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Synthesis of Partially-Deuterated 2'-Deoxyribonucleoside Blocks and their Incorporation into an Oligo-DNA for Simplification of Overcrowding and Selective Enhancement of Resolution and Sensitivity in the ^{1}H-NMR Spectra

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Abstract: The chemical synthesis of appropriately protected partially-deuterated 2'(R/S),3',5'(R/S)-$^2H_3$-2'-deoxy-ribonucleoside blocks [~43 atom % ^{2}H at C5'(R), ~57 atom % ^{2}H at C5'(S); ~15 atom % ^{2}H at C2'(R), ~85 atom % ^{2}H at C2'(S) and >99 atom % ^{2}H at C3'] is reported. The availability of these deuterium labelled blocks on large scale has enabled the chemical assemblage of the deuterio isotopomeric 12mer [d($\underline{C}^1\underline{G}^2\underline{C}^3\underline{G}^4\underline{A}^5\underline{A}^6\underline{T}^7\underline{T}^8\underline{C}^9\underline{G}^{10}\underline{C}^{11}\underline{G}^{12}$)]$_2$ DNA duplex by standard solid-phase synthesis protocol in order to demonstrate the usefulness of the new "NMR-window III" approach (see the following paper).

Introduction

The precise extraction of ^{3}J coupling constants for estimation of the torsional angles and nOe volumes for distance estimation are the two key-steps to solve the NMR structure of oligo-DNA and RNA molecules under quasi-physiological conditions[1]. With increasing molecular weight the usefulness of NMR spectroscopy becomes very restricted because of following reasons: (i) severe spectral overlap, (ii) associated line broadening arising from decreased T_2 relaxations[1e], (iii) decreased sensitivity caused by slower tumbling rate[1e], and (iv) the spin diffusion that prevents accurate nOe volume measurements[1d]. In order to overcome these intrinsic size limitation problems various isotope labelling techniques[2-10] have been developed.

The spectral overlap has been found to be substantially reduced in ^{13}C/^{15}N labelled oligo-RNA by heteronuclear multidimensional spectrum editing techniques[2]. Enzymatic syntheses have been used to produce uniformly ^{13}C/^{15}N labelled oligo-RNA[3] and oligo-DNA[4]. Additionally, such enzymatically labelled oligo-RNA synthesis does not allow labelling of a segment of interest except for using ligation techniques[5]. The preparation of uniformly ^{13}C labelled 2'-deoxynucleosides is a relatively expensive multi-step procedure[4,6]. The ^{13}C/^{15}N labelled oligonucleotides have disadvantageous relaxation properties: ^{13}C labels decrease proton T_2 relaxation decreasing the sensitivity of homonuclear J correlation techniques[2c], the short ^{13}C T_2 relaxations for proton bearing carbons[7] in nucleosides result in ^{13}C signal broadening and signal loss[2c] due to long pulse sequences of many heteronuclear experiments[2c]. Uniform heteronuclear labelling opens new relaxation pathways between neighbouring nuclei, making these relaxation-based problems even more severe[2c].

While the use of ^{13}C/^{15}N isotopes increases the number of observable resonances, deuterium labelling of oligonucleotides is based on the primary idea of supressing part(s) of the ^{1}H-NMR spectra[8a]. For this purpose,

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different schemes for the deuteration of the constituent nucleosides have been proposed[8-10]. Deuteration of C5/C6 positions of pyrimidines or C5-methyl of thymine and C8 of purine nucleobases[8b-e] removed unessential crosspeaks in the NOESY spectra of oligonucleotides. Incorporation of isotopomeric 5'(R/S) mixture of ^{2}H-labelled nucleosides[8f-h] facilitated the stereoselective assignment of the diastereotopic H5'/5" methylene resonances in oligo-DNA[8i-j]. The spectral overcrowding of aromatic to H1' region in the NOESY spectra of an RNA duplex[8k] was decreased upon selective incorporation of C1'-deuterated nucleosides. 3',4',5',5"-$^2H_4$ Labelled nucleosides were uniformly incorporated into RNA and the effect of the specific deuteration on the spectral complexity and relaxation properties were studied[8l]. The use of the non-uniform labelling ("NMR-window I" concept) in oligo-RNAs with 1',2',3',4',5',5"-$^2H_6$-β-D-ribonucleoside[9a-b] and DNAs with 1',2',2",3',4',5',5"-$^2H_7$-β-D-2'-deoxyribonucleosides[9a-b] made it possible to assign the resonances for the protons in the nondeuterated sugar residues and to determine their $^3J_{HH}$ coupling constants. Further studies from our laboratory with 12 and 20mer DNAs[9c-d] as well as 21mer and 31mer RNAs[9e-f] showed that the reduced resonance overlap in NOESY type spectra indeed helps in the assignment of the chemical shifts and extraction of nOe volumes. It was also found, that a proton vicinal to a deuteron has a ~3-10 fold increase of T_1 and ~10-30 increase of T_2[9d] resulting in highly enhanced sensitivity. This relaxation behaviour of the residual protons prompted the synthesis of an isotopomeric mixture of 2'(R[~15% ^{2}H]/S[~85% ^{2}H]), 3'[~97% ^{2}H], 4'[~65% ^{2}H], 5',5"-$^2H_5$-β-D-2'-deoxyribonucleoside blocks[10] and their site-specific incorporation into DNA oligomers. The above partial deuteration ("NMR-window II" concept) made the extraction of the $^3J_{H1',H2'}$ and $^3J_{H1',H2"}$ coupling constants in the partially-deuterated residues feasible from DQF-COSY type experiments with increased accuracy (this finding has recently been extended to other modified COSY experiments[11]). It was also shown in that work[10] that the T_2 relaxation time for the H2'(R) protons of partially-deuterated 2'-deoxynucleoside residues has increased by ~1.5 to 2 fold compared to the counterpart in the nondeuterated nucleosides. The interresidual [$(H2")_{i-1}$ - $(Ar)_i$, $(H1')_{i-1}$ - $(Ar)_i$] and intraresidual [(H2" - Ar)$_i$, (H1' - Ar)$_i$, (H1' - H2")$_i$, (H1'-H4')i, (H4'-H2")$_i$ and (H4' - Ar)$_i$] nOe volumes could be obtained with substantially reduced spin-diffusion from a HAL-NOESY experiment, which filters away crosspeaks arising from the nondeuterated nucleoside residues. Most importantly, we found[10] that despite the presence of various deuterium isotopomers at C2' in the sugar residues, the deuterium-induced chemical shift differences in the deuterio diastereomeric oligo-DNAs were negligible and nOe evolution rates at various mixing times in the NOESY experiments were non-differentiable.

The main problem encountered in the "NMR-window II" concept with ~65% deuterium enrichment at C4' in deuterio isotopomeric oligo-DNA[10] was that it reduced the intensity of those nOe crosspeaks which involved interaction with H4'. This low nOe intensity of (H1'-H4') crosspeaks in the HAL-NOESY experiment clearly hampered[10] our assessment of the sugar conformation, which prompted us to design a new synthetic route in which the H4' should be kept at natural abundance. It was also clear that the generation of a ~1:1 ratio of deuterio isotopomers at C5' would additionally help in providing constraints for β[8i-j] (through heteronuclear $^3J_{H-P}$ coupling constants) and γ[8f] (through homonuclear $^3J_{H5'-H4'}$ and $^3J_{H5"-H4'}$ coupling constants) phosphate backbone torsion angles, thereby giving most of the dihedral angle and NOE constraints essential to build a detailed picture of the local and global conformation of large oligo-DNA molecules.

We herein report on the preparation of C2' and C5' deuterio isotopomer blocks of type **A** and **B** (Fig. 1) and their incorporation into the Dickerson-Drew dodecamer[37] by solid phase synthesis in order to show the NMR improvements compared to those found in our earlier works on the "NMR-window I"[9a,c] and "NMR-window II" concepts[10].

Results and Discussion

For the synthesis of the required **A** + **B** 5'(R/S), 2'(R/S) isotopomeric mixture of the building blocks, it was considered to be advantageous to have the 2'-*O*-acyl protection in the sugar derivative during the glycosylation of the nucleobases to give preferentially β anomer, which then partially solves the problem of its tedious separation from the α anomer[12] in a large scale synthesis. The stereoselective incorporation of deuteriums at the C3 could be achieved utilising the stereocontrol of the neighbouring 1,2-bis-*O*-isopropylidene group in the reduction of a 3-carbonyl center in 1,2-bis-*O*-isopropylidene-α-D-xylo-[8f] or 1,2:5,6-bis-*O*-isopropylidene-α-D-glucofuranose[13] derivatives.

High level of deuterium incorporation at C2 is a rather difficult task both at the sugar and at the nucleoside levels. Isotopic substitution at C2 of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose in a stereoselective manner has been reported[14] but the level of deuteration achieved is only 92-94%, and the unsuitability of base-labile protecting groups in the reduction step make this alternative less attractive. On the other hand, the highly stereoselective reduction of benzyl *arabino*pyranoside derivative **9** to the respective *ribo*pyranoside achieved in our laboratory[12] paves the way for an effective C2 deuterium labelling technique. For the incorporation of deuterium at nucleoside level, the directive effect of the nucleobases in the reduction of 2'-ketonucleosides to arabinonucleosides is also well documented[12,15]. The need for the different synthetic approaches is obviated by the moderate yield oxidation-reduction reactions of the purine nucleosides[12,15], especially that of guanosine.

(i) Route A: ***Incorporation of the deuteriums at C2', C3' and C5' at the sugar level.***
1,2:5,6-Bis-*O*-isopropylidene-α-D-glucofuranose, easily available from D-glucose in large scale was chosen as starting material for our synthetic studies since the conversion of this compound exclusively to the allose epimer is well established[16]. Oxidation of this compound with pyridinium dichromate/acetic anhydride[17] in boiling dry dichloromethane followed by stereoselective reduction of the resulting ketone with $LiAlD_4$ in dry diethyl ether gave the 3-deuterated allofuranose derivative **1**[13] (85%) (Scheme 1). The incorporation of the deuterium was evident from the ^{1}H-NMR spectrum showing the H2 signal at 4.63 ppm as a doublet instead of a double doublet and the 3-OH signal as a singlet at 2.52 ppm instead of a doublet. The ^{13}C chemical shifts for the nondeuterated carbons corroborated the inversion of configuration at C3[18]. Treatment of compound **1** with 80% aqueous acetic acid at room temperature overnight cleaved the 5,6-*O*-isopropylidene group selectively affording the *cis*-diol derivative **2** (83%) after removal of the volatile materials and crystallisation from methanol. The oxidative cleavage of the vicinal *cis*-diol moiety in compound **2** was quantitatively achieved by a slight excess of $NaIO_4$ in ethanol-water mixture[19]. After careful precipitation of the inorganic salts from ethanol, the resulting crude aldehyde was directly reduced with $NaBD_4$ in ethanol to afford the 1,2-*O*-isopropylidene-α-D-

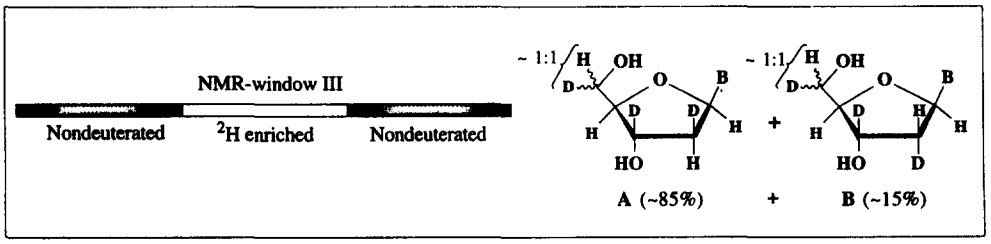
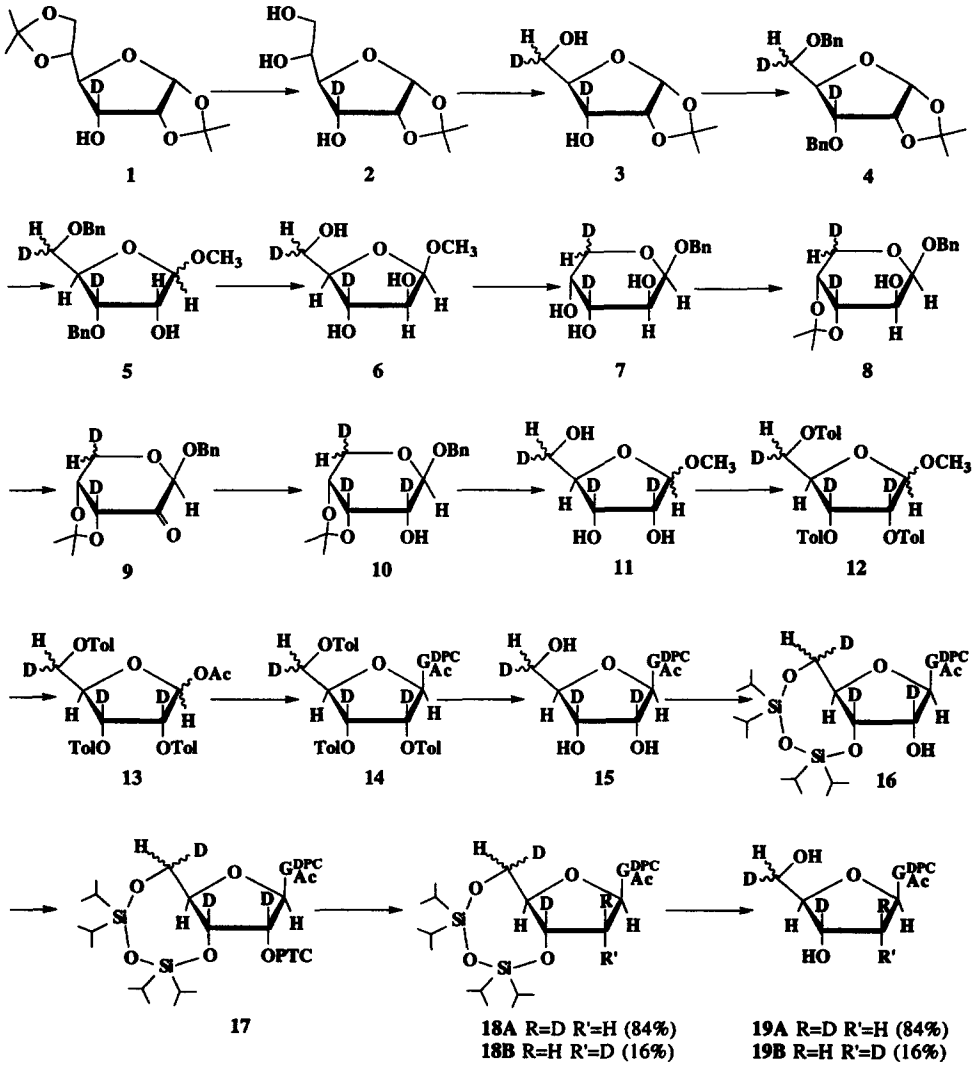


Figure 1. The "NMR-window III" concept (B = thymin-1-yl, cytosin-1-yl, adenin-9-yl or guanin-9-yl). For the "NMR-window I" and "NMR-window II" concepts, see refs 29 and 36.

ribofuranoside-3,5(R/S)-$^2H_2$ (3) (quantitative). In the proton-decoupled ^{13}C-NMR spectrum of compound 3, the partially-deuterated diastereomeric methylene-carbon appeared at 59.7 ppm as a triplet due to coupling with deuterium. Compound 3 was converted to the fully-protected deuterated methyl α,β-ribofuranoside 4 (99%) upon a treatment with benzyl bromide and sodium hydride in dry acetonitrile. The benzyl alcohol formed during hydrolysis of the excess benzyl bromide was removed in vacuo upon heating the residual oil at ~100 °C at 0.1 mbar. Treatment of compound 4 with concentrated sulfuric acid in dry methanol[20] at reflux temperature gave compound 5 (91%) as an anomeric mixture (22% α and 78% β).

Compound 5 was converted to 6 by a three-step procedure (oxidation→reduction→deprotection; overall yield: 49%): (i) Swern oxidation[21] of the 2-OH of 5 [evidenced by the disappearance of the ^{1}H-NMR signals at 4.88 (assigned to α H-1) and 3.32 ppm (assigned to the β OCH_3)] gave the crude ketone, which was directly dissolved in ethanol, and (ii) reduced stereoselectively with $NaBH_4$ to a mixture containing predominantly



Scheme 1: *Abbreviations:* Bn = benzyl, Tol = 4-toluoyl, Ac = acetyl, G = guanin-9-yl, DPC = diphenylcarbamoyl, PTC = phenoxythiocarbonyl

methyl 3,5-di-*O*-benzyl-β-D-arabinofuranoside-3,5-$^2H_2$ (identified by a new doublet at 4.86 ppm with $J_{H1,H2}$ = 4.7 Hz instead of the small coupling characteristic for *ribo* β anomer in the ^{1}H-NMR spectra as well as with a signal at 102.7 ppm in the ^{13}C-NMR spectra assigned to C1) and the α-D-*ribo* C2-epimer (characterised by the presence of a doublet at 4.88 ppm with a splitting of 4.7 Hz identical to that found for the α anomer of compound **4**) in the same 78:22 ratio as found for compound **4**, thereby suggesting that the approach of the hydride ion in the reduction of 2-ketone function is actually controlled by the nature of the anomeric configuration. (iii) Since it was impossible to cleave the benzyl groups from these isomeric compounds *via* simple catalytic hydrogenation, the removal of these groups was effected by sodium in liquid ammonia/dry toluene[22]. After neutralisation with gradually added ammonium chloride followed by removal of the inorganic salts, the methyl β-D-*arabino*furanoside-3,5-$^2H_2$ **6** was conveniently separated at this stage from the *ribo* epimer on a Dowex 1x2-400 1X2 strong anion exchange column (OH^- form)[23] in 49% yield for 3 steps. The ^{1}H-NMR spectrum of this compound revealed the presence of a multiplet at 3.94 ppm corresponding to a back-exchange at C3 (34%) *via* enolization during aqueous work-up[24] of the oxidation or during the reduction of 2-ketone under basic protic condition. In the ^{13}C-NMR spectrum, a singlet corresponding to the nondeuterated C3 appeared at 74.9 ppm, whereas the deuterated C3 appeared as a triplet at 74.6 ppm giving an isotope shift of Δδ = -0.3 ppm for this carbon signal upon deuteration.

