Total Synthesis of 2′,3′,4′,5′,5″-$^2H_5$-Ribonucleosides: The Key Building Blocks for NMR Structure Elucidation of Large RNA

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The diastereospecific chemical syntheses of uridine-2′,3′,4′,5′,5″-$^2H_5$ (**21a**), adenosine-2′,3′,4′,5′,5″-$^2H_5$ (**21b**), cytidine-2′,3′,4′,5′,5″-$^2H_5$$^2H_5$ (**21c**), and guanosine-2′,3′,4′,5′,5″-$^2H_5$ (**21d**) (>97 atom % ^2H at C2′, C3′, C4′, and C5′/C5″) have been achieved for their use in the solution NMR structure determination of oligo-RNA by the Uppsala "NMR-window" concept (refs 4a−c, 5a, 6), in which a small ^1H segment is NMR-visible, while the rest is made NMR-invisible by incorporation of the deuterated blocks **21a**−**d**. The deuterated ribonucleosides **21a**−**d** have been prepared by the condensation of appropriately protected aglycone with 1-*O*-acetyl-2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-2,3,4,5,5′-$^2H_5$ (**19**), which has been obtained via diastereospecific deuterium incorporation at the C2 center of appropriate D-ribose-$^2H_4$ derivatives either through an oxidation−reduction−inversion sequence or a one-step deuterium−proton exchange in high overall yield (44% and 24%, respectively).

Introduction

NMR is a powerful tool[1] to explore specific folding motifs and dynamics of oligo-DNA or -RNA and their complexes with various ligands under quasi-physiological condition at atomic resolution. The severe spectral overlap,[1] line broadening,[1] and the spin diffusion[2] encountered in the conformational study of large biologically functional oligonucleotides (and their complexes with various ligands), however, restricts the application of NMR to study those large molecules with specific function in natural isotopic abundance.

Various isotope labeling techniques have therefore been introduced.[3] Among these, sequence- and/or site-specific deuterium labeling of oligo-DNAs has indeed been proven to simplify the spectral crowding[4] considerably, giving coupling information[5,6b,c] and NOE cross-peaks with increased intensities with negligible spin-diffusion,[6] as well as allowing us to probe the dynamics by selective T_1 and T_2 measurements.[7] Site-specific deuteration has been proven to facilitate the NMR structure determination of relatively large oligo-RNA,[8] as evident from our study of a 21mer,[8a] 31mer,[8b] and recently a 55mer RNA.[8c] Most of these studies have been done according to our Uppsala "NMR-window" concept[4a,b] in which only a small ^1H segment of the RNA is NMR-visible, while the rest is made NMR-invisible by incorporation of the appropriate deuterated blocks. The level of deuterium incorporation into each nucleoside building block (>97 at. % at C2′, C3′, and C5′, ∼35−50 at. % at C4′, ∼0−20 at. % at C1′) was adequate to perform the sequential assignment of up to a 55nt long oligoRNA,[8c] but not for quantitative structure determination. *The bottleneck[4] of our "Uppsala NMR-window" approach was*

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the residual ~50−65 at. % proton at C4′. This causes substantial resonance overlap in the regions where important aromatic proton to H-2′/3′/4′/5′ and anomeric proton to H-2′/3′/4′/5′ NOEs appear. Consequently, the stray NOE cross-peaks from the residual H4′ made the estimation of desired cross-peak volumes erroneous, which seriously hampers the quality of the solution structure determination of larger functional RNAs.

Clearly, we needed to develop a high-yielding synthetic procedure that gave >97 atom % deuteration at C4′ (as well as retaining those high deuterium incorporations at C2′, C3′, and C5′) of all ribonucleosides. We herein report diastereospecific chemical syntheses of uridine-2′,3′,4′,5′,5″-$^2H_5$ (**21a**), adenosine-2′,3′,4′,5′,5″-$^2H_5$ (**21b**), cytidine-2′,3′,4′,5′,5″-$^2H_5$ (**21c**), and guanosine-2′,3′,4′,5′,5″-$^2H_5$ (**21d**) (>97 atom % ^2H at C2′, C3′, C4′, and C5′/C5″) through the condensation of appropriately protected aglycone with 1-*O*-acetyl-2,3,5-tri-*O*-(4-toluoyl)-α/*β*-D-ribofuranose-2,3,4,5,5′-$^2H_5$ (**19**).

The various deuteration strategies developed so far has been reviewed by us recently.[9] A close scrutiny of these methods shows that 3′,4′,5′,5″-$^2H_4$-nucleosides[10] would be a plausible choice as a starting material for the synthesis of the corresponding 2′,3′,4′,5′,5″-$^2H_5$ derivatives, if a suitable way for deuterium incorporation at C2′ could be found: (i) One way is to oxidize the 2′-OH of 3′,5′-bis-*O*-protected ribonucleosides followed by subsequent reduction to give predominantly the *ara* epimer,[6b,11] followed by inversion of the configuration of C2′. This approach,[6b] however, becomes complicated owing to the concomitant loss of the 3′,5′-*O*-protection, which is usually 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl group. It has also been found in this laboratory that this procedure gives a mixture of products in case of guanosine derivatives.[12] (ii) The deuterium incorporation at C2 at the sugar level followed by nucleoside synthesis is an alternative procedure since the oxidation of 3,4-*O*-ispropylidene-*β*-D-arabinopyranoside by CrO_3/acetic anhydride/pyridine followed by reduction with $LiAl^2H_4$ (LAD) furnishes ribopyranoside derivative with high stereoselectivity.[12] (iii) A similar oxidation−reduction sequence starting with 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose[13] results in deuterium enrichment (92−94 at. % ^2H), which is insufficient for high-resolution NMR study. The instability of the benzoyl protecting groups during the reduction also complicates this pathway. (iv) In a recent study, the Swern oxidation of methyl 3,5-di-*O*-benzyl-α,*β*-D-ribofuranoside[14] and subsequently the reduction of the resulting C2-ketosugar has been reported by us to give a mixture of predominant ara and minor ribo derivatives, which could be converted to the 2-*O*-(4-toluoyl) derivatives and separated by silica gel column chromatography. The benzyl protection is stable during the synthesis of the C2 epimeric ribo derivative during inversion of the configuration of this center. (v) Alternatively, a large scale single-step deuterium−proton exchange reaction[15] at C2 of 2,3-*O*-isopropylidene-α,*β*-D-ribofuranose-$^2H_4$ should be easily achievable, since we have already shown[14,16] that 2,3-*O*-isopropylidene-α,*β*-D-ribofuranose itself undergoes isotope exchange at C2 (>97 at. % ^2H) in a completely diastereoselective manner during equilibration in a mixture of 1,4-dioxane/tetrahydrofurane/triethylamine/^2H_2O at reflux temperature.

Results and Discussion

Since 1,2-*O*-isopropylidene-3-*O*-benzyl-α-D-ribofuranose-3,4,5,5′-$^2H_4$ (**1**) (Scheme 1) is an intermediate during the large-scale synthesis of $^2H_4$-ribonucleosides,[10] it has been chosen as the starting material in the present preparation of $^2H_5$-ribonucleosides. This compound can easily be converted either to deuterium labeled methyl 3,5-di-*O*-benzyl-α,*β*-D-ribofuranoside or to 2,3-*O*-isopropylidene-α,*β*-D-ribofuranose described above as appropriate intermediate for deuterium incorporation at C2.

Route A: Introduction of Deuterium at C2 at the Sugar Level Starting from 3-O-Benzyl-1,2-O-isopropylidene-α-D-ribose-3,4,5,5′-$^2H_4$ (1). The deuterated precursor **1** is easily available in multigram scale (~300 mmol) in seven steps from 1,2:5,6-di-*O*-isopropylidene-α-D-glucose.[17] The fully protected intermediate **2** was obtained (94%) upon a treatment of **1** with benzyl bromide in the presence of NaH in dry acetonitrile. Treatment of this product with concentrated sulfuric acid in dry methanol furnished the anomeric mixture of block **3** (90%). Crucial in this synthetic strategy (Scheme 1) is the proper selection of the oxidation−reduction procedures. The back exchange[6b] at C3 observed upon the oxidoreduction transformation during the Swern oxidation[19a,b] and sodium borodeuteride reduction rules out their application. Hence, compound **3** was subjected to oxidation with pyridinium dichromate (PDC) and acetic anhydryde[18] in dry dichloromethane at reflux temperature (the disappearance of the signal at δ 3.32 for the OCH_3 group at the anomeric center confirmed the completion of the reaction) to obtain ketone **4** (72%). A further drop of yield (68%) occurred in the subsequent reduction with LAD in dry diethyl ether. This reduction step afforded an epimeric mixture of C2-deuterated arabino- (stereoselectively from the *β*-anomer of **3**) and ribofuranoside **5** (from the α-anomer) in a ratio of ~7:3 as evidenced by ^1H NMR. The moderate overall yield in the two-step oxidation−reduction (i.e., **3** → **4** → **5** in 47% overall yield) indicated that the PDC oxidation of **3** is suitable for producing the ketone on a large scale (~11 g). The overall poor yield is most probably owing to insufficient stability of **3** under the oxidation condition (reflux). Additionally, it was not entirely possible to remove the residual oxidizing agent from crude **4** by simple precipitation procedure, which created further complication in the reduction step. This prompted us to employ the Dess−Martin reagent[19c] for oxidation of compound **3** in dry dichloromethane at room temperature (3 mmol scale) to afford the desired ketone **4** in 99% yield.

