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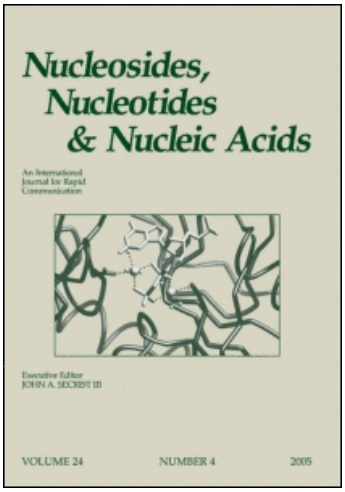

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THE SYNTHESIS OF 2'-*C*-FUNCTIONALISED NUCLEOSIDES FOR INCORPORATION INTO CATALYTIC RNA

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ABSTRACT. Five 2'-*C*-functionalized nucleosides (**1-5**) have been prepared and incorporated into dinucleoside monophosphates. The effect of the functionality on the stability of the adjacent phosphodiester bond toward hydrolysis by nuclease enzymes and extremes of pH has been assessed.

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INTRODUCTION The discovery in the early 1980's that RNA could catalyse reactions was particularly exciting due to the lack of functionality which RNA possesses.[1] In preliminary studies to probe and moderate the activity of ribozymes (catalytic RNA), we have prepared dinucleoside monophosphates containing types of functionality found in proetein based enzymes (FIG. 1), linked to the 2'-centre through a C-C bond.

The 2'-position was chosen as the site of modification for the following reasons. (i) It enables the regular 3'-5' phosphodiester linkages and the Watson-Crick hydrogen bonding scheme to be maintained. (ii) The same modification can be applied to both purine and pyrimidine nucleosides. (iii) It has been shown that introduction of 2'-α-alkyl substituents into oligonucleotides increases their resistance to degradation by nucleases.

RESULTS AND DISCUSSION Initially monomers **1-5** were prepared and protected. The synthesis of **1**, **2** and **3** has been described in a previous communication.[2]

The hydroxymethyl analogue (**4**) was prepared by an ene reaction of the allyl derivative[3] **6** in order to migrate the double bond which was cleaved with ozone. The alcohol was obtained by reductive ($NaBH_4$) work up of the intermediate ozonide. The alcohol was protected as its acetyl derivative (**10**) (FIG. 2).

The allyl compound **6** underwent Sharpless asymmetric dihydroxylation[4] to afford diol (**5**) as an 82:18 mixture of diastereoisomers. Derivatisation as a dinitrobenzoyl ester (**11**) allowed separation of the diastereoisomers by recrystallisation. The major isomer was protected as the diacetyl derivative **12** (FIG. 3).

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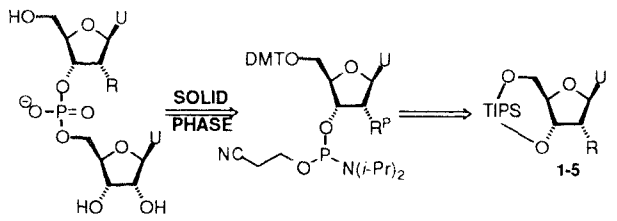

FIG. 1. Retrosynthesis of the dinucleoside monophosphates. R=CH_2COOH (**1**), CH_2CONH_2 (**2**), CH_2CH_2OH (**3**), CH_2OH (**4**), $CH_2CH(OH)CH_2OH$ (**5**). R^P = protected.

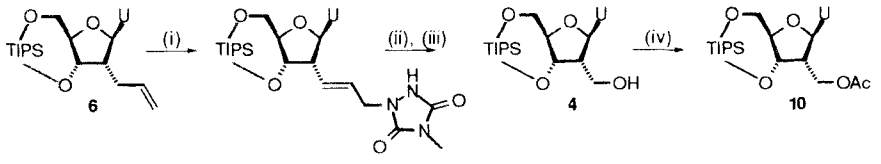

FIG. 2. *Reagents and Conditions*; (i) 4-methyl-1, 2, 4-triazoline-3, 5-dione (1.5 eq.), CH_2Cl_2, 16 h; (ii) O_3, CH_2Cl_2, 10 mins; (iii) $NaBH_4$ (8 eq.), methanol, 90% (3 steps); (iv) Ac_2O (20 eq.), pyridine, 80%.