Compound **6** was deprotected to arabinose-3,5(R/S)-$^2H_2$ by a treatment with Dowex 50 WX8 cation exchange resin (H^+ form) in water at 70 °C for 6h. The resin was filtered away and the deuterated arabinose was carefully dried over P_2O_5. The resulting powder was suspended in freshly distilled benzyl alcohol and converted to the benzyl glycoside **7** (59%) upon treatment with dry HCl[25]. Compound **7** was subsequently protected as 3,4-*O*-isopropylidene derivative **8**[25] (99%). This compound was oxidised using CrO_3/pyridine/acetic anhydride[12,15b] complex in CH_2Cl_2 to ketone **9** (95%) as evidenced by lack of any H2 signal in the ^{1}H-NMR spectrum and the disappearance of C2 signal in the ^{13}C-NMR and the presence of the carbonyl signal at 198.6 ppm. Ketone **9** was subjected to enolisation in pyridine-^2H_2O at room temperature until the diappearance of the ^{1}H-NMR signal corresponding to the residual H-3 proton at 4.70 ppm. Solvents were removed and after a few coevaporations with toluene, compound **9** was stereoselectively reduced with $LiAlD_4$ in dry THF[12] to the desired β-D-ribopyranoside derivative **10** (77%) after crystallisation from petroleum ether. The Δδ = 2.5 ppm downfield shift of the C1 carbon signal in **10** proved the inversion of configuration at C2. Removal of the benzyl protection from **10** *via* catalytic hydrogenation over Pd/charcoal[25] in dry ethanol followed by further treatment with 80% aqueous acetic acid and glycosidation in dry methanol in presence of catalytic amount of concentrated sulfuric acid resulted in the key methyl α,β-D-ribofuranoside-2,3,5(R/S)-$^2H_3$ (**11**) in 79 % yield for three steps. The methyl ribofuranoside **11** was treated with 4-toluoyl chloride in dry pyridine[9a] to obtain the protected methyl glycoside **12** (92%). Acetolysis of this derivative in dry dichloromethane with a precooled mixture of acetic anhydride, acetic acid and conc. sulfuric acid at 0 °C for 12 min gave deuterated ribufuranose derivative **13** (quantitative). Integration of the residual H3' and H2' resonances at 500 MHz (Fig. 2, Panel A) of this compound showed >99 and ~99 atom % deuterium incorporations at these positions, respectively. Trimethylsilyl trifluoromethanesulfonate catalysed condensation of sugar **13** with silylated N^2-acetyl-O^6-diphenylcarbamoylguanine in dry toluene[26] afforded the fully protected 2'-deoxyguanosine-2',3',5'(R/S)-$^2H_3$ derivative **14** (60%) (Fig. 3, Panel C), which was converted[10] to the nucleobase-protected 2'-deoxyguanosine-2'(R/S),3',5'(R/S)-$^2H_3$ analogue **19** in four steps in 63% overall yield. Integration of the appropriate signals of the ^{1}H-NMR spectrum of compound **19** at 500

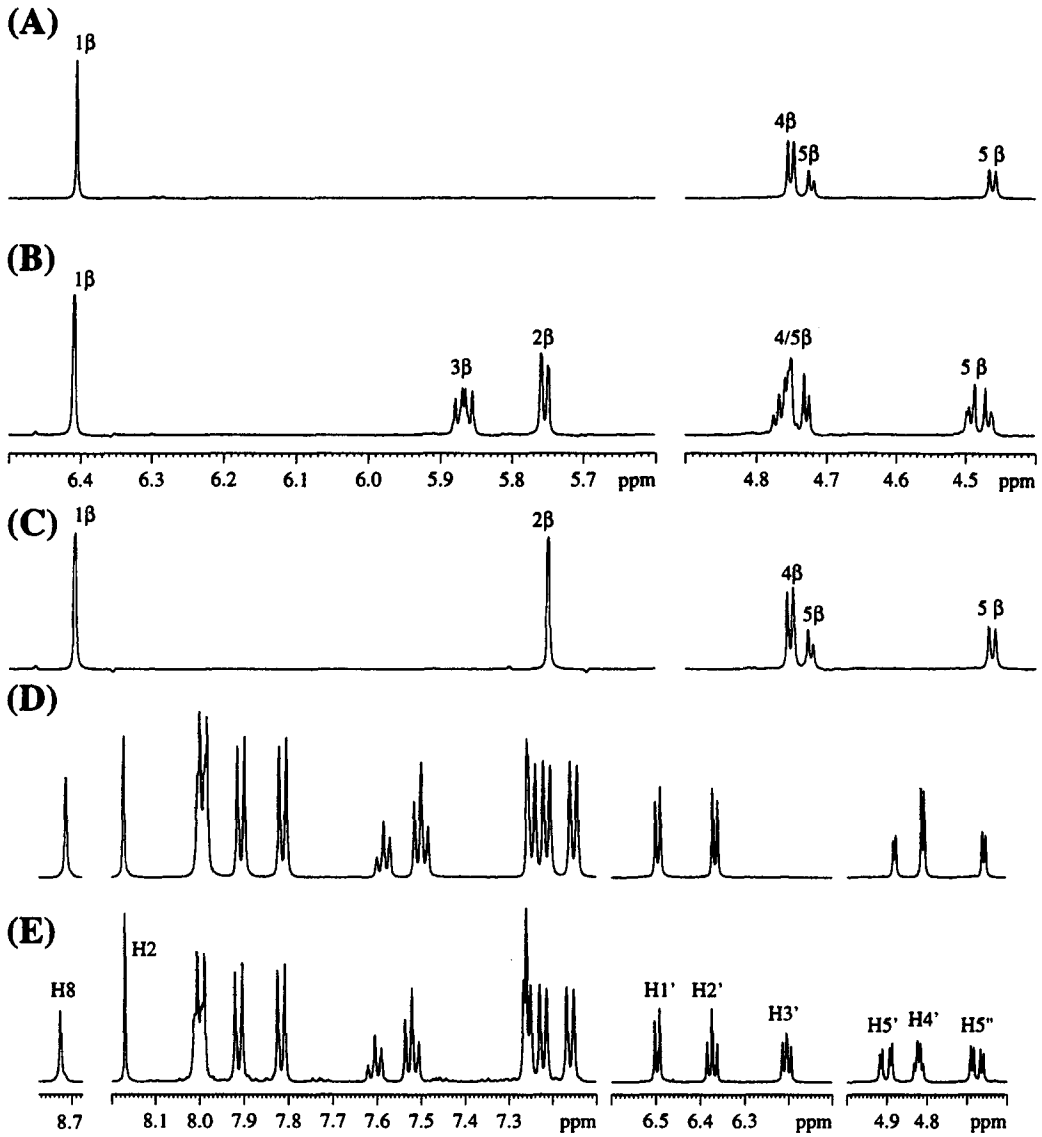


Figure 2: The sugar region of the 500 MHz ^{1}H-NMR spectra of 1-*O*-acetyl-2,3,5-tri-*O*-(4-toluoyl)-β-D-ribofuranose-2,3,5(R/S)-$^2H_3$ (**13**) (Panel A), its natural-abundance counterpart (Panel B) and 1-*O*-acetyl-2,3,5-tri-*O*-(4-toluoyl)-β-D-ribofuranose-3,5(R/S)-$^2H_2$ (**22**) (Panel C). The aromatic and sugar regions of the 500 MHz ^{1}H-NMR spectra of 2',3',5'-*O*-tri-(4-toluoyl)-N^6-benzoyladenosine-3',5'(R/S)-$^2H_2$ (**24**) (Panel D) and its natural-abundance counterpart (Panel E).

MHz (Fig. 11, Panel C) revealed a 5'R(^{2}H):5'S(^{2}H) ratio of 43:57 whereas the 2'R(^{2}H):2'S(^{2}H) ratio was found to be 14:86.

(ii) Route B: ***Incorporation of the deuterium at C2' at nucleoside level.***

Methyl α,β-D-ribofuranoside-3,5(R/S)-$^2H_2$ (**20**) (90%) was obtained upon removal of the 1,2-*O*-isopropylidene protection from the deuterated ribose derivative **3** with 80% aqueous acetic acid at 80 °C

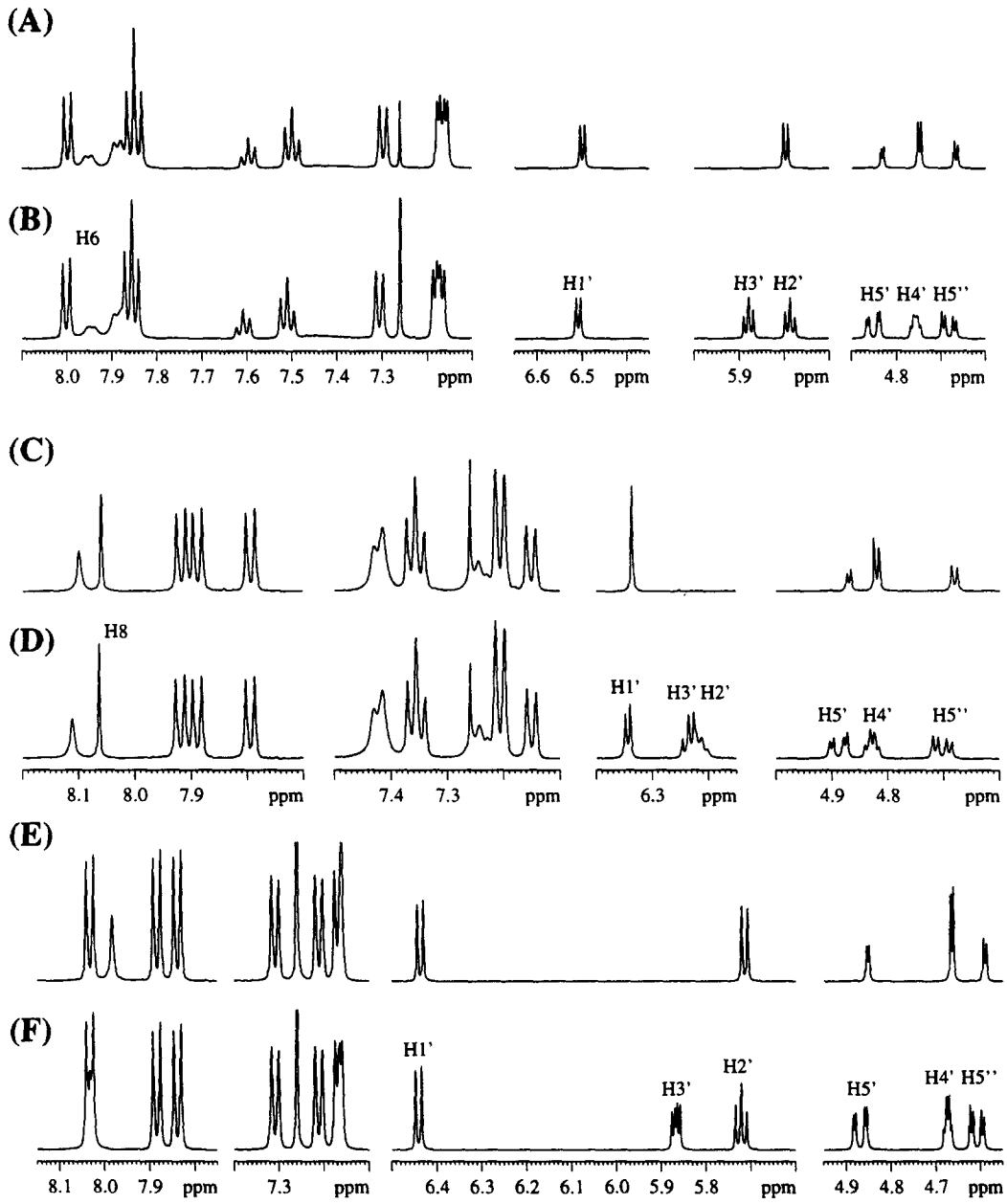


Figure 3: Aromatic and sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleoside derivatives and their natural-abundance counterparts: 2',3',5'-*O*-tri-(4-toluoyl)-N^4-benzoylcytidine-3',5'(R/S)-$^2H_2$ (**25**) (Panel A) and its natural-abundance counterpart (Panel B); 2',3',5'-tri-*O*-(4-toluoyl)-N^2-acetyl-O^6-diphenylcarbamoylguanosine-2',3',5'(R/S)-$^2H_3$ (**14**) (Panel C) and its natural-abundance counterpart (Panel D); 1-(2',3',5'-tri-*O*-(4-toluoyl)-β-D-ribofuranosyl)-thymine-3',5'(R/S)-$^2H_2$ (**23**) (Panel E); its natural-abundance counterpart (Panel F).

overnight, followed by subsequent treatment of the resulting deuterated ribose with conc. H_2SO_4 in dry methanol. Compound **22** (96%) (Fig. 2, Panel C) was prepared from this deuterated methyl ribofuranoside upon treatment with a slight excess of 4-toluoyl chloride to give compound **21** (quantitative) followed by acetolysis[9a]. Condensation of the sugar derivative **21** with silylated thymine, N^6-benzoyladenine and N^4-benzoylcytosine afforded nucleoside derivatives **23-25** (96, 72 and 78%), respectively, from which the deuterated nucleosides **26-28** (99, 100 and 99%) were obtained *via* an overnight treatment with saturated methanolic ammonia. The deuterated cytidine was regioselectively N^4-acetylated with acetic anhydride in boiling methanol[27] to give compound **29** (72%). The deuterated nucleoside derivatives **26, 27** and **29** were reacted with a small excess of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[28,29] (TPDS-Cl_2) in dry pyridine to afford compounds **30-32** (84, 77 and 72%). Swern oxidation of thymidine derivative **30** and oxidation of **31-32** with CrO_3-acetic anhydride-pyridine complex[12,15b] yielded the corresponding 2'-ketonucleosides, which were directly reduced with $NaBD_4$ in ethanol to the 2',3',5'-$^2H_3$ *arabino*nucleosides **33-35** in 65, 58 and 82% yields, respectively. Although the incorporation of deuterium at C2' made the use of $J_{H1,H2}$ coupling information impossible (compare Fig. 6 Panel F to Fig. 8 Panel B), the characteristic 0.3 - 0.2 ppm downfield shift of the H1' proton signal and the H5',5"/H4' pattern with upfield shifted H4' (Fig. 7, Panels E-F and Fig. 8 Panels A-D) evidenced the inversion of configuration at C2'. Further evidence regarding the configuration of C2' comes from the ^{13}C-NMR spectra where the C1' signals move upfield by ~6 - 7 ppm as a result of the inversion.

Since the reduction of the 2'-*O*-phenoxythiocarbonyl derivative of *arabino*thymidine **33** with tributyltin hydride failed in our hands, we pursued alternative ways for the reductive incorporation of hydrogen at C2'. The 2'-OH groups in compounds **33-35** were reacted with trifluoromethansulfonic anhydride[15d,30] in dry dichloromethane in presence of pyridine and DMAP to give the 2'-*O*-trifluoromethansulfonyl compounds **36-38** in 74, 80 and 81% yield, respectively. Although the lack of 2' ^{1}H and ^{13}C signals made it difficult to check the outcome of the reaction, the high-resolution mass spectra unequivocally proved the presence of the triflate group in these compounds. In case of the thymidine derivative **36**, a nucleophilic displacement of the triflate leaving group with cesium propionate[30] in dry *N,N*-dimethylformamide gave the desired *ribo*nucleoside-$^2H_3$ derivative **39** in 96% yield. The attempted removal of the 2'-*O*-propionyl protection *via* treatment with methanolic ammonia overnight furnished the expected *ribo*thymidine derivative **42** (67%). The close similarity of the ^{1}H- and ^{13}C-NMR spectra of this compound to that of compound **30** proved the *ribo* configuration of C-2'. Compound **42** was subsequently treated with phenoxythiocarbonyl chloride in dry dichloromethane with 1-*N*-methylimidazole as nucleophilic catalyst[9a] to give compound **43** (96%), which was readily reduced with tributyltin hydride in presence of 2,2'-azobis(2-methyl-propionitrile) (AIBN) as free radical initiator to the isotopomeric mixture of the deuterated 2'-deoxyribonucleoside derivatives **44A,B**[10] (95%) (Fig. 10, Panel A). The 2'R(^{2}H):2'S(^{2}H) ratio was integrated to be 11:89.

Since the above reaction sequence did not work well for the adenosine derivative **37** giving low yield during removal of the 2'-O-propionyl group because of the partial loss of the TPDS protection, compounds **37** and **38** were converted to the 2'-bromo-2'-deoxyribonucleoside derivatives **40** and **41** through displacement of the triflate group with LiBr in dry DMF[31] in 92 and 87% yields, respectively. The constitution of these derivatives was corroborated by measuring their exact molecular mass (see experimental). The *ribo* configuration of C2' is supported by the observed carbon chemical shifts of 90.7 and 91.9 ppm for C1'[34] of **40** and **41**, respectively. Reduction of these 2'-halogenonucleosides with tributyltin hydride under the usual thermal

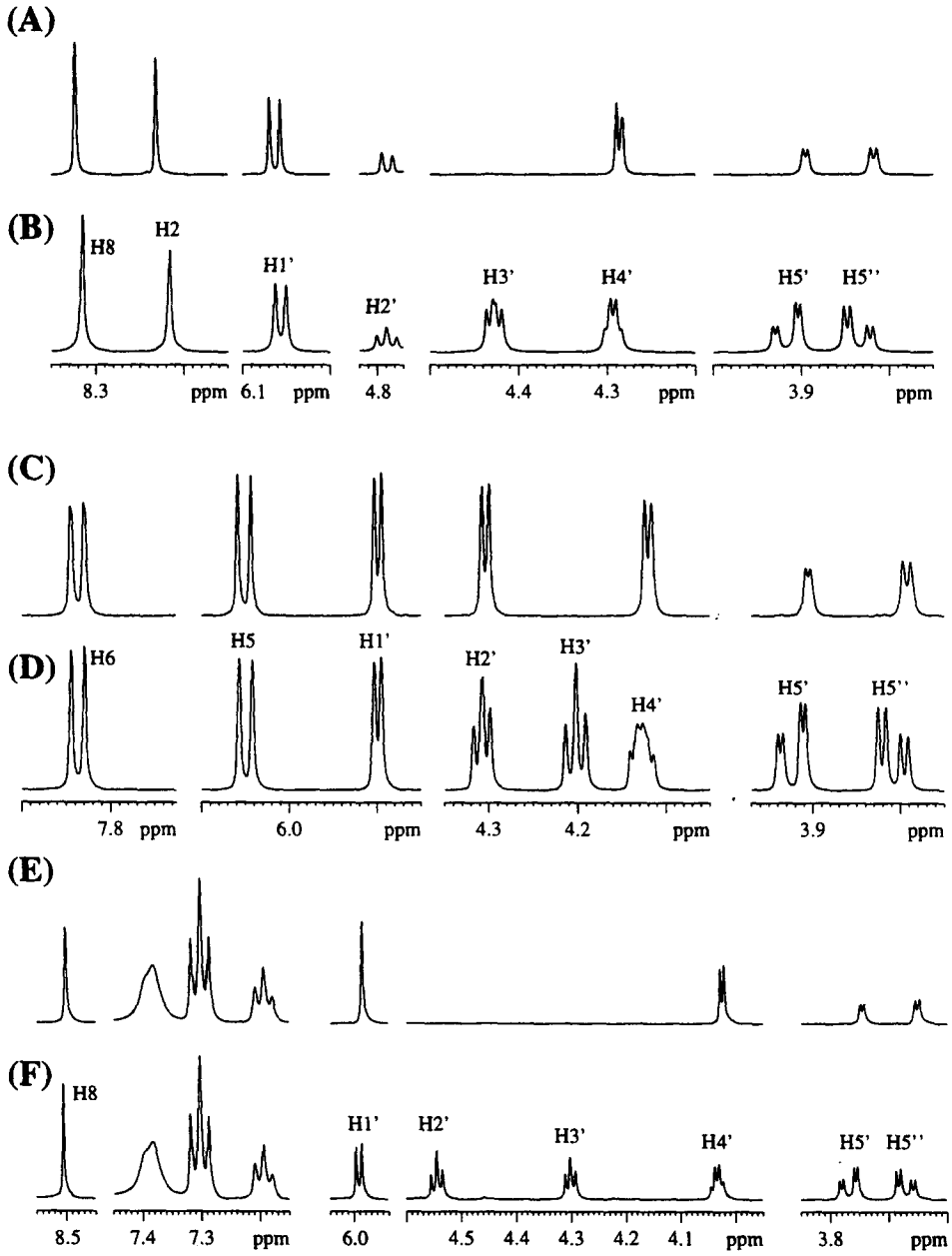
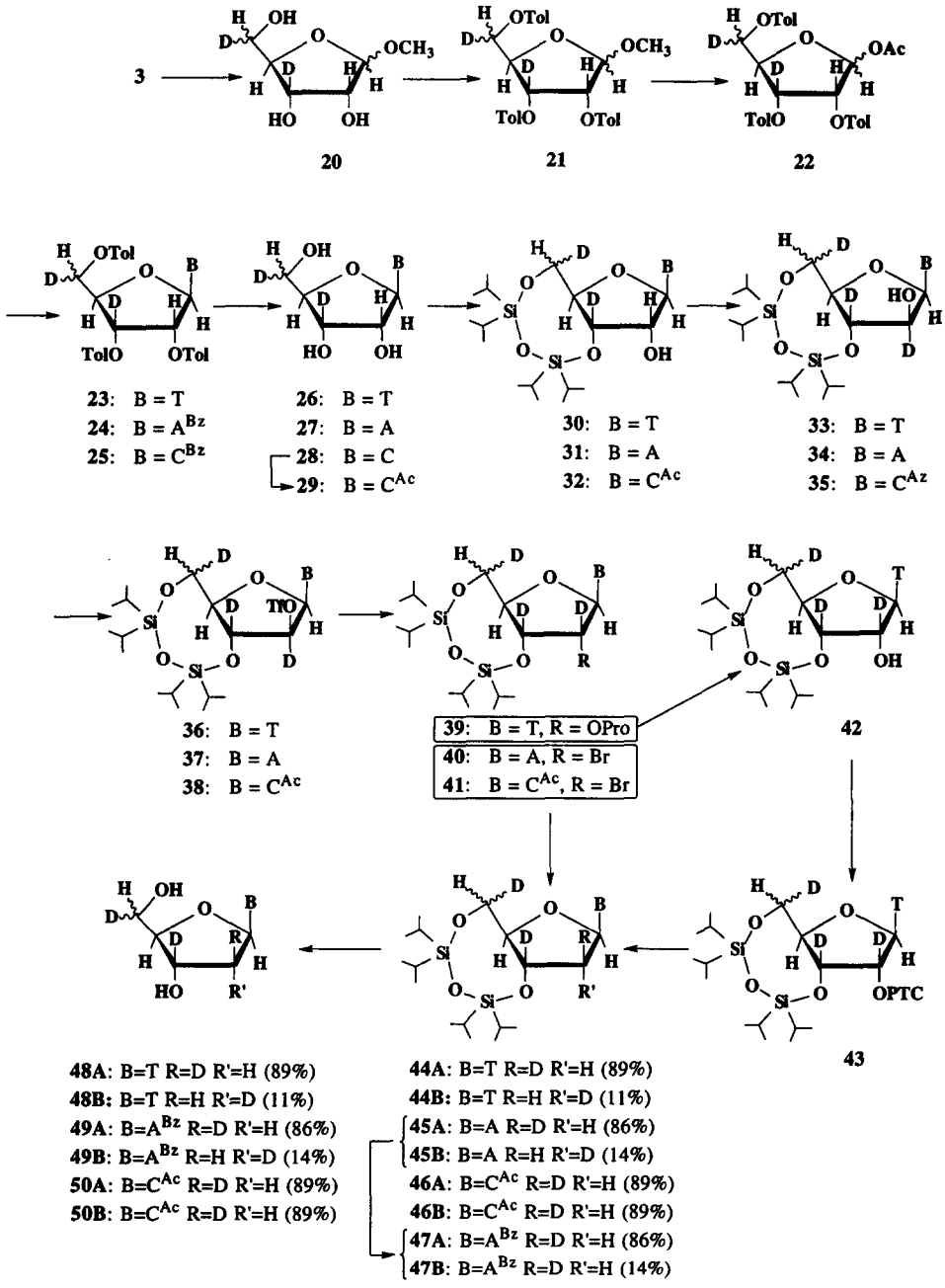
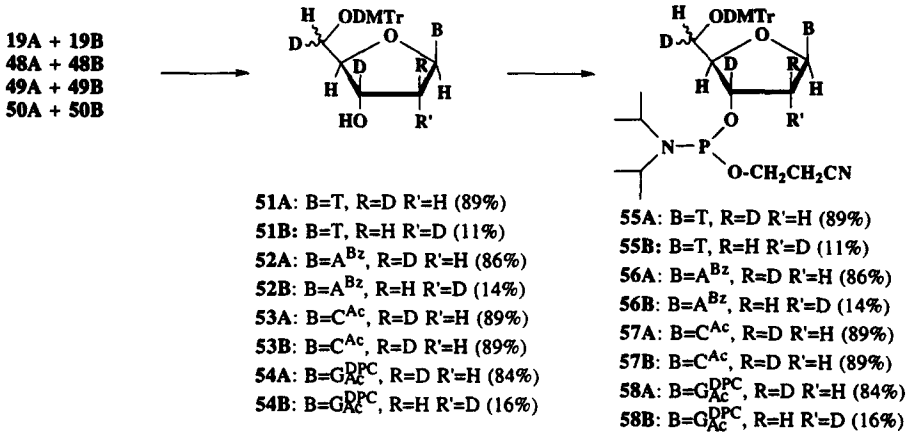


