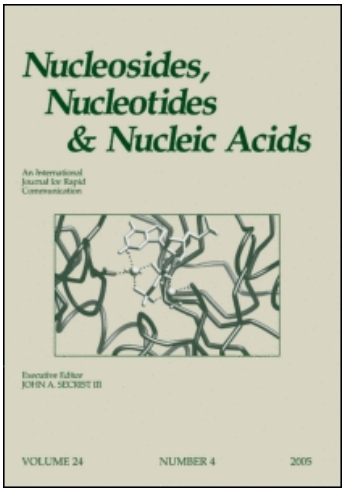

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SYNTHETIC STUDIES TO IMPROVE THE DEUTERIUM LABELLING IN NUCLEOSIDES FOR FACILITATING STRUCTURAL STUDIES OF LARGE RNAS BY HIGH-FIELD NMR SPECTROSCOPY

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ABSTRACT

Synthetic studies to prepare ribonucleosides deuterated at C2′ and the application of the developed procedures for the synthesis of $^{2}H_{5}$-ribonucleosides from 1,2-*O*-isopropylidene-3-*O*-benzyl-ribofuranose-3,4,5,5′-$^{2}H_{4}$ have been reported.

INTRODUCTION

Amongst isotope labelling techniques, site-specific deuteration has been proven to facilitate the NMR structure determination of large RNAs (1) by the "NMR-window" concept (2) in which only a small segment of the RNA is

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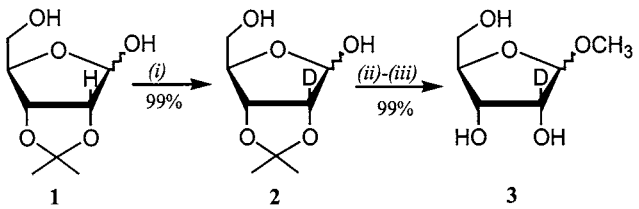
NMR-visible. The deuterium incorporation achieved into the nucleoside building blocks (>97 atom% at C2′, C3′ and C5′, ~35–50 atom% at C4′, ~0–20 atom% at C1′) was adequate to allow sequential assignment of up to 55nt long oligoRNAs (1c). The residual ~50 atom% proton at C4′ causes substantial resonance overlap in important nOe regions hampering the determination of the solution structure of long oligomers. This prompted us to seek for appropriate synthetic ways for a reliable high level deuterium incorporation at C4′.

We envisioned that the synthesis of 3′,4′,5′,5″-$^{2}H_4$-nucleosides (3) could be extended to 2′,3′,4′,5′,5″-$^{2}H_5$ derivatives provided a suitable method for deuterium incorporation at C2′ could be found. We here report the preparation of 2′-$^{2}H_1$-nucleoside block (4) by introducing deuterium right at the sugar level because it is problematic to introduce the 2′-^{2}H at the nucleoside level due to partial loss of the 3′,5′-*O*-protecting group (6b) (which is commonly 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl).

The scale-up of equilibration of **1** (5) (Scheme 1) to ~22 mmol has been achieved with excellent level of isotope incorporation (>97 atom%). The procedure is easy to carry out and the deuteronucleoside precursor **3** can be obtained in only 3 steps.

The second procedure (Scheme 2), based on the oxidation and subsequent reduction of C2-OH of compound **4**, afforded a mixture of D-arabinose-$^{2}H_1$ and D-ribose-$^{2}H_1$ derivatives **5**. Protection of the hydroxyl with 4-toluoyl group has made the separation of epimers **6** and **7** feasible. The major arabino derivative **7** has subsequently been converted to 1-*O*-methyl-β-D-ribofuranose-$^{2}H_1$ (**3**) (>97 atom%) *via* inversion of the configuration at C2 (6) using the displacement of the 2′-triflate leaving group in compound **9** with cesium propionate. Compound **3** has been further converted to the 1-*O*-acetyl-2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-$^{2}H_1$ (**13**), which has been used in the coupling reaction with the protected persilylated nucleobases to obtain fully protected 2′-deuterated nucleosides **14a–d**. The subsequent deprotection in methanolic ammonia gave the final nucleosides-$^{2}H_1$ (**15a–d**).

Finally both methods have been used for the synthesis of 2′,3′,4′,5′,5″-$^{2}H_5$-ribonucleosides taking the previously described 3′,4′,5′,5″-$^{2}H_4$ analogue of **4** as starting material. The quality of the deuterium substitution is exemplified in Figure 1 for the appropriate cytidine derivatives.



1 2 3

Scheme 1. *Abbreviation:* Tol = 4-toluoyl. *Conditions:* (i) dioxane/THF/triethylamine/$^{2}H_2O$ (24/24/12/16 mL, v/v/v/v), 90°C, 5 days; (ii) acetic acid, 90°C, 3 days; (iii) methanol, conc. H_2SO_4, 4°C, 12 h.

