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Diastereospecific Chemical Synthesis of Ribonucleosides-3',4',5',5"-d_4

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Abstract: The diastereospecific chemical syntheses of uridine-3',4',5',5"-d_4 , cytidine-3',4',5',5"-d_4 , adenosine-3',4',5',5"-d_4 and guanosine-3',4',5',5"-d_4 (>97 atom % ^{2}H at C3', C4' and C5') have been achieved by condensation of appropriately protected aglycone with 1-*O*-acetyl 2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-3,4,5,5'-d_4 (**27**), which has been obtained in an overall yield of 20 % in 11 steps starting from 50 mmol of 2:5,6-Di-*O*-isopropylidene-α-D-glucose.

Introduction

One of the major problems of the structure elucidation of functional oligo-RNA by NMR is the severe overlap of the sugar proton resonances, which normally appear within a very small stretch between 6.5-4.0 ppm.[1] In order to remedy this problem, we have earlier advocated the Uppsala "NMR-window" concept,[2] in which partially-deuterated sugar residues are non-uniformly incorporated into either oligo-DNA or -RNA for simplification of spectral crowding[2c,e,f,h,13] and coupling patterns,[2c,e,f,h,13] increasing NOE intensities,[2d,13] probing dynamics by selective T_1 and T_2 measurements,[2i,j] reducing the line-broadening[2d] associated with ^{1}H dipolar relaxation as well as the spin diffusion.[3,13] Segmental ^{2}H labelling (with or without ^{13}C enriched blocks)[2j,k] at a specific site in an oligonucleotide molecule ("NMR-window") can be easily achieved[2,4] today in a non-uniform manner using chemospecifically synthesised deuterated nucleoside blocks followed by the solid phase synthesis protocol in an automated manner. These ^{2}H/^{13}C labelled blocks have also found to be useful for the enzymatic synthesis[5] to give large RNA and DNA molecules, producing both uniformly[17] and non-uniformly[38] labelled oligo-DNA and -RNA for solution structure elucidation by isotope-edited multidimensional NMR.

In a recent study, Tolbert and Williamson[5b] have developed a combination of chemical and enzymatic syntheses of D-ribonucleoside-5'-triphosphates-3,4,5,5'-d_4 . In this procedure, a mixture of D-ribose-3,4,5,5'-d_4 and L-lyxose-3,4,5,5'-d_4 was chemically synthesised starting from glycerol-d_8 in 24% yield in 10 steps (D-ribose-d_4 in 12% yield), which was subsequently used for enzymatic synthesis of 3',4',5',5"-d_4 -ribonucleoside triphosphates (75-90 % calculated on the estimated D-ribose-d_4 content). These labelled triphosphates, obtained in 213-495 μmol scale, were then uniformly incorporated into HIV-2 TAR 30mer RNA by *in vitro* transcription with deuterated triphosphates by T7 RNA polymerase. The resulting partially-deuterated RNA showed the following NMR features: (1) The spectral crowding in the ribose region of the deuterated RNA was dramatically reduced leaving only H2' signals observed between 4 and 5 ppm. (2) The H1'-H2' nOe cross-

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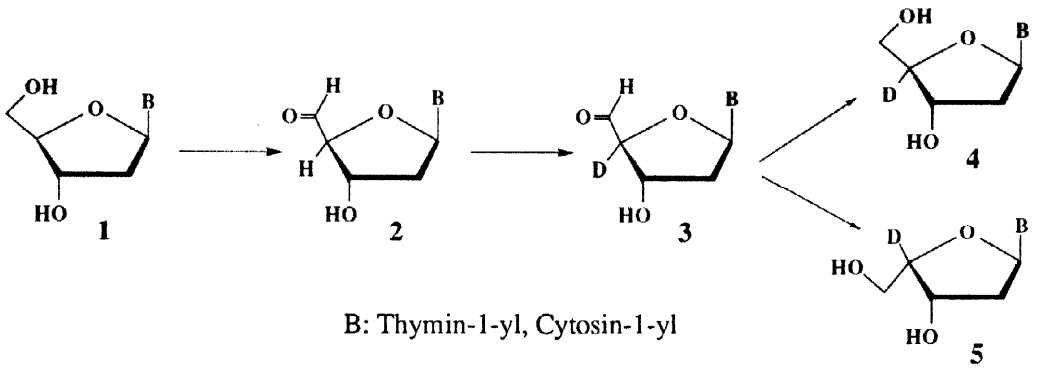
peaks could unambiguously be identified. (3) The effective T_1 and T_2 relaxation times for the d_4-RNA were increased by a factor of 2 compared to unlabelled RNA in accordance with our earlier findings.[2d] (4) It was suggested that this 50% reduction of the relaxation efficiency could significantly improve the NOESY spectra of larger RNAs.

It was clear to us that the usefulness of these ribonucleosides-3',4',5',5"-d_4 in the NMR structure elucidation of large RNA molecules as well as in the mechanistic studies[6] would increase immensely if one could find a convenient route for their diastereospecific synthesis in a large scale with a high overall yield. This would facilitate the preparation of the corresponding phosphiteamidites or H-phosphonates for incorporation of the deuterium labelled nucleotides at specific sites of an oligo-RNA molecule by the solid-phase synthesis.[39] In this paper, we report on a large scale chemical synthesis of the 3',4',5',5"-deuterated analogues (>97 atom % ^{2}H) of all four natural D-ribonucleosides, which potentially can be scaled up rather easily, and can be used for non-uniform deuterium labelling in our "NMR-window" approach.[2]

Results & Discussion

Several procedures have been so far reported in the literature[2,5b,6a-15,25,36,37] for incorporation of deuterium in a chemoselective or chemospecific manner. More than 95 atom % ^{2}H incorporation has been accomplished at C3' of adenosine with virtually complete stereoselectivity upon reduction of the 2'-*O*-tert-butyldimethysilyl-3'-ketonucleoside by sodium borodeuteride in acetic acid,[7] but this method was not extended to other nucleosides. Deuterium was incorporated at C3' (97 atom % ^{2}H) of adenosine upon stereoselective reduction of 1,2:5,6-di-*O*-isopropylidene-α-D-hexofuranos-3-ulose to 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose-3-d_1 (**16**)[6a] using sodium borodeuteride. In a recent study 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-pentafuranose-3-ulose was reduced stereoselectively to the 3-deuterated ribose derivative which was subsequently converted to thymidine-3-d_1 and its ribo counterpart.[8] 4',5'-Unsaturated pyrimidine nucleosides could be reduced by B_2D_6/THF followed by oxidation by H_2O_2/OH^- to give isomeric β-D-ribofuranosyl and α-L-lyxofuranosyl pyrimidine nucleoside in 1:3 ratio.[9] Intramolecular hydrogen atom abstraction was used for relatively high level of deuterium incorporation (70 atom % ^{2}H) upon treatment of 5'-*O*-benzyl-3'-*O*-(1-bromo-2-methyl-2-propyl)-thymidine by Bu_3SnD.[14] The attempts to introduce deuterium to the 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose *via* bromination of C4 by Br_2/CCl_4, *hv*, followed by reduction with Bu_3SnD gave D-ribo and L-lyxo epimers in 1:4 ratio.[15] The deuterium incorporation at C4 of a 2,3,5-*O*-protected D-ribose derivative was obtained by $LiAlD_4$ reduction of (3*S*,4*S*)-1-*O*-[1,1-dimethylethyl)dimethylsilyl]-2-hydroxy-6-methyl-3,4-*O*-(1-methylethylidene)-hept-5-en-4-one, followed by separation of isomers, ozonolysis and reductive workup.[15] However it gave an unfavourable ratio of α- and β-cytidine in the coupling reaction. Equilibration of 4-formyl nucleoside derivatives, such as **2**, under base-catalysed conditions in D_2O were also exploited for the deuterium incorporation at C4' (as in **3**) (Scheme 1). Thus, the 4'-formyl derivative of 2'-deoxycytidine (dC) or thymidine (T) in D_2O/pyridine at elevated temperatures[11] followed by reduction with $NaBH_4$ gave dC-4-d_1 -and T-4-d_1 (as in **4**) in good yields. During this exchange process, the α-L-lyxo by-product **5** was also formed, requiring HPLC purification. Additionally, this exchange process at the nucleoside level was found to be rather restricted because the deuteration of C4' of purine nucleosides was not successful. A similar procedure for 4'-deuteration of 2'-deoxy-4'-thionucleosides *via* equilibration of the 4'-sulfoxide gave even more α-L-lyxo by-product.[36] On the other hand, the deuteration of methyl thymidine-5'-carboxylate upon



