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THE SYNTHESIS OF DEUTERIONUCLEOSIDES†

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Abstract: *The synthesis of deuterionucleosides for site-specific incorporation into oligo-DNA or -RNA is herein reviewed for NMR or biological studies. The review covers the following aspects: (i) deuteration of the aglycone; (ii) single-site chemical deuteration of the sugar residues; (iii) multiple-site chemical deuteration of the sugar residues; (iv) enzymatic synthesis of deuterated nucleosides or nucleotides; and (v) synthesis of labelled nucleosides with multiple isotopes.*

INTRODUCTION

The structural studies of large biologically functional DNA or RNA molecules in solution are important to understand how their conformational characteristics and how variations of their local structure may translate in specific interactions and recognitions that finally culminate into specific biological function[1]. In this regard, Nuclear Magnetic Resonance (NMR) spectroscopy has emerged as a powerful tool in studies on the stereochemistry of interactions, recognitions, as well as dynamics of global and local structural changes, because it provides conformational data under quasi-physiological conditions[2-4]. Many studies have been performed with oligo-DNA[5-7], whereas, relatively very few studies have been performed on the conformation of oligo-RNA[3,8-9] because of two intrinsic problems: (i) poorer dispersion of chemical shifts of the ribose sugar, and (ii)

† *In memory of Professor Alexander Krayevsky*

the chemical synthesis of oligo-RNA on a preparative scale is more problematic compared to the oligo-DNA counterpart. In general, the NMR structure determination of both biologically functional oligo-DNA and RNA is plagued by the fact that the relaxation properties of protons dramatically change, signals broaden and the overlap owing to the repeating sugar units becomes severe, thereby adequate extraction of NMR information is very difficult, if not impossible. Clearly, many of these inherent problems are not easily overcome by simply increasing the field strength of the NMR-magnet. Here, the chemistry for specific isotope enrichment plays a more important role[3,5-7,9,12].

The full determination of the tertiary structure of DNA or RNA molecules by NMR spectroscopy requires two types of complementary information: (1) the conformation of each nucleotide unit described in terms of torsional angles, and (2) the spatial proximity between the nucleotidyl protons as interproton distance information. These are obtained from homonuclear proton-proton and heteronuclear proton-phosphorus, carbon-phosphorus coupling constants translated into torsion angle constraints[10] and from the nuclear Overhauser effect (nOe) translated into distance constraints[11]. Subsequently, these constraints are used for molecular model building. To collect this information optimally, it is ideal to have as many clearly separated resonance lines and crosspeaks from different NMR experiments as possible. Although, such coupling and distance information is available from the 2D and 3D NMR experiments for a smaller oligonucleotide, it is simply impossible to collect all of them in a non-prejudicial manner for large oligomers due to spectral overlap and line broadening which make the conformational analysis of a large, biologically functional DNA or RNA a formidable task. The overcrowding follows from the monotonous primary structure of nucleic acids and it is particularly severe for the resonances arising from the repeating pentofuranose moieties of RNA since all H2', H3', H4' and H5'/H5" protons absorb in a narrow chemical shift region (from 5.5 to 3.7 ppm). The line broadening is an intrinsic consequence of the increasing molecular size and it is associated with the more effective relaxation due to multiple pathways[12], which also gives rise to the spin diffusion hindering the accurate nOe volume determination for a given crosspeak. In order to overcome these difficulties various isotope labelling techniques have been devised.

Uniform labelling with $^{13}C/^{15}N$[13,14] has found widespread use especially in RNA structural studies, and the methods for the preparation of labelled DNA[15-17] have also been

recently published. The enzymatic syntheses[18-21] and heteronuclei edited multidimensional NMR experiments[22-25] are extensively reviewed. One of the potential drawbacks of the uniform labelling technique is the possible overcrowding of the heterodomain of the spectra since these nuclei are NMR active. To avoid this, it is important to explore economically viable ways for the *site-specific* incorporation of the $^{13}C/^{15}N$ labelled monomer units to generate short uniformly isotope enriched stretches, separated by non-enriched stretches, in a DNA or a RNA molecule by chemical[26,27] or enzymatic means[28,29], simply to disperse the signals optimally and to reduce overcrowding.

We were the first to show[30,31,116,137,211] that the substitution of proton by deuteron in a stereospecific manner at the pentose sugar residue removes not only the proton signal, it also simplifies the multiplicity of spin-spin interactions by reducing the complexity of ensemble of signals belonging to the spin-coupled neighboring proton(s). We have also subsequently shown that site specific deuteration[31-37a,38-43] is beneficial for several reasons: (i) It eliminates unessential resonance lines thus decreasing the spectral overcrowding in various regions of 1D and 2D homo- and heteronuclear correlation spectra of oligo-DNA and RNA[31-37a,38], (ii) it helps to identify coupling patterns as well as enable more precise determination of coupling values[33,35-37a,38,39] (iii) it enhances structurally important nOe intensities with diminished spin diffusion whereas removing insignificant ones[32,33,38,40], (iv) it helps to probe the dynamics of oligonucleotides by selective T_1 and T_2 measurements[38,41-43], and (v) it also reduces the line-broadening[40] associated with ^1H dipolar relaxation.

Despite reports showing the importance of deuterium substitution in structural studies of DNA and RNA, a summary of synthetic methods for chemospecific deuterium incorporation at single or multiple sites into nucleosides is clearly missing. The need for such an account is further obviated by the demand for deuterium (or tritium) labelled nucleosides in other spectroscopic investigations or in mechanistic studies on the action of various enzymes. In the present review, different synthetic methodologies that have been so far used for deuteration are discussed in details.

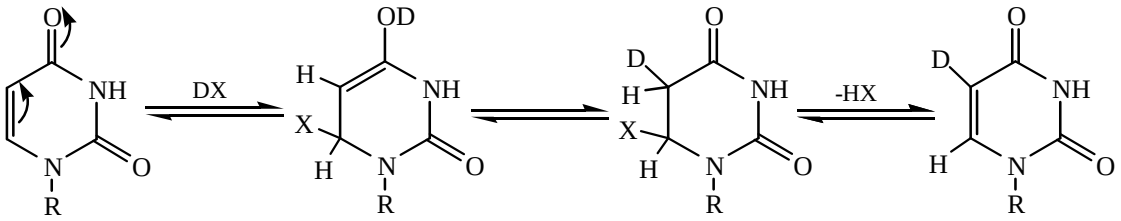
1.0 Deuteration of the aglycone

Deuteration of the C2 and C8 of the purine and C5 and/or C6 of pyrimidine base residues can be used for the simplification of overcrowded regions in NOESY or DQF-COSY type spectra of large DNA and RNA oligomers by eliminating crosspeaks appearing from the H5-H6 interaction or from cross-relaxation of the base protons and H1' of the sugar ring. This makes the NMR spectral assignment easier for structural

studies[44-51]. Base deuterated molecules can also be used in conformational investigations by Raman[52-54] or infrared[55] spectroscopy, in biological studies[56-61] and studies on hydroxyl radical reactions with nucleosides[62,63].

1.1 Deuteration at C5 of uracil and cytosine derivatives

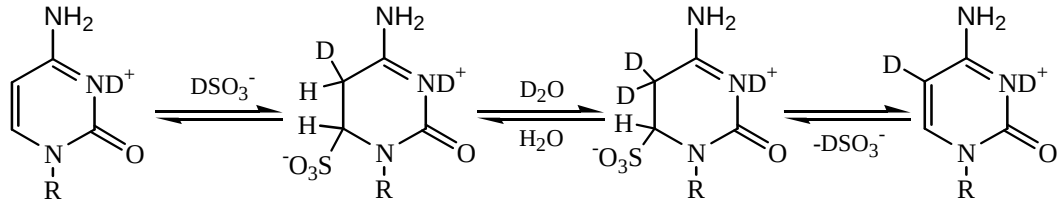
In the pyrimidine nucleosides and nucleotides, deuteration of the C5 position is based on the susceptibility of the 5,6-double bond to 1,4-nucleophilic addition reactions. The mechanism of this reaction is considered to be a 1,4-addition of the catalyst with the participation of the C4 carbonyl group of the heterocycle as shown in Scheme 1. For uracil derivatives the reaction takes place only under basic conditions[64-73].Sodium bisulfite



Scheme 1. R = H or ribofuranos-1-yl

is the most commonly used reagent to achieve C5 deuterium exchange in uracil compounds. It adds across the 5,6-double bond producing stable 5,6-dihydro-6-sulfonate derivatives[67-70]. The mechanism of this process under basic conditions at elevated or at room temperature has been investigated in details[58,65,68,70-72] and it is found that the bisulfite incorporation is dependent on the pH of the reaction medium, and the exchange is most efficient at pH 7-9[68,70]. Regeneration of uracil from the sulfonate adduct can be achieved by a treatment at pH >9[70]. It has been observed that ammonium sulfite could increase the rate of the exchange reaction[68]. Kinetic studies have shown that the rate of the hydrogen-deuterium exchange is linearly dependent on both bisulfite and amine concentrations. It is also found that amines accelerate the rate of the reversible addition of bisulfite[68,70]. Methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine, quiniclidine and imidazole are also effective as ammonia, whereas triethylamine or guanidine is not. Unfortunately, when this method is used for deuterium labelling of uracil derivatives, it gives insufficient level of isotope incorporation (30-60 atom% ^{2}H)[68]. However, there are other examples where the use of ammonium bisulfite/$^{2}H_2O$ system at pD 7.7 at 65 ?C for 72 hours exchanges proton to deuteron at C5 in 90 atom%[44,46,47,49].

Deuterium incorporation at C5 of cytidine derivatives can be achieved by the equilibration in ^2H_2O with bisulfite[67,69,70,74,75] as catalyst, under both basic and acidic conditions. The mechanism involves 1,4- nucleophilic addition[64,67,69,70,74,75] as for uridine.



Scheme 2. R = H or ribofuranos-1-yl

However, in alkaline medium the hydrolysis of the *N*-glycosidic bond[64] and deamination[64,67,69,70] can take place. The use of $(NH_4)_2SO_3$ instead of $NaHSO_3$ results in more rapid deamination[70]. The possibility to exchange H5 of cytidine monophosphate has been examined in ^2H_2O/1M HSO_3^- mixture at pH 7.7 and 37 ?C and it is found that the rate of H5 exchange with deuterium is faster than the rate of deamination[70]. This characteristic is found at all pD values in range 4-8[76]. It has also been noted that the optimum value for H5 exchange is exactly the same as the optimum for deamination (pD 5.4)[76].

The use of amines for the C5 deuteration is not recommended because of the possibility of transamination of the exocyclic NH_2 group of the cytosine-bisulfite adduct[70]. Certain amines, like imidazole, morpholine, ?-naphthalinamine and *p*-phenylazoaniline however do not cause transamination[70] and the use of imidazole buffer has also been published[75]. Nevertheless, successful applications of the bisulfite method that provides 90-98 atom% deuterium exchange at C5 for cytosine have been reported[44,46,47].

The exchange of the proton at C5 of cytidine has been studied in acidic media. In a citrate buffer at 95 ?C in ^2H_2O[64], the rate of exchange increases with increasing concentration of the buffer and reaches a maximum at pD 4.9.

Sulfhydryl compounds have been shown to be effective nucleophiles for the proton-deuteron exchange reaction. Uridine-5-*^2H* can be isolated in good yield with high isotope incorporation upon treatment with 2-mercaptoethylamine in ^2H_2O either at room or at elevated temperature[64,65]. Similar catalytic activity has been observed for glutathione[57] and cysteine[56,61] and the exchange is almost complete (>99 atom% *^2H*) in 7 days[56], or >90 atom% *^2H* in 48 hours at 80 ?C[61]. Thymidylate synthetase catalyses the C5 exchange reaction[59] upon formation of a C6 adduct with 2'-deoxyuridine monophosphate.

It has been observed that when the nucleosides have free hydroxyl groups, which can easily participate in the saturation of the 5,6-double bond, the base-catalyzed

exchange proceeds at a higher rate and the mechanism involves the formation of 6-*O*-anhydro cyclonucleosides[58,66]. As an example, when 2',3'-*O*-isopropylideneuridine is treated with 0.5N CH_3ONa/MeO^2H solution at 60 ?C, H5 is exchanged with deuterium more than 80 atom% ^2H in 2.5 hours. A similar result is obtained in case of 1-(5'-deoxy-?-D-arabinofuranosyl)-uracil under the same conditions, which is explained by the formation of the 6,2'-*O*-cyclonucleoside. An oxy-anion also participates in the exchange of H5 of 1-(3-hydroxypropyl)-uracil, where the rate is almost 6 times faster than for uridine and 2'-deoxyuridine[58]. The use of NaO^2H in ^2H_2O[37a] or the replacement of the NaO^2H with K_2CO_3[37b] for the deuteration of uridine results in high level deuterium incorporation at C5 (>95 atom% ^2H).

In a recently reported synthesis, the phenylthio group has served as protecting group at C6 during the lithiation of C5 with lithium 2,2,6,6-tetramethylpiperidide[77] in 2'-deoxy- and ribouridines. 5-Substitution can be obtained by the reaction of the lithiated species with different electrophiles followed by removal of the C6 protecting group with tributyltin hydride. Using methanol-$^2H_4$ as electrophile it is possible to incorporate deuterium at C5.

1.2 C5/C6-Di- and C6-monodeuteration of uridine and cytidine

When 2',3'-*O*-isopropylidene-uridine or cytidine is treated at elevated temperature with DMSO-$^2H_6/NaO^2H/^2H_2O$ or DMSO-$^2H_6/NaOCH_3/CH_3O^2H$, respectively, the corresponding 5,6-dideuterio derivatives are furnished[66]. 90 Atom% deuteration of the thymine nucleobase at C6 has been achieved upon heating thymidine in DMSO-$^2H_6/NaO^2H/^2H_2O$[66] or DMSO-$^2H_6/NaOH/H_2O$[48] mixtures at 135 ?C giving ~95 atom% deuterium incorporation. One possible mechanism of H6 exchange involves the formation of an anion at C6 by direct abstraction of H6 by a base[66]. This is supported by the rapid formation of 6-$^2H_1$-5-fluorouracil derivatives upon treatment with 0.5N $NaO^2H/^2H_2O$ at 60 ?C[73].

The possibility of exchange in 5'-*O*-DMTr-2'-deoxynucleosides has also been investigated. It is reported that during the equilibration of 5'-*O*-DMTr-N^4-benzoyl-2'-deoxycytidine in a solution of DMSO-$^2H_6/NaOCH_3$/methanol-$^2H_4$ at 95 ?C for 60 hours the appropriate 6-$^2H_1$ derivative is obtained with high deuterium incorporation[78]. Since this is the same reaction condition as discussed previously, and it is well known that in the ^1H NMR spectrum of 5'-*O*-DMTr-N^4-benzoyl-2'-deoxycytidine the H5 signal disappears under the other aromatic resonances, the product should likely be the 5,6-$^2H_2$ derivative.