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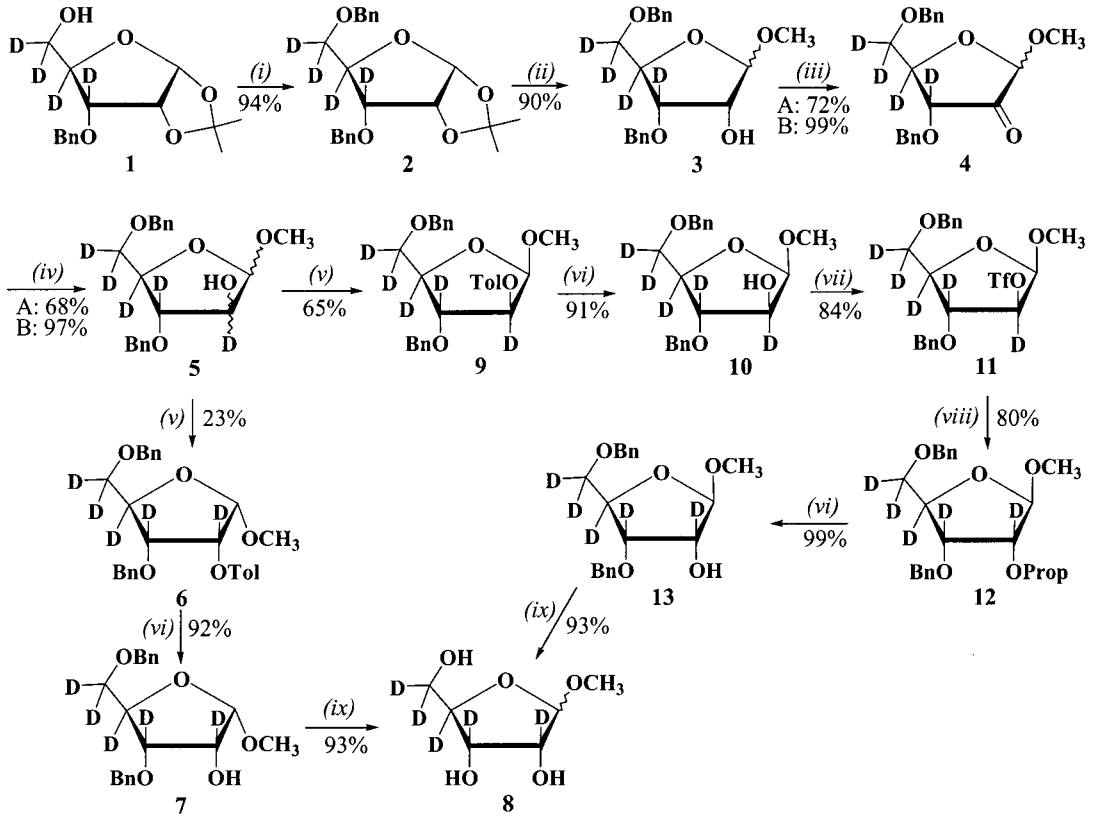
Scheme 1[a]



[a] Abbreviations: Bn = benzyl; Tf = trifluoromethanesulfonyl; Prop = propionyl; Tol = 4-toluoyl. Conditions: (i) BnBr and NaH in dry acetonitrile, rt, 2 h; (ii) concd H_2SO_4 in dry methanol, reflux, 3 h; (iii) A: pyridinium dichromate, acetic anhydride in dry CH_2Cl_2, reflux, 3 h; B: Dess−Martin reagent in dry CH_2Cl_2, rt, overnight; (iv) $LiAl^2H_4$ in dry diethyl ether, rt, 6 h; A: ketone from iiiA, B: ketone from iiiB; (v) TolCl, pyridine, rt; (vi) $NaOCH_3$ in methanol, rt, 30 min; (vii) Tf_2O, DMAP, pyridine, CH_2Cl_2, 0 °C, 3 h; (viii) cesium propionate, DMF, rt, 36 h; (ix) Pd/C, hydrogen in ethanol, rt, overnight.

The following identical reduction step (iv, Scheme 1) proceeded also with a high yield (97%). The resulting mixture of C2-deuterated arabino and ribo compounds **5** were successfully separated after toluoylation to afford methyl 3,5-di-*O*-benzyl-2-*O*-(4-toluoyl)-α-ribofuranoside-2,3,4,5,5′-$^2H_5$ (**6**) (23%) and methyl 3,5-di-*O*-benzyl-2-*O*-toluoyl-*β*-arabinofuranoside-2,3,4,5,5′-$^2H_5$ (**9**) (65%). Due to the lack of distinctive ^1H NMR signals because of the deuterated nature of the sugar, the C2 configuration of these compounds has been corroborated by the appropriate optical rotation values ($[\alpha]^{26}_D$ +98° (*c* 0.67, $CHCl_3$) and $[\alpha]^{26}_D$ −74° (*c* 0.25, $CHCl_3$) for **6** and **9**, respectively[14]). The 4-toluoyl group from **6** was cleaved by the action of methanolic sodium methoxide to yield the pure methyl α-ribofuranoside derivative **7** with >97 at. % deuterium incorporation at C2.

The inversion of configuration at C2 of the *β*-arabino compound **9** was accomplished in four steps involving the removal of the 2-*O*-toluoyl group of **9** by a brief treatment with 1.0 M sodium methoxide in methanol at room temperature to give the hydroxy block **10** (91%), which was converted to the 2-*O*-triflyl derivative **11** (84%).[6b,11b] This compound **11** was converted to the 2-*O*-propionyl **12** via displacement of the triflate leaving group with Cs-propionate[6b,20] in DMF at room temperature under an inert atmosphere (80%). The shift of the anomeric proton resonance at *δ*4.99 in **11** to *δ*4.87 in **12** proved that the S_N2 type inversion at C2 has indeed taken place. Also, the change of the appropriate specific rotations (from $[\alpha]^{26}_D$ −64° (*c* 0.74, $CHCl_3$) for **11** to $[\alpha]^{27}_D$ +14° (*c* 0.71, $CHCl_3$) for compound **12**[14]) corroborated the inversion to ribo configuration at C2. The 2-*O*-propionyl group in **12** was cleaved upon treatment with methanolic sodium methoxide to furnish compound **13** (99%). This was followed by removal of the benzyl groups from the combined carbohydrates **7** and **13** in a catalytic hydrogenation process over 10% Pd/C in ethanol to afford C2,3,4,5,5′-pentadeuterated 1-*O*-methyl-ribofuranose **8** (93%). Subsequently, **8** was 4-toluoylated to afford **18** followed by acetylation and crystallization using standard procedures[5a] to give the required 1-*O*-acetyl-2,3,5-tri-*O*-toluoyl-α,*β*-D-ribofuranose-2,3,4,5,5′-$^2H_5$ precursor (**19**). When the NMR spectrum of the crystalline *β*-anomer is compared to the spectrum of the natural counterpart (Figures 1A,B), the presence of the only singlet in the sugar region at *δ* 6.41 ppm establishes the high degree of deuterium incorporation (>97 at. % ^2H) at the remaining carbon centers of the pentofuranose moiety. The measured specific rotation also proved the identity of this compound (specific rotation for *β*-anomer of **19**: +62° (*c* 1.04, $CHCl_3$), for authentic sample: +63°).

Route B: Introduction of Deuterium at C2 at the Sugar Level Starting from D-Ribose-3,4,5,5′-$^2H_4$ (14). Earlier, we have reported[14,16] that it is possible to obtain >97 at. % diastereospecific exchange of the proton with

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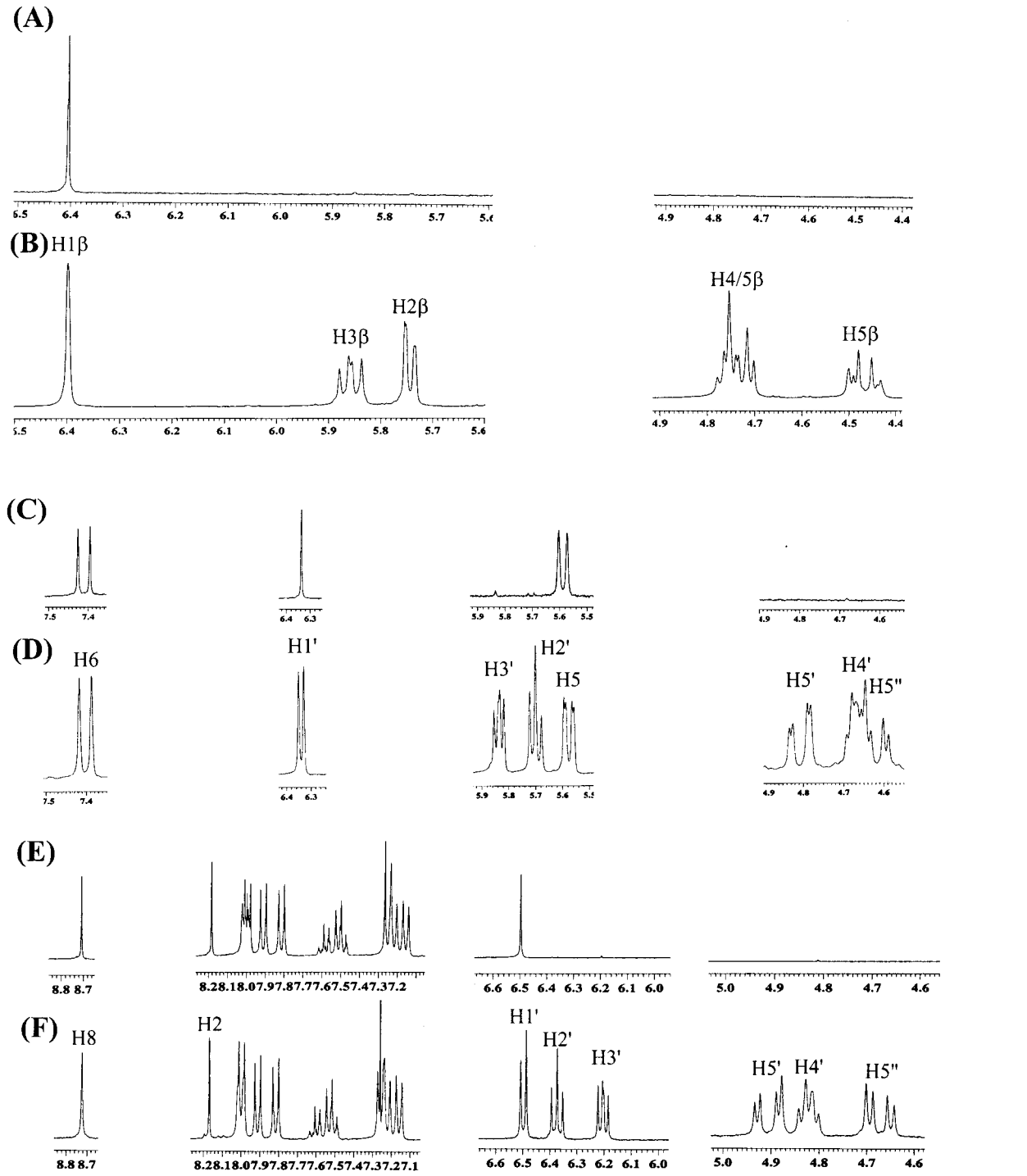

