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Synthesis of Potentially Prebiotic RNA Precursors: Cytosine and Guanine Derivatives

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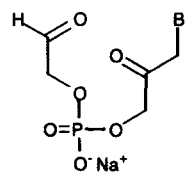
Abstract The chemical synthesis of two potentially prebiotic monomers of RNA containing cytosine and guanine is reported.

Introduction

The enigma of the origins of life has stimulated much research in the area of prebiotic chemistry.[1] The recent discovery of the catalytic properties of RNA reinforces some persuasive circumstantial evidence for the existence of the "RNA world"[2] in which RNA acts as both the purveyor of genetic information and as a catalyst for its own replication. In order to study the proposed aldol polymerisation based prebiotic synthesis of RNA first suggested by this laboratory,[3] a chemical synthesis of proposed monomers **1** - **4** was required, **Fig. 1**.



1 B = adenine
2 B = uracil
3 B = cytosine
4 B = guanine

Fig. 1 Potentially prebiotic monomers of RNA

Since base-pairing is considered of vital importance in the prebiogenesis of RNA we proposed, the monomers have been synthesised as base-pairing complements. In an earlier publication[4] we outlined efficient synthetic routes to adenine and uracil derivatives **1** and **2** and now wish to report synthesis of the corresponding cytosine and guanine variants **3** and **4**, *via* the furan double oxidolysis strategy previously described.

Results and Discussion

In an analogous manner to the formerly established routes, retrosynthetic analysis (**Fig. 2**) initially reduces targets **3** and **4** to cyclic phosphodiesters **5** and **6** respectively, the reactive nature of the two carbonyl groups making late-stage formation of these functionalities preferable. **5** and **6** should be readily accessible from base-protected diols **7** and **8** respectively, which in turn might derive from base-protected furan derivatives **9** and **10**, *via* reaction with singlet oxygen followed by hydride reduction of the first-formed ozonide equivalent.

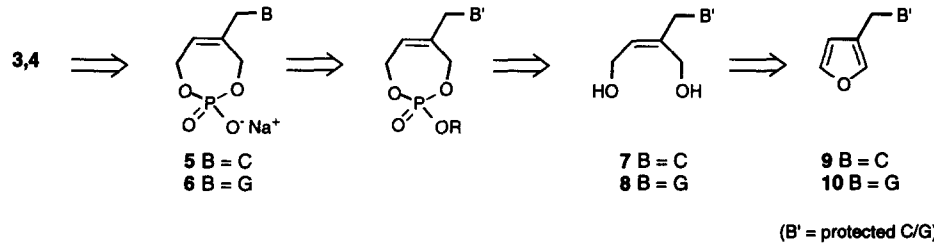
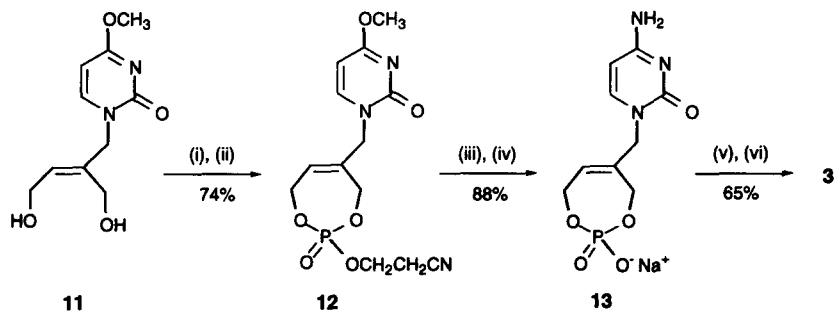


Fig. 2 Retrosynthetic analysis

Base-protected diol **11** previously prepared as an intermediate in the synthesis of uracil monomer **2**[4] provided an ideal starting point for synthesis of cytosine monomer **3**. It was reasoned that the aminopyrimidine would be accessible by displacement of the 4-methoxy substituent by ammonia at a later stage based on the work of Shen *et al.*.[5]



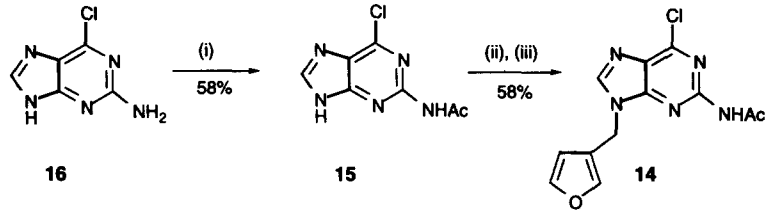
(i) $NCCH_2CH_2OP(NMe_2)_2$, 1-H tetrazole, MeCN; (ii) ^tBuOOH (70% aq. solution), MeCN; (iii) NH_4OH, 75°C; (iv) sodium Dowex, H_2O; (v) O_3 (1 eq.), MeOH, -78°C; (vi) Me_2S, MeOH, -78°C

Fig. 3 Synthetic route to cytosine monomer 3

In order to maintain conformity of protecting groups β-cyanoethoxy-*N,N,N',N'*-tetramethylphosphorodiamidite was chosen as the phosphitylating agent. This was generated by adaptation of the general procedure of Hargis and Alley[6] involving initial preparation of chloro-*N,N,N',N'*-tetramethylphosphorodiamidite[7] and its subsequent treatment with β-cyanoethanol and triethylamine. 1-H tetrazole mediated phosphitylation was followed by *in situ* oxidation using *t*-butylhydroperoxide to furnish cyclic phosphotriester **12** in good yield. Deprotection and nucleophilic aromatic substitution was accomplished by the action of concentrated aqueous ammonia at 75°C and subsequent treatment with the sodium form of Dowex-50WX8-200 furnished cytosine cyclophosphate salt **13**.