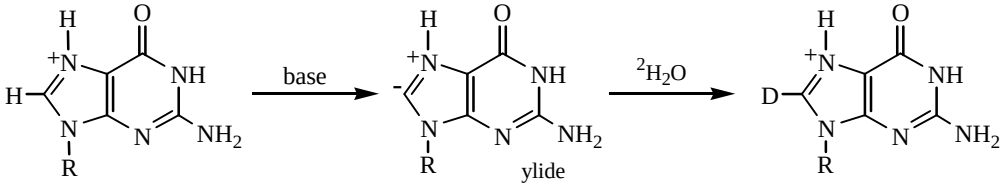
Under the conditions in which the C6 exchange is relatively slow, the back exchange of the 5,6 dideuterated U or C derivatives in protic $NaOH/H_2O$ or in $NaOCH_3/CH_3OH$ leaves 80 atom% ^2H at C6 and 30 atom% ^2H at C5 of U, or leaves C6 completely deuterated in *ara*-C[66].

In a further study on this base catalyzed deuterium exchange of the pyrimidine nucleobases, it has been found that high level isotope exchange can be achieved at C6 with a relatively lower level of C5 deuteration using DMSO-$^2H_6$ as the deuterium source[48] in the presence of a non-deuterated base.

Deuteration of the parent heterocycles is also feasible. C5, C6-$^2H_2$ uracil and cytosine can be synthesized using deuterium gas and a metal ion catalyst[79]. When uracil and cytosine are treated with $^2H_2$ in alkaline media in the presence of a Pd catalyst, 6-^2H compounds are mostly formed (deuterium incorporation: 96 atom% for uracil, 79 atom% for cytosine), whereas 5-bromouracil and 5-bromocytosine produce mostly the 5-^2H derivatives. It should be however noted that the yield of these reactions is rather moderate.

1.3 Deuteration at C8 of adenine and guanine derivatives

Protons linked to C8 of purine derivatives can be exchanged more rapidly compared to C5 and C6 of pyrimidines and C2 of purines[64]. The first observation of ex-



Scheme 3. H or ribofuranos-1-yl

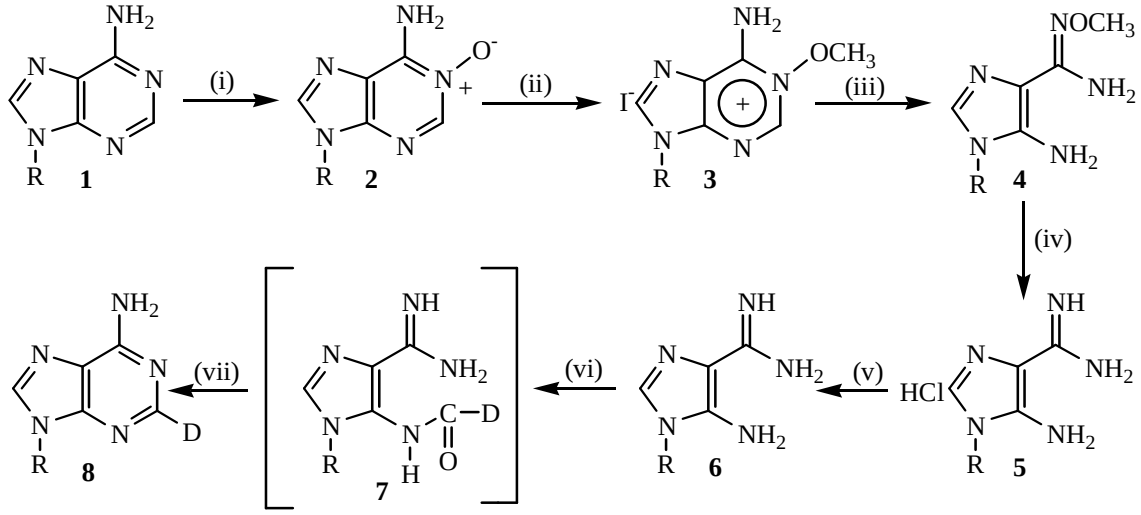
change of the proton attached to C8 with a deuteron has been done in ^2H_2O at >100 ?C[80,81]. This reaction consists of the OH^- catalyzed abstraction of the hydrogen from the N7-protonated purine giving rise to an ylide type intermediate, which then is "reprotonated" at C8. In the case of guanosine, the protonation of the N7 can also occur due to the formation of a zwitterion[82-85] (Scheme 3).

The pH-dependent kinetic studies of exchange process have also been performed[52,71,83,85-88]. It has been found, that within a certain pH range, the structure of the purine could be unchanged (neither protonated nor deprotonated). If the acidity of the media is higher than their pK_a (<4), the rate of the exchange is slower. In case of N9 substituted purines the rate of the exchange reaction sharply increases at pH higher than 12[88]. Therefore, the reaction is usually carried out in a slightly alkaline (pH = 7.8)[52] or neutral media using the published procedures[87,88]. This method has been used for deuteration of C8 of guanosine triphosphate and guanosine monophosphate (incubation of the guanosine derivatives in ^2H_2O at 50 ?C for 24 hours), and the level of deuterium incorporation is satisfactory for NMR studies of the RNA oligomer[49,53].

The methods useful for deuterium exchange at C5 and C6 in pyrimidines can also be applied to purine derivatives. C8 of adenosine and guanosine can be deuterated using a solution of triethylamine/^2H_2O/DMSO-$^2H_6$ at 60 ?C for 57 hours (95 and 99 atom% exchange for adenosine and guanosine, respectively)[48]. 5'-*O*-DMTr- and base-protected 2'-deoxyadenosine and 2'-deoxyguanosine are deuterated at C8 in DMSO-$^2H_6$/^2H_2O solution at elevated temperature[78]. The sodium bisufite/^2H_2O system under mild basic conditions (pH = 7.7-8.0) and at elevated temperature provides deuterium incorporation at C8 in >90 atom% ^2H[44,46,47].

1.4 Deuteration at C2 of adenine derivatives

The proton attached to C2 of adenines does not undergo exchange in ^2H_2O in a facile manner as that of the C8[64]. Deuterium can be introduced at C2 of adenine *via* different routes[89-91]. The reaction of aminomalondiamidine dihydrochloride with deuterated ortho esters in dimethylformamide yields 2-deuterated adenines. C2-deuterated adenine can be prepared in 76% yield by the reaction of aminomalondiamidine dihydrochloride[89] or aminoimidazolecarboxamidine hydrochloride[90] with deuterated ethyl orthoformate. A similar procedure has been applied for 9-substituted adenines 8[91]



Scheme 4. *Abbreviations*: R = ribofuranos-1-yl or 2-deoxyribofuranos-1-yl. *Conditions*: (i) *m*-chloroperbenzoic acid in CH_3OH at 30 ?C for 7 h or aqueous H_2O_2/AcOH at elevated temperature overnight; (ii) CH_3I, dimethylacetamide at 14 ?C for 6.5 h; (iii) NaOH/H_2O, reflux, 15 min; (iv) H_2O, 1 N HCl, Raney Ni W-2, 25 ?C for 7h; (v) Amberlite IRA-402 (OH^-), H_2O; (vi) and (vii) 1-(formyl-$^2H_1$)-2(1*H*)-pyridone in dimethylacetamide at r.t. for 4.5 h.

(Scheme 4). 9-Substituted 2-deuterioadenines can be prepared from aminoimidazolecarboxamidines **6** or their hydrochlorides **5** by cyclization incorporating a deuterated carbon unit such as deuterated formic acid or 1-(formyl-^2H)-2(1*H*)-pyridone. The required monocycles are available from 9-substituted adenines **1** in four steps starting with N1-oxidation to give 1-*N*-oxides **2**. Subsequently, 1-*N*-*O*-alkylation furnishes 1-alkoxy derivatives **3**, followed by hydrolytic ring opening to give the *N*′-alkoxyimidazole-4-carboxamidines **4**, and hydrogenolytic dealkoxylation.

1.5 Preparation of deuterated nucleosides using platinum catalyst

In earlier studies the possibility of the introduction of isotope by catalytic exchange has been investigated[92]. Labelled adenine is prepared by reducing platinum with $^2H_2$ in ^2H_2O followed by treating the mixture with adenine for 18 hours at 100 ?C. 8-Deuterio-adenosine is derived from 8-bromoadenosine[93] by catalytic debromination with $^2H_2$ gas in acetic acid/^2H_2O mixture using platinum as catalyst[88]. The use of a modified general procedure[92] makes the incorporation of deuterium possible at the C2 and C8 of adenosine, C5 and C6 of uridine and cytidine and the methyl group of thymidine. The deuteration level depends on the substrate to catalyst ratio, and can be as high as 100 atom% ^2H. C8 of adenosine and C5 of uridine and cytidine can be exchanged back by equilibration in H_2O under conditions where only C5 is exchangeable[94]. The method has

been used for the preparation of different base modified ribonucleosides[95] and for the preparation of deuterated single-stranded oligo-DNA[45].

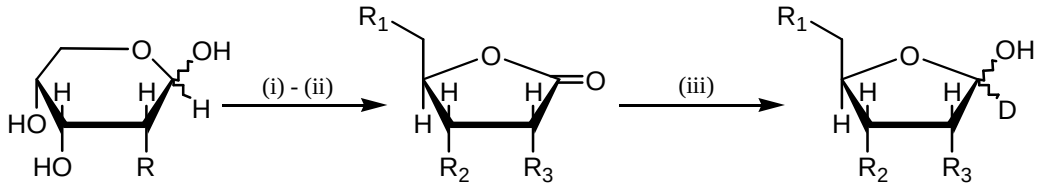
1.6 Postsynthetic deuteration of the aglycone in oligonucleotides

Since the H8 atoms of purines are very labile even at room temperature, it is necessary to perform all further manipulations of the deuterated blocks in deuterated media to avoid the back exchange to proton. Postsynthetic deuteration resolves this problem; thus in this case deuteration is the last step in the preparation of the labelled oligomers. The DNA sample can be deuterated in ^2H_2O under acidic conditions[55], or at pD 7 by addition of NaO^2H at 80 ?C[54], or using deuterioammonium bisulfite/^2H_2O solution at pD 7.8 and 65 ?C for 48 hours[44]. Deuterium exchange of oligo-RNA can be achieved at neutral pD by incubation of the substrate in ^2H_2O solution at elevated temperature[50,54], but this experiment is not always satisfactory because of the partial degradation of RNA-oligomers under the exchange conditions[50].

2.0 Single-site chemical deuteration of the sugar residues

2.1 Deuteration of the anomeric C1' of the D-nucleosides

A very serious obstacle during NMR based structural studies of oligo-DNA or RNA is the cross-relaxation between H8 or H6 protons of purine or pyrimidine nucleobases, respectively, with the respective C1' proton of the constituent sugar residue. This results in strong intraresidual H8/H6 to H1' crosspeaks causing severe spectral overlap in the aromatic to H1' nOe crosspeak region as the number of repeating nucleotide residues increases with the increase of the molecular weight of oligo-DNA or –RNA, rendering the extraction of nOe volumes inaccurate or impossible. This has posed a great demand for C1' deuterated nucleosides[96], which has been further stimulated by studies on reaction mechanisms[72,97-103] or other physico-chemical studies[53].



9: R = OH

10: R_1 = OH, R_2 = OH, R_3 = OH
10a: R_1 = OH, R_2 = OH, R_3 = H
11: R_1 = OTBDMS, R_2 = R_3 = O-Ip
12: R_1 = R_2 = R_3 = OBz
13: R_1 = R_2 = OTBDMS, R_3 = H

14: R_1 = OH, R_2 = H, R_3 = OH
15: R_1 = OTBDMS, R_2 = R_3 = O-Ip
16: R_1 = R_2 = R_3 = OBz
17: R_1 = R_2 = OTBDMS, R_3 = H

Scheme 5. *Abbreviations:* TBDMS = *tert*-butyldimethylsilyl, Ip = isopropylidene, Bz = benzoyl. *Conditions*: (i) Br_2 in H_2O followed by Na_2CO_3 for 10; then HBr, AcOH followed by Bu_3SnH for **10a**; (ii)

conc. H_2SO_4 in dry acetone then TBDMS-Cl, triethylamine, DMAP in dry CH_2Cl_2 or Bz-Cl in dry pyridine or TBDMS-Cl, $AgNO_3$, dry pyridine in dr THF; (iii) Na-amalgam in ^2H_2O, pH 3.5-4 or diisoamylborane-$^2H_1$ in THF or DIBAL-$^2H_1$ in THF, ~-78 ?C.

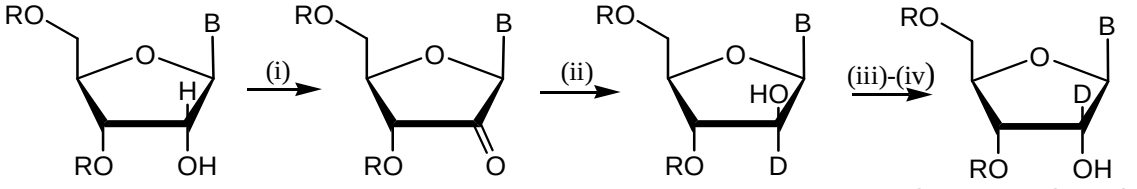
Since the base catalyzed exchange reaction at the ?-position of the appropriate 2-oxo ribofuranoside or ribopyranoside gives rise to H3 instead of H1 isotope exchange in ^2H_2O[140], all reported C1' deuterations of nucleosides start with the reduction of D-?-ribonolactone (**10**) or its 2-deoxy counterpart **10a**. These are commercially available, or they can be prepared from D-ribose (**9**) relatively easily in high yield[104,105]. The reduction of **10** to D-ribose-1-$^2H_1$ (**14**) has been achieved by the use of various reducing agents. Reduction with sodium amalgam[53,72,97,101,102,106] in a mixture of ^2H_2O and $^2H_2SO_4$ at carefully controlled pH (3.5 - 4) and temperature (below 10 ?C) gives a moderate yield of the desired deuterio ribose. On the other hand, it does not require protection of the hydroxyls and it is suitable for large scale synthesis (up to 1 mol) giving excellent deuterium enrichment (?99-98 atom% ^2H). Although the reduction with sodium borohydride[107-109] or lithium aluminum hydride (LAH)[110,111] is known for various lactones, their use for deuterium labelling has attracted little attention most probably due to difficulties in the control of overreduction to ribitol as well as in large scale purification of the reaction mixture. Nevertheless, LAD reduction of 2,3-*O*-isopropylidene-5-*O*-TBDMS-D-ribonolactone (**11**) to **15** in cold anhydrous tetrahydrofuran (THF) proceeds with 58% yield with 98 atom% ^2H enrichment[112]. Reduction of 2,3,5-tri-*O*-benzoyl-D-ribonolactone (**12**) with the hindered diisoamylborane-$^2H_1$[98,100,103,113] in THF at -15 ?C gives the deuterated ribose derivative **16** as part of the crude product. Purification of this has been achieved by 1-*O*-acetylation of the mixture to afford the desired 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose-1-$^2H_1$ in 46% yield. Reduction of 3,5-*O*-TBDMS-2-deoxyribonolactone (**13**) with DIBAL-$^2H_1$[114] has been carried out in dichloromethane at -78 ?C to furnish the C1 deuterated 2-deoxyribose **17** in 83% yield with ? 99 atom% ^2H substitution as determined by ^1H NMR analysis. The C1 deuterated ribose derivatives **14**-**17** can be further converted to the appropriate 1'-$^2H_1$-nucleosides by known procedures[31a,112,114].

