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Chemical Synthesis of ^{13}C-labelled Monomers for the Solid-Phase and Template Controlled Enzymatic Synthesis of DNA and RNA Oligomers

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Abstract: The preparation of ^{13}C-labelled ribonucleosides starting from [$^{13}C_6$]-glucose **1** and the corresponding nucleobases **5a-e** or **6a-e** (N^6-benzoyl-adenine, N^2-acetyl-guanine, N^4-benzoyl-cytosine, uracil and thymine) in 47 - 66% overall yield is described. Their subsequent transformation into 5'-*O*-dimethoxytrityl protected DNA-phosphoramidites and 5'-*O*-dimethoxytrityl-2'-*O*-trialkylsilyl protected RNA-phosphor-amidites for the solid phase synthesis of DNA- and RNA-oligomers and to 5'-*O*-ribo- and deoxyribo-nucleosidetriphosphates for template controlled enzymatic synthesis (polymerase- or reverse transcriptase reaction) has been carried out.

The availability of ^{13}C and ^{15}N labelled proteins[1] has stimulated the NMR spectroscopic structure elucidation of proteins over the last years. Powerful multidimensional methods relying on ^{13}C and ^{15}N labelled proteins[2,3,4,5] have been developed to automate the spectral assignment of resonances[6,7,8] and to extract conformationally relevant NMR parameters such as NOE[9,10] and coupling constants[11,12,13,14,15,16] even for proteins with molecular weights exceeding 25 kDa. A transfer of these methods to labelled DNA and RNA offers a promising tool for future high resolution DNA and RNA structure determination. Because of the low chemical shift dispersion of DNA and even more dramatic RNA oligonucleotides,[17] the possibility to label individual monomers gives advantage over uniform labelling techniques.

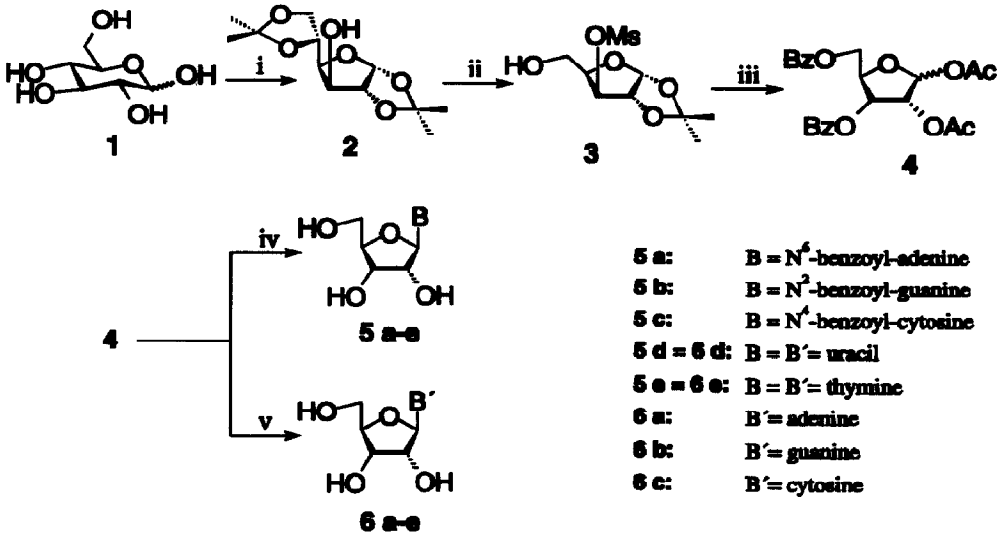
The preparation of ^{13}C,^{15}N labelled ribonucleoside monophosphates (NMP) from bacteria grown on minimal medium has been reported.[18,19,20,21] These compounds can be converted by enzymatic phosphorylation[22] into ribonucleoside triphosphates (NTP) and incorporated into oligoribonucleotides in e.g. the T7-polymerase reaction.[23] The yield of isolation of NMP obtained by this method is ~ 4% relative to [$^{13}C_6$]-glucose. Since the abundance of DNA in cells is only about 15% of that of RNA, isolation from cells is much less an economical way to labelled DNA.