Figure 4: Aromatic and sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides and their natural-abundance counterparts: adenosine-3',5'(R/S)-$^2H_2$ (**27**) (Panel A) and its natural-abundance counterpart (Panel B); N^4-acetylcytidine-3',5'(R/S)-$^2H_2$ (**29**) (Panel C) and its natural-abundance counterpart (Panel D); N^2-acetyl-O^6-diphenylcarbamoylguanosine-2',3',5'(R/S)-$^2H_3$ (**15**) (Panel E) and its natural-abundance counterpart (Panel F).



Scheme 2: Abbreviations: Tol = toluoyl, Ac = acetyl, Bz = benzoyl, T = thymin-1-yl, C = cytosin-1-yl, A = adenin-9-yl, Tf = trifluoromethanesulfonyl, Pro = propionyl, PTC = phenoxythiocarbonyl

free-radical conditions[9a,29] resulted in the inseparable diastereotopic mixtures of **45A,B** (quantitative) and **46A,B** (67%) with 2'R(²H):2'S(²H) ratios of 14:86 and 18:82, respectively. The 2'-deoxyadenosine derivative **45A,B** was treated with benzoyl chloride in dry pyridine to give the N^6-benzoyl derivative **47A,B** (76%). Removal of the TPDS protection from compounds **44**, **46** and **47** with tetrabulylammonium fluoride in THF



Scheme 3. Abbreviations: DMTr = 4,4'-Dimethoxytrityl, Ac = acetyl, Bz = benzoyl, T = thymin-1-yl, C = cytosin-1-yl, A = adenin-9-yl, DPC = diphenylcarbamoyl

afforded the desired isotopomeric mixture of the base-protected 2'-deoxyribonucleosides **48-50** (83, 99 and 99%) (Fig. 10, Panel C-F and Fig. 11, Panel A-B).

The deuterated 2'-deoxynucleoside blocks **19, 48-50** were further converted (Scheme 3) first to the corresponding 5'-*O*-DMTr derivatives **51A,B-54A,B** (91, 35, 98 and 58%, respectively), followed by phosphitylation of the 3'-hydroxyl groups with (2-cyanoethoxy)bis(*N,N*-diisopropylamino)phosphine[35] in presence of *N,N*-diisopropylammonium tetrazolide[36] as catalyst to afford the phosphoramidite derivatives **55A,B-58A,B** in 83, 55, 86 and 87 % yields, respectively.

These selectively deuterated phosphoramidite building blocks have been subsequently used for the synthesis of the Dickerson-Drew DNA dodecamer[37] [d($\underline{\underline{C}}^1\underline{\underline{G}}^2\underline{\underline{C}}^3\underline{\underline{G}}^4\underline{\underline{A}}^5\underline{\underline{A}}^6\underline{\underline{T}}^7\underline{\underline{T}}^8\underline{\underline{C}}^9\underline{\underline{G}}^{10}\underline{\underline{C}}^{11}\underline{\underline{G}}^{12}$)]$_2$ (duplex **I**) (double-underlined nucleotides are of type **A** and **B** deuterated blocks according to the "NMR-window III" concept as schematically shown in Fig. 1) on an automatic DNA synthesiser using CPG solid support (35.1 μmol/g loading) in 19 % yield (379 o.d. units, the analytical ion exchange HPLC profile of the purified oligomer is shown in Fig. 5).

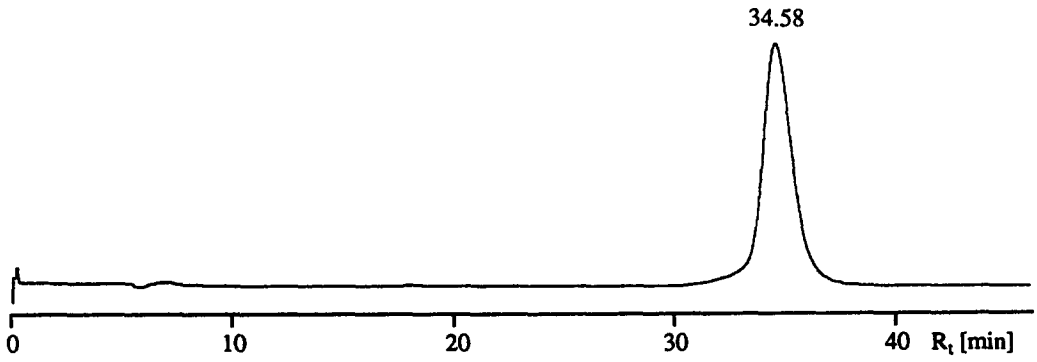


Figure 5. Analytical HPLC profile of dodecamer **I** (Millipore Protein Pak^{TM} Q 15HR 1000Å 8μm column (10x100 mm) under denaturing condition with a linear gradient of 45% → 60% buffer B (1.0 M NaCl in 0.01 M NaOH) in buffer A (0.01 M NaOH) over a period of 40 min.)

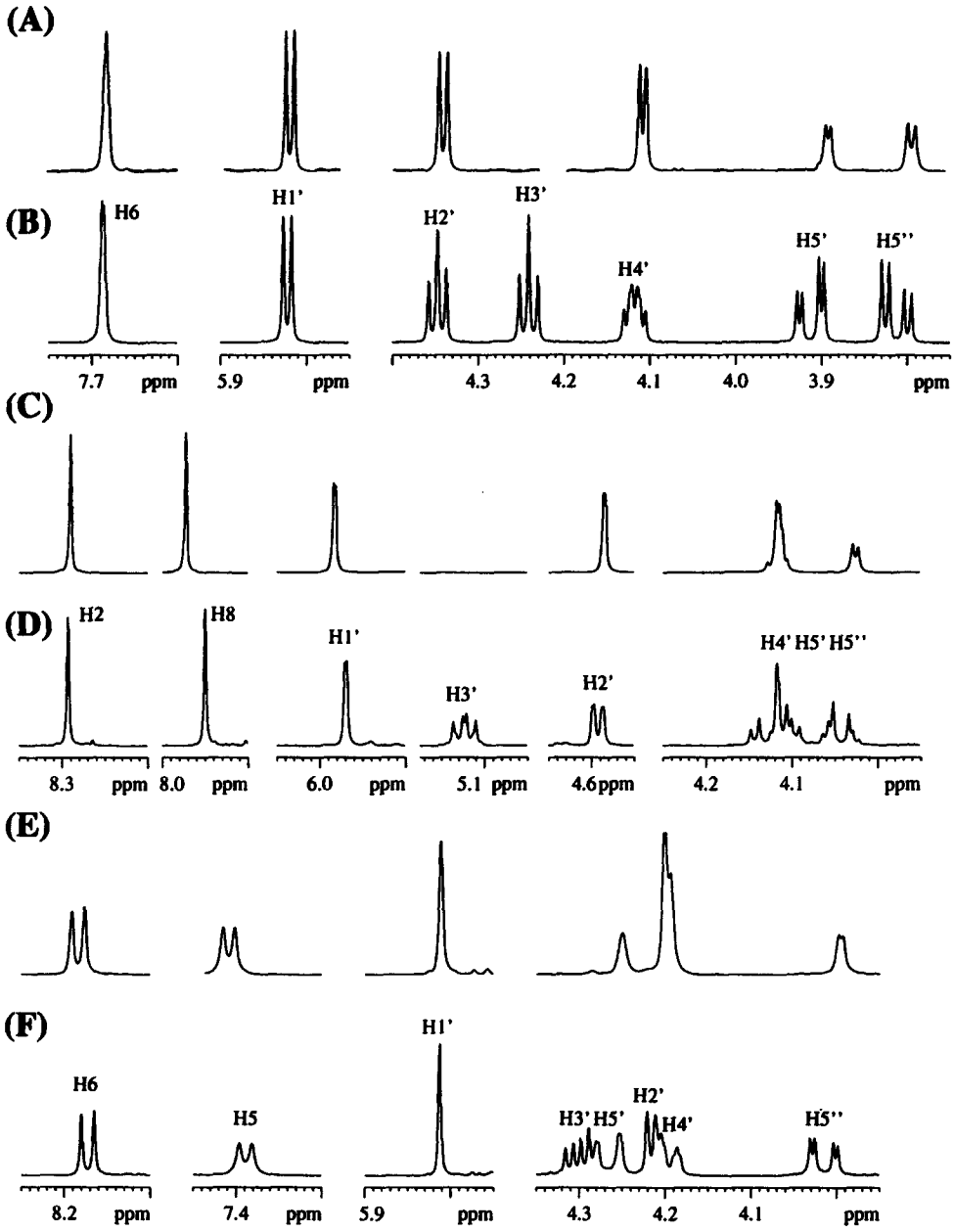


Figure 6: Aromatic and sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides and their natural-abundance counterparts: 1-(β-D-ribofuranosyl-3',5'(R/S)-$^2H_2$)-thymine (**26**) (Panel A) and its natural-abundance counterpart (Panel B); 3',5'-*O*-TPDS-adenosine-3',5'(R/S)-$^2H_2$ (**31**) (Panel C) and its natural-abundance counterpart (Panel D); 3',5'-*O*-TPDS-N^4-acetylcytidine-3',5'(R/S)-$^2H_2$ (**32**) (Panel E) and its natural-abundance counterpart (Panel F).

The NMR properties of this specifically deuterium labelled duplex **I** are published in comparison with its natural counterpart [d($C^1G^2C^3G^4A^5A^6T^7T^8C^9G^{10}C^{11}G^{12}$)]$_2$ (**II**) as well as with an analogous duplex [d($C^1G^2\underline{C}^3G^4\underline{A}^5A^6T^7\underline{T}^8C^9\underline{G}^{10}C^{11}G^{12}$)]$_2$ (**III**) deuterium labelled according to the "NMR-window II" concept[10] (the specific site of incorporations of of 2'(R[~15%]),/S[~85%]),3',4'[~65%],5',5"-$^2H_5$-β-D-2'-deoxyribonucleoside blocks[10] blocks are shown by bold-underlined letter) in the adjoining paper.

Experimental Section

AIBN, chlorotrimethylsilane (TMS-Cl), 1,1,1,3,3,3-hexamethyldisilazane (HMDS), acetic anhydride, dry diethyl ether, trimethylsilyl trifluoromethanesulfonate, LiBr, trifluoromethanesulfonic anhydride and 4-toluoyl chloride were purchased from Merck. Amberlyst A-21 weak and Dowex 1x2-400 strong anion exchange resins, $LiAlD_4$ (98 atom % ^2H), $NaBD_4$ (98 atom % ^2H), tributyltin hydride and phenyl chlorothionoformate were purchased from Aldrich. Dowex 50 WX8 strong cation exchange resin was from C. Roth Gmbh. TPDS-Cl_2 was prepared using literature procedure[28]. Pyridine and toluene were distilled after reflux over calcium hydride for 3 - 4 h, 1,2-dichloroethane and dichloromethane (DCM) were stirred with P_2O_5 overnight followed by distillation under nitrogen, *N,N*-dimethylformamide (DMF) was distilled from P_2O_5 then dried sequentally over molecular sieves (3 Å). Thin layer chromatograpic (TLC) analyses were carried out on Merck pre-coated silica gel 60 F_{254} glass backed plates developed in the following solvent systems: (A) methanol-chloroform (5:99, v/v), (B) methanol-DCM (10:90, v/v), (C) ethyl acetate, (D) ethyl acetate-cyclohexane (30:70, v/v), (E) ethyl acetate-propanol-water (30:18:6, v/v/v). Short column chromatography was done using Merck G60 silica gel. ^1H-NMR spectra were recorded with Jeol FX 90 Q, Jeol GX 270 (if nothing else is indicated) and Bruker AMX 500 spectrometers at 90, 270 and 500 MHz, respectively, using TMS (0.0 ppm) or acetonitrile (^2H_2O solutions, set at δ = 2.0 ppm) as internal standards. ^{13}C-NMR spectra were taken with a Jeol FX 90 Q spectrometer at 27.7 MHz, a Jeol GX 270 MHz spectrometer at 67.9 MHz (if nothing is specified) or Bruker AMX 500 spectromer at 125.8 MHz with the central peak of the solvents (76.9 ppm for $CDCl_3$, 39.6 ppm for DMSO-d_6) as internal reference for solutions other than ^2H_2O in case of which CH_3CN (set at δ = 1.3 ppm) was used as internal reference. Chemical shifts are reported in ppm (δ scale). Two-dimensional NMR experiments (^1H-^1H COSY and ^1H-^{13}C HETCOR) were performed on a Jeol GX 270 or a Bruker AMX 500 MHz spectrometer using standard microprograms. Proton coupled ^{13}C INEPT experiments were done on a Jeol GX 270 MHz specrometer. Nano-electrospray ionisation (EI) mass spectra were taken on an AutoSpec oaTOF-FPD spectrometer (Micromass Ltd., Manchester, UK). Fast-atom bombardment (FAB) mass spectra were obtained on a VG-7070 MS mass spectrometer (VG. Analytical Ltd., Manchester, UK).

1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose-3-$^2H_1$ (1). Pyridinium dichromate (27.18 g, 72.2 mmol) was taken in dry dichloromethane (210.0 ml) and acetic anhydride was added (21.45 ml, 227.3 mmol) to the suspension. 1,2:5,6-Di-*O*-isopropylidene-α-D-glucose (18.6 g, 71.5 mmol) was added in a minimal amount of the same solvent and the mixture was boiled at ~75 °C for 3 h. The mixture was diluted with ethyl acetate and the precipitate was filtered. The solvent was evaporated and coevaporated with toluene. Diethyl ether was added, and the solution was filtered again. After evaporation of the solvent, this procedure was repeated once more to give an oily product (15 g, 58.1 mmol, 81%). ^1H-NMR ($CDCl_3$): 6.14 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 4.4 Hz, 1H) H-1; 4.4-3.9 (*m*, 5H) H-2,4,5,6,6'; 1.46, 1.44, 1.34 (3x*s*, 12H) 4xCH_3. ^{13}C-NMR ($CDCl_3$): 208.8 (C-3); 114.3 (1,2-O-*C*[CH_3]$_2$); 110.4 (5,6-O-*C*[CH_3]$_2$); 103.1 (J_{CH} = 187.9 Hz, C-1); 79.0 (J_{CH} = 151.2 Hz, C-4/5); 77.3 (J_{CH} = 160.4 Hz, C-2); 76.4 (C-4/5); 65.8 (J_{CH} = 148.7 Hz, C-6); 27.6, 27.2, 26.0, 25.3 (4xCH_3). The residue was dissolved in dry diethyl ether (130 ml) and reduced with $LiAlD_4$ (1.22 g, 29.1 mmol) added at 0 °C followed by stirring at RT overnight. Water was added and the mixture was extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and evaporated to give compound **1** (12.5 g, 47.8 mmol, 82%). R_f: 0.72 (System B). ^1H-NMR ($CDCl_3$): 5.82 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 3.8 Hz, 1H) H-1; 4.62 (*d*, 1H) H-2; 4.31 (*m*, 1H) H-5; 4.1 - 4.0 (*m*, 2H) H-6,6'; 3.82 (*d*, $J_{H\text{-}3,H\text{-}4}$ = 4.6 Hz, 1H) H-4; 2.52 (*s*, 1H) OH; 1.58, 1.47, 1.39, 1.37 (4x*s*, 12H) 4xCH_3. ^{13}C-NMR[18] ($CDCl_3$): 112.8 (1,2-O-*C*[CH_3]$_2$); 109.8 (5,6-O-*C*[CH_3]$_2$); 103.8 (J_{CH} = 182.4 Hz, C-1); 79.6 (J_{CH} = 146.6 Hz, C-4); 78.8 (J_{CH} = 161.3 Hz, C-2); 75.5 (J_{CH} = 148.5 Hz, C-5); 65.8 (J_{CH} = 150.3 Hz, C-6); 26.5, 26.2, 25.2 (4xCH_3).