2.2 Deuteration of C2' of D-nucleosides

Amongst the most complicated NMR spectral regions for a large oligo-DNA or –RNA, the H2' in ^{1}H and C2' in ^{13}C NMR spectroscopy have been proven to be the most complicated. This is the result of large number of interacting protons in the vicinity of these nuclei as well as the stacking interaction of nucleobases resulting in numerous intra- and interresidual nOe interactions. As a consequence, the deuterium substitution at C2' has been of primary interest. It is known to be one of the most difficult tasks[115,116]. The methodologies have been reported so far include incorporation of deuterium at both sugar or nucleoside levels either by enzymatic or chemical means to afford the required ribo- or 2'-deoxyribonucleosides.

2.2.1 Synthesis *via* deuteration of C2' at nucleoside level

The syntheses of C2'-deuterated ribonucleosides are carried out by a sequence of oxidation of appropriately 3',5'-*O*-protected nucleosides **18a-e** (Scheme 6) with CrO_3/pyridine/acetic anhydride (Ac_2O) or DMSO/Ac_2O or Dess-Martin reagent followed by reduction of the resulted 2'-ketonucleoside **19** with sodium borodeuteride or LAD to afford the corresponding *arabino* epimer **20** as the predominant product along with the deuterated *ribo* analogues in variable ratios with varying yields depending on the nature of aglycone[115-117]. Recently, after the selective removal of the 5'-*O*-TBDMS protection from



18a: R = TBDMS, B = U
18b: R = TPDS, B = U
18c: R = TPDS, B = A
18d: R = TPDS, B = C^{Ac}
18e: R = TBDMS, B = A

19a: R = TBDMS, B = U
19b: R = TPDS, B = U
19c: R = TPDS, B = A
19d: R = TPDS, B = C^{Ac}
19e: R = TBDMS, B = A

20a: R = TBDMS, B = U
20b: R = TPDS, B = U
20c: R = TPDS, B = A
20d: R = TPDS, B = C^{Ac}
20e: R = TBDMS, B = A

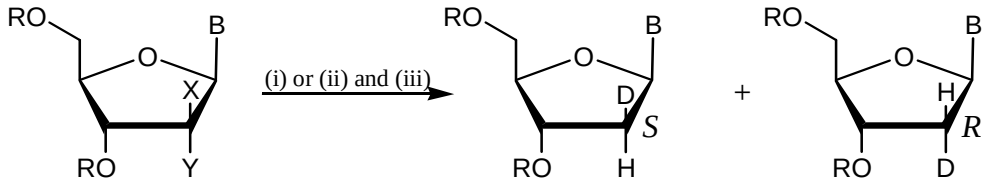
21c: R = TPDS, B = A

Scheme 6. *Abbreviations*: U = uracil-1-yl, A = adenin-9-yl, C = cytosin-1-yl, Ac = acetyl, TPDS = 1,1,3,3-tetraisopropyldisiloxan-1,3-diyl. *Conditions*: (i) CrO_3/pyridine/Ac_2O (1:2:1) in CH_2Cl_2, r.t., 45-60 min. or Dess-Martin periodinane in CH_2Cl_2, r.t., ?4 h or DMSO/Ac_2O, r.t., 22 h; (ii) $LiAl^2H_4$ in dry ether or NaB^2H_4 in ethanol; (iii) trifluoromethanesulfonic anhydride, pyridine, DMAP in dry CH_2Cl_2, 0 ?C, 2.5 h; (iv) cesium propionate in dry DMF, r.t., ?3-4 h.

19e, the ketone has been reduced[118,119] with sodium triacetoxyborodeuteride directly to the *ribo* epimer with 99:1 stereoselectivity. Compound **20c** has been converted to its

2'-*O*-triflate derivative in order to invert the configuration to obtain **21c**[117]. Alternatively, the deuterated *arabino* compounds **20b,c,d** have been subsequently deoxygenated with tributyltin deuteride after introducing a 2'-*O*-phenoxythiocarbonyl (Ptc) protection to afford 2',2''-$^{2}H_{2}$-2'-deoxynucleosides[116] in moderate yield (38 - 75%).

The diastereoselectivity of the synthesis of 2'*(R)*-$^{2}H_{1}$-2'-deoxynucleosides **23R** has been studied[120] taking the corresponding 3',5'-*O*-bis-protected 2'-halo or 2'-phenyl-thiocarbonyloxy nucleosides **22** (Scheme 7) for treatment with tributyltin deuteride in the presence of (a) AIBN under sonication or usual stirring, or (b) using triethylborane as free



22a: B = U, R = Bz, X = H, Y = OPtc
22b: B = U, R = Bz, X = H, Y = Br or Cl
22c: B = A, R = Bz, X = H, Y = OPtc
22d: B = A, R = TPDS, X = H, Y = OPtc
22e: B = A, R = TPDS, X = OPtc, Y = H
22f: B = U, R = TPDS, Y = Br
22g: B = A, R = TPDS, Y = Br or Cl
22h: B = T, R = TPDS, Y = Br
22i: B = A^{Tms}, R = Tms, X = H, Y = Cl
22j: B = A^{Tms}, R = Tms, X = Cl, Y = H

23a: B = U, R = Bz
23b: B = A, R = Bz
23c: B =U, R = H
23d: B = A, R = H
23e: B = T, R = H

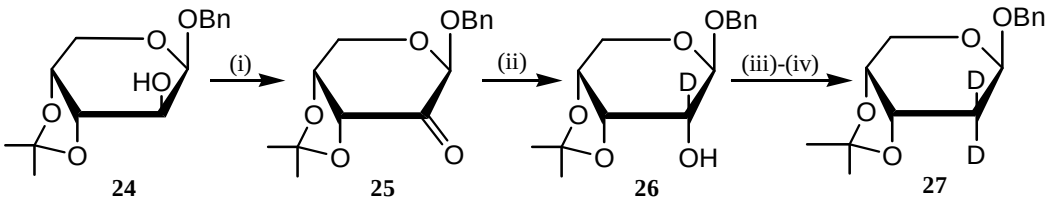
Scheme 7. *Abbreviations*: U = uracil-1-yl, A = adenin-9-yl, T = thymin-1-yl, Bz = benzoyl, Ptc = phenoxythiocarbonyl, Tms = trimethylsilyl, TPDS = 1,1,3,3-tetraisopropyldisiloxan-1,3-diyl. *Conditions:* (i) $Bu_3Sn^2H_1$, AIBN at various temperatures; (ii) $Bu_3Sn^2H_1$, triethylborane, THF, ~-70 ?C, sonication; (iii) TBAF in dry THF

radical initiator. The combination of condition (b) and 2'-bromonucleosides results in excellent stereoselectivity (**23***R*:**23***S* ?98:2)[121] allowing the preparation of the four 2'*(R)*-$^{2}H_{1}$-2'-deoxynucleosides. The use of this system with a nondeuterated reducing agent and deuterated 2'-bromo-2'-deoxynucleosides affords the 2'*(S)*-$^{2}H_{1}$-2'-deoxynucleosides with excellent diastereoselectivity[122,123] but with a surprisingly low level (?93%) of deuteration in the case of the guanosine derivative. The deuterium labelling at the C2' of 3',5'-*O*-TPDS-2'-bromonucleosides with the tris(trimethylsilyl)-silane-$^{2}H_{1}$-triethylborane system in tetrahydrofuran has been reported[124] to afford 2'*(S)*-$^{2}H_{1}$-2'-deoxynucleosides.

2.2.2 The synthesis of C2 deuterated sugar precursors

The above (Scheme 6) oxidation-reduction sequence has been used at the sugar

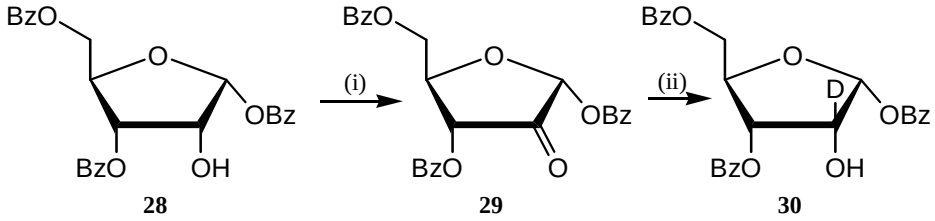
level to prepare 2',2''-dideuterio-2'-deoxynucleosides[116] (Scheme 8). The route consists of the oxidation of 3,4-*O*-isopropylidene-?-D-arabinopyranoside **24** to the corresponding ketosugar **25** followed by reduction with LAD to furnish 2*(R)*-$^{2}H_{1}$-alcohol **26**. This compound is converted to the 2-*O*-(methylthio)thiomethyl derivative and is subsequently



Scheme 8. *Abbreviation*: Bn = benzyl. *Conditions*: (i) CrO_3/pyridine/Ac_2O (1:2:1) in CH_2Cl_2, r.t., 15 min.; (ii) LAD in dry THF, crystallization from ethyl acetate; (iii) NaH in THF, reflux, 2 h, then CS_2, r.t., 2 h followed by CH_3I overnight; (iv) Bu_3Sn^2H in refluxing dry toluene, 20 h.

deoxygenated with the help of AIBN/tributyltin deuteride in toluene under reflux to afford the 2',2''-$^{2}H_{2}$-2'-deoxyribose derivative **27**. This is further transformed to the required deuterated 2'-deoxyribonucleosides *via* the coupling of 1-chloro-2-deoxyribofuranose and appropriate nucleobase derivatives.

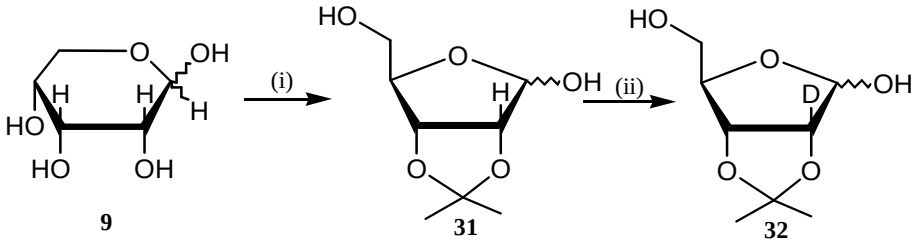
Towards the synthesis of 2'-deuterionucleosides, the oxidation-reduction procedure has also been utilized using D-ribose as starting material[125] (Scheme 9). Among several oxidizing agents, the Dess-Martin reagent is found as the most effective and convenient on 1,3,5-tri-*O*-benzoyl-?-D-ribofuranose (**28**) as substrate to afford **29** followed by reduction with NaB^2H_4 and $CeCl_3$ in THF exclusively to the corresponding 2-$^{2}H_{1}$-ribofuranoside **30** with 94-96 atom% deuterium incorporation.



Scheme 9. *Abbreviation*: Bz = benzoyl. *Conditions*: (i) Dess-Martin periodinane in CH_2Cl_2, r.t., 12 h; (ii) $CeCl_3 \cdot 7H_2O$, NaB^2H_4 in THF for 1h then quenching with AcO^2H.

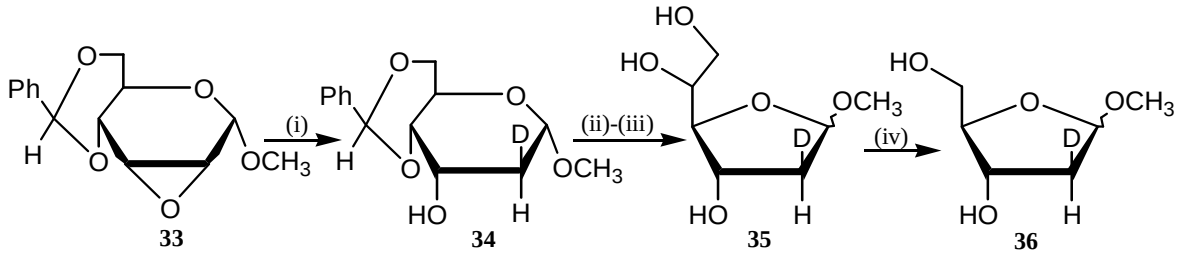
Very recently, the high yield stereoselective single step deuteration at the ?-position of an oxo-group in carbohydrates has been reported[126]. It is described that, while the hydroxyl groups are protected especially by an isopropylidene moiety, the isotope exchange reaction is stereospecific, whereas in the case of benzyl as protecting group, the epimeric mixtures are isolated. Although this methodology has been applied to a variety of oxo-compounds including 2,3:5,6-*O*-isopropylidene D-allo/L-gulofuranose and 2-deoxyribofuranose, there has not been a direct procedure for the C2-deuteration of D-ribose or its derivatives. Based on these findings it has been shown[127], that 2,3-*O*-

isopropylidene-D-ribofuranoside (**31**)[128] (Scheme 10) undergoes isotope exchange (>97 atom% *^{2}H*) at the C2 in a completely stereospecific manner in high yield to afford **32** when treated with 1,4-dioxane-tetrahydrofuran-triethylamine-$^{2}H_2O$ at reflux.



Scheme 10. *Conditions*: (i) dry acetone, conc. sulfuric acid; (ii) dioxane/THF/triethylamine/$^{2}H_2O$ (4:4:2:3, v/v), reflux for 4 days.

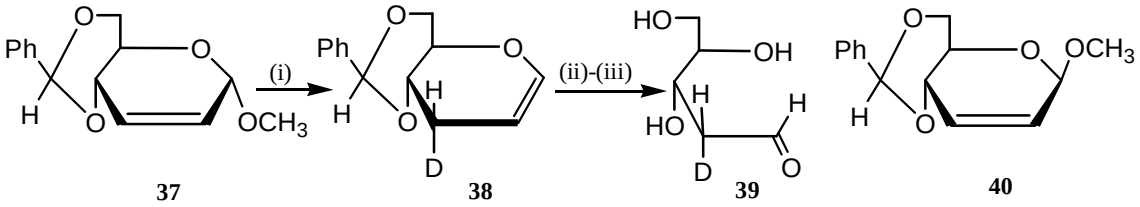
2',2''-Dideuterio-2'-deoxyguanosine and thymidine can be prepared enzymaically from 2-deoxyribose-5-phosphate using 2-deoxyribose-5-phosphate aldolase in $^{2}H_2O$ achieving ~90 atom% isotope incorporation[129].



Scheme 11. *Abbreviation*: Ph = phenyl. *Conditions*: (i) LAD in diethyl ether, reflux, 15 h; (ii) 0.1 M AcOH, 80 ?C, 24 min.; (iii) HCl (0.2 %) in methanol, r.t., 6 min.; (iv) $NaIO_4$ in ethanol/aqueous saturated $NaHCO_3$.