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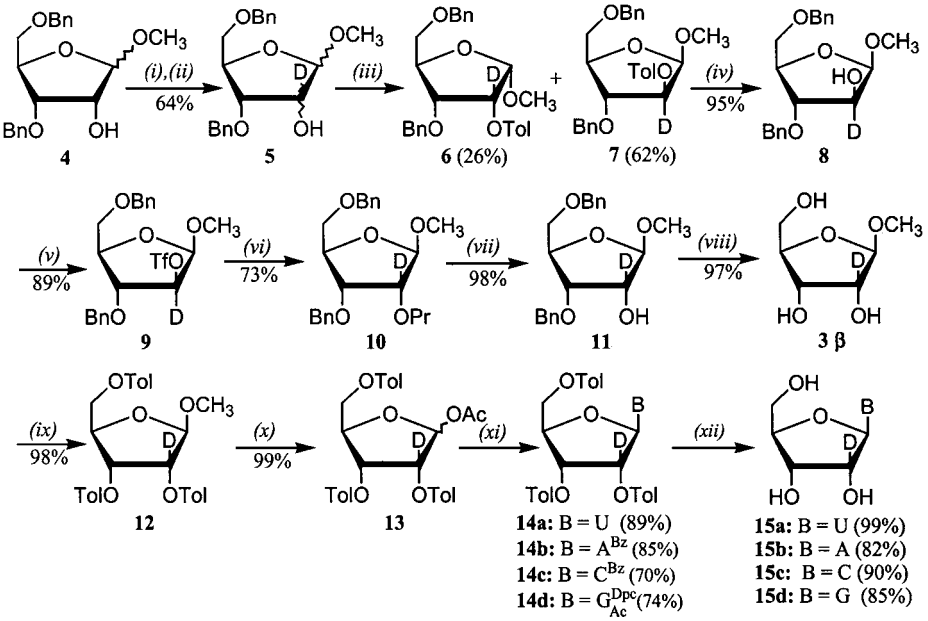

Scheme 2. *Abbreviations:* Bn = benzyl; Tf = trifluoromethanesulfonyl; Pr = propionyl; Tol = 4-toluoyl; Ac = acetyl; G = guanin-9-yl, A = adenin-9-yl, C = cytidin-1-yl, U = uracil-1-yl, Bz = benzoyl, Dpc = diphenylcarbamoyl. *Conditions:* (i) oxalyl chloride, DMSO in DCM, −70 °C; (ii) $LiAl^2H_4$ in dry diethylether or NaB^2H_4 in ethanol, r.t; (iii) TolCl, pyridine, r.t.; (iv) NH_3 in methanol, r.t.; (v) Tf_2O, DMAP, pyridine, DCM 0 °C, 3 h.; (vi) cesium propionate, DMF, r.t.; (vii) NH_3 in methanol, r.t.; (viii) Pd/C, hydrogen in ethanol, r.t.; (ix) TolCl, pyridine, r.t.; (x) Ac_2O, AcOH, conc. H_2SO_4, DCM, 0 °C, 15 min.; (xi) silylated base, TMS-Tf, 1,2-dichloroethane or toluene (14d), heating; (xii) NH_3 in methanol, r.t.

Some selected relevant data: Compound 3. $[\alpha]_D^{26}$: −38 (c 0.15, H_2O); HRMS (Ei^+): (M^+) calcd. for $C_6H_{11}DO_5$: 165.0747, found 165.0748. **Compound 6**. $[\alpha]_D^{26}$: +98 (c 0.67, $CHCl_3$); HRMS (Ei^+): (M^+) calcd. for $C_{28}H_{29}DO_6$: 463.2106, found 463.2109. **Compound 7**. $[\alpha]_D^{26}$:−74 (c 0.25, $CHCl_3$); HRMS (Ei^+): (M^+) calcd. for $C_{28}H_{29}DO_6$: 463.2106, found 463.2110. **Compound 8**. $[\alpha]_D^{26}$: −42 (c 0.71, $CHCl_3$); HRMS (Ei^+): (M^+) calcd. for $C_{20}H_{23}DO_5$: 345.1687, found 345.1695. **Compound 9**. $[\alpha]_D^{27}$: −64 (c 0.74, $CHCl_3$); HRMS (Ei^+): (M^+) calcd. for $C_{21}H_{22}DF_3O_7S$: 477.1179, found 477.1184. **Compound 10**. $[\alpha]_D^{27}$: +14 (c 0.71, $CHCl_3$); HRMS (Ei^+): (M^+) calcd. for $C_{23}H_{27}DO_6$: 401.1949, found 401.1955. **Compound 12**. $[\alpha]_D^{26}$: +75 (c 0.17, $CHCl_3$); HRMS (Ei^+): (M^+) calcd. for $C_{30}H_{29}DO_8$: 519.2004, found 519.2009. **Compound 13**. $[\alpha]_D^{26}$: +62 (c 1.04, $CHCl_3$); for natural $[\alpha]_D^{28}$: +63; HRMS (Ei^+): (M^+) calcd. for $C_{31-}H_{29}DO_9$: 547.1953, found 547.1960. **Compound 15a**. $[\alpha]_D^{26}$ +9 (c 0.2, H_2O); $[\alpha]_D^{26}$ for natural uridine +10; HRMS (Ei^+): (M^+) calcd. for $C_9H_{11}DN_2O_6$: 245.0758, found 245.0759. **Compound 15b**. $[\alpha]_D^{26}$: −53 (c 0.17, H_2O). For natural $[\alpha]_D^{26}$: −60; HRMS (Ei^+): (M^+) calcd. for $C_{10}H_{12}DN_5O_4$: 268.1030, found 268.1036. **Compound 15c**. $[\alpha]_D^{26}$: +32 (c 0.08, H_2O). For natural $[\alpha]_D^{27}$: +33; HRMS (Ei^+): (M^+) calcd. for $C_9H_{12}DN_3O_5$:

