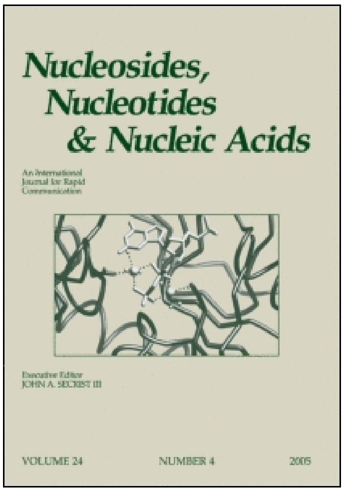

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Stereoselective Nucleoside Deuteration for NMR Studies of DNA

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STEREOSELECTIVE NUCLEOSIDE DEUTERATION FOR NMR STUDIES OF DNA

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□ *A procedure has been elaborated for stereoselective deuterium substitution of one of the diastereotopic 5′-protons in 2′-deoxynucleotides. The synthetic scheme uses the reduction of the 5-oxosugar derivative with deuterated Alpine-Borane. The resulting deuterosugar is converted into pyrimidine nucleosides and incorporated into DNA using standard protocols. Comparison of two-dimensional NMR spectra of the fully protonated and partially deuterated duplexes allowed us to assign diastereotopic 5′ protons, increasing the number of experimental restraints used for structure determination.*

Keywords Deuterated nucleosides; oligonucleotides; solution state NMR; DNA lesions

INTRODUCTION

The 5′ deoxyribose protons generate multiple inter- and intra-nucloetide nOe contacts and scalar coupling interactions that can provide valuable structural information about key sugar-phosphate backbone angles in nucleic acids. However, the usage of such data poses a serious challenge, since the 4′ and 5′ protons form a non-first order ABC spin system that is hard to assign unambiguously. Chemical shift based rules that allow the assignment of 5′ and 5′′ resonances in canonical B-form DNA are hardly applicable for non-standard DNA structures or for duplexes bearing modified nucleotides. In addition, the spectral range where 5′ sugar protons resonate is very narrow, so the 5′ proton signals have severe overlap. As a result, examples of efficient and reliable utilization of structural information derived from the nucleotide 5′ proton resonances are scarce.

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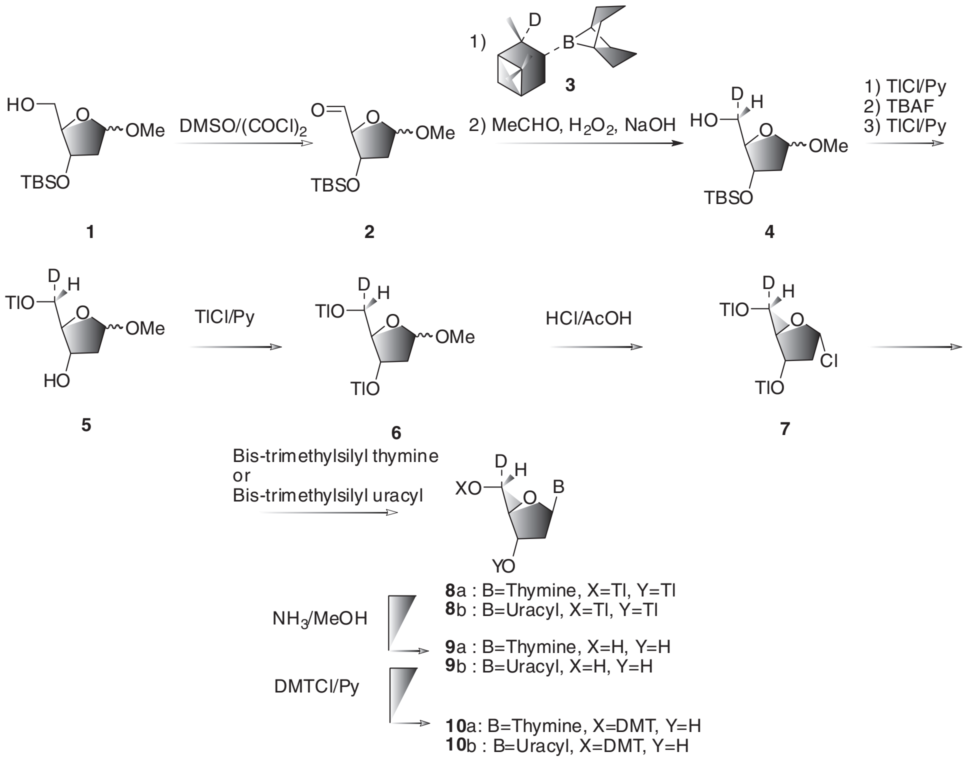
Selective deuteration as a method for masking resonances in the NMR spectra of nucleic acids has become popular during last decades.[1] In this regard, simplification of signals from the 5′-methylene group can be achieved by the selective deuteration of one of its hydrogens. This could be done by either conversion of this group into a chiral synthon (e.g., α-halogen[2] or α-selenophenyl[3] ether), followed by a reduction with a deuteride ion donor, or oxidation to the corresponding 5′ aldehyde, followed by stereospecific reduction.[4]Although the first approach allows the usage of more general reducing agents, it is usually more laborious requiring many synthetic steps that frequently have insufficient stereospecificity. Therefore, a number of approaches have been used for the reduction of deuterated 5′-oxosugar derivatives by chiral hydride donors, including (−)-isobornyloxymagnesium bromide,[4] chloro(diisopinocampheyl)borane,[5] or AlpineBorane.[6]

