Studies on the Stereoselective Synthesis of Deuterated D-Ribose Derivatives

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In view of the importance of the site-specific substitution of the H-atom by its stable isotope ^{2}H in a stereoselective/stereospecific manner at the pentose sugar residue, decreasing the spectral overcrowding in various regions of 1D and 2D homo- and heteronuclear correlation spectra of oligo-DNA and -RNA, there is always a need for the development of new methods for the incorporation of ^{2}H at different sites of a ribose. High-yielding multistep syntheses of C(2)-, and (5*R*)- and (5*S*)-3,5-deuterated ribose derivatives have been envisaged for the application of site-specific incorporation of multilabeled nucleosides into oligomers to facilitate their structure elucidation by NMR spectroscopy. All syntheses started from D-glucose after proper derivatization. In the case of C(2), >97 atom-% isotope was incorporated, employing an inversion of the configuration at C(2) as the key reaction. For C(5), two different routes were envisaged: on the one hand, deuterated achiral reagent was treated with a conformationally locked sugar moiety **15**, while, on the other, chiral protonated sources were used to transfer the H-atom to a C(5)-deuterated aldehyde **18**. In all cases, enantiomeric and isotopic purities were found to be as high as >97% as determined by NMR spectroscopy.

Introduction. – The importance of the structure–biological activity relationship of an oligo-DNA and oligo-RNA has been investigated by different physicochemical techniques, amongst which NMR spectroscopy was found to be the most powerful tool as it provides the conformational data under quasi-physiological conditions [1]. Although, with the increasing chain length, the usefulness of NMR spectroscopy becomes restricted, site-specific isotope labeling has been proven to overcome this problem in the recent past (for a review, see [2]). While, the use of ^{13}C/^{15}N isotopes increases the number of observable resonances [3][4][5–7], ^{2}H labeling of oligonucleotides is based on the primary idea of suppressing part(s) of the ^{1}H-NMR spectrum [8].

Partial or complete substitution of ^{1}H by ^{2}H in the sugar residue of a nucleoside and incorporating them into an oligomer in a sequence specific manner has helped to solve the above problem as described in '*Uppsala NMR Window*' concept [9]. Recently, the results have been reported on the chemical synthesis of (5*R*)- and (5*S*)-[3′,5′-$^2H_2$]nucleosides [9] and [3′,4′,5′,5″-$^2H_4$]nucleosides [10a] for the use in the preperation of non-uniformly labeled DNA and RNA, and in their NMR measurements. In continuation of the studies, it was envisioned that whether we can make a new window where the (5′*R*)-[3′,5′-$^2H_2$]- or (5′*S*)-[3′,5′-$^2H_2$]ribonucleoside would be incorporated inside the NMR window, whereas [2′,3′,4′,5′,5″-$^2H_5$]ribonucleosides would be placed outside the NMR window so that the spectral assignments become clear, *e.g.*, *i*) to enhance the structurally important NOE intensities with diminished spin diffusion while removing the insignificant ones, *ii*) to determine the exact ^{1}H,^{31}P coupling constants, provided

suitable general methods for ^{2}H incorporation at C(2) and C(5) ((*R*) and (*S*)) could be found. Nevertheless, this can also be implemented to prepare [2′,3′,5′(*R* and *S*)-$^{2}H_3$]-2′-deoxyribonucleosides [10b] to incorporate into the NMR-window region of a large oligo-DNA to reduce the spectral overcrowding for NMR structural determination (*Fig. 1*). In this report, detail studies on the high-yielding, efficient general methodologies towards deuteration at C(2) and C(5)(*R* and *S*) at the sugar level starting from D-glucose (considering that these protocols would be implemented in the case of the synthesis of both ^{13}C,^{2}H-labeled nucleosides) have been sought. The strategies have been envisioned in such a way that one protocol can be switched to another for further labeling in different position. Two syntheses were carried out parallel; for simplicity, C(2) labeling was performed on standard D-glucose, whereas C(5) was deuterated on a C(3)-isotope-enriched sugar derivative to check the applicability of the method to produce multi-labeled pentoses.

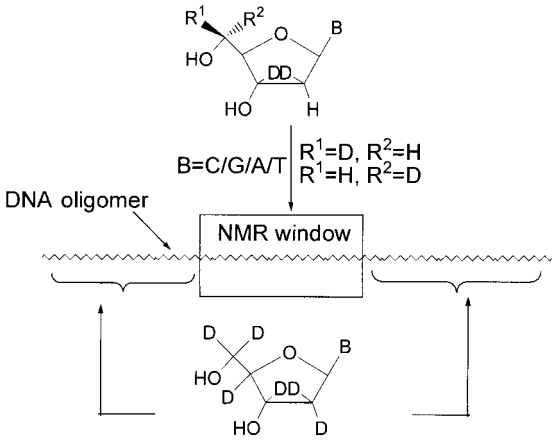
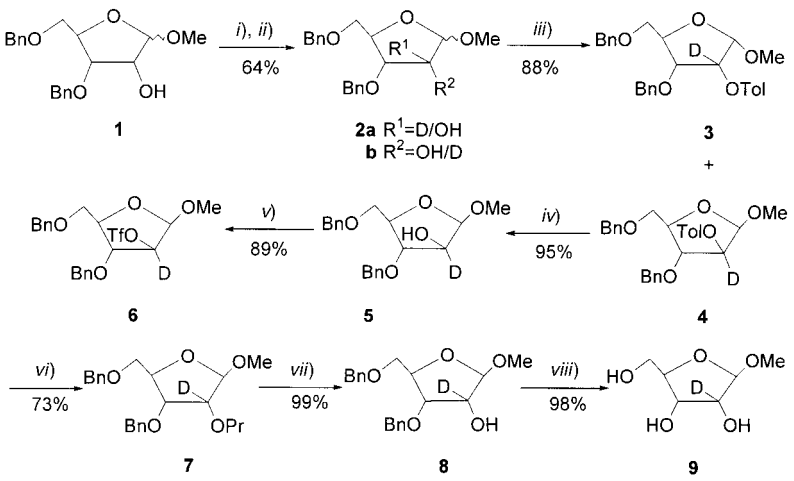


