:1) as eluent] to afford the title compound as a white solid (0.63 g, 85 %). Recrystallisation from hot ethanol provided a sample for analysis. R_f = 0.2 [EtOAc:MeOH, (4:1)]; m.p. 134 - 136°C (from EtOH); λ_{max} (MeOH)/nm 289.1, 288.4 and 260.2 ($\varepsilon/M^{-1}cm^{-1}$ 8900, 8900 and 7000); ν_{max}/cm^{-1} (MeOH) 1696m (C=O); δ_H (200 MHz, D_2O) 2.20 (3 H, s, CH_3), 4.05 (2 H, s, C(1)H_2OH), 4.14 (2 H, d, *J* 6.5, C(4)H_2OH), 4.89 (2 H, s, CH_2N), 5.67 (1 H, t, *J* 6.5, C=CH) and 8.33 (1 H, s, purine C(8)H); δ_C (125.8 MHz, D_2O) 24.13 (CH_3), 47.6 (CH_2N), 57.3 (CH_2OH), 57.4 (CH_2OH), 127.9 (purine C(5)), 131.5 (C=*C*H), 135.3 (*C*=CH), 147.7 (purine C(8)H), 150.4 (purine C(6)), 151.7 (purine C(2)), 152.7 (purine C(4)) and 173.3 (C=O); *m/z* (Electrospray, +ve) 312 (MH^+, 100%); (Found: MH^+ 312.0862. $C_{12}H_{14}N_5ClO_3$ requires MH^+ 312.0863).

t-Butyl 2-(2-acetamido-6-chloropurin-9-ylmethyl)-Z-but-2-ene-1,4-diyl cyclophosphate (**38**).

37 (0.20 g, 0.64 mmol) was first dissolved in acetonitrile (2 x 10 ml) and evaporated to remove residual water, then dried under vacuum over P_2O_5 overnight. The sample was dissolved in oligonucleotide synthesis grade acetonitrile (10 ml) and to the stirred solution under argon was added *t*-butoxy-*N,N,N',N'*-tetramethylphosphorodiamidite (0.19 g, 0.96 mmol) as a solution in acetonitrile (5 ml) followed by 1*H*-tetrazole (0.27 g, 3.8 mmol). The homogeneous mixture was stirred under argon for 3 h (monitored by t.l.c.), then *t*-butyl hydroperoxide (0.16 ml, 1.2 mmol, 70% aqueous solution) was added as a solution in acetonitrile (5 ml) and stirring continued for a further 2 h. The mixture was concentrated *in vacuo*, purified by flash column chromatography on silica gel [ethyl acetate:methanol (6:1) as eluent] and filtered through alumina to afford the title compound as a white solid (0.20 g, 83%). R_f = 0.4 [EtOAc:MeOH, (4:1)]; λ_{max} (MeOH)/nm 288.2 and 260.0 ($\varepsilon/M^{-1}cm^{-1}$ 7800 and 6400); ν_{max}/cm^{-1} (MeOH) 1712m (C=O) and 1228s (P=O); δ_H (500 MHz, CD_3OD) 1.48 (9 H, s, C(CH_3)$_3$), 2.30 (3 H, s, CH_3), 4.54 (2 H, d, *J* 16, C(1)H_2OP), 4.63 - 4.72 (2 H, m, C(4)H_2OP), 4.92 (2 H, s, CH_2N), 5.83 (1 H, t, *J* 1, C=CH), 8.39 (1 H, s, purine C(8)H); δ_C (50.3 MHz, CD_3OD) 23.5 (CO*C*H$_3$), 28.3 (C(*C*H$_3$)$_3$), 47.0 (CH_2N), 47.7 (*C*(CH$_3$)$_3$), 62.6 (d, J_{P-C} 5, CH_2OP), 63.8 (d, J_{P-C} 5, CH_2OP), 127.2 (purine C(5)), 127.4 (C=*C*H), 134.1 (*C*=CH), 142.9 (purine C(8)H), 150.2 (quaternary purine), 152.6 (quaternary purine), 152.8 (quaternary purine) and 171.2 (C=O); δ_P (101.2 MHz, CD_3OD) 0.61; *m/z* (Electrospray, +ve) 430 (MH^+, 6%), 388 (MH_2^+ - Ac, 66) and 332 (MH_3^+ - Ac - ^{t}Bu, 100).

Sodium 2-(2-amino-purin-6-on-9-ylmethyl)-Z-but-2-ene-1,4-diyl cyclophosphate (**39**).