Figure 1. Expanded regions of the 270 MHz 1D ^1H NMR spectra of 1-*O*-acetyl-2,3,5-tri-*O*-(4-toluoyl)-*β*-D-ribose-2,3,4,5,5′-$^2H_5$ (**19***β*) (Panel A) and its natural abundance counterpart (Panel B), 2′,3′,5′-tri-*O*-(4-toluoyl)-uridine-2′,3′,4′,5′,5″-$^2H_5$ (**20a**) (Panel C) and its natural abundance counterpart (Panel D), 2′,3′,5′-tri-*O*-(4-toluoyl)-N^6-benzoyladenosine-2′,3′,4′,5′,5″-$^2H_5$ (**20b**) (Panel E) and its natural abundance counterpart (Panel F).

deuterium at the C2 center of 2,3-*O*-ispropylidene-D-ribose upon equilibration in dioxane/THF/triethylamine/^2H_2O mixture at elevated temperature. The reaction sequence in this route B involves fewer steps (Scheme 2) than in route A (Scheme 1) to obtain the 4-toluoylated ribose derivative **19**. Hence we decided to check the feasibility of this route B. The starting D-ribose-3,4,5,5′-$^2H_4$ (**14**) was prepared from **1** as described previously.[10] The introduction of the isopropylidene group, however, proceeded in very moderate yield (20−27%) regardless of the procedure[21] used. The exchange reaction in a mixture of 1,4-dioxane/THF/^2H_2O/Et_3N took place with no detectable amount of side product formation to furnish the deuterated sugar **15** (98% yield) after 5 days of heating at 80 °C. The deuteration level was found to be >97 at. % as determined from the ^1H NMR spectrum. The deuterated ribose derivative **15** was converted to methyl riboside **8** by deprotection of isopropylidene group

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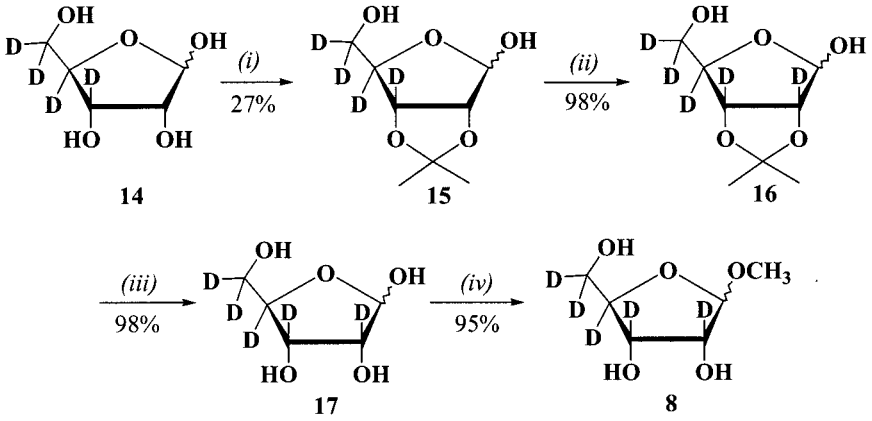
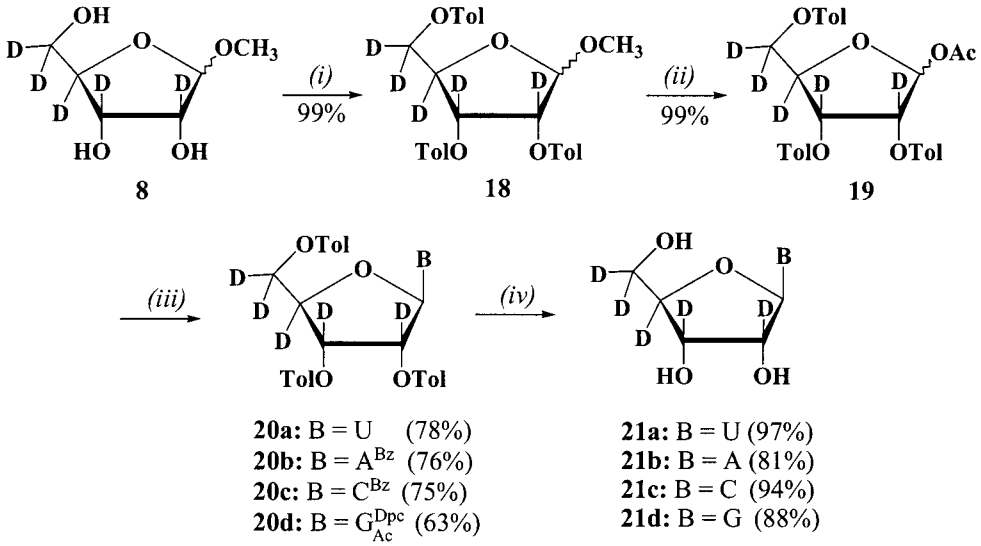
Scheme 2[a]



[a] Conditions: (i) dry acetone, concd H_2SO_4, 4 °C, overnight; (ii) Dioxane/THF/Triethylamine/^2H_2O (6/6/3/4 mL, v/v/v/v), 90 °C, 5 days; (iii) 90% aqueous TFA, rt, 30 min.; (iv) methanol, concd H_2SO_4, 4 °C, overnight.

Scheme 3[a]



[a] Abbreviations: Tol = 4-toluoyl, Ac = acetyl, G = guanin-9-yl, A = adenin-9-yl, C = cytosin-1-yl, U = uracil-1-yl, Bz = benzoyl, Dpc = diphenylcarbamoyl. Conditions: (i) TolCl, pyridine, rt, overnight; (ii) Ac_2O, AcOH, concd H_2SO_4, dry CH_2Cl_2, 0 °C, 15 min.; (iii) silylated nucleobase, trimethylsilyl trifluoromethanesulfonate, 1,2-dichloroethane or toluene (12 d), heating; (iv) NH_3 in methanol, rt.

in 90% aqueous trifluoroacetic acid (98%) followed by glycosylation in dry methanol in the presence of catalytic amount of concentrated sulfuric acid to afford compound **8** (95%). This labeled ribose derivative was further processed as described in route A.

The couplings of compound **19** 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)[22] applying trimethylsilyl trifluoromethanesulfonate as a catalyst, using modified literature procedures, were carried out following well-established methods[23] to give the corresponding fully protected pentadeuterated nucleosides **20a**−**d**. Comparison of their ^1H NMR spectra with the corresponding protected natural nucleosides proves that no proton/deuterium exchange reaction has taken place during the coupling process (Figures 1C−F and Figures 2A−D). The protecting groups were removed by treatment with NH_3 in methanol to produce the target ribonucleosides-2′,3′,4′,5′,5″-$^2H_5$ **21a**−**d** in 97, 81, 94, 88% yields, respectively (Scheme 3). The purity and percentage of isotope incorporation (>97 atom % ^2H at C2′, C3′, C4′, and C5′) of these compounds are evidenced in Figures 2E,F and 3. The identity of these nucleosides was further corroborated by high-resolution mass spectroscopy and infrared spectroscopy as well as by optical rotation measurements (see Experimental Section for details).

Conclusions

The stepwise chemical synthesis of C2′, C3′, C4′, and C5′ uniformly deuterated ribonucleosides (>97 atom % ^2H incorporation) reported herein is an improved alternative to the previous Raney-nickel-catalyzed proton−deuterium exchange[4] in that we have achieved a satisfactory level of deuteration at the C4 center. This new total synthesis of uridine-2′,3′,4′,5′,5″-$^2H_5$ (**21a**), adenosine-2′,3′,4′,5′,5″-$^2H_5$ (**21b**), cytidine-2′,3′,4′,5′,5″-$^2H_5$ (**21c**),

(22) Robins, M. J.; Zou, R. M.; Guo, Z. Q.; Wnuk, S. F. *J. Org. Chem.* **1996**, *61*, 9207.

(23) (a) Vorbrüggen, H.; Höfle, G. *Chem. Ber.* **1981**, *114*, 1256. (b) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.