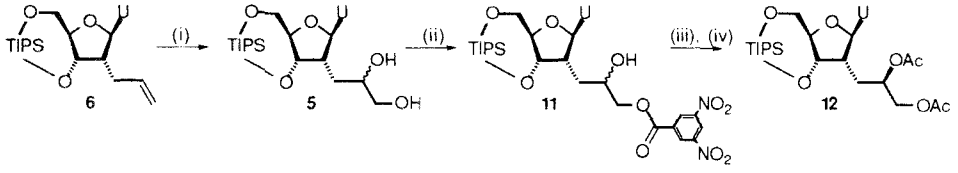

FIG. 3. *Reagents and Conditions*; (i) OsO_4 (0.01 eq.), $(DHQD)_2PYR$ (0.01 eq.), $Fe(CN)_6$, (3 eq.) K_2CO_3 (3 eq.), *t*-butanol/water (4:1), 2 h; (ii) 3, 5-dinitrobenzoylchloride (2 eq.), pyridine, 16 h; (iii) $MeOH/NH_3$; (iv) Ac_2O (20 eq.), pyridine, 16 h, 58% (3 steps).

The carboxylic acid analogue (**1**) was protected as a diphenylmethyl (benzhydryl) ester (**8**, FIG. 4), chosen due to its bulky nature in order to hinder lactonisation which was previously shown to occur with other protecting groups during desilylation.[2] The diphenylmethyl group is routinely removed under neutral (H_2, Pd/C) or acidic conditions.[5] The hydroxyethyl monomer was protected as its Fpmp ether.[2]

The monomers were derivatised as their 5'-DMT, 3'-phosphoramidites for solid-phase style introduction into oligonucleotides (FIG. 5).

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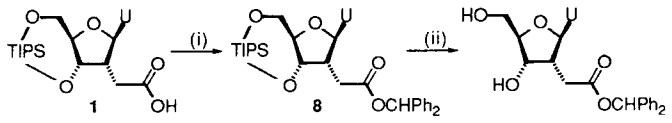

FIG. 4. *Reagents and Conditions*; (i) Ph_2CN_2 (1.4 eq.), acetone, 79%; (ii) $NEt_3 \cdot 3HF$ (10 eq.), THF, 80%.