The first chemical synthesis of specifically labelled 2'-deuterio-2'-deoxynucleosides (Scheme 11) involves the opening of 2,3-anhydro-allopyranoside **33** with LAD[130,131] to **34** followed by conversion into the corresponding furanoside **35**[132], oxidation of the diol to **36** and coupling with respective nucleobases. Another group[133,134] has reported on the stereo-selective incorporation of deuterium during the reductive rearrangement of 2,3- dehydrohexopyranoside **37** (Scheme 12) to **38** upon treatment with

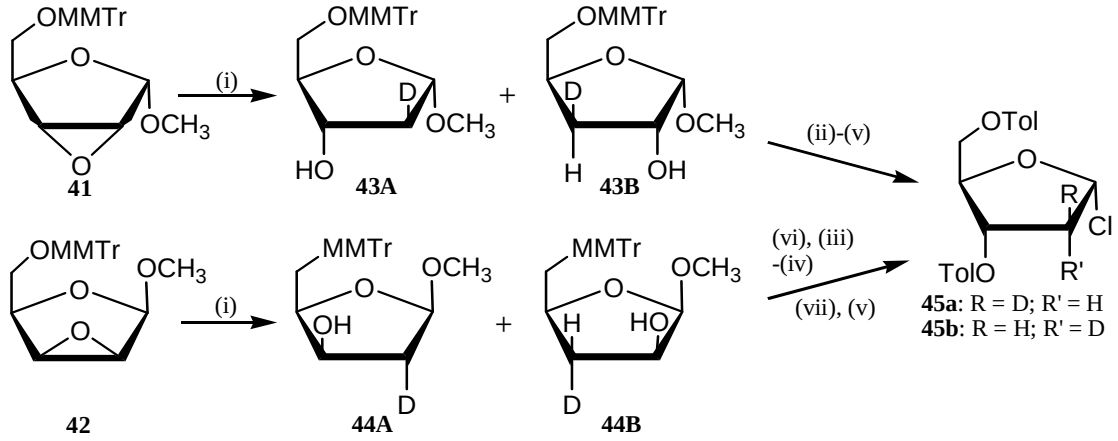
LAD[135] followed by oxidation of the resulting glycal to the pentose **39** which is further converted to 2'*(R)*-deuterio-2'-deoxycytidine. The same series of reactions taking the ?-anomer **40** as starting



Scheme 12. *Abbreviation*: Ph = phenyl. *Conditions*: (i) LAD, diethyl ether, reflux, 10 h; (ii) OsO_4 and $NaIO_4$ in dioxane/water (3:1), 0.5 h, r.t.; (iii) dilute H_2SO_4, 1.5 h, r.t.

material results in the preparation of 2'*(S)*-deuterio-2'-deoxycytidine.

Wong *et al.*[136] have reported the synthesis of 2-deoxy-1-$^{2}H_1$-D-*erythro*-pentose, 2-deoxy-2*(S)*-$^{2}H_1$-D-*erythro*-pentose and 2-deoxy-1,2*(S)*-$^{2}H_2$-D-*erythro*-pentose



Scheme 13. *Abbreviations*: MMTr = monomethoxytrityl; Tol = 4-toluoyl. *Conditions*: (i) LAD in dry THF, 6 h; (ii) Tol-Cl in dry pyridine; (iii) 80% aqueous AcOH, r.t., 4 h; (iv) Tol-Cl in dry pyridine; (v) AcOH, HCl; (vi) Ms-Cl in dry pyridine; (vii) sodium toluate in DMF-H_2O, elevated temperature, 3 days.

from D-arabinose by a sequence involving the formation and reduction of ketene dithioacetal derivatives.

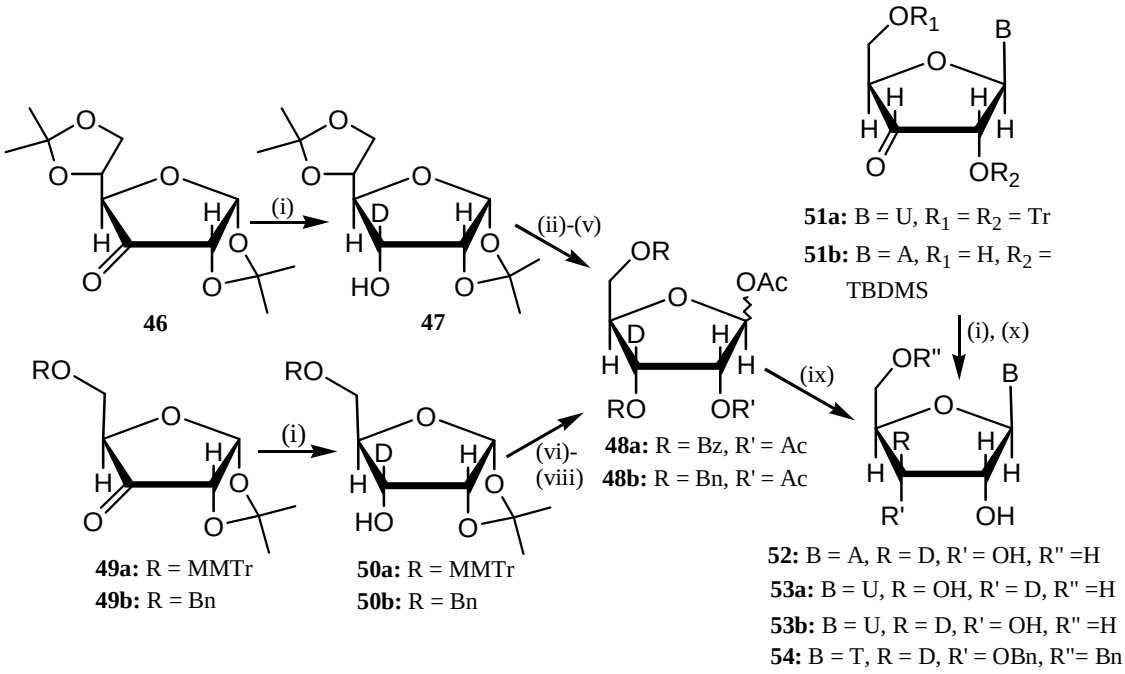
The synthesis of all eight 2'*(S)*- or 2'*(R)*-$^{2}H_1$-2'-deoxynucleosides has been published[137] by reductive opening of the appropriate methyl-2,3-anhydro-?-D-ribo **41** or ?-D-lyxofuranosides **42** (Scheme 13) to the predominant 2*(S)* or 2*(R)*-deuterio-2-deoxyribosides **43A** and **44A**, respectively, using LAD. These sugars are converted to **45a-b** for the synthesis of the target nucleosides. For the sake of completeness, it should be mentioned that the reductive elimination of a 2'-*O*-Ptc group with tributyltin

deuteride[115] proceeds with ~85% stereoselectivity for $^{2}H(R)$ incorporation, which is suitable and has been exploited for solid state ^{2}H NMR studies[138,139].

2.3 Deuterium substitution of H3' of the D-nucleosides

Deuterium incorporation at C3' position is rare. Since the ^{1}H NMR signals of these protons are in the severely overlapping H2'/4'/5'/5" region in oligo-RNAs or H4'/5'/5" region in case of oligo-DNA, a single deletion of these signals has little effect on the assignment of the NMR spectra. Such deuterium labeling at C3' however separates the H1'-H2'(2") and H4'-H5'/5" spin systems. Therefore, 3'-$^{2}H_1$ might have a serious impact on the simplification of the sugar spin-system as well as on the relaxation behavior of the coupled nuclei, which might improve both the sensitivity and resolution in a similar way as found for the H1'-H2' (or H2") protons in 2'-deuterated-2'-deoxynucleotides[33,38,39,140]. Moreover, the use of uniform ^{13}C-labelling can be restricted by the relatively limited dispersion of the C2'/C3' carbon signals. This overlapping region of the ^{13}C dimension of ^{13}C edited 2D spectra can clearly become simplified[141] upon deuteration of all or certain C3' centers. Additionally, C3' deuterated nucleosides have found their application in mechanistic studies on enzyme action[100,142,143].

The deuterium incorporation has been achieved at both sugar and nucleoside levels. For the sugar transformation, the directive effect exerted by a neighboring 1,2-*O*- isopropylidene group has been widely exploited (Scheme 14). Thus, reduction of 1,2:5,6-di-*O*-isopropylidene-?-D-hexofuranose-3-ulose (**46**) with LAD in dry ether[140,144] or with $NaB^{2}H_4$ in ethanol[142] or water-methanol (1:1) mixture[145] at room temperature affords the C3 deuterated allose derivative **47** with >97 atom% deuterium incorporation. From this, the derivatized nucleoside precursor **48** can be obtained upon homologation with $NaIO_4$. Alternatively, 5-*O*-protected 1,2-*O*-isopropylidene-?-D-pentofuranose-3-uloses **49a** or **49b** can be reduced with exclusive ?-attack by LAD in dry ether[146] or by $NaB^{2}H_4$ in ethanol[147] resulting in compounds **50a** or **50b** with >97 or >95 atom% deuterium incorporation, respectively. It is noteworthy that the benzyl protecting group used in case of compound **49b** leads to difficulties when purine nucleosides are to be deprotected by the literature protocol[147] of catalytic hydrogenation over Pd on charcoal. For the removal of this protecting group, the sodium-liquid ammonia[148] or $Pd(OH)_2$ on carbon[149] systems might be utilized but further work is needed in this



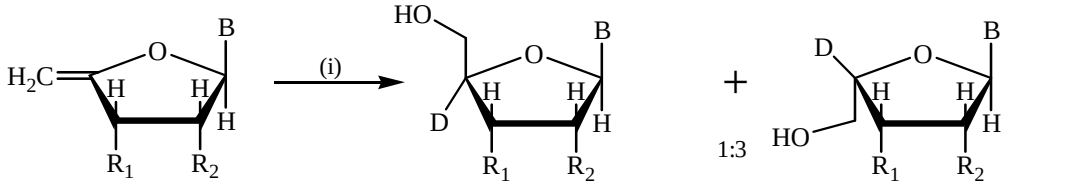
Scheme 14. *Abbreviations*: Tr = triphenylmethyl, MMTr = monomethoxytrityl, TBDMS = *t*-butyldimethylsilyl, T = thymin-1-yl, U = uracil-1-yl, A = adenin-9-yl, C = cytosin-1-yl, G = guanin-9-yl. *Conditions*: (i) NaB^2H_4 in ethanol or LAD in dry ether; (ii) Dowex 50W-X4, H^+ form in H_2O, r.t., 20 min.; (iii) $NaIO_4$ in ethanol-water then $NaBH_4$ in ethanol; (iv) Bz-Cl in dry pyridine; (v) acetolysis; (vi) BnBr, NaH in DMF; (vii) TsOH in dioxane-water; (viii) Ac_2O, pyridine, acetonitrile; (ix) silylated A or T in dry acetonitrile, $SnCl_4$ followed by full or partial deprotection; (x) sodium triacetoxyborodeuteride in acetic acid.

direction. When a 3'-ketonucleoside is reduced with $NaB(^2H)H_4$ in ethanol, the product is a mixture of appropriate *xylo* and *ribo* epimers (for U derivative **51a** this ratio was found to be **53a**/**53b** = 65:35)[150]. The 3' deuteration upon reduction of the appropriate 3'-keto derivative of adenosine **51b** with *in situ* generated[118,119] sodium triacetoxy-borodeuteride[118,119] in acetic acid is quite surprising due to the known preference for deuterium delivery from the ?-face directed by the bulky aglycone. For the rationalization of this finding the chelation of the reducing agent with the 5'-oxygen has been postulated which then results in overwhelming ?-attack by the deuterium giving **54**. The ~95 atom% deuterium incorporation, the accompanying 1-2% *xylo* impurity evidenced by reverse-phase HPLC as well as missing data regarding the other nucleosides make this isotope labelling technique somehow less attractive compared to the isotope labelling of sugar derivative **48** followed by nucleoside synthesis. From the 3'-$^2H_1$-ribonucleosides **50** the appropriate 3'-$^2H_1$-2'-deoxynucleosides can be obtained by 2'-deoxygenation[115].

2.4 Deuteration of the C4' position

The C4' deuteration of nucleosides simplifies the analysis of ^1H NMR spectra in various NMR experiments by reducing the spectral crowding in the ribose region and it is also useful in mechanistic studies on enzymatic processes[98,100,151,152].

Several procedures have been reported so far in the literature for the incorporation of deuterium at C4'. 4',5'-Unsaturated pyrimidine nucleosides **55** and **58** have been reduced by $B_2{}^2H_6$ in THF followed by oxidation using H_2O_2/OH^- to give isomeric ?-D-ribofuranosyl (**56** and **59**) and ?-L-lyxofuranosyl pyrimidine nucleosides **57** and **60** in 1:3



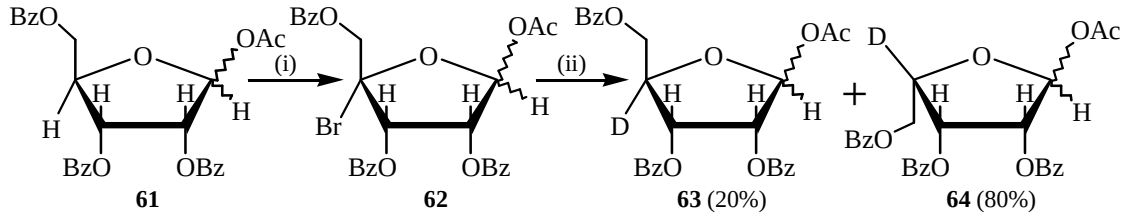
55: R_1=OAc, R_2=H, B=T
58: R_1=R_2=O-Ip, B=U

56: R_1=OAc, R_2=H, B=T
59: R_1=R_2=O-Ip, B=U

57: R_1=OAc, R_2=H, B=T
60: R_1=R_2=O-Ip, B=U

Scheme 15. *Abbreviations*: Ac = acetyl; Ip = isopropylidene; T = thymin-1-yl; U = uracil-1-yl. *Condition*: (i) $B_2{}^2H_6$ in dry THF, ~-40 ?C followed by oxidation with H_2O_2/OH^- at r.t.

ratio[153] (Scheme 15). Intramolecular hydrogen atom abstraction is used for relatively high level of deuterium incorporation (70 atom% *^2H*) upon treatment of 5'-*O*-benzyl-3'-*O*-(1-bromo-2-methyl-2-propyl)-thymidine by Bu_3Sn^2H[154]. The attempts to introduce deuterium into the 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-?-D-ribofuranose (**61**) *via* bromination at C4 by Br_2/CCl_4, *hv* to **62**, followed by reduction with Bu_3Sn^2H gives D-*ribo* and L-*lyxo* epimers **63** and **64** in a 1:4 ratio[112] (Scheme 16). The deuterium incorporation at C4 of a



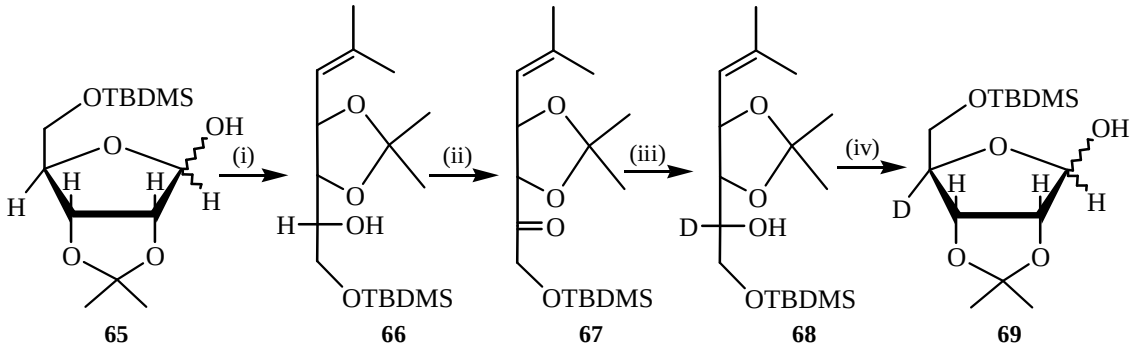
61 **62** **63** (20%) **64** (80%)

2,3,5-

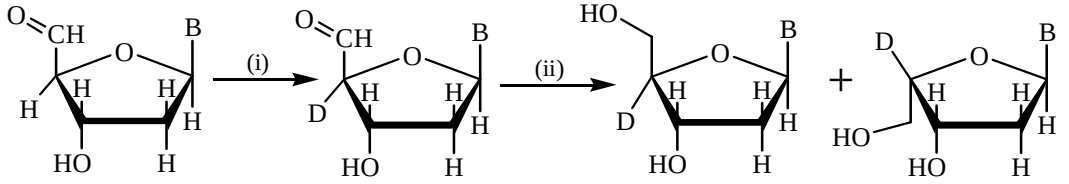
Scheme 16. *Abbreviations*: Ac = acetyl; Bz = benzoyl. *Conditions*: (i) Br_2, h? in CCl_4; (ii) Bu_3Sn^2H.

