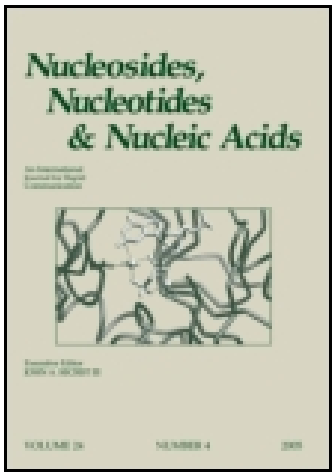

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Studies Towards the Large Scale Chemical Synthesis of the Precursors of Ribonucleosides-3′,4′,5′,5″-$^{2}H_{4}$ and -2′,3′,4′,5′,5″-$^{2}H_{5}$

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Studies Towards the Large Scale Chemical Synthesis of the Precursors of Ribonucleosides-3′,4′,5′,5″-$^2H_4$ and -2′,3′,4′,5′,5″-$^2H_5$[#]

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ABSTRACT

A summary delineating the large scale synthetic studies to prepare labeled precursors of ribonucleosides-3′,4′,5′,5″-$^2H_4$ and -2′,3′,4′,5′,5″-$^2H_5$ from D-glucose is presented. The recycling of deuterium-labeled by-products has been devised to give a high overall yield of the intermediates and an expedient protocol has been elaborated for the conversion of 3-*O*-benzyl-α,β-D-allofuranose-3,4-d_2 **6** to 1-*O*-methyl-3-*O*-benzyl-2-*O*-t-butyldimethylsilyl-α,β-D-ribofuranose-3,4,5,5′-d_4 **16** (precursor of ribonucleosides-3′,4′,5′,5″-$^2H_4$) or to 1-*O*-methyl-3,5-di-*O*-benzyl-α,β-D-ribofuranose-3,4,5,5′-d_4 **18** (precursor of ribonucleosides-3′,4′,5′,5″-$^2H_4$).

Key Words: Isotope labeling; Site specific; Stable isotope; Deuterium; Ribofuranose; Deuterated nucleosides.

[#]In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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INTRODUCTION

Amongst various physicochemical methods used to elucidate the correlation between structural motifs and biological functions of oligo-DNA or -RNA, NMR spectroscopy has emerged as particularly important since it gives insight into the structure and its time dependent change (dynamics) at atomic resolution under a quasi-physiological condition. In order to overcome molecular size related problems such as spectral overlap and the deleterious effect of nuclear relaxation, different isotope labeling techniques have been devised[1–3] and exploited.[4] Sequence specific deuteration[5] has been proven to facilitate the NMR structure determination of large RNAs[6] and DNAs.[7]

Incorporation of the 3,4,5,5′-d_4 labeled nucleoside blocks shows some considerable advantages in the NMR structure elucidation:[8] It reduces the spectral crowding in the δ 4–5 ppm ribose region. The H1′-H2′ nOe cross-peaks can unambiguously be identified and 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, which is consistent with our earlier findings on labeled oligo-DNA.[6d]

It was also expected that this reduction of the relaxation efficiency could significantly improve the NOESY spectra of larger RNAs. Later it has been shown that uniform ^{13}C- and 3,4,5,5′-d_4 double labelling[9] can be used for the simplification of the relatively crowded C2′/C3′ region in the ^{13}C dimension of ^{13}C edited 2D spectra. These results and the idea that the synthesis of 3′,4′,5′,5″-$^2H_4$-nucleosides could be extended to the stepwise synthesis of 2′,3′,4′,5′,5″-$^2H_5$ derivatives for the creation of NMR invisible stretches in our Uppsala "NMR-window" approach[5a-b,6c–e] prompted us to develop their D-glucose based preparation.[10] We herein report on the large scale preparation of the appropriately protected 3,4,5,5′-$^2H_4$-ribofuranoside precursors.

RESULTS AND DISCUSSION

The synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-ribofuranose-$^2H_4$ (**10**) follows our published procedure[10] with two important modifications (Sch. 1, the scales indicated for the particular reactions are the sums of 4–5 simultaneous reactions). Due to the long equilibration (22 days) time required to convert the C4-protio counterpart of **8** to **8**,[10] the deuterium incorporation at C4 was achieved upon a treatment of 3-ulose **2** in pyridine-^2H_2O at 95°C (see experimental section).[11] In order to achieve the required >97 atom% isotope substitution, a cycle consisting of 20 min heating at 95°C and 1-day standing at room temperature was repeated 6 times. After the third cycle of exchange, the mixture of solvents was removed in vacuo and a fresh mixture of pyridine-^2H_2O was added. This procedure is somewhat lengthier but gives significantly higher deuterium isotope incorporation (>97 atom% isotope substitution) than the published procedure which involved a cycle of 5 min heating at 95°C and 18 h standing at room temperature and change the exchange media after each cycle.[11] The latter procedure gives however only 95 atom% isotope substitution and consumes more ^2H_2O than the our present procedure. It should be mentioned that during the deuterium equilibration, the C4 center of ketone **2** is epimerized to

