Tetrahedron Vol. 48, No. 41, pp. 9033-9072, 1992
Printed in Great Britain

0040-4020/92 $5.00+.00

Synthesis of 1'#,2',3',4'#,5',5"-$^2H_6$-β-D-ribonucleosides and 1'#, 2',2",3',4'#,5',5"-$^2H_7$-β-D-2'-deoxyribonucleosides for Selective Suppression of Proton Resonances in Partially-deuterated Oligo-DNA, Oligo-RNA and in 2,5A core *(^{1}H-NMR window)*

András Földesi, Frans Peder R. Nilson, Corine Glemarec, Carlo Gioeli & Jyoti Chattopadhyaya*

Department of Bioorganic Chemistry, Box 581, Biomedical Center, University of Uppsala, S-751 23 Uppsala, Sweden

(Received in UK 3 August 1992)

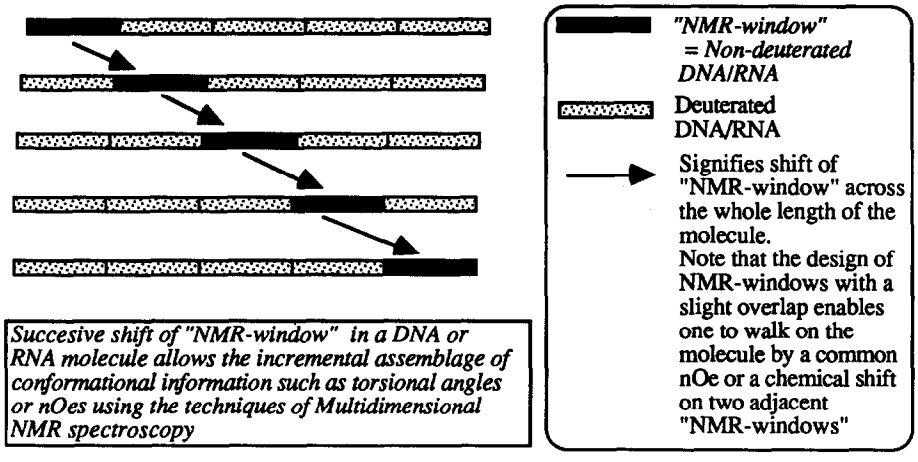
Abstract: *Raney nickel-^2H_2O exchange reaction on an epimeric mixture of methyl α/β-D-ribofuranoside [α/β = ~3:10]1 produced methyl 1#,2,3,4#,5,5'-$^2H_6$-α/β-D-ribofuranoside 2 [>97 atom % ^2H at C2, C3, C5/5'; ~85 atom % ^2H at C4 (C4#); ~20 atom % ^2H at C1(C1#)] which was obtained in 60 - 80 % yield along with epimeric xylo and arabino by-products. Toluoylation of the crude 2 in dry pyridine and a careful separation on a column of silica gel gave pure 1-O-methyl-2,3,5-tri-O-(4-toluoyl)-α/β-D-1#,2,3,4#,5,5'-$^2H_6$-ribofuranoside 4 (48%). Conversion of 4 to 1-O-acetyl-2,3,5-tri-O-toluoyl-α/β-D-1#,2,3,4#,5,5'-$^2H_6$-ribofuranoside 6 (82%) provided the crucial building block for the synthesis of deuterionucleosides for RNA or DNA synthesis. Compound 6 was then condensed with silylated uracil, N^4-benzoylcytosine, N^6-benzoyladenine, N^2-acetyl-O^6-diphenylcarbamoylguanine and thymine in anhydrous solvent using trimethylsilyl trifluoromethanesulfonate to give the corresponding isomerically pure 1#,2',3',4#,5',5"-$^2H_6$-ribonucleoside derivatives 7, 8, 9, 10, 11 in 75, 85, 60, 73 and 91% yields, respectively. 1#,2',3',4#,5',5"-$^2H_6$-ribonucleosides 13 - 16 were converted in high yields to the corresponding 1#,2',2",3',4#,5',5"-$^2H_7$-2'-deoxynucleosides 41 - 44 in the following manner: 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TPDS))-1#,2',3',4#',5',5"-$^2H_6$-nucleosides 29 - 32 were converted to the corresponding 2'-O-phenoxythiocarbonyl derivatives 33 - 36, which were deoxygenated by tri-n-butyltin deuteride to give 1#,2',2",3',4#,5',5"-$^2H_7$-2'-deoxynucleosides 37 - 40 and subsequently deprotected to give 41 - 44. Pure 1#,2',3',4#,5',5"-$^2H_6$-ribonucleoside derivatives 12 - 15, 1#,2',2",3',4#,5',5"-$^2H_7$-2'-deoxynucleoside blocks 41 - 44 and their natural-abundance counterparts were then used to assemble partially deuterated ribonucleotide-dimers (* indicates deuterated moiety): UpA* 77, CpG* 78, ApU* 79, GpC* 80, partially deuterated 2'-deoxyribonucleotide-dimers d(TpA*) 93, d(CpG*) 94, d(ApT*) 95, d(GpC*) 96 and partially deuterated 2,5A core (A*2'p5'A2'p5'A*) (109). These nine partially deuterated oligonucleotides were subsequently compared with their corresponding natural-abundance counterparts by 500 MHz ^{1}H-NMR spectroscopy to evaluate the actual NMR simplifications achieved in the non-deuterated part (^{1}H-NMR window) as a result of specific deuterium incorporation. Detailed 1D ^{1}H-NMR (500 MHz), 2D correlation spectra (DQF-COSY & TOCSY), T_1 measurements for ^{1}H-, ^{13}C- and INEPT ^{13}C-NMR spectra have been presented and discussed to assess the utility of stereospecific deuterium incorporation to create the ^{1}H- or ^{13}C- NMR window.*

Introduction. The importance of structure and dynamics of DNA and RNA in understanding the biological function has been investigated by a variety of physico-chemical techniques. Amongst these techniques, Nuclear Magnetic Resonance (NMR) spectroscopy has emerged as one of the most powerful tools[1] because it provides conformational information on the implication of variation of local structures and the dynamics under a biological condition. This has been possible due to extensive developments achieved both in hardware (increasing magnetic field, more powerful computers) and spectral editing methodologies (two[1f,2]/three[3a-c] or higher[3d]-dimensional NMR). With increasing magnetic field, the higher sensitivity reduces

the amount of an oligomer needed to obtain a good quality spectrum, and increases the dispersion of resonance signals reducing the spectral complexity due to resonance overlap (from second order J couplings to first order). Homonuclear two-dimensional (2D) correlated spectroscopy (COSY) provides a direct proof of the existence of resolved scalar couplings ($^3J_{HH}$), and correlate the chemical shifts of coupling partner through the single or multiple coherence transfer of nuclear spins from one transition to another (as in DQF-COSY) or by the migration of coherences in an oscillatory manner through the entire spin system (TOCSY) which visualize the structure of the spin system in a most direct and informative manner[1c,2]. On the other hand, 2D nuclear Overhauser enhancement (NOESY)[5] result from the transfer of magnetization due to motional processes causing cross relaxation of dipolar-coupled spins which fall off with the sixth power of the distance between two relaxing protons [$< r(t)^{-6} >^{-1/6}$], where r(t) = ensemble of distances due to interconversions of conformations when the NMR measurements were being made)[5b-e]. Thus NMR has the capabilities of yielding both interproton distances and bond torsion angles which in conjunction with various computational methods[4] (*e.g.* distance geometry, energy minimization and molecular dynamics) can give the solution structure of oligonucleotides (*i.e.* conformations of sugars, glycosidic bonds, phosphate backbone, H-bonding, stackings etc).[1d] In these efforts to collect conformational informations, it is ideal that each resonance line and cross-peak due to two interacting nuclei is clearly separated in homonuclear proton-proton, heteronuclear proton-carbon, proton-phosphorus, carbon-phosphorus, NOESY and ROESY experiments. Although such first order informations are possible to extract from the 2D and 3D NMR experiments of a smaller oligonucleotide, it is simply impossible to collect all of these informations in a non-prejudicial manner from a large molecule bigger than 14-16 mer duplex DNA and 8-12 mer single stranded RNA. These problems are associated with spectral overlap which becomes more and more complex due to overcrowding of resonances particularly from the repeating pentose moieties with increasing chain length. It is clear that any technique that simplifies spectral complexities would have a considerable impact in future structural studies on larger DNA or RNA molecules that represent specific biological function. The problem due to severe spectral overlap of proton resonances in absorption assignments and nOe volume measurements could partly be solved by chemical means by selective or complete suppression of absorptions arising from a chosen domain of an oligomer by substituting proton (^1H) with deuteron (^2H) while extracting necessary information arising from the non-deuterated part of the molecule. By incremental shift of the non-deuterated site *(^1H-NMR window)* in an oligo-DNA or RNA (see Scheme 1), one should be able to put together the total structural information of a much larger oligonucleotide than what is possible today. What is important in this concept that two *^1H-NMR windows* in two different oligomers should have at least an overlap of a nucleotide residue with specific chemical shifts in order to be able to correlate protons from both windows with respect to the same nucleotide reference point (*i.e.* same proton resonances in both NMR-windows).

The use of deuterium exchange for the spectral assignment of nucleosides and oligonucleotides is a well established technique[6,16,25,27i,j]. The deuteration of the nucleobase residue has been described (e.g. exchange of protons at C8-purine and C5-cytosine with deuterioammonium bisulfite at pD 7.8 in deoxyoligomers[6a] gave 90 - 95 atom % ^2H incorporation, and platinum-catalyzed exchange at C5-methyl of thymidine in ^2H_2O[6b] afforded 94 atom % ^2H incorporation) and its effect on 1D and 2D ^1H-NMR spectra was studied. In general, most attention has been however given to the possible simplification of the most crowded sugar part of the NMR spectra which holds important informations regarding the dynamics of both local and global conformation of the molecule. A large variety of enzymatic[20,25] and chemical methods[7-19,21-23,26-28] have been devised for deuterium incorporation at both sugar[8,15-22,27i-j,28] or nucleoside[7,9-14,25] levels which have the potential to provide

monomeric building blocks deuterated at specific carbon center(s)[7-22,27i-j,28] or perdeuterated[25]. Enzyme promoted incorporation of deuterium has been found to be rather low (~ 90%)[20] and unsatisfactory for NMR work because of the stray resonances arising as a consequence of the low level of deuteration[20,25] and cumbersome isolation of deuterated mononucleotide blocks[25]. 5',5"-$^2H_2$-Adenosine was prepared from 2',3'-O-isopropylideneadenosine-5'-carboxylic acid[7] or from methyl-2,3-isopropylidene-β-D-ribofuranosiduronic acid[8].



Scheme 1

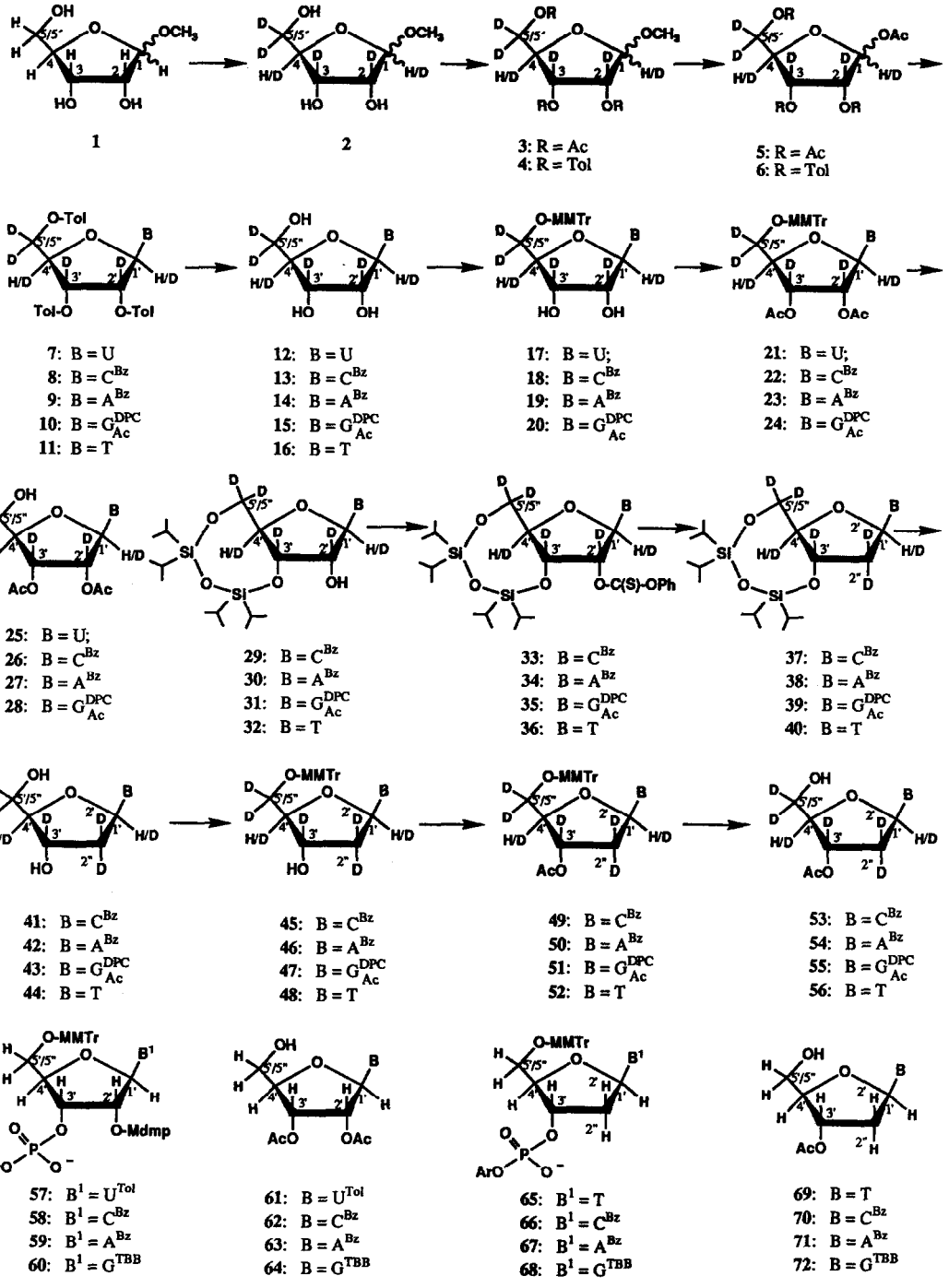
The mixtures of diastereoisomers of 5'-deuterioadenosine[9] and 5'(R/S)-deuteriothymidine (98 atom % ^2H incorporation)[10] were obtained *via* the reduction of the appropriate 5'-aldehydes by sodium borodeuteride or lithium aluminium deuteride. The 5'-aldehyde derivative of 2'-deoxyguanosine was converted to 5' or 4'-deuterio-2'-deoxyguanosine[11] by heating the aldehyde in ^2H_2O/pyridine mixture (1:1) followed by reduction of the aldehyde with $NaBH_4$. 4'-Deuterium labeled uridine and thymidine (98 atom % ^2H) was obtained upon $NaBD_4$ addition to the protected 4',5'-unsaturated nucleoside followed by oxidation[12]. Deuterium was incorporated at C3' (97 atom % ^2H) of adenosine at sugar level upon stereoselective reduction of 1,2:5,6-di-O-isopropylidene-α-D-hexofuranos-3-ulose to 1,2:5,6-di-O-isopropylidene-3-deuterio-α-D-ribohexofuranose[13] using sodium borodeuteride. Recently, more than 95 atom % ^2H incorporation has been accomplished at C3' of adenosine with virtually complete stereoselectivity upon reduction of the 2'-O-*tert*-butyldimethylsilyl(TBDMS)-3'-ketonucleoside by sodium borodeuteride in acetic acid[14]. Specifically 2'-monodeuterated (R or S)-2'-deoxycytidines were synthesized[15] from specifically 2-monodeuterated-2-deoxy-D-riboses[16] obtained upon stereospecific reduction of a 2,3-dehydro-hexopyranose with lithium aluminium deuteride and oxidation of the resulting glycal. Syntheses of 2'-deoxy-2'(S)-deuterio-uridine and cytidine were carried out by the use of 1-methyl-2-deoxy-2(S)-deuterioribofuranoside[17]. 2-Deoxy-1-deuterio-D-*erythro*-pentose, 2-deoxy 2(S)-deuterio-D-*erythro*-pentose and 2-deoxy-1,2(S)-dideuterio-D-*erythro*-pentose were obtained from D-arabinose by a reaction sequence involving the formation and $LiAlD_4$ reduction of ketene dithioacetal derivatives[18]. Detailed method was published by us about the stereospecific synthesis of all eight 2' or 2"-deuterio-2'-deoxynucleosides by reductive opening of appropriate methyl 2,3-anhydro-α-D-*ribo* or β-D-*lyxo*furanosides with $LiAlD_4$[19]. 2',2"-Dideuterio-2'-deoxyguanosine and thymidine were prepared from 2-deoxyribose 5-phosphate using 2-deoxyribose 5-phosphate aldolase enzyme in ^2H_2O achieving some 90 atom % deuteration[20]. The synthesis of

all 2',2"-dideuterio-2'-deoxynucleosides was achieved in this laboratory with deuterium incorporation at both nucleoside and sugar levels (oxidation of C2', subsequent reduction with $NaBD_4$ or $LiAlD_4$ followed by deoxygenation by tributyltin deuteride)[21]. More than 99 atom % ^2H incorporation at C1 of 2'-deoxyribose has been reported[22] recently by reduction of 3,5-bis-O-TBDMS-2-deoxyribonolactone by Dibal-D. On the other hand, the same reaction performed on the 2,2'-dideuterated ribonolactone obtained upon base-catalyzed H/D exchange at C2 of 2-deoxyribonolactone (~95% and ~85% deuteration at 2 and 2'-position, respectively) gave 1,2,2'-trideuterio-2'-deoxyribose[22].

Clearly, each position of the sugar residue can be selectively labeled, and some of these selectively deuterated nucleosides have indeed found their use in solid-state ^2H-NMR studies on the internal motions of nucleosides[23] and oligonucleotides[24]. In the temperature dependent lineshape analysis in solid-state ^2H-NMR spectroscopy, the stereoselectivity of 2' versus 2" labeling[24c] or the level of deuteration do not play a significant role. The use of specifically deuterium labeled nucleotides for the simplification of 1D and 2D ^1H-NMR spectra in solution studies has not however attracted much attention. An early and so far most extensive use of deuteration in the 1D NMR studies was performed by Danyluk *et al.* These workers isolated *perdeuterated* ^2H-labeled mononucleotides (~90 atom % ^2H incorporation)[20] in a tedious manner from RNA digest of blue-green algae grown in ^2H_2O. These *perdeuterated* nucleoside blocks were then used to obtain a wide variety of partially deuterated dimers and trimers[25] for the purpose of resonance assignments in 1D ^1H-NMR spectra (200 - 300 MHz). Synthesis of 4',5',5"-$^2H_3$-adenosine[26] was accomplished and coupled to appropriately blocked adenosine 3'-phosphite to give ApA* (pA* = 4',5',5"-$^2H_3$-pA). This dimer allowed the unequivocal measurement of nOe difference between phosphorus and H-3'[26].

A powerful alternative method of stereospecific deuteration to give polydeuterated sugars employs exchange of hydrogen with deuterium at the hydroxyl bearing carbon (*i.e.* methylene and methine protons of hydroxyl bearing carbon) using deuterated Raney nickel catalyst in ^2H_2O[27]. Detailed studies revealed structure dependent difference in exchange rates[27e,h], high level of epimerization[27d,g], significantly lower extent of deoxygenation[27h] and difficulties in the reproducibility of the level of deuteration [27h]. Despite these inherent problems in the deuterated Raney nickel-^2H_2O exchange reaction with sugars, a number of deuterated nucleosides specifically labeled at 2',3' and 4' positions were prepared for the first time by us taking advantages of this method. Our procedure[28] consisted of deuteration at 2, 3 and 4 positions of methyl β-D-arabinopyranoside by Raney nickel-^2H_2O exchange reaction[27] followed by reductive elimination of 2-hydroxyl group by tributyltin deuteride[29] to give methyl β-D-2,2',3,4-$^2H_4$-2-deoxyribopyranoside which was converted to methyl α/β-D-2,2',3,4-$^2H_4$-2-deoxyribofuranoside and glycosylated to give various 2,2',3,4-$^2H_4$-nucleosides (>97 atom % ^2H incorporation for H3' & H4'; ~94 atom % ^2H incorporation for H2 and H2'). Recently methyl β-D-erythrofuranoside was treated with deuterated Raney Ni to afford after purification methyl β-D-2,3,4(S)-$^2H_3$-erythrofuranoside (~75 atom % ^2H incorporation at C2 and C4(S) positions and 100 atom % ^2H incorporation at C3)[27i]. This sugar was converted to D-3,4,5(S)-$^2H_3$-ribose from which the four ribonucleoside were prepared. These nucleosides were subsequently reduced to the corresponding 3',4',5'(S)-$^2H_3$-2'-deoxynucleosides[27j].

Results and Discussions. During our 1D and/or 2D NMR studies on conformation of trimeric, tetrameric, pentameric, heptameric, nonameric and decameric branched RNAs[30a] and DNA duplexes[30b] we realized, that, though the assignment of given protons could be facilitated by selective deuteration at a specific site of short oligomers, *the substantial simplification of the crowded sugar domain to extract unambiguously J*


1
2
3: R = Ac
4: R = Tol
5: R = Ac
6: R = Tol
7: B = U
8: B = C^Bz
9: B = A^Bz
10: B = G^DPC_Ac
11: B = T
12: B = U
13: B = C^Bz
14: B = A^Bz
15: B = G^DPC_Ac
16: B = T
17: B = U;
18: B = C^Bz
19: B = A^Bz
20: B = G^DPC_Ac
21: B = U;
22: B = C^Bz
23: B = A^Bz
24: B = G^DPC_Ac
25: B = U;
26: B = C^Bz
27: B = A^Bz
28: B = G^DPC_Ac
29: B = C^Bz
30: B = A^Bz
31: B = G^DPC_Ac
32: B = T
33: B = C^Bz
34: B = A^Bz
35: B = G^DPC_Ac
36: B = T
37: B = C^Bz
38: B = A^Bz
39: B = G^DPC_Ac
40: B = T
41: B = C^Bz
42: B = A^Bz
43: B = G^DPC_Ac
44: B = T
45: B = C^Bz
46: B = A^Bz
47: B = G^DPC_Ac
48: B = T
49: B = C^Bz
50: B = A^Bz
51: B = G^DPC_Ac
52: B = T
53: B = C^Bz
54: B = A^Bz
55: B = G^DPC_Ac
56: B = T
57: B^1 = U^Tol
58: B^1 = C^Bz
59: B^1 = A^Bz
60: B^1 = G^TBB
61: B = U^Tol
62: B = C^Bz
63: B = A^Bz
64: B = G^TBB
65: B^1 = T
66: B^1 = C^Bz
67: B^1 = A^Bz
68: B^1 = G^TBB
69: B = T
70: B = C^Bz
71: B = A^Bz
72: B = G^TBB

Scheme 2

*couplings or nOe volumes could not be achieved in this manner.*This prompted us to reconsider the *^{1}H-NMR window* concept (Scheme 1) which in its most simple form has been proposed by Danyluk *et al.*[25]. In their work, they achieved only a low and unsatisfactory level of deuteration (~90 atom % ^{2}H incorporation)[20,25] which suffices for the purpose of assignment of resonances only. In the present concept of *^{1}H-NMR window*, we have set out to achieve a highest possible level of deuteration of nucleoside moieties *for the substantial simplification of the crowded sugar domain.* Clearly, the highest possible level of deuteration would suppress stray proton resonances from these deuterated moieties, and will help in the unambiguous extraction of conformational informations from the *^{1}H-NMR window* (creation of really ^{1}H-NMR invisible domain due to deuteration, see Scheme 1) with modern multidimensional NMR techniques. We realized that the goal for high level of deuteriation at as many carbons as possible can be achieved only by synthetic chemistry since the published enzymatic method is known to give low level of deuterium incorporation from deuterated blue green algae[20,25] unacceptable for the present concept of *^{1}H-NMR window.*

We envisioned at the outset that the preparation of a simple 2',3',5',5"-^{2}H$_4$-nucleoside with >97 atom % ^{2}H incorporations should serve well for the purpose of creating *^{1}H-NMR window* because of the fact that it would have H1' vicinal to ^{2}H-2', and H4' would be flanked by vicinal ^{2}H-3',5',5" which should block the propogation of J relays in the 2D spin-spin correlated spectroscopy such as DQF-COSY or TOCSY experiments. This means that although H1' and H4' would be detectable in 1D ^{1}H-NMR experiments, they would be completely undetectable in 2D COSY, DQF-COSY or TOCSY experiments thus simplifying the spectra for assignment purposes allowing the extraction of coupling constants and nOe volume informations from the protonated part of the molecule. It was clear to us at this stage that the H4' of 2'(2"),3',5',5"-^{2}H$_{4(5)}$-nucleotide would show coupling with the 5'-phosphorous in 1D ^{1}H-NMR experiments but this would be undetectable in 2D experiments because of the reasons said above.