Alternatively, non-deuterated 5′-oxosugars were reduced with deuterated isobornyloxymagnesium bromide or deutero-AlpineBorane,[7] with the latter approach successfully utilized for preparation of 5′-deuterated ribonucleosides.[8] Since the preparation of deuterated AlpineBorane is straightforward following hydroboration of α-pinene with in situ generated deuteroborabicyclononane (*d*-BBN),[7] we utilized this approach for our structural study of damaged DNA.

RESULTS AND DUSCUSSION

Although the synthesis of 5′-monodeuterated ribonucleosides can be successfully performed starting from 5′-oxonucleosides, the same approach worked poorly for the preparation of 2′-deoxynucleotides, presumably due to the insufficient stability of 5′-oxo-2′-deoxynucleosides (data not shown). Therefore we decided to use a sugar precursor for our synthesis. The starting material for the synthesis (Scheme 1), 3-O-*t*-butyldimethylsilyl-2-deoxy-D-*erythro*-pentofuranoside (**1**) was obtained in three steps from deoxyribose.[9] Oxidation of the 5-hydroxyl with DMSO/oxalyl chloride yielded the aldehyde (**2**), which was immediately subjected to asymmetric reduction by *B*-3-pinanyl-9-borabicyclo[3.3.1]nonane (**3**) (Alpine-Borane-*d*) without previous purification. Alpine-Borane-*d* was prepared by hydroboration of *R*-(+)-α-pinene with *B*-deutero-BBN generated in situ by treatment of commercially available *B*-methoxy-9-BBN with lithium aluminium deuteride. Treatment of the aldehyde with Alpine-Borane-*d* yielded the deuterated deoxyriboside in almost quantitative yield. The deuterated sugar (**4**) was converted into the di-*p*-toluyl ester (**6**) and then into the α-chlorosugar (**7**) with 54% overall yield according to the standard procedure (Scheme 1).[10] Coupling of the chlorosugar with bis-trimethylsilyl thymine or uracyl in standard conditions[11] produced protected thymidine or 2′-deoxyuridine (**8a**,**b**), respectively, compounds that were further converted into 5′-dimethoxytrithylated



SCHEME 1 Synthetic procedure used for preparation of partially deuterated nucleosides.

nucleosides (**10a**,**b**). High-resolution MS analysis indicated 97.7% deuterium incorporation and (Figure 1, right panel), whereas ^{1}H-NMR spectra demonstrated disappearance of the $5'H_R$ proton (configuration at C-5′ was determined according to the mechanism of reduction as proposed by Midland *et al.*[7]) with enantiomeric ratio greater than 95% (Figure 1, left panel). Proton decoupled ^{13}C-NMR spectra of the deuterated derivatives contained a triplet signal typical for deuterium-carbon spin-spin coupling ($J_{D,C} = 19.5$ Hz) that served as additional evidence for deuterium incorporation (Figure 2). Since conversion of deoxyuridine to deoxycytidine is straightforward[11], this method can be readily extended to the preparation of deuterated dC derivatives.

Subsequent phosphoramidite preparation and solid phase synthesis of deuterated oligonucleotides were performed following standard protocols without modifications.[12]

Implementation of this approach was vital for structure determination of a damaged undecamer duplex containing at its center the dA lesion of aristolochic acid II paired to thymidine. Aristolochic acids (AAs) are a family of naturally occurring nitroaromatic compounds that, upon metabolic activation to a reactive carbenium cation, react with the exocyclic amino groups