Second stage oxidolysis was carried out by controlled ozonolysis to avoid unfavourable cleavage of the pyrimidine C5-C6 double bond. The ozonisible azodye Solvent Red 19 which shows a distinct colour change upon ozonolysis,[8] was used to quantify one equivalent of ozone in a constant ozone/oxygen gas stream. Reaction of **13** with one equivalent of ozone measured in this way, furnished cytosine monomer **3** in 65% yield after purification by reverse phase HPLC.

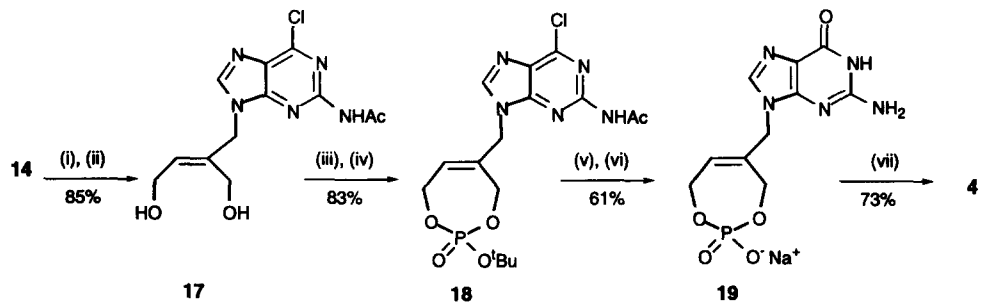
Synthesis of the base-protected furan derivative **14** as an intermediate in the preparation of guanine monomer **4** is shown in **Fig. 4**.



(i) Ac_2O, DMA, 150°C; (ii) Bu_4NOH, DCM; (iii) 3-furanmethyl bromide, DMF

Fig. 4 Route to intermediate purine-protected furan derivative

Initial attempts to protect the 2-amino substituent of **16** as an iminophosphorane (by analogy with the adenine route)[4], a phenylacetamide and an isobutyroylamide, in an effort to increase the solubility of intermediates in organic solvents were all unsuccessful. Known *N*-acetyl derivative **15** was prepared from 2-amino-6-chloropurine **16** according to the procedure of Bowles *et al.*[9] in moderate yield. Bisacchi *et al.* have recently demonstrated the use of tetra-*n*-butylammonium hydroxide in enhancing the N-9 regioselectivity in purine alkylations and in improving the solubility of the purinide anion.[10] After treatment of the so-formed anion of **15** with a freshly prepared solution of 3-furanmethyl bromide in DMF (generated by the procedure of Lohmar and Steglich)[11], 2-acetamido-6-chloro-9-(3-furanmethyl)purine **14** was isolated as the major product. A small amount of the corresponding N-7 isomer was also isolated.



(i) $^1O_2$, MeOH, EtOH, DCM, -78°C; (ii) $NaBH_4$, EtOH, -78°C; (iii) $^tBuOP(NMe_2)_2$, 1-H tetrazole (6 eq.), MeCN; (iv) ^tBuOOH (70% aqueous solution), MeCN; (v) 2M HCl, dioxane, 50°C; (vi) sodium Dowex, H_2O; (vii) OsO_4, $NaIO_4$, H_2O

Fig. 5 Synthesis of guanine monomer 4

Singlet oxygen cycloaddition followed by sodium borohydride reduction afforded diol **17** which was phosphitylated using *t*-butoxy-*N,N,N',N'*-tetramethylphosphorodiamidite (prepared as before from chloro-*N,N,N',N'*-tetramethylphosphorodiamidite, triethylamine and *t*-butanol) and 1-H-tetrazole. An excess of tetrazole was found to be required to prevent substitution of the 6-chloro substituent by liberated dimethylamine, which was thereby maintained in a protonated form. Subsequent *in situ* oxidation of the first-formed phosphite using *t*-butylhydroperoxide furnished cyclic phosphodiester **18**.

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. After much optimisation, full deprotection of **18** to give guanine cyclophosphate salt **19** was achieved by the action of aqueous hydrochloric acid in dioxane at 50°C, followed by treatment with sodium Dowex.

It was found that cleavage of the cyclophosphate double bond of **19** by ozonolysis also resulted in adverse reaction of the purine moiety. The use of osmium tetroxide with sodium periodate as cooxidant proved more favourable and enabled isolation of target compound **4** in 73% yield after purification by reverse phase HPLC.

In aqueous solution, both **3** and **4** appear as 2:1 mixtures of ketone:ketone-hydrate by nmr analysis, the aldehyde groups are fully hydrated.

Conclusion

Efficient synthetic routes to **3** and **4** have been developed which, together with the previously reported syntheses of **1** and **2**, provide the four proposed monomers required for study of a novel potentially prebiotic synthesis of RNA as earlier proposed by this laboratory.

Experiments to investigate the polymerisation behaviour of these compounds are currently underway and will be reported in due course.

Acknowledgements

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