B: Thymin-1-yl, Cytosin-1-yl

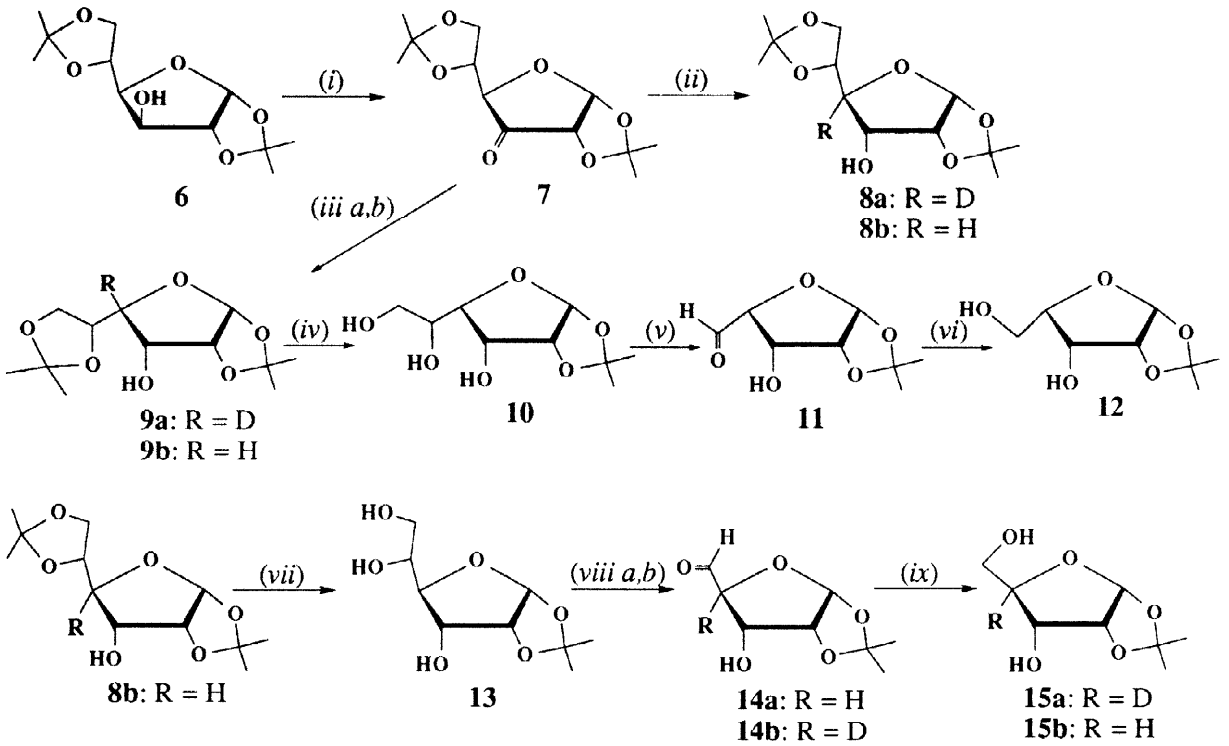
Scheme 1

proton abstraction from C4' by a mixture of lithium diisopropylamide and *n*-butyllithium at -78 °C followed by quenching with D_2O/AcOD gave 4'-deuterothymidine in an overall yield of 16 % with no detectable epimerisation.[37]

At the sugar level, the deuterium incorporation at C4 was achieved using the ability of the 1,2:5,6-di-*O*-isopropylidene-α-D-hexofuranos-3-ulose (**7**) to undergo keto-enol tautomerism (Scheme 2). Three groups of workers reported >95 atom % deuteration at C4 with undefined chiral purity by warming a solution of **7** in pyridine/D_2O (5:1 or 1:1, v/v) at 95 °C for 5 min, followed by stirring at r.t. for 18h, and repeating this sequence three times, followed by reduction[9,12,25] with $NaBH_4$. In our hands, this base catalysed equilibration of **7** in pyridine/D_2O (1:1, v/v) solution under the literature condition[9,12,25] gave 1,2:5,6-di-*O*-isopropylide-α-D-gulose-4-d_1 (**9a**) in ~12 % yield as a by-product as identified after reduction followed by a comparison with an authentic material.[26,27] In order to obtain >97 atom % ^2H labelling at C4, it required 5 cycles of reaction sequence in our hands for obtaining 1,2:5,6-di-*O*-isopropylidene-α-D-allose-4-d_1 (**8a**) in 53 % yield.

It was clear to us from the above results that it is perhaps difficult to introduce deuterium at C4' at the nucleoside level. Hence, we decided to deuterate the 3, 4, 5, 5' positions at the sugar level (see Scheme 3) in order to obtain the target 3',4',5',5''-d_4 -D-ribonucleosides (**29a-d**): 1,2:5,6-Di-*O*-isopropylidene-α-D-allose-3-d_1 **16** was obtained in two steps (**6**→**16**, overall yield of 79 %) by oxidation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucose[19] **6** by pyridinium dichromate/acetic anhydride in boiling dry dichloromethane[20] to the corresponding 3-ketone **7** (85 %), followed by stereoselective reduction by $LiAlD_4$ in dry diethyl ether to give allose derivative **16** (91 %).[6a,13] Simplification of the multiplicities of resonances corresponding to H-2 [δ 4.61 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 3.7 Hz)] and H-4 [δ 3.82 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 4.7 Hz)] in the ^1H-NMR spectrum, and appearance of a triplet at 72 ppm in the ^{13}C-NMR spectrum corresponding to deuterium coupled C3 ($^1J_{CD} \approx$ 22 Hz), clearly show that the incorporation of deuterium at C3 in **16** has indeed taken place. The 3-hydroxyl group of **16** was protected in the form of 3-*O*-benzyl (Bn) derivative **17** (88 %) in order to secure its stability under an oxidative/reductive reaction condition in a latter part of the transformation (Scheme 3). Deprotection of the 5,6-*O*-isopropylidene group by 80 % aqueous acetic acid at r.t. overnight gave **18** (99 %), which was oxidised to the corresponding 4-formyl derivative **19** by an aqueous ethanolic solution of $NaIO_4$.[21]

Since we found that the epimerization of ketone **7** had taken place during the deuteration at C4, we examined if the C4 epimerisation also takes place during the deuterium exchange at C4 of the 4-formyl 1,2-*O*-isopropylidene ribose (**14a**) (Scheme 2). Thus **14a** was subjected to a treatment in pyridine/D_2O solution (1:1, v/v) at 50 °C for 22 days for deuterium incorporation at C4. The resulting deuterated 4-formyl derivative **14b**



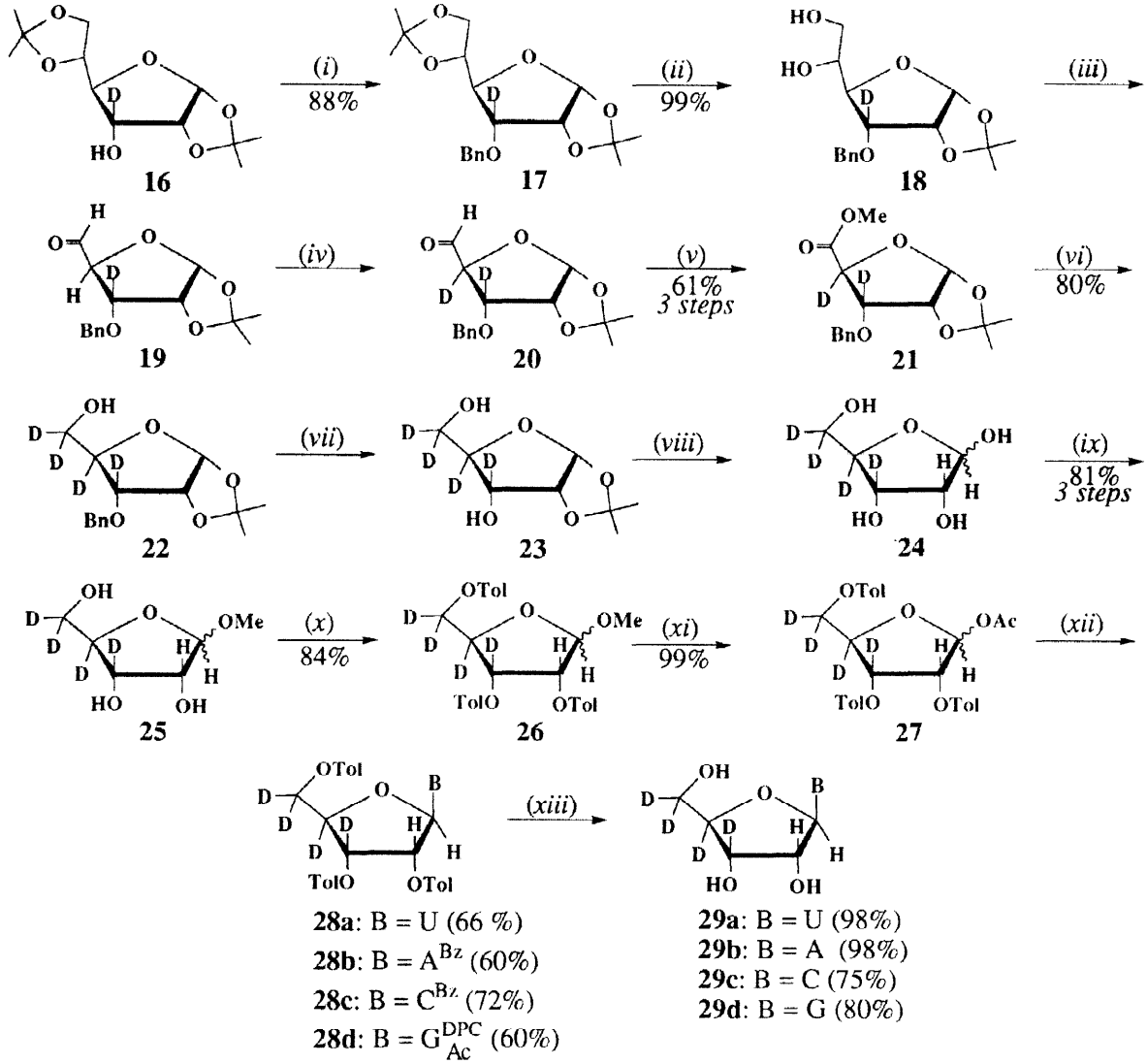
Conditions: *i*. PDC, acetic anhydride, dry DCM, reflux, 3 h.; *ii*. $LiAlD_4$ in dry diethyl ether or $NaBH_4$ in ethanol, r.t; *iii*. (a) pyridine/D_2O (1:1,v/v) at 95 °C, 5 min, additional stirring at r.t. for 18 h, followed by reduction with $LiAlD_4$ in dry diethyl ether or (b) acetic anhydride in dry pyridine, heating, followed by reduction with $NaBH_4$ in ethanol; *iv*. 80% aqueous acetic acid, r.t; *v*. $NaIO_4$, ethanol/H_2O (1:1), r.t, 3.5 h.; *vi*. $NaBH_4$ in ethanol, r.t; *vii*. 80% aqueous acetic acid, r.t, overnight; *viii*. (a) $NaIO_4$, ethanol/H_2O (1:1), r.t, 3.5 h. and (b) pyridine/D_2O (1:1), 50 °C, 22 d.; *ix*. $LiAlD_4$, dry diethyl ether or $NaBH_4$ in ethanol, r.t.