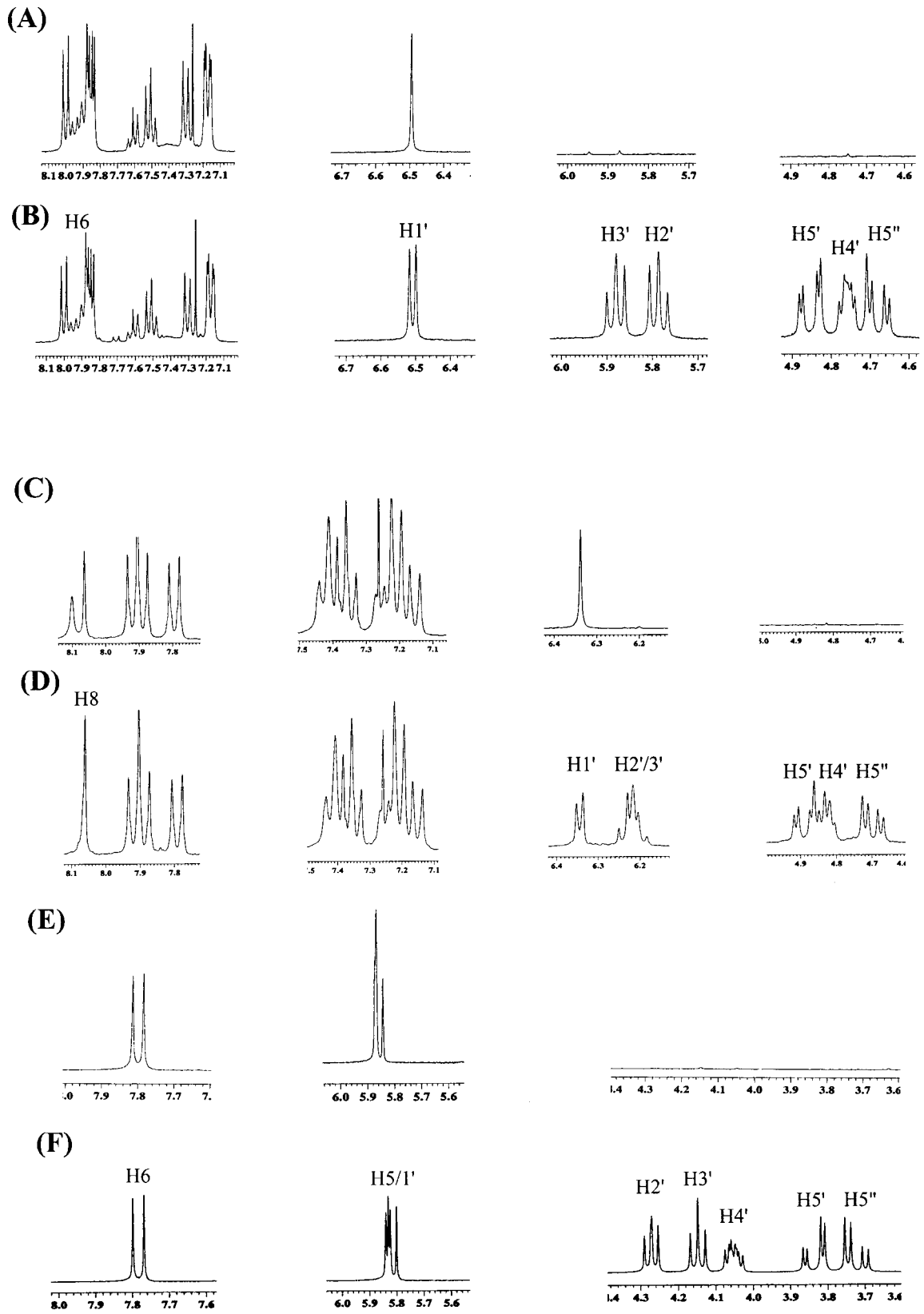


Figure 2. Expanded regions of the 270 MHz 1D ^{1}H NMR spectra of 2′,3′,5′-tri-*O*-(4-toluoyl)-N^4-benzoylcytidine-2′,3′,4′,5′,5″-$^{2}H_5$ (**20c**) (Panel A) and its natural abundance counterpart (Panel B), 2′,3′,5′-tri-*O*-(4-toluoyl)-O^6-diphenylcarbamoyl-N^2-acetylguanosine-2′,3′,4′,5′,5″-H_5 (**20d**) (Panel C) and its natural abundance counterpart (Panel D), uridine-2′,3′,4′,5′,5″-$^{2}H_5$ (**21a**) (Panel E) and its natural abundance counterpart (Panel F).

and guanosine-2′,3′,4′,5′,5″-$^{2}H_5$ (**21d**), (>97 atom % ^{2}H at C2′, C3′, C4′, and C5′/C5″), when they are deuterated at the corresponding aglycones,[8a,c] and introduced in a nonuniform manner to large oligo-RNA[8c] will successfully solve the spectral overcrowding problems, which was not earlier satisfactorily achievable owing to incomplete

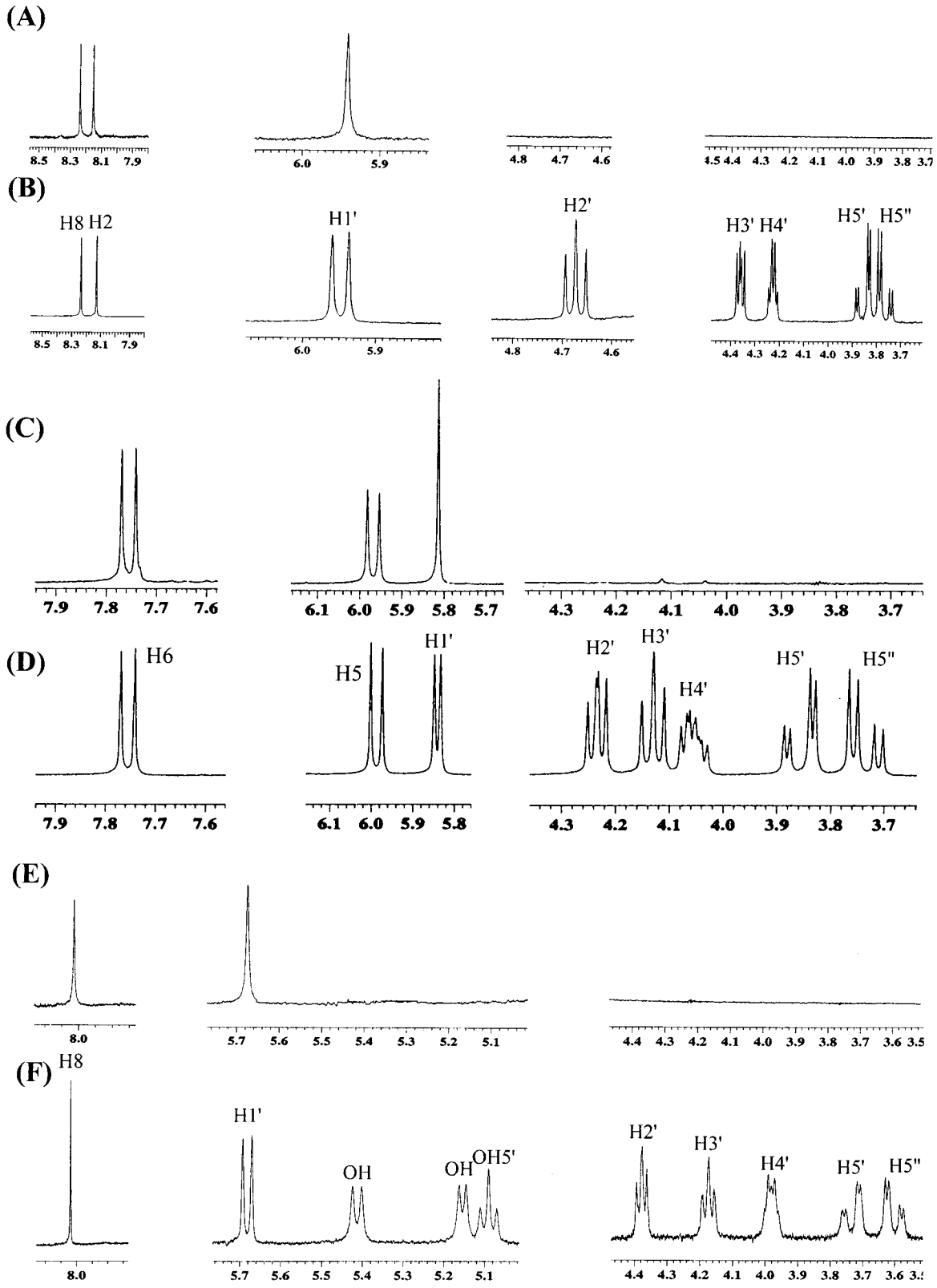


Figure 3. Expanded regions of the 270 MHz 1D ^{1}H NMR spectra of adenosine-2′,3′,4′,5′,5″-$^2H_5$ (**21b**) (Panel A) and its natural abundance counterpart (Panel B), cytidine-2′,3′,4′,5′,5″-$^2H_5$ (**21c**) (Panel C) and its natural abundance counterpart (Panel D), guanosine-2′,3′,4′,5′,5″-$^2H_5$ (**21d**) (Panel E) and its natural abundance counterpart (Panel F).

deuterium exchange at C4′ by Raney Ni/^2H_2O chemistry.[4] This report contains also diastereospecific methods for the deuterium incorporation at the C2 center of D-ribose-$^2H_4$ derivatives either via an oxidation−reduction−inversion sequence (46% overall yield from **1** to **8**) or a one-step deuterium−proton exchange (~25% overall yield from **1** to **8**). The 1-*O*-acetyl-2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-2,3,4,5,5′-$^2H_5$ (**19**) has been subsequently used for the chemical synthesis of partially deuterium labeled nucleosides which in turn can be used for the

synthesis of RNAs sequence specifically deuterated for structural studies using our Uppsala "NMR-window" concept.

Experimental Section

Dichloromethane, 1,2-dichloroethane, and acetonitrile were refluxed over phosphorus pentoxide followed by distillation under nitrogen and kept over molecular sieves (3 Å). Pyridine was stirred with calcium hydride overnight followed by distillation and stored over molecular sieves (3 Å). The chromatographic separations were performed on Merck G60 silica gel. Thin-layer chromatography was performed on Merck precoated silica gel 60 F_{254} glass-backed plates in following systems: (A) methanol−CH_2Cl_2 (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, (F) methanol−CH_2Cl_2 (10:90, v/v). (G) petroleum ether−ethyl acetate (8:2), (H) ethyl acetate−cyclohexane (30:70, v/v). ^{1}H NMR spectra were recorded with JEOL GX270 spectrometer at 270 MHz using TMS or acetonitrile (for D_2O solutions, set at δ 2.0 ppm) as internal standards. ^{13}C NMR spectra were recorded with JEOL GX270 spectrometer at 67.9 MHz using the central peak of $CDCl_3$ (δ 76.9 ppm), DMSO-d_6 (δ39.6) or CH_3CN (δ 1.3 ppm) as reference. Chemical shifts (δ) are reported in ppm. High-resolution CI(i-butane[i-Bu]) mass spectra were obtained on a VG-7035 MS mass spectrometer (VG. Analytical Ltd., Manchester, UK) equipped with an on-line VG-11-250J data system. The spectra were obtained at 40 eV with an emission current of 200 μA, source temperature 250 °C and the pressure of the chemical ionization reagent gas, isobutene, was adjusted to optimize the protonation of methyl stearate. Accurate mass measurements were carried out at a resolving power of 20000 using perfluorokerosene as a reference compound. HRMS FAB spectra were obtained on a VG-70HS spectrometer (VG. Analytical Ltd., Manchester, UK) at a resolution of $R = 8000$. Optical rotation data were measured on Perkin-Elmer 241 polarimeter. Infrared spectra were recorded with a Perkin-Elmer 298 spectrometer.

