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DISACCHARIDE NUCLEOSIDES AND THEIR ENZYMATIC AND CHEMICAL INCORPORATION INTO OLIGONUCLEOTIDES

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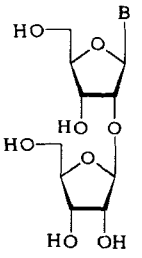
Abstract. A high yield synthesis of different *O*-ribofuranosylnucleosides has been achieved. Kinetics of the acid-catalysed hydrolysis of disaccharide nucleosides has been studied. Chemical and enzymatic incorporation of 2'-*O*-ribofuranosyl-nucleoside residue into oligonucleotides was investigated.

Purine nucleosides with additional D-ribofuranosyl residue at 2'-hydroxyl function of the natural nucleoside have been isolated from yeast methionine tRNA[1]. Several disaccharide nucleosides have shown biological activities, and some antibiotics have this type of structure[2]. Recently a general method has been developed[3-7] for the preparation of 2'/3'-*O*-ribofuranosylnucleosides **1a-e** and **2b,c**. Condensation of partially protected ribo- or deoxynucleosides with a slight excess of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose was accomplished in the presence of tin tetrachloride (dichloroethane, 0°C, under nitrogen). The glycosylation reactions stereospecifically yielded the β-anomers. The yields for the analogues **1** and **2** were in the range from 70% to 80%[4-7], which was higher than previously reported for similar condensation reactions between blocked nucleosides and monosaccharides[2]. The structure of these compounds was proven by NMR,

mass spectrometry and X-ray analysis[4,5,7]. The similar conformation of uridine derivative **1a** was observed in crystal and in solution[7]. In crystal the additional ribose residue was located nearby to the uracil moiety. NOE interactions were observed between base protons and H-5'a and H-5'b of the extra ribose residue.



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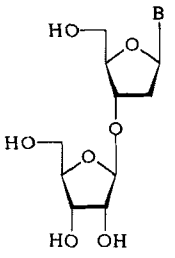

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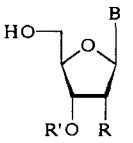
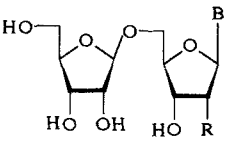
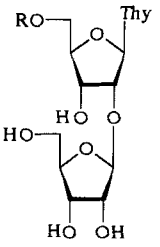
a. B=Ura **b.** B=Thy **c.** B=Cyt **d.** B=Ade **e.** B=Gua



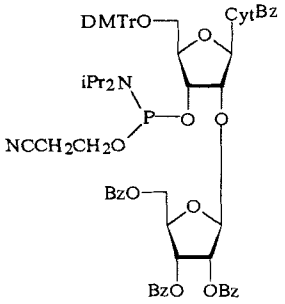
3a R=OBz, R'=Bz
3b R=OAc, R'=Ac
4b,c R=H, R'=SiPh2t-Bu
a. B=Ura **b.** B=CytBz **c.** B=Thy



5 R=OH
6 R=H
a. B=Ura **b.** B=Cyt **c.** B=Thy



7 R=P3H9O4



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To get further insight into the scope and limitations of this reaction we examined ribosylation of N,2',3'-*O*-protected ribonucleosides **3a,b** and N,3'-*O*-protected 2'-deoxynucleosides **4b,c** under the previously developed conditions[4-7]. After deprotection the desired 5'-*O*-β-D-ribofuranosylnucleosides **5a,b** and **6b,c** were obtained in high overall yields[8]. It was shown also that triflate catalyst may substitute successfully tin tetrachloride in this reaction.

First-order rate constants for the acid-catalysed hydrolysis of different ribofuranosylnucleosides have been determined[8]. The *O*-glycosidic bond is susceptible to acid-catalysed hydrolysis. The ribofuranosyl moiety is cleaved nearly as fast from the primary 5'-*O* of uridine **5a** or thymidine **6c**, and from the secondary 3'-*O* of thymidine **2b**. At 2'-hydroxyl the ribofuranosyl substitution in compound

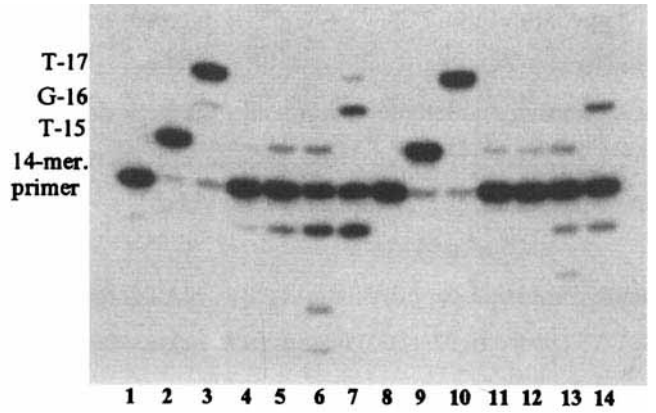

FIG. 1. Autoradiogram of [5'-^{32}P]primer elongation in the presence of dNTP and compound **7** catalysed by Klenow fragment (lanes 1-7) and reverse transcriptase of AMV (lanes 8-14): 1,8 - control ([primer + template] + enzyme); 2,9 - control + 2μM dTTP; 3,10 - control + 2μM dTTP + 2μM dGTP; 4,11 - control + 2μM of **7**; 5,12 - control + 20μM of **7**; 6,13 - control + 200μM of **7**; 7,14 - control + 200μM of **7** + 2μM dGTP.

1a is slightly, about 2 fold, more stable. With 2'-*O*-ribofuranosyladenosine **1d** depurination was found to compete with the rupture of the *O*-glycosidic bond.

Substrate properties of 1-(2-*O*-β-D-ribofuranosyl-β-D-ribofuranosyl)-thymine 5'-triphosphate **7** were studied in DNA and RNA synthesis reactions catalysed by different enzymes. It was found that **7** was a weak substrate for *E.coli* DNA polymerase I (Klenow fragment) and reverse transcriptase from avian myeloblastosis virus (Fig. 1). No incorporation of disaccharide nucleoside residue was observed in reactions catalysed by human DNA polymerases α and β and calf thymus terminal deoxynucleotidyl transferase. The same results were obtained with periodate oxidised derivative of **7**. Compound **7** and dialdehyde derivative weakly inhibited the RNA synthesis catalysed by T7 RNA polymerase (wild type), mutant Tyr639Phe and double mutant Tyr639Phe, Ser641Ala[9]. These compounds were found not to be substrates of such enzymes.

Chemical incorporation of 2'-*O*-ribofuranosylnucleosides into oligonucleotides was accomplished starting from phosphoramidite **8**. The incorporation of disaccharide nucleoside residue into oligonucleotides destabilised the duplex

formation by approximately 2-3° C. The supplementary *cis* diol group in disaccharide nucleoside derivatives and oligonucleotide may be used for introduction of a reactive dialdehyde group using the periodate oxidation reaction. Oligonucleotides with reactive dialdehyde groups are currently used as affinity labels for different polymerases and restriction enzymes.

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