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Synthesis of Functionalised 2'-*C*-Branched Nucleosides *via* Their γ-Butyrolactones

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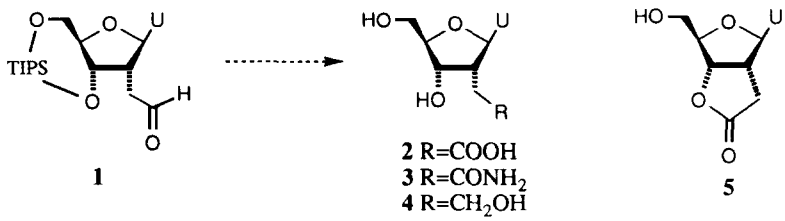
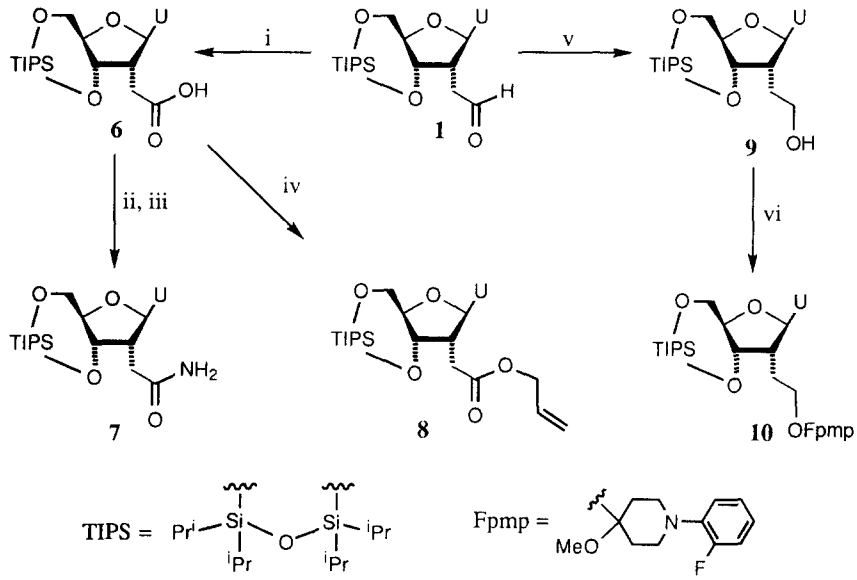
Abstract: Functionalised 2'-*C*-branched nucleosides that contain either a carboxylic acid (**2**), a primary amide (**3**) or a primary hydroxyl (**4**) group have been prepared and their protection for oligonucleotide synthesis investigated. The 2'-*C*-3'-*O*-γ-butyrolactone (**5**) was also shown to be a useful intermediate for the preparation of these analogues.

The discovery that RNA can catalyse reactions[1,2] was particularly exciting since nucleic acids do not possess the diversity of functional groups that are associated with catalytic activity in protein enzymes. To a limited extent RNA compensates for this deficiency by recruiting metal ions which are able to facilitate reactions by acting as general acid/base catalysts or Lewis acid catalysts.[3,4] Despite this assistance, the variety of reactions that RNA enzymes (ribozymes) are known to catalyse is extremely narrow in comparison to protein enzymes. It is interesting to speculate as to whether the catalytic activity and substrate diversity of ribozymes might be enhanced by the inclusion of functionality that is known to be important for protein enzymes. Robertson and Miller have recently hypothesised that functionalised RNA may have had prebiotic importance and could have been the link between the primitive RNA world and the modern DNA-protein world.[5] We have started to address these issues by preparing 2'-*C*-branched nucleosides that contain either a carboxylic acid (**2**), a primary amide (**3**) or a primary hydroxyl (**4**) group. We believe that as more becomes known about the three dimensional structure of ribozymes,[6,7] their sequence-specific modification, through the incorporation of these analogues will become an important technique for understanding and moderating their activity. In this preliminary communication we describe the synthesis of compounds **2**, **3** and **4**, from the protected aldehyde (**1**), their derivatisation with protecting groups suitable for oligonucleotide synthesis and also draw attention to the nucleoside-γ-butyrolactone (**5**) as an intermediate for the preparation of 2'-*C*-branched nucleoside analogues.