1,2-*O*-Isopropylidene-α-D-allofuranose-3-$^2H_1$ (2). Sugar derivative **1** (25.6 g, 98.0 mmol) was dissolved in 80% aqueous acetic acid and stirred for 20 h at RT. The solvent was evaporated and the residual acid was removed upon coevaporation with toluene. Crystallisation from methanol gave compound **2** (18.1 g, 82 mmol, 83%). R_f: 0.28 (System B). ^1H-NMR (D_2O): 5.80 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 3.5 Hz, 1H) H-1; 4.66 (*d*, 1H) H-2; 4.0-3.9 (*m*, 2H) H-4,5; 3.7-3.5 (*m*, 2H) H-6,6'; 1.51, 1.33 (2x*s*, 6H) 2xCH_3. ^{13}C-NMR (D_2O): 113.9 (1,2-O-

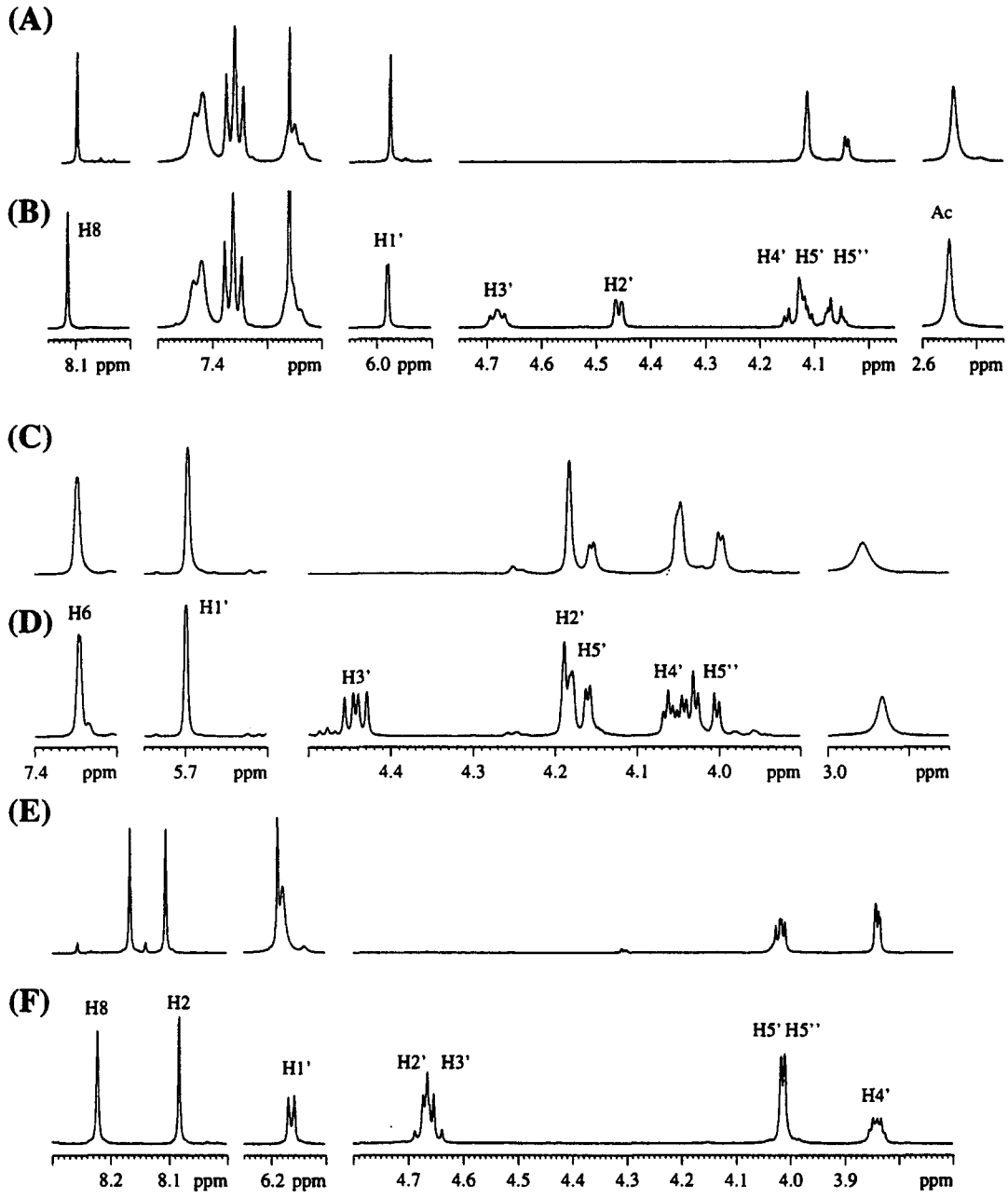


Figure 7: Aromatic and sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides and their natural-abundance counterparts: 3',5'-*O*-TPDS-N^2-acetyl-O^6-diphenylcarbamoylguanosine-2',3',5'(R/S)-$^2H_3$ (**16**) (Panel A) and its natural-abundance counterpart (Panel B); 1-(3',5'-*O*-TPDS-β-D-ribofuranosyl-3',5'(R/S)-$^2H_2$)-thymine (**30**) (Panel C) and its natural-abundance counterpart (Panel D); 9-(3',5'-*O*-TPDS-β-D-arabinofuranosyl-3',5'(R/S)-$^2H_3$)-adenine (**34**) (Panel E) and its natural-abundance counterpart (Panel F).

$C[CH_3]_2$); 104.1 (J_{CH} = 186.0 Hz, C-1); 79.9 (J_{CH} = 148.5 Hz, C-4); 79.8 (J_{CH} = 163.1 Hz, C-2); 71.2 (J_{CH} = 143.9 Hz, C-5); 62.5 (J_{CH} = 142.5 Hz, C-6); 26.0, 25.8 ($2xCH_3$).

1,2-*O*-Isopropylidene-α-D-ribofuranose-3,5(R/S)-$^2H_2$ (3). $NaIO_4$ (21.4 g, 100.1 mmol) was dissolved in ethanol (210 ml) and water (210 ml) and the solution was added to sugar **2** (21.1 g, 95.4 mmol). The mixture was stirred for 35 min, then filtered, 300 µl ethyleneglycol was added, and the filtrate was evaporated. Ethanol was added to the residue, the precipitate was filtered again and this was repeated until precipitate was not obtained. The residue was redissolved in ethanol (200 ml), $NaBD_4$ (1.46 g, 34.9 mmol) was added and stirring was maintained overnight. Then mixture was filtered, evaporated, then redissolved in water and passed through an Amberlyst A-21 ion exchange column (OH^- form). The solution was neutralised with sulfuric acid, then evaporated. The inorganic salt was precipitated from methanol to afford compound **3** in quantitative yield. R_f: 0.47 (System B). ^{1}H-NMR (D_2O): 5.80 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 3.7 Hz, 1H) H-1; 4.65 (*d*, 1H) H-2; 3.91 (*m*, 1H) H-4; 3.8 (br. s) H-5; 3.60 (*d*, $J_{H\text{-}4,H\text{-}5'}$ = 4.5 Hz) H-5'; 1.50, 1.32 (2x*s*, 6H) $2xCH_3$. ^{13}C-NMR[8f] (D_2O): 113.8 (1,2-O-$C[CH_3]_2$); 104.0 (J_{CH} = 185.1 Hz, C-1); 79.9 (J_{CH} = 144.8 Hz, C-4); 79.5 (J_{CH} = 164.0 Hz, C-2); 59.7 (t, J_{CD} = 21.5 Hz, C-5); 26.0, 25.8 ($2xCH_3$).

3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose-3,5(R/S)-$^2H_2$ (4). To compound **3** (20.9 g, 108.6 mmol) in dry acetonitrile (360 ml), benzyl bromide (31 ml, 260.6 mmol) was added followed by addition of NaH (7.84 g, 261.3 mmol) and the mixture was stirred overnight. Methanol was added and stirring was maintained for additional 1 h. The reaction mixture was partitioned between water and DCM. The organic phase was dried over $MgSO_4$ and evaporated. The residue was transferred into a distillation unit together with a second crop from a separate experiment (95 mmol scale) and heated at ~100 °C and 0.1 mbar to give compound **4** (78.4 g, 210.5 mmol, 99%). R_f: 0.51 (System D). ^{1}H-NMR ($CDCl_3$): 7.3-7.2 (*m*, 10H) *Ph*-CH_2; 5.76 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 3.8 Hz, 1H) H-1; 4.8-4.5 (*m*, 4H) Ph-CH_2; 4.55 (*d*, 1H) H-2; 4.17 (*m*, 1H) H-4; 3.75 (*d*, $J_{H\text{-}4,H\text{-}5}$ = 1.7 Hz) H-5; 3.55 (*d*, (*d*, $J_{H\text{-}4,H\text{-}5}$ = 3.7 Hz, 1H) H-5'; 1.59, 1.36 (2x*s*, 6H) $2xCH_3$. ^{13}C-NMR ($CDCl_3$): 112.8 (1,2-O-$C[CH_3]_2$); 104.0 (J_{CH} = 181.5 Hz, C-1); 77.7 (J_{CH} = 146.3 Hz, C-4); 77.2 (J_{CH} = 159.5 Hz, C-2); 73.3, 72.1 ($2xCH_2$); 67.5 (C-5); 26.7, 26.4 ($2xCH_3$).

Methyl 3,5-di-*O*-benzyl-α,β-D-ribofuranoside-3,5(R/S)-$^2H_2$ (5). Sugar derivative **4** (41.5 g, 111.4 mmol) was dissolved in dry methanol (250 ml), conc. sulfuric acid (30 drops) was added and the solution was heated at reflux for 3 h. After cooling down to room temperature, the mixture was neutralised with solid $NaHCO_3$, filtered and evaporated. The oily residue was dissolved in ether and washed with sat. $NaHCO_3$ solution. The organic phase was dried and evaporated to give ribofuranoside **5** (34.7 g, 100.2 mmol, 91%) as an anomeric mixture. R_f: 0.63 (System C). ^{1}H-NMR ($CDCl_3$): 7.4-7.2 (*m*, 10H) *Ph*-CH_2; 4.88 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 4.7 Hz, 0.22H) H-1α; 4.86 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 0.7 Hz, 0.78H) H-1β; 4.7-4.4 (*m*, 4H) Ph-CH_2; 4.22 (*m*) H-4β; 4.15 (*m*) H-4α; 4.02 (*s*) H-2; 3.6-3.4 (*m*, 1H) H-5,5'; 3.47 (*s*) OCH_3α; 3.32 (*s*) OCH_3β.

Methyl β-D-arabinofuranoside-3,5(R/S)-$^2H_2$ (6). Dry dimethylsulfoxid (16.0 ml, 225.5 mmol) was added dropwise to a solution of oxalyl chloride (9.66 ml, 110.7 mmol) in dry DCM (120 ml) at ~-70 °C under nitrogen. To this mixture, a solution of compound **5** (27.7 g, 80.0 mmol) in some dry DCM was added dropwise and the reaction mixture was stirred for 7 h. Triethylamine (68 ml, 487.9 mmol) was added in one batch and stirring was maintained for additional 1h, then the mixture was allowed to warm up to r. t. It was transferred into water and extracted with DCM. The organic phase was dried and evaporated. The resulted oil was dissolved in ethanol (500 ml) and $NaBH_4$ (2.0 g, 52.9 mmol) was added with cooling in ice-water bath. After stirring overnight, water was added and the mixture was extracted with DCM, dried and evaporated to an oil. The crude mixture (15.0 g, 43.6 mmol) was dissolved in dry toluene (40 ml) and the solution was cooled to ~-40 °C, then liquid ammonia was added (~100 ml). Sodium metal was added in small pieces to the solution until getting a persistent deep blue colour then stirring was maintained for additional 40 min. NH_4Cl was added portionwise until the blue colour disappeared, then the mixture was allowed to reach room temperature to evaporate the ammonia followed by evaporation of all volatile matters. The residue was dissolved in water, neutralised and extracted with diethyl ether. The water phase was evaporated to dryness and the inorganic materials were precipitated from methanol to leave a crude mixture of sugar derivatives. This mixture from several experiments (29.17 g, 175.6 mmol) was dissolved in water and applied on a Dowex 1x2-400 column (4.5 x 205 cm, OH^- form). Compound **6** (14.1 g, 86.5 mmol, 49%) was eluted with water. R_f: 0.54 (System E). ^{1}H-NMR[23] (D_2O): 4.83 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 4.6 Hz, 1H) H-1; 4.10 - 4.05 (*m*, 1H) H-2; 3.94 (*m*, 0.34H) H-3; 3.82 (*m*, 1H) H-4; 3.68 (*d*, $J_{H\text{-}4,H\text{-}5}$ = 3.4 Hz, 0.5H) H-5; 3.54 (*d*, $J_{H\text{-}4,H\text{-}5'}$ = 7.0 Hz, 0.5H) H-5'; 3.36 (*s*, 3H) CH_3. ^{13}C-NMR[33] (D_2O): 102.6 (J_{CH} = 174.1 Hz, C-1); 82.3 (J_{CH} = 146.6 Hz, C-4); 76.7 (J_{CH} = 144.8 Hz, C-2); 74.9 (C-3), 74.6 (C-3D); 63.2 (*t*, J_{CD} = 22.0 Hz, C-5); 55.5 (OCH_3).

Benzyl β-D-arabinopyranoside-3,5(R/S)-$^2H_2$ (7). Compound **6** (14.1 g, 86.5 mmol) was dissolved in water and Dowex 50 WX8 ion exchange resin (H^+ form, ~20 ml) was added. The mixture was stirred at 70 °C for 6 h, then the resin was filtered, washed with water. The combined filtrate and washings were evaporated and

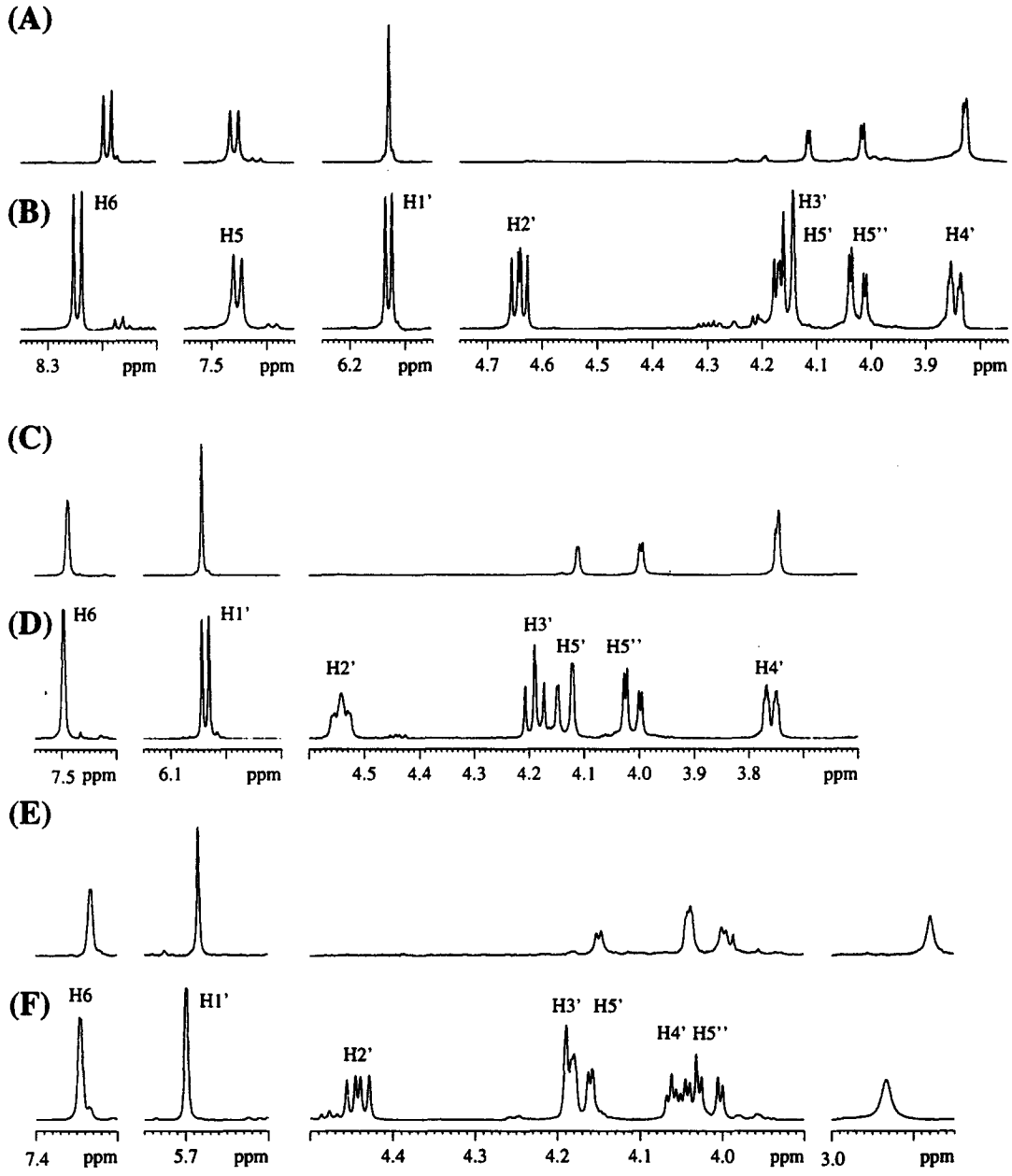


Figure 8: Aromatic and sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides and their natural-abundance counterparts: 1-(3',5'-*O*-TPDS-β-D-arabinofuranosyl-3',5'(R/S)-$^2H_3$)-N^4-acetylcytosine (**35**) (Panel A) and its natural-abundance counterpart (Panel B); 1-(3',5'-*O*-TPDS-β-D-arabinofuranosyl-3',5'(R/S)-$^2H_3$)-thymine (**33**) (Panel C) and its natural-abundance counterpart (Panel D); 1-(3',5'-*O*-TPDS-β-D-ribofuranosyl-3',5'(R/S)-$^2H_3$)-thymine (**42**) (Panel E) and its natural-abundance counterpart (Panel F).

the solid residue was dried over P_2O_5. D-Arabinose-3,5(R/S)-$^2H_2$ (16.4 g, 107.5 mmol) was suspended in freshly distilled benzyl alkohol (100 ml) and the mixture was cooled in an ice-water bath. This cold mixture was saturated with HCl, then it was stirred overnight. Ether was added and compound **7** was separated as small crystalls (15.5 g, 63.8 mmol, 59%). R_f: 0.61 (System E). ^{1}H-NMR (D_2O): 7.4 - 7.3 (*m*, 5H) *Ph*-CH_2; 4.98 (*d*, $J_{H1,H2}$ = 3.7 Hz, 1H) H-1; 4.7 - 4.5 (*m*, 2H) Ph-*CH*$_2$; 3.92 (*m*, 1H) H-4; 3.85 (s) H-5; 3.79 (m) H-2,3; 3.58 (s) H-5'. ^{13}C-NMR (D_2O, 125.8 MHz): 137.5, 129.2, 129.0, 128.8 (*Ph*-CH_2); 98.6 (C-1); 70.2 (C-4); 69.4 (C-3); 69.3 (C-2); 68.6 (Ph-*C*H_2); 63.0 (C-5).

Benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside-3,5(R/S)-$^2H_2$ (8). Compound **7** (5.78 g, 23.8 mmol) was dissolved in dry DMF (49 ml). 2,2-Dimethoxypropane (9.3 ml) was added followed by toluenesulfonic acid (70 mg) and the mixture was stirred for 3.5 h. Dry pyridine was added and the reaction mixture was evaporated then coevaporated with toluene several times. The residue was dissolved in DCM and extracted with sat. $NaHCO_3$. The organic phase was dried over $MgSO_4$, evaporated and coevaporated again with toluene to give **8** (6.73 g, 99%). R_f: 0.65 (System C). ^{1}H-NMR[12] ($CDCl_3$): 7.36 (*s*, 5H) *Ph*-CH_2; 4.93 (*d*, $J_{H-1,H-2}$ = 3.5 Hz, 1H) H-1; 4.8-4.5 (*m*, 2H) Ph-*CH*$_2$; 4.22 (m) H-3,4; 3.99 (br. s) H-5; 3.91 (br. s) H-5'; 3.80 (m) H-2; 2.28 (*d*, $J_{H-2,OH}$ = 6.4 Hz, 1H) OH; 1.53 & 1.36 (2x*s*, 6H) 2xCH_3. ^{13}C-NMR[12] ($CDCl_3$): 137.0, 128.4, 128.0, 127.9 (*Ph*-CH_2); 109.1 (3,4-O-*C*[CH_3]$_2$); 96.8 (C-1); 75.8 (C-3); 72.7 (C-4); 69.8 (C-2); 69.6 (Ph-*C*H_2); 59.5 (C-5); 27.8, 25.8 (2xCH_3).

1-*O*-Benzyl-3,4-*O*-isopropylidene-β-D-*erythro*-pentopyran-2-ulose-3,5(R/S)-$^2H_2$ (9). Sugar **8** (5.14 g, 18.2 mmol) was added to a stirred mixture of pyridinium dichromate (6.84 g, 18.2 mmol) and acetic anhydride (5.5 ml, 57.8 mmol) in dry DCM (63 ml). The resulting solution was heated under reflux for 2 h, then diluted with ethyl acetate and the slurry obtained was filtered through a silica gel column packed with ethyl acetate. Appropriate fractions were collected, evaporated and coevaporated with toluene then with dry pyridine. The ketone was subsequently dissolved in a mixture of dry pyridine (30 ml) and deuterium oxide (30 ml) and stirred for five days. All volatile materials were removed and compund **9** (4.82 g, 17.2 mmol, 95%) was obtained after a few coevaporations with toluene. R_f: 0.75 (System C). ^{1}H-NMR ($CDCl_3$): 7.4-7.1 (*m*, 5H) *Ph*-CH_2; 4.90 (*s*, 1H) H-1; 4.8-4.6 (*m*, 2H) Ph-*CH*$_2$; 4.52 (*m*, 1H) H-4; 4.28 (s) H-5; 4.09 (s) H-5'; 1.46 & 1.39 (2x*s*, 6H) 2xCH_3. ^{13}C-NMR[12] ($CDCl_3$): 198.6 (C-2); 135.8, 128.5, 128.1 (aromatic carbons); 110.3 (3,4-O-*C*[CH_3]$_2$); 98.9 (J_{CH} = 173.2 Hz, C-1); 69.9 (J_{CH} = 144.3 Hz, CH_2); 58.3 (C-5); 27.0, 26.0 (2xCH_3).