O-protected D-ribose derivative **65** (Scheme 17) has been achieved by LAD reduction of (3*S*,4*S*)-1-*O*-TBDMS-2-hydroxy-6-methyl-3,4-*O*-(1-methylethylidene)-hept-5-en-2-one (**67**), followed by the separation of the required isomer **68**, its ozonolysis and reductive work-up to afford deuterated **69**. However, this method gives an unfavorable ?/? anomeric ratio of the desired 4'-*$^2H_1$*-cytidine in the subsequent coupling reaction.

Equilibration of 5-aldehydo nucleoside derivatives **70** under base-catalyzed conditions in ^2H_2O has also been exploited for deuterium incorporation at C4' (Scheme 18). Thus, the 4'-formyl derivative **70a-b** of 2'-deoxycytidine or thymidine in ^2H_2O/pyri-



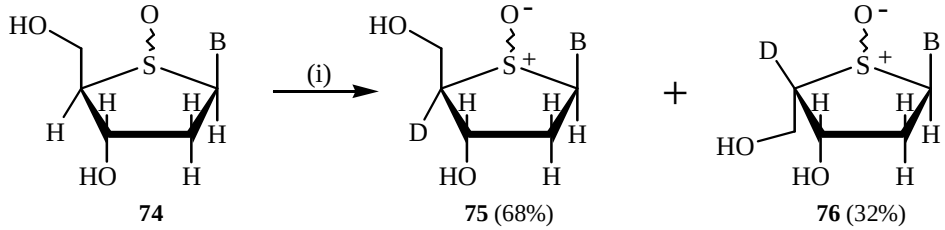
Scheme 17. *Abbreviation*: TBDMS = *t*-butyldimetylsilyl. *Conditions*: (i) *i*-propyltriphenylphosphonium iodide in THF, *n*-BuLi, overnight; (ii) Dess-Martin periodinane in CH_2Cl_2 at r.t. for 2 h; (iii) $LiAlD_4$ in dry



ether; (iv) CH_2Cl_2, O_3, -78 ?C.

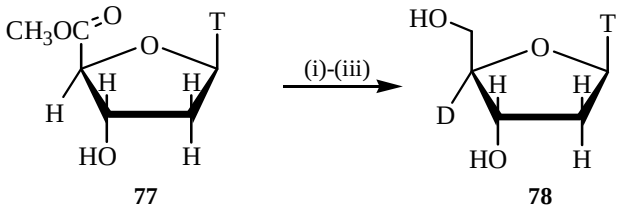
Scheme 18. *Abbreviations*: T = thymin-1-yl, C = cytosin-1-yl. *Conditions*: (i) ^2H_2O/pyridine (50:50), elevated temperature; (ii) $NaBH_4$, ethanol.

dine at elevated temperature[155] affords labelled **71a-b**, which upon reduction with $NaBH_4$ gives 4'-$^2H_1$-2'-deoxynucleosides **72a-b** in good yields. During this exchange process, the ?-L-*lyxo* byproduct **73a-b** is also formed, requiring HPLC purification. Additionally, this exchange reaction at the nucleoside level is not successful for the deuteration at C4' of purine nucleosides. A similar procedure for 4'-deuteration of 2'- deoxy-4'-thionucleosides[156] *via* equilibration of the 4'-sulfoxide **74** gives even more ?-L-*lyxo* byproduct[157] **76** (Scheme 19). On the other hand, the deuteration of methyl thymidine -5'-



Scheme 19. *Abbreviation:* B = 5-ethyluracil-1-yl. *Conditions*: (i) NaO^2H, ^2H_2O, r.t., 18 h, ^2HCl.

carboxylate **77** (Scheme 20) can be achieved through proton abstraction from C4' by a mixture of lithium diisopropylamide and *n*-butyllithium at -78 ?C followed by quenching

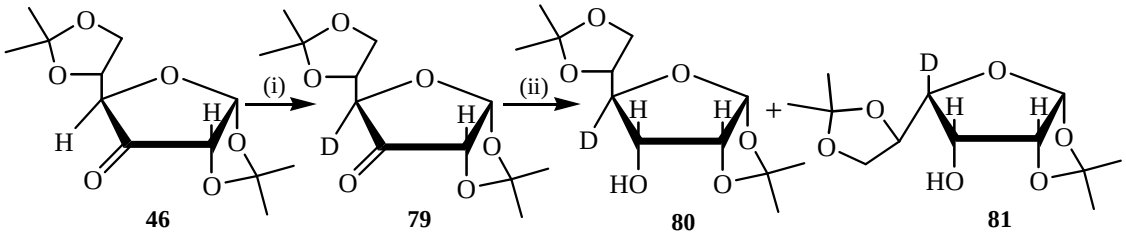


77 **78**

Scheme 20. *Abbreviation:* T = thymin-1-yl. *Conditions*: (i) a. lithium diisopropylamide in dry HMPA/THF, b. N-butyllithium, -78 ?C, 1 h, c. ^2H_2O-acetic acid-$^2H_1$; (ii) acetic anhydride in dry pyridine; (iii) $LiBH_4$ in dry THF, 65 ?C, 2 h.

with $^2H_2O/AcO^2H$ and subsequent reduction to 4'-deuteriothymidine **78** in an overall yield of 16 % with no detectable epimerisation at C4'[158].

At the sugar level, the deuterium incorporation at C4 has been achieved using the ability of the 1,2:5,6-di-*O*-isopropylidene-?-D-hexofuranos-3-ulose[159] (**46**) to undergo keto-enol tautomerism (Scheme 21). Three groups of workers have reported >95 atom%

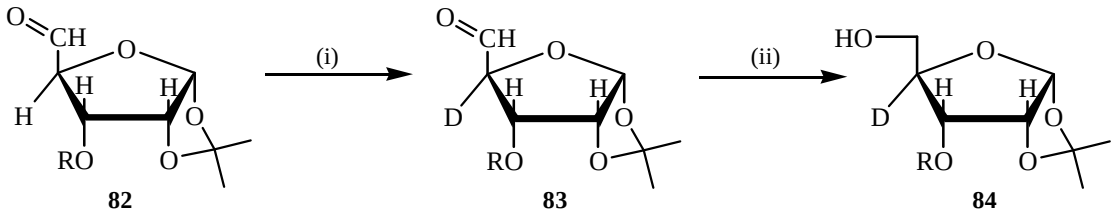


46 **79** **80** **81**

Scheme 21. *Conditions*: (i) pyridine/^2H_2O (1:1, v/v) at 95 ?C, 5 min, additional stirring at r.t. for 18 h; (ii) $NaBH_4$ in ethanol.

deuterium enrichment at C4 with undefined chiral purity by warming a solution of **46** in pyridine/^2H_2O (5:1 or 1:1, v/v) at 95 ?C for 5 min, followed by stirring at room temperature for 18h, and repeating this sequence three times, followed by reduction[160-162] of **79** with $NaBH_4$ to **80**.

Recently it has been reported[144] that this base catalyzed equilibration of ketone **46** in a pyridine/^2H_2O (1:1, v/v) solution under the literature conditions[153,160-162] followed by reduction gives 1,2:5,6-di-*O*-isopropylidene-?-D-gulose-4-$^2H_1$ (**81**) in ~12 % yield as a byproduct as identified by ^1H NMR. In order to obtain >97 atom% ^2H labelling, it requires 5 cycles of this reaction to give **80** in 53 % yield. On the other hand, when the 5-oxo 1,2-*O*-isopropylidene ribose **82** (Scheme 22) is subjected to a treatment in pyridine/^2H_2O solution (1:1, v/v) at 50 ?C for 22 days, no epimerisation takes place during

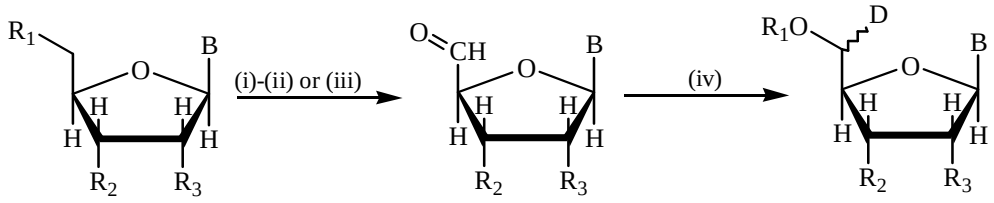


Scheme 22. *Abbreviation*: R = H or benzyl. *Conditions*: (i) pyridine/^2H_2O (1:1, v/v) at 50 ?C, 22 days; (ii) $NaBH_4$ in ethanol.

this base catalyzed exchange reaction and the target C4 deuterated derivatives **84** are obtained >97 atom% isotope enrichment[144].

2.5 5'-Mono- and dideuterionucleoside derivatives

Deuteration at C5' of the sugar moiety helps to reduce the spectral crowding in the sugar region to reduce the proton line widths by reducing the dipolar relaxation and to enhance sensitivity giving rise to an overall simplification and precise assignment of the spectra. Stereoselective 5'(*S*) or 5'(*R*) deuterium incorporation provides means to determine the exact $^3J_{H5',31P}$ and $^3J_{H5'',31P}$ coupling constants and unambiguous nOe assignment, which are essential to elucidate the conformation of the sugar-phosphate backbone of DNA/RNA oligomers[140,163-165]. They are used to probe the internal dynamics of oligonucleotides by solid phase ^2H NMR spectroscopy[166,167]. 5'-Deuterated compounds are also used in conformational[168-170] and mechanistic[171-173] studies. Most of the existing methods do not give a satisfactory level of isomerically pure products and work to develop better methods is in progress in our lab.



85: R_1 =N_3, R_2 = R_3 =O-Ip, B=A
86: R_2 = R_3 = O-Ip, B=A
87: R_1 =H, R_2 = R_3 = O-Ip, B=A
88: R_1 = N_3, R_2 = R_3 =OBn, B=U
89: R_2 = R_3 =OBn, B=U
90: R_1 =H, R_2 = R_3 =OBn, B=U
91: R_1 =OH, R_2 = R_3 =O-Ip, B=U
92: R_2 =R_3 = O-Ip, B=U
93: R_1 =H, R_2 =R_3 = O-Ip, B=U
94: R_1 =OH, R_2 =OAc, R_3 = H, B=T
95: R_2 =OAc, R_3 = H, B=T
96: R_1 =H, R_2 =OAc, R_3 = H, B=T

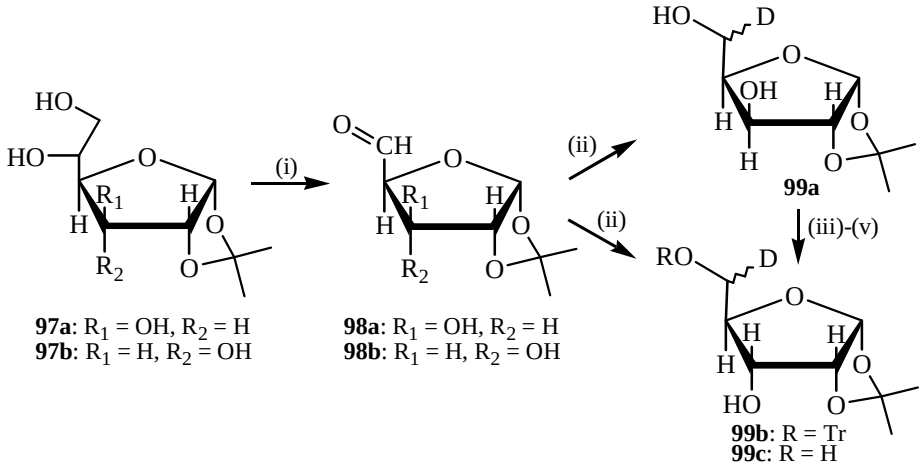
Scheme 23. *Abbreviations*: Ac = acetyl; Bn = benzyl; Ip = isopropylidene; A = adenin-9-yl; U = uracil-1-yl; T = thymin-1-yl. *Conditions*: (i) h? in dry benzene; (ii) Amberlite IR-120 (H^+ form) in water-acetone-methanol, ~25 ?C, 18 h; (iii) DMSO, DCC, dichloroacetic acid or oxalyl chloride, DMSO in CH_2Cl_2, ~-75 ?C; (iv) NaB^2H_4 in ethanol or LAD in diethyl ether.

2.5.1 5'(*S*)/5'(*R*)-monodeuterated nucleosides

In one of the earlier reports of deuterium incorporation at C5' at the nucleoside level, the procedure of reduction of 5'-aldehydo derivatives of adenosine **86** and uridine **89** with NaB^2H_4 has been described[174] (Scheme 23). The aldehydes are obtained from 5'-

azido-5'-deoxy-2',3'-*O*-isopropylideneadenosine (**85**) or 5'-azido-2',3'-*O*-benzylidene-5'-deoxy-uridine (**88**), respectively upon photolysis and mild treatment with acid. The same procedure of labelling of C5' has been used for the aldehydo derivatives of uridine **92** and thymidine **95**, which are prepared from **91** or **94**, respectively by Moffatt[175] or Swern[176,177] oxidation. The deuterium incorporation into **93** and **96** has been determined to be ~95 atom% ^{2}H by mass spectra analysis[169,178]. The level of stereoselectivity obtained by this method is usually very low. Thymidine-5'-$^{2}H_1$ **96** and 2'-deoxyuridine-5'-$^{2}H_1$ **93** can be synthesized by oxidation followed by $NaB^{2}H_4$ reduction to give a 1:1 ratio of 5'(*S*)/ 5'(*R*) (with 98 atom% ^{2}H) as determined by ^{1}H NMR spectroscopy[167,170].