Fig. 1. *Schematic representation of a typical NMR window*

Results and Discussion. – 1. *Synthesis of [2-$^{2}H_1$]Ribose Derivative* **9** (*Scheme 1*). The high degree of isotope substitution at C(2) has been found as the most difficult task either at the sugar level or at the nucleoside level as reported by *Serianni et al.* decades ago [11]. Although some selective isotope labelings to produce epimeric mixtures have already been reported, there were several limitations of those studies, *e.g.*, the use of highly toxic reagent such as KCN in the reactions usually carried out at relatively high pH. Moreover, the cyanohydrin products were only stable at pH 4.0, so that the chromatographic purifications were also performed under this condition. Considering the acid- and base-labile protecting groups present in a pentose sugar (as in our case), the application of these procedures becomes restricted. Nevertheless, for multiple labeling at different sites of a ribose sugar (*e.g.*, C(4) and C(5) together with C(3) and C(2)), the methodologies were turned out to be certainly disadvantageous. The oxidation followed by the reduction of 1,3,5-*O*-tribenzoylribofuranose has been less attractive because of the low-level incorporation of ^{2}H at C(2) as well as the presence of the base-labile protecting groups in the ribofuranose moiety [12]. Although the

Raney NiD_2O exchange reaction [9] of an anomeric mixture of methyl α/β-D-ribofuranose offers an alternative route, the slow exchange rate at specific positions (especially at the secondary C-atoms; hence, 3–4 cycles of isotope exchange are necessary) eliminates the possibility of being noteworthy. On the other hand, the highly stereoselective reduction of benzyl arabinopyranoside [13] to the corresponding ribopyranoside is proved to be an effective but lengthy procedure. Therefore, there was indeed a need for an efficient alternative synthesis that would overcome all limitations mentioned above and could be used in multi-gram-scale preparation of multilabeled deuterated nucleosides.

Scheme 1. *Synthesis of 1-*O*-Methyl-β-*D*-[2-$^2H_1$]ribofuranose* (**9**)



i) $(COCl)_2$, DMSO, CH_2Cl_2, $-70°$; *ii*) $LiAlD_4/NaBD_4$, Et_2O/EtOH, r.t. *iii*) p-TolCl, pyridine, r.t. *iv*) NH_3/MeOH, r.t. *v*) Tf_2O, DMAP, pyridine, 0°, CH_2Cl_2. *vi*) Cesium propionate, DMF, r.t. *vii*) NH_3/MeOH. *viii*) Pd-C/H_2, EtOH, r.t.

Our methodology outlined in *Scheme 1* is based on the oxidation–reduction process of methylribofuranoside derivative **1** (α/β 7:3) [14] to form C(2)-deuterated compound **2**, followed by inversion of configuration at C(2) of the arabino compound **2b** to afford the sugar derivative **7**. Thus, **1** was subjected to *Swern* oxidation at $-70°$, followed by subsequent reduction with either $LiAlD_4$ in dry Et_2O or $NaBD_4$ in EtOH at room temperature to give a unseparable epimeric mixture (7:3 in favor of *arabino*) of **2**. However, it was necessary to separate the epimers at this stage. Compound **2** was treated with *p*-toluoyl chloride (1.15 equiv.) in dry pyridine overnight at room temperature, and the toluoylated ribo and arabino derivatives **3** and **4**[1]), respectively, were successfully separated over a silica-gel column with petroleum ether/AcOEt as eluent.

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[1]) *Data of* **3**: 26%. $[\alpha]_D^{26} = +97.9$ ($c = 0.67$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 8.03 (*d*, $J = 8.1$, 2 H, Tol); 7.33–7.23 (*m*, 12 H, Tol, Bn); 5.21 (*s*, H–C(1)); 4.73–4.43 (*m*, PhCH_2); 4.30–4.24 (*m*, H–C(4)); 4.10 (*d*, $J = 4.45$, H–C(3)); 3.52–3.33 (*m*, CH_2(5), MeO); 2.42 (*s*, Me). *Data of* **4**: 62%. $[\alpha]_D^{26} = -74$ ($c = 0.246$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 7.92 (*d*, $J = 8.1$, 2 arom. H); 7.36–7.23 (*m*, 12 arom. H); 5.21 (*s*, H–C(1)); 4.66–4.59 (*m*, 2 CH_2); 4.34 (*d*, $J = 5.5$, H–C(3)); 4.25–4.19 (*m*, H–C(4)); 3.65–3.54 (*m*, CH_2(5)); 3.28 (*s*, MeO); 2.41 (*s*, Me).