38 (0.04 g, 0.2 mmol) was dissolved in dioxane (12 ml) and aqueous hydrochloric acid (2M, 2.4 ml). The mixture was stirred at 50°C for 48 h then concentrated *in vacuo* to afford a white solid. Sodium Dowex resin was freshly prepared and added gradually to a solution of the product in water (5 ml) until the pH was neutral. After stirring for 5 min the resin was removed by filtration and the filtrate freeze-dried to afford the title compound as a white solid, (0.025 g, 61%). The product was purified by reverse phase HPLC [water as eluent], retention time = 3.6 min. m.p. > 250°C; λ_{max} (MeOH)/nm 255.6 ($\varepsilon/M^{-1}cm^{-1}$ 11200); δ_H (500 MHz, D_2O) 4.39 (2 H, d, *J* 1.6, CH_2OP), 4.40 (2 H, dd, *J* 2, 16, CH_2OP), 4.52 (2 H, s, CH_2N), 5.42 (1 H, brs, C=CH) and 7.69 (1 H, s, purine C(8)H); δ_C (125.8 MHz, D_2O) 46.8 (CH_2N), 62.8 (d, $J_{P\text{-}C}$ 5.5, CH_2OP), 63.7 (d, $J_{P\text{-}C}$ 4.5, CH_2OP), 125.8 (C=*C*H), 134.4 (*C*=CH), 140.3 (purine C(8)H), 152.0 (quaternary purine), 154.7 (quaternary purine), 159.8 (quaternary purine) and 160.8 (quaternary purine); δ_P (D_2O, 101.2 MHz) 6.46; *m/z* (Electrospray, -ve) 312 (M - H^+, 100%).

Sodium 3-(2-amino-purin-6-on-9-yl)-hydroxyacetone-1-phosphoglycoaldehyde (**34**).

To a solution of **39** (0.045 g, 0.13 mmol) and sodium periodate (0.033 g, 0.15 mmol) in water (10 ml) was added osmium tetroxide (40 µl, 4% aqueous solution, 0.006 mmol) and the mixture stirred for 2 h. After this time the aqueous solution was washed with chloroform (5 x 5 ml) then concentrated *in vacuo* to afford a colourless glass. Purification by reverse phase HPLC [water as eluent], retention time = 2.4 min, afforded the title compound as a colourless glass, (0.0036 g, 73%). The product was seen by ^1H nmr spectrometry to exist in D_2O as a 2:1 mixture of ketone and ketone hydrate both with hydrated aldehyde. λ_{max} (MeOH)/nm 254.7 ($\varepsilon/M^{-1}cm^{-1}$ 17900); δ_H (500 MHz, D_2O) 3.55 - 3.75 (6 H, m, $OCH_2CH(OH)_2$ (ketone and hydrate), $CH_2C(OH)_2CH_2N$), 3.97 - 4.04 (4 H, m, $CH_2C(OH)_2CH_2N$ and CH_2COCH_2N), 4.65 (2 H, d, *J* 7.5, OCH_2COCH_2N), 5.04 (1 H, t, *J* 5, $CH(OH)_2$ (ketone)), 5.08 (1 H, t, *J* 5, $CH(OH)_2$ (hydrate)), 7.59 (1 H, s, purine C(8)H (hydrate)) and 7.72 (1 H, s, purine C(8)H (ketone)); δ_C (125.8 MHz, D_2O) 48.3 (OCH_2COCH_2N), 49.7 ($CH_2C(OH)_2CH_2N$), 67.8 - 68.6 (m, $OCH_2CH(OH)_2$ (ketone and hydrate), $OCH_2C(OH)_2CH_2N$ and OCH_2COCH_2N), 88.2 ($CH(OH)_2$ (ketone)), 88.3 ($CH(OH)_2$ (hydrate)), 93.4 ($CH_2C(OH)_2CH_2N$), 140.1 (purine C(8)H (hydrate)), 140.8 (purine C(8)H (ketone)), 151.6 (quaternary purine (hydrate)), 152.1 (quaternary purine (ketone)), 153.5 (quaternary purine (ketone)), 153.7 (quaternary purine (hydrate)), 158.7 (quaternary purine (ketone and hydrate)) and 202.7 (OCH_2COCH_2N); δ_P (101.2 MHz, D_2O) 0.57 (hydrate) and 0.75 (ketone); *m/z* (Electrospray, -ve) 380 ((M - H^+) + $2H_2O$, 62%), 362 ((M - H^+) + H_2O, 100) and 344 ((M - H^+), 98).

General Procedure for Polymerisation.