Scheme 2

was then reduced. An aqueous workup followed by an extraction with dichloromethane, and concentration of the dichloromethane phase gave chromatographically homogeneous 1,2-*O*-isopropylidene-β-D-ribose-4-d_1 (**15a**) in 77 % yield with >97 atom % deuteration. In order to prove that compound **14b** or **15a** was free of any isomeric product, we also subjected 4-formyl 1,2-*O*-isopropylidene-β-D-ribose (**14a**) to the exchange reaction in pyridine/H_2O solution under an identical condition as used for the preparation of **15a** to give 1,2-*O*-isopropylidene-β-D-ribose **15b** after reduction with $NaBH_4$. A comparison of **15b** with the corresponding isomeric α-L-lyxose derivative **12**, prepared *via* an independent way from **9b**[26] through **10** and **11** (Scheme 2) also showed that **15b** was free of the isomeric product by ^1H- and ^{13}C-NMR spectroscopy,[40] thereby suggesting that no epimerisation had taken place during base catalysed exchange at C4.

Based on the above findings, compound **19** was then deuterated at C4 using the equilibration in pyridine/D_2O (1:1, v/v) at 50 °C for 22 days to afford 3,4-deuterated sugar derivative **20**.

In order to introduce deuterium at the 5'/5" positions to prepare our target 3,4,5,5'-d_4 -D-ribonucleosides (**29a-d**), we attempted to reduce either 3-*O*-benzyl-1,2-*O*-isopropylidene-5-ribofuranosiduronic acid[16] or methyl 1,2-*O*-isopropylidene-β-D-ribofuranosiduronate. Unfortunately, the reduction of 3-*O*-benzyl-1,2-*O*-isopropylidene ribofurouronic acid by $NaBD_4$ or $LiAlD_4$ to the corresponding alcohol did not work well in our hands, hence the 3,4-deuterated aldehyde **20** was converted to the corresponding 4-carboxy methyl ester[18] **21** (61%) by oxidation with bromine in 10% aqueous methanol using sodium bicarbonate as a buffer.[22] Compound



Abbreviations: Bn = benzyl, Tol = 4-toluoyl, Ac = acetyl, Me = methyl, Bz = benzoyl, DPC = diphenylcarbamoyl, G = guanin-9-yl, U = uracil-1-yl, A = adenin-9-yl, C = cytosin-1-yl. *Conditions*: *i.* BnBr, NaH, dry acetonitrile, r.t, overnight; *ii.* 80% aqueous acetic acid, r.t, overnight; *iii.* $NaIO_4$, ethanol/H_2O (1:1, v/v), r.t, 3.5 h.; *iv.* pyridine/D_2O (1:1,v/v), 50 °C, 17 d.; *v.* bromine in methanol/H_2O/$NaHCO_3$, r.t, 4 h.; *vi.* $LiAlD_4$, dry diethyl ether, r.t, 4 h.; *vii.* 10% Pd/charcoal, H_2, ethanol, r.t, overnight; *viii.* 80% aqueous acetic acid, 80 °C, 1 d.; *ix.* methanol, conc H_2SO_4, 4 °C, overnight; *x.* 4-toluoyl chloride, dry pyridine, r.t, overnight; *xi.* acetic anhydride, acetic acid, conc H_2SO_4, dry DCM, 0 °C, 12 min; *xii.* silylated nucleobase, TMS-triflate in dry EDC or toluene, at elevated temperature; *xiii.* methanolic ammonia, r.t, overnight.

Scheme 3

21 was then reduced[18] by $LiAlD_4$ in dry ether giving the ribose-3,4,5,5'-d_4 derivative **22** (81%). The deuterium incorporation at C3, C4 and C5 of **22** was clearly evident from its ^{1}H-NMR spectrum in which only sugar resonances for H1 [δ 5.72 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 3.7 Hz)] and H2 [δ 4.56 (*d*, H2)] were visible with the disappearance of all other signals of the ribose protons. The ^{13}C-NMR experiments with both proton and deuterium decoupling showed the appropriate singlets for C4, C3 and C5 at δ78.1, 76.0 and 59.6, respectively. Upon proton decoupling only (*i.e.* deuterium coupled), we could observe the expected triplet resonances for methine C4 at δ78.1 ($^1J_{CD} \approx$ 22 Hz) and C3 at δ76.0 as well as a multiplet for methylene C5 at δ59.6. Finally, the optical rotation of **22** was found to be very similar to the non-deuterated counterpart showing that the

configurations of their chiral centers are identical: {$[\alpha]_D^{27}$ for **22**: +128 (c 1.2, $CHCl_3$); the $[\alpha]_D^{27}$ for the non-deuterated counterpart: +127 (c 1.2, $CHCl_3$)}.

The feasibility of achieving a deuterium incorporation at C4 without protecting the 3-OH was also investigated. In this effort, the C3-deuterio derivative of **14** was subjected to the exchange reaction in pyridine/D_2O as described above to afford methyl 1,2-*O*-isopropylidene-α-D-ribofuranuronate-3,4-d_2 (60%) after the bromine oxidation step. This compound, however, could not be reduced successfully by $LiAlD_4$.

Compound **22** was then directly converted to the methyl D-ribofuranoside-3,4,5,5'-d_4 (**25**) in an overall yield of 81 % in the following manner: the 3-*O*-benzyl protecting group of **22** was cleaved by catalytic hydrogenation over Pd/charcoal in dry ethanol;[23] the 1,2-*O*-isopropylidene group was then removed by treatment with 80% aqueous acetic acid at 80 °C overnight, followed by a glycosylation reaction in dry methanol in the presence of catalytic amount of conc H_2SO_4 to give methyl α,β-D-ribofuranoside-3,4,5,5'-d_4 (**25**).

Compound **25** was protected by using 4-toluoyl chloride in dry pyridine[2a] to give tri-*O*-(4-toluoyl) methyl glycoside **26** (86 %), which was subsequently converted to 1-*O*-acetyl derivative **27** (99 %) by keeping a solution of **26** in dry dichloromethane in presence of acetic acid, acetic anhydride and conc H_2SO_4 at 4 °C for 16 h. The β-anomer of 1-*O*-acetyl 2,3,5-tri-*O*-(4-toluoyl)-β-D-ribofuranoside-3,4,5,5'-d_4 (**27**) was crystallised from methanol (see experimental). A 500 MHz ^{1}H-NMR spectrum of this compound (Fig. 1A) showed only two singlets [δ 6.41, H1) and δ 5.75 (H1)] in the sugar region. A comparison of the NMR spectrum of **27** with its natural counterpart (Fig. 1B) also showed that indeed the substitution of C3, C4 and C5 centers with deuterium (>97 atom % ^{2}H at C3, C4 and C5) has taken place satisfactorily in the former. The ^{13}C-NMR experiments with both proton and deuterium decoupling showed singlets for C4, C3 and C5 at δ79.4, 70.7 and 62.8, respectively, whereas in the proton decoupled ^{13}C spectra (*i.e.* deuterium coupled), we could observe the expected triplet resonance for methine C4 at δ79.4 ($^1J_{CD} \approx 22$ Hz) and C3 at δ70.7 as well as a multiplet for methylene C5 at δ62.8 ppm. The final identity of this β-D-ribofuranoside-3,4,5,5'-d_4 **27** was established by comparing its specific rotation {$[\alpha]_D^{27}$ for β-anomer **27**: +60 (c 1, $CHCl_3$)} to that of the authentic counterpart[34] {$[\alpha]_D^{28}$: +63 (c 1, $CHCl_3$)}.

The fully protected nucleosides **28a** (66 %), **28b** (60 %), **28c** (72 %) and **28d** (60 %) were finally obtained by condensation of 1-*O*-acetyl 2,3,5-tri-*O*-(4-toluoyl)-β-D-ribofuranoside-3,4,5,5'-d_4 (**27**) with persilylated uracil (U), N^6-benzoyladenine (A^{Bz}), N^4-benzoylcytosine (C^{Bz}) and O^6-diphenylcarbamoyl-N^2-acetylguanine (G_{Ac}^{DPC}) in dry 1,2-dichloroethane (dry toluene for G)[24] applying trimethylsilyl trifluoromethanesulfonate as a catalyst using modified literature procedures.[28] That no undesirable deuterium exchange has taken place during the glycosylation step is again evident by comparison of the protected **28a**, **28b**, **28c** and **28d** with the corresponding natural counterparts (compare Figs. 1C with 1D, 1E with 1F, 2A with 2B and 2C with 2D).

The protecting groups were subsequently removed by an overnight treatment with saturated methanolic ammonia to afford nucleosides-3',4',5',5"-d_4 **29a**-**29d** in 98, 98, 75, 80% yields, respectively. Purity and deuteration level (>97 atom % ^{2}H at C3, C4 and C5) of the compounds were found to be satisfactory by comparison of the non-deuterated counterparts in a pairwise manner by ^{1}H- and ^{13}C-NMR (^{1}H at 500 MHz) (Figs. 2E/F, 3A/B, 3C/D and 3E/F), high resolution mass spectroscopy, infrared sectroscopy as well as by optical rotation measurements (see experimental section for details).