Since the glycosylation reaction of a nucleobase with the α/β mixture of sugar derivative **5** or **6** gives β-D-nucleoside in a stereospecific manner owing to the intermediacy of carboxonium ion formed with participation of the 2'-O-acyl protecting group[31a], we decided to start our Raney nickel-^{2}H$_2$O exchange reaction on a crude mixture of methyl α/β-D-ribofuranoside (Scheme 2). A mixture of methyl α/β-D-ribofuranoside (~3:10) **1** was obtained in 98% yield upon treatment of D-ribose with concentrated sulfuric acid in dry methanol[32] at ~4 °C for 24 h, followed by neutralization upon a passage through a column of Amberlyst A-21 (OH^- form) ion exchange resin. This epimeric mixture of sugars **1**, ^{2}H$_2$O and deuterated Raney nickel were heated under reflux with exclusion of atmospheric moisture (see experimenatal). The site specific exchange (rates C5 > C3 => C2 >> C4) competes with slower epimerization reactions at C2 and C3 [27]. It has been shown that complete deuteration at C5 and 30-50% deuteration at C2 and C3 could be achieved within 6 h upon boiling methyl α or β-D-ribofuranoside in ^{2}H$_2$O with deuterated Raney nickel. In the early stage of our work, the generally applied W-2 type Raney nickel[33] was used with moderate reaction time (3 - 4 days). In our experiments, however, we wished to drive the deuteration exchange reactions to as high deuterium atom content as possible for suppressing all stray resonances (monitored by 500 MHz ^{1}H-NMR). An acceptable level of deuterium incorporation (>97 atom % ^{2}H) even at the relatively slowly exchanged C2 required at least 4 - 7 days of boiling under reflux (see experimental) giving a higher level of deuterium exchange at the expense of lower yield (60 - 80 %) of the desired methyl 1#,2,3,4#,5,5'-^{2}H$_6$-α/β-D-ribofuranoside **2** (>97 atom % ^{2}H at C2, C3, C5/5'; ~85 atom % ^{2}H at C4 (C4'#); ~20 atom % ^{2}H at C1(C1'#)). The ^{1}H-NMR spectra of this crude sugar derivative **2** at 500 MHz revealed the presence of products due to side reactions[27d,g,h] (see anomeric region in Figure 1A). Subsequent

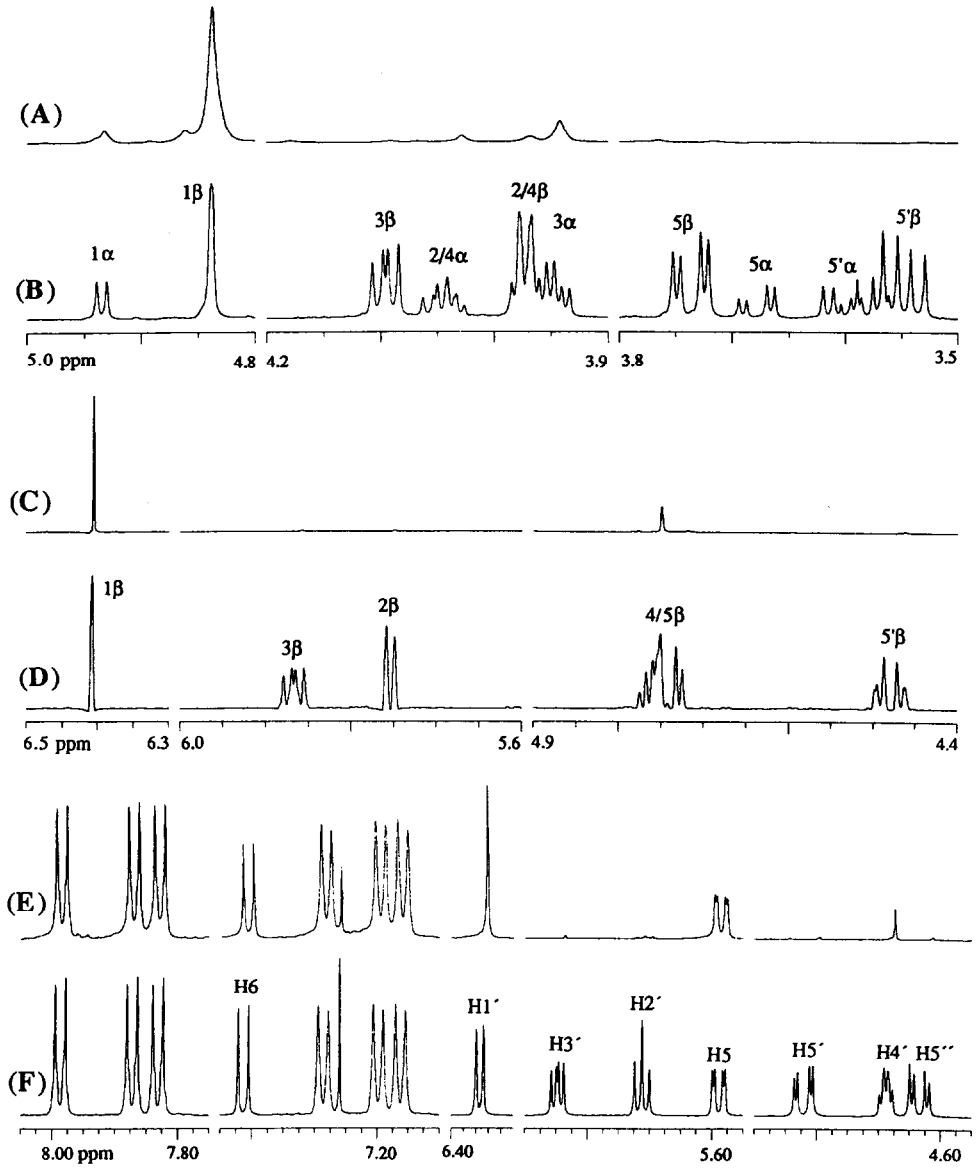


Figure 1: 500 MHz ^{1}H-NMR spectra of deuterated D-ribofuranoses (>97 atom % ^{2}H at C2, C3, C5/5'; ~85 atom % ^{2}H at C4; ~20 atom % ^{2}H at C1) and their natural-abundance counterparts (99.985 atom % ^{1}H). (A) shows the reaction mixture of deuterated Raney-Ni exchanged methyl-(α/β)-D-ribofuranoside (2) & (B) shows natural-abundance methyl-(α/β: ~3/10)-D-ribofuranoside (1). (C) shows 1-O-acetyl-2,3,5-tri-O-(4-toluoyl)-β-D-ribofuranoside (6) and (D) shows its natural-abundance counterpart. Note that *only sugar protons* are shown in the above subspectra. (E) shows 2',3',5'-O-tri-(4-toluoyl)-1'#,2',3',4'#,5',5"-$^{2}H_{6}$-uridine (7); (F) shows natural-abundance counterpart.

acetylation of this crude product **2** with acetic anhydride in dry pyridine resulted in 1-O-methyl-2,3,5-tri-O-acetyl-α/β-D-1#,2,3,4#,5,5'-$^2H_6$-ribofuranoside **3** in 82% yield, which was converted to 1,2,3,5-tetra-O-acetyl-α/β-D-1#,2,3,4#,5,5'-$^2H_6$-ribofuranoside **5** by a treatment with sulfuric acid in acetic acid-acetic anhydride for 12 h at room temperature (RT) (97%). Neither compound **3** nor **5** were possible to purify by chromatographic means. Compound **5** was therefore coupled with N-protected nucleobases[31] and purified chromatographically. The examination of ^{1}H-NMR spectra of the *chromatograpically pure* reaction product revealed that the the required β-D-nucleoside was the major component (90-95%) which was contaminated with inseparable by-products. This impurity content slowly diminished during the run through the synthetic sequences and purification steps giving pure 5'-hydroxy blocks **25** - **28** and **53** - **56**. Another striking finding was the highly variable level of deuterium exchange (~98% down to ~70 %) depending upon the given batch of the deuterated Raney-Ni catalyst used in the isotope exchange reaction. This problem could partially be solved by repetition of the deuteration reaction one more time without any further loss of yield of **2** due to isomerization reactions. This is because of the fact that the first cycle of deuteration exchange for 4-7 days gave the thermodynamic mixture of epimers which did not alter in the second cycle of deuteration exchange. In the course of synthesis of RNA dimers it turned out, that 95-96% ^{2}H incorporation is sufficient for the suppression of cross peaks in DQF-COSY and TOCSY spectra. On the contrary, the same level of deuteration was not sufficient to suppress the cross peaks in the DNA dimers because of more effective J relays through the 2'-deoxy protons (*i.e.* H2' and H2"). These findings clearly indicated the need for (i) a reliable deuterium exchange method to attain a consistent deuterium enrichment in the sugar in different batches, and (ii) since such deuterium exchange reaction will inherently produce epimeric *xylo* and *arabino* sugars as main by-products[27g], therefore the purification of the methyl α/β-D-1#,2,3,4#,5,5'-$^2H_6$-ribofuranoside **2** from the exchange reaction should be achieved.

In the pursuit for a more active catalyst, we tried to use W-5 type Raney nickel[34], which is prepared at higher temperature (50 ±4 °C). With this modification and by the use of at least 20 ml ^2H_2O/g of sugar, it was possible to achieve a *reproducible* >97 atom % deuterium incorporation at C2, C3 and C5 positions in **2**. It was also noted that C1 and C4 could be consistently deuterium enriched by ~20 and ~85 %, respectively. As it turns out from Table 1 (compound **9** *vs* others), the extent of exchange at C1 and C4 increases slowly depending on the length of reflux (~10 atom % ^{2}H at C1 and ~78 atom % ^{2}H at C4 after boiling at reflux for 4 days, and ~17 atom % ^{2}H at C1 and ~85 atom % ^{2}H at C4 after boiling at reflux for 7 days) whereas the deuteration levels at C2, C3 and C4 practically do not change. In order to obtain pure starting material for the coupling reactions with the N-protected nucleobases, we decided to apply 4-toluoyl protection for the hydroxyls, which made the UV detection possible facilitating the separation procedure. The toluoylation was carried out in dry pyridine (5 ml/mmol sugar) at ambient temperature overnight[35] to give after a careful separation on a column of silica gel the desired practically pure 1-O-methyl-2,3,5-tri-O-(4-toluoyl)-α/β-D-1#,2,3,4#,5,5'-$^2H_6$-ribofuranoside **4** in 48% yield. The conversion of 1-O-methyl **4** to 1-O-acetyl **6** provided a second opportuinity for purification. The reaction, performed in acetic acid-acetic anhydride mixture by adding sulfuric acid, gave the 1-O-acetyl-2,3,5-tri-O-toluoyl-α/β-D-1#,2,3,4#,5,5'-$^2H_6$-ribofuranoside **6** (Figure 1C) in a substantially higher yield (82%) after purification than the previous one, indicating that the most important separation was really achieved after the toluoylation reaction (**1** → **4**).

Compound **6** was condensed with silylated uracil, N^4-benzoylcytosine[36], N^6-benzoyladenine[36], N^2-acetyl-O^6-diphenylcarbamoylguanine[37] and thymine in anhydrous 1,2-dichloroethane (in case of the protected

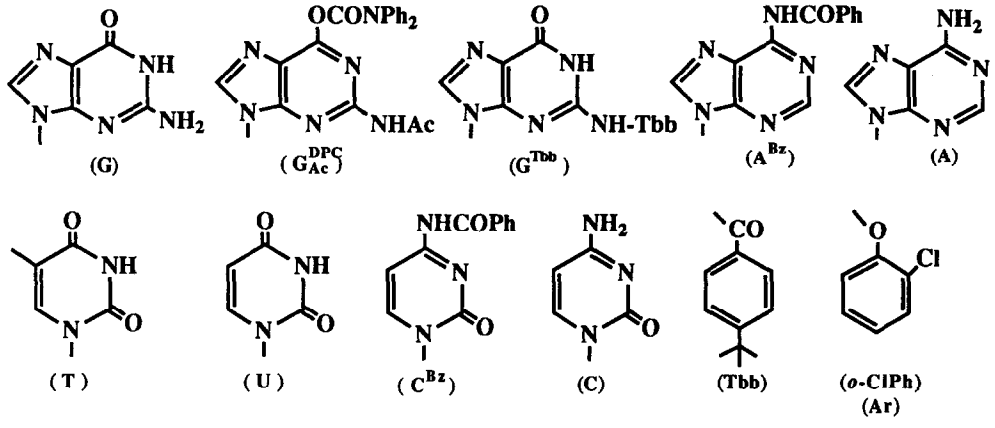
guanine dry toluene) using trimethylsilyl trifluoromethanesulfonate as Lewis acid catalyst[31,37] to prepare the corresponding 1'#,2',3',4'#,5',5"-$^2H_6$-β-D-ribonucleoside derivatives **7, 8, 9, 10, 11** in 75, 85, 60, 73 and

Table 1: Residual ^1H (%) at different carbons of deuterated sugars **4 - 6**, deuterated ribonucleosides **7 - 11** and 2'-deoxyribonucleosides **37 - 40** obtained by integration of the ^1H-NMR signals at 500 MHz.

Compound	H1'	H2'	H2"	H3'	H4'	H5'	H5"
4*	83	1	-	1	15	1	1
6*	82	1	-	1	13	1	1
7*	81	1	-	1	12	1	2
8*	83	2	-	2	13	2	2
9¥	90	1	-	1	22	1	1
10*	84	1	-	2	13	2	2
11*	84	1	-	2	11	2	2
37*	81	2	2	2	14	2	2
38*	85	2	2	2	16	1	2
39*	83	2	2	2	12	2	2
40*	84	3	2	2	15	2	2

*deuteration was performed for 7 days (see experimental); ¥deuteration was performed for 4 days (as in the experimental but was heated to reflux for 4 days). The variation in integration values in **4-8** and **10, 11, 37-40** reflect margin of error in integration of resonances at 500 MHz (Bruker AMX 500).

Abbreviations:



91% yields, respectively. The isomeric purities of these products are found to be excellent as evident through their 500 MHz ^1H-NMR spectra (Figures 1E & 2A - F & 3A). For further work, it was necessary to remove the hydroxyl protecting groups in a regioselective manner that did not knock off the N-protection(s) on the nucleobases in order to reduce the number of synthetic steps to access oligomers in a convenient manner. In order to find an optimum condition for release of 4-toluoyl groups, model experiments were carried out. 2',3',5'-Tri-O-(4-toluoyl)-N^6-benzoyladenosine was dissolved in dry methanol followed by addition of 0.2 N sodium methylate in dry methanol[38]. Tlc examination after 9 min revealed that the removal of the 2',3',5'-tris-O-(4-toluoyl) group was complete but accompanied by a ~40% loss of N^6-benzoyl group. In the second experiment, the fully protected adenosine derivative was dissolved in pyridine-ethanol (2:3 ml/ mmol) followed

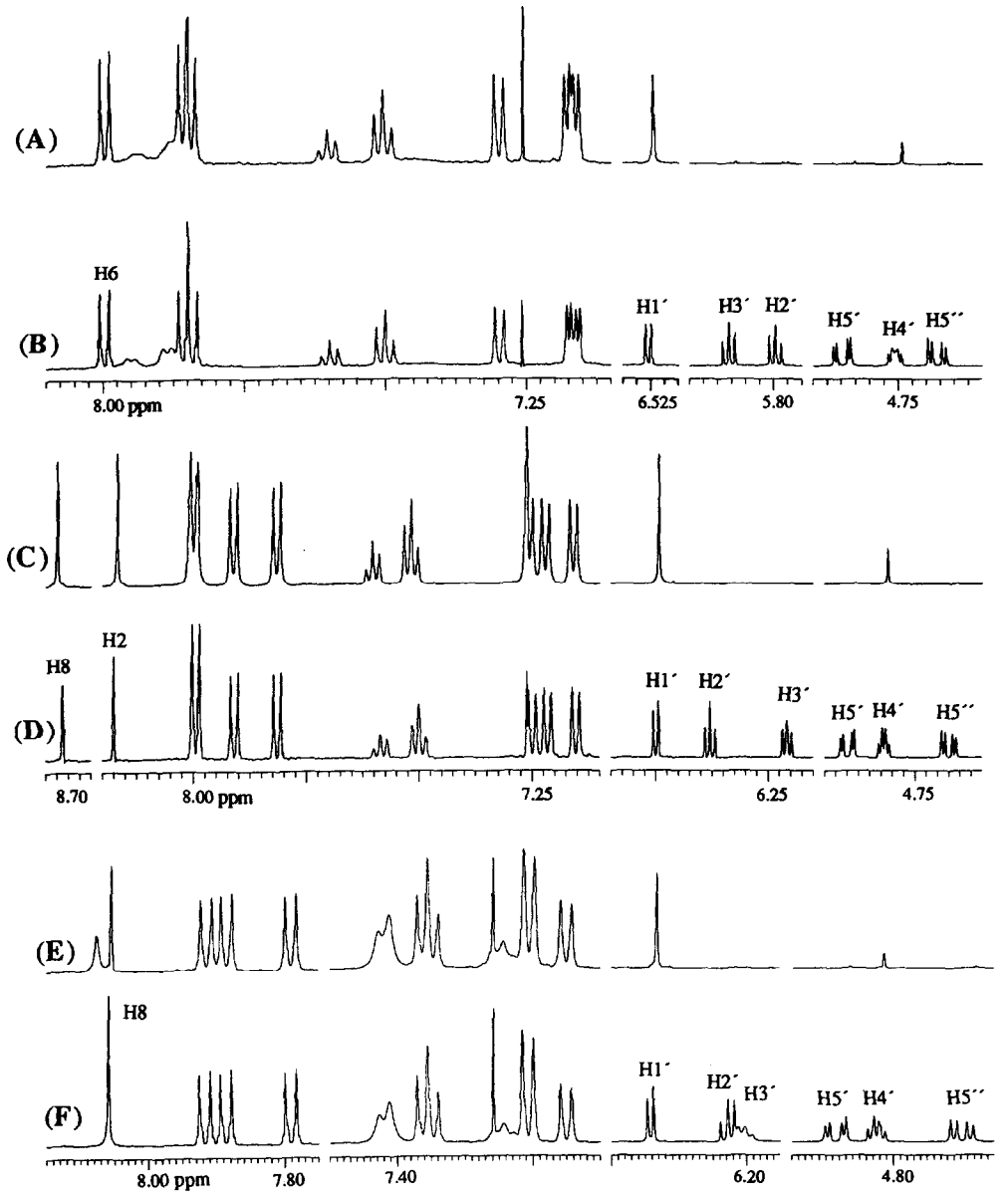


Figure 2: 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides (>97 atom % ^{2}H at C2', C3', C5'/5"; ~85 atom % ^{2}H at C4' (C4'$^{\#}$); ~20 atom % ^{2}H at C1' (C1'$^{\#}$)) and their natural-abundance counterparts (99.985 atom % ^{1}H). (A) shows 2',3',5'-O-tri-(4-toluoyl)-1'$^{\#}$,2',3',4'$^{\#}$,5',5"-$^{2}H_6$-N^{4}-benzoylcytidine (8); (B) shows natural-abundance counterpart; (C) shows 2',3',5'-O-tri-(4-toluoyl)-1'$^{\#}$,2',3',4'$^{\#}$,5',5"-$^{2}H_6$-N^{6}-benzoyladenosine (9); (D) shows natural-abundance counterpart; (E) shows 2',3',5'-O-tri-(4-toluoyl)-1'$^{\#}$,2',3',4'$^{\#}$,5',5"-$^{2}H_6$-N^{2}-acetyl-O^{6}-diphenylcarbamoylguanosine (10); (F) shows natural-abundance counterpart;

73: $B^1 = U^{Tol}$; $B = A^{Bz}$
74: $B^1 = C^{Bz}$; $B = G^{DPC}_{Ac}$
75: $B^1 = A^{Bz}$; $B = U$
76: $B^1 = G^{TBB}$; $B = C^{Bz}$

77: $B^1 = U$; $B = A$
78: $B^1 = C$; $B = G$
79: $B^1 = A$; $B = U$
80: $B^1 = G$; $B = C$

81: $B^1 = U^{Tol}$; $B = A^{Bz}$
82: $B^1 = C^{Bz}$; $B = G^{TBB}$
83: $B^1 = A^{Bz}$; $B = U^{Tol}$
84: $B^1 = G^{TBB}$; $B = C^{Bz}$

85: $B^1 = U$; $B = A$
86: $B^1 = C$; $B = G$
87: $B^1 = A$; $B = U$
88: $B^1 = G$; $B = C$

89: $B^1 = T$; $B = A^{Bz}$
90: $B^1 = C^{Bz}$; $B = G^{DPC}_{Ac}$
91: $B^1 = A^{Bz}$; $B = T$
92: $B^1 = G^{TBB}$; $B = C^{Bz}$

93: $B^1 = T$; $B = A$
94: $B^1 = C$; $B = G$
95: $B^1 = A$; $B = T$
96: $B^1 = G$; $B = C$

97: $B^1 = T$; $B = A^{Bz}$
98: $B^1 = C^{Bz}$; $B = G^{TBB}$
99: $B^1 = A^{Bz}$; $B = T$
100: $B^1 = G^{TBB}$; $B = C^{Bz}$

101: $B^1 = T$; $B = A$
102: $B^1 = C$; $B = G$
103: $B^1 = A$; $B = T$
104: $B^1 = G$; $B = C$

Scheme 3

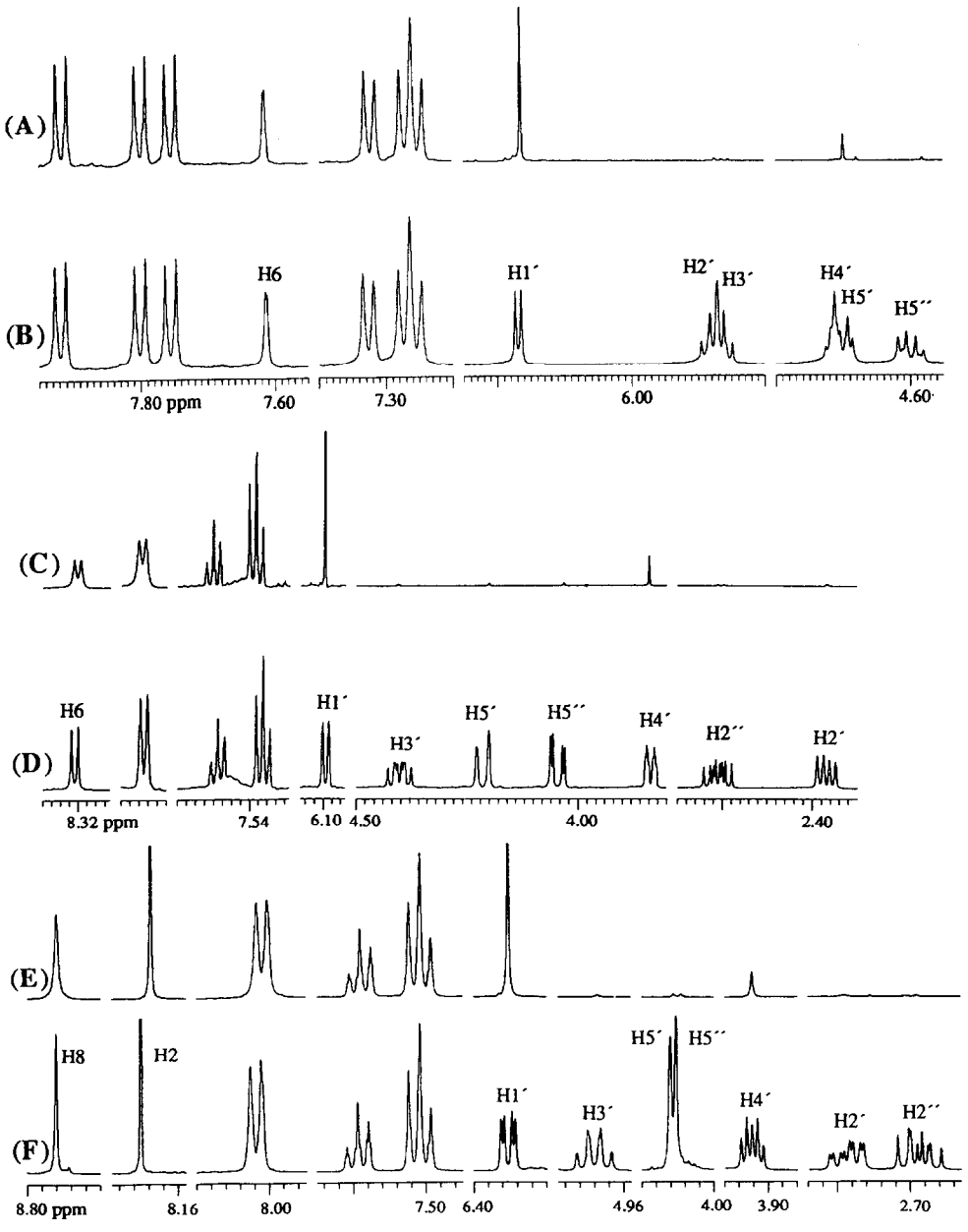


Figure 3: 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides (>97 atom % ^{2}H at C2', C3', C5'/5"; ~85 atom % ^{2}H at C4' (C4'$^{\#}$); ~20 atom % ^{2}H at C1' (C1'$^{\#}$)) and their natural-abundance counterparts (99.985 atom % ^{1}H). (A) shows 1-(2',3',5'-O-tri-(4-toluoyl)-1'$^{\#}$,2',3',4'$^{\#}$,5',5"-$^{2}H_6$-β-D-ribofuranosyl)-thymine (**11**); (B) shows natural-abundance counterpart; (C) shows 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1'$^{\#}$,2',2",3',4'$^{\#}$,5',5"-$^{2}H_7$-2'-deoxy-N^{4}-benzoylcytidine (**37**); (D) shows natural-abundance counterpart; (E) shows 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1'$^{\#}$,2',2",3',4'$^{\#}$,5',5"-$^{2}H_7$-2'-deoxy-N^{6}-benzoyladenosine (**38**); (F) shows natural-abundance counterpart.

by addition of ethanolic NaOH (6.4 ml of ethanol and 6.4 ml of 2N NaOH / mmol)[39], complete deprotection was found to take place within 5 min without any loss of base protection. The latter method was used to obtain the crude deuterated nucleosides **12, 13, 14, 15** and **16** in 99, 98, 105, 79 and 99% yields, respectively, after neutralization by Dowex cation exchange resin (H^+ form) and processing as specified in the experimental part for the different nucleobase protected nucleosides. Though these nucleosides were contaminated by toluoyl derivative (4-toluic acid) as evidenced by ^{1}H-NMR, this impurity did not however interfere in any respect with the subsequent reaction sequences. Crude **12 - 15** were treated with 4-methoxytriphenylmethyl (MMTr) chloride in dry pyridine overnight to give the 5'-O-MMTr-1'#,2',3',4#',5',5"-$^2H_6$-nucleosides **17, 18, 19** and **20** in 53, 78, 72 and 78 % yields, respectively, after column chromatography. These derivatives were acetylated by acetic anhydride treatment in dry pyridine to afford the 5'-O-MMTr-2',3'-di-O-acetyl-1'#,2',3',4'#,5',5"-$^2H_6$-nucleosides **21, 22, 23** and **24** in 85, 97, 92 and 78% yields, respectively. After removal of the 5'-O-MMTr group by a treatment with 80% aqueous acetic acid at ambient temperature overnight, the 5'-hydroxy blocks **25, 26, 27** and **28** were obtained in 95, 81, 94 and 58 % yields, respectively, which were used for dimer syntheses.