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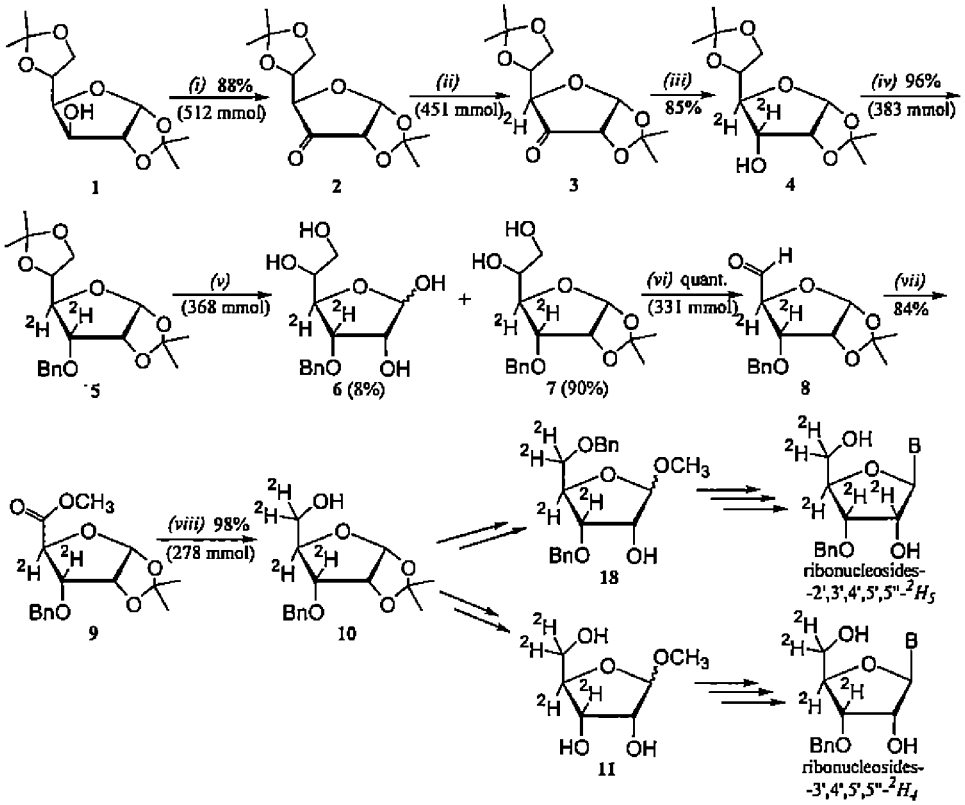
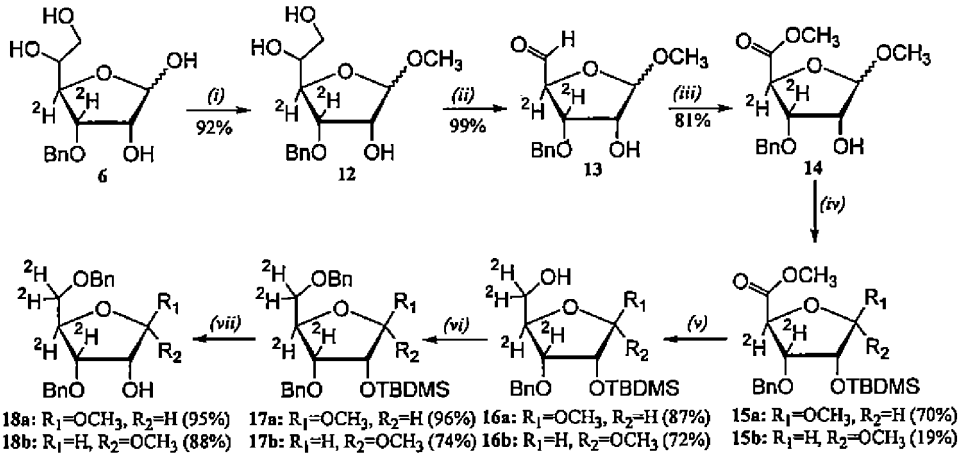


Scheme 1. *Abbreviations*: Bn = benzyl. *Conditions*: (i) PDC, acetic anhydride, dry dichloromethane (DCM), reflux, 3 h; (ii) Pyridine/^2H_2O (3.2 mL/0.8 mL/mmol), 95 °C for 20 min, then 1 day, r.t., repeated 6 times; (iii) $LiAlD_4$ in dry diethyl ether or $NaBD_4$ in ethanol, r.t.; (iv) BnCl, NaH in dry acetonitrile, r.t., overnight; (v) 80% aqueous acetic acid, r.t., 16–18 h; (vi) $NaIO_4$, ethanol/H_2O (1:1, v/v), r.t., 3.5 h; (vii) bromine in methanol/H_2O/$NaHCO_3$, r.t, 4 h; (viii) $LiAlD_4$, dry diethyl ether, r.t, 4 h.