3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose-3,4,5,5′-$^2H_4$ (2). Compound **1** (10.37 g, 37.5 mmol) was dissolved in dry acetonitrile (160 mL). Benzyl bromide (5.35 mL, 45 mmol) and NaH (1.43 g, 47.7 mmol) were added to the solution at 0 °C, and the reaction mixture was stirred at room temperature overnight. Methanol was added, and stirring was maintained for an additional 2 h. The reaction mixture was partitioned between aqueous sat. $NaHCO_3$ and CH_2Cl_2. The organic phase was separated, dried over $MgSO_4$, and then evaporated. The residue was purified by column chromatography to afford compound **2** as yellow syrup (13.3 g, 35.4 mmol, 94%). R_f: 0.51 (System H). $[\alpha]^{28}_D$ +85° (*c* 0.24, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.4−7.2 (m, 10H) 2 × Ph-CH_2; 5.75 (d, $J_{H1,H2} = 3.5$ Hz, 1H) H-1; 4.75−4.47 (m, 4H) 2 × Ph-CH_2; 4.55 (d, 1H) H-2; 1.59 and 1.36 (2 × s, 6H) CH_3. ^{13}C NMR ($CDCl_3$) δ: 138.0, 137.6, 128.3, 128.2, 127.9, 127.8, 127.6, 127.5 (2 × Ph-CH_2); 112.8 (1,2-O-*C*[CH_3]$_2$); 104.0 (C-1); 77.2 (C-2); 73.3, 72.1 (2 × Ph-CH_2); 26.7, 26.4 (2 × CH_3). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{20}H_{23}{}^2H_4O_5$: 351.2110, found 351.2114.

1-*O*-Methyl-3,5-di-*O*-benzyl-α,β-D-ribofuranose-3,4,5,5′-$^2H_4$ (3). Carbohydrate derivative **2** (13.2 g, 35.4 mmol) was dissolved in dry methanol (∼100 mL). Concd sulfuric acid (10 drops) was added and mixture was heated at reflux for 3h. Solid $NaHCO_3$ was added for neutralization. The suspension was filtered and the liquid phase was evaporated to an oil. This was dissolved in CH_2Cl_2 and washed with aqueous sat. $NaHCO_3$. The organic phase was separated, dried over $MgSO_4$ and then evaporated to compound **3** (yellow syrup) (11.4 g, 33.0 mmol, 93%). R_f: 0.64 (System E). ^{1}H NMR ($CDCl_3$) δ: 7.4−7.2 (m, 10H) 2 × Ph-CH_2; 4.88 (d, $J_{H1,H2} = 4.6$ Hz, 0.26H) H-1α; 4.86 (d, $J_{H1,H2} = 0.9$ Hz, 0.74H) H-1β; 4.75−4.42 (m, 4H) 2 × Ph-CH_2; 4.02 (br s, 0.74H) H-2β; 3.48 (s, 0.78H) $CH_3\alpha$, 3.32 (s, 2.28H) $CH_3\beta$. ^{13}C NMR ($CDCl_3$) δ: 138.0, 137.8, 137.7, 137.0, 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5 (2 × Ph-CH_2); 108.4 (C-1β); 102.8 (C-1α); 73.3, 73.2, 73.1, 72.8, 72.6, 71.6 (C-2, 2 × Ph-CH_2); 55.5 (O$CH_3\alpha$), 54.9 (O$CH_3\beta$). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{20}H_{21}{}^2H_4O_5$: 349.1953, found 349.1959.

1-*O*-Methyl-3,5-di-*O*-benzyl-α,β-D-ribofuranose-2-ulose-3,4,5,5′-$^2H_4$ (4). Method A. Pyridinium dichromate (15.64 g; 41.6 mmol) was suspended in the mixture of dry CH_2Cl_2 (320 mL) and acetic anhydride (11.8 mL; 124.8 mmol). A solution of compound **3** (11.11 g; 32.0 mmol) in dry CH_2Cl_2 was added to the suspension, and the reaction mixture was refluxed at ∼80 °C for 3 h. After the dilution with ethyl acetate a precipitate was formed, and the solution was filtered through silica gel using ethyl acetate as eluent. The solvents were evaporated, and the residue was coevaporated with toluene to give an oily product (7.93 g; 23.0 mmol; 72%), which was taken to the next step without further purification. **Method B**. Ribose derivative **3** (1.04 g, 3 mmol) was dissolved in dry CH_2Cl_2 (30 mL). Dess−Martin reagent (7.59 g, 18 mmol) was added in dry CH_2Cl_2 (30 mL), and stirring was maintained overnight at room temperature. Diethyl ether was added, and the mixture was poured into cold saturated aqueous sodium bicarbonate containing $Na_2S_2O_3$. After shaking for a while, the organic layer was separated and extracted with saturated aqueous sodium bicarbonate. The organic layer was dried over $MgSO_4$ and evaporated to give the crude ketone **4** (1.03 g, 2.98 mmol, 99%). R_f: 0.67 (System E). IR ν_{max} ($CHCl_3$): 3018, 2929, 1780, 1495, 1388, 1368, 1217, 1150, 1113, 1085, 1048 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.4−7.2 (m, 10H) 2 × Ph-CH_2; 4.97−4.47 (m, 4H) 2 × Ph-CH_2; 4.83 (s, H-1α); 4.73 (s, H-1β); 3.48 (s, $CH_3\alpha$), 3.46 (s, $CH_3\beta$). ^{13}C NMR ($CDCl_3$) δ: 207.8, (C=Oα), 207.0 (C=Oβ), 137.9, 137.7, 137.5, 137.0, 136.97, 136.8, 128.4, 128.3, 128.3, 128.0, 127.9, 127.7, 127.7, 127.6, 127.5 (benzyl), 98.9 (C-1α), 98.6 (C-1β), 73.5, 73.4, 73.1, 72.5 (CH_2-benzyl), 56.0 ($OCH_3\beta$), 55.7 ($OCH_3\alpha$). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{20}H_{19}{}^2H_4O_5$: 347.1796, found 347.1804.

1-*O*-Methyl-3,5-di-*O*-benzyl-β-D-arabino/α-D-ribofuranose-2,3,4,5,5′-$^2H_5$ (5). The crude compound **4** was dissolved in dry ether (∼100 mL), and LAD (483 mg; 11.5 mmol) was added at 0 °C. The mixture was stirred for 6 h at room temperature. Ethyl acetate was added, and the mixture was partitioned between water and CH_2Cl_2. The organic phase was concentrated, and the residue was purified by column chromatography to yield the mixture of compound **5** (5.48 g; 15.7 mmol; 68%). R_f: 0.64 (System E). ^{1}H NMR ($CDCl_3$) δ: 7.4−7.2 (m, 10H) 2 × Ph-CH_2; 4.88, 4.85 (2 × s, 1H) H-1 (ribo and ara); 4.77−4.46 (m, 4H) 2 × Ph-CH_2; 3.47, 3.41 (2 × s, 3H) CH_3 (α and β). ^{13}C NMR ($CDCl_3$) δ: 138.0, 137.9, 137.8, 137.7, 128.5, 128.3, 128.25, 127.8, 127.7, 127.6, 127.56 (benzyl), 102.8 (ribo C-1), 102.6 (ara C-1), 73.3, 72.9 (2 × CH_2, ribo-benzyl), 73.1, 71.7 (2 × CH_2, ara-benzyl), 55.5 (ribo OCH_3), 55.3 (ara OCH_3). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{20}H_{20}{}^2H_5O_5$: 350.2016, found 350.2019.

1-*O*-Methyl-3,5-di-*O*-benzyl-2-*O*-(4-toluoyl)-α-D-ribofuranose-2,3,4,5,5′-$^2H_5$ (6) and 1-*O*-Methyl-3,5-di-*O*-benzyl-2-*O*-(4-toluoyl)-β-D-arabinofuranose-2,3,4,5,5′-$^2H_5$ (9). Compound **5** (4.64 g; 13.3 mmol) was coevaporated with dry pyridine and dissolved in the same solvent (130 mL). 4-Touoyl chloride (1.94 mL; 14.65 mmol) was added, and the mixture was stirred overnight. Saturated sodium bicarbonate solution was added, and the reaction mixture was stirred for 30 min. The mixture was extracted with CH_2Cl_2, and the organic phase was dried over $MgSO_4$. After removing the solvent under reduced pressure, the crude product was subjected to column chromatography to afford sugar derivatives **6** (1.44 g; 3.1 mmol; 23%) and **9** (4.0 g; 8.57 mmol; 65%). Compound **6**: R_f: 0.60 (System B). $[\alpha]^{26}_D$ +98° (*c* 0.67, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.04−7.23 (m, 14H) toluoyl, 2 × Ph-CH_2; 5.21 (s, 1H) H-1; 4.73−4.43 (m, 4H) 2 × Ph-CH_2; 3.46 (s, 3H) OCH_3; 2.42 (s, 3H) CH_3. ^{13}C NMR ($CDCl_3$) δ: 166.0 (C=O, toluoyl), 143.8, 137.8, 129.9, 129.0, 128.3, 128.2, 128.0, 127.6, 126.8, 126.7 (benzyl, toluoyl), 102.1 (C-1), 73.3 and 72.9 (2 × CH_2, benzyl), 55.7 (OCH_3), 21.6 (CH_3, toluoyl). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{28}H_{26}{}^2H_5O_6$: 468.2435, found 468.2441. Compound **9**: R_f: 0.48 (System G). $[\alpha]^{26}_D$ −74° (*c* 0.25, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.93−7.21 (m, 14H) toluoyl, 2 × Ph-CH_2; 5.21 (s, 1H) H-1; 4.70−4.59 (m, 4H) 2 × Ph-CH_2; 3.28 (s, 3H) OCH_3;

2.42 (s, 3H) CH_3. ^{13}C NMR ($CDCl_3$) δ: 165.9 (C=O, toluoyl), 143.9, 138.0, 137.7, 129.8, 129.0, 128.3, 127.9, 127.6, 126.6 (benzyl, toluoyl), 101.3 (C-1), 73.2, 71.9 (2 × CH_2, benzyl), 55.2 (OCH_3), 21.6 (CH_3, toluoyl). HRMS (FAB^+): $(M + H)^+$ calcd for $C_{28}H_{26}{}^2H_5O_6$: 468.2435, found 468.2439.