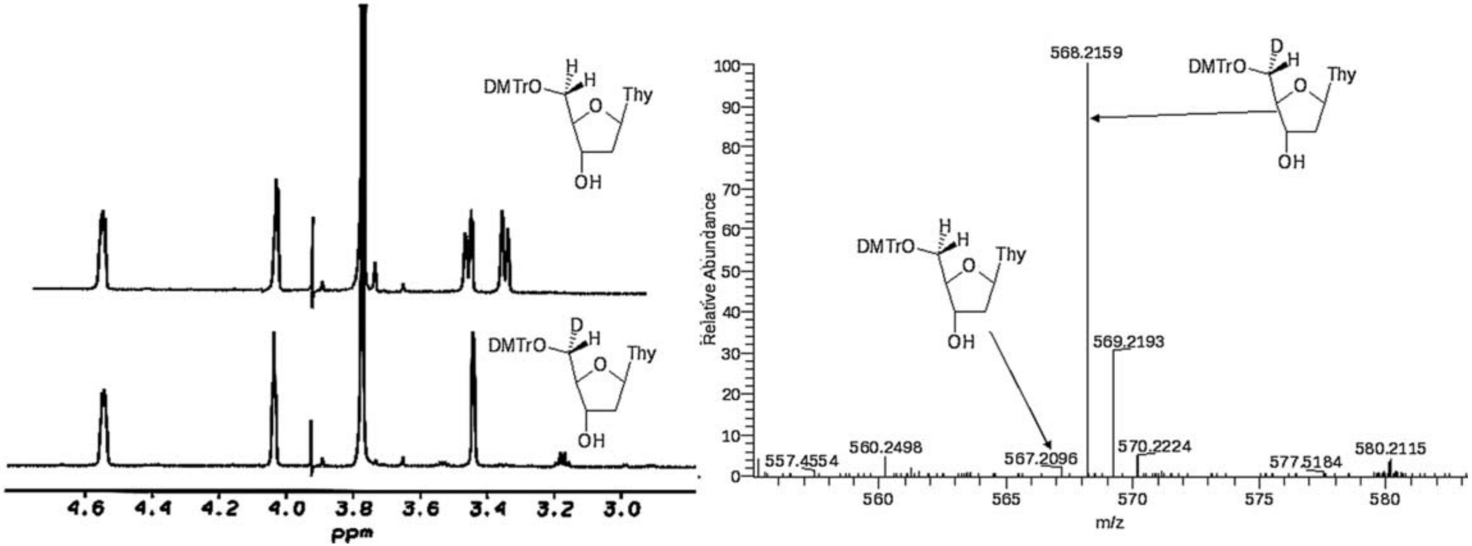


FIGURE 1 Left) Fragments of ^{1}H-NMR spectra of non-deuterated (top) and deuterated (bottom) dimethoxytritylthymidine, that demonstrate disappearance or the pro-S proton upon deuteration. Right) High-resolution mass spectrum of 5′-^{2}H-5′-dimethoxytritylthymidine (**9a**).

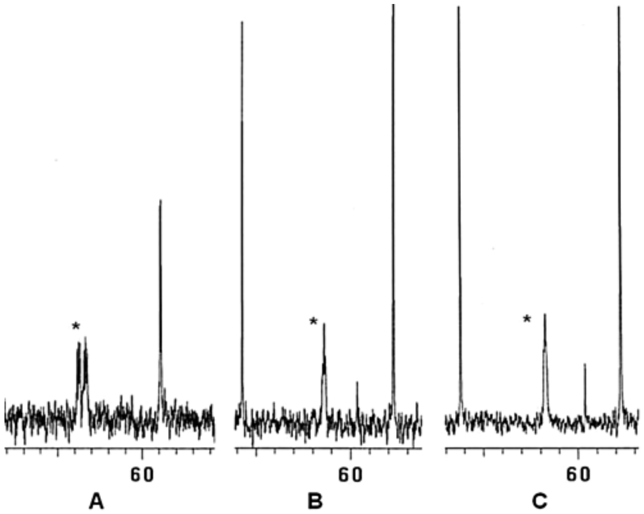


FIGURE 2 Fragments of proton decoupled ^{13}C-NMR spectra of methyl-5–^{2}H-3,5-O-*bis*-p-toluoyl-2-α,β-D-erythro-pentofuranoside (left), 5′-^{2}H-5′-dimethoxytritylthymidine (center), and 5′-^{2}H-5′-dimethoxytrityl-2′-deoxyuridine (right). The signals of C-5 (C-5′) with carbon-deuterium splitting are marked with asterisks.

of adenine and guanine forming highly mutagenic lesions.[13] In order to elucidate molecular mechanisms of lesion repair and mutagenesis, it is necessary to know the three-dimensional structure of duplex DNA containing AA lesions (Figure 3). NMR studies of the lesion containing duplex demonstrated that the thymidine complementary to the AA adduct assumed an extrahelical orientation. As a result, it exhibited a limited number of nOe contacts with adjacent nucleosides and its conformation poorly defined in the final refined structure. To overcome this problem, we prepared the duplex containing a stereoselectively deuterated thymidine residue opposite the AA lesion. Comparison of the NOESY spectra of the fully protonated and partially deuterated duplexes (Figure 4, left) allowed us to assign both

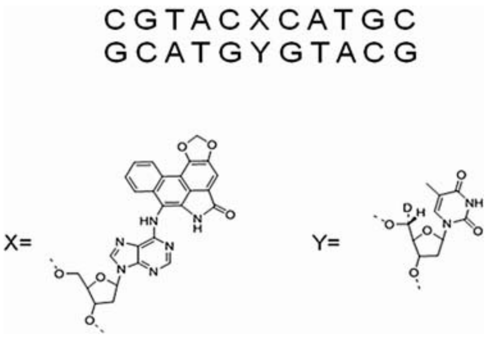


FIGURE 3 The oligonucleotide duplex bearing an ALII lesion used in the present study.