give *gulo* counterpart of **3** along with the major *allo/gluco* product **3**. After the reduction, the mixture of 1,2:5,6-di-*O*-isopropylidene-α-D-*gulo*furanose-3,4-$^2H_2$ diastereomer (ca 10%) and 1,2:5,6-di-*O*-isopropylidene-α-D-*allo*furanose-3,4,$^2H_2$ (**4**) (*allo* is formed over *gluco* owing to the hydride delivery from the β-face) was separated by column chromatography to give the desired *allo*furanose **4** (85% in 2-steps) and 1,2:5,6-di-*O*-isopropylidene-α-D-gulofuranose-3,4-$^2H_2$.

Another important modification was to separate the low R_f compound **6** formed in 8% yield from the desired product **7** in the acid deprotection of **5** [step (*v*)]. This was achieved in the following manner: after the removal of the volatile matter, the residual deprotected mixture still contained acetic acid which was first carefully removed by coevaporation with toluene. The acid-free residue was then partitioned between water and dichloromethane. From the aqueous phase the 3-*O*-benzyl derivative **6** could be recovered in substantially pure form, which is an important



Scheme 2. *Abbreviations*: Bn = benzyl, TBDMS = *tert*-butyldimethylsilyl. *Conditions*: (i) conc. H_2SO_4 in dry methanol, 4°C, 4 days; (ii) $NaIO_4$, ethanol/H_2O (1:1, v/v), r.t, 3.5 h; (iii) bromine in methanol/H_2O/$NaHCO_3$, r.t., 4 h; (iv) TBDMS-Cl, pyridine, $AgNO_3$ in dry THF; (v) $LiAlD_4$, dry diethyl ether, r.t, 4 h; (vi) BnBr, NaH in dry acetonitrile, r.t., overnight; (vii) 1.0 M TBAF in dry THF.

improvement because this by-product bears 2 deuterium labels, and in the large scale synthesis this 8% by-product accumulates to be a considerable amount (~28 mmol).

Thus, it is clear from the above discussion (Sch. 1) that for the improvement of the overall yield of either **11** or **18**, we have two possibilities: (1) recycling the C4 epimeric *gulo* counterpart of **4** or (2) finding an appropriate recycling of **6**. Since the former might be carried out through the equilibration of the gulofuranose-3-ulose, one practically looses the two deuterium labels already present in the molecule. Hence it was more attractive to find a salvage pathway for compound **6** (Sch. 2).

The recycling commences (Sch. 2) with the preparation of methyl glucofuranoside **12** (92%) (from **6**) in dry methanol with concentrated H_2SO_4 at ~4°C. The ^{13}C chemical shifts for C1 (108.9 ppm for β, 102.4 ppm for α) indicate the presence of the furanose ring. The free C5-C6 diol system of **12** was cleaved using $NaIO_4$ in ethanol-water to give the corresponding C5 aldehyde **13** (99%), which was taken to the next step without further purification. The oxidation of **13** directly to the uronic acid methyl ester **14** with bromine in methanol-water and in presence of sodium bicarbonate as buffer proceeded with a good yield (81%). According to our earlier findings[10], the protection of the C2-hydroxyl function is mandatory in order to successfully reduce the methyl ester functionality of **14**. The introduction of a 2-*O*-TBDMS group made the separation of the anomers also possible by short column chromatography giving the β-anomer **15a** as predominant product (70%) over the α-anomer **15b** (19%). From this point, subsequent reactions were carried out separately with the anomerically pure compounds. The ester group of **15a-b** was reduced with lithium aluminium deuteride in dry diethyl ether to give the partially protected tetradeuterio methyl ribofuranosides **16a** (87%) and **16b** (72%). The inspection of the ^1H-NMR spectrum showed the absence of H5/5′ signals revealing >97 atom%

incorporation of deuterium at C5. This was further corroborated by the absence of the C5 signal in the ^{13}C spectra of **16a-b**. It is necessary to emphasize here that in the IR spectra of **16a** and **16b** we observed C-D stretching bands in the region[12] of 2300–2000 cm^{-1}, which were however not observable for any other deuterated precursors of **16a**/**16b**. Compounds **16a-b** can directly be deprotected to **11** in two steps giving an additional crop of the methyl ribofuranoside-3,4,5,5′-$^{2}H_4$, (∼12 mmol). For the synthesis of methyl ribofuranoside-2,3,4,5,5′-$^{2}H_5$,[5b] which includes an oxidation-reduction-inversion sequence at C2, the C5-OH was protected as benzyl ether upon a treatment of **16a-b** with NaH and benzyl bromide in dry acetonitrile. The relatively lower yield for the α-anomer **17b** (74%) compared to that of **17a** (96%) might indicate a higher level of steric hindrance at the α-face for this series of compounds. Removal of the TBDMS protecting group with the usual treatment with 1M TBAF in dry tetrahydrofurane afforded the deuterated compounds **18a** (95%) and **18b** (88%) which are the key intermediates in the synthesis of ribonucleosides-2′,3′,4′,5′,5″-$^{2}H_5$.[5b] Compounds **18a-b** were obtained in an amount (10.5 mmol) which represents ∼3% overall improvement on the described ∼270 mmol scale synthesis of this precursor **18**.