At the sugar level, C5-deuterated ribose derivatives can be synthesized starting



Scheme 24. *Abbreviation*: Tr = Triphenylmethyl. *Conditions*: (i) $NaIO_4$ in ethanol-water; (ii) $NaB^{2}H_4$ in ethanol or water; (iii) Tr-Cl in dry pyridine; (iv) DMSO, DCC; (v) $NaBH_4$ in ethanol

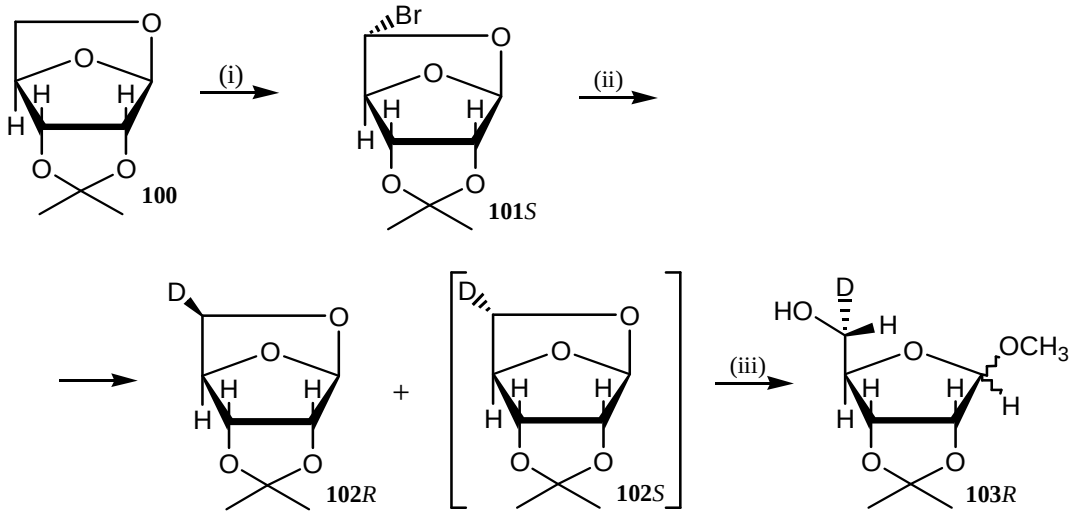
from D-glucose[179,180] by converting it into 1,2:5,6-*O*-di-*O*-isopropylidene-D-glucose[181], then (i) inverting the configuration at C3 by oxidation-reduction[159,181] procedure, followed by (ii) chemoselective deprotection of the 5,6-*O*-isopropylidene group by aqueous acetic acid to **97b**, followed by subsequent (iii) oxidation with sodium metaperiodate to 5-aldehydo derivative **98b** and reduction with $NaB^{2}H_4$ or LAD to give **99c** (Scheme 24). The stereoselectivity of this reaction is also very low; the ratios of 5(*R*) and 5(*S*) isomers are ~45:55 and ~43:57 in the case of $NaB^{2}H_4$ and LAD, respectively[162]. Synthesis of 1,2-*O*-isopropylidene-D-xylofuranose-5*(R/S)*-$^{2}H_1$ **99a** by a similar strategy has also been reported[182]. From this sugar derivative, compound **99c** is prepared by protection of the 5-hydroxyl group to give **99b**, inversion of configuration at C3 *via* oxidation-reduction reactions and subsequent removal of the 5-*O* protection. The ratio of *R/S* diastereomers is found to be 2:1[168].

The reduction of 5-aldehydo derivatives of ribofuranose[183-186] and the appropriate nucleosides[187-189] has been investigated under different conditions in the presence of

different chiral reducing agents. It has been found that when the reaction is carried out using LAD and (-)*N*-methylephedrine or (+)camphor or (-)isoborneol as external chiral catalysts at low temperature in THF, the stereoselectivity of the reduction of N^6-benzoylated adenosine derivative **86** is improved to 75:25, 70:30 and 85:15, respectively, in favor of the *R* isomer[187]. In an extension of this type of studies[184] to the reduction of the 3-*O*-Bn protected derivative of **98** and its *xylo* epimer, it has been shown, that addition of LiI together with *t*-amyl alcohol and (-)isoborneol can substantially enhance the stereoselectivity. The best results are obtained with *t*-amyl alcohol/10 eq. LiI in THF giving the deuterated xylose and ribose with *S/R* ratio of 1:7 and 4:1, respectively. The 5-deuterio-D-xylose can be converted to ribose, and by mixing the two riboses of different isotopomeric purity the *S/R* isomer content can be controlled[185]. The use of the adduct of (-)-?-pinene and 9-borabicyclo-?3.3.1?nonane (9-BBN) as reducing agent results in 60 % chiral purity with predominant *S* selectivity in the reduction of the (5'-$^2H_1$)-N^6-benzoylated derivative of **86**[188]. In a recent report[189] the *S/R* ratio has been increased to 20:1 by using deuterio-9-BBN for the preparation of the adduct.

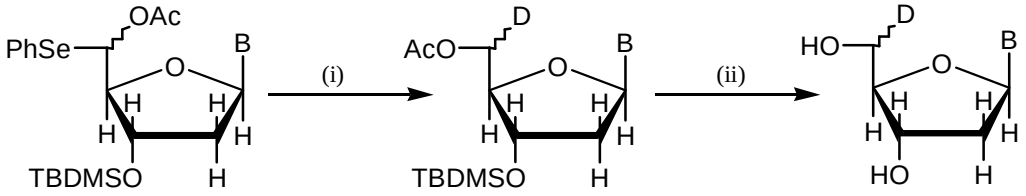
Deuteride transfer reaction using deuterium labelled (-)(2-$^2H_1$)-isobornyloxy-MgBr[183] Grignard reagent affords the 5(*S*)-labelled **99** almost exclusively with 98 atom% deuteration level [186] but with concomitant nonlabelled ribose derivative. The amount of this non-deuterated sugar varies from 2 to 15% depending on the reaction temperature. The isotopomeric 5(*R*)-ribose-$^2H_1$ derivative can be prepared from the appropriate deuterioaldehyde by reduction with non-deuterated Grignard reagent[183] circumventing the problem of getting unlabelled material.

The stereoselectivity of deuterium labelling of C5 at the sugar level can be increased *via* stereoselective photobromination of the 1,5-anhydro compound **100**[190] to **101**, followed by reductive substitution of the bromine by the use of different deuterated reagents[173,191-194] (Scheme 25) as deuteride source. Tri-*n*-butyltin deuteride with 2,2'-azobisisobutyronitrile (AIBN) initiator under various conditions yields **102** in 80% yield with an *S/R* isotopomeric ratio of 85:15. Better stereoselectivity (88:12) is achieved with triphenyltin deuteride. The reduction with LAD is less stereoselective (*R/S*=3/2)[191]. However, the reduction with lithium triethyl borodeuteride, which is known to react completely in an S_N2 manner gives 5(*R*)-1,5-anhydro-2,3-*O*-isopropylidene-(5-$^2H_1$)-?-D-ribofuranose (**102***R*) in 85% yield with 100% chiral purity[192]. The 2,3-*O*-protected ribose derivative **103***R* is obtained from the anhydro compound **102***R* by acidic hydrolysis. The *S* isotopomer can be prepared by inverting the configuration at C5[194].



Scheme 25. *Conditions*: (i) h?, NBS in CCl_4; (ii) various reducing agents, with lithium triethylborodeuteride in THF exclusive (*R*); (iii) 2,2-dimethoxypropane, toluenesulfonic acid in methanol.

The use of the phenylseleno group as a leaving group for the free-radical deuteration has also been reported[195-198] (Scheme 26). 5'-$^{2}H_{1}$-2'-Deoxynucleoside derivatives **106a-e** are synthesized by the following procedure: introduction of the PhSe group with $(PhSe)_2$ and $NaBH_4$ then oxidation with *m*-chloroperbenzoic acid, derivatization to aciloxyselenide by Pummerer rearrangement[199] using Ac_2O to give **104a-e**, followed by reductive deuteration with Bu_3Sn^2H-Et_3B[120] below -70 ?C to afford **105a-e** in an overall yield of 67-91 %. The final **106a-e** show 90-93 atom% ^{2}H isotope incorpor-



104a: B = T
104b: B = U
104c: B = C^{Bz}
104d: B = A^{Bz}
104e: B = G^{iBu}

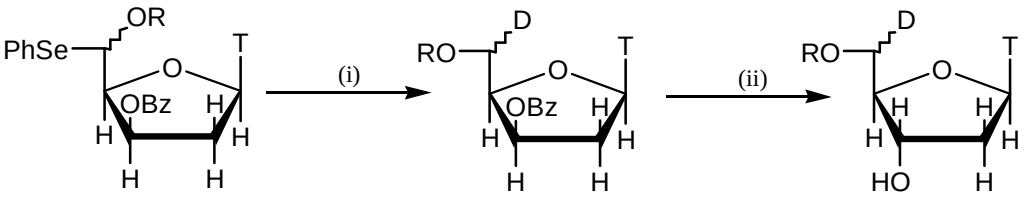
105a: B = T
105b: B = U
105c: B = C^{Bz}
105d: B = A^{Bz}
105e: B = G^{iBu}

106a: B = T
106b: B = U
106c: B = C^{Bz}
106d: B = A^{Bz}
106e: B = G^{iBu}

Scheme 26. *Abbreviations*: G = guanin-9-yl, iBu = isobutyryl, A = adenin-9-yl, Bz = benzoyl, C = cytosin-1-yl, U = uracil-1-yl, T = thymin-1-yl, Ac = acetyl, Ph = phenyl, TBDMS = *t*-butyldimethylsilyl. *Conditions*: (i) Bu_3Sn^2H, triethylborane in THF, below –75 ?C; (ii) TBAF in dry THF and NaOH in pyridine-ethanol.

ation whereas the isotopomeric 5'(*R*):5'(*S*) ratio varies from 39:61 (for A^{Bz}) to 20:80 (for G^{iBu})[195]. The 3',5'-*O*-protected 1-(?-D-xylofuranosyl)-thymines **107a-b** (Scheme 27) have also been examined for this deuteration method and in case of the 5'-*O*-acetyl protecting group, the *S*/*R* ratio is found to be 85:15, whereas the use of the 5'-*O*-benzoyl derivative

increases this ratio up to 91:9[197]. However, some 5% of the nucleosides remain unlabelled[163].



107a: R = Ac
107b: R = Bz

108a: R = Ac
108b: R = Bz

109a: R = Ac
109b: R = Bz

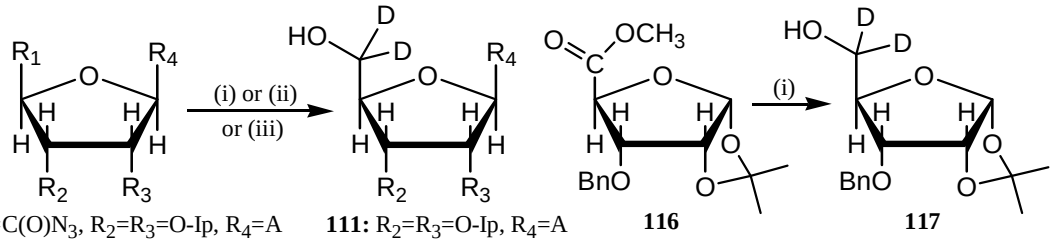
Scheme 27. *Abbreviations:* Ac = acetyl, Bz = benzoyl, Ph = phenyl, T = thymin-1-yl. *Conditions*: (i) Bu_3Sn^2H, triethylborane in THF, –78 ?C; (ii) Mitsunobu reaction.

2.5.2 Synthesis of 5',5''-dideuterated derivatives

Incorporation of two deuterium atoms at C5 at the sugar level can be achieved by reduction of the appropriate ribofuranouronic acid methyl esters **112** and **116** by LAD[112,144,183,200] (Scheme 28). The esters can be synthesized by oxidation of 4-formyl derivatives by pyridinium dichromate in methanol/DMF[183], by bromine/H_2O/methanol system with sodium bicarbonate as buffer[201] or by oxidation of the 5-hydroxyl of ribofuranoside with potassium permanganate and subsequent conversion into the methyl ester by use of diazomethane[200] or *via* oxidation of 1-*O*-methyl-2,3-*O*-isopropylidene-?-D-ribofuranoside with RuO_2 $H_2O/NaIO_4$ followed by *in situ* ester formation with diazomethane[112] followed by a reduction step to yield **112**. At the nucleoside level, 5',5''-dideuterioadenosine has been obtained by the reduction of 5'-carboxylic acid azide **110** by NaB^2H_4[202] or by reduction of thymidine 5'-carboxylic acid **114** with B^2H_3[172].

3.0 Multiple-site chemical deuteration of the sugar residues

It has clearly been demonstrated above that each position of the sugar residue can be selectively labelled. Although some of these selectively deuterated nucleosides have indeed found their use in solid-state ^{2}H NMR studies[133,134,138,139], labelling of a single site has little impact on the structural studies on oligo-DNA or RNA since the simplification of 1D and 2D ^{1}H NMR spectra is not dramatic even in the case of uniform incorporation of the monomers. Nevertheless, the synthesis of 4',5',5''-$^2H_3$–adenosine[203] has been



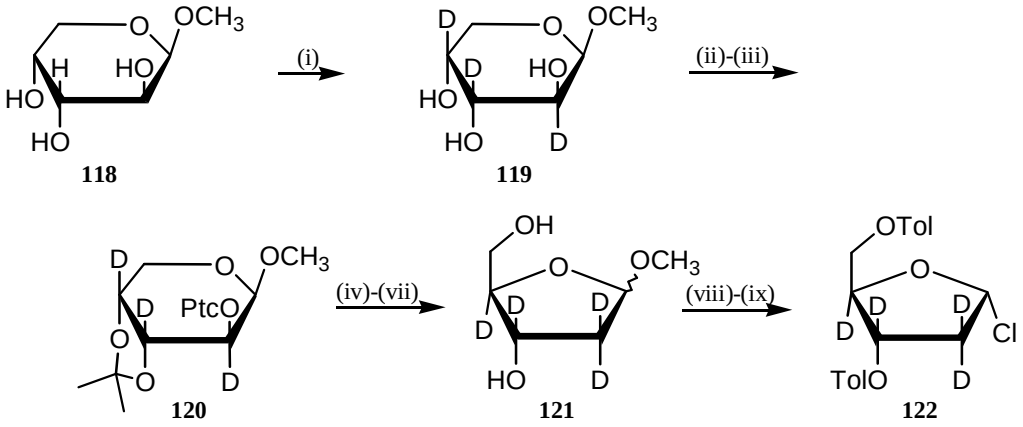
110: R_1=C(O)N_3, R_2=R_3=O-Ip, R_4=A
111: R_2=R_3=O-Ip, R_4=A
112: R_1=C(O)OCH_3, R_2=R_3=O-Ip, R_4=OCH_3
113: R_2=R_3=O-Ip, R_4=OCH_3
114: R_1=COOH, R_2=OH, R_3=H, R_4=T
115: R_2=OH, R_3=H, R_4=T
achieved

Scheme 28. *Abbreviations*: A = adenin-9-yl, T = thymin-1-yl, Ip = isopropylidene, Bn = benzyl. *Conditions*: (i) LAD in dry diethyl ether; (ii) NaB^2H_4 in methanol-water (1:1); (iii) B^2H_3 in dry THF, r.t., 20 h.

through a combination of (i) C4' isotope exchange with the 2',3'-*O*-isopropylidene 5'-carboxylic acid ester derivative of adenosine in a low-temperature process catalyzed by sodium methoxide (ii) followed by reduction with NaB^2H_4. 1',2',2''-$^2H_3$-2'-Deoxynucleosides can be synthesized combining the consecutive deuterium exchange at C2 of D-2-deoxyribonolactone (**10a**) in methanol-$^2H_1$ with sodium methoxide for 7 days (95 atom% deuterium at 2' and 87 atom% ^2H at 2'' positions) followed by reduction of the 3',5'-bis-*O*-TBDMS derivative with DIBAL-$^2H_1$[114].