1-*O*-Methyl-3,5-di-*O*-benzyl-α-D-ribofuranoside-2,3,4,5,5′-$^2H_5$ (7). Compound **6** (1.44 g; 3.1 mmol) was treated with 1.0 N sodium methoxide solution in methanol (25 mL) in the same way as compound **10** to give ribose derivative **7** (0.99 g; 2.84 mmol; 92%). R_f: 0.64 (System E). $[\alpha]^{28}_D$ +117° (*c* 0.18, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.4−7.2 (m, 10H) 2 × Ph-CH_2; 4.88 (s, 1H) H-1; 4.75−4.42 (m, 4H) 2 × Ph-CH_2; 3.48 (s, 3H) OCH_3. ^{13}C NMR ($CDCl_3$) δ: 137.8, 137.7, 128.32, 128.28, 127.8, 127.7, 127.6, 127.5 (benzyl), 102.8 (C-1), 73.3, 72.9 (2 × CH_2, benzyl), 55.5 (OCH_3). HRMS (FAB^+): $(M + H)^+$ calcd for $C_{20}H_{20}{}^2H_5O_5$: 350.2016, found 350.2022.

Methyl α/β-D-ribofuranoside-2,3,4,5,5′-$^2H_5$ (8). The benzyl groups of the combined sugars **7** and **13** (3.32 g; 9.53 mmol) were cleaved using Pd/C−H_2 (721 mg) in ethanol (50 mL) overnight at room temperature. The reagent was filtered through Celite, and the filtrate was evaporated to dryness to afford methyl ribofuranoside derivative **8** (1.50 g; 8.87 mmol, 93%). R_f: 0.58 and 0.42 (System D). ^1H NMR (D_2O) δ: 4.92 (s, 0.25H) H-1α, 4.83 (s, 0.75H) H-1β; 3.36 (s, 0.75H) $OCH_3\alpha$, 3.33 (s, 2.25H) $OCH_3\beta$. ^{13}C NMR (CD_3OD) δ: 110.2 (C-1β), 104.9 (C-1α) 55.9 ($OCH_3\alpha$), 55.7 ($OCH_3\beta$). HRMS (CI-^{i}Bu): $(M + H)^+$ calcd for $C_6H_8{}^2H_5O_5$: 170.1077, found 170.1085.

1-*O*-Methyl-*O*-3,5-di-*O*-benzyl-β-D-arabinofuranose-2,-3,4,5,5′-$^2H_5$ (10). The sugar **9** (4.0 g, 8.57 mmol) was dissolved in 1.0 N sodium methoxide in methanol (75 mL), and the solution was stirred at room temperature for 30 min. The mixture was neutralized by addition of concd acetic acid and worked up using saturated $NaHCO_3$ solution in the usual way. Removal of solvents and purification on silica gel column gave compound **10** (2.7 g; 7.75 mmol; 91%). R_f: 0.64 (System E). $[\alpha]^{26}_D$ −42° (*c* 0.71, $CHCl_3$) δ: ^1H NMR ($CDCl_3$): 7.34−7.25 (m, 10H) benzyl; 4.85 (s, 1H) H-1; 4.77−4.56 (m, 4H) CH_2-benzyl 3.41 (s, 3H) OCH_3. ^{13}C NMR ($CDCl_3$) δ: 137.93, 137.87, 128.2, 127.8, 127.60, 127.53 (benzyl), 102.5 (C-1), 73.1, 71.7 (2 × CH_2), 55.3 (OCH_3). HRMS (FAB^+): $(M + H)^+$ calcd for $C_{20}H_{20}{}^2H_5O_5$: 350.2016, found 350.2022.

1-*O*-Methyl-3,5-di-*O*-benzyl-2-*O*-trifluoromethanesulfonyl-β-D-arabinofuranoside-2,3, 4,5,5′-$^2H_5$ (11). The sugar derivative **10** (3.52 g; 10.1 mmol) was coevaporated with dry pyridine, and it was dissolved in dry CH_2Cl_2 (∼75 mL) followed by the addition of 4-*N*,*N*-(dimethylamino)pyridine (4.32 g; 35.36 mmol) and pyridine (7.5 mL). The mixture was cooled to 0 °C, triflic anhydride (2.4 mL; 14.24 mmol) was added dropwise, and the resultant mixture was stirred at the same temperature for 3 h. The reaction mixture was poured into cold sat. sodium bicarbonate solution and extracted by CH_2Cl_2. The combined organic extract was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography to give the triflyl derivative **11** (4.06 g; 8.45 mmol; 84%). R_f: 0.59 (System G). $[\alpha]^{27}_D$ −64° (*c* 0.74, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.38−7.20 (m, 10H) benzyl; 4.99 (s, 1H) H-1; 4.65−4.45 (m, 4H) CH_2-benzyl; 3.38 (s, 3H) OCH_3. ^{13}C NMR ($CDCl_3$) δ: 137.6, 136.8, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, (benzyl), 118.4 (q, J_{CF} = 319.7 Hz, CF_3), 100.3 (C-1), 73.3 and 72.5 (2 × CH_2, benzyl), 55.4 (OCH_3). HRMS (FAB^+): $(M + H)^+$ calcd for $C_{21}H_{19}{}^2H_5F_3O_7S$: 482.1509, found 482.1514.

1-*O*-Methyl-3,5-di-*O*-benzyl-2-*O*-propionyl-β-D-ribofuranose-2,3,4,5,5′-$^2H_5$ (12). Cesium propionate (2.26 g; 11.0 mmol) was added to a solution of compound **11** (4.06 g; 8.45 mmol) in dry DMF (50 mL) and stirred for 36 h at room temperature. The DMF was removed under reduced pressure, water was added, and the compound was extracted with CH_2Cl_2. After removal of volatile matters, the residue was purified on a silica gel column to give ribofuranose derivative **12** (2.7 g; 6.73 mmol; 80%). R_f: 0.54 (System G). $[\alpha]^{27}_D$ +14° (*c* 0.71, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.35−7.22 (m, 10H) benzyl; 4.87 (s, 1H) H-1; 4.61−4.38 (m, 4H) 2 × CH_2-benzyl; 3.33 (s, 3H) OCH_3; 2.40 (q, 2H) OCH_2; 1.14 (*t*, 3H) CH_3. ^{13}C NMR ($CDCl_3$) δ: 173.5 (C=O), 138.1, 137.5, 128.2, 127.8, 127.7, 127.5, 126.7 (benzyl), 106.2 (C-1), 73.1 and 72.9 (2 × CH_2); 54.9 (OCH_3), 27.3 (OCH_2), 9.0 (CH_2CH_3). HRMS (FAB^+): $(M + H)^+$ calcd for $C_{23}H_{24}{}^2H_5O_6$: 406.2278, found 406.2285.

1-*O*-Methyl-3,5-di-*O*-benzyl-β-D-ribofuranoside-2,3,4,5,5′-$^2H_5$ (13). Compound **12** (2.7 g; 6.73 mmol) was treated with 1.0 N sodium methoxide solution in methanol (∼60 mL) as described for compound **10** to give ribose derivative **13** (2.33 g; 6.69 mmol; 99%). R_f: 0.64 (System E). $[\alpha]^{27}_D$ −29° (*c* 0.71, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.37−7.25 (m, 10H) benzyl, 4.86 (s, 1H) H-1; 4.57 (d, 4H) 2 × CH_2-benzyl; 3.31 (s, 3H) OCH_3. ^{13}C NMR ($CDCl_3$) δ: 138.1, 137.0, 128.5, 128.2, 128.1, 127.8, 127.5, 108.4 (C-1), 73.1, 72.6 (2 × CH_2-benzyl), 54.9 (OCH_3). HRMS (FAB^+): $(M + H)^+$ calcd for $C_{20}H_{20}{}^2H_5O_5$: 350.2016, found 350.2024.

2,3-*O*-Isopropylidene-α/β-D-ribofuranose-3,4,5,5′-$^2H_4$ (15). The 0.1% solution of H_2SO_4 in dry acetone was added to compound **14** (12.02 g, 61.9 mmol) (HRMS (CI-^{i}Bu): $(M + H)^+$ calcd for $C_5H_7{}^2H_4O_5$: 155.0858, found 155.0867), and the mixture was kept in a refrigerator at 4 °C for 3 d, followed by stirring the reaction mixture at room temperature for 24 h. Solid Na_2CO_3 was added to the solution for 2 h after which the residual solid material was filtered away. The acetone was evaporated, and the crude mixture was subjected to silica gel chromatography to obtain compound **15** (3.2 g, 16.7 mmol, 27%). R_f: 0.53 (System F). ^1H NMR ($CDCl_3$) δ: β-anomer: 5.41 (s, 1H) H-1; 4.58 (s, 1H) H-2; 1.49, 1.33 (2 × s, 6H) 2 × CH_3; α-anomer: 5.42 (d) H-1; 4.65 (d, $J_{H1,H2}$ = 4.2 Hz) H-2; 1.58, 1.40 (2 × *s*) 2 × CH_3. ^{13}C NMR ($CDCl_3$) δ: β-anomer: 112.0 ($C[CH_3]_2$); 102.7 (C-1); 86.6 (C-2); 26.2 and 24.6 (2 × CH_3); α-anomer: 114.1 ($C[CH_3]_2$); 96.8 (C-1); 79.4 (C-2); 26.0 and 24.6 (2 × CH_3). HRMS (CI-^{i}Bu): $(M + H)^+$ calcd for $C_8H_{11}{}^2H_4O_5$: 195.1171, found 195.1184.

