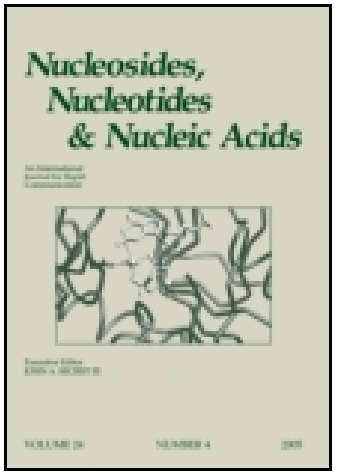

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Synthesis of ^{2}H,^{13}C-Labelled 2′-Deoxynucleosides and Their Site Specific Incorporation into Oligo-DNA for Structural Studies via Relaxation Time Measurements

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SYNTHESIS OF ^{2}H,^{13}C-LABELLED 2'-DEOXYNUCLEOSIDES AND THEIR SITE SPECIFIC INCORPORATION INTO OLIGO-DNA FOR STRUCTURAL STUDIES *VIA* RELAXATION TIME MEASUREMENTS

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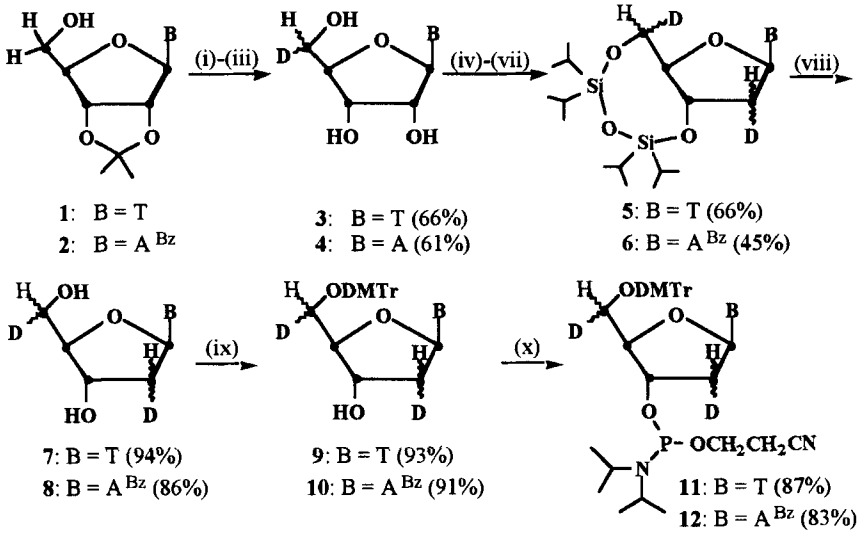
ABSTRACT: We have recently shown[1] the usefulness of ^{2}H,^{13}C-labelled 2'-deoxynucleoside building blocks for structural studies *via* relaxation time measurements. The synthesis of phosphoramidite blocks **11** and **12** for their site-specific incorporation (indicated by underlines) into the $d^{5'}(^{1}C^{2}G^{3}\underline{A}^{4}\underline{T}^{5}\underline{T}^{6}\underline{A}^{7}\underline{A}^{8}\underline{T}^{9}C^{10}G)_{2}{}^{3'}$ is briefly described for studying the T_1 and $T_{1\rho}$ relaxations of ^{2}H and ^{13}C at specific deuterated carbons in a large molecule.

At the outset of our work, it was anticipated, that the use of $^{13}C/^{2}H$ labelled 2'(R/S), 5'(R/S)-$^{2}H_{2}$-1',2',3',4', 5'-$^{13}C_{5}$-2'-deoxynucleosides makes it possible to perform ^{2}H relaxation measurements only at C2' and C5' of the double-labelled nucleosides through a $^{1}H \rightarrow {}^{13}C \rightarrow {}^{2}H \rightarrow {}^{13}C \rightarrow {}^{1}H$ polarisation transfer, since the absence of $^{2}J_{HH}$ couplings in these residues allows the filtration of all other non-double labelled ^{13}C-fragments.

The 5'-deuteration of ribonucleosides with uniformly ^{13}C-labelled sugar moiety[2] was achieved by Moffatt-oxidation[3] of the 2',3'-*O*-isopropylidene derivatives **1** and **2**, followed by reduction of the 5'-aldehyde with $NaBD_4$ to give a ~1:1 R/S mixture of deuterio-isotopomers. The cleavage of the isopropylidene group with 10% aqueous acetic acid at elevated temperature gave substantial loss of adenosine derivative most probably due to depurination. Nucleosides **3** and **4** were converted to the 3',5'-*O*-(1,1,3,3-tetraisopropyl-disiloxan-1,3-diyl)-2'-*O*-phenoxythiocarbonyl derivatives which were reduced with tributyltin deuteride[4] to afford the diastereomeric 2'-deuterio derivatives (~85% ^{2}H at R; ~15% ^{2}H at S) **5** and **6** (after additional N^{6}-benzoylation step). After removal of sugar protection upon a treatment with 1.0 M TBAF, the 2'-deoxynucleoside blocks **7** and **8** were converted to the appropriate phosphoramidite derivatives **11** and **12** through phosphitylation[5] of the 5'-*O*-dimethoxytrityl nucleosides **9** and **10**. All intermediates were

satisfactorily characterised by their ^{1}H-, ^{13}C- and ^{31}P-NMR spectra recorded on a Jeol JNM GX 270 spectrometer at 270.17, 67.94 and 109.37 MHz, respectively. The labelled 10-mer $d^{5'}(^{1}C^{2}G^{3}\underline{A}^{4}\underline{T}^{5}\underline{T}^{6}\underline{A}^{7}\underline{A}^{8}\underline{T}^{9}C^{10}G)_2{}^{3'}$ was prepared by the solid phase method on a Pharmacia LKB Gene Assembler Special synthesiser[1b].



Scheme 1. (i) DMSO, DCC, dichloroacetic acid, rt; (ii) $NaBD_4$ in ethanol, rt; (iii) 10% aq. acetic acid, ~90 °C (followed by NH_3 in methanol for compound 4); (iv) TPDS-Cl_2 in dry pyridine, rt; (v) phenoxythiocarbonyl chloride, methylimidazole in dry dichloromethane, rt; (vi) tributyltin deuteride, AIBN in dry toluene, ~85 °C; (vii) (to get compound 6) benzoyl chloride in dry pyridine; (viii) TBAF in dry THF, rt; (ix) DMTr-Cl in dry pyridine, rt; (x) (2-cyanoethoxy)bis(N,N-diisopropylamino)phosphine, N,N-diisopropylammonium tetrazolide in dry dichloromethane, rt.

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