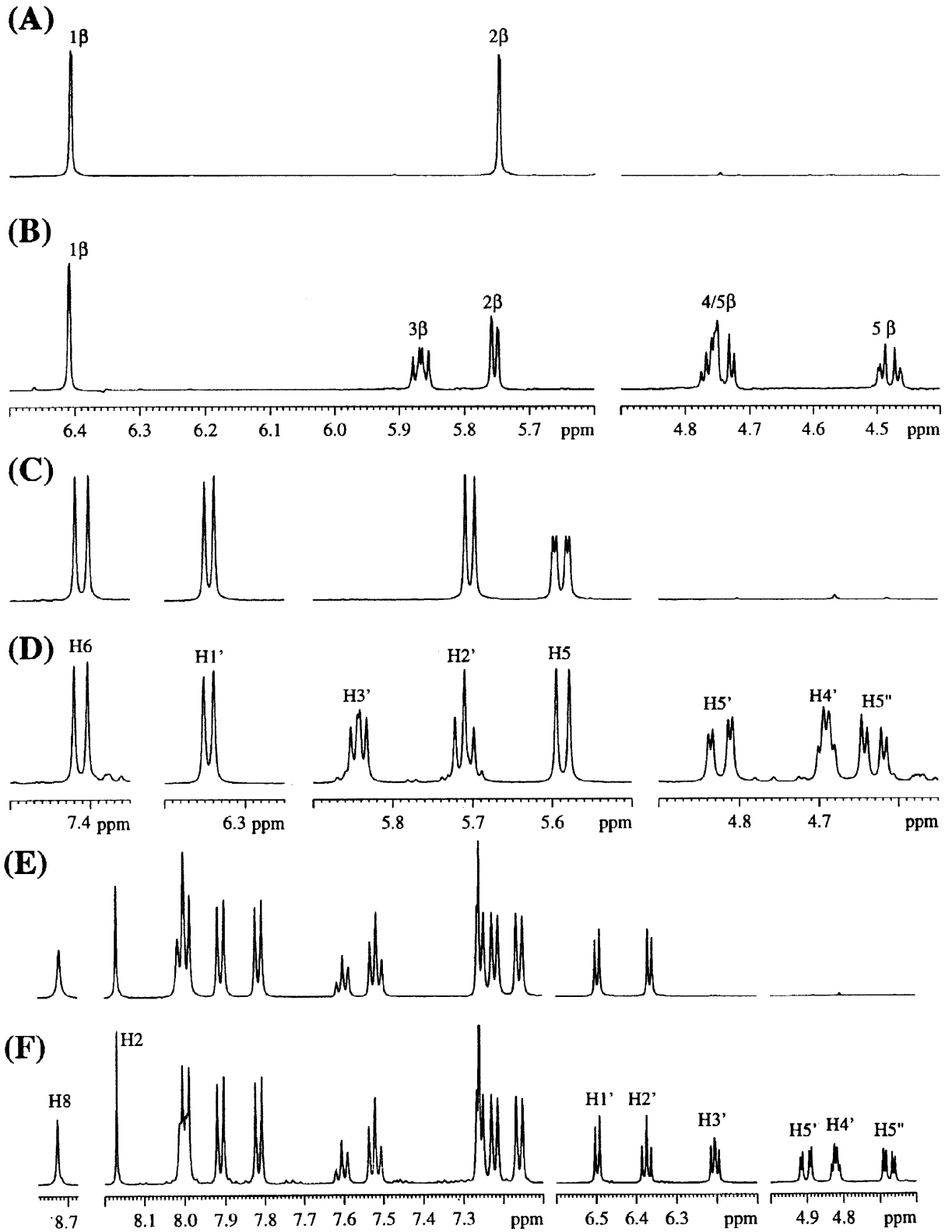


Figure 1: The sugar region of the 500 MHz ^{1}H-NMR spectra of 1-*O*-acetyl-2,3,5-tri-*O*-(4-toluoyl)-β-D-ribofuranose-3,4,5,5'-d_4 (**27**) (Panel A) and its natural-abundance counterpart (Panel B). Aromatic and the sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated β-D-nucleoside derivatives and their natural-abundance counterparts: 2',3',5'-tri-*O*-(4-toluoyl)-uridine-3',4',5',5"-d_4 (**28a**) (Panel C) and its natural-abundance counterpart (Panel D); 2',3',5'-tri-*O*-(4-toluoyl)-N^6-benzoyladenosine-3',4',5',5"-d_4 (**28b**) (Panel E) and its natural-abundance counterpart (Panel F).

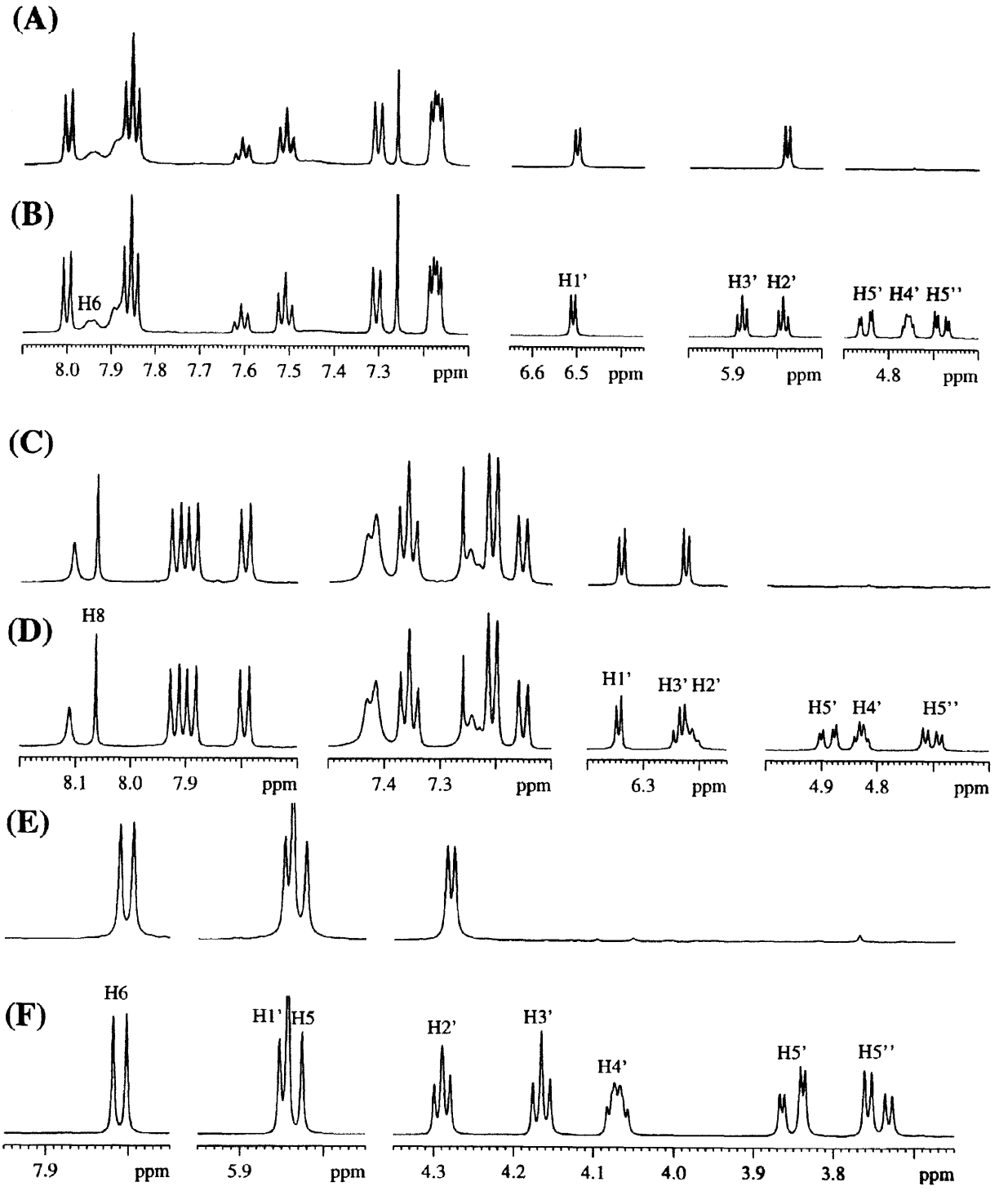


Figure 2: Aromatic and the sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated β-D-nucleoside derivatives and their natural-abundance counterparts: 2',3',5'-tri-*O*-(4-toluoyl)-N^4-benzoylcytidine-3',4',5',5"-d_4 (**28c**) (Panel A) and its natural-abundance counterpart (Panel B); 2',3',5'-tri-*O*-(4-toluoyl)-N^2-acetyl-O^6-diphenylcarbamoylguanosine-3',4',5',5"-d_4 (**28d**) (Panel C) and its natural-abundance counterpart (Panel D). Aromatic and the sugar regions of the 500 MHz ^{1}H-NMR spectra of uridine-3',4',5',5"-d_4 (**29a**) (Panel E) and its natural-abundance counterpart (Panel F).