We report the chemical synthesis of ^{13}C-labelled ribonucleosides with good yields opening the opportunity to further transform the ribonucleosides to DNA and RNA monomers both for the solid phase and template controlled enzymatic synthesis of DNA and RNA oligomers.[24,25,26,27,23]

Key compounds in the chemical synthesis of both forms of monomer building-blocks are the ribosyl ^{13}C-labelled nucleosides **6a-e** that we obtain in an overall yield of 47-66% in nine steps from **1** (Scheme 1).

1,2:5,6-Di-*O*-isopropyliden-D-glucose **2** is obtained in 93% yield from [$^{13}C_6$]-glucose **1** in a similar procedure as reported by Glenn et al.[28] After mesylation and selective deprotection of the 5,6-acetonide[29] 1,2-*O*-isopropylidene-3-*O*-mesyl-D-glucofuranose was obtained in >99%. This compound can be converted in 90% yield to **3** in a one flask reaction by periodate cleavage and reduction.[30] Subsequent benzoylation with benzoyl chloride yields 5-*O*-benzoyl-1,2-*O*-isopropylidene-3-*O*-mesyl-D-xylofuranose in 98%. After deprotection of the 1,2-acetonide[31] and subsequent acetylation of the resulting diol, 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-*O*-mesyl-xylofuranose is obtained in 97%. The inversion at C3 with tetrabutylammoniumbenzoate in toluene/water to the ribosyl derivative gives 3,5-di-*O*-benzoyl-1,2-di-*O*-acetyl-ribofuranose **4** in 71%

overall yield (based on 1). We also tried a different way from compound 2 to 4, mentioned in the literature: The oxidation of 2 to the analog 3-keto compound[32] and subsequent reduction[33] give inversion to 1,2-isopropylidene-allofuranose in 81% yield. Selective deprotection of the 5,6-acetonide[34], periodate cleavage and reduction[30] yields 1,2-isopropylidene-ribofuranose in 53%. Treatment with benzoyl chloride and acetolysis[31] gives 4 in 40% overall yield. 4 is used as sugar component in a Vorbrüggen glycosylation.[35,36] Using either sodium hydroxide/ methanol/pyridine or methanolic NH_3 leads to the different nucleosides **5a-e** or **6a-e**, respectively.



i: $ZnCl_2$, H_3PO_4, acetone;
ii: 1. methanesulfonyl chloride (MsCl), DMAP, NEt_3, CH_2Cl_2; 2. HCl 2N, methanol; 3. $NaIO_4$, water/$NaBH_4$, ethanol;
iii: 1. benzoyl chloride (BzCl), DMAP, pyridine; 2. AcOH, Ac_2O, H_2SO_4, NaOAc; 3. tetrabutylammoniumbenzoate, toluene/water;
iv: 1. silylated nucleobase, trimethylsilyltriflate or $SnCl_4$, dichloroethane; 2. NaOH/methanol/pyridine
v: 1. silylated nucleobase, trimethylsilyltriflate or $SnCl_4$, dichloroethane; 2. NH_3/methanol

Scheme 1

The nucleosides **5a-e** or **6a-e** are further transformed by standard procedures to obtain monomers for RNA or DNA synthesis. For template controlled enzymatic synthesis 5'-nucleoside triphosphates are used as substrates for polymerase reaction. In solid-phase synthesis RNA and DNA phosphoramidites are of common use.

5'-Ribonucleoside triphosphates can be obtained by chemical 5'-*O*-monophosphorylation of **6a-d** by Yoshikawa's method[37] and subsequent enzymatical triphosphorylation.[20]

5'-Deoxyribonucleoside triphosphates are accessible after 2'-deoxygenation[38] by a direct chemical route.[39]

RNA phosphoramidites can be synthesized starting from the base protected nucleosides **5a-d**. After dimethoxytritylation, silylation with trialkylsilyl chloride as reported by Ogilvie[40] gives the 2'-silyl compounds **7a-d** (Fig.1) which are subsequently phosphitylated to obtain the phosphoramidites. The undesired 3'-silyl isomer which always is built in various amounts can be deoxygenated at C2' without further protection and subsequently phosphitylated to yield DNA phosphoramidites.