EXPERIMENTAL SECTION

Pyridine was distilled after refluxing with calcium hydride and it was kept over molecular sieves (4Å). Dichloromethane, 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 (10:90, v/v), (B) ethyl acetate-cyclohexane (1:3, v/v) (C) methanol-DCM (5:95), (D) ethyl acetate-cyclohexane (1:1, v/v). ^{1}H-NMR spectra were recorded with Jeol GX 270 spectrometer at 270.17 MHz, using TMS (0.0 ppm) peak as internal standards. ^{13}C-NMR spectra were recorded with Jeol GX 270 spectrometer at 67.9 MHz using the central peak of $CDCl_3$ (76.9 ppm) as internal standard. Chemical shifts are reported in ppm (δ scale). 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-3,4-$^{2}H_2$ (4). Pyridinium dichromate (46.0 g, 72.2 mmol) was added to dry dichloromethane (420.0 mL) followed by acetic anhydride (37.0 mL, 227.3 mmol). To the stirred suspension 1,2:5,6-di-*O*-isopropylidene-α-D-glucose (**1**) (31.7 g, 121.8 mmol) was added and the mixture was boiled at ∼75°C for 3.5 h. The mixture was cooled to room temperature, diluted with ethyl acetate and the precipitate was filtered. The filtered solid was washed with ethyl acetate then the solvent was evaporated and the oil was repeatedly co-evaporated with toluene. Diethyl ether was added, and the solution was filtered again. After evaporation of the solvent, this procedure was repeated once more to give an oily product (25.7 g, 82%). ^{1}H-NMR ($CDCl_3$): 6.14 (*d*, $J_{H1,H2} = 4.4$ Hz, 1H) H-1; 4.4–3.9 (*m*, 5H) H-2,4,5,6,6′; 1.46, 1.44, 1.34 (3x*s*, 12H) 4xCH_3. The procedure was

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repeated on scales (18.6 g, 31.7 g and 2 × 25.6 g, altogether 512.0 mmol) of **1** giving altogether 116.6 g (451.6 mmol) ketone **2**. 3-Ulose 2 (33.9 g, 131.2 mmol) was dissolved in a mixture of dry pyridine (420 mL and ^2H_2O (105 mL). The solution was heated at ~95 °C for 20 min, then kept at room temperature till next day. This was repeated twice more, then the solvent was removed and the same mixture of fresh solvent was added. After three heating an wait periods, reduction of an aliquot with $LiAlD_4$ showed appropriate level of deuteration (>97 atom%). Solvents were removed, the residual oil was co-evaporated with dry toluene (3x), then it was dissolved in dry ether (300 mL) and reduced with $LiAlD_4$ (2.05 g, 48.8 mmol) to give compound **4** (30.9 g, 90%). ^{1}H-NMR ($CDCl_3$): 5.82 (*d*, $J_{H1,H2} = 3.8$ Hz, 1H) H-1; 4.61 (*d*, 1H) H-2; 4.31 (*t*, 1H) H-5; 4.11–3.99 (*m*, $J_{H6,H5} = 6.6$ Hz, $J_{H6',H5} = 6.6$ Hz, $J_{H6,H6'} = 8.5$ Hz, 2H) H-6/6′; 2.54 (*s*, 1H) OH; 1.58, 1.47, 1.39, 1.38 (4x*s*, 12H) 4xCH_3. ^{13}C-NMR ($CDCl_3$): 112.7 (1,2-O-*C*[CH_3]$_2$); 109.7 (5,6-O-*C*[CH_3]$_2$); 103.8 (C-1); 78.8 (C-2); 75.4 (C-5); 65.7 (C-6); 26.5, 26.4, 26.2, 25.2, (4x*C*H_3).