As mentioned, the strategy of this study was to invert the configuration at C(2) of the arabino compound **4** to that of the ribo counterpart **7**, and to use **3** and **7** (after proper derivatization) for base-coupling reaction to prepare the deuterated nucleosides. Here, we made use of cesium-propionate, which acts as an effective nucleophile in a S_N2 reaction provided there is a good leaving group such as triflate at the reaction center. Therefore, the sugar derivative **4** was detoluoylated by methanolic NH_3 at room temperature in 2 days, followed by the removal of solvent and chromatographic purification over silica gel, to afford methyl β-3,5-di-*O*-benzyl[2-$^2H_1$]arabinofuranoside (**5**) in 95% yield[2]). The 2-*O*-triflate **6** was then obtained from **5** on treatment with triflic anhydride in the presence of 4-(dimethylamino)pyridine (DMAP) and pyridine in CH_2Cl_2. (It should be noted that, in the first instance, this reaction was carried out with **2** itself. Although, the ribo and arbino derivatives were also separated and the arabino-triflate compound **6** could be obtained directly for nucleophilic substitution reaction, we prefered the toluoylation and detoluoylation steps, as the ribo triflate could not be deprotected cleanly for further use, leading to a low yield of the [2-$^2H_1$]sugar. On the other hand, detoluoylation gave almost quantitative conversion.) Then, the arabino-triflate was converted to ribo propionate **7** with cesium-propionate in DMF [15] at room temperature under inert atmosphere. The change of the chemical shift from 5.0 to 4.87 for the anomeric H-atom confirmed that the displacement was indeed S_N2. Also, the change of the peak positions of C(3) and C(4) (from 80.8 (C(3)) and 79.6 (C(4)) of compound **6** to 80.3 (C(4)) and 77.7 (C(3)) of compound **7** as observed in the ^{13}C-NMR spectrum and confirmed by H,C-correlation experiment) showed that the inversion of configuration at C(2) was very successful. Considering that 1-*O*-acetate provides better coupling with the silylated base, and 2-*O*-toluoyl also helps to afford exclusively the β-anomer, the deuterated ribo-propionate **7** was converted to 1-*O*-methyl-β-D-[2-$^2H_1$]-ribose **9** as a precursor of the acetate. The appearance of a clean *doublet* (J(3,4)) at 4.08 ppm corresponding to H–C(3) demonstrated the high-level (> 97 atom-%) incorporation of ^{2}H at C(2). The compound **9** can be easily converted to the corresponding acetate, followed by coupling with silylated bases according to well-established methods [9], to give [2-$^2H_1$]ribonucleosides.

2. *Synthesis of (5*R*)- and (5*S*)-[3,5-$^2H_2$]Ribose Derivatives* (*Scheme 2*). Stereoselective (5′*S*)- or (5′*R*)-$^2H_1$ incorporation provides means to determine the exact ^3J(5′,P) and ^3J(5″,P) coupling constants and unambiguous NOE assignments, which are essential to elucidate the conformation of sugar phosphate backbone of DNA oligomers [16–19]. They are used to probe the internal dynamics of oligonucleotides by solid-phase ^{2}H-NMR spectroscopy [20][21]. C(5)-Deuterated compounds are also used in conformational and mechanistic studies [22–27]. We herein report our studies on the synthesis of (5*R*)- and (5*S*)-[3,5-$^2H_2$]ribose derivatives and development of the procedures on C(5)-deuteration at sugar level.

The synthesis was carried out starting from 1,2:5,6-*O*-diisopropylidene-[3-$^2H_1$]-D-glucose (**10**), which was converted to compound **11** by a sequence of steps [9] (*Scheme 3*). Towards our aim in synthesizing both (5*R*)- and (5*S*)-3,5-dideuterated ribose, two different synthetic routes have been envisaged. In the first instance, achiral