For the conversion of our deuterated ribonucleosides **13 - 16** to the corresponding 2'-deoxynucleosides **41 - 44**, the convenient route devised by Robins *et al.* was chosen[30, 21, 28]. Treatment of compounds **13 - 16** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[40] in dry pyridine resulted in 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl(TPDS))-1'#,2',3',4'#,5',5"-$^2H_6$-nucleosides **29, 30, 31** and **32** in 85, 77, 83 and 66 % yields, respectively, obtained as white foams. The reaction of 2'-hydroxyls in 3',5'-O-(TPDS)-nucleosides with phenoxythiocarbonyl chloride in dry acetonitrile using N,N-dimethylaminopyridine as catalyst did not proceed smoothly. In a set of control experiments nondeuterated counterparts of **33** and **34** were obtained in ~60% yield and complete loss of compound occured in case of the nondeuterated guanosine derivative **31**. Since the guanosine derivative proved to be the most sensitive under the above condition, we took the nondeuterated counterpart of **31** as a starting material for further model experiments to devise condition for the introduction of phenoxythiocarbonyl group to the 2'-OH. Overnight treatment of nondeuterated **31** with phenoxythiocarbonyl chloride in dry pyridine gave nondeuterated **35** (61 %), after a work-up with saturated sodium bicarbonate and column chromatography, but the reaction mixture as well as the product had a strong brown colour. Treatment of the same compound in the same manner but in dry dichloromethane using 1-methylimidazole as catalyst proved to be more successful to give nondeuterated **35** in 77 % yield. Application of this method for introduction of phenoxythiocarbonyl group in deuterated **29 - 32** gave the corresponding 2'-phenoxythiocarbonate derivatives **33, 34, 35** and **36** in 87, 88, 89 and 89 % yields, respectively. 2'-Deoxygenation of these compounds by tributyltin deuteride in presence of 2,2'-azobis(2-methyl-propionitrile) (AIBN) in dry toluene at 75 °C proceeded without any problem to give compounds **37, 39** and **40** in 75, 96 and 95 % yields respectively, except for the adenosine derivative **34**, in which case simultaneous partial loss of benzoyl group had occured as it was revealed by the ^{1}H-NMR spectra. The re-benzoylation of the 3',5'-O-TPDS-1'#,2',2",3',4'#,5',5"-$^2H_7$-2'-deoxyadenosine was done easily to give an additional crop of the desired derivative increasing the original 77% yield to 92%. The deuterium incorporation at the 2" was >97 % as evidenced by integration of ^{1}H-NMR spectra at 500 MHz (Figures 3C - F & 4A - D) of the residual proton resonance, which is consistent with the isotope content of $LiAlD_4$ (98 atom % ^2H) used in the reduction of tributyltin chloride[41] to tributyltin deuteride which was used as the reagent. After removal of TPDS protection by a treatment with tetrabutylammonium fluoride in dry tetrahydrofuran, the 1'#,2',2",3',4'#,5',5"-$^2H_7$-2'-

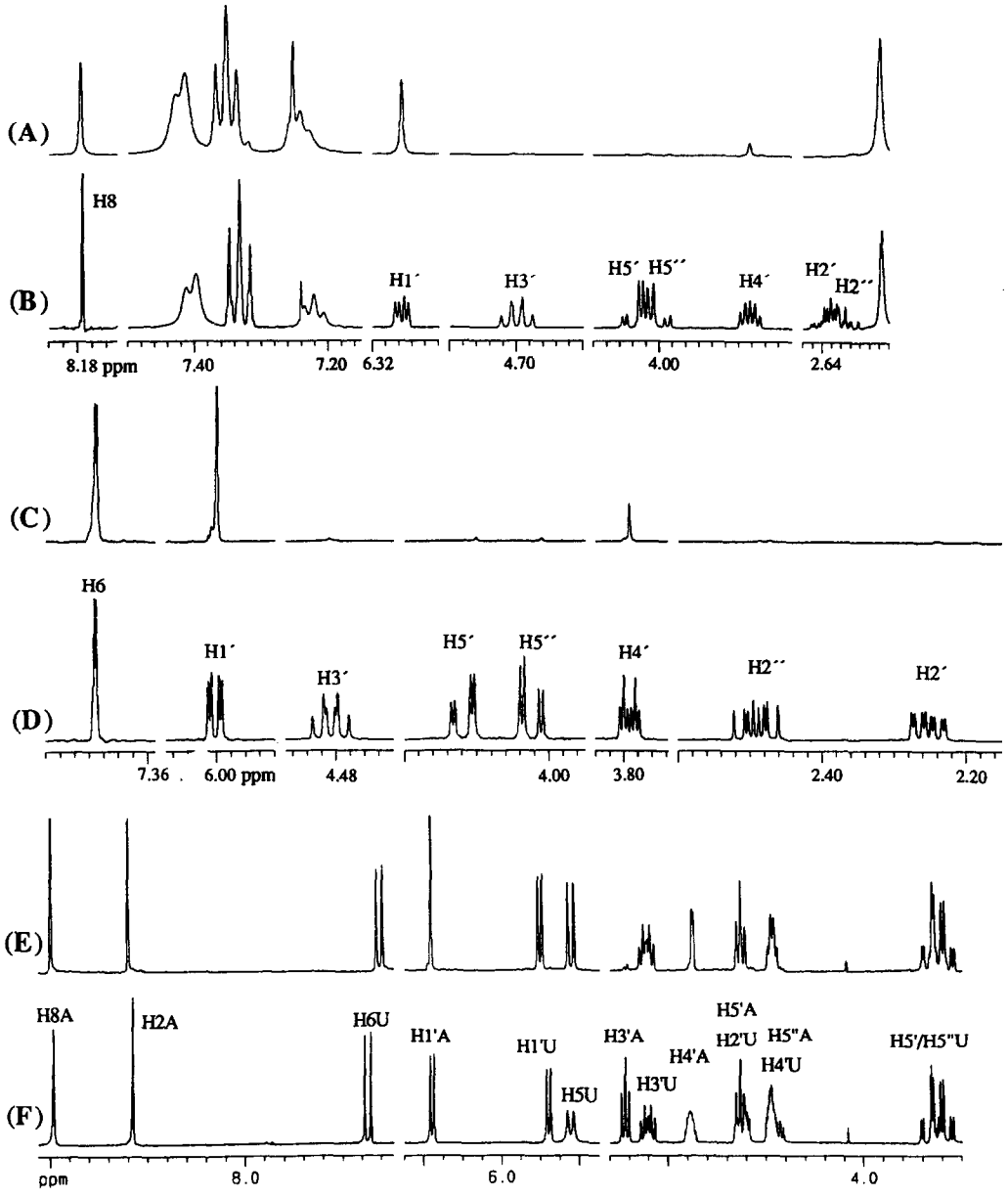


Figure 4: 500 MHz ^{1}H-NMR spectra of deuterated-β-D-nucleosides (>97 atom % ^{2}H at C2', C3', C5'/5"; ~85 atom % ^{2}H at C4' (C4$^{\#}$); ~20 atom % ^{2}H at C1' (C1$^{\#}$)) and their natural-abundance counterparts (99.985 atom % ^{1}H). (A) shows 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1$^{\#}$,2',2",3',4$^{\#}$,5',5"-^{2}H$_7$-2'-deoxy-N^2-acetyl-O^6-diphenylcarbamoylguanosine (**39**); (B) shows natural-abundance counterpart; (C) 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1$^{\#}$,2',2",3',4$^{\#}$,5',5"-^{2}H$_7$-thymidine (**40**); (D) shows natural-abundance counterpart. ^{1}H-NMR spectra of partially deuterated and natural diribonucleoside-(3'→5')-monophosphates in D_2O at 298 K. (E): UpA* where the 1$^{\#}$,2',3',4$^{\#}$ and 5'/5" protons of the adenosine (pA*) residue are exchanged with ^{2}H, (F): natural UpA.

deoxynucleosides **41**, **42**, **43** and **44** were obtained in 88, 93, 80 and 83 % yields, respectively. These compounds were further transformed to the 5'-O-MMTr derivatives **45**, **46**, **47** and **48** (87, 90, 84 and 88%, respectively), followed by acetylation of the 3'-hydroxyls as documented above for the ribo compounds to afford the fully protected heptadeuterio nucleosides **49**, **50**, **51** and **52** in 89, 77, 94 and 83 % yields, respectively. It should be noted here that acetylation of 3'-hydroxyl group of 2'-deoxyadenosine derivative **46** had to be carried out at low temperature (4 °C) with small excess of acetic anhydride (1.3 equiv) in order to avoid the formation of bis-N^6-protected adenine (N^6-benzoyl, N^6-acetyl) derivative. This diminished regioselectivity of 3'-O-acetylation reaction in **46** is presumably owing to the lack of the inductive effect of the missing 2'-OH group[42], which also enhances the basicity of N^6. Removal of 5'-O-MMTr group was achieved by a short treatment with 2 % benzenesulfonic acid in dichloromethane-methanol (7:3, v/v) mixture to give the 5'-hydroxy blocks **53**, **54**, **55** and **56** in 86, 90, 78 and 69 % yields, respectively. Though the harsher acid treatment in case of 2'-deoxypurine nucleosides (especially N^6-benzoyladenosine[43]) can be potentially harmful despite the fact that the glycosyl bonds in **50** and **51** are stabilized by 3'-O-acetyl group[42], the shortened treatment gave 5'-hydroxy blocks with yields comparable to those obtained for ribo compounds using milder acid treatment (with 80% acetic acid).

Preparation of Partially Deuterated Dinucleotides & Trinucleotide. In order to investigate the effect of deuteration on 1D and 2D ^{1}H-NMR such as DQF-COSY, HOHAHA (TOCSY) and NOESY, two sets of dimers were synthesized using phosphotriester chemistry[43]. The 5'-OH group of 1'#,2',3',4'#,5',5"-$^2H_6$-ribonucleoside blocks **25**, **26**, **27** and **28** as well as the nondeuterated **61**, **62**, **63** and **64** were coupled to the triethylammonium salt of 2'-O-(3-methoxy-1,5-dicarbomethoxypentane-3-yl(MDMP))-5'-O-MMTr-ribonucleoside 3'-(2-chlorophenyl)-phosphates[43-46] **57**, **58**, **59** and **60** according to the reaction Schemes 2 & 3 in dry pyridine in the presence of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT)[45] to give the fully protected partially-deuterated dimers UpA* (* denotes for deuterated nucleoside moiety) **73** (72%), CpG* **74** (95%), ApU* **75** (79%), GpC* **76** (40%), and their natural counterparts UpA **81** (91%), CpG **82** (84%), ApU **83** (65%) and GpC **84** (84%) after saturated sodium hydrogen carbonate work-up and column chromatography. The deprotection of the dimers above was carried out using a well established literature procedure[44a,46] and subsequent purification on Sephadex A-25 culumn using a linear gradient of ammonium bicarbonate to obtain the deprotected partially deuterated dimers UpA* **77** (53%), CpG* **78** (49%), ApU* **79** (77%), GpC* **80** (49%), and their nondeuterated natural counterparts UpA **85** (76%), CpG **86** (76%), ApU **87** (56%) and GpC **88** (85%), which were lyophylised from ^2H_2O before they were subjected to NMR studies. The 5'-OH group of 1'#,2',2",3',4'#,5',5"-$^2H_7$-2'-deoxyribonucleoside blocks **53** - **56** and the nondeuterated **69** - **72** were coupled with the 3'-phosphotriester blocks **65** - **68** as described above[43-46] to give the fully protected partially deuterated di-(2'-deoxynucleoside)monophosphates d(TpA*) **89** (86%), d(CpG*) **90** (75%), d(ApT*) **91** (77%), d(GpC*) **92** (80%), and their natural counterparts d(TpA) **97** (86%), d(CpG) **98** (83%), d(ApT) **99** (90%) and d(GpC) **100** (89%). Subsequently, the deprotected partially deuterated 2'-deoxyribonucleotide dimers d(TpA*) **93** (62%), d(CpG*) **94** (92%), d(ApT*) **95** (78%), d(GpC*) **96** (80%), and their natural counterparts d(TpA) **101** (80%), d(CpG) **102** (90%), d(ApT) **103** (89%) and d(GpC) **104** (71%) were obtained after deprotection and purification procedures reported for the diribonucleoside-monophosphates dimers[43-46].

In order to further evaluate the actual NMR simplification that has taken place in the *^{1}H-NMR window* part as a result of specific deuterium incorporation we decided to prepare the shortest oligomer in which a central nondeuterated unit (*^{1}H-NMR window*) is sandwiched between two deuterated units, *i.e.* a trinucleotide. An

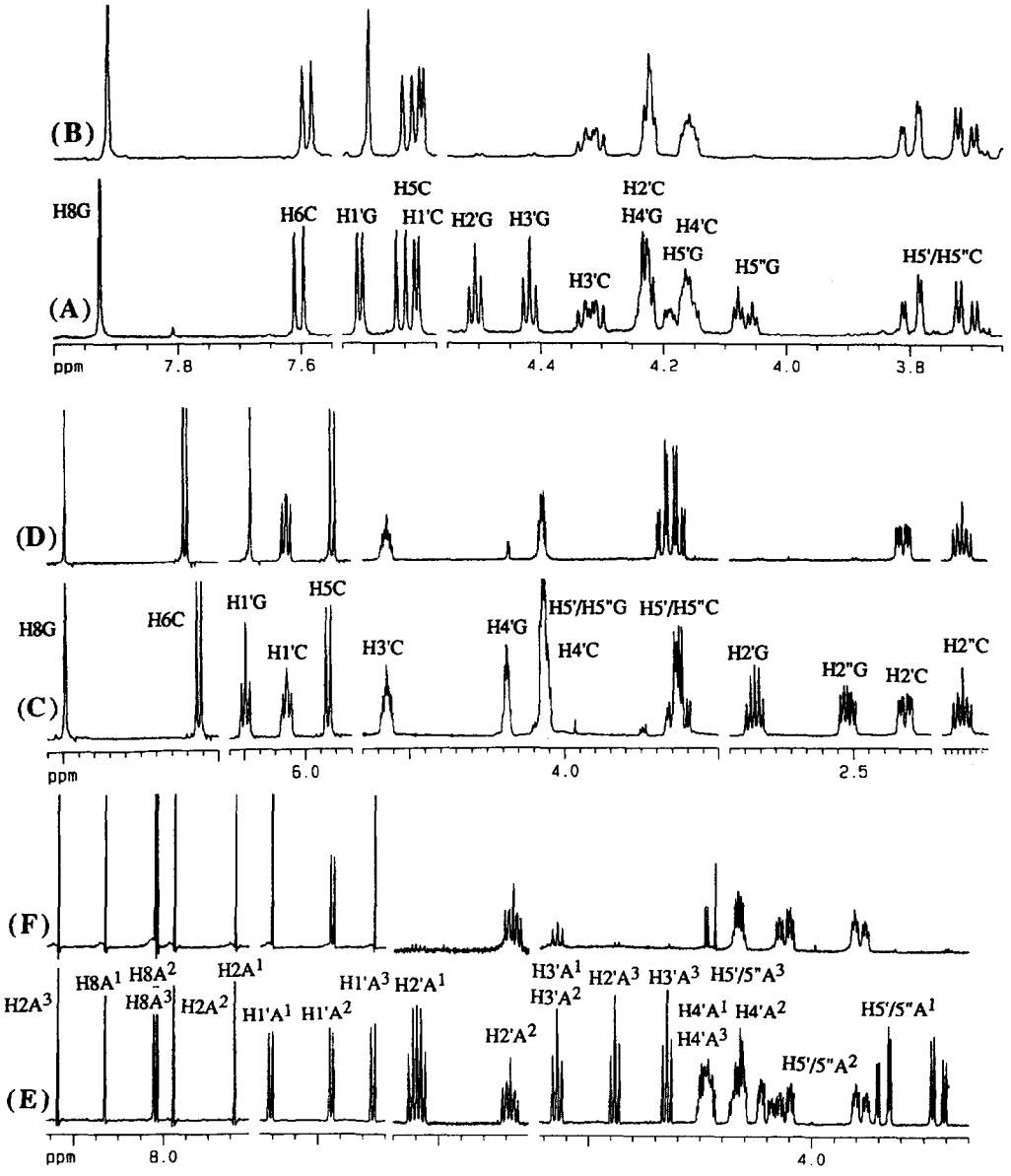
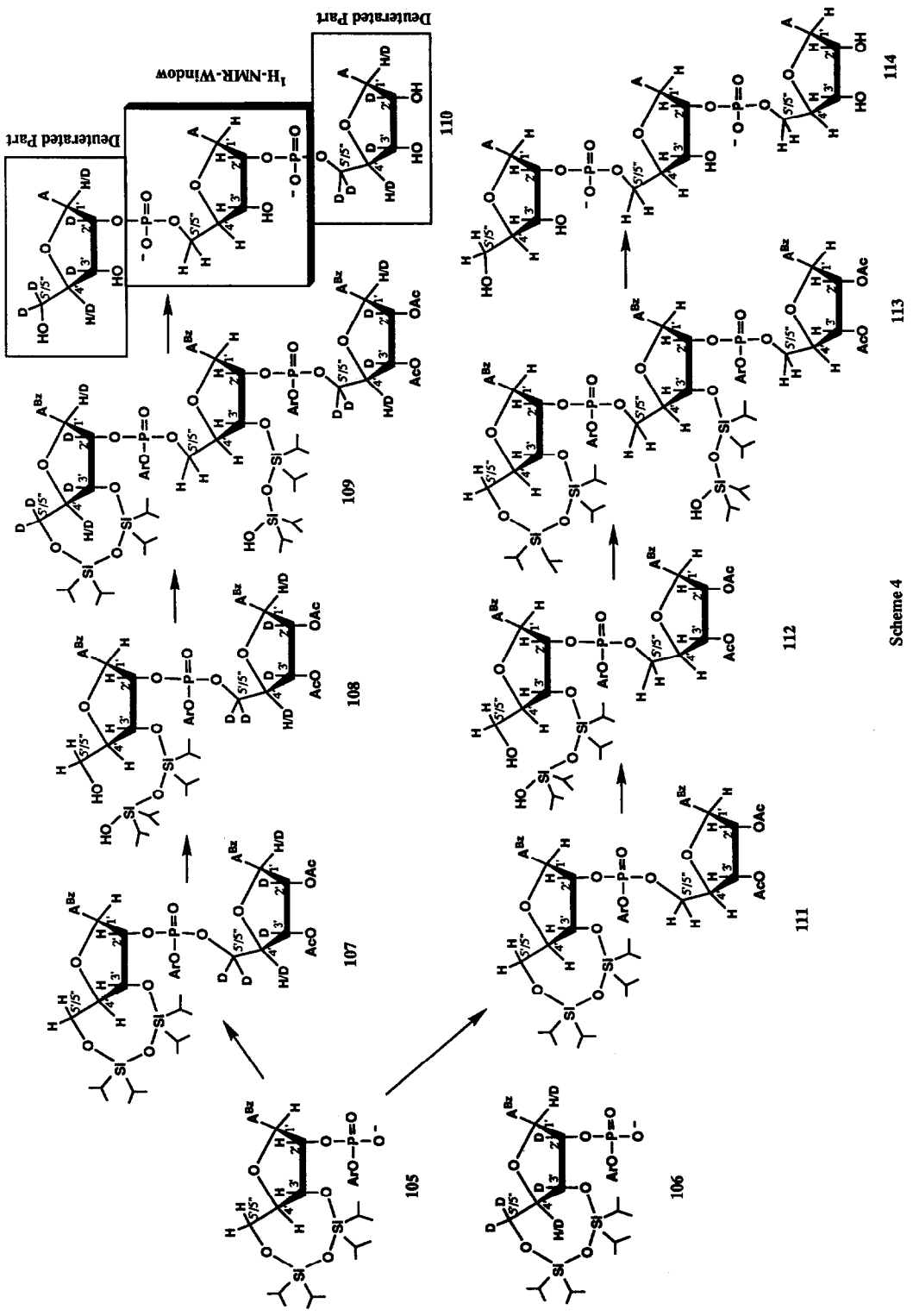


Figure 5: ^{1}H-NMR spectra of natural and partially deuterated diribonucleoside-(3'→5')-monophosphates, di(2'-deoxyribonucleoside)-(3'→5')-monophosphates and 2,5A core in D_2O at 298 K. (A): natural CpG, (B): CpG* where the 1'#,2',2",3',4'# and 5'/5" protons of the guanosine (pG*) residue are exchanged with ^{2}H. (C): natural d(CpG), (D): d(CpG*) where the 1'#,2',2",3',4'# and 5'/5" protons of the guanosine (pG*) residue are exchanged. (E) $A^1(2'\rightarrow5')A^2(2'\rightarrow5')A^3$, (F): $A^{1*}(2'\rightarrow5')A^2(2'\rightarrow5')A^{3*}$ where the 1'#,2',3',4'# and 5'/5" protons of the 5'-terminal A^1 and 3'-terminal A^3 residues are exchanged with ^{2}H. The H1'# of A^{1*} and A^{3*} residues appear as singlet. The H4'# of A^{1*} appears as a singlet while the H4'# of A^{3*} appears as a doublet due to its coupling to the phosphorus of the $A^2(3'\rightarrow5')A^{3*}$ phosphate linkage.


Deuterated Part
^{1}H-NMR-Window
Deuterated Part
105
106
107
108
109
110
111
112
113
114

Scheme 4

obvious choice was the 2,5A core (A2'p5'A2'p5'A) **114** and its partially deuterated counterpart (A*2'p5'A2'p5'A*) **110** because it mimics the antiviral properties of interferon[47], and the detailed structural studies of the 2,5A core **114** have been performed by ^{1}H-NMR spectroscopy[48]. In the present synthesis of A2'p5'A2'p5'A **114** and its partially deuterated counterpart A*2'p5'A2'p5'A* **110**, we have employed the same strategy which was devised earlier[49b] in this laboratory, and based on the use of simultaneous 5' & 3' protection by the 1,3-dichloro-1,1,3,3-tetraisopropyl-disiloxane[49b] (Scheme 4). The eight partially deuterated dinucleotides and the trinucleotide were subsequently compared with the corresponding natural counterparts *to evaluate the actual NMR simplifications achieved* in the *^{1}H-NMR window* part as a result of *specific deuterium incorporation* [69]. A few 1 and 2D ^{1}H-NMR spectra at 500 MHz (UpA*, CpG*, d(CpG*), A*2'p5'A2'p5'A* and their natural abundance conterparts) are shown in Figures 4E - F & 5 - 12 as representative examples.

The properties and application of deuterated nucleoside blocks in the NMR spectroscopy. The main source of information used to solve 3D structures of nucleic acids by NMR spectroscopy resides in short (<5Å) interproton distance data. To obtain these distances, the ^{1}H-NMR spectrum must first be assigned using the two-dimensional correlation and nOe experiments which show through bond and through space connectivities respectively. Correlation experiments serve to group together protons belonging to the same sugar residue, while the detection of nOes serve to connect one residue with its immediate neighbours dependending upon their dipolar relaxation rates which are inversely proportional to the sixth power of their distance. The simplest experiment to delineate the spin systems *via* scalar correlation is the COSY experiment[55] which shows direct through bond connectivities. COSY can however show some serious limitations due to spectral overlap in the 1', 2', 3', 4' and 5'/5" region. The coupling network is then best identified by means of Hartmann-Hahn (HOHAHA) spectroscopy[56] which shows both the direct and the relayed through bond connectivities along the H1'-H2'-H3'-H4'-H5'/H5" pathway in each sugar unit. In the 2D COSY or HOHAHA spectrum, the intensity of a cross peak depends on the magnitude of the J-coupling constant[57]. A sequential assignment can also be performed using nOe spectroscopy[58,59]. The base and sugar moieties of the same nucleotide are connected *via* the intraresidue H1'/H2'/H3'(i)-H8/H6(i) cross relaxation pathway. The through space connectivities along the H1'/H2'(i-1)-H8/H6(i)-H1'/H2'(i) and H8/H6(i)-H5(i+1) pathway will give the sequential assignment along one strand.

In addition to the interproton distances derived from the nOe data, the vicinal ^{3}J spin-spin coupling constants [*i.e.* $^3J_{1',2'}$, $^3J_{1',2''}$, $^3J_{2',3'}$, $^3J_{2'',3'}$, $^3J_{3',4'}$, $^3J_{4',5'}$, $^3J_{4',5''}$] provide useful information regarding the phase angle of the sugar pucker and the conformation about the C4'-C5' bond. Various proton-proton torsion angles in the nucleotide can be estimated from the magnitude of these ^{3}J-couplings which are easily obtained by analyzing the multiplet pattern in COSY-like spectra such as DQF-COSY[60] or E-COSY[61].

However, the assignments by HOHAHA or NOESY experiments, or the determination of vicinal coupling constants by DQF-COSY are seriously restricted to cases where the spectral overlap of the proton resonances is not too critical and where the linewidths are not significantly larger than the magnitude of the J-couplings. In the NMR spectra with overlapping resonances, it becomes increasingly difficult to assign the chemical shifts, and to extract data from the nOe experiments, or to identify the proton spin system to extract the torsional informations, and thus, it becomes impossible to solve the solution structure of biologically functional oligonucleotides as the size of the molecule increases. The problem of spectral overlap is particularly critical in the proton NMR spectra of RNA as seen from the few reported RNA structural studies[62] compared to DNA studies[1e]. The exchange of some of the sugar hydrogens with deuterium in selected nucleotides indeed simplifies the NMR spectrum by

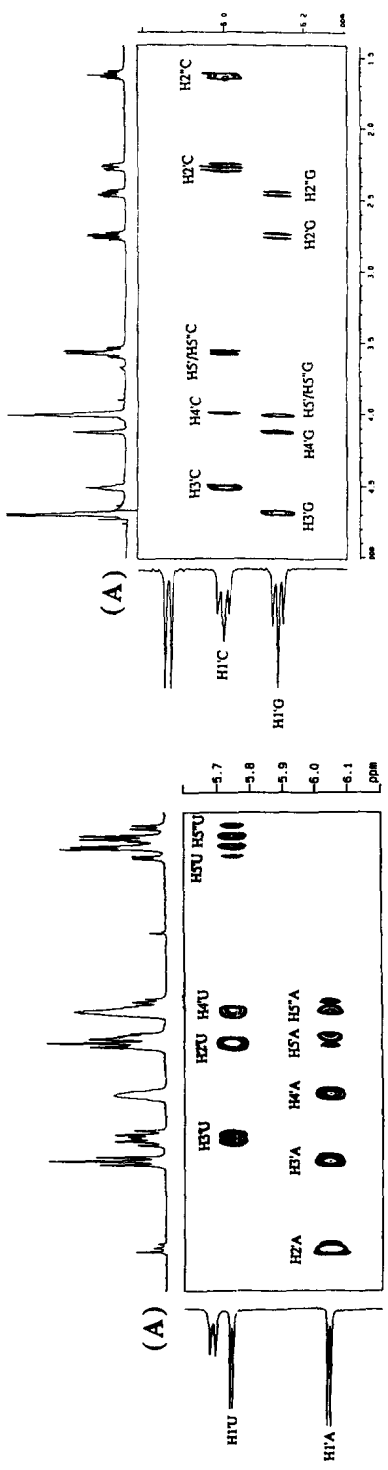


Figure 6: 2D homonuclear Hartmann Hahn (HOHAHA) spectra of the natural and partially deuterated Uridylyl-(3'→5')-adenosine (UpA) in D_2O at 298K. **Panel A** represents the 2D spectrum for the non-deuterated UpA. **Panel B** represents the 2D spectrum of the partially deuterated UpA* where the 1'#, 2', 3', 4'# and 5'/5" protons of the adenosine (pA*) residue are exchanged. In the 1D spectrum, the H1' appears as a singlet at 6.05 ppm while the H4' appears as a doublet at 4.29 ppm due to its coupling to the phosphorus of the 3'→5' phosphate linkage. In the 2D spectrum, the J-network for the 3'-terminal residue (pA*) has vanished.