2,3-*O*-Isopropylidene-α/β-D-ribofuranose-2,3,4,5,5′-$^2H_5$ (16). The deuterated 2,3-*O*-isopro-pylidene-α/β-D-ribose (**15**) (2.6 g; 13.5 mmol) was coevaporated with ^2H_2O and dissolved in the system dioxane/THF/triethylamine/^2H_2O (16/16/8/11 mL, v/v/v/v). The solution was heated at 90 °C for 5 days, and then volatile matters were evaporated to give compound **16** (2.55 g; 13.3 mmol; 98%) as brown oil. R_f: 0.53 (System F). ^1H NMR ($CDCl_3$) δ: β-anomer: 5.42 (s, 1H) H-1; 1.49, 1.32 (2 × *s*) 2 × CH_3; α-anomer: 5.43 (*s*) H-1; 1.58, 1.40 (2 × *s*) 2 × CH_3. ^{13}C NMR ($CDCl_3$) δ: β-anomer: 112.0 ($C[CH_3]_2$); 102.6 (C-1); 26.2, 24.6 (2 × CH_3); α-anomer: 114.1 ($C[CH_3]_2$); 96.8 (C-1); 26.0, 24.6 (2 × CH_3). HRMS (CI-^{i}Bu): $(M + H)^+$ calcd for C_8H_{10}-$^2H_5O_5$: 196.1232, found 196.1239.

Methyl α/β-D-ribofuranoside-2,3,4,5,5′-$^2H_5$ (8). Method A. The benzyl groups of the combined sugars **7** and **13** (3.32 g; 9.53 mmol) were cleaved using Pd/C−H_2 (721 mg) in ethanol (50 mL) overnight at room temperature. The reagent was filtered through Celite, and the filtrate was evaporated to dryness to afford the deuterated methyl ribofuranoside derivative **8** (1.50 g; 8.87 mmol, 93%) as an oil. **Method B.** The deuterated ribose derivative **16** (1.72 g; 9.05 mmol) was dissolved in 90% aqueous trifluoroacetic acid (27 mL) and stirred at room temperature for 1 h. The aqueous phase was evaporated, and the residual acid was removed by repeated coevaporations with water. The obtained crude compound **17** (1.41 g, 8.92 mmol, 98%) (HRMS (CI-^{i}Bu): $(M + H)^+$ calcd for $C_5H_6{}^2H_5O_5$: 156.0920, found 156.0928) was dissolved in dry methanol (30 mL) and a few drops of concd H_2SO_4 were added at 0 °C. The solution was kept in a refrigerator at 4 °C overnight and then neutralized passing through an Amberlist A-21 column (OH^- form) using methanol as an eluant. The methanol was evaporated to give compound **8** (1.46 g; 8.47 mmol; 95%) as a thick light yellow syrup. R_f: 0.59 and 0.42 (β and α, System D). ^1H NMR (D_2O) δ: 4.92 (s, 0.3H) α-H-1; 4.83 (s, 1H) β-H-1; 3.36 (s, 0.9H) $CH_3\alpha$; 3.33 (s, 3H) $CH_3\beta$. ^{13}C NMR (D_2O) δ: β-anomer: 108.1 (C-1); 55.3 (OCH_3); α-anomer: 103.3 (C-1); 55.6 (OCH_3).

1-*O*-Methyl-2,3,5-tri-*O*-(4-toluoyl)-α/β-D-ribofuranose-2,3,4,5,5′-$^2H_5$ (18). Compound **8** (1.50 g; 8.87 mmol) was coevaporated with dry pyridine and dissolved in the same solvent (80 mL). 4-Toluoyl chloride (4.6 mL, 34.6 mmol) was added under stirring. The mixture was kept overnight at ambient temperature. Saturated sodium bicarbonate solution was added, and stirring was maintained for 3 h. The compound

was extracted with CH_2Cl_2 from water. Volatile matters were removed under reduced pressure to give chromatographically homogeneous compound **18** (5.01 g; 8.87 mmol; 99%). R_f: 0.81 (System C). ^{1}H NMR ($CDCl_3$) δ: 7.98−7.10 (m, 12H) toluoyl; 5.36 and 5.13 (2 × s, 1H) H-1; 3.47 and 3.40 (2 × s, 3H) OCH_3; 2.41, 2.39, 2.36 (3 × s, 9H) CH_3. ^{13}C NMR ($CDCl_3$) δ: 166.3, 166.2, 166.0, 165.50, 165,3, 165.2 (C═O, toluoyl), 144.4, 144.1, 144.0, 143.9, 143.7, 130.1, 129.9, 129.8, 129.74, 129.70, 129.1, 128.9, 127.0, 126.82, 126.8, 126.5, 126.4, 126.2, 106.4 (C-1β), 101.9 (C-1α), 55.6 ($OCH_3\alpha$), 55.2 ($OCH_3\beta$), 21.6 ($CH_3\alpha$+β, toluoyl). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{30}H_{26}{}^2H_5O_8$: 524.2333, found 524.2337.

1-*O*-Acetyl-2,3,5-tri-*O*-(4-toluoyl)-α/*β*-D-ribofuranose-2′,3′,4′,5′,5″-$^2H_5$ (19). A cold mixture of acetic anhydride (5.0 mL), acetic acid (4.0 mL), and concd sulfuric acid (0.8 mL) was added to a solution of compound **18** (5.01 g; 8.87 mmol) in dry CH_2Cl_2 (25 mL) at 0 °C and stirred for 15 min. The reaction mixture was slowly poured into cold saturated $NaHCO_3$ solution, and stirring was maintained for 3 h. The acetyl derivative was extracted with CH_2Cl_2 from the water phase and dried over magnesium sulfate. The solvent was evaporated, and coevaporation with toluene furnished compound **19** (4.84 g, 8.78 mmol; 99%). The β-anomer was crystallized from methanol as a white solid (2.92 g; 5.3 mmol; 60%). R_f: 0.75 (System C). $[\alpha]^{26}_D$ +62° (*c* 1.04, $CHCl_3$). For natural $[\alpha]^{28}_D$ +63°. IR ν_{max} (KBr): 3062, 3039, 3015, 2920, 2848, 1750, 1758, 1738, 1620, 1410, 1370, 1285, 1274, 1220, 1177, 1110, 1092, 1070, 973 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 7.98−7.10 (m, 12H) toluoyl; 6.41 (s, 1H) H-1; 2.41, 2.40, 2.37 (3 × s, 9H) 3 × CH_3 toluoyl; 2.01 (s, 3H) CH_3 acetyl. ^{13}C NMR ($CDCl_3$) δ: 169.0 (C═O, acetyl), 166.0, 165.3, 164.9 (C═O, toluoyl), 144.3, 144.2, 143.8, 129.8, 129.7, 129.1, 129.0, 126.8, 126.0, 125.9 (toluoyl), 98.4 (C-1), 21.59, 21.54 (CH_3, toluoyl), 20.8 (CH_3, acetyl). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{31}H_{26}{}^2H_5O_9$: 552.2282, found 552.2287.

2′,3′,5′-Tri-*O*-(4-toluoyl)uridine-2′,3′,4′,5′,5″-$^2H_5$ (20a). Uracil (146 mg; 1.30 mmol) was suspended in hexamethyldisilazane (2.4 mL), and trimethylchlorosilane (0.25 mL) was added. The reaction mixture was stirred at 120 °C in nitrogen atmosphere for 4 h. The volatile materials were evaporated, and the residue was kept on oil pump for 20 min. Sugar **19** (552 mg; 1.0 mmol) was dissolved in dry 1,2-dichloroethane (12 mL), and this solution and trimethylsilyl trifluoromethanesulfonate (0.25 mL) were added to the persilylated nucleobase. The reaction was kept overnight at 32 °C in nitrogen atmosphere. Workup by saturated sodium bicarbonate solution and separation on silica gel column gave compound **20a** (534 mg; 0.89 mmol; 89%) as a white foam. R_f: 0.74 (System E). $[\alpha]^{26}_D$ −74° (*c* 0.75, $CHCl_3$). IR ν_{max} (KBr): 3028, 2920, 1728, 1690, 1609, 1451, 1376, 1280, 1210, 1179, 1108, 1092, 1019 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.47 (br d, 1H) N−H; 8.00−7.15 (m, 12H) toluoyl; 7.41 (d, 1H) H-6; 6.34 (s, 1H) H-1′; 2.43, 2.41, 2.38 (3 × s, 9H) 3 × CH_3 (toluoyl). ^{13}C NMR ($CDCl_3$) δ: 166.0, 165.3, 165.2 (3 × C═O); 162.1 (C-4); 149.8 (C-2); 144.60, 144.51, 144.5 (toluoyl); 139.3 (C-6); 129.9, 129.8, 129.6, 129.4, 129.1, 126.3, 125.8 125.5 (toluoyl); 103.2 (C-5); 87.4 (C-1′); 21.6 (CH_3 toluoyl). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{33}H_{26}{}^2H_5N_2O_9$: 604.2343, found 604.2349.

2′,3′,5′-Tri-*O*-(4-toluoyl)-*N*^{6}-benzoyladenosine-2′,3′,4′,5′,5″-$^2H_5$ (20b). N^6-Benzoyladenine (232 mg; 0.98 mmol) was condensed with sugar **19** (414 mg; 0.75 mmol) as described for compound **20a** to give **20b** as white foam (417 mg; 0.57 mmol; 76%). R_f: 0.68 (System E). $[\alpha]^{26}_D$ −97° (*c* 0.27, $CHCl_3$). IR ν_{max} (KBr): 3060, 3038, 2920, 1718, 1609, 1580, 1508, 1480, 1451, 1409, 1284, 1179, 1093, 1019 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 9.15 (s, 1H) NH, 8.71 (s, 1H) H-8; 8.19 (s, 1H) H-2; 8.04−7.15 (m, 17H) toluoyl, benzoyl; 6.50 (s, 1H) H-1′; 2.42, 2.41, 2.38 (3 × s, 9H) CH_3, toluoyl. ^{13}C NMR ($CDCl_3$) δ: 166.1, 165.3, 165.1 (3 × C═O, toluoyl), 164.4 (C═O, benzoyl), 152.6 (C-2), 151.7 (C-6), 149.5 (C-4), 144.6, 144.5, 144.2 (toluoyl), 141.5 (C-8), 133.4, 132.7 (benzoyl), 129.8, 129.7, 129.3, 129.2, 129.1, 128.8, 127.8, 127.3, 126.5, 125.9, 125.5 (toluoyl, benzoyl), 123.3 (C-5), 86.7 (C-1′), 21.6 (CH_3, toluoyl). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{41}H_{31}{}^2H_5N_5O_8$: 731.2878, found 731.2885.