To obtain deoxyribonucleosides selectively, the ribonucleosides **5a-c,e** can be transformed according to Robins et al.[38] to **8a-c,e**. (Fig.1) 5'-*O*-dimethoxytritylation and phosphitylation lead to the DNA phosphoramidites.

7 a-d **8 a-c,e**

Figure 1

All described synthetic procedures have been optimized with unlabelled material. If not explicitely shown the yields are as given in the literature. No difference in yields have been observed with labelled material. 400MHz nmr spectra of ($^{13}C_5$-ribo)-N4-benzoyl-5'-*O*-dimethoxytrityl-2'-*O*-(*t*-butyl-dimethyl-silyl)-cytidine **7c** and ($^{13}C_5$-deoxyribo)-thymidine **8e** are shown in figure 2.

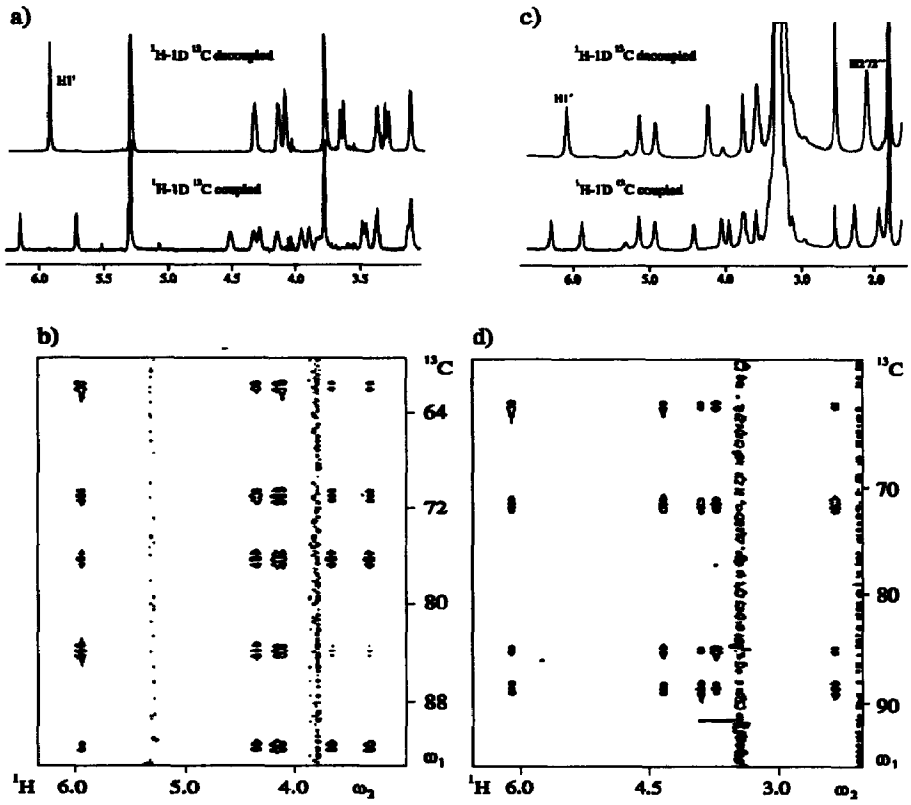


Figure 2

400MHz nmr spectra of ($^{13}C_5$-ribo)-N4-benzoyl-5'-*O*-dimethoxytrityl-2'-*O*-(*t*-butyl-dimethyl-silyl)-cytidine **7c** (left) and ($^{13}C_5$-deoxyribo)-thymidine **8e** (right). 1D-^{1}H spectra ^{13}C coupled and decoupled are shown in a) and c). HCCH-TOCSY spectra in b) and d).

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