Figure 7: HOHAHA spectra of the natural and partially deuterated 2'-deoxycytidylyl-(3'→5')-2'-deoxyguanosine [d(CpG)] in D_2O at 298K. **Panel A** represents the 2D spectrum for the non-deuterated d(CpG). **Panel B** represents the 2D spectrum of the partially deuterated d(CpG*) where the 1'#, 2', 3', 4'# and 5'/5" protons of the guanosine (pG*) residue are exchanged. In the 1D spectrum, the H1' appears as a singlet at 6.19 ppm while the H4' appears as a doublet at 4.10 ppm due to its coupling to the phosphorus of the 3'→5' phosphate linkage. In the 2D spectrum, the J-network for the 3'-terminal residue has vanished.

significantly reducing the spectral overlap. This work has shown that it is possible to suppress the proton resonances of some selected sugar residues by specific deuteration in an oligo-DNA or oligo-RNA through the creation of *^{1}H-NMR window*. Such specific deuteration of sugar moieties in a large DNA or RNA molecule should create an ^{1}H-NMR invisible part, and allows the study of structurally functional region(s) in a large RNA or DNA molecule in the ^{1}H-NMR window part. Clearly, the reduced number of protons makes it possible to assign the protons of the residual sugar units in the ^{1}H-NMR window, which, in turn allows the measurement of both the vicinal J-couplings necessary to derive the torsion angles and the nOe volumes for distance measurements. We have substituted the 2', 2", 3' and 5'/5" protons in 2'-deoxyribonucleosides, or the 2', 3' and 5'/5" protons of the sugar residues in ribonucleosides with deuterons (>97 % ^{2}H). Note that the H1' and H4' in these 2'-deoxyribonucleosides and ribonucleosides are also ~20 % and ~85 % deuterated (H1'$^\#$, H4'$^\#$), respectively. In these deuterated nucleosides, the H1'$^\#$ appears as a clear singlet in the 1D ^{1}H-NMR spectra. The H4'$^\#$ however appears as a doublet in the 1D spectrum due to its coupling with the phosphorus of the 5'-phosphate linkage. To show the efficiency of the methods for simplification of the ^{1}H-NMR spectra in the ^{1}H-NMR window, several natural abundance and partially-deuterated di-[ribonucleoside](3'→5')monophosphates (UpA* **73**, CpG* **74**, ApU* **75**, GpC* **76**) and di-[2'-deoxyribonucleoside](3'→5')monophosphates (d(TpA*) **89**, d(CpG*) **90**, d(ApT*) **91**, d(GpC*) **92**), and trimer (A*2'p5'A2'p5'A*) **110** have been synthesized (*vide supra*) and their ^{1}H 1D NMR , 2D HOHAHA and 2D DQF-COSY spectra have been studied[69]. A few representative examples of 2D NMR simplifications are shown in Figures 4E - F, 5 A - E, 6 - 9 and 10 - 12. In 2D HOHAHA experiments, the number of nuclei to which the magnetization is distributed is controlled through the duration of the spin lock period. Large mixing times give rise to relayed and multiple relay peaks. The transfer of magnetization is therefore possible across the total spin system. Since the 2', (2"), 3', 5' and 5" protons are >97 % exchanged with deuterium, the relay between the H1'$^\#$ and H4'$^\#$ is interrupted. In the 2D HOHAHA spectra, no H1'-H4' cross peak is visible and the network for the deuterated sugar is totally absent. In Figures 6 - 8, the HOHAHA spectra of natural and partially deuterated dimers are represented where the H1'$^\#$, H2' (H2"), H3', H4'$^\#$ and H5'/H5" of the 3'-terminal residue are exchanged with deuterium. In the 1D spectrum of these partially-deuterated dimers and trimers, the H1' from the deuterated sugar moiety appears as a singlet while the H4' (H4' of 3' terminal) appears as a doublet due to its coupling with the phosphorus of the phosphate linkage (Figs. 4E,F and 5A-E). In the 2D spectrum, however, the network for the 3'-terminal residue (and 5' terminal residue of trimer) is totally absent making the assignment of the protonated sugar residue easier. In the DQF-COSY experiment, the magnetization is transferred from one proton to the other proton which is coupled to it. A cross-peak is generated between these two coupled spins. Therefore, in the DQF-COSY spectra, despite the fact that the H1'$^\#$ and H4'$^\#$ are not fully deuterated, all the cross peaks originating from the deuterated sugar residue have vanished (Figs. 10 - 12). In Figures 10 - 12 are represented the DQF-COSY spectra of natural and partially deuterated dimers where the H1'$^\#$, H2' (H2"), H3', H4'$^\#$ and H5'/H5" of the 3'-terminal residue are deuterated. It can be seen that all cross peaks originating from the 3'-terminal deuterated sugar residue are absent in the off diagonal region. The crowded H1'$^\#$, H2' (H2"), H3', H4'$^\#$ and H5'/H5" region close to the diagonal in panel A is simplified in panel B and it becomes possible to extract the residual cross peaks and to measure the vicinal J-couplings. An example is shown in Figure 10, Panel B which represents the cross peaks present in the box 2. The box 2 contains four cross peaks namely the H2'-H3' and H3'-H4' cross peaks for the uridine residue and the H4'-H5' and H4'-H5" cross peaks for the adenosine residue. A vertical slice through these pattern is shown in Figure 10, panel C. The overlap of the cross peaks

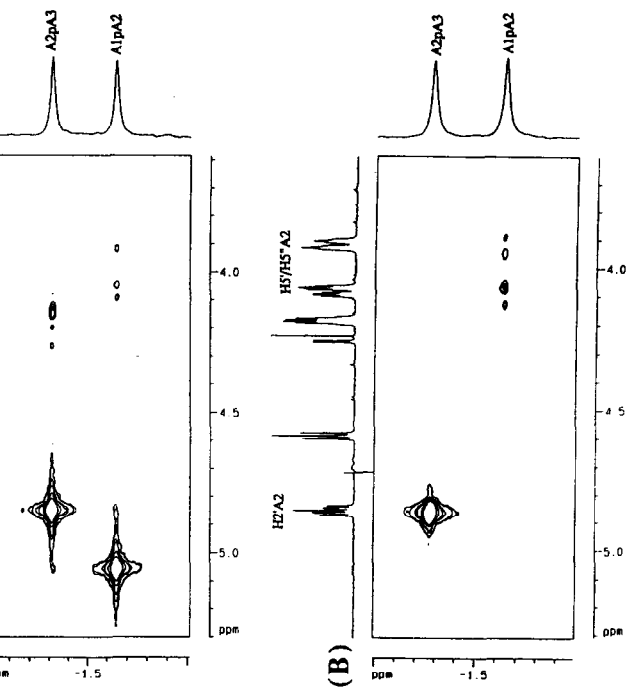


Figure 9: 2D ^{31}P-^1H chemical shift correlation spectra of the natural and partially-deuterated 2,5A core $A^1(2'\rightarrow5')A^2(2'\rightarrow5')A^3$ in D_2O at 308 K. **Panel A** represents the 2D spectrum for the non-deuterated $A^1(2'\rightarrow5')A^2(2'\rightarrow5')A^3$. The $A^1(2'\rightarrow5')A^2$ phosphorus shows a cross peak with the H2'A^1 and H5'/H5"A^2. The $A^2(2'\rightarrow5')A^3$ phosphorus shows a cross peak with the H2'A^2 and H5'/H5"A^3. **Panel B** represents the 2D spectrum of the partially deuterated $A^{1*}(2'\rightarrow5')A^2(2'\rightarrow5')A^{3*}$ where the $1^{\#}$, 2', 3', $4^{\#}$ and 5'/5" protons of the A^{1*} and A^{3*} residues are exchanged. The cross peaks between the $A^{1*}(2'\rightarrow5')A^2$ phosphorus and H2'A^{1*} and between the $A^2(2'\rightarrow5')A^{3*}$ phosphorus and H5'/H5"A^{3*} have vanished.

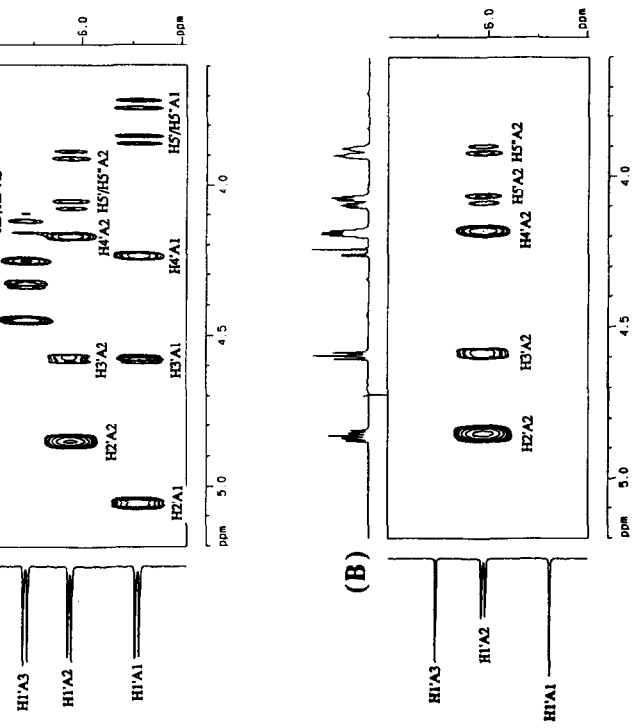


Figure 8: HOHAHA spectra of the natural and partially deuterated 2,5A core $A^1(2'\rightarrow5')A^2(2'\rightarrow5')A^3$ in D_2O at 308 K. **Panel A** represents the 2D spectrum for the non-deuterated $A^1(2'\rightarrow5')A^2(2'\rightarrow5')A^3$. **Panel B** represents the 2D spectrum of the partially deuterated $A^{1*}(2'\rightarrow5')A^2(2'\rightarrow5')A^{3*}$ where the $1^{\#}$, 2', 3', $4^{\#}$ and 5'/5" protons of the A^{1*} and A^{3*} residues are exchanged. In the 1D spectrum, the H1' of A^{1*} and A^{3*} appears as a singlet at 6.1 and 5.9 ppm respectively. The H4' of A^{1*} appears as a singlet at 4.2 ppm while the H4' of A^{3*} appears as a doublet at 4.25 ppm due to its coupling to the phosphorus of the $A^2(3'\rightarrow5')A^3$ phosphate linkage. In the 2D spectrum, the J-network for the 5'-terminal residue A^{1*} and 3'-terminal residue A^{3*} has vanished.

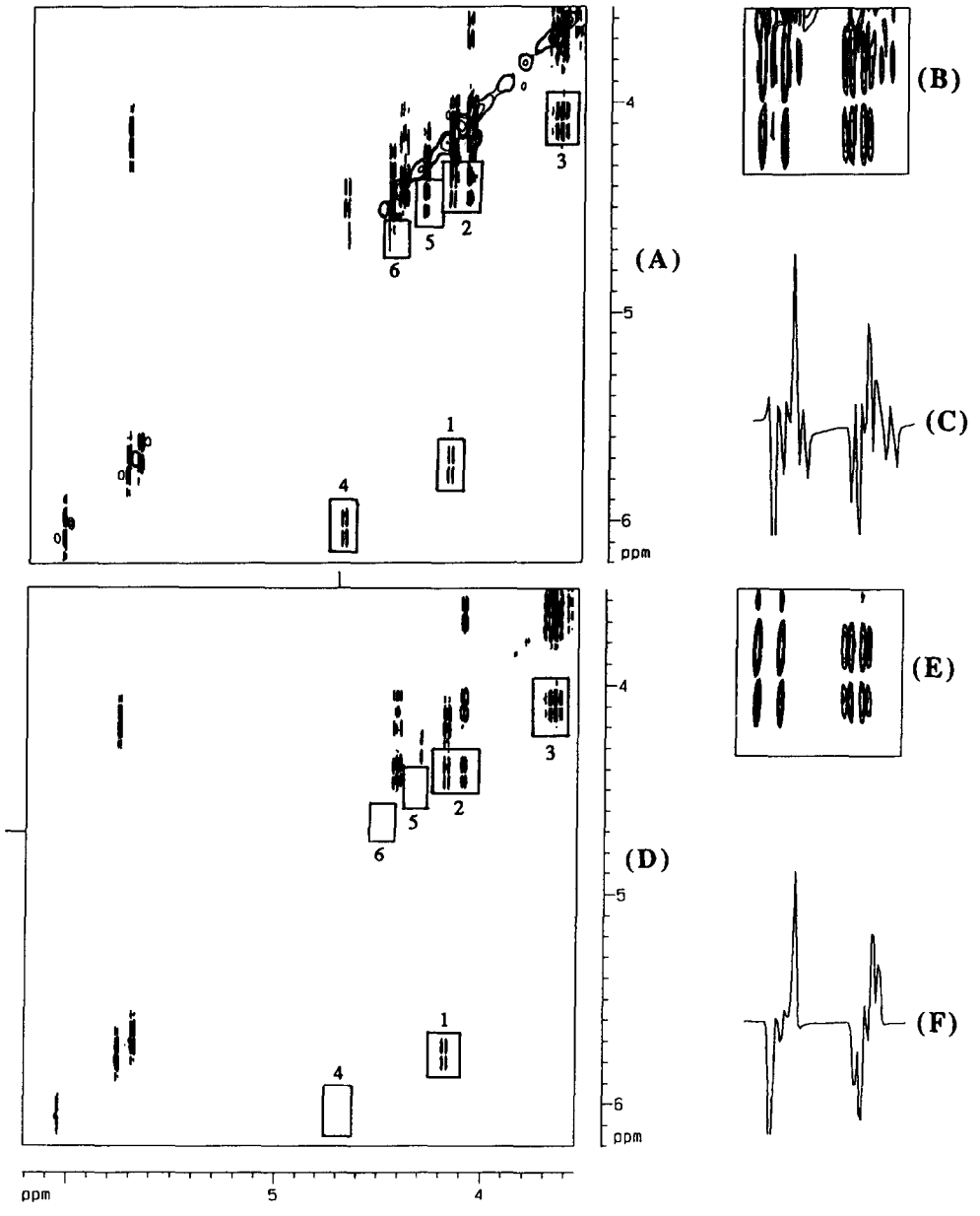


Figure 10: DQF-COSY spectra of the natural and partially deuterated Uridylyl-(3'→5')-adenosine (UpA) in D_2O at 298K. **Panel A**: 2D spectrum of the natural UpA. The cross peaks used for the determination of the vicinal $^3J_{HH}$ coupling constants are shown in the numbered boxes: (1) H1'U-H2'U, (2): H2'U-H3'U, H3'U-H4'U cross peaks, and H4'A-H5'A, H4'A-H5"A, (3) H4'U-H5'U, H4'U-H5"U, (4) H1'A-H2'A, (5) H3'A-H4'A, (6) H2'A-H3'A. **Panel B**; Expansion of the cross peak in box 2. **Panel C**: Vertical slice through the cross peak at the site indicated by an arrow. The determination of the J-couplings is complicated due to the overlap of the H2'U-H3'U with the H4'A-H5'A cross peaks. Similarly, The H3'U-H4'U cross peak overlap with the H4'A-H5"A cross peak. **Panel D**: 2D spectrum of UpA* where the 1'#,2', 3', 4'# and 5'/5" of pA* have been exchanged with deuterium. The empty boxes show that all cross peaks involving the adenosine residue have vanished. **Panel E**: Expansion of the cross peak in box 2. **Panel F**: Vertical slice through the cross peak at the site indicated by an arrow, which now contains only the H2'U-H3'U and H3'U-H4'U allowing an easy extraction of the coupling constants.

makes the measurement of the J-coupling difficult. Panel E and F represent the same region but for the deuterated dimer. Only the H2'-H3' and H3'-H4' cross peaks of the uridine residue are present which makes the determination of the J-couplings easy and straightforward.

Dipolar coupling which operates through space is responsible for the dominating mechanism of relaxation in solution. It appears in the 1D NMR spectrum as line broadening. It also generates the mutual relaxation between spatially close nuclei, the cross relaxation which gives rise to the nOe. Upon random deuteration, each of the remaining protons in the molecule will be surrounded by fewer other protons and will have fewer pathway for cross relaxation. This will result in longer relaxation times and therebye narrower line widths. Upon selective deuteration of the protons of some sugars, the overlap problem can be overcome, but the relaxation time of the remaining protons in other non-deuterated sugar moiety(ies) will not be much affected due to cross relaxation. For example, upon selective deuteration of the H2', H2", H3', H4' and H5'/H5" of the cytidine residue (C*) in the dimer d(GpC*), the relaxation time of the H1' of G is little affected [T_1 = 1.7s in the naturel d(GpC) and T_1 = 1.8s in the partially deuterated d(GpC*)]. On the other hand, the relaxation time T_1 of the H1'# of the C* changes from 2s in the natural dimer to 3.9s in the partially-deuterated d(GpC*). In the natural trimer $A^1$2'p5'$A^2$2'p5'A^3, the T_1 for the H1' are as follows: 2.1s for A^1, 2.0s for A^2 and 2.5s for A^3. In the partially deuterated trimer A*^{1}2'p5'$A^2$2'p5'A*^{3}, where the H2', H3', H4' and H5'/H5" of the A^1 and A^3 residues have been >97% exchanged with deuterium and H1'# and H4'# are respectively ~20 and 80 % deutered, the T_1 for H1'# in A^1 and A^3 are 3s and 4s, respectively, but the T_1 for H1' in A^2 is 2.1s which is basically unaltered from the natural counterpart.

Information on the conformation of the sugar ring can also be obtained from ^{13}C-NMR chemical shifts and coupling constants[63]. The conformation about the C3'-O3' bond (ε) and C5'-O5' bond (β) in nucleic acids can be monitored by carbon-phosphorus coupling constants, $^3J_{C4'P3'}$, $^3J_{C2'P3'}$ for ε and $^3J_{C4'P5'}$ for β. In large oligomers, the assignment of the carbon resonances becomes more complicated as the size of the molecule increases. Also, the measurement of the carbon-phosphorus coupling constants from the $^{13}C\{^1H\}$ spectrum is difficult due to the *overlap* of the carbon resonances. In the $^{13}C\{^1H\}$ spectrum with nOe, the carbon bonded to a deuterium atom should have a much lower intensity than a carbon bonded to a proton because of the fact that nOe effect does not arise through deuterium, and the intensity is also considerably decreased because of the splitting due to the ^1J coupling with deuterium. The T_1 relaxation time for the carbons of four natural and partially deuterated 2'-deoxynucleosides dA, dG, dC and T have been measured using inversion recovery experiments. The carbons bonded to a deuterium atom C2', C3' and C5' have longer relaxation time than the carbons bonded to hydrogen. (In natural 2'-deoxyadenosine, the T_1 of the sugar carbons are: C1' = 0.65s, C2' = 0.39s, C3' = 0.71s, C4' = 0.65s and C5' = 0.33s. In *deuterated 2'-deoxyadenosine*, the T_1 of the sugar carbons are: C1' = 0.6s, C2' = 1.76s, C3' = 1.5s, C4' = 0.6s and C5' = 2.1s. In natural 2'-deoxycytidine, the T_1 of the sugar carbons are: C1' = 0.65 s, C2' = 0.41s, C3' = 0.68s, C4' = 0.62s and C5' = 0.35s. In *deuterated 2'-deoxycytidine*, the T_1 of the sugar carbons are: C1' = 0.61s, C2' = 1.1s, C3' = 1.2s, C4' = 0.6s and C5' = 2.5s. In natural thymidine, the T_1 of the sugar carbons are: C1' = 0.7 s, C2' = 0.41s, C3' = 0.71s, C4' = 0.65s and C5' = 0.36s. In *deuterated thymidine*, the T_1 of the sugar carbons are: C1' = 0.7s, C2' = 1.82s, C3' = 1s, C4' = 0.66s and C5' = 1.58s. In natural 2'-deoxyguanosine, the T_1 of the sugar carbons are: C1' = 0.61s, C2' = 0.4s, C3' = 0.62s, C4' = 0.62s and C5' = 0.31s. In *deuterated 2'-deoxguanosine*, the T_1 of the sugar carbons are: C1' = 0.6s, C2' = 1.3s, C3' = 1.1s, C4' = 0.6s and C5' = 1.6s.) Consequently, it should be possible to

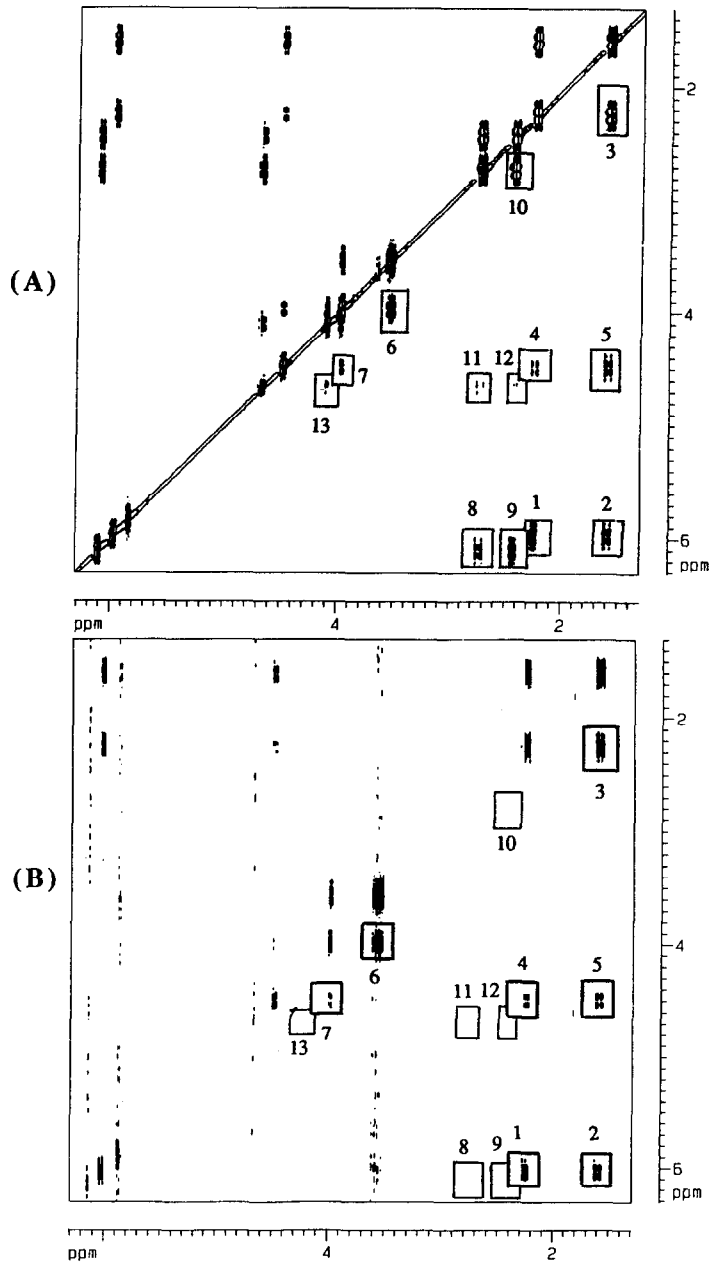


Figure 11: DQF-COSY spectra of natural and partially deuterated 2'-deoxycytidylyl-(3'→5')-2'-deoxyguanosine [d(CpG)] in D_2O at 298K. **Panel A**: 2D spectrum of the natural d(CpG). The cross peaks used for the determination of the vicinal $^3J_{HH}$ coupling constants are shown in the numbered boxes: (1) H1'-H2'C, (2) H1'-H2"C, (3) H2'-H2"C, (4) H2'-H3'C, (5) H2"-H3'C, (6) H4'-H5', H4'-H5"C, (7) H3'-H4'C, (8) H1'-H2'G, (9) H1'-H2"G, (10) H2'-H2"G, (11) H2'-H3'G, (12) H2"-H3'G, (13) H3'-H4'G. **Panel B**: 2D spectrum of d(CpG*) where the $1'^{\#}$, 2', 2", 3', $4'^{\#}$ and 5'/5" of G* have been exchanged with deuterium. The empty boxes show that all cross peaks involving the pG* residue have vanished.

invert or suppress particular carbon resonances selectively by the appropriate choice of the relaxation delay, and thereby to distinguish between the carbons which are bonded to a hydrogen or to a deuterium.

A straightforward and convenient means to observe the carbon chemical shifts and to measure the carbon-phosphorus coupling constants in oligo-DNA or oligo-RNA is to use a $^{13}C\{^1H\}$ INEPT experiment[64,65,66] where the proton spin polarization is transferred to carbon. This experiment acts as a filter eliminating effectively the resonances of the deuterated carbons. ^{13}C-NMR INEPT experiments, with and without proton decoupling, have been performed on natural and partially deuterated 2'-deoxynucleotides, dA, dG, dC and T. In the INEPT spectra of deuterated nucleosides (dA*, dG*, dC* and T* where the 2', 2", 3', 5', 5" are exchanged with >97% deuterium, and 1'# and 4'# are respectively ~20 and 80 % deuterated), the transfer of polarization from ^1H to ^{13}C arises only for the C1' and C4' carbons. The resonances of the carbons bonded to deuterium, C2', C3' and C5' are effectively eliminated (Figures 13 and 14). One should also try to get the maximum possible information from the deuterated part of the molecule. The sensitivity of the carbon bearing deuterium can be selectively enhanced by using a $^{13}C\{^2H\}$ INEPT experiment where the ^2H spin polarization is selectively transferred to ^{13}C nucleus[67,68]. The sensitivity of this experiment is comparable to the normal proton decoupled ^{13}C experiment with nOe. The quadrupolar ^2H nucleus relaxes faster than the ^1H nucleus and since the rate of repetition of the INEPT is determined by the relaxation T_1 of ^2H, faster repetition rates can be used. Hence, depending upon whether the spin polarization to carbon nucleus is transfered through *proton* [$^{13}C\{^1H\}$ INEPT experiment] or *deuterium* [$^{13}C\{^2H\}$ INEPT experiment] in *partially deuterated* DNA or RNA (such as **77-80**, **93-96** or **110**), one can visualize *either* ^1H-bonded carbon or ^2H-bonded carbon *selectively* thus creating effective *^{13}C-NMR window*.