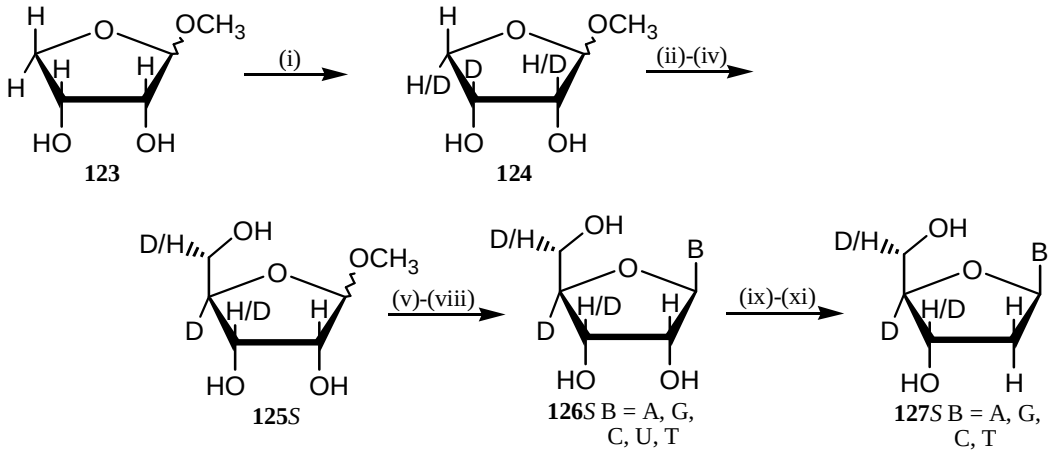
Some 20 years ago deuterated Raney nickel catalyzed deuteron-proton exchange in ^2H_2O at the OH bearing carbons of carbohydrates emerged as a powerful means for one-pot isotope incorporation at multiple sites[204-210]. This method has been used for high level isotope substitution in nucleosides starting at the sugar level (the exchange with nucleosides gives rise to base deuterated analogues[204]). In an attempt to reduce the overcrowding in the 2',2'',3',4' sugar region of the ^{1}H NMR spectra of oligo-DNA, the methyl arabinopyranoside **118** (Scheme 29) has been subjected[211] to the exchange resulting in arabinopyranoside **119** (?97 atom% ^2H enrichment at C3 and C4; ?94 atom% enrichment at C2). This is converted to the 2-*O*-phenoxythiocarbonyl derivative **120** for

free-radical incorporation of the second deuterium at C2 by Bu_3Sn^2H followed by conversion to methyl-2-deoxyribofuranoside-$^2H_4$ **121** and finally to the required labelled



Scheme 29. *Abbreviations*: Tol =4-toluoyl, Ptc = phenoxythiocarbonyl. *Conditions*: (i) Deuterated Raney Ni in ^2H_2O, reflux, 15 h; (ii) 2,2-dimethoxypropane, TSA in dry DMF, r.t., 3 h; (iii) Ptc-Cl, DMAP in dry acetonitrile, r.t., overnight; (iv) Bu_3Sn^2H, AIBN in dry toluene, 80 ?C, 2h; (v) 80% aqueous acetic acid, r.t. 15h; (vi) hydrochloric acid, r.t., 40h; (vii) methanolic HCl; (viii) Tol-Cl in dry pyridine; (ix) HCl in acetic acid.

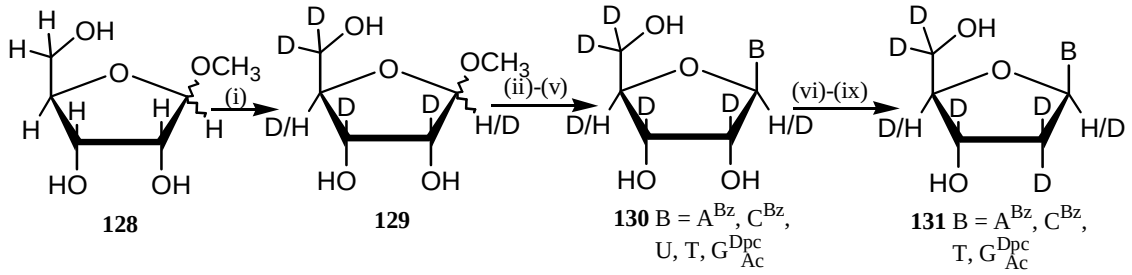
chloro sugar **122**. This is used for the synthesis of the four 2',2'',3',4'-$^2H_4$-2'-deoxynucleosides. When ?-D-erythrofuranoside **123** is treated with deuterated Raney nickel, deuterium incorporation is found at the C2, C3 and C4(*S*) positions in **124** (75 atom% ^2H at C2 and C4 and 100 atom% ^2H at C3)[212] (Scheme 30). This sugar is



Scheme 30. *Abbreviations*: G = guanin-9-yl, A = adenin-9-yl, C = cytosin-1-yl, U = uracil-1-yl, T = thymin-1-yl, Bz = benzoyl, Ac = acetyl, Ptc = phenoxythiocarbonyl. *Conditions*: (i) Deuterated Raney Ni in ^2H_2O, reflux; (ii) 0.1 N H_2SO_4, reflux, 30 min; (iii) cyanohydrin reaction; (iv) conc. H_2SO_4 in dry methanol; (v) Bz-Cl in dry pyridine; (vi) Ac_2O, AcOH, conc. H_2SO_4; (vii) silylated nucleobases, TMS-triflate in dry acetonitrile; (viii) methanolic ammonia; (ix) TPDS-Cl_2 in dry pyridine (for C^{Ac}); (x) Ptc-Cl, DMAP in dry acetonitrile; (xi) Bu_3SnH, AIBN in dry toluene, ~75 ?C, 3 h (14 h for C); (xii) 1M TBAF in dry THF followed by methanolic ammonia for C.

converted to D-3,4,5(*S*)-$^2H_3$ -ribofuranoside **125***S* from which ribonucleosides **126***S* are prepared. They are subsequently reduced to the corresponding 3',4',5'(*S*)-$^2H_3$-2'-deoxyribofuranosides **127***S*[213].

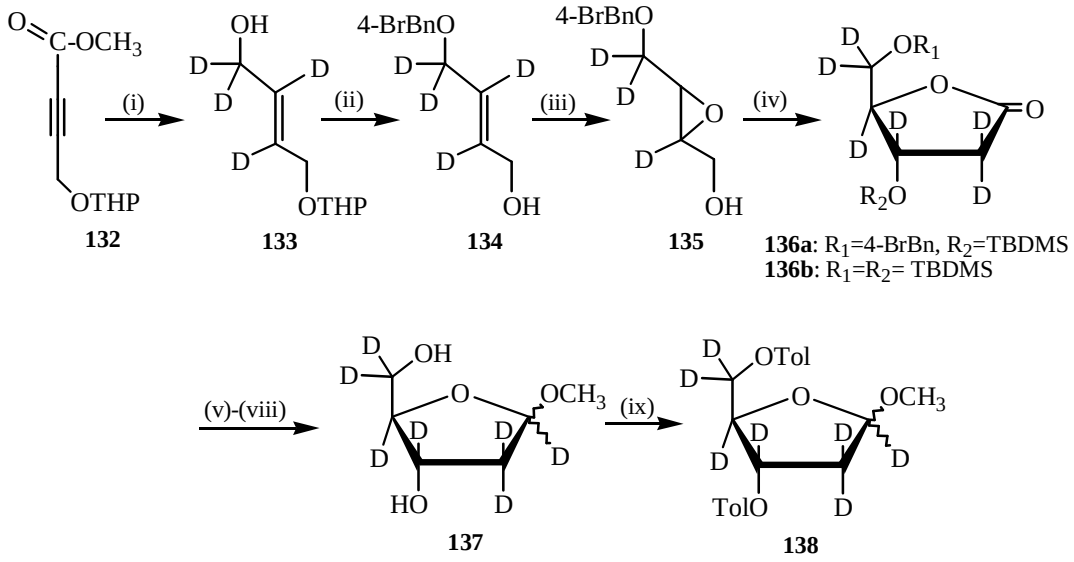
In the preparation of suitable building blocks for the most extensive use of the segmental deuteration in NMR studies on oligonucleotides (the Uppsala NMR-window



Scheme 31. *Abbreviations*: G = guanin-9-yl, A = adenin-9-yl, C = cytosin-1-yl, U = uracil-1-yl, T = thymin-1-yl, Bz = benzoyl, Ac = acetyl, Dpc = diphenylcarbamoyl. *Conditions*: (i) Deuterated Raney Ni in ^2H_2O, reflux, 7 d; (ii) Tol-Cl in dry pyridine; (iii) Ac_2O, AcOH, conc. H_2SO_4 in dry CH_2Cl_2, 0 ?C, 12 min.; (iv) silylated nucleobases, TMS-triflate in dry 1,2-dichloroethane or toluene, ~75 ?C; ~4h; (v) 1N NaOH in pyridine-ethanol, r.t., 6 min. (0 ?C, 10-12 min for G derivative); (vi) TPDS-Cl_2 in dry pyridine; (vii) Ptc-Cl, 1N-mehylimidazole in dry CH_2Cl_2, overnight; (viii) Bu_3Sn^2H, AIBN in dry toluene, ~75 ?C, 3.5 h; (ix) 1M TBAF in dry THF.

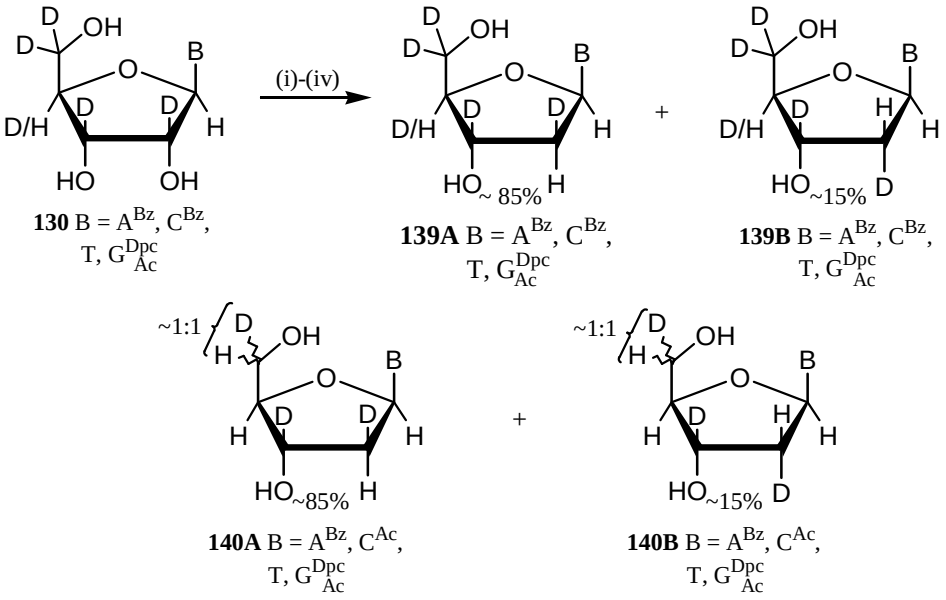
approach)[31], an epimeric mixture of methyl ?/?-D-ribofuranosides (**128**) (Scheme 31) is subjected to the Raney nickel-^2H_2O exchange reaction to produce methyl 1,2,3,4,5,5'-$^2H_6$ ribofuranosides[31] **129** along with epimeric *arabino* and *xylo* derivatives as byproducts with high percentage (>97 atom% ^2H) deuterium incorporation at the C2, C3, C5 centers, whereas at the C4, and C1 the isotope exchange is relatively low (~85 and 20 atom% ^2H, respectively). The deuterated sugars thus obtained are used in the synthesis of deuterated ribonucleosides **130** and 1',2',2'',3',4',5',5''-$^2H_7$-2'-deoxyribonucleosides **131**.

In order to mask all unwanted resonance lines or crosspeaks in the ^1H NMR spectra, it seems necessary to prepare sugar perdeuterated nucleosides. This goal has been achieved for DNA starting with the readily available protected butynoate **132** (Scheme 32). The synthetic strategy[214] consists of reduction with LAD and ^2H_2O quenching to afford **133** stereoselectively. After a protection-deblocking procedure, the Sharpless asymmetric epoxidation of alcohol **134** affords optically pure crystalline **135**. This is converted to the lactone derivatives **136** for reduction with DIBAL-$^2H_1$ at –90 ?C. After forming methyl furanoside and subsequent removal of the hydroxyl protecting groups the obtained **137** is toluoylated to the perdeuterated 2'-deoxyribofuranoside derivative **138**.



Scheme 32. *Abbreviations*: THP = tetrahydropyran-2-yl, Tol = 4-toluoyl, Bn = benzyl, TBDMS = *t*-butyldimethylsilyl. *Conditions*: (i) LAD in dry THF followed by ^2H_2O; (ii) NaH, 4-BrBn-Br in THF:DMF followed by PPTs in methanol; (iii) 4 Å molecular sieves, Ti-isopropoxide, D-(-)-diisopropyl tartrate, tert-butyl hydroperoxide in CH_2Cl_2, -23 ?C, overnight then quenching with H_2O; (iv) NaCN in EtO^2H:^2H_2O (2:3) then lactonization in boiling toluene; (v) a. TBDMS-NO_3, pyridine in THF or b. H_2 over Pd on charcoal in ethanol then TBDMS-NO_3, pyridine in THF; (vi) DIBAL-$^2H_1$ in CH_2Cl_2, -90 ?C, 2 h; (vii) methanolic HCl; (viii) a. 1M TBAF in THF or b. 1M TBAF in THF followed by H_2 over $Pd(OH)_2$ on charcoal in ethanol; (ix) Tol-Cl, DMAP in dry pyridine.

^{1}H NMR studies of 20mer DNA duplexes using the "Uppsala ^{1}H NMR-window" approach have shown[32,40] that the appropriate labelling of the ^{1}H NMR-window part of an oligonucleotide should be engineered such that the dihedral information, in addition to the spin-diffusion free nOe information, from this part should be obtainable from the COSY-type experiments. Thus, the incorporation of proton instead of deuteron by the use of tributyltin hydride during the deoxygenation of the selectively deuterated ribonucleosides **130** affords the isotopomeric mixture of **139A-B**[33] (Scheme 33) due to the restricted stereoselectivity. In order to improve the quality of H1'-H4' nOe crosspeaks as well as to obtain additional $^3J_{H5',31P}$ and $^3J_{H5'',31P}$ coupling constants, a mixture of the partially deuterated isotopomeric nucleosides **140A-B** has also been synthesized starting from D-glucose derivative **97**[140]. The synthetic route follows the steps outlined previously for compound **47**, followed by reduction as from **98** ? **99**. The C3 deuterated **99** is further