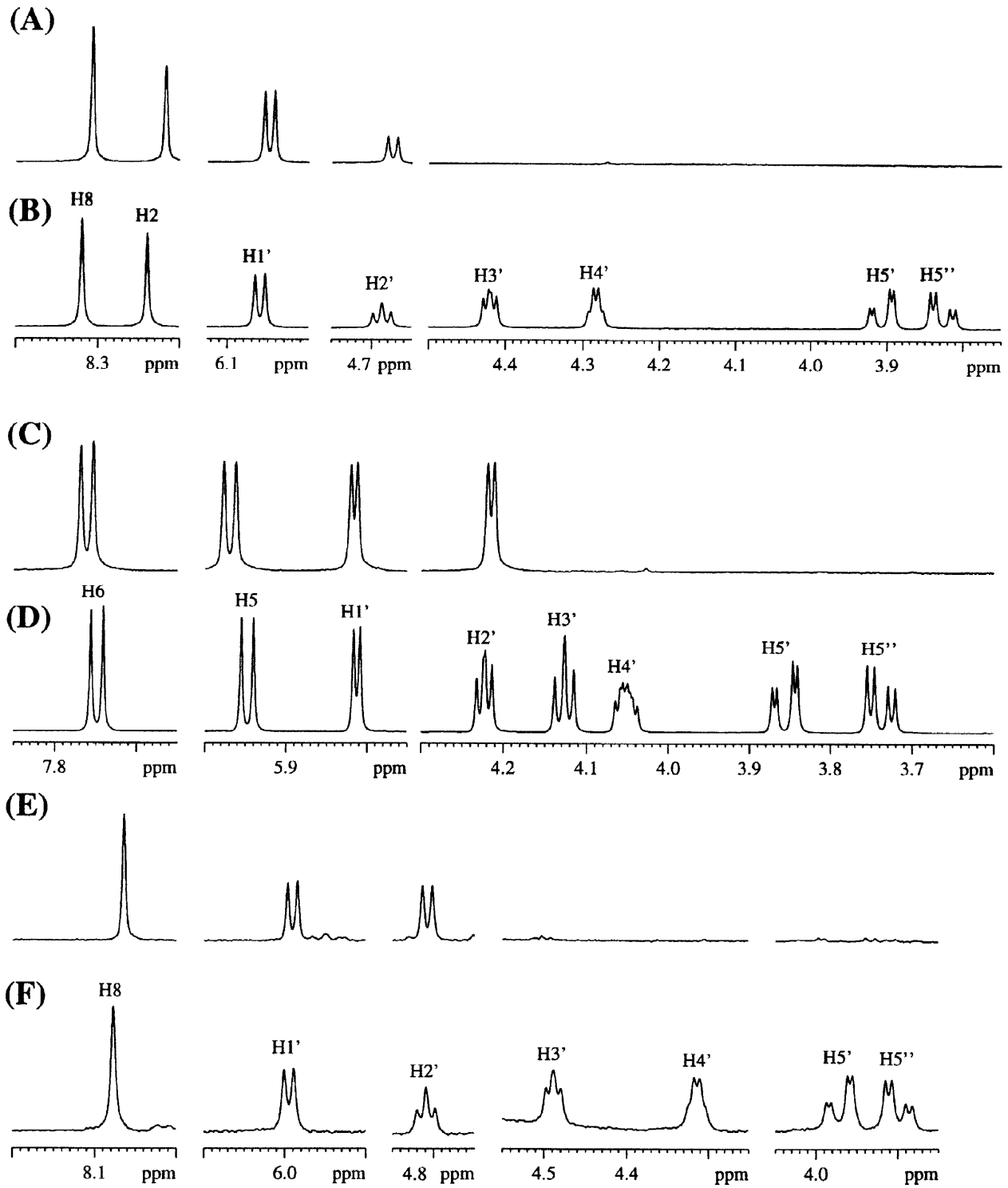


Figure 3: Aromatic and the sugar regions of the 500 MHz ^{1}H-NMR spectra of deuterated β-D-nucleosides and their natural-abundance counterparts: adenosine-3',4',5',5"-d_4 (**29b**) (Panel A) and its natural-abundance counterpart (Panel B); cytidine-3',4',5',5"-d_4 (**29c**) (Panel C) and its natural-abundance counterpart (Panel D); guanosine-3',4',5',5"-d_4 (**29d**) (Panel E) and its natural-abundance counterpart (Panel F).

Conclusions

We have herein reported on the chemical synthesis of C3', C4' and C5' deuterated ribonucleosides (>97 atom % ^2H), which can be potentially used for the preparation of non-uniformly labelled RNA oligomers using standard solid-phase chemistry. The report contains a diastereospecific method for the deuterium-proton exchange at the C4 of D-ribose derivative. 1-*O*-acetyl-2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-3,4,5,5'-d_4 (**27**), obtained by 11-step synthesis starting from D-glucose with an overall yield of 20 %, has been subsequently used for the chemical synthesis of partially deuterated nucleosides in 60-75 % yield. Clearly, the main advantage of the present synthetic route is that it can be easily scaled-up for large scale production of appropriately protected nucleosides-3',4',5',5''-d_4 for any RNA synthesis for structural studies using our "NMR-window" concept.

Experimental Section

Pyridine was distilled after refluxing with 4-tosyl chloride (first distillation) and calcium hydride (second distillation). Dichloromethane (DCM), 1,2-dichloroethane and acetonitrile were stirred with phosphorus pentoxide overnight and distilled in nitrogen atmosphere. Toluene was refluxed with calcium hydride followed by distillation. The chromatographic separations were performed on Merck G60 silica gel. Thin layer chromatography (TLC) was performed on Merck pre-coated silica gel 60 F_{254} glass backed plates developed in following systems: (A) methanol-DCM (5:95, v/v), (B) ethyl acetate-cyclohexane (1:1, v/v), (C) ethyl acetate-cyclohexane (70:30, v/v), (D) ethyl acetate-propanol-water (30:18:6, v/v/v), (E) ethyl acetate. ^1H-NMR spectra were recorded with Jeol GX 270 (if nothing else is indicated) and Bruker DRX 500 spectrometers at 270 and 500 MHz, using TMS (0.0 ppm) or acetonitrile (for D_2O solutions, 2.0 ppm) peaks as internal standards. ^{13}C-NMR spectra were recorded with Jeol GX 270 spectrometer at 67.9 MHz using central peak of $CDCl_3$ (76.9 ppm) as internal standard or CH_3CN (δ = 1.3 ppm) as a reference for D_2O solutions. Chemical shifts are reported in ppm (δ scale). Deuterium decoupled ^{13}C-NMR experiments were done on a Bruker DRX 500 MHz spectrometer.[2i] Electrospray ionisation (EI^+) mass spectra were taken with LCT™ oa-TOF mass spectrometer (Micromass, Manchester, UK). Fast-atom bombardment (FAB) mass spectra were obtained on a VG-7070 MS mass spectrometer (VG. Analytical Ltd., Manchester, UK). Optical rotation data were measured on Perkin-Elmer 241 polarimeter. Infrared spectra were recorded with a Perkin-Elmer 298 spectrometer.

1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose-3-d_1 (16). Pyridinium dichromate (18.8 g, 50.5 mmol) was suspended in dry DCM (172 ml) and acetic anhydride (15.2 ml, 151.9 mmol) was added to the suspension. A DCM solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucose (13.01 g, 50 mmol) was then added. Reaction mixture was boiled at ~ 78 °C for 3 h, then it was diluted with ethyl acetate. Precipitate was filtered through a silica gel column, using ethyl acetate as eluent. The solvent was evaporated and the residue was coevaporated with toluene to give an oily product (11.06 g, 42.8 mmol, 86 %). ^1H-NMR ($CDCl_3$): 6.14 (*d*, $J_{H-1,H-2}$ = 4.5 Hz, 1H) H-1; 4.40-4.31 (*m*, 3H) H-2,4,5; 4.04-4.02 (*m*, 2H) H-6,6'; 1.46, 1.43, 1.34 (3x*s*, 12H) 4xCH_3. ^{13}C-NMR ($CDCl_3$): 208.8 (C=O); 114.3 (1,2-O-*C*[CH_3]$_2$); 110.4 (6,5-O-*C*[CH_3]$_2$); 103.2 (C-1); 79.0 (C-4); 77.4 (C-2); 76.4 (C-5); 64.3 (C-6) ; 27.9, 27.2, 26.0, 25.3 (4xCH_3). This product was dissolved in dry diethyl ether (110 ml) and $LiAlD_4$ (98 atom % ^2H, 0.9 g, 21.4 mmol) was added at 0 °C and stirred at r.t. for 2 h. Water was added and the mixture was extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and evaporated to give compound **16** as a white solid (10.26 g, 39.27 mmol, 92 %). R_f: 0.48 (System A). $[\alpha]_D^{26}$ +36 (c 1.5, $CHCl_3$). IR ν_{max}($CDCl_3$): 3556, 2988, 2934, 2882, 1452, 1382, 1373, 1249, 1216, 1162, 1070, 1009 cm^{-1}. ^1H-NMR[6a] ($CDCl_3$): 5.82 (*d*, $J_{H-1,H-2}$ = 3.7 Hz, 1H) H-1; 4.61 (*d*, 1H) H-2; 4.35-4.28 (*m*, 1H) H-5; 4.11-3.99 (*m*, 2H) H-6,6'; 3.82 (*d*, $J_{H-4,H-5}$ = 4.7 Hz, 1H) H-4; 2.52 (br.*s*, 1H) OH; 1.58, 1.47, 1.38 (3x*s*, 12H) 4xCH_3. ^{13}C-NMR[29,13a] ($CDCl_3$):112.8 (1,2-O-*C*[CH_3]$_2$); 109.8 (6,5-O-*C*[CH_3]$_2$); 103.9 (C-1); 79.6 (C-4); 79.0 (C-2); 75.5 (C-5); 65.7 (C-6); 26.6, 26.5, 26.3 (4xCH_3).