Experimental Section

Materials and Methods. Nickel-aluminium alloy powder (50/50%), 2,2'-azobis(2-methyl-propionitrile) (AIBN), ammonia solution 32%, chlorotrimethylsilane, acetic anhydride, Amberlyst A-21 ion exchanger, 1,1,3,3-tetramethylguanidine, 1-methylimidazole and toluoyl chloride were purchased from Merck. D-Ribose, 4-methoxytriphenylmethyl chloride, diphenylcarbamyl chloride, benzoyl chloride, lithium aluminium deuteride (98 atom % ^2H), tri-n-butyltin chloride, 1,1,1,3,3,3-hexamethyldisilazane and phenyl chlorothionoformate were purchased from Aldrich. ^2H_2O (99.9 atom % D) for the deuteration experiments was purchased from Goss Sci. Inst. Ltd. Sodium hydroxide was purchased from Eka Nobel AB, Sweden, ammonium hydrogen carbonate from BDH. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane ($TPDSCl_2$)[40], *o*-chlorophenylphosphoro-bis-(1,2,4-triazolide)[44b], *syn*-4-nitrobenzaldoxime[45] and 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MS-NT)[45] were prepared using literature procedures. Pyridine and toluene were distilled after being refluxed over calcium hydride for 3 - 4 h, 1,2-dichloroethane and dichloromethane was stirred with phosphorus pentoxide overnight followed by distillation under nitrogen. TLC was carried out using Merck pre-coated silica gel 60 F_{254} plates in the following solvent systems: (A) methanol-dichloromethane (1:99, v/v), (B) methanol-dichloromethane (5:95, v/v), (C) methanol-dichloromethane (10:90, v/v), (D) acetonitrile-water (70:30, v/v). The short column chromatographic separations were done using Merck G60 silica gel. DEAE-Sephadex A-25 (Pharmacia) was used for the anion excange chromatography. After purification on DEAE-Sephadex column, the ammonium counterions in dimers and trimers were replaced with Na^+ by passing the compounds through a Dowex 50 WX 8 (C. Roth Gmbh) (Na^+ form) column, then they were repeatedly freeze-dried from ^2H_2O. ^1H-NMR spectra were recorded with a Jeol FX 90 Q and Bruker AMX 500 spectrometer at 90 and 500 MHz, respectively, using TMS (0.0 ppm) or acetonitrile peak for ^2H_2O solutions (set at $\delta = 2.0$ ppm) as the internal standards. ^{13}C-NMR spectra were taken with a Jeol FX 90 Q spectrometer at 27.7 MHz with TMS as internal reference for solutions other than ^2H_2O in case of which CH_3CN (set at $\delta = 1.3$ ppm) was used as internal reference. ^{31}P-NMR spectra were recorded at 36 MHz and 202 MHz in the same solvent as for ^1H-NMR spectra using 85 % phosphoric acid (0.0 ppm) or cAMP (-2.1 ppm) as external standard. Chemical shifts are reported in ppm (δ scale). The two-dimensional NMR experiments were performed on a Bruker AMX-500 MHz spectrometer. The DQF-COSY and Hartmann-Hahn spectra were recorded in pure-phase absorption mode with the time proportional incrementation

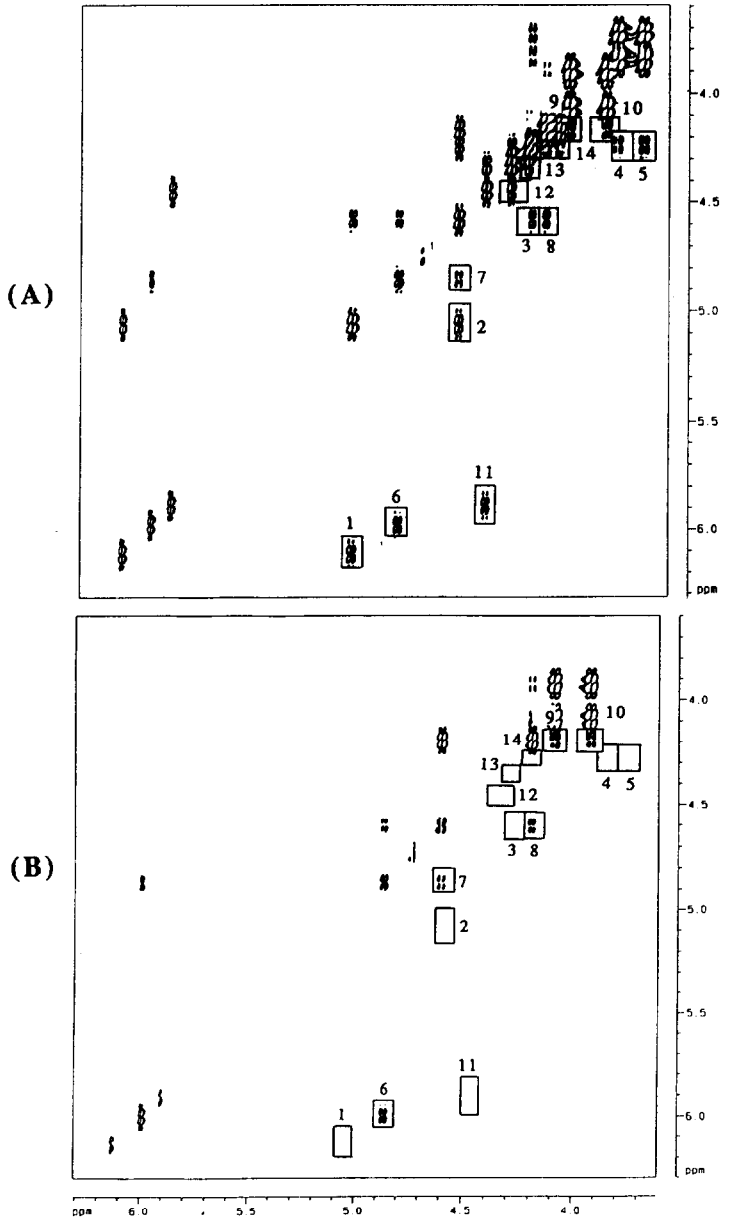


Figure 12: DQF-COSY spectra of natural and partially deuterated 2,5A core $A^1(2'\rightarrow5')A^2(2'\rightarrow5')A^3$ in D_2O at 308 K. **Panel A** represents the 2D spectrum for the non-deuterated $A^1(2'\rightarrow5')A^2(2'\rightarrow5')A^3$. The cross peaks used for the determination of the vicinal $^3J_{HH}$ coupling constants are shown in the numbered boxes: (1) H1'-H2'A^1, (2) H2'-H3'A^1, (3) H3'-H4'A^1, (4) H4'-H5'A^1, (5) H4'-H5"A^1, (6) H1'-H2'A^2, (7) H2'-H3'A^2, (8) H3'-H4'A^2, (9) H4'-H5'A^2, (10) H4'-H5"A^2, (11) H1'-H2'A^3, (12) H2'-H3'A^3, (13) H3'-H4'A^3, (14) H4'-H5', H4'-H5"A^3. **Panel B** represents the 2D spectrum of the partially deuterated $A^{1*}(2'\rightarrow5')A^2(2'\rightarrow5')A^{3*}$ where the 1'#, 2', 3', 4'# and 5'/5" of A^{1*} and A^{3*} have been exchanged with deuterium. The empty boxes show that all cross peaks involving the A^{1*} and A^{3*} residues have vanished.

method (TPPI) and with low power preirradiation of the residual HDO peak during the relaxation delay. The DQF-COSY[52] spectra were acquired with 4096 complex data points in t_2 and 256 points in t_1. The data were zero filled to give a 4096 x 1024 point matrix and a sine-square bell window was applied in both directions before Fourier transformation. The Hartmann-Hahn[53] spectra were acquired with 2048 complex data points in t_2 and 256 points in t_1. The data were zero filled to give a 2048 x 1024 point matrix and a sine-square bell window was applied in both directions before Fourier transformation. The ^1H-^{31}P chemical shift correlation experiment[54] was performed in the absolute magnitude mode. A 1024 x 128 matrix data set was zero filled to 1024 x 512 data points a sine-square bell multiplication was applied in both directions before Fourier transformation. The ^{13}C NMR INEPT experiments with and without proton decoupling and the ^{13}C NMR inversion recovery experiments were performed on a Jeol GX 270 MHz spectrometer operating at 67.8 MHz for carbon. IR absorption spectra were recorded with a Perkin-Elmer 298 spectrometer. *The chemical shifts of all deuterated compounds have been compared to the ^1H- and ^{13}C-NMR spectra of corresponding natural-abundance counterpart in order to delineate (i) site and level of deuteration, and (ii) isomeric purity.*

Tri-n-butyltin deuteride was prepared *via* a modified method[41]. Lithium aluminium deuteride (1 g, 23.8 mmol) was suspended in dry diethyl ether (75 ml) and freshly distilled tri-n-butyltin chloride (16 ml) was added dropwise (30 min) at RT under argon. After an additional stirring for 3 h, the mixture was cooled in an ice-bath and water (~50 ml) was slowly added. The etheral phase was washed with water (2 x 50 ml), dried over $MgSO_4$ and evaporated. Residue was subjected to vacuum distillation and fractions collected up to 75 °C were used in deuteration reactions. ^{13}C-NMR (neat): 30.2 C-β (taken as reference[50]); 27.38 C-γ; 13.79 C-δ; 8.15 C-α. IR (neat): $\nu_{Sn\text{-}D}$= 1298 cm^{-1} (litr.[51]: $\nu_{Sn\text{-}D}$= 1300 cm^{-1}).

Methyl α/β-D-ribofuranoside (1). A solution of D-ribose (8 g, 53.3 mol) in dry methanol (120 ml) was treated at 0 °C with concentrated sulfuric acid (0.5 ml), dissolved in 3-4 ml ice-cold dry methanol and added in a portionwise manner, then stored in refrigerator at ~4 °C for 24 h. The solution was neutralized by passage through a bed of Amberlyst A-21 (OH^- form) (300 g) resin pre-washed with distilled methanol. After eluting the column with 1 L distilled methanol, eluant was evaporated and residue coevaporated with deionized water followed by drying on oilpump to give the mixture of title compound **1** [α/β = ~3:10] as a syrup (8.57 g, 98%). ^1H-NMR (D_2O): 4.92 (d, $J_{1,2}$ = 3.2 Hz, 1H) H-1 (α); 4.83 (s, 1H) H-1 (β); 4.04 - 3.28 (m, 5H) H-2, H-3, H-4 & H-5 (α+β); 3.36 (s, 3H) OMe (α); 3.33 (s, 3H) OMe (β). ^{13}C-NMR (α): 103.5 C1; 84.9 C4; 71.5 C2; 70.0 C3; 61.9 C5; 55.8 OCH_3; (β) 108.3 C1; 83.2 C4; 74.6 C2; 71.2 C3; 63.2 C5; 55.5 OCH_3.

Preparation of the Raney-nickel catalyst (W-5). A one-liter Erlenmeyer flask containing a five-centimeter long teflon-coated magnet, was placed in a plastic beaker on top of a magnetic stirrer. A thermometer was fixed along the inner wall of the flask. The flask was filled with deionized-water (192 ml) and when slowly stirring, NaOH-pellets (51.2 g) were added in one portion. After all pellets had dissolved, some ice-water was filled into the plastic beaker letting the temperature decrease to 50 °C. Addition Ni-Al-alloy was started *keeping the temperature at* 50 ±4 °C. The total amount of Ni-Al-alloy (40.0 g) should be added within approximately 30 min starting with small portions and ending with up to 1 ml per portion.The temperature is adjusted by the cooling-rate and by the addition speed. After completion of the addition, the flask was heated in an oil-bath at 50 °C for approximately 60 min. The flask was left for 1 h to cool down to room temperature. Deionized-water (3x 1L) was added and decanted. The particles were transferred to a 250 ml filtering flask with hose connection. A 20-cm long PVC-tube was connected on the hose of the flask. The flask was placed on a magnetic stirrer and the tube ended freely over a one liter beaker (for safety if to many particles are washed out). While stirring slowly, water (totally 4 L) was added slowly,while liquid containing very small particles went into the beaker, and the flask could be left overnight, for continued washing the next day. Washing was continued for a whole day (using totally 20 L of water, during the last 10 L, a funnel was put into the filtering flask). In the beginning stirring was used between the washings while in the end stirring was used continously. After the washing *the pH was checked in the following manner:* most of the liquid was decanted from the flask, the particles were standing in a small volume of water for 10 min, then the pH of the remaining water in the flask was measured. If the pH was 6.5 - 7.0 and the liquid was almost clear, then the deuteration of the catalyst could be started.

Preparation of the deuterated Raney-nickel catalyst. The catalyst particles were transferred to a 50 ml serum bottle containing a two-centimeter long teflon-coated magnet. The bottle was sealed using a rubber stopper and the suspension was stirred for one minute, then after settling of the particles, water was removed with a pasteurpipette. After repeating this procedure a few times, deuteriumoxide (approximately 1.5 ml) was added, the bottle was flushed with nitrogen whereafter it was stoppered and sealed with parafilm. Stirring was maintained for 30 min ("1st wash"). The liquid was removed as before, ^2H_2O was added (same amount), the bottle was flushed again with nitrogen, sealed with parafilm and stirred for half an hour. (At this stage the bottle could be left without stirring overnight,"2nd wash"). This washing procedure was repeated a few times. From

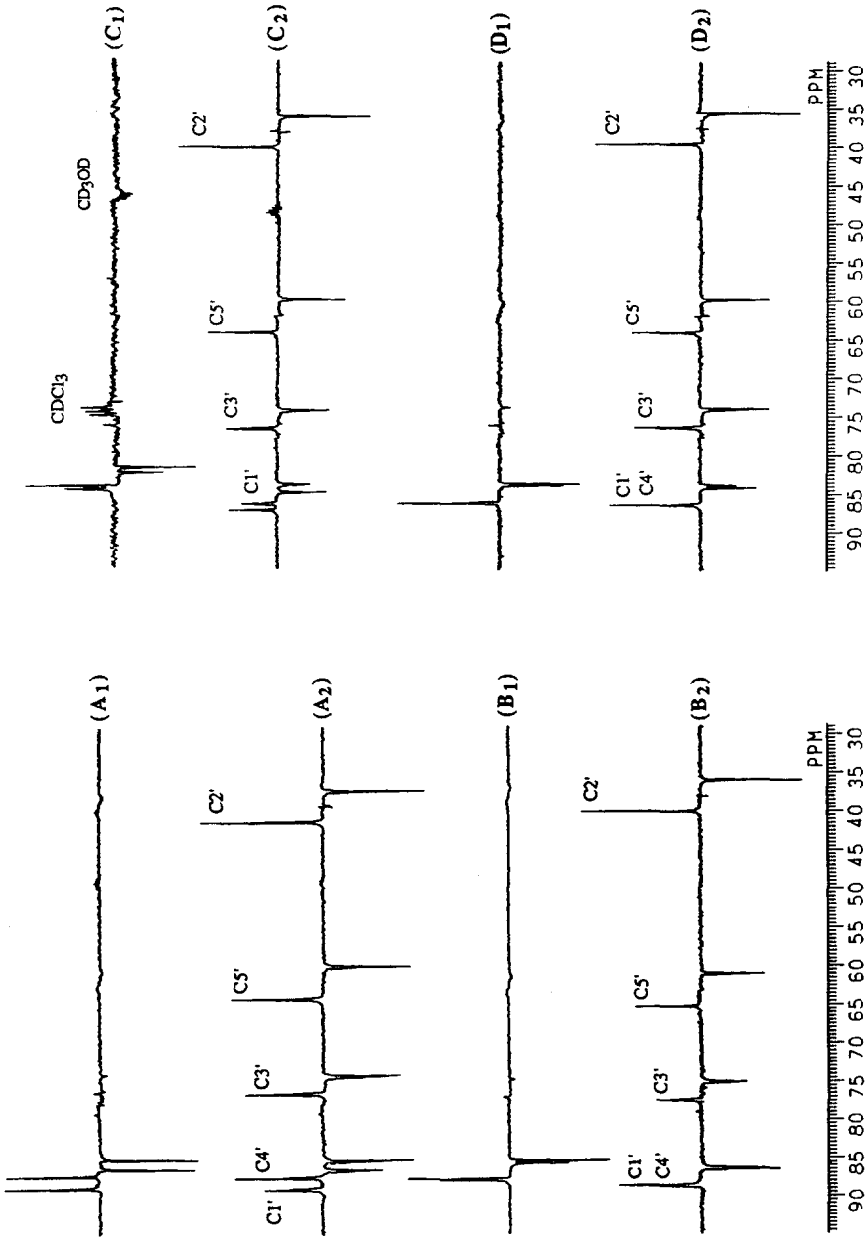


Figure 13: ^{13}C INEPT NMR spectra of natural and partially deuterated deoxynucleosides at 298K in $CDCl_3$. (A_2): Natural 2'-deoxycytidine [dC], (A_1): Deuterated 2'-deoxycytine [dC*], (B_2): Natural 2'-deoxyadenosine [dA], (B_1): Deuterated 2'-deoxyadenosine [dA*], (C_2): Natural 2'-deoxyguanosine [dG], (C_1): Deuterated 2'-deoxyguanosine [dG*], (D_2): Natural thymidine (T), (D_1): Deuterated thymidine [T*]. In dC*, dA*, dG* and T*, the 2', 2", 3', 5' and 5" protons have been exchanged with deuterium (>97% ^2H). The H1' is ~ 20% deuterated while the H4' is ~ 85% deuterated. In dC*, dA*, dG* and T*, the transfer of polarization from ^1H to ^{13}C arises only for the C1' and C4' carbons. The resonances of the carbons covalently bonded to deuterium, C2', C3' and C5', are effectively eliminated.

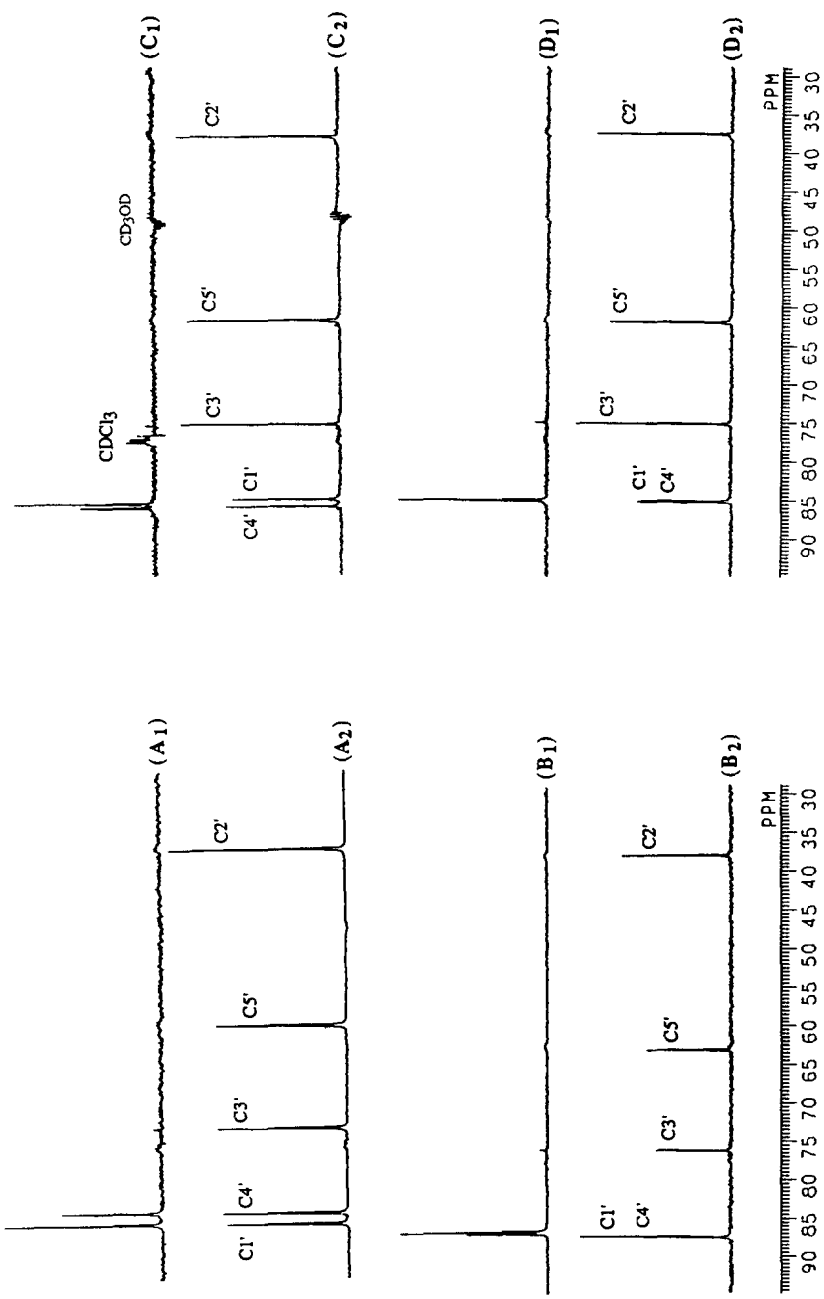


Figure 14: ^{13}C {^{1}H} INEPT NMR experiments of natural and partially deuterated deoxynucleosides at 298K in $CDCl_3$. (A2): Natural 2'-deoxycytidine [dC], (A1): Deuterated 2'-deoxycytine [dC*], (B2): Natural 2'-deoxyadenosine [dA], (B1): Deuterated 2'-deoxyadenosine [dA*], (C2): Natural 2'-deoxyguanosine [dG], (C1): Deuterated 2'-deoxyguanosine [dG*], (D2): Natural thymidine [T], (D1): Deuterated thymidine (T*). In dC*, dA*, dG* and T*, the 2', 2", 3', 5' and 5" protons have been exchanged with deuterium (>97% ^{2}H). The H1' is ~ 20% deuterated while the H4' is ~ 85% deuterated. In dC*, dA*, dG* and T*, the transfer of polarization from ^{1}H to ^{13}C arises only for the C1' and C4' carbons. The resonances of the carbons covalently bonded to deuterium, C2', C3' and C5', are effectively eliminated.

the 4th wash to the 16th wash a twofold amount of ^2H_2O was used with stirring for at least one hour if at daytime, or add a threefold amount without stirring if overnight. Usually 3-4 overnight washes were used. Gas evolved during the washes and during the last washes a strong white color appeared. After the 16th wash the catalyst was ready for use (total ^2H_2O used ~50 ml).

Methyl α/β-D-ribofuranoside-1#,2,3,4#,5,5'-$^2H_6$ (2) (# denotes partial deuteration). To a mixture of methyl α/β-ribofuranoside **1** (1.4 g, 8.5 mmol) coevaporated two times with deuterium oxide (99.8 % atom D, ~ 6 ml each) and dissolved in the same solvent (28 ml), deuterated Raney-Ni (settled volume: 14.5 ml) was added using a graduated pipette and after bubbling nitrogen through the mixture for 10 min, it was boiled to reflux with stirring, at 110 °C oil bath, for 7 days in an atmosphere of nitrogen. The mixture was filtered through a bed of celite, washed with water, and the combined aqueous phases were evaporated to give a syrup (0.84 g, 82%). ^{1}H-NMR (D_2O) of main components **2**. ^{1}H-NMR (D_2O) (α): 4.92 (s, 1H) H-1; 4.02 (s, 0.3H) H-4; 3.37 (s, 3H) OCH_3; (β) 4.83 (s, 1H) H-1; 3.93 (s, 0.7H) H-4; 3.33 (s, 3H) OCH_3. ^{13}C -NMR (D_2O) (α): 103.5 C1; 84.9 C4; 55.8 OCH_3; (β): 108.3 C1; 83.1 C4; 55.52 OCH_3.

1-O-Methyl-2,3,5-tri-O-acetyl-α/β-D-ribofuranoside-1#,2,3,4#,5,5'-$^2H_6$ (3). To a solution of methyl α/β-D-ribofuranoside-1#,2,3,4#,5',5"-$^2H_6$ (2) (6.3 g, 37.2 mmol) in dry pyridine (62.5 ml), acetic anhydride (25 ml) was added with cooling in an ice-bath and stirring, the stirring was continued for 2 days at room temperature. Usual work-up and drying on oil pump gave a thick syrup (9 g, 82%). ^{1}H-NMR ($CDCl_3$) of main components **3**: (β): 4.87 (s, 1H) H-1; 4.09 (s) H-4#; 3.40 (s, 3H) OCH_3; (α): 5.10 (s, 1H) H-1; 4.29 (s) H-4#; 3.37 (s, 3H) OCH_3; 2.11 - 2.06 (3xs, 9H) 3xOAc (α+β).

1-O-Methyl-2,3,5-tri-O-(4-toluoyl)-α/β-D-ribofuranoside-1#,2,3,4#,5,5'-$^2H_6$ (4). Methyl α/β-D-ribofuranoside-1#,2,3,4#,5',5"-$^2H_6$ (2) (1.49 g, 8.8 mmol) was coevaporated with dry pyridine 5 times to give a dry thick syrup which subsequently was dissolved in dry pyridine (44 ml). This solution was cooled to 0 °C in an ice-bath, then toluoyl chloride (7 ml, 53 mmol) was added in one portion with stirring. Stirring was maintained for 20 min at bath temperature, then at room temperature overnight, when Tlc showed complete consumption of starting sugar. After addition of methanol and additional stirring for 5 min, usual work-up resulted in an oily residue which was subjected to short column chromatography. First column (120 g silica) was packed, loaded and washed with 75 % petroleum ether in CH_2Cl_2 until complete removal of reagent residue then the polarity was increased. Mixed fractions were re-chromatographed starting with 20 % petroleum ether in dichloromethane followed by sequential increase of polarity (a series of similar colums was necessary on mixed fractions). After four columns, sufficiently pure compound **4** was obtained as a thick syrup (2.19 g, 48%). R_f: 0.50 (System A). ^{1}H-NMR ($CDCl_3$): 8.0 - 7.1 (m, 9H) toluoyl (α+β); (β): 5.13 (s, 1H) H-1; 4.70 (s) H-4#; 3.40 (s, 3H) OCH_3; (α): 5.37 (s, 1H) H-1; 4.61 (s) H-4#; 3.47 (s, 3H) OCH_3; 2.39 & 2.36 (2xs, 9H) 3x toluoyl-CH_3 (α+β).

1,2,3,5-Tetra-O-acetyl-α/β-D-ribofuranoside-1#,2,3,4#,5,5'-$^2H_6$ (5). 1-O-Methyl-2,3,5-tri-O-acetyl-α/β-D-ribofuranoside-2,3,4,5,5'-$^2H_4$ (3) (9 g, 30.5 mmol) was dissolved in acetic acid (75 ml) and acetic anhydride (17.5 ml) and treated with sulfuric acid (3.75 ml) with ice-cooling. After 12 h at room temperature the solution was stirred with ice-water, extracted with dichloromethane, washed with water, then aqueous sodium hydrogen carbonate, dried over $MgSO_4$ and evaporated to give compound **5** as pale yellow syrup (9.6 g, 97 %). ^{1}H-NMR of main components ($CDCl_3$): (β): 6.16 (s, 1H) H-1; 4.36 (s) H-4#; (α): 6.42 (s, 1H) H-1; 4.44 (s) H-4#; 2.2 -2.1 (4xs, 12H) 4xOAc (α+β).

1-O-Acetyl-2,3,5-tri-O-(4-toluoyl)-α/β-D-ribofuranoside-1#,2,3,4#,5,5'-$^2H_6$ (6). 1-O-Methyl-2,3,5-tri-O-toluoyl-α/β-D-ribofuranoside-2,3,4#,5,5'-$^2H_4$ (4) (1.57 g, 3 mmol) was dissolved in acetic acid (4.3 ml) and acetic anhydride (1.0 ml) and treated with sulfuric acid (0.23 ml) with ice-cooling. Resulting mixture was stirred overnigt at room temperature, then ice-water was added followed by usual work-up. Short column chromatography as described for compound **4** gave **6** as a white foam (1.36 g, 82 %). R_f: 0.47 (System A). ^{1}H-NMR ($CDCl_3$): 8.1 - 7.1 (m, 9H) toluoyl (α+β); (β): 6.41 (s, 1H) H-1; 4.75 (s) H-4#; 2.01 (s, 3H) acetyl; (α) 6.68 (s, 1H) H-1; 2.13 (s, 3H) acetyl; 2.41 & 2.37 (2xs, 9H) 3x toluoyl-CH_3 (α+β).