Benzyl 3,4-*O*-isopropylidene-β-D-ribopyranoside-2,3,5(R/S)-$^2H_3$ (10). Compound **9** (8.0 g, 28.7 mmol) was dissolved in dry THF (60 ml) and cooled down to 0 °C in an ice-water bath. $LiAlD_4$ (600 mg, 14.3 mmol) was added and stirring was maintained for 23 h. Water was added to the suspension, then the mixture was transferred into a separation funnel containig water and extracted with DCM. The organic phase was dried and evaporated. Crystallisation from petroleum ether afforded compound **10** (6.29 g, 22.2 mmol, 77%). R_f: 0.61 (System C). ^{1}H-NMR[12] ($CDCl_3$): 7.4-7.3 (*m*, 5H) *Ph*- CH_2; 4.85 (*s*, 1H) H-1; 4.9-4.5 (*m*, 2H) Ph-*CH*$_2$; 4.27 (*m*, 1H) H-4; 3.84 (*d*, $J_{H-4,H-5}$ = 3.3 Hz) H-5; 3.73 (*d*, $J_{H-4,H-5'}$ = 2.5 Hz) H-5'; 1.54 & 1.37 (2x*s*, 6H) 2xCH_3. ^{13}C-NMR[12] ($CDCl_3$, 125.8 MHz): 137.3, 128.4, 127.82, 127.77 (*Ph*-CH_2); 109.7 (3,4-O-*C*[CH_3]$_2$); 99.3 (C-1); 72.4 (C-4); 69.8 (Ph-*C*H_2); 61.3 (C-5); 26.4, 25.2 (2xCH_3). HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{15}H_{18}D_3O_5$: 284.1577, found 284.1579.

Methyl α/β-D-ribofuranoside-2,3,5(R/S)-$^2H_3$ (11). Compound **10** (6.29 g, 22.2 mmol) was dissolved in dry ethanol (100 ml) and palladium/charcoal (10% Pd) (2.5 g) was added to the solution. The flask was fitted with a balloon filled with hydrogen and the reaction was stirred overnight. The mixture was filtered through a layer of Celite, the filter was washed with ethanol and the filtrate and washings were eveporated to give an oil. An additional crop (1.75 g, 6.2 mmol) was deprotected in the same way. The 3,4-*O*-isopropylidene-D-ribose-2,3,5(R/S)-$^2H_3$ (5.35 g, 27.7 mmol) obtained was dissolved and stirred in 80% aqueous acetic acid for 4 days. The solution was evaporated then coevaporated with toluene and methanol and dried on oil pump to give D-ribose-2,3,5(R/S)-$^2H_3$ in quantitative yield. This was dissolved in dry methanol (60 ml) and conc. H_2SO_4 (0.2 ml) was added at 0 °C. The solution was kept in refrigerator at ~4 °C overnight, then neutralised by passing through an Amberlyst A-21 column (OH^- form) with methanol as eluant. The resulted oil was separated on a Dowex 1x2-400 (2.8 x 135 cm, OH^- form) column to give compound **11** (3.74 g, 22.4 mmol, 79% for 3 steps). R_f: 0.62 & 0.48 (β & α, System E). ^{1}H-NMR[9a] (D_2O) for β-anomer: 4.84 (*s*, 1H) H-1; 3.95 (*m*, 1H) H-4; 3.72 (*d*, $J_{H4,H5}$ = 3.3 Hz) H-5; 3.54 (*d*, $J_{H4,H5'}$ = 6.6 Hz) H-5', for α-anomer: 4.93 (*s*, 1H) H-1; 4.03 (*m*, 1H) H-4; 3.67 (*d*, $J_{H4,H5}$ = 3.2 Hz) H-5; 3.59 (*d*, $J_{H4,H5'}$ = 4.6 Hz) H-5'. ^{13}C-NMR[9a] (D_2O) β anomer: (22.7 MHz): 108.3 (C-1); 83.1 (C-4); 61.7 (*t*, J_{CD} = 22.0 Hz, C-5); 55.5 (O*C*H_3), α anomer: 103.6 (C-1); 84.8 (C-4); 61.7 (*t*, J_{CD} = 21.8 Hz, C-5); 55.8 (O*C*H_3).

1-*O*-Methyl-2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-2,3,5(R/S)-$^2H_3$ (12). Sugar **11** (3.74 g, 22.4 mmol) was coevaporated with dry pyridine 4 times, and it was redissolved in the same solvent (200 ml). The solution was cooled to 0 °C in an ice-water bath, then toluoyl chloride (9.6 ml, 72.7 mmol) was added in

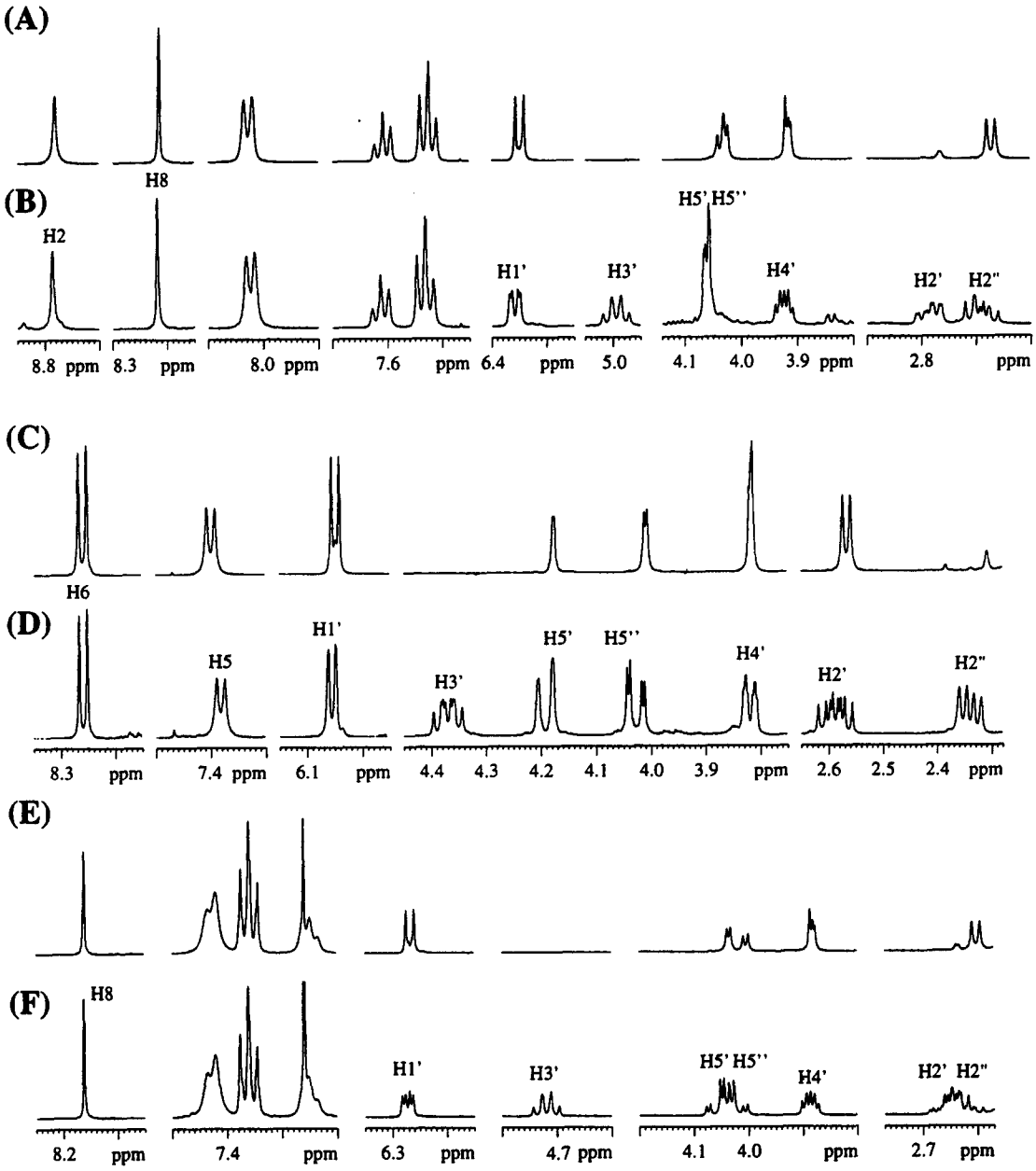


Figure 9: Aromatic and sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides and their natural-abundance counterparts: 3',5'-*O*-TPDS-2'-deoxyadenosine-2'(R/S),3',5'(R/S)-$^{2}H_3$ (**45**) (Panel A) and its natural-abundance counterpart (Panel B); 3',5'-*O*-TPDS-N^4-acetyl-2'-deoxycytidine-2'(R/S),3',5'(R/S)-$^{2}H_3$ (**46**) (Panel C) and its natural-abundance counterpart (Panel D); 3',5'-*O*-TPDS-N^2-acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine-2'(R/S),3',5'(R/S)-$^{2}H_3$ (**18**) (Panel E) and its natural-abundance counterpart (Panel F).

one portion with stirring. Stirring was maintained for 20 min at bath temperature, then at RT overnight, when Tlc showed complete consumption of starting sugar. The reaction mixture was poured into cooled saturated solution of $NaHCO_3$ and stirred for 2h. After extraction with DCM and evaporation of volatile materials followed by coevaporations with toluene, compound **12** (10.73 g, 20.6 mmol, 92%) was obtained. R_f: 0.55 (System D). ^{1}H-NMR[9a] ($CDCl_3$): 8.0 - 7.1 (*m*, 12H) toluoyl (α+β); 5.37 (*s*, 0.3H) H-1(α); 5.13 (*s*, 1H) H-1(β); 4.71 -4.69 (m) H-4(β), 5(α+β); 4.62 (m) H-4(α); 4.57 (*d*, $J_{H-4,H-5'}$ = 3.2 Hz) H-5'(α); 4.46 (*d*, $J_{H-4,H-5'}$ = 5.2 Hz) H-5'(β); 3.47 (*s*, 3H) OCH_3(α);. 3.40 (*s*, 3H) OCH_3(α); 2.42, 2.40 & 2.37 (3x*s*, 9H) CH_3 (α+β).

1-*O*-Acetyl-2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-2,3,5(R/S)-$^2H_3$ (13). Compound **12** (10.73 g, 20.6 mmol) was dissolved in dry DCM (52 ml) and the mixture was cooled to 0 °C in ice-water bath. Acetic acid (10.0 ml), acetic anhydride (12.0 ml) and conc. sulfuric acid (2.0 ml) were mixed in a separate ice-water bath, then added to the cold solution of sugar. The resulting mixture was stirred at 0 °C for 12 min, then poured carefully into ice cold saturated sodium bicarbonate solution for neutralisation of the acids. Extraction, drying on $MgSO_4$ and evaporation gave compound **13** (11.3 g, 20.6 mmol, quantitative). From the oil, the pure β-anomer was crystallised from methanol. R_f: 0.47 (System D). ^{1}H-NMR[9a] ($CDCl_3$): 8.0 -7.1 (*m*, 12H) toluoyl; 6.41 (*s*, 1H) H-1'; 4.8-4.7 (m) H-4',5'; 4.46 (*d*, $J_{H-4',H-5''}$ = 4.5 Hz) H-5"; 2.41, 2.40 & 2.37 (3x*s*, 9H) 3xCH_3; 2.00 (*s*, 3H) acetyl. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{31}H_{28}D_3O_9$: 550.2156, found 550.2161.

2',3',5'-Tri-*O*-(4-toluoyl)-N^2-acetyl-O^6-diphenylcarbamoylguanosine-2',3',5'(R/S)-$^2H_3$ (14). N^2-acetyl-O^6-diphenylcarbamoylguanine (9.5 g, 24.5 mmol) was suspended dry 1,2-dichloroethane (150 ml) followed by addition of bis(trimethylsilyl)acetamide (8.0 ml) and the mixture was heated at ~83 °C under nitrogen for 6 h. The volatile matters were evaporated, the oil was coevaporated with dry toluene then kept on oil pump for ~20 min. Compound **13** (10.34 g, 18.8 mmol) was dissolved in dry toluene (200 ml) and some 1,2-dichloroethane and added to the persilylated nucleobase followed by addition of trimethylsilyl trifluoromethanesulfonate (5.0 ml). The mixture was stirred at ~70 °C in nitrogen atmosphere for 6 h then at RT overnight. Work-up with sat. $NaHCO_3$ solution and short column chromatography yielded compound **14** (9.84 g, 11.3 mmol, 60%). R_f: 0.72 (System C). ^{1}H-NMR ($CDCl_3$): 8.20 (br. *s*, 1H) N-H ; 8.06 (s. 1H) H-8; 7.9 -7.1 (*m*, 22H) toluoyl, phenyls; 6.33 (*s*, 1H) H-1'; 4.86 (*d*, $J_{H4',H5'}$ = 3.5 Hz) H-5'; 4.82 (*d*, 1H) H-4'; 4.68 (*d*, $J_{H4',H5'}$ = 5.0 Hz) H-5"; 2.46 (*s*, 3H) N^2-acetyl; 2.42 & 2.38 (2x*s*, 9H) 3x toluoyl-CH_3. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{49}H_{40}D_3N_6O_{10}$: 878.3229, found 878.3233.

N^2-Acetyl-O^6-diphenylcarbamoylguanosine-2',3',5'(R/S)-$^2H_3$ (15). Compound **14** (9.84 g, 11.3 mmol) was dissolved in a mixture of pyridine (46 ml) and ethanol (94 ml) and the solution was cooled in an ice-water bath. Precooled 2N NaOH solution (65 ml) was added. After stirring for ~12 min at 0 °C, the solution was neutralised by addition acetic acid. The mixture was poured into sat. $NaHCO_3$ solution and extracted with DCM. The organic phase was dried and evaporated. After repeated coevaporation with toluene, short column chromatography gave compound **15** (4.64 g, 8.9 mmol, 79%). ^{1}H-NMR ($CDCl_3$): 8.93 (br.*s*, 1H) N-H; 8.13 (*s*, 1H) H-8; 7.4 - 7.2 (*m*, 10H) phenyls; 5.84 (*s*, 1H) H-1'; 4.15 (*s*, 1H) H-4'; 3.73 (*d*, $J_{H4',H5'}$ = 2.2 Hz) H-5'; 3.61 (*d*, $J_{H4',H5''}$ = 3.6 Hz) H-5"; 1.98 (*s*, 3H) N^2-acetyl. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{25}H_{22}D_3N_6O_7$: 524.1973, found 524.1978.

3',5'-*O*-TPDS-N^2-acetyl-O^6-diphenylcarbamoylguanosine-2',3',5'(R/S)-$^2H_3$ (16). Compound **15** (4.62 g, 8.9 mmol) was coevaporated with dry pyridine three times then dissolved in the same solvent (89 ml). After addition of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.1 ml, 9.8 mmol), the mixture was stirred under dry condition for 2h, followed by normal work-up. The resulting syrup was purified on a short column of silica gel to afford compound **16** (5.41 g, 7.1 mmol, 80%) as a foam. R_f: 0.57 (System C). ^{1}H-NMR ($CDCl_3$): 8.14 (*s*, 1H) H-8; 8.08 (br. s., 1H) N-H; 7.4 - 7.2 (*m*, 10H) phenyls; 5.99 (*s*, 1H) H-1'; 4.13 (m) H-4',5'; 4.04 (*d*, $J_{H4',H5''}$ = 3.2 Hz) H-5"; 2.50 (*s*, 3H) N^2-acetyl; 1.1 - 1.0 (*m*, 24H) methyls of TPDS.

3',5'-*O*-TPDS-2'-*O*-phenoxythiocarbonyl-N^2-acetyl-O^6-diphenylcarbamoylguanosine-5'(R/S), 3',2'-$^2H_3$ (17). To the mixture compound **16** (5.41 g, 7.1 mmol) and 1-*N*-methylimidazole (1.2 ml, 15.0 mmol) in dry DCM (40 ml), phenyl chlorothionoformate (1.63 ml, 11.8 mmol) was added and the solution was stirred overnight. The reaction mixture was poured into sat. $NaHCO_3$ solution and extracted with DCM. Pooled organic phases were washed with sat. citric acid solution, dried with $MgSO_4$, evaporated and the residue was purified on a short column of silica gel to afford compound **17** (6.17 g, 6.87 mmol, 97%). R_f: 0.71 (System C). ^{1}H-NMR ($CDCl_3$): 8.18 (*s*, 1H) H-8; 7.92 (br. *s*, 1-H) N-H; 7.5 - 7.1 (*m*, 15H) aromatics; 6.20 (*s*, 1H) H-1'; 4.19 (*d*, $J_{H-4',H-5'}$ = 2.7 Hz) H-5'; 4.17 (*m*, 1H) H-4'; 4.06 (*d*, $J_{H-4',H-5''}$ = 3.0 Hz) H-5"; 2.58 (*s*, 3H) N^2-acetyl; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{44}H_{52}D_3N_6O_9Si_2S$: 902.3478, found 902.3482.

3',5'-*O*-TPDS-N^2-acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine-2'(R/S),3',5'(R/S)-$^2H_3$ (18A+18B). Compound **17** (6.17 g, 6.87 mmol) was coevaporated with dry toluene and dissolved in the same solvent (45 ml) then AIBN (220 mg, 1.34 mmol) and tri-n-butyltin hydride (2.8 ml, 10.4 mmol) were added.

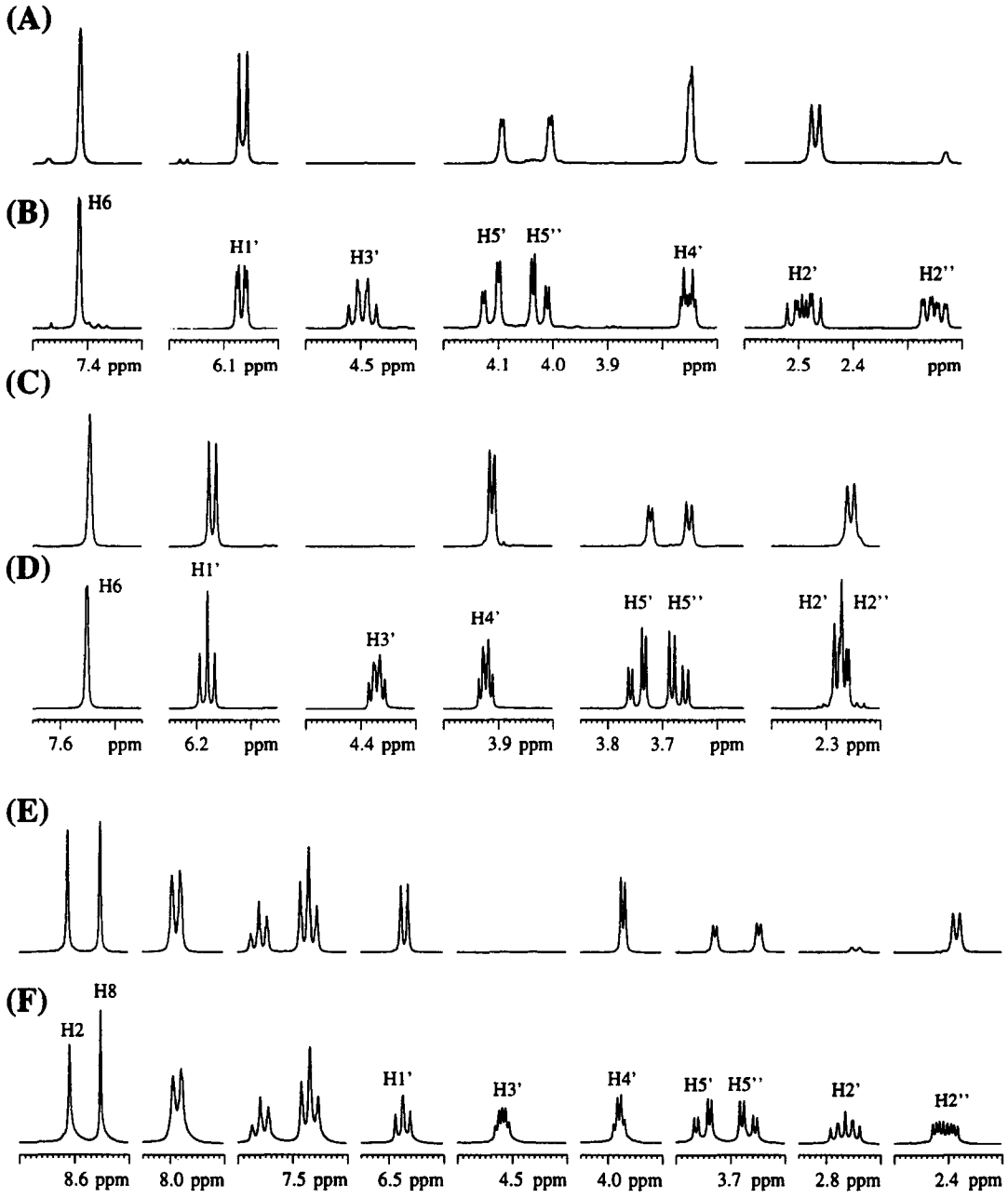


Figure 10: Aromatic and sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides and their natural-abundance counterparts: 3',5'-*O*-TPDS-thymidine-2'(R/S),3',5'(R/S)-$^{2}H_3$ (**44**) (Panel A) and its natural-abundance counterpart (Panel B); thymidine-2'(R/S),3',5'(R/S)-$^{2}H_3$ (**48**) (Panel C) and its natural-abundance counterpart (Panel D); N^6-benzoyl-2'-deoxyadenosine-2'(R/S),3',5'(R/S)-$^{2}H_3$ (**49**) (Panel E) and its natural-abundance counterpart (Panel F).