1-*O*-Methyl-3-*O*-benzyl-α,β-D-allofuranose-3,4-d_2 (12). Sugar derivative **6** (7.73 g, 28.6 mmol) was dissolved in dry methanol (100 mL) and conc. sulfuric acid (500 μL) was added at 0°C. The reaction mixture was kept in a refrigerator at 4°C for 4 days. Solid $NaHCO_3$ was added for neutralization of the acid. After filtering away the excess solid salt, the methanol was evaporated, the residue was taken up in dichloromethane and extracted with aqueous sat. sodium bicarbonate. The organic phase was dried over $MgSO_4$, filtered and evaporated to obtain **12** (7.55 g, 92%) as a white solid. R_f: 0.55 (System A). ^{1}H-NMR ($CDCl_3$): 7.42–7.30 (*m*, 5H) benzyl; 4.86 (d, $J_{H1,H2} = 4.6$ Hz,) H-1 α; 4.85 (d, $J_{H1,H2} = 0.6$ Hz), H-1 β; 4.77–4.55 (*m*, 2H) CH_2-benzyl; 4.09 (*m*) H-2 α; 4.02 (*s*) H-2 β; 3.83–3.55 (*m*, 3H) H-5, H-6,6′; 3.46 (*s*) OCH_3 α; 3.38 (*s*) OCH_3 β. ^{13}C-NMR ($CDCl_3$): 137.5, 136.7, 128.6, 128.4, 128.3, 128.1, 128.0, 127.9 (benzyl), 108.9 (C-1 β), 102.4 (C-1 α), 73.2 (C-2), 72.9 (Ph-*C*H_2, β), 72.8 (Ph-*C*H_2, α), 72.2 (C-5 β), 71.6 (C-5 α), 63.2 (C-6), 55.6 (O*C*H_3).

1-*O*-Methyl-3-*O*-benzyl-α,β-D-*ribo*-pentodialdo-1,4-furanose-3,4-d_2 (13). $NaIO_4$ (5.9 g, 27.7 mmol) was dissolved in a mixture of ethanol (75.0 mL) and water (75.0 mL). This solution was added to sugar **12** (7.55 g, 26.4 mmol) and the reaction mixture was stirred for 3.5 h at ambient temperature. The white precipitate was filtered and a few drops of ethylene glycol were added to the filtrate. The precipitate was filtered again and the solution phase was evaporated. Ethanol was added to the residue and the precipitate was filtered and this procedure was repeated until no further precipitation occurred. The oily residue was kept on an oil pump to give aldehyde **13** (6.68 g, 99%).

Methyl 1-*O*-Methyl-3-*O*-benzyl-α-β-D-ribofuranuronate-3,4-d_2 (14). The crude deuterated aldehyde **13** (6.68 g, 26.3 mmol) was dissolved in mixture of methanol (51.5 mL) and H_2O (5.7 mL) to obtain a 0.5 *M* solution. $NaHCO_3$ (91.6 g, 114.4 mmol) was added followed by a solution of bromine (14.7 mL) in a mixture of methanol (129 mL) and H_2O (14 mL). The reaction mixture was stirred for 4 h at ambient temperature, 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

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$MgSO_4$, filtered and evaporated. The residue was separated on silica gel column yielding compound **14** as yellow oil (6.0 g, 81%). R_f: 0.51 (α), 0.60 (β) (System D). IR ν_{max} (neat): 3490, 3082, 3059, 3025, 2994, 2950, 2926, 2835, 1745, 1493, 1450, 1434, 1279, 1192, 1140, 1110, 1080, 1050, 989, 960 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.4–7.3 (*m*, 5H) *Ph*-CH_2; 5.02 (d, $J_{H1,H2} = 4.8$ Hz, 1H) H-1 α; 4.94 (d, $J_{H1,H2} = 0.6$ Hz, 1H) H-1 β; 4.75–4.63 (*m*, 2H) Ph-CH_2; 4.14 (*dd*, 1H) H-2 α; 4.02 (*d*, 1H) H-2 β; 3.77 (*s*, 3H) C(O)OCH_3 β; 3.74 (*s*, 3H) C(O)OCH_3 α; 3.50 (*s*, 3H) OCH_3 α; 3.40 (*s*, 3H) OCH_3 β; 2.91 (*d*, $J_{OH,H2} = 11.4$ Hz, 1H) OH α; 2.74 (*d*, $J_{OH,H2} = 2.6$ Hz, 1H) OH β. ^{13}C-NMR ($CDCl_3$): 171.7 (C=O β), 170.6 (C=O α), 137.0, 136.7, 128.5, 128.2, 127.9 (*Ph*-CH_2); 109.0 (C-1 β); 103.1 (C-1 α); 73.2 (C-2), 73.0, 72.9, 71.2 (Ph-CH_2); 55.9 (OCH_3 α), 55.2 (OCH_3 β), 52.4 (C(O)OCH_3 α), 52.2 (C(O)OCH_3 β).