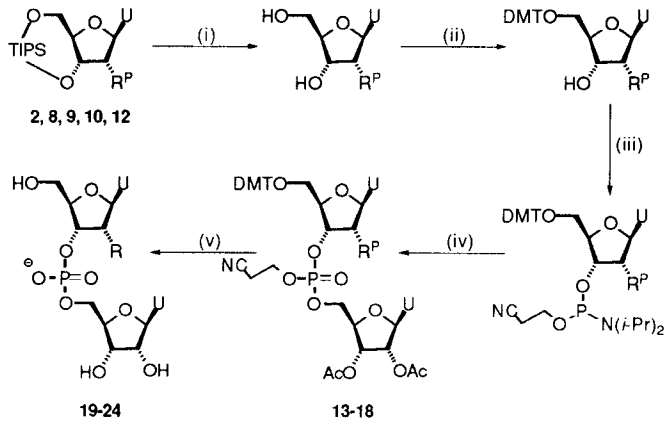

FIG. 5. (**2/13/19**) $R^P/R = CH_2CONH_2$, (**14/20**) $R^P/R = CH_2CN$; (**8/15**) $R^P = CH_2COOCHPh_2$, (**21**) R = CH_2COOH; (**9/16**) $R^P = CH_2CH_2OFpmp$, (**22**) R = CH_2CH_2OH; (**10/17**) $R^P = CH_2OAc$, (**23**) R = CH_2OH; (**12/18**) $R^P = CH_2CH(OAc)CH_2OAc$, R = $CH_2CH(OH)CH_2OH$. *Reagents and Conditions*; (i) $NEt_3 \cdot 3HF$ (10 eq.), THF, 70-90%; (ii) DMT-Cl (1.5 eq.), pyridine, CH_2Cl_2, 1.5 h, 65-90%; (iii) β-cyanoethylbis-*N, N, N' ,N'*-diisopropylaminophosphoramidite (1.1 eq.), diisopropylammonium tetrazolide (0.5 eq), CH_2Cl_2, 29-90%; (iv) 2', 3'-di-*O*-acetyluridine (0.75 eq.), 1*H*-tetrazole (5 eq.), CH_3CN; (v) deprotection.

The coupling of the amide containing phosphoramidite with di-*O*-acetyluridine led to a mixture of two products; that arising from the expected reaction to produce the amide containing dimer (**13**) and that arising from dehydration of the amide functionality to produce the respective nitrile containing dimer (**14**). The dehydration is believed to take place during oxidation of the intermediate dinucleoside phosphite, a proposed mechanism for this transformation is shown in FIG. 6.

The product composition could be largely controlled by the choice of oxidant. Standard oxidation using 4% I_2 in lutidine/water/THF (1:1:8) gave a 1.5:1 mixture in favour of the amide product (**13**). Omission of the water from the oxidant gave a ratio of

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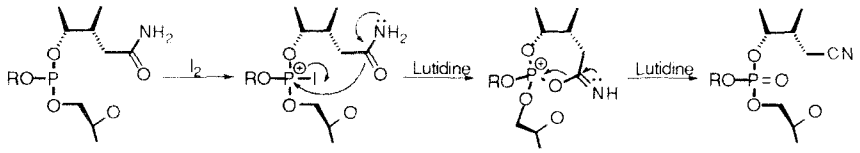

FIG. 6. Proposed mechanism for the dehydration of the amide functionality during oxidation.

9:1 in favour of the nitrile (**14**) whilst oxidation using either 70% aqueous *t*-butylhydroperoxide or 10% tetrabutylammonium Oxone® in CH_3CN gave ratios in excess of 20:1 favouring the amide (**13**).

The dinucleoside monophosphates were tested for their stability toward hydrolysis by both nuclease enzymes and extremes of pH and compared to the wild-type UpU (R = OH, relative $t_{1/2}$ = 1 in all cases). The nitrile (**20**), acid (**21**) and hydroxymethyl (**23**) containing dimers showed modest resistance toward snake venom phosphodiesterase (SVPD) (relative $t_{1/2}$ of 18, 29 and 39 respectively). The amide (**19**) showed reasonable resistance (relative $t_{1/2}$ = 84) whilst both the hydroxyethyl (**22**) and diol (**24**) containing dimers showed a large increase (relative $t_{1/2}$ = 129, 120 respectively). Only the hydroxymethyl dimer (**22**) showed signs of hydrolysis with nuclease P1 (relative $t_{1/2}$ >32). All dimers were stable to ribonuclease A after incubation for 1 week and also showed no signs of phosphodiester hydrolysis under acidic conditions (2M HCl, 25°C, 4 days). In terms of phosphodiester hydrolysis, only the hydroxymethyl dimer (**22**) showed signs of hydrolysis ($t_{1/2}$ ~ 16 h, 2M NaOH, 50°C) however, both the amide (**19**) and nitrile (**20**) were quantitatively converted to the acid (**21**) after treatment with 2M NaOH for 4 days at 25°C.

In conclusion, six 2'-*C*-functionalised dinucleoside monophosphates containing either a primary amide (**19**), a nitrile (**20**), a carboxylic acid (**21**), a primary alcohol (**22**, **23**) or a diol (**24**) have been prepared. All the modified dimers have proved to be more resistant to both nucleases and extremes of pH than UpU.

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