The solution was degassed by nitrogen bubbling (~20 min), followed by heating at ~75 °C in nitrogen atmosphere for 3.1 h. Volatile materials were evaporated, and the oily residue was subjected to column chromatography to obtain compound **18** (4.22 g, 5.66 mmol, 82%). R_f: 0.63 (System C). ^{1}H-NMR ($CDCl_3$): 8.16 (*s*, 1H) H-8; 7.97 (br. s., 1H) N-H; 7.4 - 7.2 (*m*, 10H) phenyls; 6.27 (*m*, 1H) H-1'; 4.03 (*d*, $J_{H-4',H-5'}$ = 2.2 Hz) H-5'; 4.00 (*d*, $J_{H-4',H-5''}$ = 4.5 Hz) H-5''; 3.89 (*m*, 1H) H-4'; 2.61 (*d*, $J_{H-1',H-2'}$ = 7.2 Hz) H-2'(R); 2.54 (*s*, 3H) N^2-acetyl; 1.1 - 1.0 (*m*, 24H) methyls of TPDS.

N^2-Acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine-2'(R/S),3',5'(R/S)-$^2H_3$ (19A+19B). Compound **18** (4.22 g, 5.66 mmol) was dissolved in dry THF (56 ml) and 1.0 M TBAF solution in dry THF (5.7 ml) was added. After stirring for 5 min, volatile materials were evaporated and the residue was purified on silica gel to give compound **19** (2.82 g, 5.6 mmol, 99%). ^{1}H-NMR ($CDCl_3$:methanol-d_4, 500 MHz): 8.36 (*s*, 1H) H-8; 7.5 - 7.3 (*m*, 10H) phenyls; 6.42 (*m*, 1H) H-1'; 4.05 (*d*, 1H) H-4'; 3.83 (*d*, $J_{H-4',H-5'}$ = 3.5 Hz) H-5'; 3.75 (*d*, $J_{H-4',H-5''}$ = 3.46 Hz) H-5''; 2.77 (*d*, $J_{H-1',H-2'}$ = 6.4 Hz, 0.14H) H-2'(S); 2.45 (*d*, $J_{H-1',H-2'}$ = 6.4 Hz, 0.86H) H-2'(R); 2.37 (*s*, 3H) N^2-acetyl. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{25}H_{22}D_3N_6O_6$: 508.2024, found 508.2030.

Methyl α/β-D-ribofuranoside-3,5(R/S)-$^2H_2$ (20). Compound **3** (28.7 g, 147.7 mmol) was dissolved in 80% aq. acetic acid (400 ml) and stirred at 80 °C for 19 h. After evaporation of the volatile matters and coevaporations with water and toluene the residue was dissolved in dry methanol (340 ml) and treated at 0 °C with concentrated sulfuric acid (1.47 ml), dissolved in 3-4 ml ice-cold dry methanol, then stored in refrigerator for 19.5 h. The solution was neutralised by passing through an Amberlyst A-21 resin (OH^- form, 300 g) pre-washed with methanol. After eluting the column with methanol, eluant was evaporated and the residue was dried on an oil pump to give the anomeric mixture of compound **20** (22.2 g, 133.4 mmol, 90%). R_f: 0.62 & 0.48 (β & α, System E). ^{1}H-NMR (D_2O) β-anomer: 4.84 (*d*, $J_{H1,H2}$ = 1.3 Hz, 1H) H-1; 3.97 (*d*, 1H) H-2; 3.94 (*m*, 1H) H-4; 3.72 (*d*, $J_{H-4,H-5}$ = 3.3 Hz) H-5; 3.53 (*d*, $J_{H-4,H-5'}$ = 6.5 Hz) H-5'; 3.34 (*s*, 3H) CH_3, α-anomer: 4.94 (*d*, $J_{H-1,H-2}$ = 4.4 Hz, 1H) H-1; 4.05 (*d*, 1H) H-2; 4.03 (*m*, 1H) H-4; 3.66 (*d*, $J_{H-4,H-5}$ = 3.2 Hz, 0.44H) H-5; 3.59 (*d*, $J_{H-4,H-5'}$ = 4.4 Hz, 0.56H) H-5'. ^{13}C-NMR (D_2O) β anomer: 108.3 (C-1); 83.1 (C-4); 74.5 (C-2); 55.5 (OCH_3), α anomer: 103.4 (C-1); 84.9 (C-4); 71.4 (C-2); 55.6 (OCH_3).

1-O-Methyl-2,3,5-tri-O-(4-toluoyl)-α/β-D-ribofuranose-3,5(R/S)-$^2H_2$ (21). Sugar **20** (22.2 g, 133.4 mmol) treated with toluoyl chloride (62.5 ml, 473 mmol) in dry pyridine (440 ml) as described for **12** to obtain compound **21** (69.3 g, 133.1 mmol, quantitative). R_f: 0.55 (System D). ^{1}H-NMR ($CDCl_3$): 8.0 - 7.1 (*m*, 12H) toluoyl (α+β); 5.64 (*s*, 0.7H) H-2(β); 5.37 (*d*, $J_{H-1,H-2}$ = 4.5 Hz, 0.3H) H-1(α); 5.29 (*d*, $J_{H-1,H-2}$ = 4.4 Hz, 0.3H) H-2 (α); 5.13 (*s*, 0.7H) H-1(β); 4.70 -4.67 (m) H-4(β), 5(α+β); 4.61 (m) H-4(α); 4.57 (*d*, $J_{H-4,H-5'}$ = 3.7 Hz) H-5'(α); 4.47 (*d*, $J_{H-4,H-5'}$ = 5.1 Hz) H-5'(β); 3.47 (s) OCH_3(α, 0.9H); 3.40 (*s*, 2.1H) OCH_3(β); 2.42, 2.41, 2.40, 2.39 & 2.36 (*s*, 9H) CH_3 (α+β).

1-O-Acetyl-2,3,5-tri-O-(4-toluoyl)-α/β-D-ribofuranose-3,5(R/S)-$^2H_2$ (22). Compound **21** (69.3 g, 133.1 mmol) was reacted with a mixture of acetic acid (64.0 ml), acetic anhydride (78.0 ml) and concentrated sulfuric acid (13.0 ml) at 0 °C in dry DCM (350 ml) as described for compound **13** to afford compound **22** (70.3 g, 128.2 mmol, 96%). Pure β-anomer (37 g) was cristallysed from methanol. R_f: 0.47 (System D). ^{1}H-NMR ($CDCl_3$): 8.0 -7.1 (*m*, 12H) toluoyl; 6.41 (*s*, 1H) H-1'; 5.75 (*s*, 1H) H-2; 4.8-4.7 (m) H-4,5; 4.47 (*d*, $J_{H-4',H-5'}$ = 4.5 Hz) H-5'; 2.41, 2.40 & 2.37 (3x*s*, 9H) 3xCH_3; 2.00 (*s*, 3H) acetyl. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{31}H_{29}D_2O_9$: 549.2094, found 549.2096.

1-(2',3',5'-Tri-O-(4-toluoyl)-β-D-ribofuranosyl)-thymine-3',5'(R/S)-$^2H_2$ (23). A suspension of thymine (2.65 g, 21.0 mmol) in HMDS (25.0 ml) and TMS-Cl (4.0 ml) was heated at reflux at ~120 °C under nitrogen until clearness. The solution was evaporated, coevaporated with dry toluene, then the oily residue was kept on an oil-pump for ~20 min. Sugar **22** (8.77 g, 16.0 mmol) was dissolved in dry 1,2-dichloroethane (130.0 ml) and added to the persilylated thymine followed by addition of trimethylsilyl trifluoromethanesulfonate (4.2 ml). The mixture was heated at ~70 °C under nitrogen for 4 h. Work-up and short column chromatography yielded compound **23** (9.42 g, 15.3 mmol, 96%). R_f: 0.64 (System C). ^{1}H-NMR ($CDCl_3$, 500 MHz): 8.48 (br.*s*, 1H) N-H; 8.0 - 7.2 (*m*, 12H) Ar; 7.16 (m) H-6; 6.45 (*d*, $J_{H-1',H-2'}$ = 6.7 Hz, 1H) H-1'; 5.72 (*d*, 1H) H-2'; 4.85 (*d*, $J_{H-4',H-5'}$ = 2.7 Hz) H-5'; 4.66 (*m*, 1H) H-4'; 4.59 (*d*, $J_{H-4',H-5''}$ = 3.3 Hz) H-5''; 2.43, 2.41 & 2.38 (3x*s*, 3H) 3x toluoyl-CH_3; 1.57 (*d*, $J_{H-5,CH3}$ = 1.3 Hz, 3H) 5-CH_3. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{34}H_{31}D_2N_2O_9$: 615.2312, found 615.2317.

2',3',5'-Tri-O-(4-toluoyl)-N^6-benzoyladenosine-3',5'(R/S)-$^2H_2$ (24). N^6-Benzoyladenine (2.72 g, 11.35 mmol) and deuterated sugar **22** (4.79 g, 8.73 mmol) were condensed as described for compound **23** to give compound **24** (4.6 g, 6.32 mmol, 72%). R_f: 0.58 (System C). ^{1}H-NMR ($CDCl_3$): 9.07 (br. *s*, 1H) N-H ; 8.71 (s. 1H) H-2; 8.18 (*s*, 1H) H-8; 8.0-7.1 (*m*, 17H) toluoyl, benzoyl; 6.50 (*d*, $J_{H-1',H-2'}$ = 5.5 Hz, 1H) H-1'; 6.37 (*d*, 1H) H-2'; 4.89 (*d*, $J_{H-4',H-5'}$ = 3.1 Hz) H-5'; 4.82 (*m*, 1H) H-4'; 4.66 (*d*, $J_{H-4',H-5''}$ = 4.0 Hz) H-5''; 2.41, 2.40 & 2.37 (3x*s*, 9H) 3x toluoyl-CH_3. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{41}H_{34}D_2N_5O_8$: 728.2689,

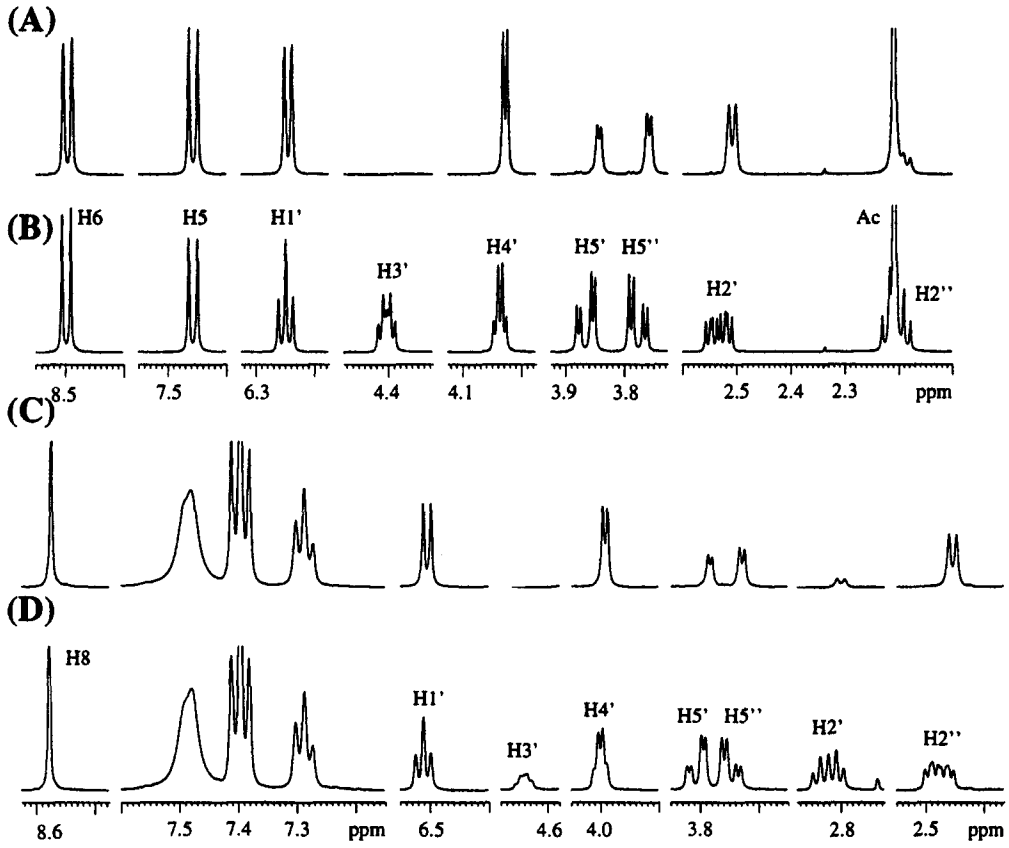


Figure 11: Aromatic and sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides and their natural-abundance counterparts: N^4-acetyl-2'-deoxycytidine-2'(R/S),3',5'(R/S)-$^2H_3$ (**50**) (Panel A) and its natural-abundance counterpart (Panel B); N^2-acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine-2'(R/S),3',5'(R/S)-$^2H_3$ (**19**) (Panel C) and its natural-abundance counterpart (Panel D).

found 728.2694.

2',3',5'-Tri-*O*-(4-toluoyl)-N^4-benzoylcytidine-3',5'(R/S)-$^2H_2$ (25). N^4-Benzoylcytosine (5.59 g, 26.0 mmol) was condensed with sugar **22** (11.3 mg, 20.6 mmol) as described for compound **23** to afford compound **25** (10.8 g, 16.0 mmol, 78%). ^{1}H-NMR ($CDCl_3$): 8.70 (br. *s*, 1H) N-H ; 8.0 -7.2 (*m*, 19H) toluoyl, benzoyl, H-5 & H-6; 6.51 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 5.0 Hz, 1H) H-1'; 5.80 (*d*, 1H) H-2'; 4.84 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 2.7 Hz) H-5'; 4.76 (*m*, 1H) H-4'; 4.67 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 3.8 Hz) H-5''; 2.43, 2.39 & 2.38 (3x*s*, 9H) 3x toluoyl-CH_3. HR-MS (FAB^+): $(M+H)^+$ calc. for $C_{40}H_{34}D_2N_3O_9$: 704.2577, found 704.2581.

1-(β-D-Ribofuranosyl-3',5'(R/S)-$^2H_2$)-thymine (26). Compound **23** (9.42 g, 15.3 mmol) was stirred in methanolic ammonia (~150 ml) at room temperature overnight. After evaporating the solvent, the residue was dissolved in water and DCM and the water phase was extracted with DCM twice then with ether. Evaporation of the water phase gave compound **26** (3.99 g, 99%). ^{1}H-NMR (D_2O): 7.62 (*m*, 1H) H-6; 5.83 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 4.8 Hz, 1H) H-1'; 4.27 (*d*, 1H) H-2'; 4.04 (*m*, 1H) H-4'; 3.83 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 2.8 Hz) H-5'(S); 3.73 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 4.3 Hz) H-5''(R); 1.83 (*d*, $J_{H\text{-}5,CH3}$ = 1.2 Hz, 3H) 5-CH_3. ^{13}C-NMR (D_2O): 152.3 (C-2); 166.9 (C-4); 137.8 (C-6); 111.9 (C-5); 89.4 (J_{CH} = 169.5 Hz, C-1'); 84.4 (J_{CH} = 149.4 Hz, C-4'); 73.7 (J_{CH} = 152.1 Hz, C-2'); 60.8 (C-5'); 12.0 (CH_3). HR-MS (FAB^+): $(M+H)^+$ calc. for $C_{10}H_{13}D_2N_2O_6$: 261.1055, found 261.1058.

Adenosine-3',5'(R/S)-$^2H_2$ (27). Compound **24** (10.0 g, 13.7 mmol) was deprotected by the procedure used for compound **26** to afford adenosine **27** (3.68 g, quantitative). ^{1}H-NMR (D_2O): 8.31 (*s*, 1H) H-8; 8.17 (*s*, 1H) H-2; 6.06 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 6.1 Hz, 1H) H-1'; 4.80 (*d*, 1H) H-2'; 4.33 (*m*, 1H) H-4'; 3.95 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 2.6 Hz) H-5'(S); 3.86 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 3.7 Hz) H-5''(R). ^{13}C-NMR[15b] (D_2O): 156.1 (C-6); 153.0 (C-2); 148.9

(C-4); 141.0 (C-8); 119.6 (C-5); 88.8 (J_{CH} = 165.9 Hz, C-1'); 86.1 (J_{CH} = 149.4 Hz, C-4'); 74.1 (J_{CH} = 149.4 Hz, C-2') 61.6 (C-5'). HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{10}H_{12}D_2N_5O_4$: 270.1171, found 270.1170.

Cytidine-3',5'(R/S)-$^2H_2$ (28). Protecting groups from compound **25** (10.8 g, 16.0 mmol) were removed by the procedure used for compound **26** to afford the deuterated nucleoside **28** (3.9 g, 15.9 mmol, 99%). ^{1}H-NMR (D_2O): 7.77 (*d*, $J_{H-5,H-6}$ = 7.6Hz, 1H) H-6; 5.98 (*d*, 1H) H-5; 5.83 (*d*, $J_{H-1',H-2'}$ = 4.0 Hz, 1H) H-1'; 4.23 (*d*, 1H) H-2'; 4.05 (*m*, 1H) H-4'; 3.84 (*d*, $J_{H-4',H-5'}$ = 2.7 Hz) H-5'(S); 3.72 (*d*, $J_{H-4',H-5''}$ = 4.5 Hz) H-5"(R). ^{13}C-NMR[15b] (D_2O): 158.1 (C-2); 166.7 (C-4); 142.1 (C-6); 96.5 (C-5); 90.8 (J_{CH} = 169.5 Hz, C-1'); 84.1 (J_{CH} = 149.4 Hz, C-4'); 74.3 (J_{CH} = 153.1 Hz, C-2'); 60.8 (C-5'). HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_9H_{12}D_2N_3O_5$: 246.1059, found 246.1061.

N^4-Acetylcytidine-3',5'(R/S)-$^2H_2$ (29). Compound **28** (3.9 g, 15.9 mmol) in dry methanol (300 ml) was heated at reflux. Acetic anhydride was added in portions (3.0 ml) in each hour (5 times). After cooling down, half of the solvent was evaporated to get crystalls, then the suspension was kept in a refrigerator. The crystalls were filtered to obtain compound **29** (3.3 g, 11.5 mmol, 72%). ^{1}H-NMR (DMSO-d_6): 8.47 (*d*, $J_{H-5,H-6}$= 7.5 Hz, 1H) H-6; 7.28 (*d*, 1H) H-5; 5.90 (*d*, $J_{H-1',H-2'}$ = 3.4 Hz, 1H) H-1'; 4.11 (*d*, 1H) H-2'; 4.02 (*m*, 1H) H-4'; 3.82 (*d*, $J_{H-4',H-5'}$ = 2.9 Hz) H-5'; 3.69 (*d*, $J_{H-4',H-5''}$ = 3.3 Hz) H-5"; 2.22 (s) C(O)CH_3. ^{13}C-NMR (DMSO-d_6): 171.1 (C=O); 162.3 (C-4); 154.8 (C-2); 145.5 (C-6); 95.3 (C-5); 90.3 (J_{CH} = 171.4 Hz, C-1'); 84.2 (J_{CH} = 146.3 Hz, C-4'); 74.5 (J_{CH} = 151.2 Hz, C-2'); 59.6 (C-5'); 24.4 (C(O)CH_3).