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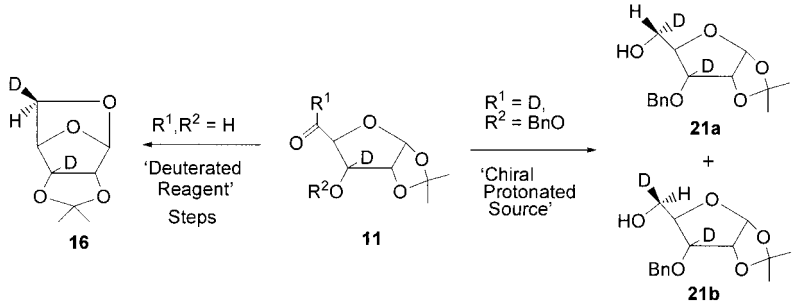
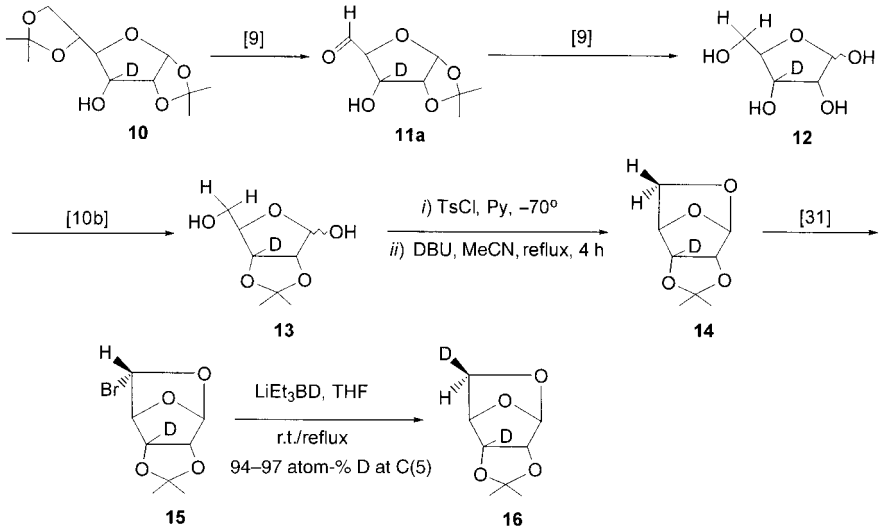
[2]) *Data of* **5**: ^{1}H-NMR ($CDCl_3$): 7.34–7.25 (*m*, 10 arom. H); 4.85 (*s*, H–C(1)); 4.77–4.56 (*m*, 2 CH_2); 4.26–4.06 (*m*, H–C(4)); 3.84 (*d*, J = 5.5, H–C(3)); 3.55–3.52 (*m*, CH_2(5)); 3.41 (*s*, MeO).

Scheme 2. *Strategies towards the Derivatives of (5*R*)- and (5*S*)-[3,5-$^2H_2$]Ribose*



Scheme 3. *Synthesis of (5*R*)-1,5-Anhydro-2,3-*O*-isopropylidene-*D*-[3,5-$^2H_2$]ribose* (**16**)

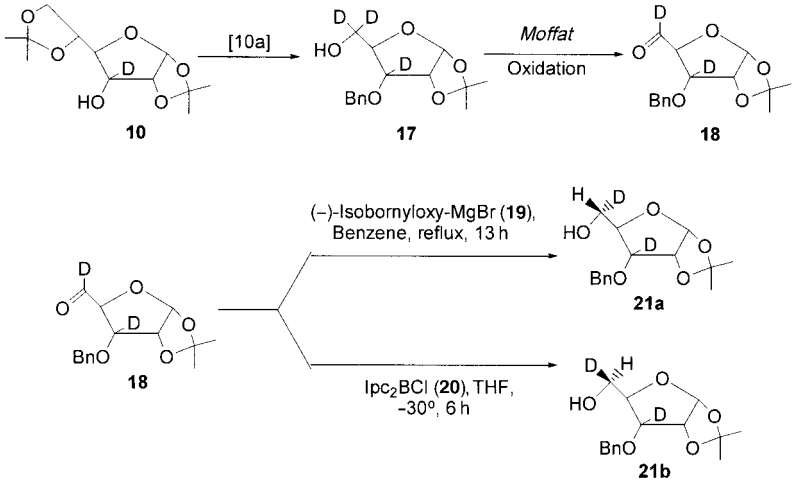


deuterated reagent was used to introduce ^2H at C(5) of a conformationally locked sugar moiety, while in the other, a H-atom was transferred from external protonated chiral reagents to a C(5)-deutero aldehyde **18** (cf. *Scheme 4*).

i) Usual reduction with $NaBD_4$ or $LiAlD_4$ of the corresponding C(5)-aldehyde produced a *ca.* 1:1 mixture of the unseparable (5*R*)- and (5*S*)-isomers [28] (*Scheme 4*). The use of LiI and *tert*-pentyl alcohol in the presence of (−)-isoborneol improved the selectivity; however, the best result provided only a *ca.* 4:1 mixture in favor of the (5*R*)-isomer [29]. In a recent report, it has been shown that ^2H can be transfered easily from (−)-[2-$^2H_1$]isobornyloxymagnesium bromide in *ca.* 100% enantiomer purity but with concomittant nonlabeled ribose derivative up to 15% [30].

The absolute stereoselectivity and isotopic purity at C(5) of the ribose moiety at the sugar level can be increased *via* photobromination [31] of 1,5-anhydro-2,3-*O*-isopropylidene-D-ribose, followed by reductive elimination of bromine with different deuteride reagents as isotope source amongst which 'super deuteride' was found to be the best. Encouraged by these findings, we thought of using this strategy for C(5)-

Scheme 4. *Use of Protonated Chiral Reagents: Synthesis of (5*R*)- and (5*S*)-3-*O*-Benzyl-1,2-*O*-isopropylidene-*D*-[3,5-$^2H_2$]ribose* (**21a** and **21b**, respectively)