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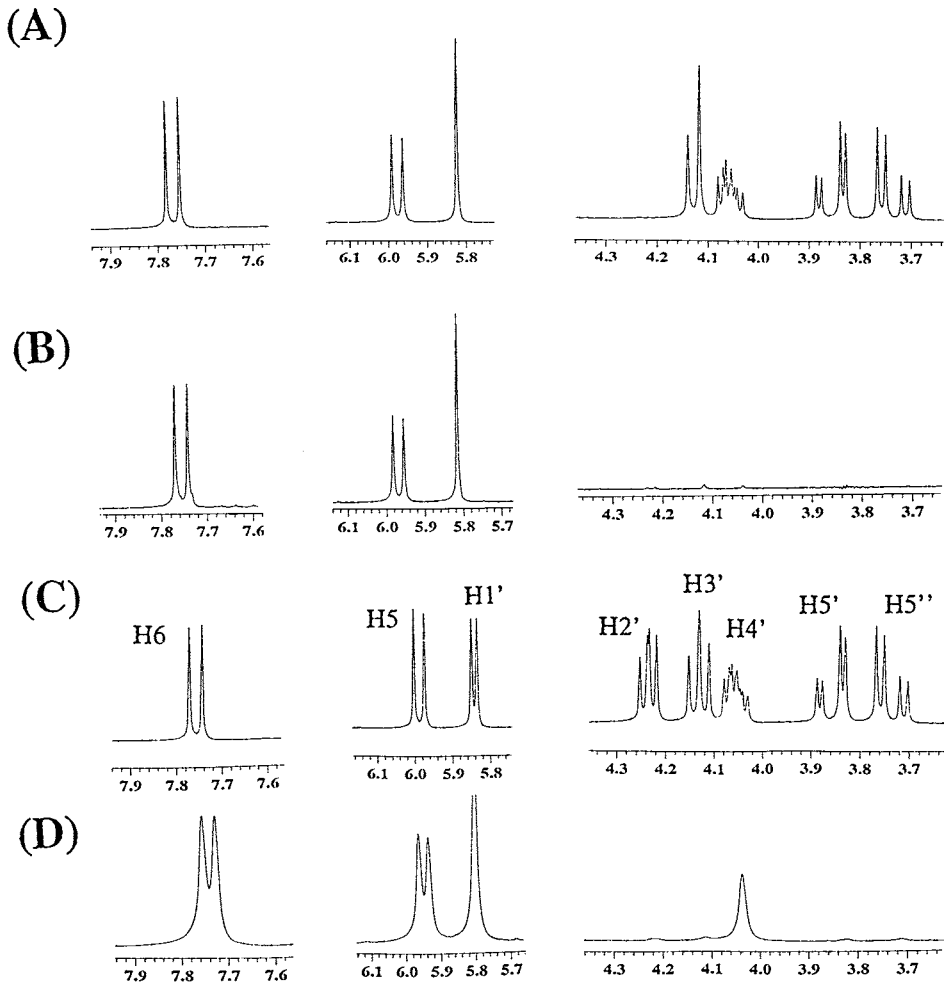

Figure 1. Expanded regions of the 270 MHz 1D ^{1}H NMR spectra of 2′-$^2H_1$-cytidine (Panel **A**), 2′,3′,4′,5′,5″-$^2H_5$-cytidine (Panel **B**), their natural counterpart (Panel **C**) and the 1′$^{\#}$,2′,3′,4′$^{\#}$,5′,5″-$^2H_6$-cytidine[2] (Panel **D**).

244.0918, found 244.0922. **Compound 15d**. $[\alpha]_D^{26}$ −36 (c 0.04, H_2O); $[\alpha]_D^{26}$ for natural guanosine −37; HRMS (Ei$^+$): (M^+) calcd. for $C_{10}H_{12}DN_5O_5$: 284.0979, found 284.0983.

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