3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose-3-*d*$_1$ (17). Compound **16** (9.93 g, 38 mmol) was coevaporated with dry acetonitrile and dissolved in 127 ml of the same solvent. Benzyl bromide (5.4 ml, 45.6 mmol) and NaH (1.37 g, 45.6 mmol) were added to the solution at 0 °C and the reaction mixture was stirred overnight at r.t. Methanol was added and the solution was stirred for additional 2 h. The reaction mixture was partitioned between water and DCM, organic phase was separated, dried over $MgSO_4$ and then evaporated. The residue was purified by column chromatography giving compound **17** as a yellow syrup (11.74 g, 33.4 mmol, 88%). R_f: 0.64 (System B). $[\alpha]_D^{28}$ +112 (c 0.7, $CHCl_3$). IR $\nu_{max}(CDCl_3)$: 2986, 2934, 2886, 1452, 1382, 1373, 1249, 1216, 1160, 1095, 1062, 1018 cm^{-1}. ^{1}H-NMR[30] ($CDCl_3$): 7.48-7.26 (*m*, 5H) *Ph*-CH_2; 5.75 (*d*, J_{H1-H2} = 3.7 Hz, 1H); 4.80-4.57 (*m*, J_{AB} = 11.4 Hz, 2H) Ph-*CH*$_2$; 4.58 (*d*, 1H) H-2; 4.40-4.34 (*m*, 1H) H5; 4.14 (*d*, $J_{H-4,H-5}$ = 2.97Hz, 1H) H-4; 4.04-3.93 (*m*, 2H) H-6,6'; 1.59 (*s*, 3H) CH_3; 1.39 (*s*, 3H) CH_3; 1.37 (*s*, 3H) CH_3; 1.36 (*s*, 3H) CH_3. ^{13}C-NMR (CDCl3): 137.6, 128.5, 128.3, 128.0 (*Ph*-CH_2); 112.9 (1,2-O-*C*[CH_3]$_2$); 109.7 (6,5-O-*C*[CH_3]$_2$); 104.0 (C-1); 78.1 (C-4); 77.8 (C-2); 74.9 (C-5); 72.2 (Ph-*CH*$_2$); 65.1 (C-6); 27.0, 26.9, 26.3, 25.2 (4xCH_3). HRMS (FAB$^+$): $(M+H)^+$ calcd. for $C_{19}H_{26}D_1O_6$: 352.1870, found 352.1878.

3-*O*-Benzyl-1,2-*O* -isopropylidene-α-D-allofuranose-3-*d*$_1$(18). The sugar derivative **17** (11.74 g, 33.4 mmol) was treated with 144 ml of 80% aqueous acetic acid overnight at r.t. Solvent was evaporated and acetic acid was removed by coevaporation with toluene giving oily compound **18** in a 99% yield (10.4 g). R_f: 0.26 (System C). $[\alpha]_D^{27}$ +91 (c 1, $CHCl_3$). IR $\nu_{max}(CDCl_3)$: 3440, 2982, 2935, 1723, 1451, 1382, 1373, 1215, 1154, 1095, 1024 cm^{-1}. ^{1}H-NMR[31] ($CDCl_3$): 7.42-7.18 (*m*, 5H) *Ph*-CH_2; 5.77 (*d*, J_{H1-H2} = 3.7 Hz, 1H) H-1; 4.78 (*d*, J_{AB} = 11.4 Hz, 1H) Ph-*CH*$_2$; 4.60-4.55 (*m*, 2H) Ph-*CH*$_2$, H-2; 4.12 (*d*, $J_{H-4,H-5}$ = 3.2 Hz, 1H) H-4; 4.04-3.99 (*m*, 1H) H-5; 3.70-3.68 (*m*, 2H) H-6,6'; 3.04 (*brs*, 2H) 5-OH, 6-OH; 1.61 (*s*, 3H) CH_3; 1.38 (*s*, 3H) CH_3. ^{13}C-NMR ($CDCl_3$): 137.3, 129.1, 128.6, 128.3, 128.2 (*Ph*-CH_2); 113.1 (1,2-O-*C*[CH_3]$_2$); 104.2 (C-1); 79.0 (C-4); 77.5 (C-2); 72.2 (C-5); 71.3 (Ph-*CH*$_2$); 63.1 (C-6); 26.9, 26.7 (2xCH_3). HRMS (FAB$^+$): $(M+H)^+$ calcd. for $C_{16}H_{22}D_1O_6$: 312.1557, found 312.1584.

Methyl 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranuronate-3,4-*d*$_2$ (21). $NaIO_4$ (8.06 g, 37.7 mmol) was dissolved in the mixture of ethanol (112 ml) and water (112 ml) and the solution was added to the sugar **18** (10.4 g, 33.4 mmol). The reaction mixture was stirred for 3.5 h, then precipitate was filtered and a few drops of ethylene glycol were added. The precipitate was filtered again and the filtrate was evaporated. Ethanol was added to the residue and the precipitate was filtered and this procedure was repeated until no further precipitate appeared. The solvent was evaporated and residue was coevaporated 3 times with dry pyridine, once with a mixture of dry pyridine and D_2O and then with dry pyridine. The residue was dissolved in a mixture of dry pyridine (65 ml) and D_2O (65 ml). The solution was stirred at r.t. for 120 h, and then at 50 °C for 17 days in nitrogen atmosphere. After completion of deuteration (NMR), solvents were evaporated and residual pyridine was removed by coevaporation with toluene. The deuterated aldehyde was separated on silica gel (8.42 g, 30.0 mmol) and dissolved in mixture of MeOH (54 ml) and H_2O (6 ml) to obtain 0.5 *M* solution. $NaHCO_3$ (100.8 g, 1200 mmol) was added to this solution followed by 2 *M* solution of bromine in MeOH (135 ml) and H_2O (15 ml). The reaction mixture was stirred at r.t. for 4 h, then a small amount of Na_2SO_3 was added and the reaction mixture was partitioned between water and DCM. The organic phase was dried over $MgSO_4$, filtered and evaporated. Residue was separated on silica gel column yielding compound **21** as yellow oil (5.67 g, 18.2 mmol, 61%). R_f: 0.83 (System A). $[\alpha]_D^{25}$ +93 (c 1.1, $CHCl_3$). IR $\nu_{max}(CDCl_3)$: 3015, 2994, 2950, 2926, 2863, 1746, 1452, 1436, 1383, 1372, 1303, 1217, 1205, 1164, 1094, 1069, 1023 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.4-7.3 (*m*, 5H) *Ph*-CH_2; 5.84 (*d*, $J_{H-1,H-2}$ = 3.5 Hz, 1H) H-1; 4.76-4.59 (*m*, J_{AB} = 12.1 Hz, 2H) Ph-*CH*$_2$; 4.57 (*d*, 1H) H-2; 3.76 (*s*, 3H) OCH_3; 1.60(*s*, 3H) CH_3; 1.36(*s*, 3H) CH_3. ^{13}C-NMR ($CDCl_3$): 170.6 (C=O), 137.2, 128.5, 128.2, 128.0 (*Ph*-CH_2); 113.7 (1,2-*O*-C[CH_3]$_2$); 104.8 (C-1); 77.8 (C-2); 72.5 (Ph-*CH*$_2$); 52.5 (*C*H_3COO); 26.9, 26.6 (2xCH_3). HRMS (FAB$^+$): $(M+H)^+$ calcd. for $C_{16}H_{19}D_2O_6$: 311.1464, found 311.1473.

3-*O*-Benzyl-1,2-isopropylidene-α-D-ribofuranose-3,4,5,5'-*d*$_4$ (22). Compound **21** (5.67 g, 18.2 mmol) was dissolved in dry diethyl ether (60 ml) and $LiAlD_4$ (0.46 g, 7.28 mmol) was added at 0 °C and the mixture was stirred at r.t. for 4 h. Then water was added and the mixture was extracted with DCM. The organic

phase was dried over $MgSO_4$ and evaporated. The residue was purified using column chromatography to obtain compound **22** as a colourless oil (4.17 g, 14.7 mmol, 80%). R_f: 0.41 (System A). $[\alpha]_D^{25}$ +128 (c 1.2, $CHCl_3$). IR $\nu_{max}(CDCl_3)$: 3486, 2984, 2935, 2863, 1716, 1451, 1383, 1373, 1275, 1216, 1165, 1096, 1017 cm^{-1}. ^{1}H-NMR[32] ($CDCl_3$): 7.4-7.3 (*m*, 5H) *Ph*-CH_2, 5.72 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 3.7 Hz, 1H) H-1; 4.78-4.56 (*d*, J_{AB} = 12.1 Hz, 2H) Ph-*CH_2* ; 4.56 (*d*, 1H) H-2; 1.87 (*brs*, 1H) OH; 1.59 (*s*, 3H) CH_3; 1.36 (*s*, 3H) CH_3. ^{13}C-NMR[32] ($CDCl_3$): 137.66, 128.55, 128.12, 128.06 (*Ph*-CH_2); 113.17 (1,2-O-*C*$[CH_3]_2$); 104.20 (C-1); 77.63 (C-2); 72.38 (Ph-*CH_2*); 26.90, 26.60 (2xCH_3). HRMS (FAB$^+$): $(M+H)^+$ calcd. for $C_{15}H_{17}D_4O_5$: 285.1640, found 285.1651.

Methyl α/β-D-ribofuranoside-3,4,5,5'-*d*$_4$ (25). Compound **22** (4.17 g, 14.7 mmol) was dissolved in dry ethanol (66 ml) and palladium/charcoal (10% Pd) (1.7 g) was added. The reaction flask was fitted with a balloon containing hydrogen and stirred overnight. The reaction mixture was filtered through a layer of Celite, the Celite was washed with ethanol, the solvent was evaporated giving an oily product. This was dissolved in 80% aqueous acetic acid (65 ml) and stirred for 3 days at r.t. and for 1 day at 80 °C. Solvents were evaporated and residue was coevaporated with toluene to remove acetic acid. The residue was dissolved in water and extracted 3 times with DCM and then with diethyl ether. The aqueous phase was evaporated. The product was dissolved in dry methanol (34 ml) and few drops of conc H_2SO_4 were added at 0 °C. The solution was kept in refrigerator at 4 °C overnight, then neutralised passing through an Amberlist A-21 column (OH^- form) using methanol as an eluent. The methanol was evaporated to leave compound **25** (1.87 g, 11.3 mmol, 81 %) as a thick light yellow syrup. R_f: 0.59 & 0.42 (α & β, System D). ^{1}H-NMR[33] (methanol-d_4): 4.90 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 4.5 Hz, 1H) α-H-1; 4.80 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 1.0 Hz, 1H) β-H-1; 4.04 (*d*, 1H) α-H-2; 3.92 (*d*, 1H) β-H-2; 3.47-3.46 (*m*, 3H) α-CH_3; 3.40-3.39 (*m*, 3H) β-CH_3. HRMS (FAB$^+$): $(M+H)^+$ calcd. for $C_6H_9D_4O_5$: 169.1014, found 169.1023.