deuteration on our [3-$^2H_1$]sugar derivative as shown in *Scheme 3*. Hence, compound **11a** was reduced with $NaBH_4$ in EtOH, followed by boiling with 80% AcOH in H_2O to give [3-$^2H_1$]ribose derivative **12** [9]. Acetone treatment [10b] of **12** in the presence of catalytic amount of conc. H_2SO_4 at room temperature, followed by neutralization with solid Na_2CO_3, gave compound **13** in 50% yield[3]). The acetonide **13** was then tosylated at $-70°$ in dry pyridine with 1.1 equiv. of TsCl to give only C(5)-monotosyl compound, which, after workup, was refluxed in dry MeCN in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 4 h. Purification by silica-gel column chromatography with AcOEt/hexane afforded 1,5-anhydro [3-$^2H_1$]compound **14** in 72% yield (after two steps)[4]). The photobromination step was achieved according to [31]. The appearance of signals at 5.76 and 5.75 (2*s*, H–C(1), H–C(5)), 4.90 (*s*, H–C(4)), and 4.27 (*s*, H–C(2)) indicated that photobromination was indeed stereoselective. ^2H was then introduced at C(5) in dry THF with $LiEt_3BD$ (*Aldrich*; 1.0M in THF, 2 equiv.) as ^2H source. High-field NMR spectroscopy revealed that the (*R*)-**16** was formed (62%) with >99% chiral purity[5]). Interestingly, during the deuteration reaction in our hand, we observed the presence of small amount (3–6 atom-%) of nonlabeled anhydro compound depending on the reaction conditions.

At this point, it was clear that, although we could obtain compound **16** only as (5*R*)-isomer, the level of deuterium incorporation at C(5) was not satisfactory. Nevertheless, the photobromination step was low-yielding (30–40% (also mentioned in [31]), which

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[3]) *Data of* **13** (β-anomer): ^{1}H-NMR ($CDCl_3$): 5.42 (*d*, H–C(1)); 4.58 (*s*, H–C(2)); 4.41 (*t*, H–C(4)); 3.75–3.71 (*m*, CH_2(5)); 1.49, 1.32 (2*s*, Me). ^{13}C-NMR ($CDCl_3$): 112.0; 102.9 (C(1)); 87.6 (C(4)); 86.7 (C(2)); 63.5 (C(5)); 26.3; 24.6.

[4]) *Data of* **14**: ^{1}H-NMR ($CDCl_3$): 5.44 (*s*, H–C(1)); 4.70 (*d*, *J* = 3.7, H–C(4)); 4.28 (*s*, H–C(2)); 3.42 (*dd*, *J* = 3.8, 7.2, 1 H–C(5) (*R*)); 3.30 (*d*, *J* = 7.2, 1 H–C(5) (*S*)).

[5]) ^{1}H-NMR ($CDCl_3$): 5.45 (*s*, H–C(1)); 4.70 (*s*, H–C(4)); 4.28 (*s*, H–C(2)); 3.41 (*d*, *J* = 3.8, H–C(5) (*R*)). ^{13}C-NMR ($CDCl_3$): 112.0; 99.7 (C(1)); 81.1 (C(2)); 77.3 (C(4)); 25.8; 25.1.

would result in the reduction of overall yield), and we cannot afford this especially considering that we were working with valuable C(3)-deuterated compound. Instead, if we could use C(5)-deuterated aldehyde **11b** in lieu of the derivative **11a** as substrate, and incorporate ^1H from protonated chiral reagents, the ^1H contamination would definitely vanish. We wish to report our findings on this aspect as below.

ii) Protected [3-$^2H_1$]aldehyde **11b** ($R^1 = H$, $R^2 = Bn$) was converted to [3,5,5-$^2H_3$]ribose moiety **17** (*Scheme 4*) according to [10a]. The trideutero derivative **17** was then subjected to *Moffat* oxidation [32] to give the [3,5-$^2H_2$]aldehyde **18**, which was ready for proton-transfer reactions. We used two different chiral reagents. First, the *Grignard* reagent **19** [33] (obtained from (+)-camphor after reduction with $LiAlH_4$, followed by the addition to a solution of BuMgBr in dry Et_2O) was tried. When the aldehyde **18** was refluxed with (−)-isobornyloxymagnesium bromide (**19**) in benzene overnight, (5*R*)-3-*O*-benzyl-1,2-*O*-isopropylidene[3,5-$^2H_2$]ribose (**21a**; 61% yield after 2 steps (oxidation and reduction)) was obtained[6]). High-field ^1H-NMR spectroscopy revealed that there was no $^1H/^2H$ exchange taking place during the reaction, and isotopic purity was >97 atom-% (*Fig. 2*).

Terpenes, being replenishable source of chiral carbon compounds available both in cyclic (*e.g.*, (+)-α-pinene) and acyclic forms, and displaying a plethora of conformational features, have been recognized as wealth of stereochemical attributes. Decades ago, *Brown et al.* have shown the usefulness of pinene in synthesizing various chiral reagents (*e.g.*, chloro(diisopinocampheyl)borane (Ipc_2BCl, **20**) for the reductions of carbonyl compounds with unprecedented stereoselectivity [34]. Thus, by the addition of Ipc_2BCl (2.5 equiv.) to a solution of **18** in dry THF at $-30°$ under N_2, (5*S*)-isomer **21b** was obtained (45%, after 2 steps). The ^1H-NMR signals in $CDCl_3$ at 5.73 (*d*, $J = 3.8$, H−C(1)); 4.76, 4.60 (2*d*, $J = 10.3$, PhCH_2); 4.56 (*d*, $J = 3.6$, H−C(2)); 4.11 (*d*, $J = 2.8$, H−C(4)); and 3.61 (br. *s*, H−C(5) (*S*)) were in accordance with the structure (*Fig. 2*). The formation of two different enantiomers by using two different chiral reagents could well be understood from the proposed models as shown (*Fig. 3*).