2',3',5'-Tri-O-(4-toluoyl)-1'#,2',3',4'#,5',5"-$^2H_6$-uridine (7). Uracil (73 mg, 0.65 mmol) was coevaporated with dry toluene (2x) then suspended in 1,1,1,3,3,3-hexamethyldisilazane (3.9 ml) followed by addition of chlorotrimethylsilane (0.39 ml) and the heterogeneous mixture was heated at reflux at 120 °C under an atmosphere of nitrogen for 60 min. The clear solution resulted was evaporated, then coevaporated with dry

toluene (2x) to remove traces of hexamethyldisilazane. 1-O-Acetyl-2,3,5-tri-O-toluoyl-α/β-D-ribofuranoside-1#,2,3,4#,5,5'-$^2H_6$ **6** (276 mg, 0.5 mmol) was coevaporated three times with dry toluene, dissolved in 1,2-dichloroethane (6.5 ml) and added to the oily persilylated uracil followed by addition of trimethylsilyl trifluoromethanesulfonate (0.125 ml). The mixture was heated at 70 °C in nitrogen atmosphere for 4 h. Usual work-up and short column chromatography yielded compound **7** (0.23 g, 75%). R_f: 0.45 (System B). ^{1}H-NMR ($CDCl_3$): 8.81 (br. s, 1H) N-H ; 8.0 -7.1 (m, 12H) toluoyl; 7.42 (d, $J_{5,6}$ = 8.1 Hz, 1H) H-6; 6.35 (s, 1H) H-1'#; 5.59 (dd, $J_{NH,5}$ = 2.2 Hz, 1H) H-5; 4.68 (s) H-4'#; 2.42, 2,41 & 2.38 (3xs, 9H) 3x toluoyl-CH_3.

2',3',5'-Tri-O-(4-toluoyl)-1'#,2',3',4'#,5',5"-$^2H_6$-N^4-benzoylcytidine (8). N^4-Benzoylcytosine (140 mg, 0.65 mmol) was condensed with sugar **6** (276 mg, 0.5 mmol) as described for compound **7** (0.42 g, 85%). R_f: 0.56 (System B). ^{1}H-NMR ($CDCl_3$): 8.71 (br. s, 1H) N-H ; 8.1 -7.1 (m, 19H) toluoyl, benzoyl, H-5 & H-6; 6.50 (s, 1H) H-1'#; 4.75 (s) H-4'#; 2.44 & 2.20 (2xs, 9H) 3x toluoyl-CH_3.

2',3',5'-Tri-O-(4-toluoyl)-1'#,2',3',4'#,5',5"-$^2H_6$-N^6-benzoyladenosine (9). N^6-Benzoyladenine (650 mg, 2.71 mmol) and deuterated sugar **6** (1.36 g, 2.47 mmol) were condensed as described for compound **7** (1.2 g, 60%). R_f: 0.53 (System B). ^{1}H-NMR ($CDCl_3$): 8.98 (br. s, 1H) N-H ; 8.72 (s. 1H) H-8; 8.17 (s, 1H) H-2; 8.1 -7.1 (m, 17H) toluoyl, benzoyl; 6.50 (s, 1H) H-1'#; 4.82 (s) H-4'#; 2.42 & 2.38 (2xs, 9H) 3x toluoyl-CH_3.

2',3',5'-Tri-O-(4-toluoyl)-1'#,2',3',4'#,5',5"-$^2H_6$-N^2-acetyl-O^6-diphenylcarbamoyl guanosine (10). N^2-acetyl-O^6-diphenylcarbamoylguanine (253 mg, 0.65 mmol) and deuterated sugar derivative **6** (276 mg, 0.5 mmol) were condensed by the method described by Robins[37] (0.32 g, 73%). R_f: 0.65 (System B). ^{1}H-NMR ($CDCl_3$): 8.10 (br. s, 1H) N-H ; 8.05 (s. 1H) H-8; 8.0 -7.1 (m, 22H) toluoyl, phenyls; 6.33 (s, 1H) H-1'#; 4.82 (s) H-4'#; 2.48 (s, 3H) acetyl; 2.42 & 2.38 (2xs, 9H) 3x toluoyl-CH_3.

1-(2',3',5'-Tri-O-(4-toluoyl)-1'#,2',3',4'#,5',5"-$^2H_6$-β-D-ribofuranosyl)-thymine (11). Thymine (82 mg, 0.65 mmol) was condensed with deuterated sugar derivative **6** (276 mg, 0.5 mmol) as described for compound **7** (0.28 g, 91%). R_f: 0.48 (System B). ^{1}H-NMR ($CDCl_3$): 8.35 (br.s, 1H) N-H; 8.05 (s, 1H) H-8; 7.37 - 7.30 (m, 10H) Ar.; 6.09 (s, 1H) H-1'#; 4.45 (s) H-4'#; 2.46 (s, 3H) Ac-N^2; 2.15 - 1.88 (3xs, 9H) 3xOAc.

1'#,2',3',4'#,5',5"-$^2H_6$-Uridine (12). Compound **7** (264 mg, 0.43 mmol) was treated as written for compound **14**, but partition between water and dichloromethane followed by dichloromethane wash of water (3x) phase was used for purification. Evaporation of water phase gave compound **12** (87 mg, 99%). ^{1}H-NMR (D_2O): 7.82 (d, $J_{5,6}$= 8.0 Hz, 1H) H-6; 5.84 (s, 1H) H-1'#; 5.83 (d, 1H) H-5; 4.05 (s) H-4'#.

1'#,2',3',4'#,5',5"-$^2H_6$-N^4-Benzoylcytidine (13). Compound **8** (0.77 g, 1.1 mmol) was treated as written for compound **14** to give crude compound **13** with minor impurities (0.375 g, 98%). ^{1}H-NMR ($CDCl_3$/methanol-d_4/DMSO-d_6): 8.53 (d, $J_{5,6}$ = 7.7 Hz, 1H) H-6; 8.1 - 7.4 (m, 5H) benzoyl; 7.44 (d, $J_{5,6}$ = 7.7 Hz, 1H) H-5; 5.88 (s, 1H) H-1'#; 4.03 (s) H-4'#.

1'#,2',3',4'#,5',5"-$^2H_6$-N^6-Benzoyladenosine (14). Compound **9** (1.17 g, 1.6 mmol) was dissolved in a mixture of pyridine (3.2 ml, 2.0 ml/mmol) and ethanol (4.8 ml, 3.0 ml/mmol), and ethanolic sodium hydroxide solution (prepared from 2 N aqueous sodium hydroxide (6.4 ml) and 6.4 ml ethanol) was added. After stirring for 5 min at room temperature, the solution was neutralized by addition of Dowex ion exchange resin (H^+ form). Resin was filtered off, then washed with ethanol (200 ml), the combined filtrate and washings were evaporated. Repeated coevaporation with toluene gave a powder which was triturated with diethylether, filtered then rinsed with diethylether again. The dry crude **14** (0.63 g, 105%) was sufficiently pure for being taken in the next step. ^{1}H-NMR ($CDCl_3$/methanol-d4): 8.73 (s, 1H) H-8; 8.37 (s, 1H) H-2; 8.1 - 7.4 (m, 5H) benzoyl; 6.01 (s, 1H) H-1'#; 4.25 (s) H-4'#.

1'#,2',3',4'#,5',5"-$^2H_6$-N^2-Acetyl-O^6-diphenylcarbamoylguanosine (15). Compound **10** (316 mg, 0.36 mmol) was treated as described for compound **14**, but instead of trituration, a short column of silica was run to afford compound **15** (150 mg, 79%). ^{1}H-NMR ($CDCl_3$/methanol-d4): 8.58 (s, 1H) H-8; 7.4 - 7.2 (m, 10H) phenyls; 6.08 (s, 1H) H-1'#; 4.13 (s) H-4'#; 2.28 (s, 3H) N^2-OAc.

1-(1'#,2',3',4'#,5',5"-$^2H_6$-β-D-Ribofuranosyl)-thymine (16). Compound **11** (264 mg, 0.43 mmol) was treated as written for compound **12** to obtain crude compound **16** (112 mg, 99%). ^{1}H-NMR (D_2O): 7.61 (d, $J_{5CH3,6}$= 1.2 Hz, 1H) H-6; 5.83 (s, 1H) H-1'#; 4.03 (s) H-4'#, 1.81 (d, 3H) 5-CH_3.

***Procedure A:* 5'-O-MMTr-1'#,2',3',4'#,5',5"-$^2H_6$-uridine (17).** To a solution of compound **12** (110 mg, 0.44 mmol), in dry pyridine (5 ml), MMTr-Cl (178 mg, 0.575 mmol) was added and stirred overnight at room temperature. After usual-work up and purification on short silica gel column compound **17** (121 mg, 53

%) was obtained. ^{1}H-NMR ($CDCl_3$): 8.0 (d, $J_{5,6}$ = 8.06 Hz, 1H) H-6; 7.5 - 6.8 (m, 14 H) MMTr; 5.88 (s, 1H) H-1'#; 5.33 (d, 1H) H-5; 4.15 (s) H-4'#; 3.77 (s, 3H) OCH_3 .

5'-O-MMTr-1'#,2',3',4'#,5',5"-$^2H_6$-N^4-benzoylcytidine (18). Compound **13** (116 mg, 0.33 mmol) was treated with MMTr-Cl (122 mg, 0.39 mmol.) to give **18** (161 mg, 78 %) according to Procedure A. ^{1}H-NMR ($CDCl_3$) : 8.84 (br.s, 1H) N-H; 8.26 (d, $J_{5,6}$ = 7.57 Hz, 1H) H-6; 8.0 - 6.8 (m, 19H) MMTr + benzoyl; 5.87 (s, 1H) H-1'#; 4.39 (s) H-4'#; 3.78 (s, 3H) OCH_3.

5'-O-MMTr-1'#,2',3',4'#,5',5"-$^2H_6$-N^6-benzoyladenosine (19). Compound **14** (150 mg, 0.4 mmol) was treated with MMTr-Cl (185 mg, 0.6 mmol) to give **19** (186 mg, 72 %) as in Procedure A. ^{1}H-NMR ($CDCl_3$): 9.28 (br. s, 1H) NH; 8.66 (s, 1H) H-8; 8.24 (s, 1H) H-2; 8.1 - 6.7 (m, 19H) MMTr + benzoyl; 6.07 (s, 1H) H-1'#; 4.38 (s) H-4'#; 3.75 (s, 3H) OCH_3.

5'-O-MMTr-1'#,2',3',4'#,5',5"-$^2H_6$-N^2-acetyl-O^6-diphenylcarbamoylguanosine (20). Procedure A was applied to compound **15** (145 mg, 0.275 mmol) and MMTr-Cl (102 mg, 0.33 mmol) to give compound **20** (172 mg, 78 %). ^{1}H-NMR ($CDCl_3$): 8.54 (s, 1H) N-H; 8.19 (s, 1H) H-8; 7.5 - 6.7 (m, 24H) MMTr + phenyls; 5.92 (s, 1H) H-1'#; 4.49 (s) H-4'#; 3.75 (s, 3H) OCH_3; 2.23 (s, 3H) N^2-OAc.

Procedure B: **5'-O-MMTr-2',3'-di-O-acetyl-1'#,2',3',4'#,5',5"-$^2H_6$-uridine (21).** Compound **17** (108 mg, 0.207 mmol) was coevaporated with dry pyridine (3x) then re-dissolved in dry pyridine (4 ml) and treated with acetic anhydride (63.5 mg, 2.5 mol eq.) and stirred at RT overnight. After usual work-up and purification on silica gel column, compound **21** (106 mg, 85 %). Yield: 106 mg (85 %). ^{1}H-NMR ($CDCl_3$): 8.25 (br. s, 1H) N-H; 7.69 (d, $J_{5,6}$ = 8.3 Hz, 1H) H-6; 7.4 - 6.8(m, 14 H) MMTr; 6.23 (s, 1H) H-1'#; 5.32 (d, 1H) H-5 ; 4.22 (s) H-4'#; 3.81 (s, 3H) OCH_3; 1.60 (s,6H) OAc.

5'-O-MMTr-2',3'-di-O-acetyl-1'#,2',3',4'#,5',5"-$^2H_6$-N^4-benzoylcytidine (22). Compound **18** (142 mg, 0.227 mmol) was treated as described in Procedure B to yield compound **22** (157 mg, 97 %). ^{1}H-NMR ($CDCl_3$): 8.25 (d, 1H) H-6; 8.0 - 6.8 (m, 19H) MMTr + benzoyl.; 6.32 (s, 1H) H-1'#; 4.30 (s, 1H) H-4'; 3.82 (s, 3H) OMe; 2.12, 2.07 (2xs, 6H) 2xOAc.

5'-O-MMTr-2',3'-di-O-acetyl-1'#,2',3',4'#,5',5"-$^2H_6$-N^6-benzoyladenosine (23). Treatment of compound **19** (200 mg, 0.3 mmol) as described in Procedure B afforded compound **23** (208 mg, 92 %). ^{1}H-NMR ($CDCl_3$): 8.59 (s, 1H) H-8; 8.25 (s, 1H) H-2; 7.7 - 6.8 (m, 19H) MMTr + benzoyl; 6.30 (s, 1H) H-1'#; 4.33 (s) H-4'#; 3.78 (s, 3H) OMe; 2.12, 2.05 (2xs, 6H) OAc.

5'-O-MMTr-2',3'-di-O-acetyl-1'#,2',3',4'#,5',5"-$^2H_6$-N^2-acetyl-O^6-diphenylcarbamoyl-guanosine (24). Compound **20** (60 mg, 0.075mmol) was acetylated according to Procedure B to obtain compound **24** (52 mg, 78 %). ^{1}H-NMR ($CDCl_3$): 8.06 (s, 1H) H-8; 7.4 - 6.8 (m, 24H) MMTr + phenyls; 6.17 (s, 1H) H-1'#; 4.32 (s) H-4'#; 3.75 (s, 3H) OMe; 2.36 - 2.05 (3xs, 9H) 3x Ac.

Procedure C: **2',3'-Di-O-acetyl-1'#,2',3',4'#,5',5"-$^2H_6$-uridine (25).** Compound **21** (100 mg, 0.165 mmol) was dissolved in 80 % aqueous acetic acid (8 ml) at room temperature and stirred overnight then volatile materials were evaporated and residue coevaporated with toluene twice. Purification on silica gel column gave compound **25** (52 mg, 95%). ^{1}H-NMR ($CDCl_3$): 8.62 (br.s, 1H) N-H; 7.72 (d, $J_{5,6}$ = 8.06 Hz, 1H) H-6; 6.04 (s, 1H) H1'; 5.78 (d, 1H) H-5; 4.21 (s) H-4'#; 2.14, 2.09 (2xs, 6H) 2x OAc.

2',3'-Di-O-acetyl-1'#,2',3',4'#,5',5"-$^2H_6$-N^4-benzoylcytidine (26). Compound **22** (140 mg, 0.198 mmol) was subjected to Procedure C to obtain compound **26** (70 mg, 81%).^{1}H-NMR ($CDCl_3$): 8.29 (d, 1H) H-6; 7.9 -7.5 (m, 6H) benzoyl + H-5; 6.12 (s, 1H) H-1'#; 4.27 (s) H-4'#; 2.11, 2.08 (2xs, 6H) 2x OAc.

2',3'-Di-O-acetyl-1'#,2',3',4'#,5',5"-$^2H_6$-N^6-benzoyladenosine (27). Procedure C was performed on compound **23** (215 mg, 0.28 mmol) to give compound **27** (132 mg, 94 %). ^{1}H-NMR ($CDCl_3$): 9.27 (br. s, 1H) N-H; 8.76 (s, 1H) H-8; 8.13 (s, 1H) H-2; 8.1 - 7.5 (m, 5H) benzoyl; 6.13 (s, 1H) H-1'#; 4.36 (s) H-4'#; 2.17 -2.02 (2xs, 6H) 2x OAc.

2',3'-Di-O-acetyl-1'#,2',3',4'#,5',5"-$^2H_6$-N^2-acetyl-O^6-diphenylcarbamoylguanosine (28). Compound **24** (100 mg, 0.113mmol) was treated according to Procedure C to give compound **28** (40 mg, 58%). ^{1}H-NMR ($CDCl_3$): 8.05 (s, 1H) H-8; 7.4 -7.3 (m, 10H) phenyls; 6.03 (s, 1H) H-1'#; 4.31 (s) H-4'#; 2.39 - 2.02 (3xs, 9H) 3xAc.

Procedure D: **1-(3',5'-O-(TPDS)-1'#,2',3',4'#,5',5"-$^2H_6$-β-D-ribofuranosyl)-thymine (32).** Compound **16** (0.53 g, 1.5 mmol) was repeatedly coevaporated with dry pyridine and re-dissolved in the same solvent (15 ml). After addition of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.62 ml, 1.95 mmol, 1.3 mol eq.), the mixture was stirred under dry condition for 1h, followed by normal work-up. The resulting syrup was

chromatographed on a short column of silica gel to give compound **32** (0.46 g, 61 %) as a foam. ^{1}H-NMR ($CDCl_3$): 9.31 (br. s, 1H) N-H; 7.44 (d, $J_{5CH3,6}$= 1.2 Hz, 1H) H-6; 5.71 (s, 1H) H-1'#; 3.99 (s) H-4'#; 1.90 (br. s, 3H) 5-CH_3; 1.1 - 1.0 (m, 24H) methyls of TPDS.

3',5'-O-(TPDS)-1'#,2',3',4'#,5',5"-$^2H_6$-N^4-benzoylcytidine (29). Procedure D was applied to compound **18** (0.66 g, 1.88 mmol) to get compound **29** (0.96 g, 85%). ^{1}H-NMR ($CDCl_3$): 8.28 (d, $J_{5,6}$= 7.6 Hz, 1H) H-6; 8.0 - 7.4 (m, 5H) benzoyl; 5.86 (s, 1H) H-1'#; 4.23 (s) H-4'#; 1.1 - 1.0 (m, 24H) methyls of TPDS.

3',5'-O-(TPDS)-1'#,2',3',4'#,5',5"-$^2H_6$-N^6-benzoyladenosine (30). Procedure D was carried out on compound **19** (0.57 g, 1.52 mmol) to afford compound **30** as a foam (0.72 g, 77%). ^{1}H-NMR ($CDCl_3$): 9.26 (br. s., 1H) N-H; 8.74 (s, 1H) H-8; 8.15 (s, 1H) H-2; 8.1 - 7.4 (m, 5H) Bz; 6.03 (s, 1H) H-1'#; 4.11 (s) H-4'#; 1.1 - 1.0 (m, 24H) methyls of TPDS.

3',5'-O-(TPDS)-1'#,2',3',4'#,5',5"-$^2H_6$-N^2-acetyl-O^6-diphenylcarbamoylguanosine (31). Treatment of compound **20** (152 mg, 0.29 mmol) according to Procedure D gave compound **31** (185 mg, 83%). ^{1}H-NMR ($CDCl_3$): 8.29 (br. s., 1H) N-H; 8.18 (s, 1H) H-8; 7.5 - 7.2 (m, 10H) phenyls; 6.01 (s, 1H) H-1'#; 4.12 (s) H-4'#; 2.49 (s, 3H) Ac; 1.1 - 1.0 (m, 24H) methyls of TPDS.

Procedure E: **1-(2'-O-Phenoxythiocarbonyl-3',5'-O-(TPDS)-1'#,2',3',4'#,5',5"-$^2H_6$-β-D-ribofuranosyl)-thymine (36).** To the mixture of vacuum-dried **32** (0.46 g, 0.92 mmol) and 1-methylimidazole (0.147 ml, 1.84 mmol, 2.0 mol eq.) in dry dichloromethane (10 ml), phenyl chlorothionoformate (0.179 ml, 1.29 mmol, 1.4 mol eq) was added and stirred in nitrogen atmosphere at room temperature overnight. After dilution with dichloromethane, the reaction mixture was poured into saturated sodium hydrogen carbonate solution and extracted with dichloromethane. Pooled organic phases were washed with saturated citric acid solution (2 x 30 ml), dried with $MgSO_4$, evaporated and the residue was purified on a short column of silica gel to afford compound **32** as a foam (0.58 g, 89 %). R_f: 0.51 (System B). ^{1}H-NMR ($CDCl_3$): 8.66 (br. s., 1H) N-H; 7.6 - 7.0 (m, 6H) H-6 + phenoxy; 5.92 (s, 1H) H-1'#; 4.05 (s) H-4'#; 1.92 (d, $J_{5CH3,6}$=1.2 Hz, 3H) 5-CH_3; 1.1 - 1.0 (m, 24H) methyls of TPDS.

2'-O-Phenoxythiocarbonyl-3',5'-O-(TPDS)-1'#,2',3',4'#,5',5"-$^2H_6$-N^4-benzoylcytidine (33). Compound **29** (0.92 g, 1.55 mmol) was converted to compound **33** (0.98 g, 87%) using Procedure E. R_f: 0.60 (System B). ^{1}H-NMR ($CDCl_3$): 8.88 (br. s., 1H) N-H; 8.27 (d, $J_{5,6}$= 7.6 Hz, 1H) H-6; 8.0-7.1 (m, 11H) phenoxy + benzoyl + H-5; 6.08 (s, 1H) H-1'#; 4.19 (s) H-4'#; 1.1 - 1.0 (m, 24H) CH_3 of TPDS.

2'-O-Phenoxythiocarbonyl-3',5'-O-(TPDS)-1'#,2',3',4'#,5',5"-$^2H_6$N^6-benzoyladenosine (34). Compound **30** (0.72 g, 1.16 mmol) was subjected to Procedure E to get compound **34** (0.77 g, 88%). R_f: 0.59 (System B). ^{1}H-NMR ($CDCl_3$): 9.21 (br. s., 1H) N-H; 8.78 (s, 1H) H-8; 8.19 (s, 1H) H-2; 8.1 - 7.1 (m, 10H) phenoxy + benzoyl; 6.24 (s, 1H) H-1'#; 4.14 (s) H-4'#; 1.1 - 1.0 (m, 24H) methyls of TPDS.

2'-O-Phenoxythiocarbonyl-3',5'-O-(TPDS)-1'#,2',3',4'#,5',5"-$^2H_6$-N^2-acetyl-O^6-diphenylcarbamoylguanosine (35) Treatment of compound **32** (0.28 g, 0.37 mmol) as described in Procedure E gave compound **35** (0.30 g, 89%). R_f: 0.68 (System B). ^{1}H-NMR ($CDCl_3$): 8.12 (s, 1H) H-8; 7.94 (br. s, 1-H) N-H; 7.6 - 7.0 (m, 15H) phenoxy; 6.20 (s, 1H) H-1'#; 4.17 (s) H-4'#; 2.58 (s, 3H) Ac; 1.1 - 1.0 (m, 24H) methyls of TPDS.

Procedure F: **3',5'-O-(TPDS)-1'#,2',2",3',4'#,5',5"-$^2H_7$-thymidine (40).** After coevaporation with dry toluene, compound **36** (0.70 g, 1.12 mmol) was dissolved in the same solvent (20 ml), AIBN (37 mg, 0.23 mmol, 0.2 mol eq.) and tri-n-butyltin deuteride (0.46 ml, 1.71 mmol, 1.5 mol eq.) were added. The solution was degassed by nitrogen (20 min), followed by heating at 75 °C under nitrogen atmosphere for 3.5 h. Volatile materials were evaporated, the residual oil was subjected to column chromatography to afford compound **40** (0.52 g, 95 %). R_f: 0.44 (System B). ^{1}H-NMR ($CDCl_3$): 9.04 (br. s., 1H) N-H; 7.42 (d, $J_{5CH3,6}$= 1.2 Hz, 1H) H-6; 6.08 (s, 1H) H-1'#; 3.74 (s) H-4'#; 1.92 (d, 3H) 5-CH_3; 1.1-1.0 (m, 24H) methyls of TPDS.

3',5'-O-(TPDS)-1'#,2',2",,4'#,5',5"-$^2H_7$-N^4-benzoyl-2'-deoxycytidine (37). Procedure F was used for 14 h to convert compound **33** (0.29 g, 0.39 mmol) to compound **37** (0.17 g, 75%). R_f: 0.41 (System B). ^{1}H-NMR ($CDCl_3$): 8.78 (br. s., 1H) N-H; 8.33 (d, $J_{5,6}$= 7.7 Hz, 1H) H-6; 8.0 - 7.4 (m, 6H) benzoyl + H-5; 6.08 (s, 1H) H-1'#; 3.83 (s) H-4'#; 1.1 - 1.0 (m, 24H) methyls of TPDS.

3',5'-O-(TPDS)-1'#,2',2",3',4'#,5',5"-$^2H_7$-N^6-benzoyl-2'-deoxyadenosine (38) Treatment of compound **34** (0.76 g, 1.01 mmol) according to Procedure F gave compound **38** (0.47 + 0.07 g; 77 ~ 15%). R_f: 0.45 + 0.28 (System B). ^{1}H-NMR ($CDCl_3$): 9.13 (br. s., 1H) N-H; 8.77 (s, 1H) H-8; 8.17 (s, 1H) H-2; 8.1 - 7.4 (m, 5H) benzoyl; 6.04 (s, 1H) H-1'#; 4.12 (s) H-4'#; 1.1 - 1.0 (m, 24H) methyls of TPDS.

3',5'-O-(TPDS)-1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^2-acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine (39). Compound **35** (0.66 g, 0.73 mmol) was treated as in Procedure F to afford compound **39** (0.53 g, 96%). R_f: 0.50 (System B). ^{1}H-NMR ($CDCl_3$): 8.21 (s, 1H) H-8; 8.03 (br. s., 1H) N-H; 7.6 - 7.1 (m, 10H) phenyls; 6.30 (s, 1H) H-1'#; 3.89 (s) H-4'#; 2.55 (s, 3H) Ac; 1.1 - 1.0 (m, 24H) methyls of TPDS.

***Procedure G:* 1'#,2',2",3',4'#,5',5"-$^{2}H_7$-Thymidine (44).** Compound **40** (0.49 g, 1.0 mmol) was dissolved in dry tetrahydrofurane (10 ml) and 1.0 M TBAF solution in dry THF (1 ml, 1.0 mmol, 1.0 mol. eq.) was added. After stirring for 5 min, volatile materials were evaporated and the residue was purified on silica gel to give compound **44** (0.22 g, 83 %). ^{1}H-NMR ($CDCl_3$/MeOH-d_4): 7.70 (d, $J_{5CH3,6}$= 1.2 Hz, 1H) H-6; 6.26 (s, 1H) H-1'#; 3.92 (s) H-4'#; 1.92 (d, 3H) 5-CH_3.