Methyl 1-*O*-Methyl-3-*O*-benzyl-2-*O*-*t*-butyldimethylsilyl-β-D-ribofuranuronate-3,4-d_2 (15a) and Methyl 1-*O*-Methyl-3-*O*-benzyl-2-*O*-*t*-butyldimethylsilyl-α-D-ribofuranuronate-3,4-d_2 (15b). The ester **14** (4.61 g, 16.3 mmol) was dissolved in dry THF (130 mL). Dry pyridine (7.3 mL) was added, followed by silver nitrate (4.23 g, 27.5 mmol). This mixture was stirred for 15 min, then *t*-butyldimethylsilyl chloride (3.9 g, 25.9 mmol) was added. The reaction mixture was stirred in darkness for 4 h, when more silver nitrate (1.27 g, 8.3 mmol) and *t*-butylmethylsilyl chloride (0.74 g, 4.9 mmol) were added. After overnight stirring the solution was filtered through a Celite bed, which was washed with DCM and the filtrates were evaporated. The residue was dissolved in DCM, and washed with sat. $NaHCO_3$, water and dried over $MgSO_4$. The solvent was evaporated and the residue was subjected to short column chromatography to give compounds **15a** (4.52 g, 69.6%) and **15b** (1.22 g, 18.8%). Compound **15a**: R_f: 0.60 (System B). $[\alpha]_D^{25} + 13$ (c, 0.39, $CHCl_3$). IR ν_{max} (neat): 3082, 3060, 3025, 2992, 2950, 2922, 2854, 1752, 1732, 1491, 1460, 1451, 1432, 1385, 1359, 1286, 1252, 1185, 1139, 1110, 1084, 1052, 1002, 962, 890, 835, 776 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.4–7.3 (*m*, 5H) *Ph*-CH_2; 4.79 (*d*, $J_{H1,H2} =$ 0.99 Hz, 1H) H-1; 4.69–4.52 (*m*, 2H) Ph-CH_2; 4.12 (*d*, 1H) H-2; 3.74 (*s*, 3H) C(O)OCH_3; 3.41 (*s*, 3H) OCH_3; 0.90 (*s*, 9H) Si-C$(CH_3)_3$; 0.09 (2x*s*, 6H) Si-$(CH_3)_2$. ^{13}C-NMR ($CDCl_3$): 172.2 (*C*=O), 137.5, 128.2, 127.7 (*Ph*-CH_2); 109.1 (C-1); 74.4 (C-2), 72.4 (Ph-*C*H_2); 55.1 (OCH_3), 52.0 (C(O)OCH_3), 25.6 (Si-C$(CH_3)_3$), 18.1 (Si-*C*$(CH_3)_3$), −4.8 & −4.9 (Si-$(CH_3)_2$). Compound **15b**: Rf: 0.43 (System B). $[\alpha]_D^{25} + 103$ (c 0.28, $CHCl_3$). IR ν_{max} (neat): 3082, 3060, 3025, 2946, 2922, 2852, 1747, 1491, 1460, 1451, 1432, 1387, 1359, 1280, 1252, 1192, 1139, 1113, 1080, 1064, 1032, 958, 872, 835, 776 cm^{-1}. ^{1}H-NMR ($CDCl_3$) 7.4–7.3 (*m*, 5H) *Ph*-CH_2; 4.92 (*d*, $J_{H1,H2} = 4.5$ Hz, 1H) H-1; 4.82–4.70 (m, 2H) Ph-CH_2; 4.07 (*d*, 1H) H-2; 3.71 (*s*, 3H) C(O)OCH_3; 3.48 (*s*, 3H) OCH_3; 0.93 (*s*, 9H) Si-C$(CH_3)_3$; 0.08 & 0.11 (2x*s*, 6H) Si-$(CH_3)_2$. ^{13}C-NMR ($CDCl_3$): 171.0 (C=O), 137.6, 128.1, 128.0, 127.5 (*Ph*-CH_2); 103.8 (C-1); 72.7 (C-2), 72.1 (Ph-*C*H_2); 55.9 (OCH_3), 52.2 (*C*(O)OCH_3), 25.7 (Si-C(*C*$H_3)_3$), 18.2 (Si-*C*$(CH_3)_3$); −4.9 & −5.0 (Si-$(CH_3)_2$).

1-*O*-Methyl-3-*O*-benzyl-2-*O*-*t*-butyldimethylsilyl-β-D-ribufuranose-3,4,5,5′-d_4 (16a). Compound **15a** (4.6 g, 11.4 mmol) was dissolved in dry diethyl ether (∼100 mL) and $LiAlD_4$ (239 mg, 5.7 mmol) was added at 0°C. The mixture was stirred at r.t. for 2 h, then water was added and the mixture was extracted with DCM. The organic phase was dried over $MgSO_4$. After evaporation of the volatile matters,

the residue was purified by column chromatography to obtain compound **16a** as colourless oil (3.68 g, 87%). R_f: 0.70 (System C). $[\alpha]_D^{25} + 34$ (c 0.36, $CHCl_3$). IR ν_{max} (neat): 3462, 3085, 3060, 3026, 2950, 2924, 2855, 2198, 2188, 2094, 1494, 1470, 1461, 1452, 1387, 1359, 1301, 1251, 1172, 1135, 1102, 1080, 1062, 980, 935, 889, 838, 775 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.4–7.3 (*m*, 5H) *Ph*-CH_2; 4.73 (*d*, $J_{H1,H2} = 0.99$ Hz, 1H) H-1; 4.69–4.43 (*m*, 2H) Ph-*CH_2*; 4.15 (*d*, 1H) H-2; 3.39 (*s*, 3H) O*CH_3*; 0.91 (*s*, 9H) Si-C(*CH_3*)$_3$; 0.12 & 0.11 (2x*s*, 6H) Si-(*CH_3*)$_2$. ^{13}C-NMR ($CDCl_3$): 137.8, 128.3, 127.7, 127.6 (*Ph*-CH_2); 109.1 (C-1); 74.5 (C-2), 72.3 (Ph-*C*H_2); 55.5 (O*C*H_3), 25.6 (Si-C(*C*H_3)$_3$), 18.1 (Si-*C*$(CH_3)_3$), −4.8 (Si-(*C*H_3)$_2$).