1-*O*-Methyl-2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-3,4,5,5'-*d*$_4$ (26). Sugar **25** (1.87 g, 11.3 mmol) was coevaporated with dry pyridine 3 times and dissolved in pyridine (150 ml). 4-Toluoyl chloride (4.9 ml, 37.3 mmol) was added in 5 portions at 0 °C and the reaction mixture was stirred overnight. Then the reaction mixture was poured into a saturated solution of $NaHCO_3$ and stirred for 2 h followed by extraction with DCM. Solvents were evaporated and the residual pyridine was removed by coevaporation with toluene to afford compound **26** (4.94 g, 9.45 mmol, 84 %) as an oil. R_f: 0.81 (System C). IR $\nu_{max}(CDCl_3)$: 2924, 2848, 1723, 1611, 1274, 1179, 1105, 1022 cm^{-1}. ^{1}H-NMR[34] ($CDCl_3$): 7.99-7.08 (*m*, 12H) toluoyl (α+β); 5.64 (*s*, 0.7H) H-1 (β); 5.37 (*d*, $J_{H\text{-}1,H\text{-}2}$ = 4.5, 0.3H) H-1 (α); 5.30 (*d*, 0.3H) H-2 (α); 5.14 (*s*, 0.7H) H-2 (β); 3.47 (*s*, 0.9H) OCH_3 (α); 3.40 (*s*, 2.1H) OCH_3 (β); 2.40-2.35 (*m*, 9H) CH_3 (α+β). ^{13}C-NMR ($CDCl_3$): β-anomer: 144.3, 144.2, 143.9 (C=O, toluoyl); 136.5, 129.9, 129.2, 129.1, 126.7 (C-H, toluoyl); 106.4 (C-1); 75.3 (C-2); 55.4 (OCH_3); 21.7 (CH_3). HRMS (FAB$^+$): $(M+H)^+$ calcd. for $C_{30}H_{27}D_4O_8$: 523.2270, found 523.2284.

1-*O*-Acetyl-2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-3,4,5,5'-*d*$_4$ (27). Compound **26** (4.94 g, 9.45 mmol) was dissolved in dry DCM (23 ml) and cooled in an ice bath. Acetic anhydride (5.2 ml), acetic acid (4.4 ml) and conc H_2SO_4 (0.9 ml) were mixed together separately, cooled and added to the solution of sugar **26**. The reaction mixture was stirred at 0 °C for 12 min. Saturated $NaHCO_3$ solution was prepared and cooled to 0 °C. The reaction mixture was poured carefully to this solution and stirred in the beginning at 0 °C and then at r.t. for 3h. The solution was extracted by DCM, the organic phase was dried over $MgSO_4$, evaporated and coevaporated with toluene, giving compound **27** (5.17 g, 9.37 mmol, 99%). The β-anomer was recrystallised from methanol (3.0 g, 5.5 mmol) to obtain white crystals. R_f: 0.75 (System C). $[\alpha]_D^{27}$ β-anomer:[34] +60 (c 1, $CHCl_3$). IR $\nu_{max}(CDCl_3)$: 3036, 2920, 2848, 1725, 1611, 1364, 1310, 1274, 1225, 1177, 1110, 1096, 1018, 966 cm^{-1}. ^{1}H-NMR[34] (500 MHz; $CDCl_3$) β-anomer: 8.0-7.1 (*m*, 12H) toluoyl; 6.41 (*s*, 1H) H-1; 5.75 (*s*, 1H) H-2; 2.41-2.37 (*3xs*, 9H) 3xCH_3 (toluoyl); 2.00 (*s*, 3H) CH_3 (acetyl). ^{13}C-NMR ($CDCl_3$) β-anomer: 169.2 (C=O, acetyl); 166.2, 165.5, 165.2 (C=O, toluoyl); 144.5, 144.4, 144.0, 130.0, 129.9, 129.3, 129.2, 127.1, 126.2, 126.1 (C-H, toluoyl); 98.6 (C-1); 74.9 (C-2); 21.7 (CH_3, toluoyl), 21.0 (CH_3, acetyl). HRMS (FAB$^+$): $(M+H)^+$ calcd. for $C_{31}H_{27}D_4O_9$: 551.2219, found 551.2228.

2',3',5'-Tri-*O*-(4-toluoyl)-uridine-3',4',5',5"-*d*$_4$ (28a). Uracil (146 mg, 1.3 mmol) was suspended in hexamethyldisilazane (2.3 ml) and trimethylchlorosilane (0.2 ml) was added. The reaction mixture was stirred

at 120 °C in nitrogen atmosphere for 4 h. The volatile materials were evaporated and residue was kept on oil pump for 20 min. Sugar **27** (551 mg, 1.0 mmol) was dissolved in dry 1,2-dichloroethane (13 ml) and this solution and trimethylsilyl trifluoromethanesulfonate (0.3 ml) were added to the persilylated nucleobase. The reaction was kept overnight at 40 °C in nitrogen atmosphere. Work up by saturated sodium bicarbonate solution and separation on silica gel column gave compound **28a** (360 mg, 66%) as white foam. R_f: 0.74 (System E). $[\alpha]_D^{27}$ -76 (c 0.75, $CHCl_3$). IR ν_{max}($CDCl_3$): 1724, 1693, 1609, 1453, 1379, 1283, 1269, 1244, 1179, 1092, 1019 cm^{-1}. ^{1}H-NMR (500 MHz; $CDCl_3$): 8.27 (br.*s*, 1H) N-H; 8.00-7.16 (*m*, 12H) toluoyl; 7.41 (*d*, 1H) H-6; 6.35 (*d*, 1H) H-1'; 5.60 (*d*, 1H) H-2'; 5.58 (*d*, 1H) H-5; 2.43, 2.41, 2.38 (3x*s*, 9H) 3xCH_3 (toluoyl). ^{13}C-NMR ($CDCl_3$): 166.0, 165.6, 165.4 (3 x C=O); 162.7 (C-4); 150.2 (C-2); 144.72, 144.65, 144.60 (C-4 toluoyl); 139.5 (C-6); 130.04, 129.97, 129.74, 129.57, 129.31 (C-2, C-3 toluoyl); 126.5, 126.0 125.7 (C-1 toluoyl); 103.5 (C-5); 87.7 (C-1'); 73.6 (C-2'); 21.8 (3xCH_3). HRMS (ES^+) $(M+H)^+$: calcd. for $C_{33}H_{27}D_4N_2O_9$: 603.2280, found 603.2292.

2',3',5'-Tri-*O*-(4-toluoyl)-N^6-benzoyladenosine-3',4',5',5"-d_4 (28b). N^6-benzoyladenine (309 mg, 1.3 mmol) was condensed with sugar **27** (551 mg, 1.0 mmol) as it was described for compound **28a**. The reaction was carried out at 70 °C for 3h to yield nucleoside **28b** (434 mg, 60%) as white foam after work up and separation. R_f: 0.67 (System E). $[\alpha]_D^{27}$ -112 (c 1.02, $CHCl_3$). IR ν_{max}($CDCl_3$): 1722, 1609, 1585, 1456, 1284, 1179, 1093, 1019 cm^{-1}. ^{1}H-NMR (500 MHz; $CDCl_3$): 8.99 (br.*s*, 1H) N-H; 8.72 (*s*, 1H) H-8; 8.17 (*s*, 1H) H-2; 8.02-7.15 (*m*, 17H) toluoyl, benzoyl; 6.50 (*d*, 1H) H-1'; 6.37 (*d*, 1H) H-2'; 2.42, 2.41, 2.38 (3x*s*, 9H) 3xCH_3 (toluoyl). ^{13}C-NMR ($CDCl_3$): 166.1, 165.3, 165.0 (3xC=O, toluoyl); 164.4 (C=O, benzoyl); 152.9 (C-2); 151.6 (C-6); 149.6 (C-4); 144.5, 144.4, 144.1 (toluoyl); 141.4 (C-8); 133.5, 132.6 (benzoyl); 129.7, 129.6, 129.1, 128.7, 127.7, 126.4, 125.9, 125.5, (toluoyl, benzoyl); 123.4 (C-5); 86.7 (C-1'); 73.7 (C-2'); 21.6 (3xCH_3). HRMS (ES^+) $(M+H)^+$: calcd. for $C_{41}H_{32}D_4N_5O_8$: 730.2815, found 730.2818.