1-(3',5'-*O*-TPDS-β-D-ribofuranosyl-3',5'(R/S)-$^2H_2$)-thymine (30). Compound **26** (1.27 g, 4.88 mmol) was reacted with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.0 ml, 6.34 mmol), as written for compound **16** to give compound **30** (2.07 g, 4.11 mmol, 84%). R_f: 0.46 (System A). ^{1}H-NMR ($CDCl_3$): 8.27 (br. *s*, 1H) N-H; 7.35 (*m*, 1H) H-6; 5.70 (*d*, $J_{H-1',H-2'}$ = 1.1 Hz, 1H) H-1'; 4.18 (*d*, 1H) H-2'; 4.16 (*d*, $J_{H-4',H-5'}$ = 2.6 Hz) H-5'; 4.05 (*m*, 1H) H-4'; 4.00 (*d*, $J_{H-4',H-5''}$ = 2.9 Hz) H-5"; 1.91 (*d*, $J_{H-5,CH3}$ = 1.2 Hz, 3H) 5-CH_3; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. ^{13}C-NMR ($CDCl_3$): 150.0 (C-2); 163.8 (C-4); 135.8 (C-6); 110.6 (C-5); 91.2 (J_{CH} = 174.1 Hz, C-1'); 81.8 (J_{CH} = 147.5 Hz, C-4'); 74.9 (J_{CH} = 156.7 Hz, C-2'); 60.2 (C-5'); 17.4, 17.2, 17.1, 17.0 (CH(CH_3)$_2$), 13.4, 13.0, 12.7, 12.5 (CH_3, CH(CH_3)$_2$). HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{22}H_{39}D_2N_2O_7Si_2$: 503.2578, found 503.2588.

3',5'-*O*-TPDS-adenosine-3',5'(R/S)-$^2H_2$ (31). After repeated coevaporation with dry pyridine, compound **27** (3.12 g, 11.5 mmol) was dissolved in dry pyridine (110 ml) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.94 ml, 12.5 mmol) was added dropwise. The solution was stirred for 4 h, followed by processing as described for compound **16** to afford compound **31** as a foam (4.51 g, 8.8 mmol, 77%). R_f: 0.30 (System A). ^{1}H-NMR ($CDCl_3$): 8.29 (*s*, 1H) H-8; 7.95 (*s*, 1H) H-2; 5.97 (*d*, $J_{H-1',H-2'}$ = 1.2 Hz, 1H) H-1'; 4.60 (*d*, 1H) H-2'; 4.11 (m) H-4',5'; 4.03 (*d*, $J_{H-4',H-5''}$ = 3.3 Hz) H-5"; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. ^{13}C-NMR[15d] ($CDCl_3$): 155.6 (C-6); 153.1 (C-2); 149.4 (C-4); 139.6 (C-8); 120.5 (C-5); 89.8 (J_{CH} = 166.8 Hz, C-1'); 82.2 (J_{CH} = 148.5 Hz, C-4'); 75.2 (J_{CH} = 159.5 Hz, C-2'); 61.8 (C-5'); 17.44, 17.36, 17.2, 17.0, (CH(CH_3)$_2$); 13.4, 13.1, 12.9, 12.8 (CH(CH_3)$_2$).

3',5'-*O*-TPDS-N^4-acetylcytidine-3',5'(R/S)-$^2H_2$ (32). Compound **29** (3.3 g, 11.5 mmol) was silylated in dry prydine (100 ml) upon addition of 1.0 ml portions of the silylating reagent (4.0 ml, 12.7 mmol) in 15 min intervals to get compound **32** (4.36 g, 72%). R_f: 0.35 (System A). ^{1}H-NMR ($CDCl_3$): 8.19 (*d*, $J_{H-5,H-6}$ = 7.6 Hz, 1H) H-6; 7.44 (*d*, 1H) 5H; 5.82 (*s*, 1H) H-1'; 4.25 (s) H-5'; 4.20 (*s*, 2H) H-2',4'; 3.99 (*d*, $J_{H-4',H-5''}$ = 2.8 Hz) H-5"; 2.30 (*s*, 3H) C(O)CH_3; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. ^{13}C-NMR ($CDCl_3$): 170.8 (C=O); 163.0 (C-4); 154.9 (C-2); 144.2 (C-6); 96.4 (C-5); 91.4 (J_{CH} = 176.9 Hz, C-1'); 81.8 (J_{CH} = 147.6 Hz, C-4'); 75.1 (J_{CH} = 158.5 Hz, C-2'); 59.4 (C-5'); 24.8 (C(O)CH_3); 17.33, 17.26, 17.2, 16.9, 16.8, 16.7 (CH(CH_3)$_2$); 13.4, 13.2, 12.8 (CH(CH_3)$_2$).

1-(3',5'-*O*-TPDS-β-D-arabinofuranosyl-2',3',5'(R/S)-$^2H_3$)-thymine (33). Oxalyl chloride (0.66 ml, 7.57 mmol) was dissolved in dry DCM (7.0 ml) and this mixture was cooled down to ~-70 °C. Dry DMSO (1.18 ml, 16.63 mml) was added dropwise over a period of ~15 min and the solution was stirred for 5 min. Nucleoside **30** (2.07 g, 4.11 mmol) was dissolved in dry DCM (8.0 ml) and it was added dropwise to the solution of the reagent. Stirring was maintained for 2.8 h, then triethylamine (3.5 ml, 25.1 mmol) was added with additional stirring for 1 h when the cooling bath was removed and after reaching RT, the reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with DCM. After evaporation, the oily residue was dissolved in ethanol, cooled in ice-water bath and reduced with $NaBD_4$ (80 mg, 1.91 mmol) for 1 h. Work-up with sat. aqueous $NaHCO_3$ followed by short column chromatography provided compound **33** (1.35 g, 2.68 mmol, 65%). R_f: 0.39 (System A). ^{1}H-NMR ($CDCl_3$): 8.79 (br. *s*, 1H) N-H; 7.46 (*m*, 1H) H-6; 6.03 (*s*, 1H) H-1'; 4.10 (*d*, $J_{H4',H5'}$ = 2.1 Hz) H-5'; 4.01 (*d*, $J_{H4',H5''}$ = 3.1 Hz) H-5"; 3.74 (*m*, 1H) H-4'; 1.90 (*d*, $J_{H-5,CH3}$ = 1.2 Hz, 3H) 5-CH_3 1.1 - 1.0 (*m*, 24H) methyls of TPDS. ^{13}C-NMR ($CDCl_3$): 151.4 (C-2); 164.1 (C-4); 136.3 (C-6); 110.2 (C-5); 84.1 (J_{CH} = 169.5 Hz, C-1'); 80.5 (J_{CH} = 149.4 Hz, C-4'); 17.5, 17.3, 17.1, 17.0, 16.9 (CH(CH_3)$_2$), 13.6, 13.0, 12.8, 12.6, 12.4 (CH_3, CH(CH_3)$_2$). HR-MS (ES$^+$): $(M+Na)^+$ calc. for

$NaC_{22}H_{37}D_3N_2O_7Si_2$: 526.2460, found 526.2465.

9-(3',5'-*O*-TPDS-β-D-arabinofuranosyl-2',3',5'(R/S)-$^2H_3$)-adenine (34). Nucleoside **31** (6.57 g, 12.8 mmol) was added to a premixed solution of CrO_3 (3.84 g), pyridine (6.4 ml) and acetic anhyride (3.84 ml) in dry DCM (60 ml) and the reaction mixture was stirred at RT for 1.5 h when Tlc showed complete reaction. Ethyl acetate was added and the resulting slurry was filtered through a short silica gel column, packed in ethyl acetate. Evaporation of appropriate fractions gave the crude keto compound (6.0 g, 11.7 mmol) which was reduced with $NaBD_4$ (244 mg, 5.8 mmol) as outlined for compound **33** to obtain compound **34** (3.84 g, 7.49 mmol, 58%). R_f: 0.23 (System A). ^{1}H-NMR[12] ($CDCl_3$): 8.15 (*s*, 1H) H-8; 8.12 (*s*, 1H) H-2; 6.30 (br. *s*, 2H) NH_2; 6.20 (*s*, 1H) H-1'; 4.04 (*d*, $J_{H4',H5'}$ = 3.8 Hz) H-5'; 4.02 (*d*, $J_{H4',H5''}$ = 3.2 Hz) H-5''; 3.85 (*m*, 1H) H-4'; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. ^{13}C-NMR[12,15d] ($CDCl_3$): 155.7 (C-6); 152.5 (C-2); 149.3 (C-4); 140.2 (C-8); 119.6 (C-5); 84.0 (J_{CH} = 166.8 Hz, C-1'); 81.1 (J_{CH} = 146.6 Hz, C-4'); 61.1 (C-5'); 17.5, 17.4, 17.0, ($CH(CH_3)_2$); 13.6, 13.1, 12.9, 12.5 ($CH(CH_3)_2$).

1-(3',5'-*O*-TPDS-β-D-arabinofuranosyl-2',3',5'(R/S)-$^2H_3$)-N^4-acetylcytosine (35). Compound **32** (4.36 g, 8.23 mmol) was subjected to the oxidation-reduction reactions according to the procedure described for compound **34** to get the ara-C derivative **38** (3.57 g, 6.73 mmol, 82%). R_f: 0.31 (System A). ^{1}H-NMR ($CDCl_3$): 9.72 (br. *s*, 1H) N-H; 8.19 (*d*, $J_{H-5,H-6}$ = 7.4 Hz, 1H) H-6; 7.46 (*d*, 1H) H-5; 6.13 (*s*, 1H) H-1'; 4.12 (*d*, $J_{H4',H5'}$ = 2.3 Hz) H-5'; 4.02 (*d*, $J_{H4',H5''}$ = 2.9 Hz) H-5''; 3.83 (*m*, 1H) H-4'; 2.25 (*s*, 3H) C(O)CH_3; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. ^{13}C-NMR ($CDCl_3$): 170.8 (C=O); 162.3 (C-4); 156.4 (C-2); 145.3 (C-6); 96.6 (C-5); 85.6 (J_{CH} = 175.0 Hz, C-1'); 81.2 (J_{CH} = 146.6 Hz, C-4'); 60.0 (C-5'); 24.8 (C(O)CH_3); 17.4, 17.3, 17.24, 17.2, 16.9, 16.8, 16.7 ($CH(CH_3)_2$); 13.4, 12.9, 12.3 ($CH(CH_3)_2$).

1-[3',5'-*O*-TPDS-2'-*O*-trifluoromethanesulfonyl-β-D-arabinofuranosyl-2',3',5'(R/S)-$^2H_3$)-thymine (36). After repeated coevaporation with dry pyridine, compound **33** (1.35 g; 2.68 mmol) was dissolved in dry DCM (68.0 ml) then DMAP (1.07 g, 8.8 mmol) and dry pyridine (1.82 ml) were added and the solution was kept at 0 °C. Trifluoromethanesulfonic anhydride (0.74 ml, 4.5 mmol) was added dropwise followed by stirring of the reaction mixture with cooling for 2.5h. The mixture was poured into cold saturated aqueous sodium bicarbonate and extracted with DCM. The combined organic phases were dried over $MgSO_4$, evaporated and the residue was purified by short column chromatography to provide compound **36** (1.22 g; 1.92 mmol; 74%). ^{1}H-NMR ($CDCl_3$, 90 MHz): 8.88 (br. *s*, 1H) N-H; 7.18 (*m*, 1H) H-6; 6.30 (*s*, 1H) H-1'; 4.1 - 4.0 (*m*, 1H) H-5',5''; 3.83 (*m*, 1H) H-4'; 1.94 (*d*, $J_{H-5,6CH3}$ = 1.5 Hz, 1H); 1.1-1.0 (*m*, 24H) methyls of TPDS. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{23}H_{33}D_3F_3N_2O_9Si_2S$: 632.1820, found 632.1834.

9-(3',5'-*O*-TPDS-2'-*O*-trifluoromethanesulfonyl-β-D-arabinofuranosyl-2',3',5'(R/S)-$^2H_3$)-adenine (37). Compound **34** (3.84 g; 7.49 mmol) was converted to compound **37** (3.83 g; 5.99 mmol; 80%) using the procedure written for compound **36**. R_f: 0.40 (System A). ^{1}H-NMR ($CDCl_3$): 8.33 (*s*, 1H) H-8; 7.93 (*s*, 1H) H-2; 6.38 (*s*, 1H) H-1'; 5.72 (br. *s*, 2H) NH_2; 4.21 (*d*, $J_{H4',H5'}$ = 6.22 Hz) H-5'; 4.07 (*d*, $J_{H4',H5''}$ = 3.2 Hz) H-5''; 3.95 (*m*, 1H) H-4'; 1.2-1.1 (*m*, 24H) methyls of TPDS. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{23}H_{36}D_3F_3N_5O_7Si_2S$: 645.2249, found 645.2255.

1-(3',5'-*O*-TPDS-2'-*O*-trifluoromethanesulfonyl-β-D-arabinofuranosyl-2',3',5'(R/S)-$^2H_3$)-N^4-acetylcytosine (38). Compound **35** (3.57 g, 6.73 mmol) was subjected to the procedure described for compound **36** to get nucleoside derivative **38** (3.59 g, 5.42 mmol, 81%). R_f: 0.49 (System A). ^{1}H-NMR ($CDCl_3$): 9.91 (br. *s*, 1H) N-H; 7.85 (*d*, $J_{H-5,H-6}$ = 7.7 Hz, 1H) H-6; 7.52 (*d*, 1H) 5-H; 6.29 (*s*, 1H) H-1'; 4.15 (*d*, $J_{H-4',H-5'}$ = 3.7 Hz) H-5'; 3.98 (m) H-4',5''; 2.29 (*s*, 3H) C(O)CH_3; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{24}H_{38}D_3F_3N_3O_9Si_2S$: 663.2242, found 663.2248.

1-(3',5'-*O*-TPDS-2'-*O*-propionyl-β-D-ribofuranosyl-2',3',5'(R/S)-$^2H_3$)-thymine (39). Compound **36** (1.22 g, 1.92 mmol) was dissolved in dry DMF (3.0 ml) and cesium propionate (523 mg, 2.54 mmol) was added. The reaction mixture was stirred for 4.5 h, then volatile matters were evaporated and the residue was partitioned between water and DCM. The organic phase was dried over $MgSO_4$, evaporated and the residual oil was purified on a short silica gel column to provide compound **39** (1.03 g, 1.84 mmol, 96%). R_f: 0.55 (System A). ^{1}H-NMR ($CDCl_3$, 90 MHz): 9.04 (br. *s*, 1H) N-H; 7.37 (*m*, 1H) H-6; 5.86 (*s*, 1H) H-1'; 4.20 (*m*, 1H) H-4'; 4.19 (*d*, $J_{H4',H5'}$ = 1.5 Hz) H-5'; 3.97 (m) H-4',5''; 2.43 (q, J = 7.1 Hz, 2H) $CH_3CH_2C(O)$; 1.92 (*d*, $J_{H-5,CH3}$ = 1.2 Hz, 3H) 5-CH_3; 1.17 (*t*, 3H) $CH_3CH_2C(O)$; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{25}H_{42}D_3N_2O_8Si_2$: 560.2903, found 560.2912.

9-(3',5'-*O*-TPDS-2'-bromo-2'-deoxy-β-D-ribofuranosyl-2',3',5'(R/S)-$^2H_3$)-adenine (40). Product **37** (3.83 g, 5.99 mmol) was dissolved in dry DMF (60 ml). LiBr (0.78 g; 8.98 mmol) was added and the mixture was stirred for 24 h. Volatile materials were evaporated and the residue was partitioned between water and DCM. Organic phase was dried, filtered and evaporated. Short column chromatography afforded compound **40** (3.27 g; 5.5 mmol, 92%). R_f: 0.39 (System A). ^{1}H-NMR ($CDCl_3$): 8.32 (*s*, 1H) H-8; 8.08 (*s*, 1H) H-2; 6.39 (*s*, 1H) H-1'; 5.73 (br. *s*, 2H) N-H; 4.20 (*d*, $J_{H-4',H-5'}$ = 2.7 Hz) H-5'; 4.19 (*m*, 1H) H-4'; 4.04

(*d*, $J_{H\text{-}4',H\text{-}5''}$ = 2.7 Hz) H-5"; 1.1 -1.0 (*m*, 24H) TPDS. ^{13}C-NMR ($CDCl_3$): 155.4 (C-6); 153.2 (C-2); 149.0 (C-4); 139.0 (C-8); 120.3 (C-5); 90.7 (C-1'); 82.6 (C-4'); 59.9 (C-5'); 17.4, 17.3, 17.1, 17.0, 16.9 ($CH(CH_3)_2$); 13.3, 13.0, 12.8, 12.6 ($CH(CH_3)_2$). HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{22}H_{36}D_3BrN_5O_4Si_2$: 575.1912, found 575.1918.

1-(3',5'-*O*-TPDS-2'-bromo-2'-deoxy-β-D-ribofuranosyl-2',3',5'(R/S)-$^2H_3$)-*N*^{4}-acetylcytosine (41). Compound **38** (3.59 g, 5.42 mmol) was converted to nucleoside derivative **41** (2.81 g, 4.73 mmol, 87%) using the procedure described for compound **40**. R_f: 0.49 (System A). ^{1}H-NMR ($CDCl_3$): 10.15 (br. *s*, 1H) N-H; 8.31 (*d*, $J_{H\text{-}5,H\text{-}6}$ = 7.4 Hz, 1H) H-6; 7.44 (*d*, 1H) 5-H; 6.13 (*s*, 1H) H-1'; 4.27 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 3.0 Hz) H-5'; 4.26 (m) H-4'; 3.98 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 2.5 Hz) H-5"; 2.29 (*s*, 3H) C(O)CH_3; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. ^{13}C-NMR ($CDCl_3$, 125.8 MHz): 170.7 (C=O); 163.1 (C-4); 154.9 (C-2); 143.6 (C-6); 96.4 (C-5); 91.9 (C-1'); 82.9 (C-4'); 58.8 (C-5'); 24.9 (C(O)CH_3); 17.35, 17.28, 17.2, 17.1, 16.9, 16.8, 16.7 ($CH(CH_3)_2$); 13.2, 13.0, 12.7, 12.4 ($CH(CH_3)_2$). HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{23}H_{38}D_3BrN_3O_6Si_2$: 593.1906, found 593.1913.

1-(3',5'-*O*-TPDS-β-D-ribofuranosyl-2',3',5'(R/S)-$^2H_3$)-thymine (42). Compound **39** (1.03 g, 1.84 mmol) was dissolved in methanolic ammonia and the solution was stirred for 21 h at room temperature. Volatile matters were evaporated and the residue was purified by short column chromatography to provide compound **42** (0.62 g, 1.23 mmol, 67%). R_f: 0.46 (System A). ^{1}H-NMR ($CDCl_3$, 90 MHz): 8.91 (br. *s*, 1H) N-H; 7.42 (*m*, 1H) H-6; 5.71 (*s*, 1H) H-1'; 4.18 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 1.7 Hz) H-5'; 4.19 (*m*, 1H) H-4'; 4.00 (*d*, *d*, $J_{H\text{-}4',H\text{-}5''}$ = 1.7 Hz) H-5"; 1.92 (*d*, $J_{H\text{-}5,CH3}$ = 1.0 Hz, 3H) 5-CH_3; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. ^{13}C-NMR ($CDCl_3$): 149.9 (C-2); 163.7 (C-4); 135.9 (C-6); 110.6 (C-5); 91.2 (J_{CH} = 171.4 Hz, C-1'); 81.9 (J_{CH} = 148.5 Hz, C-4'); 60.2 (C-5'); 17.40, 17.35, 17.3, 17.0 ($CH(CH_3)_2$), 13.4, 13.0, 12.7, 12.6 (CH_3, $CH(CH_3)_2$). HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{22}H_{38}D_3N_2O_7Si_2$: 504.2641, found 504.2648.