2′,3′,5′-Tri-*O*-(4-toluoyl)-*N*^{4}-benzoylcytidine-2′,3′,4′,5′,5″-$^2H_5$ (20c). N^4-Benzoylcytosine (280 mg; 1.3 mmol) and deuterated sugar **19** (550 mg, 1.0 mmol) were condensed following the above procedure at 70 °C overnight. After usual workup and purification, compound **20c** (526 mg; 0.75 mmol; 75%) was isolated as a white foam. R_f: 0.72 (System E). $[\alpha]^{26}_D$ −68° (*c* 0.43, $CHCl_3$). IR ν_{max} (KBr): 3060, 3035, 2920, 1722, 1669, 1625, 1610, 1550, 1480, 1279, 1246, 1179, 1109, 1091, 1019 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.78 (br s, 1H), NH; 8.02−7.83 (m, 9H) H-6, toluoyl, benzoyl; 7.64−7.48 (m, 3H) benzoyl; 7.32−7.15 (m, 7H) H-5, toluoyl, benzoyl; 6.49 (s, 1H) H-1′; 2.44, 2.40, 2.38 (3 × s, 9H) CH_3, toluoyl. ^{13}C NMR ($CDCl_3$) δ: 166.1, 165.3, 165.2 (C═O, toluoyl), 162.2 (C-4), 153.8 (C-2); 144.44, 144.4, 144.38 (toluoyl), 144.32 (C-6), 133.2, 129.9, 129.8, 129.6, 129.4, 129.1, 128.9, 127.6, 126.4, 125.8, 125.7 (toluoyl, benzoyl), 97.3 (C-5), 88.9 (C-1′), 21.6 (CH_3, toluoyl). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{40}H_{31}{}^2H_5N_3O_9$: 707.2765, found 707.2771.

2′,3′,5′-Tri-*O*-(4-toluoyl)-*N*^{2}-acetyl-*O*^{6}-diphenylcarbamoylguanosine-2′,3′,4′,5′,5″-$^2H_5$ (20d). N^2-Acetyl-O^6-diphenylcarbamoyl-guanine (378 mg; 0.98 mmol) was suspended in dry 1,2-dichloroethane (6.0 mL), and bis(trimethylsilyl)acetamide (0.32 mL) was added. The mixture was heated at ∼90 °C under nitrogen for 1 h. The volatile materials were evaporated, and after a coevaporation with dry toluene the residue was kept on an oil pump for 20 min. Sugar derivative **19** (414 mg; 0.75 mmol) was added in dry toluene (11.0 mL) to the persilylated nucleobase followed by trimethylsilyl trifluoromethanesulfonate (0.2 mL). The reaction was kept at ∼83 °C overnight. After $NaHCO_3$ workup, crude nucleoside derivative was subjected to column chromatography to yield pure compound **20d** (426 mg; 0.47 mmol; 63%) as white foam. R_f: 0.75 (System E). $[\alpha]^{26}_D$ −36° (*c* 1.03, $CHCl_3$). IR ν_{max} (KBr): 3061, 3039, 2920, 1725, 1610, 1589, 1508, 1489, 1450, 1409, 1370, 1278, 1210, 1178, 1093, 1058, 1018, 979 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.10 (br s, 1H) N−H; 8.06 (s, 1H) H-8; 7.93−7.13 (m, 22 H) phenyl, toluoyl; 6.33 (s, 1H) H-1′; 2.47 (s, 3H) N^2−C(O)CH_3; 2.41, 2.37 (2 × s, 9H) 3 × CH_3 (toluoyl). ^{13}C NMR ($CDCl_3$) δ: 170.0 (C(O)-CH_3); 166.1, 165.2, 165.0 (3 × C═O, toluoyl); 156.3 (C-6); 154.3 (C-4); 152.2 (C-2); 150.1 (DPC); 144.6, 144.4, 144.1 (toluoyl); 142.1 (C-8); 141.6 (DPC); 129.7, 129.6, 129.2, 129.1; 126.8, 126.4, 125.9, 125.5 (DPC, toluoyl); 121.1 (C-5); 87.1 (C-1′); 25.0 (C(O)CH_3); 21.6 (CH_3, toluoyl). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{49}H_{38}{}^2H_5N_6O_{10}$: 880.3354, found 880.3359.

Uridine-2′,3′,4′,5′,5″-$^2H_5$ (21a). Nucleoside **20a** (500 mg; 0.87 mmol) was dissolved in methanolic ammonia (50 mL) and stirred at room temperature for 3 days. Solvent was evaporated, and the residue was dissolved in water and extracted two times with CH_2Cl_2 and then with diethyl ether. Evaporation of aqueous phase gave uridine **21a** (215 mg; 0.86 mmol; 99%). $[\alpha]^{26}_D$ +9° (*c* 0.2, H_2O); $[\alpha]^{26}_D$ for natural uridine +10°. IR ν_{max} (KBr): 3346, 3103, 2918, 2798, 1776, 1670, 1467, 1418, 1390, 1358, 1320, 1263, 1212, 1178, 1150, 1126, 1085, 1060, 1038, 975, 961, 950, 900, 825, 766 cm^{-1}. ^{1}H NMR (D_2O) δ: 7.79 (d, $J_{H5,H6}$ = 8.1 Hz, 1H) H-6; 5.83 (s, 2H) H-1′, H-5. ^{13}C NMR (D_2O) δ: 166.4 (C-4); 151.9 (C-2); 142.0 (C-6); 102.5 (C-5); 89.6 (C-1′). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_9H_8{}^2H_5N_2O_6$: 250.1087, found 250.1091.

Adenosine-2′,3′,4′,5′,5″-$^2H_5$ (21b). Nucleoside **21b** (112 mg; 0.41 mmol; 82%) was obtained as white powder after deprotection of **20b** (365 mg; 0.5 mmol) in methanolic ammonia. $[\alpha]^{26}_D$ −58° (*c* 0.2, H_2O). For natural $[\alpha]^{26}_D$ −60°. IR ν_{max} (KBr): 3422, 3160, 2798, 1678, 1659, 1603, 1573, 1474, 1417, 1379, 1338, 1292, 1245, 1208, 1177, 1160, 1130, 1111, 1073, 992, 970, 843, 819, 792, 752, 730 cm^{-1}. ^{1}H NMR (D_2O) δ: 8.32 (s, 1H) H-8; 8.24 (s, 1H) H-2; 6.06 (s, 1H) H-1′. ^{13}C NMR (DMSO-d_6) δ: 156.2 (C-6), 152.4 (C-2), 149.1 (C-4), 140.0 (C-8), 119.4 (C-5), 87.9 (C-1′). HRMS (FAB$^+$): $(M + H)^+$ calcd for $C_{10}H_9{}^2H_5N_5O_4$: 273.1359, found 273.1363.

Cytidine-2′,3′,4′,5′,5″-$^2H_5$ (21c). Compound **20c** (493 mg; 0.7 mmol) was stirred for 3 days in methanolic ammonia followed by removal of methanol. The residue was dissolved in water and extracted with CH_2Cl_2 and diethyl ether to afford **21c** (156.mg; 0.63 mmol; 90%). $[\alpha]^{26}_D$ +32° (*c* 0.08, H_2O). For natural $[\alpha]^{27}_D$ +33°. IR ν_{max} (KBr): 3332, 3200, 2920, 2780,

1645, 1602, 1525, 1488, 1400, 1369, 1289, 1208, 1132, 1050, 969, 784 cm^{-1}. ^1H NMR (D_2O) δ: 7.76 (d, $J_{H5,H6} = 7.6$ Hz, 1H) H-6; 5.97 (d, 1H) H-5; 5.81 (s, 1H) H-1′. ^{13}C NMR (D_2O) δ: 166.4 (C-4), 157.8 (C-2), 141.9 (C-6), 96.4 (C-5), 90.5 (C-1′). HRMS (FAB^+): $(M + H)^+$ calcd for $C_9H_9{}^2H_5N_3O_5$: 249.1247, found 249.1252.

Guanosine-2′,3′,4′,5′,5″-$^2H_5$ (21d). Compound **20d** (363 mg, 0.4 mmol) was deprotected upon stirring in methanolic ammonia (50 mL) for 3 days at room temperature. After evaporation of the methanol, the residue was dissolved in water and extracted with CH_2Cl_2 (2×) and then with diethyl ether. Evaporation of the aqueous phase gave compound **21d** as white solid (99 mg; 0.34 mmol; 85%). $[\alpha]^{26}_D$ −36° (*c* 0.04, H_2O); $[\alpha]^{26}_D$ for natural guanosine −37°. IR ν_{max} (KBr): 3470, 3320, 3203, 2850, 2730, 1738, 1690, 1620, 1531, 1482, 1421, 1383, 1330, 1241, 1180, 1138, 1079, 1058, 1018, 982, 970, 910, 887, 820, 681 cm^{-1}. ^1H NMR (DMSO-d_6/D_2O) δ: 8.11 (s, 1H) H-8; 5.99 (s, 1H) H-1′. ^{13}C NMR (DMSO-d_6) δ: 156.8 (C-6); 153.7 (C-2); 151.4 (C-4); 135.6 (C-8); 116.8 (C-5); 86.4 (C-1′). HRMS (FAB^+): $(M + H)^+$ calcd for $C_{10}H_9{}^2H_5N_5O_5$: 289.1309, found 289.1314.

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