1-*O*-Methyl-3-*O*-benzyl-2-*O*-*t*-butyldimethylsilyl-α-D-ribofuranose-3,4,5,5′-*d_4* (16b). Compound **15b** (1.22 g, 3.1 mmol) was reduced with $LiAlD_4$ (65 mg, 1.5 mmol) as described for **15a** to get compound **16b** as colourless oil (0.83 g, 72%). R_f: 0.65 (System C). $[\alpha]_D^{25} + 149$ (c 0.4, $CHCl_3$). IR ν_{max} (neat): 3498, 3082, 3060, 3022, 2945, 2922, 2852, 2198, 2188, 2092, 1494, 1470, 1462, 1453, 1387, 1361, 1251, 1190, 1170, 1111, 1080, 1065, 970, 876, 838, 778 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.4–7.2 (*m*, 5H), *Ph*-CH_2; 4.79 (*d*, $J_{H1,H2} = 4.3$ Hz, 1H) H-1; 4.88–4.53 (*dd*, 2H) Ph-*CH_2*; 4.03 (*d*, 1H) H-2; 3.46 (*s*, 3H) O*CH_3*; 0.95 (*s*, 9H) Si-C(*CH_3*)$_3$; 0.134 & 0.127 (2x*s*, 6H) Si-(*CH_3*)$_2$. ^{13}C-NMR ($CDCl_3$): 138.5, 128.2, 127.7, 127.5 (*Ph*-CH_2); 103.7 (C-1); 73.5 (C-2), 72.6 (Ph-*C*H_2); 55.5 (O*C*H_3), 25.7 (Si-C(*C*H_3)$_3$), 18.3 (Si-*C*$(CH_3)_3$), −4.7, −5.0 (Si-(*C*H_3)$_2$).

1-*O*-Methyl-3,5-di-*O*-benzyl-2-*O*-*t*-butyldimethylsilyl-β-D-ribofuranose-3,4,5,5′-*d_4* (17a). Compound **16a** (3.68 g, 9.91 mmol) was coevaporated with dry acetonitrile then dissolved in the same solvent (33 mL). NaH (286 mg, 11.9 mmol) was added followed by benzyl bromide (1.42 mL, 11.9 mmol). The mixture was stirred overnight at r.t., then methanol was added and the stirring was maintained for additional 1 h. The reaction mixture was poured into sat. $NaHCO_3$ and extracted with DCM. The pooled organic phase was dried over $MgSO_4$, filtered and evaporated to a syrup. Purification by column chromatography afforded compound **17a** as colourless oil (4.41 g, 96%). R_f: 0.72 (System B). $[\alpha]_D^{25} + 27$ (c 1.23, $CHCl_3$). IR ν_{max} (neat): 3082, 3060, 3022, 2945, 2922, 2848, 2821, 2175, 2150, 2070, 1491, 1468, 1460, 1450, 1383, 1358, 1310, 1250, 1200, 1184, 1145, 1050, 959, 890, 832, 772 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.33–7.3 (*m*, 10H) 2x*Ph*-CH_2; 4.74 (*d*, $J_{H1,H2} = 1.2$ Hz, 1H) H-1; 4.66–4.40 (*m*, 4H) 2xPh-*CH_2*; 4.12 (*d*, 1H) H-2; 3.33 (*s*, 3H) O*CH_3*; 0.91 (*s*, 9H) Si-C(*CH_3*)$_3$; 0.10 & 0.096 (2x*s*, 6H) Si-(*CH_3*)$_2$. ^{13}C-NMR ($CDCl_3$): 138.3, 137.9, 128.2, 127.6, 127.5, 127.46, 127.3 (2x*Ph*-CH_2); 108.6 (C-1); 74.1 (C-2), 73.0, 72.1 (2xPh-*C*H_2); 55.0 (O*C*H_3), 25.6 (Si-C(*C*H_3)$_3$), 18.1 (Si-*C*$(CH_3)_3$); −4.82, −4.86 (Si-(*C*H_3)$_2$).