1-(3',5'-*O*-TPDS-2'-*O*-phenoxythiocarbonyl-β-D-ribofuranosyl)-2',3',5'(R/S)-$^2H_3$-thymine (43). To the mixture of compound **42** (1.15 g, 2.28 mmol) and 1-*N*-methylimidazole (0.73 ml, 9.16 mmol) in dry DCM (20 ml), phenyl chlorothionoformate (0.95 ml, 6.87 mmol) was added and the mixture was stirred at RT overnight. The reaction mixture was poured into sat. $NaHCO_3$ solution and extracted with DCM. Pooled organic phases were washed with sat. citric acid solution, dried with $MgSO_4$, evaporated and the residue was purified by short column chromatograpy to provide compound **43** (1.41 g, 2.19 mmol, 96%). R_f: 0.58 (System A). ^{1}H-NMR ($CDCl_3$, 90 MHz): 8.49 (br. s., 1H) N-H; 7.6 - 7.0 (*m*, 6H) H-6, phenoxy; 5.91 (*s*, 1H) H-1'; 4.22 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 1.5 Hz) H-5'; 4.1-4.0 (s) H-4',H-5"; 1.93 (*d*, $J_{5CH3,6}$ = 1.0 Hz, 3H) 5-CH_3; 1.1 - 1.0 (*m*, 24H) methyls of TPDS. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{29}H_{42}D_3N_2O_8Si_2S$: 640.2624, found 640.2631.

3',5'-*O*-TPDS-thymidine-2'(R/S),3',5'(R/S)-$^2H_3$ (44A + 44B). Compound **43** (0.74 g, 1.16 mmol) was deoxygenated as described for guanosine derivative **18**. Short column chromatography afforded compound **44** (0.54 g, 1.1 mmol, 95%). R_f: 0.53 (System A). ^{1}H-NMR ($CDCl_3$): 8.57 (br. s., 1H) N-H; 7.42 (*m*, 1H) H-6; 6.07 (*m*, 1H) H-1'; 4.10 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 2.3 Hz) H-5'; 4.01 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 2.9 Hz) H-5"; 3.75 (*m*, 1H) H-4'; 2.48 (*d*, $J_{H\text{-}1',H\text{-}2''}$ = 7.5 Hz, 0.89H) H-2"; 2.23 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 2.0 Hz, 0.11H) H-2'; 1.92 (*d*, $J_{H\text{-}5,CH3,6}$ = 1.2 Hz, 3H) 5-CH_3; 1.1-1.0 (*m*, 24H) methyls of TPDS. ^{13}C-NMR ($CDCl_3$): 150.0 (C-2); 163.4 (C-4); 135.2 (C-6); 110.5 (C-5); 84.8 (J_{CH} = 146.6 Hz, C-4'); 83.8 (J_{CH} = 172.3 Hz, C-1'); 59.9 (C-5'); 39.5 (C-2'); 17.5, 17.3, 17.1, 17.0, 16.9 ($CH(CH_3)_2$), 13.6, 13.0, 12.8, 12.6, 12.4 (CH_3, $CH(CH_3)_2$).

3',5'-*O*-TPDS-2'-deoxyadenosine-2'(R/S),3',5'(R/S)-$^2H_3$ (45A + 45B). Compound **40** (3.27 g; 5.5 mmol) was reduced with tributyltin hydride (2.2 ml, 8.18 mmol) and AIBN (176 mg, 1.07 mmol) as free radical initiator in dry toluene (50 ml) at ~80 °C for 2 h. Processing as written for compound **18** gave compound **45** (2.72 g; 5.5 mmol, quantitative). R_f: 0.33 (System A). ^{1}H-NMR ($CDCl_3$): 8.32 (*s*, 1H) H-8; 8.03 (*s*, 1H) H-2; 6.29 (*m*, 1H) H-1'; 5.92 (br. *s*, 2H) N-H; 4.04 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 5.0 Hz) H-5'; 4.03 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 3.7 Hz) H-5"; 3.89 (*m*, 1H) H-4'; 2.71 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 2.3 Hz, 0.14H) H-2'; 2.63 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 5.6 Hz, 0.86H) H-2"; 1.1 - 1.0 (*m*, 24H) methyls of TPDS.

3',5'-*O*-TPDS-*N*^{4}-acetyl-2'-deoxycytidine-2'(R/S),3',5'(R/S)-$^2H_3$ (46A + 46B). Compound **41** (2.77 g, 4.66 mmol) was deoxygenated as described for compound **45** to furnish compound **46** (1.59 g, 3.1 mmol, 67%). R_f: 0.43 (System A). ^{1}H-NMR ($CDCl_3$): 9.97 (br. *s*, 1H) N-H; 8.25 (*d*, $J_{H\text{-}5,H\text{-}6}$ = 7.4 Hz, 1H) H-6; 7.41 (*d*, 1H) H-5; 6.03 (*m*, 1H) H-1'; 4.17 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 1.4 Hz) H-5'; 4.00 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 2.7 Hz) H-5"; 3.80 (m) H-4'; 2.55 (*d*, $J_{H\text{-}1',H\text{-}2''}$ = 6.9 Hz, 0.82H) H-2"; 2.27 (*s*, 3H) C(O)CH_3; 1.1 - 1.0 (*m*, 24H) methyls of TPDS.

3',5'-*O*-TPDS-*N*^{6}-benzoyl-2'-deoxyadenosine-2'(R/S),3',5'(R/S)-$^2H_3$ (47A + 47B). After repeated coevaporation with dry pyridine, compound **45** (2.78 g, 5.5 mmol) was treated with benzoyl chloride (1.9 ml, 16.5 mmol) in dry pyridine (50 ml) for 2h. Aqueous ammonia solution was added and stirring was maintained for 20 min. After extraction from aqueous $NaHCO_3$ with DCM, the organic phase was separated, dried with $MgSO_4$, filtered and evaporated. Pyridine was removed by coevaporation with toluene. Short column

chromatography afforded product **47** (2.51 g, 4.18 mmol, 76%). R_f: 0.61 (System A). ^{1}H-NMR ($CDCl_3$): 9.06 (br. *s*, 1H) N-H; 8.78 (*s*, 1H) H-2; 8.22 (*s*, 1H) H-8; 8.0 - 7.5 (*m*, 5H) benzoyl; 6.36 (*m*, 1H) H-1'; 4.05 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 3.3 Hz) H-5'; 4.04 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 4.9 Hz) H-5"; 3.92 (*m*, 1H) H-4'; 2.76 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 2.1 Hz) H-2'; 2.68 (*d*, $J_{H\text{-}1',H\text{-}2''}$ = 7.7 Hz) H-2"; 1.1 - 1.0 (*m*, 24H) TPDS.

Thymidine-2'(R/S),3',5'(R/S)-$^2H_3$ (48A + 48B). Compound **44** (0.54 g, 1.1 mmol) was dissolved in dry THF (11 ml) and 1.0 M TBAF solution in dry THF (1.1 ml) was added. After stirring for 5 min, volatile materials were evaporated and the residue was purified on silica gel column to give compound **48** (0.22 g, 83%). ^{1}H-NMR (D_2O): 7.56 (*m*, 1H) H-6; 6.20 (*m*, 1H) H-1'; 3.94 (*m*, 1H) H-4'; 3.74 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 3.4 Hz) H-5'; 3.67 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 4.88 Hz) H-5"; 2.28 (*d*, $J_{H\text{-}1',H\text{-}2''}$ = 6.6 Hz, 0.89H) H-2"; 1.82 (*d*, $J_{H\text{-}5,CH3,6}$ = 1.2 Hz, 3H) 5-CH_3. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{10}H_{12}D_3N_2O_5$: 246.1169, found 246.1173.

N^6-Benzoyl-2'-deoxyadenosine-2'(R/S),3',5'(R/S)-$^2H_3$ (49A + 49B). Compound **47** (2.51g, 4.18 mmol) was subjected to the treatment described for compound **48** to provide compound **49** (1.48 g, 4.13 mmol, 99%). ^{1}H-NMR ($CDCl_3$): 9.05 (br. *s*, 1H) N-H; 8.79 (*s*, 1H) H-2; 8.10 (*s*, 1H) H-8; 8.0 -7.5 (*m*, 5H) benzoyl; 6.41 (*d*, 1H) H-1'; 4.24 (*m*, 1H) H-4'; 3.98 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 1.6 Hz) H-5'; 3.81 (br. s) H-5"; 2.37 (*d*, $J_{H\text{-}1',H\text{-}2''}$ = 5.2 Hz) H-2". HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{17}H_{15}D_3N_5O_4$: 359.1547, found 359.1550.

N^4-Acetyl-2'-deoxycytidine-2'(R/S),3',5'(R/S)-$^2H_3$ (50A + 50B). Compound **46** (3.16 g, 5.14 mmol) was deprotected as written for derivative **48** to get compound **50** (1.39 g, 5.10 mmol, 99%). ^{1}H-NMR ($CDCl_3$/methanol-d_4): 8.45 (*d*, $J_{H\text{-}5,H\text{-}6}$ = 7.4 Hz, 1H) H-6; 7.40 (*d*, 1H) H-5; 6.16 (*m*, 1H) H-1'; 3.99 (m) H-4'; 3.85 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 3.0 Hz) H-5'; 3.82 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 3.0 Hz) H-5"; 2.50 (*d*, $J_{H\text{-}1',H\text{-}2''}$ = 6.4 Hz) H-2"; 2.20 (*s*, 3H) C(O)*CH*$_3$. HR-MS (FAB$^+$): $(M+H)^+$ calc. for $C_{11}H_{13}D_3N_3O_5$: 273.1278, found 273.1280.

5'-O-DMTr-thymidine-2'(R/S),3',5'(R/S)-$^2H_3$ (51A + 51B). Compound **48** (0.65 g, 2.63 mmol) were repeatedly coevaporated with dry pyridine (3x), then it was treated with DMTr-Cl (1.07 g, 3.16 mmol) in the same solvent (13 ml) at RT for 2 h. The reaction mixture was poured into sat. $NaHCO_3$ solution and extracted with DCM. After drying over $MgSO_4$, the organic phase was evaporated, coevaporated with toluene and the residual foam was chromatographed on silica gel to obtain compound **51** (1.31 g, 2.39 mmol, 91%). ^{1}H-NMR ($CDCl_3$ + DABCO): 7.59 (*m*, 1H) H-6; 7.4 - 6.8 (*m*, 13H) DMTr; 6.42 (*m*, 1H) H-1'; 4.05 (*m*, 1H) H-4'; 3.78 (*s*, 6H) 2xOCH$_3$; 3.45 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 3.2 Hz) H-5'; 3.34 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 3.0 Hz) H-5"; 2.39 (*d*, $J_{H1',H2''}$ = 5.9 Hz) H-2"; 1.46 (*d*, $J_{H\text{-}5,CH3,6}$ = 1.1 Hz, 3H) 5-CH_3.

5'-O-DMTr-N^6-benzoyl-2'-deoxyadenosine-2'(R/S),3',5'(R/S)-$^2H_3$ (52A + 52B). Compound **49** (1.48 g, 4.13 mmol) was treated according to the procedure described for **51** to afford compound **52** (0.95 g, 1.44 mmol, 35%). ^{1}H-NMR ($CDCl_3$ + DABCO): 9.20 (br. s., 1H) N-H; 8.72 (*s*, 1H) H-2; 8.15 (*s*, 1H) H-8; 8.0 - 6.8 (*m*, 18H) DMTr + benzoyl; 6.47 (*d*, 1H) H-1'; 4.15 (*d*, 1H) H-4'; 3.76 (*s*, 6H) 2xOCH$_3$; 3.38 (*d*, 1H) H-5'/5"; 2.53 (*d*, $J_{H\text{-}1',H\text{-}2''}$ = 6.4 Hz) H-2".

5'-O-DMTr-N^4-acetyl-2'-deoxycytidine-2'(R/S),3',5'(R/S)-$^2H_3$ (53A + 53B). Upon subjecting compound **50** (1.39 g, 5.10 mmol) to a treatment described for the preparation of **51**, compound **53** (2.88 g, 5.0 mmol, 98%) was obtained. ^{1}H-NMR ($CDCl_3$ + DABCO): 9.41 (br. s., 1H) N-H; 8.24 (*d*, $J_{H5,H6}$ = 7.4 Hz, 1H) H-6; 7.4 - 6.8 (*m*, 14H) DMTr + H-5; 6.30 (*m*, 1H) H-1'; 4.14 (*m*, 1H) H-4'; 3.80 (*s*, 6H) 2xOCH$_3$; 3.46 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 3.2 Hz) H-5'; 3.39 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 4.0 Hz) H-5"; 2.78 (*d*, $J_{H1',H2''}$ = 6.2 Hz) H2"; 2.18 (*s*, 3H) C(O)*CH*$_3$.

5'-O-DMTr-N^2-acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine-2'(R/S),3',5'(R/S)-$^2H_3$ (54A + 54B). Compound **19** (2.44 g, 5.66 mmol) was converted to compound **54** (4.14 g, 3.28 mmol, 58 %) by procedure described for **51**. ^{1}H-NMR ($CDCl_3$ + DABCO): 8.15 (br. s., 1H) N-H; 8.08 (*s*, 1H) H-8; 7.5 - 6.8 (*m*, 23H) DMTr + DPC; 6.41 (*m*, 1H) H-1'; 4.13 (*m*, 1H) H-4'; 3.73 (*s*, 6H) 2xOCH$_3$; 3.36 (*d*, $J_{H\text{-}4',H\text{-}5'}$ = 4.7 Hz) H-5'; 3.30 (*d*, $J_{H\text{-}4',H\text{-}5''}$ = 4.0 Hz) H-5"; 2.49 (*d*, $J_{H1',H2''}$ = 6.4 Hz) H-2"; 2.35 (*s*, 3H) N^2-Ac.

5'-O-DMTr-thymidine-2'(R/S),3',5'(R/S)-$^2H_3$ 3'-(2-(cyanoethyl)-*N,N*-diisopropylamino) phosphoramidite (55A + 55B). To compound **51** (0.76 g, 1.39 mmol) in dry DCM (12 ml), (2-cyanoethoxy)bis(*N,N*-diisipropylamino)phosphine (0.62 ml, 1.96 mmol) was added followed by N,N-diisopropylammonium tetrazolide (119 mg, 0.69 mmol) and stirring was maintained overnight. The reaction mixture was diluted with ethyl acetate, poured into sat. $NaHCO_3$ and extracted. After two additional washings with brine, the organic phase was dried over $MgSO_4$, filtered and evaporated. The residue was subjected to short column chromatography to give phosphoramidite **55** (0.86 g, 1.15 mmol, 83%) as a foam. ^{1}H-NMR ($CDCl_3$ + DABCO): 8.55 (*m*, 1H) N-H; 7.63 & 7.58 (2xbr. s., 1H) H-6; 7.6 - 6.8 (*m*, 13H) DMTr; 6.40 (*m*, 1H) H-1'; 4.17, 4.13 (2x*d*, 1H) H-4'; 3.79 (2x*s*, 6H) 2xOCH$_3$; 3.7 - 3.3 (*m*, 3H) -*CH*$_2$CH$_2$ CN and H-5'/5"; 2.5 - 2.4 (*m*, 3H)CH$_2$*CH*$_2$CN and H-2'/2"; 1.43 (*s*, 3H) 5-CH_3; 1.2 -1.1 (*m*, 14H) 2x isopropyl. ^{31}P-NMR: 148.87, 148.42. HR-MS (ES$^+$): $(M+Na)^+$ calc. for $C_{40}H_{46}D_3N_4O_8PNa$: 770.3374, found 770.3355.

5'-O-DMTr-N^6-benzoyl-2'-deoxyadenosine-2'(R/S),3',5'(R/S)-$^2H_3$ 3'-(2-(cyanoethyl)-*N,N*-diisopropylamino) phosphoramidite (56A + 56B). Compound **52** (0.92 g, 1.4 mmol) was phosphity-

lated as described for compound **55** to afford phosphoramidite **56** (0.66 g, 0.77 mmol, 55%). ^{1}H-NMR ($CDCl_3$ + DABCO): 9.1 (br. s., 1H) N-H; 8.74 (*s*, 1H) H-2; 8.21 & 8.19 (2x*s*, 1H) H-8; 8.1 - 6.7 (*m*, 18H) DMTr + benzoyl; 6.50 (*d*, $J_{H1',H2''}$ = 5.9 Hz, 1H) H-1'; 4.32 (*m*, 1H) H-4'; 3.9 - 3.3 (*m*, 3H) CH_2 CH_2CN, H-5'/5"; 3.77 (*s*, 6H) 2xOCH_3; 2.7 - 2.4 (*m*, 3H) CH_2CH_2 CN, H-2'/2"; 1.3 - 1.1 (*m*, 14H) 2x isopropyl. ^{31}P-NMR: 149.48, 149.36. HR-MS (ES$^+$): $(M+Na)^+$ calc. for $C_{47}H_{49}D_3N_7O_7PNa$: 861.3932, found 861.3963.

5'-O-DMTr-N^4-acetyl-2'-deoxycytidine-2'(R/S),3',5'(R/S)-$^2H_3$ 3'-(2-(cyanoethyl)-*N*,*N*-diisopropylamino) phosphoramidite (57A + 57B). Compound **53** (1.44 g, 2.5 mmol) were converted to compound **57** (1.66 g, 2.14 mmol, 86%) according to the procedure used for preparation of phosphoramidite **55**. ^{1}H-NMR ($CDCl_3$ + DABCO): 8.29 and 8.20 (2x*d*, $J_{H5,H6}$ = 7.7 Hz, 1H) H-6; 7.5 - 6.8 (*m*, 19H) benzoyl, H-5 and DMTr; 6.25 (*m*, 1H) H-1'; 4.20 (*m*, 1H) H-4'; 3.80 (2x*s*, 6H) 2xOCH_3; 3.8 - 3.3 (*m*, 3H) CH_2CH_2 CN, H-5'/5"; 2.8 - 2.4 (*m*, 3H) CH_2CH_2CN and H-2'/2"; 2.25 (s) C(O)CH_3; 1.2 - 1.1 (*m*, 14H) 2x isopropyl. ^{31}P-NMR: 149.17, 148.56. HR-MS (ES$^+$): $(M+Na)^+$ calc. for $C_{41}H_{47}D_3N_5O_8PNa$: 797.3483, found 797.3503.

5'-O-DMTr-N^2-acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine-2'(R/S),3',5'(R/S)-$^2H_3$ 3'-(2-(cyanoethyl)-*N*,*N*-diisopropylamino) phosphoramidite (58A + 58B). Compounds **54** (2.01 g, 2.5 mmol) were treated as described for compound **55** to obtain compound **58** (2.18 g, 2.17 mmol, 87%). ^{1}H-NMR ($CDCl_3$ + DABCO): 8.12 & 8.10 (2x*s*, 1H) H-8; 7.96 & 7.89 (2xbr. s., 1H) N-H; 7.5 - 6.7 (*m*, 23H) DMTr + phenyls; 6.37 (*d*, $J_{H1',H2''}$ = 5.9 Hz, 1H) H-1'; 4.30 & 4.27 (2x*d*, 1H) H-4'; 3.9 - 3.3 (*m*, 3H) CH_2 CH_2CN and H-5'/5"; 3.75 (2x*s*, 6H) 2xOCH_3; 2.7 - 2.4 (*m*, 6H) CH_2CH_2 CN, H-2'/2", N^2-Ac; 1.2 - 1.1 (*m*, 14H) 2x isopropyl. ^{31}P-NMR: 148.91, 148.73. HR-MS (ES$^+$): $(M+Na)^+$ calc. for $C_{55}H_{56}D_3N_8O_9PNa$: 1032.4229, found 1032.4237.

DNA Synthesis and Purification. The deuterium labelled 12-mer (**I**) was prepared by the solid phase phosphoramidite method on a Pharmacia LKB Gene Assembler Special synthesiser. The standard programs for 1.3 μmol scale synthesis from Pharmacia were modified to have 2 min. coupling time. After deprotection in 32% aqueous ammonia solution for 7 days at RT, the solvent was evaporated. The residue was partitioned between water and DCM and finally diethyl ether. Purification was carried out by preparative ion exchange HPLC (Gilson system: Model 305 & 306 Pumps, 811C Dynamic Mixer and 118 UV Detector) on a Millipore Protein Pak™ Q 15HR 1000Å 8μm column (10x100 mm) at pH = 12 with a linear gradient of 45% → 60% buffer B (1.0 M NaCl in 0.01 M NaOH) in buffer A (0.01 M NaOH) over a period of 40 min applying 50 o.d. units of crude DNA per injection. Appropriate peaks were collected, concentrated and desalted on a Sephadex G 25 gel filtration column. Finally, the purified sample [379 o.d. units, 19 %] was lyophilised together with the appropriate buffer used for NMR spectroscopy from D_2O (99.9 % D atom).

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