130 B = A^{Bz}, C^{Bz}, T, G^{Dpc}_{Ac}

139A B = A^{Bz}, C^{Bz}, T, G^{Dpc}_{Ac}

139B B = A^{Bz}, C^{Bz}, T, G^{Dpc}_{Ac}

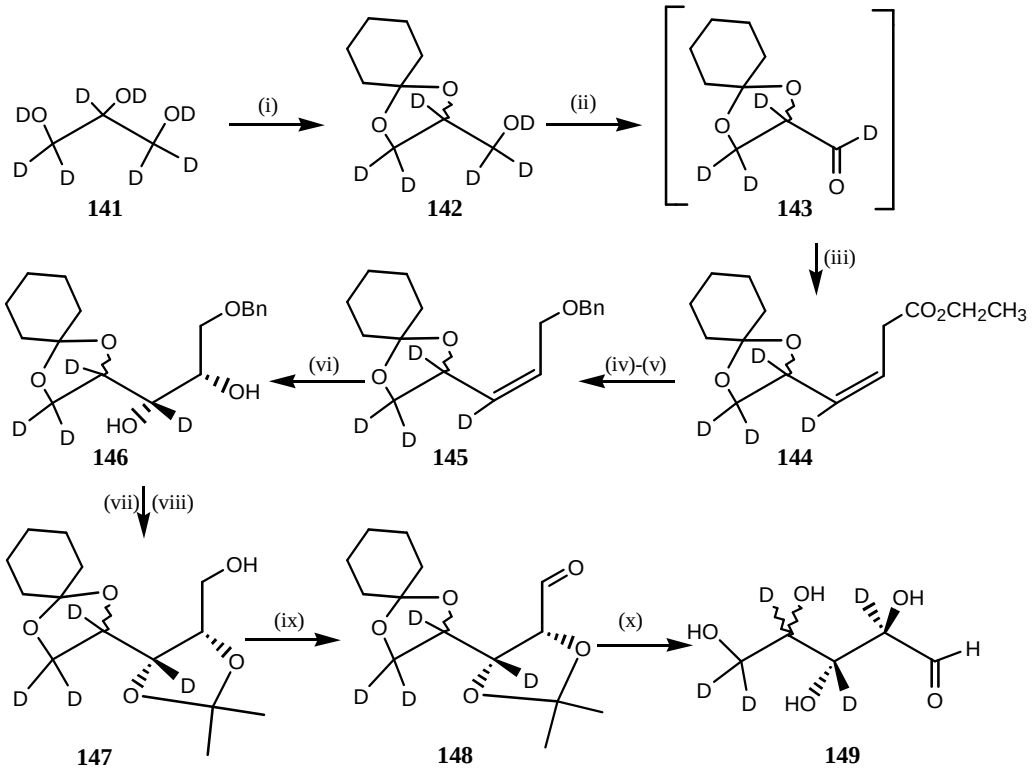
140A B = A^{Bz}, C^{Ac}, T, G^{Dpc}_{Ac}

140B B = A^{Bz}, C^{Ac}, T, G^{Dpc}_{Ac}

Scheme 33. *Abbreviations*: G = guanin-9-yl, A = adenin-9-yl, C = cytosin-1-yl, U = uracil-1-yl, T = thymin-1-yl, Bz = benzoyl, Ac = acetyl, Dpc = diphenylcarbamoyl. *Conditions*: (i) TPDS-Cl_2 in dry pyridine; (ii) Ptc-Cl, 1*N*-mehylimidazole in dry CH_2Cl_2, overnight; (iii) Bu_3SnH, AIBN in dry toluene, ~75 ?C, 3.5 h; (iv) 1M TBAF in dry THF.

converted to 5'(*R*/*S*),3'-$^{2}H_{2}$- nucleosides. The third deuterium is subsequently incorporated into the dideuterionucleosides by the chemistry outlined in Scheme 7. Alternatively, after benzylation of the 5- and 3-hydroxyls the resulting 3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-? -D-ribofuranose-5(*R*/*S*),3-$^{2}H_{2}$ is converted by an oxidoreduction pathway and reglycosylation to the 5(*R*/*S*),3-deuterated analogue of arabinoside **24** for the incorporation of the third deuterium as outlined in Scheme 8. This is followed by the subsequent synthesis of the 5'(*R*/*S*),3',2'-$^{2}H_{3}$-nucleosides.

Recently the C4 epimeric mixture of D-ribose and L-lyxose **149** has been synthesized starting from glycerol-$^{2}H_{8}$ **141**[215] (Scheme 34). Protection of **141** to yield **142** is followed by Swern oxidation resulting in D,L-glyceraldehyde-1,2,3,3'-$^{2}H_{4}$ ketal **143**. This is subjected to Wittig reaction with $Ph_3P{=}CHCO_2Et$ in methanol at 0 ?C, to form the Z olefin **144** in a 76 % yield. Reduction of the ester with DIBAL-H and protection of the



Scheme 34. *Abbreviations:* Bn = benzyl. *Conditions*: (i) cyclohexanone, $(CH_3O)_3CH$, H^+; (ii) oxalyl chloride, DMSO, triethylamine; (iii) $(C_5H_6)_3P{=}CHCO_2C_2H_5$, methanol; (iv) DIBAL-H, CH_2Cl_2,; (v) BnBr, NaH, $(n\text{-}C_4H_9)_4NI$, THF; (vi) OsO_4, *N*-methylmorpholine *N*-oxide, acetone:H_2O (8:1); (vii) 2-methoxypropene, H^+; (viii) Pd on C, H2; (ix) oxalyl chloride, DMSO, triethylamine; (x) H^+, THF, H_2O.

resulting alcohol with a benzyl group affords the benzyl ether **145**. Dihydroxylation with osmium tetroxide results in protected ribitol and lyxitol diastereomers in a 7:3 ratio. After chromatographic separation of **146**, protection of the *cis*-diol fragment and the removal of the benzyl ether afford the partially protected alditol **147**. Swern oxidation gives the protected ribose **148**. The hydrolysis of crude aldehyde furnishes the crude mixture of 3,4,5,5'-deuterated D-ribose and L-lyxose **149** (12 % overall yield), which is suitable for enzymatic nucleoside synthesis. Both the overall yield and the chiral purity of the final deuterio sugar have been improved by the chemical synthesis of 3,4,5,5'-$^2H_4$-deuterated D-ribose[144] starting from **47**. After conversion to the aldehyde **83** (R=Bn) the deuterium is incorporated as described for **84** (Scheme 22).

4.0 Enzymatic synthesis of deuterated nucleosides or nucleotides

In a very early attempt to establish the production of deuterium labelled RNA building blocks and labelled RNAs, *Synechococuus lividius* blue-green algae has been grown in ^2H_2O[216,217]. After harvesting the cells, the fully deuterated nucleic acid content is hydrolyzed by a base treatment followed by the removal of protein and DNA. The four isomeric pairs of 2' and 3'-rNMPs are separated on a Dowex-50 NH_4^+ column and converted to cyclic 2',3'-cNMPs. These are converted into appropriately protected 3'-

monophosphates that are useful for chemical synthesis of short oligomers by diester chemistry or are used directly in enzymatic syntheses of various short oligomers[218-221]. An 11mer RNA part of a hammerhead ribozyme has been synthesized applying a similar strategy but growing *E. coli* in 100% $^{2}H_2O$ and converting the isolated fully deuterated rNMPs to rNTPs which are used in T7 polymerase transcription[34]. In a recent paper[141] the synthesis of different deuterium labelled NTPs has been described starting with phosphorylation of *ul-*$^{2}H_7$-D-glucose by hexokinase. In the synthesis of uniformly sugar deuterated ATP, GTP and UTP, this is followed by the use of 6-phosphogluconic dehydrogenase and ribose-5-phosphate isomerase. 3',4',5',5''-$^{2}H_4$,$^{13}C_5$-UTP is prepared from the phosphorylated *ul-*$^{2}H_7$,$^{13}C_6$-D-glucose in H_2O by the action of glucose-6-phosphate isomerase, 6-phosphogluconic dehydrogenase and ribose-5-phosphate isomerase. The reactions are conducted in a one-pot fashion followed by boronate chromatography.

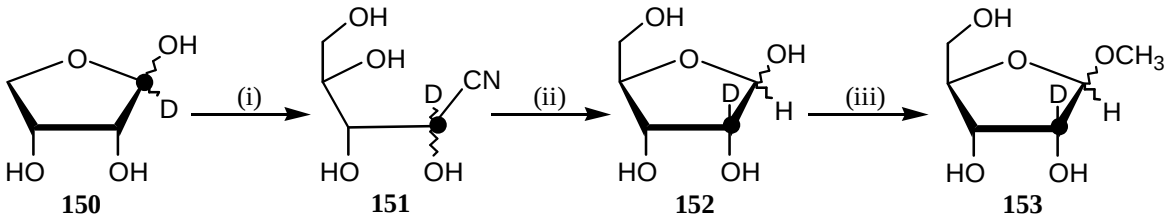
5.0 Synthesis of labelled nucleosides with multiple isotopes

The purpose of preparing nucleosides with multiple labelling patterns is to facilitate the performance of various heteronuclear correlation experiments. For examples, labelling the prochiral 5'-methylene moiety for stereospecific assignment and through this to measure vicinal ^{1}H-^{31}P coupling constants[163]; to improve the accuracy of coupling data extracted from ^{1}H-^{13}C HSQC spectra[222]; to eliminate crosspeaks completely from crowded regions of 2D spectra[141] or to aid relaxation time measurements[43,46,223,228,229] can be recalled.

5.1 Synthesis of ^{2}H,^{13}C labelled nucleosides

5.1.1 Chemical syntheses

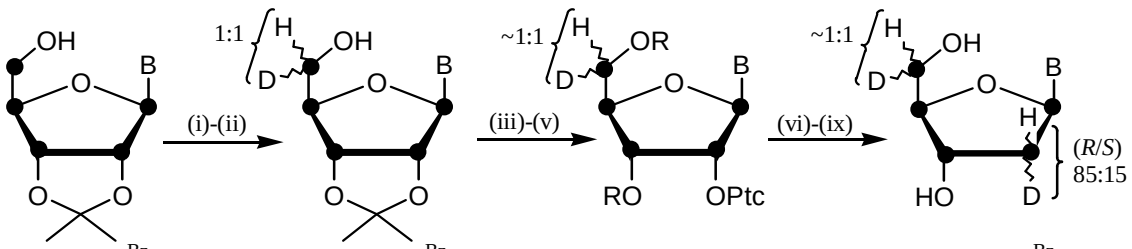
The stereoselective deuteration of the C5' carbon (Scheme 26) has been applied to a thymidine nucleoside having a uniformly ^{13}C labelled 2'-deoxyribose ring[26,224] to afford 5'(*R*/*S*)-$^{2}H_1$-1',2',3',4',5'-$^{13}C_5$-thymidine. This compound has been prepared subsequently from the uniformly carbon labelled glucose derivative **97**, which is first converted to uniformly carbon labelled **98** (Scheme 23). This is subjected to the stereoselective reduction described[186] for 5(*S*) deuterated **99**. D-2-$^{2}H_1$,^{13}C-Ribose **152** and its anomeric methylglycosides **153** have been prepared from D-1-$^{2}H_1$,^{13}C-erythrose (Scheme 35)[225]. The latter can be prepared from D-glyceraldehyde and separated[209] from its *threo* epimer



150 **151** **152** **153**

Scheme 35. *Conditions*: (i) KCN in H_2O, pH 7.8-8.0 for 9 min then to pH 4.0 with AcOH and finally to pH 3.0?0.1 with HCl; (ii) Pd/H_2, 20 psi, 25 ?C followed by separation on Dowex 1x2 (OH^- form); (iii) conc. H_2SO_4 in dry methanol.

using the condensation (with $K^{13}CN$) ↴ hydrogenolysis (with $^2H_2$/Pd) ↴ ion exchange chromatography sequence outlined in Scheme 35. The method has the potential to label any position within the ribofuranose moiety of a nucleoside. Taking into consideration the labour required, it is especially well suited for the incorporation of site specific ^{13}C labels. The Moffatt oxidation of the 2',3'-*O*-isopropylidene-1',2',3',4',5'-$^{13}C_5$-nucleosides **154a-d** (Scheme 36), which are again prepared from uniformly (*ul*)-$^{13}C_6$-labelled D-glucose[27,226,227], followed by reduction with NaB^2H_4 affords 5'(*R*/*S*)-$^2H_1$-1',2',3',4',5'-$^{13}C_5$ double labelled derivatives[228] **155a-d** which are further converted to 5'(*R*/*S*),2'(*R*/*S*)-$^2H_2$-1',2',3',4',5'-$^{13}C_5$-2'-deoxynucleosides **157a-e** *via* free-radical deoxygenation of **156a-d** using tributyltin deuteride. The 2'-deoxycytidine **157e** is prepared from **157c** *via* the established O^4-(2-nitrophenyl) procedure[37]. Taking advantage of the ^2H_2O/pyridine equilibration of the *ul*-$^{13}C_6$-derivative of **46** followed by reduction to carbon labelled **80** (Scheme 21) or that of the reduction to carbon labelled **47** (Scheme 13) and subsequent reduction of the appropriate *ul*-$^{13}C_5$-4-$^2H_1$ or 3-$^2H_1$-analogues of **83** double-labelled nucleoside precursors have been synthesised[162,180]. From these compounds 5'(*R*/*S*),4'-$^2H_2$-1',2',3',4',5'-$^{13}C_5$- or 5'(*R*/*S*),3'-$^2H_2$-1',2',3',4',5'-$^{13}C_5$-adenosine are obtained.



154a: B = A^{Bz}
154b: B = T
154c: B = U
154d: B = G^{Dpc}_{Ac}

155a: B = A^{Bz}
155b: B = T
155c: B = U
155d: B = G^{Dpc}_{Ac}

156a: R = TPDS, B = A
156b: R = TPDS, B = T
156c: R = TPDS, B = U
156d: R = TPDS, B = G^{Ac}

157a: B = A^{Bz}
157b: B = T
157c: B = U
157d: B = G^{Ac}
157e: B = C

Scheme 36. *Conditions*: (i) DMSO, DCC, dichloroacetic acid, r.t.; (ii) $NaBD_4$ in ethanol, r.t.; (iii) 10% aq. acetic acid, ~90 °C (followed by NH_3 in methanol for compound **152a**) or 90% aq. TFA; (iv) TPDS-Cl_2 in dry pyridine, r.t.; (v) Ptc-Cl, methylimidazole in dry CH_2Cl_2, r.t.; (vi) Bu_3Sn^2H, AIBN in dry toluene, ~85 °C; (vii) (to get compound **154a**) Bz-Cl in dry pyridine; (viii) (to get **154e** from **153c**) a. mesitylenesulfonyl chloride, triethylamine, DMAP in dry CH_2Cl_2, followed by 2-nitrophenol and DABCO b. liq. NH_3 in dry THF; (ix) TBAF in dry THF, r.t.

5.1.2 Enzymatic syntheses

Double labelled 3',4',5',5''-$^{2}H_{4}$-1',2',3',4',5'-$^{13}C_{5}$-ATP, GTP and UTP have been prepared[141] from *ul*-$^{2}H_{7}$,$^{13}C_{6}$-D-glucose phosphate in H_2O by the action of glucose-6-phosphate isomerase, 6-phosphogluconic dehydrogenase and ribose-5-phosphate isomerase. The UTP is converted to CTP with CTP synthetase.

5.2 Synthesis of ^{2}H,^{15}N labelled nucleotides

Uniformly ^{2}H,^{15}N labelled 5'-nucleoside triphosphates have been prepared by growing *Escherichia coli* on minimal medium containing 88% $^{2}H_2O$, ^{15}N-ammonium sulfate as the sole nitrogen source and sodium acetate-$^{2}H_3$ as the sole carbon source[223]. The labelled rNTPs have been isolated from the labelled biomass and used in transcription with T7 RNA polymerase to give uniformly labelled RNA oligomer.

5.3 Synthesis of ^{2}H,^{15}N,^{13}C triple labelled nucleotides

The synthesis of uniformly ^{2}H,^{15}N,^{13}C triple labelled 5'-nucleoside triphosphates has been achieved *via* a similar method described above. *Escherichia coli* has been grown on minimal medium containing 90% $^{2}H_2O$, ^{15}N-ammonium sulfate as the sole nitrogen and sodium acetate-$^{13}C_2$ as the sole carbon source[46]. Alternatively[47], uniformly ^{15}N,^{13}C labelled 5'-rNMPs are subjected to deuterium exchange at C5 of pyrimidines with metabisulfite anion as catalyst[44] giving ?98% completion upon incubating for 102 h at 65 ?C.

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