1-*O*-Methyl-3,5-di-*O*-benzyl-2-*O*-*t*-butyldimethylsilyl-α-D-ribofuranose-3,4,5,5′-*d_4* (17b). Compound **16b** (0.83 g, 2.23 mmol) was coevaporated with dry acetonitrile three times. After dissolving in the same solvent (8 mL), NaH (80 mg, 3.35 mmol) was added followed by benzyl bromide (0.40 mL, 3.35 mmol). The mixture was stirred for 4 h at r.t., then the reaction mixture was poured into sat. $NaHCO_3$ and extracted with DCM. The pooled organic phase was dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography afforded

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compound **17b** as colourless oil (0.76 g, 74%). R_f: 0.52 (System B). $[\alpha]_D^{25} + 114$ (c 1.13, $CHCl_3$). IR ν_{max} (neat): 3082, 3059, 3022, 2945, 2920, 2845, 2170, 2155, 2070, 1491, 1468, 1460, 1450, 1383, 1358, 1248, 1194, 1170, 1113, 1086, 1065, 1042, 960, 878, 832, 774 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.35–7.21 (*m*, 10H) 2x*Ph*-CH_2; 4.79 (*d*, $J_{H1,H2} = 4.4$ Hz, 1H) H-1; 4.81–4.40 (*m*, 4H) 2xPh-*CH_2*; 4.07 (*d*, 1H) H-2; 3.45 (*s*, 3H) O*CH_3*; 0.94 (*s*, 9H) Si-C(*CH_3*)$_3$; 0.12 & 0.10 (2x*s*, 6H) Si-(*CH_3*)$_2$. ^{13}C-NMR ($CDCl_3$): 138.6, 137.9, 128.2, 128.1, 127.8, 127.5, 127.3 (2x*Ph*-CH_2); 103.5 (C-1); 73.3 (Ph-*C*H_2), 73.2, (C-2); 72.3 (Ph-*C*H_2), 55.5 (O*C*H_3), 25.8 (Si-C(*C*H_3)$_3$), 18.3 (Si-*C*$(CH_3)_3$); −4.8, −5.0 (Si-(*C*H_3)$_2$).

1-*O*-Methyl-3,5-*O*-dibenzyl-β-D-ribofuranose-3,4,5,5′-*d_4* (18a). Compound **17a** (4.41 g, 9.55 mmol) was dissolved in dry tetrahydrofuran (95 mL). 1 M TBAF in dry THF (9.55 mL) was added and stirring was maintained for 10 min at ambient temperature. The mixture was evaporated to an oil. Purification on silica gel afforded compound **18a** (3.15 g, 95%) as colourless oil). R_f: 0.82 (System A). $[\alpha]_D^{25}$ −27 (c 0.71, $CHCl_3$). IR ν_{max} (neat): 3450, 3081, 3059, 3022, 2922, 2856, 2824, 2178, 2150, 2072, 1491, 1450, 1385, 1366, 1203, 1148, 1050, 1115, 955, 870 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.38–7.23 (*m*, 10H) 2x*Ph*-CH_2; 4.86 (*d*, $J_{H1,H2} = 0.87$ Hz, 1H) H-1; 4.57 & 4.568 (2x*s*, 4H) 2xPh-*CH_2*; 4.02 (*br.s*, 1H) H-2; 3.32 (*s*, 3H) O*CH_3*; 2.70 (*br.d*, $J_{OH,H2} = 2.5$ Hz, 1H) OH. ^{13}C-NMR ($CDCl_3$): 138.1, 137.0, 128.5, 128.2, 128.1, 127.8, 127.5 (2x*Ph*-CH_2); 108.5 (C-1); 73.2, (C-2); 73.1, 72.7 (2xPh-*C*H_2), 54.9 (O*C*H_3).

1-*O*-Methyl-3,5-*O*-dibenzyl-α-D-ribofuranose-3,4,5,5′-*d_4* (18b). Compound **17b** (0.75 g, 1.62 mmol) was treated in dry tetrahydrofuran (16 mL) with 1 M TBAF in dry THF (1.62 mL) as described for **18a**. Column chromatography afforded compound **18b** (497 mg, 88%) as colourless oil. R_f: 0.83 (System A). $[\alpha]_D^{25} + 121$ (c 0.18, $CHCl_3$). IR ν_{max} (neat): 3552, 3080, 3059, 3022, 2922, 2856, 2824, 2170, 2140, 2070, 1491, 1450, 1408, 1383, 1366, 1320, 1188, 1148, 1045, 959 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.37–7.22 (*m*, 10H) 2x*Ph*-CH_2; 4.88 (*d*, $J_{H1,H2} = 4.7$ Hz, 1H) H-1; 4.73 & 4.57 and 4.51 & 4.44 (2x*q*, $J_{AB} = 12.4$ & 12.1 Hz, 4H) 2xPh-*CH_2*; 4.11 (*dd*, 1H) H-2; 3.47 (*s*, 3H) O*CH_3*; 2.94 (*d*, $J_{OH,H2} = 11.1$ Hz, 1H) OH. ^{13}C-NMR ($CDCl_3$): 137.8, 137.7, 128.3, 128.28, 127.8, 127.7, 127.6, 127.5 (2x*Ph*-CH_2); 102.9 (C-1); 73.3, 72.9 (2xPh-*C*H_2), 71.6 (C-2); 55.5 (O*C*H_3)

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