1 **2** R=COOH, **3** R=$CONH_2$, **4** R=CH_2OH **5**

For the first step in the preparation of the carboxylic acid (**2**) the previously reported aldehyde[8] (**1**) was oxidised using $NaClO_2$ in the presence of KH_2PO_4 and 2-methyl-2-butene in aqueous t-butanol, to give **6** (**Scheme 1**) in essentially quantitative yield.[9,10] Conversion to the primary amide (**7**) was achieved *via* the intermediate *N*-hydroxysuccinimide ester.[11] Thus treatment of acid (**6**) with *N*-hydroxysuccinimide and DCC in dioxane overnight, followed by the addition of excess concentrated aqueous ammonia, cleanly produced **7** in 79% yield after purification by column chromatography.



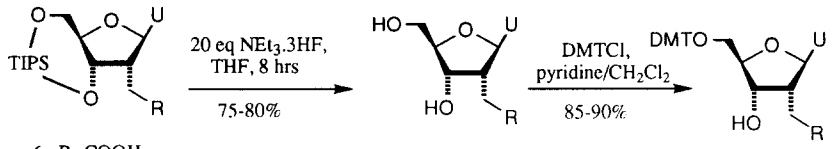
Scheme 1 - *Reagents and conditions:* i) 15eq $NaClO_2$, 10eq KH_2PO_4, 20eq 2-methyl-2-butene, ^tBuOH:water (3:1); ii) 1.3eq NHS, 1.3eq DCC, 0.3eq DMAP, dioxane; iii) excess conc aq. NH_3; iv) 1.1eq CH_2=$CHCH_2OH$, 1.1eq DCC, 1.1eq DMAP, THF; v) 3eq $NaBH_4$, MeOH, 0°C; vi) 1.6eq 1-(2-fluorophenyl)-4-methoxy-1,2,3,6-tetrahydropyridine, 0.5eq camphorsulfonic acid, CH_2Cl_2.

Whilst it is envisaged that incorporation of **3** into oligonucleotides will not require protection of the amide function, a blocking group for the carboxylic acid function is clearly necessary in the case of **2**. There is no precedent for the protection of a comparable carboxylic acid function in oligonucleotide chemistry, but since it is likely that this functionality could assist the hydrolysis of the vicinal phosphodiester bond at extremes of pH, it was considered prudent to use a blocking group that could be removed under neutral conditions. Therefore the allyl group, (generally removed using palladium)[12] was introduced using allyl alcohol and DCC to give the ester (**8**) in 80% yield. Alternatively **8** could be prepared by treatment of the *N*-hydroxysuccinimide ester with allyl alcohol and DBU.

For the preparation of a suitably protected derivative of the hydroxyethyl nucleoside (**4**) the aldehyde (**1**) was reduced using $NaBH_4$ in MeOH to give the alcohol (**9**, **Scheme 1**) in quantitative yield.[8] The 1-(2-fluorophenyl)-4-methoxypiperidinyl (Fpmp) group was chosen to protect the primary hydroxy group.[13,14] This achiral acetal group has been extensively used in RNA synthesis and unlike the rival *t*-butyldimethylsilyl group, it is compatible with the removal of the cyclic TIPS ether. Thus,

treatment of the hydroxyethyl nucleoside (**9**) with excess 1-(2-fluorophenyl)-4-methoxy-1,2,3,6-tetrahydropyridine in the presence of camphorsulfonic acid gave the Fpmp-protected nucleoside (**10**) in 73% yield.

Removal of the TIPS protecting group (**Scheme 2**) was achieved using $NEt_3 \cdot 3HF$ complex as a fluoride source. Although deprotection with this reagent is slower than that achieved with the more commonly used TBAF, the NEt_3 residues could be removed relatively easily by flash column chromatography, although in the case of the allyl ester (**8**) desilylation resulted in partial lactonisation. However, compounds **3** and **12** were subsequently converted to their 5'-*O*-dimethoxytrityl derivatives (compounds **13** and **14** respectively), in preparation for oligonucleotide synthesis. The synthesis and properties of oligo- and dinucleotides containing **2**, **3** and **4** is currently under investigation.



