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EFFICIENT SYNTHESIS OF D-[1'-¹³C]-RIBONUCLEOSIDES FOR INCORPORATION INTO OLIGO-RNA

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NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4-7), 937-940 (2001)

EFFICIENT SYNTHESIS OF D-[1'-¹³C]-RIBONUCLEOSIDES FOR INCORPORATION INTO OLIGO-RNA

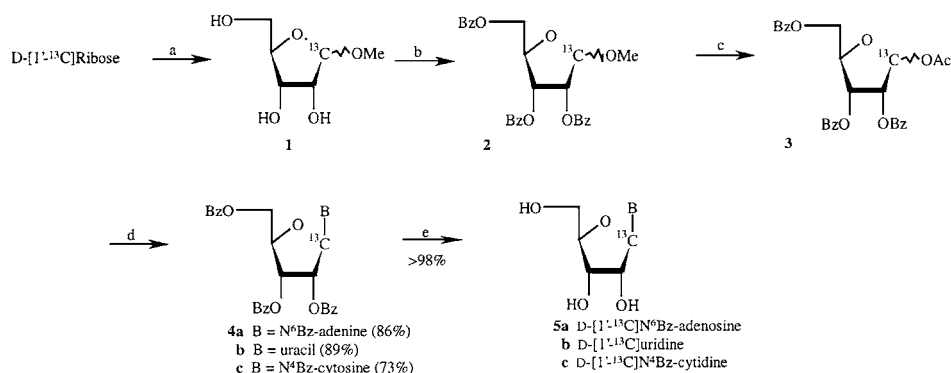
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ABSTRACT

Syntheses of the monomer building blocks used for the solid-phase synthesis of specifically 1'-¹³C-labeled oligoribonucleotides from the D-[1-¹³C]ribose is presented. Procedure has been used for the selective formation of 2'-*O*-silylated ribonucleosides. After 5'-*O*-dimethoxytritylation, the synthesis of D-[1'-¹³C]ribonucleoside phosphoramidites has been achieved.

The utility of ¹³C-labeled RNAs and DNAs for the heteronuclear multidimensional NMR studies for nucleic acids has been shown in several reports (1). One of the major problems in the chemical synthesis of ¹³C enriched nucleoside derivatives is the price of the starting compound that requires optimized reactions. Standard procedures for preparation of nucleoside synthons used in solid-phase synthesis of oligoribonucleotides include protection of the amino groups on the nucleobases, protection of the 5'- and 2'-hydroxyl groups as dimethoxytrityl (DMTr) and TBDMS ether, respectively, and phosphorylation of the 3'-oxygen to the β-cyanoethyl *N,N*-diisopropylphosphoramidite (Ogilvie's approach) (2). In designing the synthesis of monomer for labeled oligo-RNA, we considered a regioselective protection of the ribose hydroxyls in such a way as to obtain solely the 2'-*O*- *tert*-butyldimethylsilyl derivative and to circumvent the ever aggravating problem of the 2'- vs. 3'-silylation. This paper describes the syntheses of the [1'-¹³C]-labeled building blocks for the preparation of oligo-RNA. A selective and efficient procedure for the 2'-*O*-silylation has been applied for the synthesis of [1'-¹³C]-ribonucleoside phosphoramidites. The D-[1-¹³C]ribose was converted into the D-[1'-¹³C]ribonucleosides following well known procedure (Scheme 1).



Scheme 1. Reagents and Conditions: (a) MeOH, H₂SO₄; (b) BzCl, pyridine, rt; (c) Ac₂O, AcOH, conc. H₂SO₄, 0°C to rt; (d) Base, TMSOTf, DCE, reflux; (e) 1N NaOH pyridine/EtOH (for **4a** and **4c**) or NH₃/MeOH (for **4b**) then amberlite H⁺.

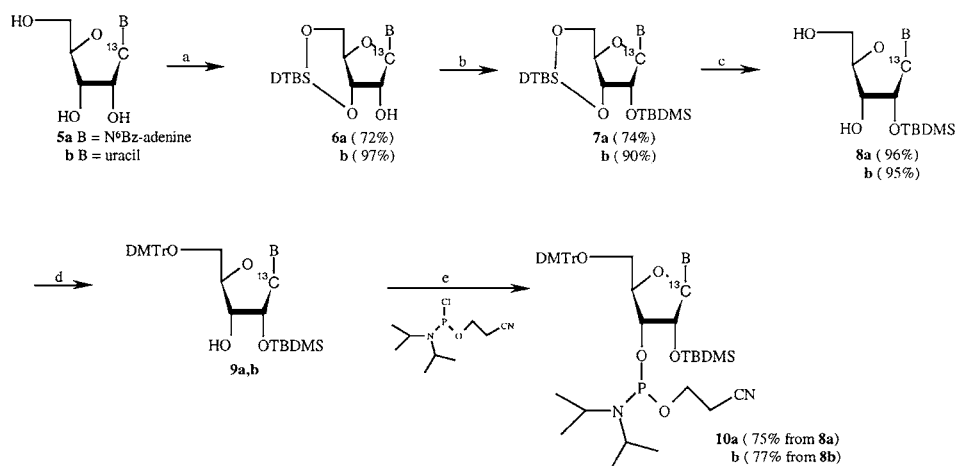
Compound **3** was subjected to a coupling reaction (3) with persilylated nucleobases (persilylated N⁶-benzoyladenine, uracil, and N⁴-benzoylcytosine) to give 2,3,5-*O*-tri-benzoyl-[1'-¹³C]ribonucleoside derivatives **4a–c** with fairly good yields (86%, 57% and 73% respectively). In case of the synthesis of protected uridine **4b**, the temperatures must be kept under 45°C to avoid undesired side product. Finally, the selective deprotection of *O*-acyl groups from **4a–c** was achieved with aqueous 1N NaOH-ethanol-pyridine (for **4a** and **4c**) or NH₃/MeOH (for **4b**).