In conclusion, we have demonstrated different methodologies for the synthesis of the [2-$^2H_1$]-, and (5*R*)- and (5*S*)-[3,5-$^2H_2$]ribose derivatives with high isotope enrichment and high optical purity. These derivatives can be easily converted to the deuterated nucleosides according to already established reaction conditions. It is worth noting that, by using two different protonated chiral reagents, in the case of C(5)-labeling, **18** afforded two different isomers, (5*R*) and (5*S*), which is certainly advantageous for us as we would obtain two different sets of informations about ^3J(5,P) couplings in our NMR analysis of oligo-deoxyribonucleic acids. The use of these protocols in large-scale production of (*R*)- and (*S*)-3,5-dideutero ribonucleosides and (5′*R*)- and (5′*S*)-2′-deoxy[2′,3′,5′-$^2H_2$]ribonucleosides (in combination of C(2)- and C(5)-deuteration procedures) and their incorporation into the oligomer together with their structural studies will be our following target.

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[6]) *Data of* **21a**: ^1H-NMR ($CDCl_3$): 5.73 (*d*, $J = 3.7$, H−C(1)); 4.77, 4.59 (2*d*, $J = 11.9$, PhCH_2); 4.56 (*d*, $J = 3.7$, H−C(2)); 4.11 (*d*, $J = 2.3$, H−C(4)); 3.89 (br. *s*, H−C(5) (*R*)).

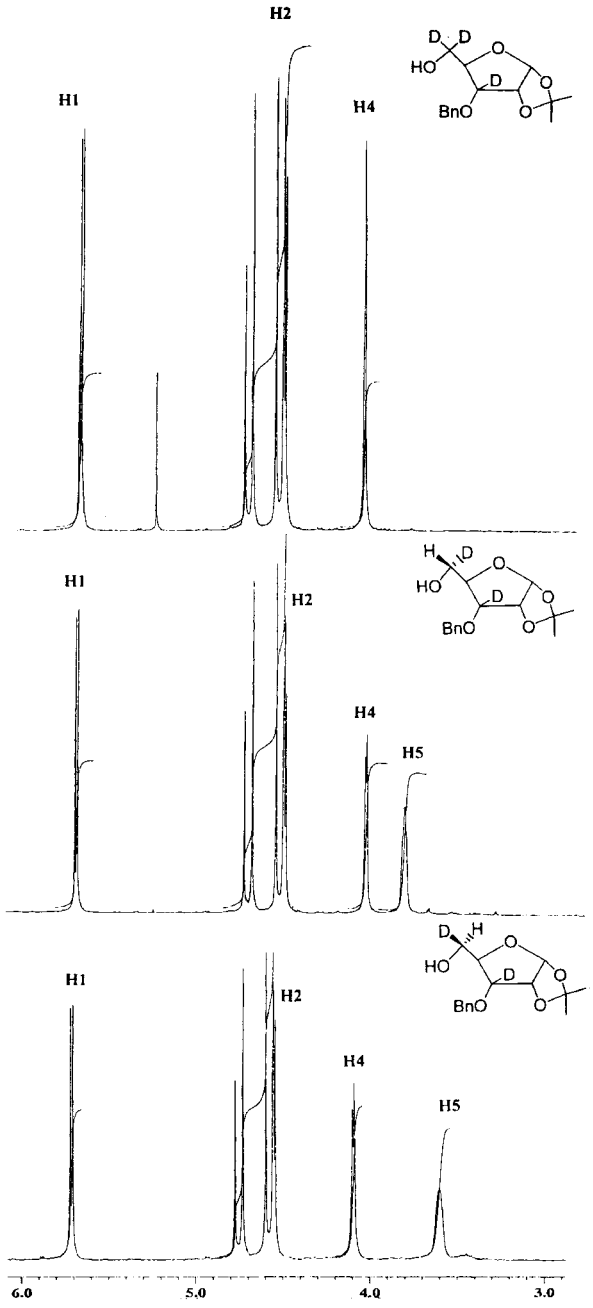

Fig. 2. *Comparison of the 270-MHz NMR spectrum* (sugar region) *of the* D-*[3,5-$^2H_2$]ribose derivatives* **17**, **21a**, *and* **21b**

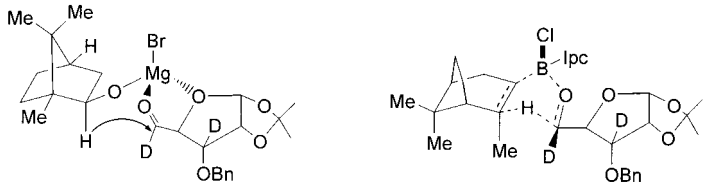

Fig. 3. *Induction of chirality: proposed mechanism of ^{1}H-transfer to the aldehyde* **18**

Experimental Part

General. All dry solvents were prepared according to standard procedures and stored over molecular sieves. The chromatographic separations were performed on *Merck G60* silica gel with AcOEt/petroleum ether. TLC: *Merck* pre-coated silica-gel *60 F254* glass baked plates. ^{1}H- and ^{13}C-NMR spectra: *Jeol GX 270* spectrometer at 270 and 67.9 MHz, resp., with TMS or MeCN as reference; chemical shifts are reported in ppm. Electron-spray ionization (ESI) mass spectra: *LCT^{TM} oa-TOF* mass spectrometer (*Micromass*, Manchester, UK).