1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^4-Benzoyl-2'-deoxycytidine (41) When compound **37** (153 mg, 0.26 mmol) was treated according to Procedure G, compound **41** (77 mg, 88%) was obtained. ^{1}H-NMR ($CDCl_3$/MeOH-d_4): 8.53 (d, $J_{5,6}$= 7.6 Hz, 1H) H-6; 8.1 - 7.5 (m, 6H) benzoyl + H-5; 6.23 (s, 1H) H-1'#; 4.03 (s) H-4'#.

1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^6-Benzoyl-2'-deoxyadenosine (42). Compound **38** (0.51g, 0.84 mmol) was subjected to the treatment described in Procedure G to give compound **42** (0.28 g, 93%). ^{1}H-NMR ($CDCl_3$/MeOH-d_4): 8.75 (s, 1H) H-8; 8.34 (s, 1H) H-2; 8.1 - 7.4 (m, 5H) benzoyl; 6.46 (s, 1H) H-1'#; 4.17 (s) H-4'#.

1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^2-Acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine (43). Upon treatment of compound **39** (0.21 g, 0.27 mmol) with TBAF as described in Procedure G compound **43** (0.22 g, 80%) was obtained. ^{1}H-NMR ($CDCl_3$): 8.89 (br. s., 1H) N-H; 8.20 (s, 1H) H-8; 7.5 - 7.1 (m, 10H) phenyls; 6.26 (s, 1H) H-1'#; 3.97 (s) H-4'#; 2.30 (s, 3H) N^2-acetyl.

5'-O-MMTr-1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^4-benzoyl-2'-deoxycytidine (45) Upon subjecting compound **41** (0.15 g, 0.44 mmol) to a treatment described in Procedure A compound **45** (0.23 g, 87%) was obtained. ^{1}H-NMR ($CDCl_3$): 8.82 (br. s., 1H) N-H; 8.28 (d, $J_{5,6}$= 7.3 Hz, 1H) H-6; 8.1 - 6.7 (m, 20H) MMTr + benzoyl + H-5; 6.30 (s, 1H) H-1'#; 4.18 (s) H-4'#; 3.79 (s, 3H) OCH_3.

5'-O-MMTr-1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^6-benzoyl-2'-deoxyadenosine (46). Compound **42** (0.27 g, 0.75 mmol) was treated according to Procedure A to obtain compound **46** (0.43 g, 90%). ^{1}H-NMR ($CDCl_3$): 9.14 (br. s., 1H) N-H; 8.71 (s, 1H) H-8; 8.14 (s, 1H) H-2; 8.1 - 6.7 (m, 19H) MMTr + benzoyl; 6.46 (s, 1H) H-1'#; 4.15 (s) H-4'#; 3.76 (s, 3H) OCH_3.

5'-O-MMTr-1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^2-acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine (47). Compound **43** (0.11 g, 0.22 mmol) was converted into compound **47** (0.15 g, 84%) according to Procedure A. ^{1}H-NMR ($CDCl_3$): 8.20 (br. s., 1H) N-H; 8.08 (s, 1H) H-8; 7.5 - 6.7 (m, 24H) MMTr + phenyls; 6.42 (s, 1H) H-1'#; 4.14 (s) H-4'#; 3.73 (s, 3H) OCH_3; 2.36 (s, 3H) N^2-Ac.

5'-O-MMTr-1'#,2',2",3',4'#,5',5"-$^{2}H_7$-thymidine (48). Compound **44** (0.22 g, 0.88 mmol) was treated as described in Procedure A to obtain compound **48** (0.4 g, 88 %) as a foam. ^{1}H-NMR ($CDCl_3$): 9.45 (br. s., 1H) N-H; 7.60 (d, $J_{5CH3,6}$= 1.2 Hz, 1H) H-6; 7.5-6.78 (m, 14H) MMTr; 6.42 (s, 1H) H-1'#; 4.07 (s) H-4'#; 3.78 (s, 3H) OCH_3; 1.45 (d, 3H) 5-CH_3.

3'-O-Acetyl-5'-O-MMTr-1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^4-benzoyl-2'-deoxycytidine (49) Upon treating compound **45** (0.23 g, 0.38 mmol) as described in Procedure B, compound **49** was obtained (0.22 g, 89%). ^{1}H-NMR ($CDCl_3$): 8.75 (br. s., 1H) N-H; 8.16 (d, $J_{5,6}$= 7.6 Hz, 1H) H-6; 8.0 - 6.8 (m, 20H) MMTr + benzoyl + H-5; 6.31 (s, 1H) H-1'#; 4.26 (s) H-4'#; 3.8 (s, 3H) OCH_3; 2.08 (s, 3H) OAc.

3'-O-Acetyl-5'-O-MMTr-1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^6-benzoyl-2'-deoxyadenosine (50). Treatment of compound **46** (0.28 g, 0.45 mmol) as described in Procedure B with 1.2 eq. of acetic anhydride at ~ 4 °C for 24 h afforded compound **50** (0.23 g, 77%). ^{1}H-NMR ($CDCl_3$): 9.12 (br. s., 1H) N-H; 8.74 (s, 1H) H-8; 8.18 (s, 1H) H-2; 8.1 - 6.7 (m, 19H) MMTr + benzoyl; 6.52 (s, 1H) H-1'#; 4.30 (s) H-4'#; 3.78 (s, 3H) OCH_3; 2.12 (s, 3H) OAc.

3'-O-Acetyl-5'-O-MMTr-1'#,2',2",3',4'#,5',5"-$^{2}H_7$-N^2-acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine (51). Compound **47** (0.25 g, 0.32 mmol) was reacted according to Procedure B to obtain compound **51** (0.25 g, 94%). ^{1}H-NMR ($CDCl_3$): 8.10 (s, 1H) H-8; 7.94 (br. s., 1H) N-H; 7.5 - 6.7 (m, 24H) MMTr + phenyls; 6.37 (s, 1H) H-1'#; 4.25 (s) H-4'#; 3.75 (s, 3H) OCH_3; 2.42 & 2.11 (ds, 2x3H) N^2 & OAc.

3'-O-Acetyl-5'-O-MMTr-1'#,2',2",3',4'#,5',5"-$^{2}H_7$-thymidine (52). Procedure B was applied to compound **48** (0.4 g, 0.77 mmol) to give compound **52** (0.36 g, 83 %) as a foam. ^{1}H-NMR ($CDCl_3$): 9.15

(br. s., 1H) N-H; 7.61 (d, $J_{5CH3,6}$= 1.2 Hz, 1H) H-6; 7.4 - 6.8 (m, 14H) MMTr; 5.29 (s, 1H) H-1'#; 4.12 (s) H-4'#; 3.80 (s, 3H) OCH_3; 2.08 (s, 3H) Ac; 1.41 (d, 3H) 5-CH_3.

Procedure H: **3'-O-Acetyl-1'#,2',2",3',4'#,5',5"-$^2H_7$-thymidine (56).** Compound **52** (0.34 g, 0.6 mmol) was dissolved in 12 ml (20ml/mmol) of 2 % benzenesulfonic acid in dichloromethane-methanol (70/30 v/v) at room temperature. After 6 min this solution was neutralized with triethylamine, volatile matters were evaporated and the residue was subjected to column chromatography to give compound **56** (0.12 g, 69 %) as a foam. ^{1}H-NMR ($CDCl_3$/MeOH-d_4): 7.75 (d, $J_{5CH3,6}$= 1.2 Hz, 1H) H-6; 6.31 (s, 1H) H-1'#; 4.08 (s) H-4'#; 2.12 (s, 3H) OAc; 1.92 (d, 3H) 5-CH_3.

3'-O-Acetyl-1'#,2',2",3',4'#,5',5"-$^2H_7$-N^4-benzoyl-2'-deoxycytidine (53). Procedure H was carried out on compound **49** (0.21 g, 0.32 mmol) to yield compound **53** (104 mg, 86%). ^{1}H-NMR ($CDCl_3$): 8.27 (d, $J_{5,6}$= 7.3 Hz, 1H) H-6; 8.0 - 7.4 (m, 6H) benzoyl + H-5; 6.27 (s, 1H) H-1'#; 4.19 (s) H-4'#; 2.11 (s, 3H) OAc.

3'-O-Acetyl-1'#,2',2",3',4'#,5',5"-$^2H_7$-N^6-benzoyl-2'-deoxyadenosine (54) Upon treatment of compound **50** (0.17 g, 0.25 mmol) as described in Procedure H, compound **54** was obtained (90 mg, 90%). ^{1}H-NMR ($CDCl_3$): 9.11 (br. s., 1H) N-H; 8.78 (s, 1H) H-8; 8.11 (s, 1H) H-2; 8.1 - 7.4 (m, 5H) benzoyl; 6.36 (s, 1H) H-1'#; 4.28 (s) H-4'#; 2.14 (s, 3H) OAc.

3'-O-Acetyl-1'#,2',2",3',4'#,5',5"-$^2H_7$-N^2-acetyl-O^6-diphenylcarbamoyl-2'-deoxyguanosine (55). Compound **51** (0.21 g, 0.26 mmol) was treated as described in Procedure H to obtain compound **55** (112 mg, 78 %). ^{1}H-NMR ($CDCl_3$): 8.18 (br. s., 1H) N-H; 8.10 (s, 1H) H-8; 7.5 - 7.2 (m, 10H) Ph; 6.26 (s, 1H) H-1'#; 4.19 (s) H-4'#; 2.40 & 2.12 (ds, 2x3H) N^2 & OAc.

General procedure I: **Fully protected UpA* (73) (* denotes for deuterated nucleoside).** Diester **57** (120 mg, 0.13mmol) and 5'-hyroxy block **23** (50 mg, 0.1mmol) were coevaporated with dry pyridine, redissolved in the same solvent (2 ml) then MSNT (88mg, 0.3 mmol, 3 mol eq.) was added and the mixture was stirred under dry condition for 60 min. Usual work-up and short column chromatography gave dimer **73** (89 mg, 72%). ^{1}H-NMR ($CDCl_3$): 9.29 (br.s, 1H) N-H A; 8.78 (s, 1H) H-8 A; 8.26 & 8.21 (s, 1H) H-2 A; 8.04 - 7.07 (m, 14H) toluoyl, *o*-chlorophenyl, benzoyl + H-6 U; 6.27 & 6.22 (2x s, 1H) H-1'#A; 6.04 (d, 1H) H-1'U; 5.62 (d, $J_{5,6}$ = 8.0 Hz, 1H) H-5 U; 5.24 - 3.57 (m, 8H) H-2'& H-3'& H-4', H-5'& H-5"U + H-4'#A + Thp; 2.39 (s, 6H) Me-Tol; 2.12 & 2.04 (2xs,6H) acetyls; 1.54 (m, 6H) Thp. ^{31}P-NMR ($CDCl_3$): -7.47, -7.71.

Fully protected CpG* (74). Compound **58** (47 mg. 0.041 mmol) and compound **24** (21 mg, 0.034 mmol) were condensed according to Procedure I to give compound **74** (52 mg, 95 %). ^{1}H-NMR ($CDCl_3$) : 8.85 - 8.67 (2xbr. s, 2H) N-H G; 8.14 - 6.81 (m, 35H) MMTr, benzoyl, *o*-chlorophenyl, phenyls + H-6 & H-5 C; 6.42 (d, 1H) H-1'C; 6.08 & 5.96, (2xs, 1H) H-1'#G; 5.13 - 4.43 (m, 3H) H-2'& H-3'& H-4'C; 4.11 (s, 1H) H-4'#G; 3.79 (s, 3H) OMe (MMTr); 3.54 - 3.52 (s,s,m, 8H) MDMP-methyl esters, H-5'& H-5"C; 3.06 (s, 3H) OMe (MDMP); 2.37 - 1.83 (m, 8H) acetyls, methylenes (MDMP). ^{31}P-NMR ($CDCl_3$): -7.15, -7.69.

Fully protected ApU* (75). Condensation of compound **59** (208 mg,0.18 mmol) and compound **21** (50 mg, 0.15 mmol) was carried out in the same manner as described in Procedure I using MSNT (133 mg, 0.45 mmol) to give compound **75** (163 mg, 79%). ^{1}H-NMR ($CDCl_3$/D_2O): 8.72 (s, 1H) H-8 A; 8.24 (s, 1H) H-2 A; 8.05 - 6.78 (m, 24H) Arom.+ H-6 U; 6.27 (d, 1H) H-1'A; 5.94 (s, 1H) H-1'#U; 5.72 (d, $J_{5,6}$ = 8.0Hz, 1H) H-5'U; 5.6 - 4.6 (m, 3H) H-2'& H-3'& H-4'A; 4.27 (d, 1H) H-4'#U; 3.78 (s, 3H) OMe(MMTr); 3.67 - 3.53 (s, s, m, 8 H) MDMP-methyl esters, H-5'& H-5"A; 3.16 (s, 3H) OMe (MDMP); 2.50 - 1.60 (m, 8H) methylenes (MDMP); 2.11 & 2.08 (2xs, 6H) acetyls U. ^{31}P-NMR ($CDCl_3$): -6.30, -7.35.

Fully protected GpC* (76). A mixture of compound **60** (145 mg, 0.12 mmol) and compound **22** (50 mg, 0.11 mmol) was treated in the same manner as described in Procedure J to give dimer **76** (70 mg, 40%). ^{1}H-NMR ($CDCl_3$): 8.01 (s, 1H) H-8 G; 7.87 - 6.68 (m, 30H) Arom.+ H-2 G + H-6 & H-5 C; 6.21 - 4.20 (m, 6H) H-1'#& H-4'#C + H-1'& H-2'& H-3'& H-4' G; 3.79 (s, 3H) OMe (MMTr); 3.64 - 3.48 (m s, 8H) MDMP-methyl esters & H-5'& H-5" G; 3.05 (s, 3H) OMe (MDMP); 2.78 - 1.1 (s, m, 23H) methylenes (MDMP)+ acetyls C+TBB. ^{31}P-NMR ($CDCl_3$): -6.29, -7.25.

Procedure J: **Dimer UpA* (77).** Compound **73** (50 mg, 0.04 mmol) was dissolved in a mixture of dioxane : water (1:1 v/v , 5 ml). *Syn*-4-nitrobenzaldoxime (46.5 mg, 0.4 mmol) was added, followed by 1,1,3,3-tetramethylguanidine (50 µl, 0.4 mmol) and stirred at room temperature for 24 h. Aqueous ammonia (32%, 15 ml) was added and the stirring was continued for 2 days at 50 °C. After evaporation and coevaporation with distilled water, the residue was treated with 80 % aqueous acetic acid (10 ml) overnight, evaporated, coevaporated with dist.water, re-dissolved in distilled water and extracted with dichloromethane. The water phase was evaporated, taken up in distilled water (5 - 6 ml) and applied on a DEAE-Sephadex A-25 column (2 x

20 cm) using ammonium hydrogen carbonate (0.001 M - 0.12 M, linear gradient) as eluant. The center part of the product peak was collected, concentrated on a rotavapor, and co-evaporated with re-distilled water until free of salt, exchanged into it's sodium form and dried. Yield: 353 A_{260} units (53%). ^{31}P-NMR (D_2O): -0.61.

Dimer CpG* (78). Compound **74** (52 mg, 0.03 mmol) was treated as described in Procedure J to give compound **78** (282 A_{260} units, 49%). ^{31}P-NMR (D_2O): -0.68.

Dimer ApU* (79). Compound **75** (51 mg, 0.037 mmol) was treated according to Procedure J to afford dimer **79** (672 A_{260} units, 77%). ^{31}P-NMR (D_2O): -0.73.

Dimer GpC* (80). Compound **76** (52 mg, 0.032 mmol) was deprotected as described in Procedure J to give dimer **80** (282 A_{260} units, 49 %). ^{31}P-NMR (D_2O): -0.85.

Fully protected UpA (81). 3'-Phosphodiester block **57** (0.21 g, 0.18 mmol) and 5'-OH block **63** (76 mg, 0.17 mmol) were condensed as described in Procedure I to get dimer **81** (0.225 g, 91%). ^{1}H-NMR ($CDCl_3$): 9.11 & 9.04 (2s, 1H) NH; 8.78 & 8.76 (2s, 1H) H-8; 8.22 & 8.05 (2s, 1H) H-2; 8.1 - 6.8 (m, 28H) MMTr, *o*-chlorophenyl, toluoyl, benzoyl and H-6; 6.3 - 6.1 (m, 2H) H-1'A & U; 6.0 - 5.5 (m, 2H) H-2' & 3'A; 5.38 & 5.34 (2d, 9.00, 1H) H-5; 5.2-4.7 (m, 2H); H-2' & 3'U; 4.6 - 4.3 (m, 4H) H4'-A & U, H-5'A; 3.80 (s, 3H) MMTr methoxy; 3.60 & 3.58, 3.54 & 3.51, 3.26 & 3.22 (6s, 9H) MDMP methyl esters and methoxy; 3.33 (m, 2H) H-5'U ; 2.41 (s, 3H) toluoyl; 2.5 - 1.6 (m, 8H) MDMP methylenes; 2.10 & 2.08, 2.04 & 2.05 (4s, 6H) acetyls. ^{31}P-NMR ($CDCl_3$): -6.86, -7.35.

Fully protected CpG (82). Condensation of diester block **58** (202 mg, 0.18 mmol) with 5'-OH block **64** (79 mg, 0.15 mmol) was carried out as described in Procedure I to get dimer **82** (192 mg, 84%). ^{1}H-NMR ($CDCl_3$): 8.2 - 6.6 (m, 30H) MMTr, benzoyl, H-5, H-6, H-8, Tbb, *o*-chlorophenyl; 6.48 (m, 1H) H-1'C; 6.17 & 6.09 (2d, 5.4, 1H) H1'G; 6.0 - 5.6 (m, 2H) H-2'& 3'G; 5.0 - 4.3 (m, 6H) H-2'& 3' & 4'C, H-4'& 5'G; 3.77 (s, 3H) MMTr methoxy; 3.58 & 3.54 & 3.41 (3s, 6H) MDMP methyl ester; 3.5 - 3.3 (m, 2H) H-5'C; 3.01 & 2.98 (2s, 3H) MDMP methoxy; 2.4 - 1.6 (m, 6H) MDMP methylenes; 2.17 & 2.08 & 2.00 (3s, 6H) acetyls; 1.24 & 1.19 (2s, 9H) Tbb. ^{31}P-NMR ($CDCl_3$): -6.96, -8.57.

Fully protected ApU (83). Condensation of diester block **59** (320 mg, 0.28 mmol) with 5'-OH block **61** (89 mg, 0.20 mmol) was carried out using MSNT (207 mg, 0.71 mmol) as described in Procedure I to get dimer **83** (191 mg, 65%). ^{1}H-NMR ($CDCl_3$): 9.01 (s, 1H) NH; 8.67 & 8.62 (2s, 1H) H-8; 8.27 & 8.25 (2s, 1H) H-2; 8.1 - 6.8 (m, 28H) MMTr, *o*-chlorophenyl, toluoyl, benzoyl and H-6; 6.3 - 6.0 (m, 2H) H-1'A & U; 5.86 & 5.76 (2d, 8.3, 1H) H-5; 5.7 - 5.2 (m, 4H) H-2'& 3'U, H-2'& 3'A; 4.7 - 4.2 (m, 4H) H-4'A & U, H-5'A; 3.78 (s, 3H) MMTr methoxy; 3.55 & 3.53 (2s, 6H) MDMP methyl esters; 3.8 - 3.2 (m, 2H) H-5'A; 2.76 & 2.64 (2s, 3H) MDMP methoxy; 2.41 (s, 3H) toluoyl; 2.5 - 1.4 (m, 8H) MDMP methylenes; 2.13 & 2.09 & 2.07 & 2.05 (4s, 6H) acetyls. ^{31}P-NMR ($CDCl_3$): -6.30, -7.20.

Fully protected GpC (84). Diester block **60** (189 mg, 0.15 mmol) was coupled to the 5'-OH block **62** (49 mg, 0.12 mmol) as described in Procedure I to afford dimer **84** (153 mg, 84%). ^{1}H-NMR ($CDCl_3$): 8.01 (s, 1H) H-8; 7.9 - 6.6 (m, 29H) MMTr, benzoyl, H-5, H-6, Tbb, *o*-chlorophenyl; 6.30 & 6.11 (2d, 4.15, 1H) H-1'G; 5.96 (d, 7.2, 1H) H1'C; 5.8 - 5.6 (m, 4H) H-2'& 3'G, H-2'& 3'C; 4.6 - 4.1 (m, 4H) H-4'& 5'C, H-4'G; 3.64 (s, 3H) MMTr methoxy; 3.58 & 3.56 & 3.53 & 3.48 (3s, 6H) MDMP methyl ester; 3.5 - 3.1 (m, 2H) H-5'G; 2.81 & 2.78 (2s, 3H) MDMP methoxy; 2.4 - 1.6 (m, 6H) MDMP methylenes; 2.11 & 2.10 & 2.08 (3s, 6H) acetyls; 1.26 (s, 9H) Tbb. ^{31}P-NMR ($CDCl_3$): -6.18, -7.18.

Dimer UpA (85). Deprotection of dimer **81** (100 mg, 67 μmol) was done according to Procedure J to give dimer **85**. (1206 A_{260} o.d. units, 76 %). ^{31}P-NMR (D_2O): -0.68.

Dimer CpG (86) Upon treatment of dimer **82** (107 mg, 74 μmol) as described in Procedure J, compound **86** (1017 A_{260} o.d. units, 76 %) was obtained. ^{31}P-NMR (D_2O): -0.71.

Dimer ApU (87). Procedure J was applied to dimer **83** (107 mg, 73 μmol) to afford dimer **87** (954 A_{260} o.d. units, 56 %). ^{31}P-NMR (D_2O): -0.43.

Dimer GpC (88). Dimer **84** (111 mg, 73.2 μmol) was treated as described in Procedure J to give dimer **88** (1116 A_{260} o.d. units, 85 %). ^{31}P-NMR (D_2O): -0.54.

Fully protected d(TpA*) dimer 89. Phosphodiester block **65** (0.21 g, 0.26 mmol) and 5'-hyroxy block **54** (80 mg, 0.20 mmol) were condensed using MSNT (191 mg, 0.65 mmol) as described in Procedure I to obtain dimer **89** (0.14 g, 86 %). ^{1}H-NMR ($CDCl_3$): 9.62 & 9.23 (2xs, 2H) NH; 8.81 (s, 1H) H-8; 8.33 & 8.24 (2xs, 1H) H-2; 8.1 - 6.8 (m, 24H) MMTr, *o*-chlorophenyl, benzoyl and H-6; 6.53 & 6.50 (2xs, 1H) H-1'#A; 6.5 - 6.2 (m, 1H) H-1'T; 5.3 - 5.1 (m, 2H) H-3'T; 4.4 - 4.1 (m, 1H) H-4'T & 4'#A; 3.76 (s, 3H) MMTr methoxy; 3.5 - 3.2 (m, 2H) H-5'T ; 2.6 - 2.3 (m, 2H) H-2'2"T; 2.12 & 1.90 (2xs, 3H) acetyl; 1.34 & 1.25 (2xs, 3H) 5CH_3. ^{31}P-NMR ($CDCl_3$): -7.25, -7.32.

Fully protected dimer d(CpG*) (90). Phosphodiester block **66** (161 mg, 0.18 mmol) and 5'-hydroxy block **55** (83 mg, 0.15 mmol) were treated with MSNT (133 mg, 0.45 mmol) as reported in Procedure I to give

dimer **90** (149 mg, 75%). ^{1}H-NMR ($CDCl_3$): 8.75 & 8.71 (2xbr. s, 2H) NH; 8.2 - 6.8 (m, 36H) MMTr, benzoyl, H-5, H-6, H-8, phenyls, *o*-chlorophenyl; 6.3 - 6.1 (m, 1H) H-1'C; 6.33 & 6.27 (2xs, 1H) H1'#G; 5.3 - 5.0 (m, 1H) H-3'C; 4.5 - 4.1 (m, 1H) H-4'C & G; 3.77 (s, 3H) MMTr methoxy; 3.5 - 3.3 (m, 2H) H-5'C; 3.2 - 2.0 (m, 2H) H-2',2"C; 2.38, 2.32, 2.13 & 2.11 (4xs, 6H) acetyls. ^{31}P-NMR ($CDCl_3$): -7.10, -7.69.

Fully protected dimer d(ApT*) (91). Phosphodiester block **67** (239 mg, 0.26 mmol) and 5'-hydroxy block **56** (57 mg, 0.2 mmol) were treated as reported in Procedure I to give compound **91** (168 mg, 77%). ^{1}H-NMR ($CDCl_3$): 9.88, 9.60, 9.47 & 9.47 (4xbr. s, 2H) NH; 8.72 (s, 1H) H-8; 8.17 (s, 1H) H-2; 8.1 - 6.7 (m, 24H) MMTr, *o*-chlorophenyl, benzoyl and H-6; 6.6 - 6.2 (m, 1H) H-1'A; 6.29 (s, 1H) H-1'#T; 5.5 - 5.2 (m, 1H) H-3'A; 4.7 - 4.1 (m, 1H) H-4'A & 4'#T; 3.76 (s, 3H) MMTr methoxy; 3.6 - 2.4 (m, 4H) H-5'A, H-2',2"A; 2.10 (s, 3H) acetyl; 1.82 (s, 3H) $5CH_3$. ^{31}P-NMR ($CDCl_3$): -7.15, -7.71.

Fully protected dimer d(GpC*) (92). Phosphodiester block **67** (233 mg, 0.24 mmol) and 5'-hydroxy block **56** (69 mg, 0.18 mmol) were treated with MSNT (174 mg, 0.59 mmol) in the same manner as described in Procedure J to afford dimer **92** (182 mg, 80%). ^{1}H-NMR ($CDCl_3$): 10.50, 9.88 & 8.81 (3xbr. s, 2H) NH; 8.25 & 8.13 (2xd, $J_{5,6}$= 7.7 Hz, 1H) H-6; 7.9 - 6.7 (m, 29H) MMTr, benzoyl, H-5, H-8, Tbb, *o*-chlorophenyl; 6.32 & 6.25 (2xs, 1H) H-1'#C; 6.1 - 5.9 (m, 1H) H-1'G; 5.5 - 5.3 (m, 1H) H-3'G; 4.5 - 4.2 (m, 1H) H-4'#C & H-4'G; 3.72 & 3.71 (2xs, 3H) MMTr methoxy; 3.4 - 2.1 (m, 4H) H-5'G, H-2',2"G; 2.08 & 2.06 (2xs, 3H) acetyl; 1.32 (s, 9H) Tbb. ^{31}P-NMR ($CDCl_3$): -6.89, -7.42.