6 R=COOH
7 R=$CONH_2$
8 R=COOAllyl
9 R=CH_2OH
10 R=CH_2OFpmp

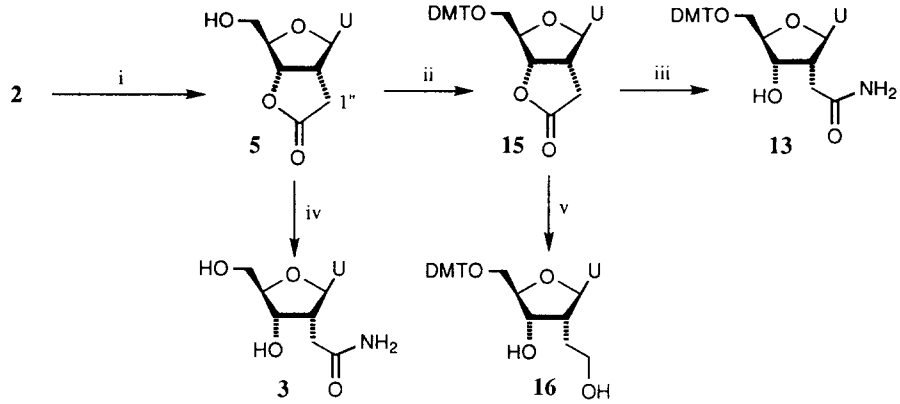
2 R=COOH
3 R=$CONH_2$
4 R=CH_2OH
11 R=COOAllyl
12 R=CH_2OFpmp

13 R=$CONH_2$
14 R=CH_2OFpmp

Scheme 2

The formation of 5-membered ring lactones is extremely favourable and often spontaneous, thus we were somewhat surprised to find that the fully deprotected carboxylic acid (**2**) was relatively stable, and could easily be purified by column chromatography. However, in solution, tlc analysis revealed that both the acid (**2**) and the amide (**3**) underwent slow cyclisation to the lactone (**5**). Preparatively, a variety of conditions for the formation of the fused-ring lactone were investigated. The most efficient conversion, in terms of speed of reaction and ease of purification, was achieved by warming the acid (**2**) at 60°C in acetic acid:methanol (80:20) for 2 hrs, and under these conditions the lactone was obtained quantitatively (**Scheme 3**).[15]

Our interest in the lactone (**5**) was stimulated by a recent report by Camarasa and co-workers[16], which describes the synthesis of some 1''-alkyl nucleoside-γ-butyrolactones through a radical cyclisation. Their studies on the susceptibility of these lactones to ring-opening showed that they were resistant to aminolysis under standard conditions and these reactions could only be achieved in the presence of aluminium chloride. However, treatment of the lactone (**5**) with 15% aqueous ammonia at 0°C led to quantitative formation of the primary amide (**3**) in less than 10 min. In order to explore the reactions of this lactone (**5**) more fully it was first converted to the more soluble dimethoxytrityl derivative (**15**). This protected lactone could also be converted to the corresponding primary amide (**13**) with methanolic ammonia. Gratifyingly reduction of the protected lactone (**15**) could be achieved using DIBAL in THF, to give the alcohol (**16**) quantitatively. The lactone route to this alcohol is particularly useful since it provides a facile means of distinguishing between the two primary hydroxyl groups of the fully deprotected triol (**4**), and this will be beneficial in the further manipulation of this compound.



Scheme 3 - *Reagents and conditions:* i) AcOH:MeOH (80:20), 60°C; ii) 1.5eq DMTCl, pyridine:CH_2Cl_2 (65:35); iii) sat NH_3/MeOH; iv) excess conc aq. NH_3; v) 3eq DIBAL, THF, -78°C to rt.

In conclusion, three functionalised 2'-*C*-branched nucleosides for incorporation into oligonucleotides have been prepared. We have also shown that the nucleoside-γ-butyrolactone (**5**) is a very useful intermediate for the preparation of this type of nucleoside analogue and we are currently investigating the functionalization of this important building block.

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