Some Representative Procedures. 3,5-Di-O-*benzyl-1*-O-*methyl-α/β*-D-*[2-$^2H_1$]arabino/ribofuranose* (**2**). In a two-necked round bottom flask fitted with a dropping funnel and N_2-gas inlet was placed oxalyl chloride (3.7 ml, 42.3 mmol) in dry CH_2Cl_2 (15 ml) at $-70°$. A soln. of DMSO (6 ml, 84.6 mmol) in CH_2Cl_2 (15 ml) was added slowly, followed by the dropwise addition of a soln. of **1** (11.25 g, 32.7 mmol, in 35 ml of CH_2Cl_2), and the resultant mixture was stirred for 4 h at the same temp. Et_3N (23 ml, 163 mmol) was added to quench the reaction, and then the mixture was heated slowly to r.t. H_2O was added, and the compound was extracted with CH_2Cl_2. The org. layer was successively washed with brine and H_2O, dried ($MgSO_4$), and concentrated. The residue was dissolved in dry Et_2O (100 ml), $LiAlD_4$ (800 mg, 19 mmol) was added in portions at 0°, and the mixture was stirred overnight at r.t. H_2O was added slowly to quench the reaction, and the mixture was extracted with CH_2Cl_2. Removal of the solvent and purification by column chromatography (CC) gave compound **2** (7.2 g, 64%, after two steps). ^{1}H-NMR ($CDCl_3$): 7.34–7.27 (*m*, 10 arom. H); 4.88, 4.85 (2*s*, H–C(1) (α and β)); 4.77–4.56 (*m*, PhCH_2); 4.26–4.22, 4.16–4.06 (2*m*, H–C(4) (α and β)); 3.84, 3.78 (2*d*, H–C(3) (α and β)); 3.54–3.52 (*m*, CH_2(5)); 3.47, 3.41 (*s*, Me (α and β)). ^{13}C-NMR ($CDCl_3$): 137.9; 129.7; 129.6; 128.2; 127.8; 127.6; 127.5; 102.5 (C(1)); 84.5 (C(3)); 80.7 (C(4)); 73.2 (CH_2); 71.9 (C(5)); 71.7 (CH_2); 55.3 (Me).

3,5-Di-O-*benzyl-1*-O-*methyl-2*-O-*(trifluoromethylsulfonyl)-β*-D-*[2-$^2H_1$]arabinofuranose* (**6**). The ribose derivative **5** (4.18 g, 12.11 mmol) was co-evaporated with dry pyridine and dissolved in dry CH_2Cl_2 (90 ml), followed by the addition of 4-(dimethylamino)pyridine (DMAP; 5.18 g, 42.4 mmol) and pyridine (9 ml). The mixture was cooled to 0° and triflic anhydride (2.8 ml, 16.96 mmol) was slowly added, and the resultant mixture was stirred for 3 h at the same temp. The reaction mixture was then poured into cold sat. $NaHCO_3$ soln., and CH_2Cl_2 layer was separated. The H_2O phase was extracted again with CH_2Cl_2, and the combined CH_2Cl_2 layer was dried ($MgSO_4$) and concentrated. Chromatographic purification yielded **6** (5.18 g, 89.5%). $[\alpha]_D^{27} = -64.2$ ($c = 0.74$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 7.35–7.20 (*m*, 10 arom. H); 5.00 (*s*, H–C(1)); 4.76–4.47 (*m*, 2 CH_2); 4.29 (*d*, $J = 5.4$, H–C(3)); 4.17–4.11 (*m*, H–C(4)); 3.58–3.44 (*m*, CH_2(5)); 3.38 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 137.6; 136.8; 128.4; 128.3; 127.7; 127.6 (arom. C); 100.3 (C(1)); 80.8 (C(3)); 79.6 (C(4)); 73.4 (CH_2); 72.5 (CH_2); 71.3 (C(5)); 55.4 (Me). HR-EI-MS: 477.1184 (M^+; calc. 477.1179).

3,5-Di-O-*benzyl-1*-O-*methyl-2*-O-*propanoyl-β*-D-*[2-$^2H_1$]ribofuranose* (**7**). Cesium propanoate (2.9 g, 14.12 mmol) was added to a soln. of **6** (5.18 g, 10.86 mmol) in dry DMF (60 ml), and the mixture was stirred for 36 h at r.t. DMF was removed under reduced pressure, H_2O was added, and the compound was extracted with CH_2Cl_2. After removal of CH_2Cl_2, the residue was chromatographed over silica gel to give **7** (3.2 g, 73.5%). $[\alpha]_D^{26} = +14$ ($c = 0.713$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 7.33–7.25 (*m*, 10 arom. H); 4.87 (*s*, H–C(1)); 4.61–4.38 (*m*, 2 CH_2); 4.25–4.19 (*m*, H–C(4)); 4.12 (*d*, $J = 7.6$, H–C(3)); 3.63–3.59, 3.53–3.47 (2*dd*, CH_2(5)); 3.33 (*s*, MeO); 2.44–2.36 (*q*, CH_2O); 1.13 (*t*, Me). ^{13}C NMR ($CDCl_3$): 173.0 (CO); 138.0; 137.4; 128.2; 127.8; 127.7; 127.5; 106.2 (C(1)); 80.3 (C(4)); 77.7 (C(3)); 73.1, 72.9 (CH_2); 71.0 (C(5)); 54.9; 27.3; 8.9. HR-EI-MS: 401.1955 (M^+; calc. 401.1949).