***General procedure K:* Dimer d(TpA*) (93).** Dimer **89** (65 mg, 60 μmol) was treated as described in procedure K. After extraction with dichloromethane, the aqueous phase was evaporated and the residue was purified on two preparative TLC plates (System D). Appropriate band was cut out and eluted and washed with water. After evaporation of the solvent, the residue was applied on a DEAE-Sephadex A-25 column using a linear gradient of ammonium bicarbonate buffer as eluant (0.001 M - 0.12 M, 500 ml each). Appropriate fractions were collected and evaporated then desalted by repeated coevaporation with water giving dimer **93** (859 A_{260} o.d. units, 62%). ^{31}P-NMR (D_2O): -0.98.

Dimer d(CpG*) (94). Dimer **90** (75 mg, 55 μmol) was treated according to Procedure K to get compound **94** (910 A_{260} o.d. units, 92%) ^{31}P-NMR (D_2O): -0.95.

Dimer d(ApT*) (95). Treatment of dimer **91** (86 mg, 79 μmol) as described in Procedure K afforded compound **95** (1418 A_{260} o.d. units, 78%) ^{31}P-NMR (D_2O): -0.93.

Dimer d(GpC*) (96). Dimer **92** (69 mg, 55 μmol) was deprotected and purified as written in Procedure K to give dimer **96** (570 A_{260} o.d. units, 58%) ^{31}P-NMR (D_2O): -0.76.

Fully protected d(TpA) dimer (97). Phosphodiester block **65** (157 mg, 0.2 mmol) and 5'-hydroxy block **71** (60 mg, 0.15 mmol) were condensed as described in Procedure I to give dimer **97** (140 mg, 86%). ^{1}H-NMR ($CDCl_3$): 9.68 & 9.57 (2xbr. s, 2H) NH; 8.83 (s, 1H) H-8; 8.35 & 8.26 (2xs, 1H) H-2; 8.1 - 6.8 (m, 24H) MMTr, *o*-chlorophenyl, benzoyl and H-6; 6.6 - 6.2 (m, 2H) H-1'A & T; 5.6 - 5.1 (m, 2H) H-3'A & T; 4.6 - 4.1 (m, 4H); H-4'A & T, H-5'A; 3.77 (s, 3H) MMTr methoxy; 3.6 - 2.1 (m, 6H) H-5'A , H-2',2"T & A; 2.14 & 2.13 (2xs, 3H) acetyl; 1.35 (s, 3H) $5CH_3$. ^{31}P-NMR ($CDCl_3$): -7.30, -7.40.

Dimer d(CpG) (98). Phosphodiester block **66** (220 mg, 0.24 mmol) and 5'-hydroxy block **72** (93 mg, 0.2 mmol) were treated as reported in Procedure I to obtain dimer **98** (182 mg, 83%). ^{1}H-NMR ($CDCl_3$): 12.46, 10.38, 10.22 (3xbr. s, 2H) NH; 8.2 - 6.7 (m, 30H) MMTr, benzoyl, H-5, H-6, H-8, Tbb, *o*-chlorophenyl; 6.5 - 6.0 (m, 2H) H-1'C & G; 5.6 -4.8 (m, 2H) H-3'G & C; 4.7 - 3.8 (m, 4H) H-4'C & G, H-5'G; 3.7 & 3.75 (2xs, 3H) MMTr methoxy; 3.5 - 1.4 (m, 6H) H-5'C, H-2',2"C & G; 2.14 & 2.13 (2xs, 3H) acetyl; 1.30 & 1.25 (2xs, 9H) Tbb. ^{31}P-NMR ($CDCl_3$): -7.74, -8.52.

Dimer d(ApT) (99). Phosphodiester block **67** (239 mg, 0.26 mmol) and 5'-hydroxy block **69** (57 mg, 0.2 mmol) were treated in the same manner as reported in Procedure I to afford dimer **99** (196 mg, 90%). ^{1}H-NMR ($CDCl_3$): 10.12, 9.98, 9.58 (3xbr. s, 2H) NH; 8.70 (s, 1H) H-8; 8.18 (s, 1H) H-2; 8.1 - 6.7 (m, 24H) MMTr, *o*-chlorophenyl, benzoyl and H-6; 6.6 - 6.2 (m, 2H) H-1'A & T; 5.6 - 5.1 (m, 2H) H-3'A & T; 4.6 - 4.0 (m, 4H) H-4'A & T, H-5'T; 3.76 (s, 3H) MMTr methoxy; 3.6 - 3.2 (m, 2H) H-5'A; 3.2 - 2.1 (m, 4H) H-2',2"A & T; 2.11 (s, 3H) acetyl; 1.83 (s, 3H) $5CH_3$. ^{31}P-NMR ($CDCl_3$): -7.15, -7.69.

Dimer d(GpC) (100). Diester block **68** (258 mg, 0.26 mmol) and 5'-hydroxy block **69** (75 mg, 0.2 mmol) were coupled according to Procedure I to give dimer **100** (219 mg, 89%). ^{1}H-NMR ($CDCl_3$): 10.25, 9.84 (2xbr. s, 2H) NH; 8.26 & 8.12 (2xd, $J_{5,6}$= 7.6 Hz, 1H) H-6; 7.9 - 6.7 (m, 29H) MMTr, benzoyl, H-5, H-8, Tbb, *o*-chlorophenyl; 6.4 - 5.9 (m, 2H) H-1'G & C; 5.5 - 5.2 (m, 2H) H-3'G & C; 4.6 - 4.2 (m, 4H) H-4'& 5'C, H-4'G; 3.72 & 3.71 (2xs, 3H) MMTr methoxy; 3.5 - 2.1 (m, 6H) H-5'G, H-2',2"C & G; 2.09 & 2.06 (2xs, 3H) acetyl; 1.32 (s, 9H) Tbb. ^{31}P-NMR ($CDCl_3$): -6.74, -7.45.

Dimer d(TpA) (101). Procedure K was applied to dimer **97** (86 mg, 79 μmol) to afford dimer **101** (1461 A_{260} o. d. units, 80%). ^{31}P-NMR (D_2O): - 1.00.

Dimer d(CpG) (102). Upon treatment of dimer **98** (98 mg, 79 mmol) as described in Procedure K, compound **102** (1422 A_{260} o. d. units, 90%) was obtained. ^{31}P-NMR (D_2O): -0.88.

Dimer d(ApT) (103). Dimer **99** (86 mg, 79 μmol) was deprotected according to Procedure K to give dimer **103** (1616 A_{260} o. d. units, 89%). ^{31}P-NMR (D_2O): -0.95.

Dimer d(GpC) (104). Dimer **100** (99 mg, 79 μmol) was deprotected as described in Procedure K to give compound **104** (1011 A_{260} o. d. units, 71%). ^{31}P-NMR (D_2O): -0.90.

Conversion of (3',5'-O-(TPDS)-1',2',3',4#',5',5"-$^2H_6$-N^6-benzoyladenosine (30) into the triethylammonium salt of its 3'-(*o*-chlorophenyl)-phosphate (106). After repeated coevaporation with dry pyridine, compound **30** (99 mg, 0.16 mmol) was dissolved in the same solvent (2 ml) and *o*-chlorophenylphosphorobis-(1,2,4-triazolide) (1.3 ml, 0.25 M in acetonitrile) was added. After 90 min excess of reagent was hydrolysed by triethylamine-water mixture and worked up in the usual manner to give compound **106** (142 mg, 97%) as a foam. ^{1}H-NMR ($CDCl_3$): 8.68 (s, 1H) H-8; 8.17 (s, 1H) H-2; 8.1 - 7.1 (m, 9H) benzoyl, *o*-chlorophenyl; 6.34 (s, 1H) H-1'#; 4.15 (s) H-4'#; 3.2-2.8 (m, 6H) CH_2CH_3; 1.23 (t, J= 7.3 Hz, 9H) CH_2CH_3; 1.08, 1.01 & 0.99 (3xs, 24H) methyls of TIPSI. ^{31}P-NMR: -5.42.

Fully protected dimer (A2'p5'A*) (107). Diester block **105** (270 mg, 0.30 mmol) and 5'-hydroxy block **27** (105 mg, 0.23 mmol) were condensed according to Procedure I to get compound **107** (210 mg, 74%). ^{1}H-NMR ($CDCl_3$): 9.08 (br. s, 2H) N-H; 8.78, 8.75, 8.69 & 8.68 (4xs, 2H) H-8; 8.27, 8.24 & 8.19 (3xs, 2H) H-2; 8.1 - 7.4 (m, 10H) Bz; 7.27 & 7.23 (2xs, 4H) *o*-chlorophenyl; 6.30 & 6.09 (2xs, 2H) H-1'A & H-1'#A*; 5.6-5.3 (m, 1H) H-3'A; 5.1 - 4.9 (m, 1H) H-2'A; 4.3 - 3.9 (m, 3H) H-4' & H-5'A; 2.16, 2.13, 2.07 & 2.02 (4xs, 6H) acetyls; 1.08, 1.03, 0.97 & 0.94 (4xs, 24H) methyls of TIPSI. ^{31}P-NMR: -6.66, -6.93.

5'-OH-(A2'p5'A*) (108). Fully protected dimer **107** (203 mg, 0.16 mmol) was dissolved in 0.2 M HCl solution in dioxane-water (4:1) (1.67 ml) and the resulting clear solution was stirred for 2 h at RT, when Tlc showed complete conversion of starting material to a compound of lower R_f. Usual work-up and short column chromatography gave dimer **108** (171 mg, 85%) as a foam. ^{1}H-NMR ($CDCl_3$): 9.36 & 9.17 (2xbr. s, 2H) N-H; 8.79, 8.76, 8.68 (3xs, 2H) H-8; 8.36, 8.30, 8.22 & 8.13 (4xs, 2H) H-2; 8.1 - 7.4 (m, 10H) benzoyls; 7.3 - 6.8 (m, 4H) *o*-chlorophenyl; 6.26 & 6.17 (2xs, 2H) H-1'A & H-1'#A*; 5.8 - 5.5 (m, 1H) H-2'A; 5.0 - 4.9 (m, 1H) H-3'A; 4.3-4.2 (m, 1H) H-4'A; 4.0 - 3.7 (m, 2H) H-5'A; 2.12, 2.09, 2.06 & 2.03 (4xs, 6H) acetyls; 1.03 & 1.00 (2xs, 24H) methyls of TIPSI. ^{31}P-NMR: -7.25, -7.42.

Fully protected trimer (A*2'p5'A2'p5'A*) (109). Condensation of diester block **106** (115 mg, 0.13 mmol) and 5'-hydroxy block **108** (145 mg, 0.13 mmol) was carried out as described in Procedure I to give compound **109** (211 mg, 90%). ^{1}H-NMR ($CDCl_3$): 9.33, 9.17, 9.14 & 9.00 (4x br. s, 3H) N-H; 8.75, 8.72, 8.70, 8.61, 8.60 & 8.58 (6xs, 3H) H-8; 8.26, 8.22, 8.19 & 8.17 (4xs, 3H) H-2; 8.1 - 6.8 (m, 19H) benzoyls, *o*-chlorophenyl; 6.3 - 6.1 (m, 3H) H-1'#A* & A & A*; 5.8 -5.4 (m, 1H) H-3'A; 5.2 - 4.9 (m, 1H) H-2'A; 4.7 - 4.2 (m, 3H) H-4' & H-5'A; 2.11, 2.09, 2.04 & 1.87 (4xs, 6H) acetyls; 1.05, 1.00, 0.95, 0.91 & 0.88 (5xs, 36H) methyls of TIPSI. ^{31}P-NMR: -6.76, -6.81, -6.88, -7.08, -7.13.

Deprotection of fully protected trimer (109) to trimer (A*2'p5'A2'p5'A*) (110). Trimer **109** (113 mg, 55 μmol) and *syn*-4-nitrobenzaldoxime (183 mg 1.1 mmol) were dissolved in dioxane (4 ml) then water was added (2 ml) followed by 1,1,3,3-tetramethylguanidine (137 ml, 1,1 mmol) and the mixture was stirred for 20 h at room temperature. Ammonia solution (32%) (20 ml) was added and stirring was maintained for additional 51 h. Volatile matters were evaporated and after three coevaporations with water, 0.3 M TBAF (THF:pyridine 4:1) (1.1 ml) was added. After stirring for 6 h, solvent was evaporated, residue was coevaporated with water (2x), dissolved in water (25 ml) and extracted with dichloromethane. Water phase was concentrated and applied on a Sephadex A-25 column eluted with ammonium hydrogen carbonate buffer (0.001 - 0.15 & 0.15 - 0.30 M, 500 ml each). After evaporation of appropriate fractions and desalting with coevaporations with water trimer **110** (1871 A_{260} o.d. units, 78%) was obtained. ^{31}P-NMR (D_2O): -1.27, -1.63.

Fully protected dimer (A2'p5'A) (111). Condensation of diester block **105** (0.59 g, 0.65 mmol) and 5'-hydroxy block **63** (0.23 g, 0.50 mmol) was carried out as described in Procedure I to give compound **111** (0.56 mg, 90%), ^{31}P-NMR: -6.66, -6.96.

5'-OH-(A2'p5'A*) (112). Dimer **107** (0.54 g, 0.43 mmol) was treated as described in case of compound **108** to give dimer **112** (0.49 g, 91%) as a foam. ^{31}P-NMR: -7.25, -7.47.

Fully protected trimer (A2'p5'A2'p5'A) (113). Condensation of diester block **105** (152 mg, 0.17 mmol) and 5'-hydroxy block **112** (177 mg, 0.14 mmol) was carried out as described in Procedure I to give compound **113** (226 mg, 79%). ^{31}P-NMR: -6.84, -6.88, -6.93, -7.10, -7.18.

Deprotection of fully protected trimer (113) to trimer (A2'p5'A2'p5'A) (114) Trimer **113** (113 mg, 55 μmol) was deprotected in the same manner as described for trimer **109** to give trimer **114** (2005 A_{260} o.d. units, 84%). ^{31}P-NMR (D_2O): -1.32, -1.65.

Acknowledgements: Authors thank Swedish Board for Technical Development (NUTEK) and Swedish Natural Science Research Council for generous financial support. Authors also thank Wallenbergstiftelsen, University of Uppsala, Forskningsrådsnämnden (FRN) for financial supports for purchasing the 500 MHz NMR spectrometer.

References

1. (a) Jardetzky, O.; Roberts, G. C. K. *NMR in Molecular Biology;* Academic Press: New York, 1981; Chapter 13. (b) Wemmer, D. E.; Reid, B. R. *Ann. Rev. Phys. Chem.* **1985**, *36*, 105. (c) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986. (d) Reid, B. R. *Q. Rev. Biophys.* **1987**, *20*, 1. (e) Van de Ven, F. J. M.; Hilbers, C. W. *Eur. J. Biochem.* **1988**, *178*, 1. (f) Hosur, R. V.; Govil, G.; Miles, H. T. *Magn. Reson. Chem.* **1988**, *26*, 927.
2. (a) Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Domensions*, Clarendon Press: Oxford, 1987. (b) Oppenheimer, N. J.; James, T. L. Eds. *Methods Enzymol.* **1989**, *176*, Chapter 1 & 2.
3. (a) Vuister, G.W.; Boelens, R. *J. Magn. Reson.* **1987**, *73*, 328. (b) Mooren, M. M. W.; Hilbers, C. W.; Van Der Marel, G. A.; Van Boom, J. H.; Wijmenga, S. S. *J. Magn. Reson.* **1991**, *94*, 101. (c) Majumdar, A.; Hosur, R. V. *J. Biomol. NMR*, **1991**, 1, 205. (d) Sörensen, O. W. *J. Magn. Reson.* **1990**, *90*, 433.
4. (a) Weiner, P. K.; Kollman, P. A. *J. Comp. Chem.* **1981**, *2*, 287. (b) (a) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comp. Chem.* **1986**, *7*, 230.
5. (a) Neuhaus, D.; Williamson, M. P. *The Nuclear Overhauser Effect in Structural and Conformational Analysis;* VHC Publishers: New York, 1989. (b) James, T. L. *Current Opinion in Structural Biology* **1991**, *1*, 1042. (c) Torda, A. E.; Scheek, R. M.; van Gunsteren, W. F. *J. Mol. Biol.* **1990**, *214*, 223. (d) Kaluarachchi, K.; Meadows, R. P.; Gorenstein, D. G. *Biochemistry* **1991**, *30*, 8785. (e) Pearlman, D. A.; Kollman, P. A. *J. Mol. Biol.* **1991**, *220*, 457.
6. (a) Brush, C. K.; Stone, M. P.; Harris, T. M. *Biochemistry* **1988**, *27*, 115. (b) Brush, C. K.; Stone, M. P.; Harris, T. M. *J. Am. Chem. Soc.* **1988**, *110*, 4405.
7. Schmidt, R. R.; Scholz, U; Schwille, *D. Chem. Ber.* **1968**, *101*, 590.
8. Schmidt, R. R.; Heermann, D.; Jung, K.-H. *Liebigs Ann. Chem.* **1974**, 1856.
9. Dupre, M.; Gaudemer, A. *Tetrahedron Lett.* **1978**, 2783.
10. Kintanar, A.; Alam, T. M.; Huang, W.-C.; Schindele, D. C.; Wemmer, D. E.; Drobny, G. *J. Am. Chem. Soc.* **1988**, *110*, 6367.
11. Berger, A.; Shaw, A.; Cadet, J. *Nucleosides & Nucleotides* **1987**, *6*, 395.
12. Ajmera, S.; Massof, S.; Kozarich, J. W. *J. Labelled Compd.* **1986**, *23*, 963.
13. Sinhababu, A. K.; Bartel, R. L.; Pochopin, N.; Borchardt, R. T. *J. Am. Chem. Soc.* **1985**, *107*, 7628.
14. Robins, M. J.; Samano, V.; Johnson, M. D. *J. Org. Chem.* **1990**, *55*, 410.
15. Fraser-Reid, B.; Radatus, B. *J. Am. Chem. Soc.* **1971**, *93*, 6342.
16. Radatus, B.; Yunker, M.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1971**, *93*, 3086.
17. (a) David, S.; Eustache, J.; *Carbohyd. Res.* **1971**, *16*, 469. (b) David, S.; Eustache, J.; *Carbohyd. Res.* **1971**, *20*, 319.
18. Wong, M. Y. H.; Gray, G. R. *J. Am. Chem. Soc.* **1978**, *100*, 3548.
19. Pathak, T.; Bazin, H.; Chattopadhyaya, J. *Tetrahedron* **1986**, *42*, 5427.
20. Roy, S.; Hiyama, Y.; Torchia, D. A.; Cohen, J. S. *J. Am. Chem. Soc.* **1986**, *108*, 1675.
21. Wu, J.-C.; Bazin, H.; Chattopadhyaya, J. *Tetrahedron* **1987**, *43*, 2355.
22. Hodge, R. P.; Brush, C. K.; Harris, C. M.; Harris, T. M. *J. Org. Chem.* **1991**, *56*, 1553.
23. Hiyama, Y.; Roy, S.; Cohen, J. S.; Torchia, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 8609.
24. (a) Alam, T. M.; Drobny, G. *Biochemistry* **1990**, *29*, 3421. (b) Alam, T. M.; Orban, J.; Drobny, G. *Biochemistry* **1990**, *29*, 9610. (c) Huang, W.-C.; Orban, J.; Kintanar, A.; Reid, B. R.; Drobny, G. P. *J. Am. Chem. Soc.* **1990**, *112*, 9059. (d) Drobny, G. P.; et al. *Biochemistry* **1991**,*30*, 9229.
25. (a) Kondo, N. S.; Danyluk, S. S. *J. Am. Chem. Soc.* **1972**, *94*, 5121. (b) Kondo, N. S.; Leung, A.; Danyluk, S. S. *J. Labelled Compd.* **1973**, *9*, 497. (c) Kondo, N. S.; Ezra, F.; Danyluk, S. S. *FEBS Lett.* **1975**, *53*, 213. (d) Kondo, N. S.; Danyluk, S. S. *Biochemistry*, **1976**, *15*, 756. (e) Lee, C.-H.; Ezra, F.; Kondo, N. S.; Sarma, R. H.; Danyluk, S. S. *Biochemistry*, **1976**, *15*, 3627. (f) Ezra, F. S.; Lee, C.-H.; Kondo, N. S.; Danyluk, S. S.; Sarma, R. H. *Biochemistry*, **1977**, *16*, 1977.
26. Yang, J. J.-H. *Diss. Abstr. Int. B.* **1980**, *41*, 1726.

27. (a) Koch, H. J.; Stuart, R. S. *Carbohydr. Res.* **1977**, *59*, C1. (b) Balza, F.; Cyr, N.; Hamer, G. K.; Perlin, A. S.; Koch, H. J.; Stuart, R. S. *Carbohydr. Res.* **1977**, *59*, C7. (c) Koch, H. J.; Stuart, R. S. *Carbohydr. Res.* **1978**, *64*, 127. (d) Koch, H. J.; Stuart, R. S.; *Carbohydr. Res.* **1978**, *59*, 341. (e) Balza, F.; Perlin, A. S. *Carbohydr. Res.* **1982**, 107, 270. (f) Angyal, S. J.; Odier, L. *Carbohydr. Res.* **1983**, *123*, 13. (g) Wu, G. D.; Serianni, A. S.; Barker, R. *J. Org. Chem.* **1983**, *48*, 1750. (h) Angyal, S. J.; Stevens, J. D.; Odier, L. *Carbohydr. Res.* **1986**, *157*, 83. (i) Kline, P. C.; Serianni, A. S. *Magn. Reson. Chem.* **1988**, *26*, 120. (j) Kline, P. C.; Serianni, A. S. *Magn. Reson. Chem.* **1990**, *28*, 324.
28. Pathak, T.; Chattopadhyaya, J. *Tetrahedron* **1987**, *43*, 4227.
29. Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.
30. (a) Sund, C.; Agback, P.; Koole, L. H.; Sandström, A.; Chattopadhyaya, J. *Tetrahedron* **1992**, *48*, 695. and references therein (b) Maltseva, T.; Sandström, A.; Zarytova, V.; Chattopadhyaya, J. manus. in prep.
31. (a) Vorbrüggen, H.; Höfle, G. *Chem. Ber.* **1981**, *114*, 1256. (b) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.
32. Barker, R.; Fletcher Jr., H. G. *J. Org. Chem.* **1961**, *26*, 4605.
33. (a) Mozingo, R. Org. Synth. 1985, 3, 181. (b) Augustine, R. L. "Catalytic Hydrogenation"; Marcell Dekker: NY, 1965; p 147.
34. Adkins, H.; Billica, H. R. *J.Am.Chem.Soc.* **1948**, *70* , 695.
35. Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777.
36. Davoll, J.; Lowy, B. A. *J.Am.Chem.Soc.* **1951**, *73*, 1650.
37. Zou, R.; Robins, M. J. *Can. J. Chem.* **1987**, *65*, 1436.
38. Altermatt, R.; Tamm, C. *Helv. Chim. Acta* **1985**, *68*, 475.
39. Brown, E. L.; Belagaje, R.; Ryan, M. J.; Khorana, H. G. *Methods Enzymol.* **1979**, *68*, 109.
40. Markiewicz, W. T. *J. Chem. Research (S).* **1979**, 24.
41. Van der Kerk, G. J. M.; Noltes, J. G.; Luijten, J. G. A. *J. Appl. Chem.* **1957**, *7*, 366.
42. Remaud, G.; Zhou, X.-X.; Chattopadhyaya, J.; Oivanen, M.; Lönnberg, H. *Tetrahedron* **1987**,*43*, 4453.
43. Reese, C. B. *Tetrahedron* **1978**, *34*, 3143.
44. (a) Sandström, A.; Kwiatkowski, M.; Chattopadhyaya, J. *Acta Chem. Scand.* **1985**, *B39*, 273. (b) Chattopadhyaya, J.; Reese, C. B. *Tetrahedron Lett.* **1979**, 5059. (c) Kwiatkowski, M.; Heikkilä, J.; Björkman, S.; Chattopadhyaya, J. *Chemica Scripta* **1983**, *22*, 30.(d) Zhou, X. X.; Sandström, A.; Chattopadhya, J. *Chemica Scripta* **1986**, *26*, 241.
45. Jones, S. S.; Rayner, B.; Reese, C. B.; Ubasawa, A.; Ubasawa, M. *Tetrahedron* **1980**, *36*, 3075.
46. Reese, C. B.; Titmus, R. C.; Yau, L. *Tetrahedron Lett.* **1978**, 2727.
47. (a) Stewart, W. E. *The Interferon System* 2nd Ed. Springer Verlag, New York 1981. (b) Lengyel, P. *Ann. Rev. Biochem.* **1982**, *51*, 251. (c) Johnston, M. I.; Torrence, P. F. In: Friedman, R. M.; Ed., Finter, N. B., Series Ed. *Interferon, Mechanisms of Production and Action* Elsevier Science, Amsterdam 1984, Vol. 3, Chapter 7., pp. 189-298.
48. Doornbos, J.; Den Hartog, J. A. J.; Van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1981**, *116*, 403.
49. (a) Chattopadhyaya, J. B. *Tetrahedron Lett.* **1980**, *21*, 4113. (b) Gioeli, C.; Kwiatkowski, M.; Öberg, B.; Chattopadhyaya, J. B. *Tetrahedron Lett.* **1981**, *22*, 1741. (c) Nyilas, Á.; Vrang, L.; Drake, A.; Öberg, B.; Chattopadhyaya, J. *Acta Chem. Scand.* **1986**, *B40*, 678.
50. Mitchell, T. N. *J. Organometal. Chem.* **1973**, *59*, 189.
51. Potter, P. E.; Pratt, L.; Wilkinson, G. *J. Chem. Soc.* **1964**, 524.
52. Piantini, O. W.; Sorensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.***1982**, *104*, 6800.
53. Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301.
54. Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501.
55. (a) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542. (b) Aue, W. P.; Batholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.
56. Edwards, M. W.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 918.
57. Kessler, H; Gehrke, M; Griesinger, C. *Angew. Chem. Int. Ed. Engl.* **1988**, *27* , 490.
58. Jeener, J.; Meier, B. H.; Bachmann P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546.
59. Macura, S.; Huang, Y.; Suter D.; Ernst, R. R. *J. Magn. Reson.* **1981**, *43*, 259.
60. Piantini, O. W.; Sorensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800.
61. Griesinger, C.; Sorensen, O. W.; Ernst, R. R. *J. Magn. Reson.* **1987**, *75*, 474.
62. Varani, G.; Tinoco, I. *Quart. Rev. Biophys.* **1991**, *4*, 479.
63. Varani, G.; Tinoco, I. *J. Am. Chem. Soc.* **1991**, *113*, 9349.
64. Morris, G. A.; Freeman, R. *J. Am. Chem. Soc.* **1979**, *101*, 760.
65. Bolton, P. H. *J. Magn. Reson.* **1980**, *41*, 287.
66. Burum, D. P.; Ernst, R. R. *J. Magn. Reson.* **1980**, *39*, 163.
67. Rinaldi, P. L.; Baldwin, N. J. *J. Am. Chem. Soc.* **1983**, *105*, 7523.
68. Rinaldi, P. L.; Baldwin, N. J. *J. Am. Chem. Soc.* **1982**, *104*, 5791.
69. Földesi, A; et al., *J. Biochem. Biophys. Methods* (submitted)