103 (0.02 g, 0.06 mmol) was dissolved in water (0.30 ml) and the pH adjusted to 9.5 by treatment with aqueous sodium hydroxide (0.1 M). The mixture was allowed to stand at room temperature and samples (10 µl) were removed at intervals for analytical purposes. The pH was regularly checked and readjusted to 9.5 when necessary. After 63 h the mixture was acidified to *ca.* pH 5.0 by treatment with aqueous hydrochloric acid (0.1 M) and the mixture analysed directly by ^1H and ^{31}P nmr spectrometry, HPLC, gel electrophoresis and mass spectrometry.

HMBC/HMQC nmr Data .

Tables 1-7 show HMBC and HMQC nmr data used as an aid to structural assignment. In all cases only selected nmr signals are shown with assignments made by comparison with known compounds where necessary. Observed one-bond correlations (^{1}J) are marked + and two and three-bond correlations ($^{2,3}J$) are marked x.

	δ_H (ppm)	3.99	5.32
δ_C (ppm)	Assignment	OCH_3	OCH_2
110.9	furan C(4)		x
120.9	furan C(3)		x
141.8	furan C(2)		x
165.0	pyrimidine C(2)		x
171.8	pyrimidine C(4)	x	

Table 1 HMBC nmr data for 2-(3-furanmethoxy)-4-methoxypyrimidine **10**

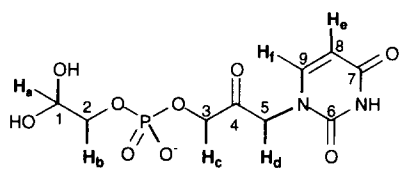
	δ_H (ppm)	3.97	4.90	7.41
δ_C (ppm)	Assignment	OCH_3	NCH_2	C(6)H
43.7	NCH_2			x
54.4	OCH_3			
96.0	C(5)			x
110.5	furan C(4)		x	
120.0	furan C(3)		x	
141.7	furan C(2)		x	
146.1	pyrimidine C(6)		x	
157.0	pyrimidine C(2)		x	x
172.5	pyrimidine C(4)	x		x

Table 2 HMBC nmr data for *N*-1-(3-furanmethyl)-4-methoxypyrimidin-2-one **11**

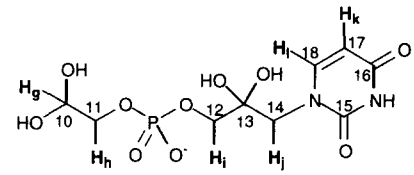
	δ_H (ppm)	5.34	8.12	8.81
δ_C (ppm)	Assignment	NCH_2	purine C(8)H	purine C(2)H
110.1	furan C(4)	x		
119.3	furan C(3)	x		
131.6	purine C(5)		x	
141.5	furan C(2)	x		
144.7	furan C(5)			
145.0	purine C(8)	x		
151.2	purine C(6)			x
151.7	purine C(4)	x	x	x

Table 3 ^1H - ^{13}C HMBC nmr data for 6-chloro-9-(3-furanmethyl)purine **24**

Table 4 shows the ^1H - ^{13}C HMQC data (1-bond correlations) and ^1H- ^{13}C HMBC data (2- and 3-bond correlations) for the carbon skeletons of **40** and **41**. **Table 5** similarly shows HMQC and HMBC data for the pyrimidine heterocycle. **Table 6** shows the ^1H - ^{31}P HMBC data for the carbon skeletons.



40



41

	δ_H (ppm)	3.72 - 3.84		3.94 - 3.98		4.66		5.12 - 5.16	
	Assignment	H_b, H_h, H_i		H_d, H_j		H_c		H_a, H_g	
δ_C (ppm)	Assignment	^1J	^2J, ^3J	^1J	^2J, ^3J	^1J	^2J, ^3J	^1J	^2J, ^3J
49.0	C_5, C_{14}	+		+	x				
67.3 - 68.4	C_2, C_3, C_{11}, C_{12}	+	x	+	x	+			
82.7	C_{13}		x		x				
88.2	C_1		x					+	
89.2	C_{10}		x					+	
203.9	C_4						x		

Table 4 ^1H - ^{13}C HMQC and HMBC nmr data for uracil monomer **18** (carbon skeleton)

	δ_H (ppm)	5.81		5.84		7.49		7.64	
	Assignment	H_k		H_e		H_f		H_l	
δ_C (ppm)	Assignment	^1J	^2J, ^3J	^1J	^2J, ^3J	^1J	^2J, ^3J	^1J	^2J, ^3J
101.1	C_{17}	+							x
101.6	C_8			+			x		
147.0	C_9				x	+			
148.0	C_{18}		x					+	
151.9	C_6						x		
152.6	C_{15}								x
166.6	C_7, C_{16}		x		x		x		x