3,5-Di-O-*benzyl-1*-O-*methyl-β*-D-*[2-$^2H_1$]ribofuranose* (**8**): Compound **7** (2.7 g, 6.73 mmol) was treated with NH_3/MeOH under stirring at r.t. for 30 h. Usual workup, followed by removal of the solvent and washing with H_2O, gave chromatographically homogeneous **8** (2.3 g, 99%). $[\alpha]_D^{26} = -28.7$ ($c = 0.71$, $CHCl_3$). ^{1}H-NMR

($CDCl_3$): 7.37–7.25 (*m*, 10 arom H); 4.86 (*s*, H–C(1)); 4.57 (*s*, 2 CH_2); 4.26–4.20 (*q*, H–C(4)); 4.07 (*d*, $J = 6.2$); 3.54 (*d*, $J = 5.3$, $CH_2(5)$); 3.31 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 138.0; 137.0; 128.5; 128.2; 127.5; 108.4 (C(1)); 80.5 (C(4)); 79.4 (C(3)); 73.2 (Ph*C*H_2); 72.7 (Ph*C*H_2); 71.5 (C(5)); 54.9 (Me).

*1-*O*-Methyl-β-*D*-[2-$^2H_1$]ribofuranose* (**9**). The Bn groups of **8** (2.1 g, 6.08 mmol) were cleaved with Pd/C–H_2 (450 mg) in EtOH (40 ml) during 3 h at r.t. The reagent was filtered over *Celite*, and the filtrate was concentrated to dryness to afford **9** (980 mg, 98%). $[\alpha]_D^{25} = -38$ ($c = 0.15$, H_2O). ^{1}H-NMR (D_2O): 4.83 (*s*, H–C(1)); 4.08 (*d*, $J = 6.9$, H–C(3)); 3.97–3.91 (*m*, H–C(4)); 3.76–3.70, 3.57–3.50 (2*dd*, $J = 3.3$, 12.2, and 6.4, 12.2, $CH_2(5)$); 3.33 (*s*, Me). ^{13}C-NMR (D_2O): 107.7 (C(1)); 82.6 (C(4)); 70.5 (C(3)); 62.6 (C(5)); 54.9 (Me). HR-EI-MS: 165.0748 (M^+; calc. 165.0747).

*(5*R*)-3-*O*-Benzyl-1,2-*O*-isopropylidene-*D*-[3,5-$^2H_2$]ribofuranose* (**21a**). A soln. of BuBr (2.9 ml, 27.7 mmol) in dry Et_2O (10 ml) was added dropwise to Mg (650 mg, 27.2 mmol) in dry Et_2O (10 ml) under N_2. After complete dissolution of Mg, a soln. of isoborneol (3.9 g, 25.5 mmol) in dry Et_2O (10 ml) was added slowly. A white precipitate was observed immediately. Benzene (30 ml) was added, and Et_2O was distilled under N_2.

The trideutero compound **17** (570 mg, 2.0 mmol) was dissolved in dry DMSO (4 ml), followed by the addition of *N,N′*-dicyclohexylcarbodiimide (DCC; 1.24 g, 6 mmol) and $Cl_2CHCOOH$ (80 µl, 1 mmol). The mixture was stirred for 2 h at r.t. Oxalic acid (570 mg) in MeOH (7 ml) was added to quench, and precipitate was formed upon addition of AcOEt, which was filtered off. The filtrate was concentrated, redissolved in AcOEt, and the org. layer was washed with sat. $NaHCO_3$ soln. and H_2O. AcOEt layer was then dried ($MgSO_4$) and concentrated to give **18**, which was used directly without further purification.

The aldehyde **18** (475 mg, 1.7 mmol) was co-evaporated with dry benzene three times, dissolved in 5 ml of benzene, and added to the *Grignard* reagent prepared as described above. The mixture was refluxed for 13 h. After cooling to r.t., 0.1N HCl was added, and the mixture was stirred for 10 min. The org. compounds were extracted with $CHCl_3$ (200 ml) and washed successively with sat. $NaHCO_3$ (2 × 100 ml), brine (1 × 100 ml), and H_2O (1 × 100 ml). The org. layer was then dried ($MgSO_4$), concentrated, and the residue was subjected to CC to give **21a** (61%).

*(5*S*)-3-*O*-Benzyl-1,2-*O*-isopropylidene-*D*-[3,5-$^2H_2$]ribofuranose* (**21b**). A soln. of chloro(diisopinocampheyl)borane (Ipc_2BCl; 1.6 g, 5 mmol) in dry THF (5 ml) was added to a soln. of **18** (2 mmol; prepared as described above for **21a**) at −30° dropwise, and the mixture was stirred for 6 h under inert atmosphere. Then, the mixture was allowed to warm to r.t. slowly. The volatile materials were removed *in vacuo*, and the residue was dissolved in Et_2O (10 ml), followed by the addition of 2,2′-iminobis[ethanol] (1.2 ml), and the mixture was stirred for 2 h again at r.t. After filtering the precipitate formed, the filtrate was concentrated, and the residue was purified over silica gel to give **21b** (45%).

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