In all cases, deacylated nucleosides **5a–c** were directly engaged into the next reaction. A principal consideration in the chemical syntheses of the ribonucleoside phosphorimidates is contamination of the desired 2'-*O*-silyl-3'-*O*-phosphoramidite with undesired 3'-*O*-silyl-2'-*O*-phosphoramidite. Thus, the classical procedure to introduce *tert*-butyldimethylsilyl as a 2'-OH protecting group presents the problem of optimizing the yield of the desired 2'-*O*-tBDMS isomer, consuming valuable labeled ribonucleosides and lowering the yield of desired labeled ribonucleoside phosphoramidites. This is mainly due to the fact that during the silylation reaction, the 2'- and 3'-hydroxyl groups are not markedly distinguishable; these interconversions are base catalyzed. To circumvent this problem, we applied a three steps procedure involving the initial and regioselective protection of 3',5'-hydroxyl using the Markiewicz disiloxane reagent (4). As a result, we obtain the desired compounds avoiding by-products as obtained by the classical approach (5). Thus treatment of D-[1'-¹³C]ribonucleosides **5a,b** with di-*tert*-butyldichlorosilane in the presence of AgNO₃ in DMF afforded the corresponding 3',5'-di-*O*-*tert*-butylsilyl derivatives (6) **6a** (72%) and **6b** (97%), respectively, (Scheme 2). Treatment with *tert*-butyldimethylsilyl chloride (TBDMSiCl) and silver nitrate in DMF led 2'-*O*-tBDMS ether **7a** and **7b** in 74% and 90% yield, respectively.

The di-*tert*-butylsilylene group was then removed by treatment with pyridinium poly(hydrogen fluoride) at -20°C to afford **8a** and **8b** with 96% and 95% yield, respectively. One hour in the presence of DMTrCl and AgNO₃ was

SYNTHESIS OF D-[1'-¹³C]-RIBONUCLEOSIDES

939



Scheme 2. Reagents and Conditions: (a) DTBSCl, AgNO₃, DMF; (b) TBDMSiCl, AgNO₃, DMF; (c) HF-Py, -20°C; (d) DMTrCl, AgNO₃, DMF; (e) 2,4,6-collidine, CH₂Cl₂, *N*-methylimidazole.

sufficient to convert **8a,b** to **9a,b**. Under these conditions, no migration of the 2'-*O*-TBDMS group to the 3'-oxygen was observed. Finally, the synthesis of the phosphoramidites **10a,b** by isomerisation free procedure (7), was accomplished using (*N,N*-diisopropylamino)(cyanoethyl)-phosphonamidic chloride as the phosphorylating agent. And with 2,4,6-collidine as a base and *N*-methylimidazole as it has been reported to not isomerize the 2'-*O*-silyl into 3'-*O*-silyl derivatives. The desired monomers for oligo-RNA, the labeled D-[1'-¹³C]-ribonucleoside phosphoramidites **10a,b** (8) were thus obtained.

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6. **General Procedure for the Sequential Transformation of ribonucleosides to 5'-DMTr-2'-O-Silylribonucleosides:** D-[1'-¹³C]-3',5'-O-(Di-*tert*-butylsilanediyl)-N⁶-benzoyladosine (6a): To a solution of **5a** (0.58 g, 1.54 mmol) and AgNO₃ (0.78 g, 4.6 mmol) in anhydrous DMF (9 mL) at 0°C was added dropwise di-*tert*-butyldichlorosilane (0.5 mL, 2.37 mmol) with vigorous stirring. The mixture was warmed to room temperature and subsequently stirred for 15 min. Triethylamine (0.65 mL, 4.64 mmol) was added, and the mixture was stirred for an additional 5 min. The solvent was removed under reduced pressure. Water (15 mL) was added and the resulting solution extracted with CH₂Cl₂. The organic layer was separated, washed with brine, dried over Na₂SO₄, and evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography to give **7a** (72%). All NMR analyses were in agreement with the structure. D-[1'-¹³C]-2'-O-*tert*-butyldimethylsilyl-3',5'-O-di-*tert*-butylsilanediyl-N⁶-benzoyladosine (7a): To a solution of **6a** (0.41 g, 0.81 mmol) and catalytic amount of DMAP in anhydrous pyridine (10 mL) was added dropwise *tert*-butyldimethylsilyl chloride (2 eq, 0.12 g, 0.78 mmol). The mixture was refluxed overnight. After evaporation under reduced pressure, the resulting mixture was diluted with CH₂Cl₂, and the organic phase washed with brine and dried over Na₂SO₄. After a column chromatography on silica gel, the desired product **7a** was isolated with 74% yield. All NMR analyses were in agreement with the structure. D-[1'-¹³C]-2'-O-*tert*-butyldimethylsilyl-N⁶-benzoyladosine (8a): A solution of HF.pyridine (60 μL, 2.34 mmol; commercially available) was carefully diluted with anhydrous pyridine (0.31 mL) and then added dropwise to a solution of **7a** (0.36 g, 0.58 mmol) in THF (3 mL) at 0°C. The mixture was warmed to room temperature, stirred for 5 min. and then diluted with pyridine (0.5 mL). Water (5 mL) was added and the extraction was performed with CH₂Cl₂. The organic layer was collected, washed with 5% NaHCO₃ and dried over Na₂SO₄. After evaporation under reduced pressure, the residue was purified by a column chromatography on silica gel to yield the desired compound **8a** with 96% yield.
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8. All synthesized products have been fully characterized by ¹H, ¹³C NMR and MS.



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