Table 5 ^1H - ^{13}C HMQC and HMBC nmr data for uracil monomer **18** (pyrimidine)

δ_H (ppm)	3.72 - 3.84	3.94 - 3.98	4.66	5.12 - 5.16
Assignment	H_b, H_h, H_j	H_d, H_i	H_c	H_a, H_g
^{31}P - ^1H correlation	yes	yes	yes	no

Table 6 ^1H - ^{31}P HMBC nmr data for uracil monomer **18** (carbon skeleton)

References and Footnotes

1 Gilbert, W. *Nature* **1986**, *319*, 618.

2 Guerrier-Takada, C.; Gardiner, K.; Marsh, T.; Pace, N.; Altman, S. *Cell* **1983**, *35*, 849.

3 Kruger, K.; Grabowski, P. J.; Zaug, A. J.; Sands, J.; Gottschling, D. E.; Cech, T. R. *Cell* **1982**, *31*, 147.

4 Joyce, G. F. *Current Opinions in Structural Biology* **1994**, *4*, 331.

5 Illangesekare, M.; Sanchez, G.; Nickles, T.; Yarus, M. *Science* **1995**, *267*, 643.

6 Dai, X.; De Mesmaeker, A. D.; Joyce, G. F. *Science* **1995**, *267*, 237.

7 Wilson, C.; Szostak, J. W. *Nature* **1995**, *374*, 777.

8 Hodge, J. E. *Adv. Carbohyd. Chem.* **1955**, *10*, 169.

9 Stryer, L. *Biochemistry*; W. H. Freeman: New York, 1981, pp 496.

10 Hommel, U.; Lustig, A.; Kirschner, K. *European J. Biochem.* **1989**, *180*, 33.

11 Whitfield, J. N. *Studies on a Potentially Prebiotic Synthesis of RNA*; Part II Thesis, Oxford University, 1993.

12 Sutherland, J. D.; Weaver, G. W. *Tetrahedron Lett.* **1994**, *35*, 9109.

13 Kenner, G. W.; Reese, C. B.; Todd, A. R. *J. Chem. Soc.* **1955**, 855.

14 Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
15 Hargis, J. H.; Alley, W. D. *J. Am. Chem. Soc.* **1974**, *96*, 5927.
16 Nöth, H.; Vetter, H.-J. *Chem. Ber.* **1961**, *94*, 1505.
17 Veysoglu, T.; Mitscher, L. A.; Swayze, J. K. *Synthesis* **1980**, 807.
18 Shen, T. Y.; Lewis, H. M.; Ruyle, W. V. *J. Org. Chem.* **1965**, *30*, 835.
19 Eckstein, F. *Oligonucleotides and Analogues - A Practical Approach*; Oxford University Press: New York, 1991.
20 Lohmar, R.; Steglich, W. *Chem. Ber.* **1980**, *113*, 3706.
21 The solid-state tetrazole structure has been confirmed by X-ray crystallographic analysis, J. Chuhan and K. Prout unpublished results.
22 Bowles, W. A.; Schneider, F. H.; Lewis, L. R.; Robins, R. K. *J. Med. Chem.* **1963**, *6*, 471.
23 Bisacchi, G.; Singh, J.; Jr., J. D. G.; Kissick, T. P.; Mitt, T.; Malley, M. F.; DiMarco, J. D.; Gougoutas, J. Z.; Mueller, R. H.; Zahler, R. *J. Org. Chem.* **1995**, *60*, 2902.
24 Sutherland, J. D.; Weaver, G. W. *Tetrahedron Lett.* **1994**, *35*, 9113.
25 Whitney, P. S. *Studies on Potentially Prebiotic Chemistry*; Part II Thesis, Oxford University, 1996.
26 Cook, S. D.; Sutherland, J. D. *Tetrahedron Lett.* **1996**, *37*, 5779.
27 McCloskey, J. A.; Crain, P. F. *Intl. J. Mass Spectrom., Ion Processes* **1992**, *118/119*, 593-615.
28 Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal. Chem.* **1990**, *62*, 882.
29 Baldwin, J. E.; Fleming, M. D.; Sutherland, J. D.; Martin, S. L. *Pharmacia FPLC News* **1989**.
30 Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
31 Bretherick, L. *Bretherick's Handbook of Reactive Chemical Hazards*; Butterworth and Co.: London, 1990.

(*Received in UK* 8 *April* 1997; *accepted* 24 *April* 1997)