2',3',5'-Tri-*O*-(4-toluoyl)-N^4-benzoylcytidine-3',4',5',5"-d_4 (28c). N^4-Benzoylcytosine (280 mg, 1.3 mmol) and deuterated sugar **27** (551 mg, 1.0 mmol) were condensed using a procedure used for the preparation of compound **28a**, but at 70 °C for 5 h (white foam, 508 mg, 72%). R_f: 0.73 (System E). $[\alpha]_D^{27}$ -65 (c 1.04, $CHCl_3$). IR ν_{max}($CDCl_3$): 1722, 1668, 1626, 1610, 1556, 1479, 1271, 1246, 1179, 1109, 1091, 1019 cm^{-1}. ^{1}H-NMR (500 MHz; $CDCl_3$): 8.95 (br.*s*, 1H) N-H; 8.01-7.84 (*m*, 9H) H-6, toluoyl, benzoyl; 7.61-7.50 (*m*, 3H) benzoyl; 7.31-7.16 (*m*, 7H) H-5, benzoyl; 6.50 (*d*, 1H) H-1'; 5.78 (*d*, 1H) H-2'; 2.44, 2.40, 2.39 (3x*s*, 9H) 3xCH_3 (toluoyl). ^{13}C-NMR ($CDCl_3$): 166.3, 165.4, 165.3 (C=O, toluoyl); 162.6 (C-4); 153.7 (C-2); 144.36, 144.31, 144.30 (toluoyl); 144.01 (C-6); 133.3, 132.9 (benzoyl); 129.8, 129.6, 129.4, 129.0, 128.9, 127.5, 126.4, 125.83, 125.76 (toluoyl, benzoyl); 97.4 (C-5); 89.1 (C-1'); 74.6 (C-2'); 21.8 (CH_3). HRMS (ES^+) $(M+H)^+$: calcd. for $C_{40}H_{32}D_4N_3O_9$: 706.2702, found 706.2703.

2',3',5'-Tri-*O*-(4-toluoyl)-N^2-acetyl-O^6-diphenylcarbamoylguanosine-3',4',5',5"-d_4 (28d). N^2-Acetyl-O^6-diphenylcarbamoylguanine (504 mg, 1.3 mmol) was suspended in dry 1,2-dichloroethane (7.8 ml) and bis(trimethylsilylacetamide) (0.4 ml) was added to the suspension. The mixture was heated at 83 °C in nitrogen atmosphere for 1h. Then volatile materials were evaporated and the residue was kept on oil pump for 20 min. Toluoylated sugar **27** (551 mg, 1 mmol) was dissolved in dry toluene (14.3 ml) and some drops of 1,2-dichloroethane and added to the persilylated nucleobase. Trimethylsilyl trifluoromethanesulfonate (0.4 ml) was added as a catalyst. The reaction was kept at 70 °C for 5h. The reaction was quenched by adding an aqueous solution of saturated sodium bicarbonate, from which the product was extracted by DCM. The organic phase was dried over $MgSO_4$ and the solvent was evaporated. The product was separated by column chromatography to yield compound **28d** (671 mg, 60%) as off white foam. R_f: 0.75 (System E). $[\alpha]_D^{27}$ -34 (c 1.03, $CHCl_3$). IR ν_{max}($CDCl_3$): 1726, 1610, 1589, 1511, 1489, 1411, 1370, 1282, 1178, 1108, 1092, 1018, 976 cm^{-1}. ^{1}H-NMR (500 MHz; $CDCl_3$): 8.10 (*s*, 1H) N-H; 8.06 (*s*, 1H) H-8; 7.92-7.14 (*m*, 22 H) phenyl, toluoyl; 6.34 (*d*, 1H) H-1'; 6.23 (*d*, 1H) H-2'; 2.48 (*s*, 2H) N^2-C(O)CH_3; 2.42, 2.38 (2x*s*, 9H) 3xCH_3 (toluoyl). ^{13}C-NMR ($CDCl_3$): 169.9 (*C*(O)CH_3); 166.1, 165.1, 165.0 (3 x C=O, toluoyl); 156.2 (C-6); 154.2 (C-4); 152.2 (C-2); 150.0 (DPC); 144.5, 144.3, 144.0 (toluoyl); 142.2 (C-8); 141.5 (DPC); 129.7, 129.5, 129.13, 129.05; 126.8, 126.3, 125.9, 125.5 (toluoyl, DPC); 121.1 (C-8); 87.2 (C-1'); 73.8 (C-2'); 24.9 (C(O)*C*H_3; 21.5 (CH_3, toluoyl). HRMS (ES^+) $(M+H)^+$: calcd. for $C_{49}H_{39}D_4N_6O_{10}$: 879.3291, found 879.3292.

Uridine-3',4',5',5"-d_4 (29a). Nucleoside **28a** (0.36 g, 0.85 mmol) was dissolved in methanolic ammonia (50 ml) and stirred at room temperature overnight. Solvent was evaporated, the residue was dissolved in water and extracted 3 times with DCM and then with diethyl ether. Evaporation of aqueous phase gave uridine **29a** (0.21 g, 98 %). Sample for analysis was recrystallised from H_2O to afford white crystals. $[\alpha]_D^{26}$ +9 (c 0.2, H_2O); $[\alpha]_D^{26}$ for natural uridine +10 (c 0.2, H_2O). IR[41] ν_{max}(KBr): 3346, 3112, 2967, 2880, 2796, 1776, 1665, 1467, 1394, 1360, 1311, 1267, 1186, 1147, 1103, 1061, 1038, 991, 971, 962, 950, 829, 773, 763 cm^{-1}. ^{1}H-NMR (D_2O): 7.80 (*d*, $J_{H\text{-}5,H\text{-}6}$ = 8.2 Hz, 1H) H-6; 5.84 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 4.5 Hz, H-1) H-1'; 5.82 (*d*, 1H) H-5; 4.27 (*d*, 1H) H-2'. ^{13}C-NMR[35] (D_2O): 166.4 (C-4); 151.9 (C-2); 142.0 (C-6); 102.5 (C-5); 89.6 (C-1'); 73.8 (C-2'). HRMS (ES^+) $(M+H)^+$: calcd. for $C_9H_9D_4N_2O_6$: 249.1024, found 249.1021.

Adenosine-3',4',5',5"-d_4 (29b). Compound **28b** (0.382 g, 0.53 mmol) was deprotected using the same procedure as described for compound **28a** giving adenosine **29b** (0.14 g, 98 %). Sample was recrystallised from water to give white powder. $[\alpha]_D^{26}$ -57 (c 0.2, H_2O); $[\alpha]_D^{26}$ for natural adenosine -60 (c 0.2, H_2O). IR[42] ν_{max}(KBr): 3480, 3200, 2928, 2848, 2768, 1642, 1601, 1570, 1474, 1417, 1369, 1384, 1298, 1249, 1211, 1174, 1139, 1108, 1092, 1070, 988, 957, 824, 794, 765, 716 cm^{-1}. ^{1}H-NMR (500 MHz; D_2O): 8.31 (*s*, 1H) H-8; 8.22 (s, 1H) H-2; 6.05 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 6.1 Hz, 1H) H-1'; 4.77 (*d*, 1H) H-2'. ^{13}C-NMR (DMSO-d_6): 156.3 (C-6); 152.5 (C-2); 149.2 (C-4); 140.0 (C-8); 119.5 (C-5); 88.1 (C-1'); 73.5 (C-2'). HRMS (ES^+) $(M+H)^+$: calcd. for $C_{10}H_{10}D_4N_5O_4$: 272.1297, found 272.1300.

Cytidine-3',4',5',5"-d_4 (29c). For deprotection of nucleoside **28c** (0.44 g, 0.72 mmol) the same conditions were used as for compound **28a**. After purification on a column of Dowex 1x2-400 (OH^- form) using deionised water as eluent, the deuterated cytidine **29c** was obtained (0.13 g, 75 %) as white powder. $[\alpha]_D^{27}$ +32 (c 0.7, H_2O); $[\alpha]_D^{27}$ for natural cytidine: +33 (c 0.7, H_2O). IR[43] ν_{max}(KBr): 3332, 3195, 2920, 2778, 1642, 1604, 1535, 1486, 1400, 1371, 1289, 1226, 1202, 1147, 1168, 970, 784 cm^{-1}. ^{1}H-NMR (H_2O): 7.76 (*d*, $J_{H\text{-}5,H\text{-}6}$ = 7.6 Hz, 1H) H-6; 5.95 (*d*, 1H) H-5; 5.81 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 4.0 Hz, 1H) H-1'; 4.22 (*d*, 1H) H-2'. ^{13}C-NMR (H_2O): 166.5 (C-4); 157.9 (C-2); 141.9 (C-6); 96.5 (C-5); 90.6 (C-1'); 74.1(C-2'). HRMS (ES^+) $(M+H)^+$: calcd. for $C_9H_{10}D_4N_3O_5$: 248.1184, found 248.1183.

Guanosine-3',4',5',5"-d_4 (29d). Compound **28d** (0.5 g, 0.57 mmol) was deprotected using the same procedure as used for deprotection of compound **28a** to afford deuterated guanosine **29d** (0.13 g, 80 %) as white powder. Sample for analysis was recrystallised from water. $[\alpha]_D^{26}$ -37 (c 0.04, H_2O); $[\alpha]_D^{26}$ for natural guanosine -37 (c 0.04, H_2O). IR[44] ν_{max}(KBr): 3400, 3336, 3211, 2926, 2850, 2732, 1684, 1637, 1484, 1412, 1358, 1228, 1170, 1147, 1079, 1061, 1014, 970, 814, 774, 678 cm^{-1}. ^{1}H-NMR (500 MHz; D_2O): 8.06 (*s*, 1H) H-8; 5.99 (*d*, $J_{H\text{-}1',H\text{-}2'}$ = 5.9 Hz, 1H) H-1'; 4.81 (*d*, 1H) H-2'. ^{13}C-NMR (DMSO-d_6): 157.0 (C-6); 153.8 (C-2); 151.4 (C-4); 135.8 (C-8); 116.8 (C-5); 86.6 (C-1'); 73.8 (C-2'). HRMS (ES^+) $(M+H)^+$: calcd. for $C_{10}H_{10}D_4N_5O_5$: 288.1246, found 288.1250.

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($CDCl_3$): 114.9 (1,2-O-*C*[CH_3]$_2$); 109.1 (5,6-O-*C*[CH_3]$_2$); 105.2 (C-1); 84.2 (C-4); 79.8 (C-2); 75.4 (C-5); 69.6 (C-3); 66.2 (C-6); 27.0, 26.6, 25.1 CH_3.

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