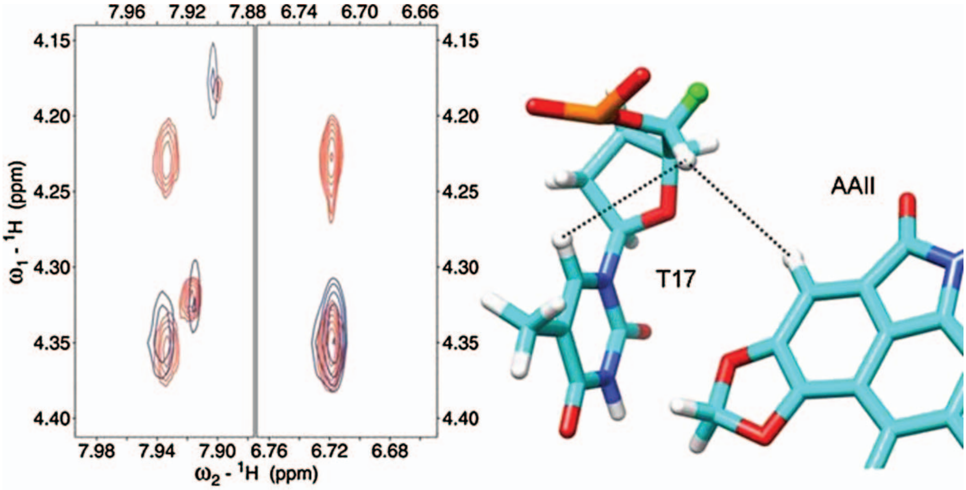


FIGURE 4 Left) Superimposed regions of two-dimensional ^{1}H-NOESY spectra of DNA duplexes containing an aristolochic acid II generated lesion. The spectrum of the non-deuterated duplex is shown in red and that of the 5′-monodeuterated duplex is in blue. Right) Close up view of the lesion site structure after energy minimization of the damageded duplex. Distances corresponding to the cross-peaks visible only on the deuterated duplex's spectrum are shown as dashed lines. The 5″-Deuterium is shown in green.

diastereotopic 5′ protons unequivocally, increasing the number of experimental restraints used for structure determination. Energy minimized three-dimensional structure of the duplex (Figure 4, right)[14] demonstrated that the nOe distances derived from the 5′ and 5″ protons provided valuable information for defining the thymidine glycosidic χ and sugar γ torsion angles, as well as the relative localization of the thymine and the aristolochic acid residues in the duplex.

CONCLUSION

We developed a simple and robust scheme for the preparation of stereoselectively deuterated 5′-pyrimidine nucleosides, in quantities suitable for chemical synthesis of oligonucleotides in amounts needed for NMR studies. We demonstrated that the use of the stereoselective deuterium labeling lead to considerable simplification of NMR spectra and facilitated access to otherwise inaccessible information used for determination of the duplex conformation. A manuscript describing the complete NMR structure determination of duplex DNA having an AAII-dA adduct will be submitted elsewhere.

EXPERIMENTAL

General Methods

NMR spectra were recorded on Varian Inova (400, 500, or 600 MHz, Varian Inc., CA, USA) and Brucker Avance (600 or 800 MHz, Bruker BioSpin, MA, USA) spectrometers. Chemical shifts are reported in parts per million (ppm) relative to the residual proton signal (7.26 ppm) of deuterated chloroform or to the central carbon peak (77.0 ppm) for proton and carbon spectra, respectively. Analytical thin layer chromatography (TLC) was performed with E. Merck precoated TLC plates, silica gel 60F-254, layer thickness 0.25 mm. Flash chromatography separations were performed on E. Merck Kieselgel 60 (230–400) mesh silica gel.

Methyl-5–^{2}H-5-O-p-toluoyl-3-O-tert-butyldimethylsilyl-2-α,β-D-erythro-pentofuranoside (4)

A mixture of dimethyl sulfoxide (71 mL, 100 mmol) in 20 mL of *dry* CH_2Cl_2 was added drop wise to a cooled (−55°C) solution of oxalyl chloride (40 mL, 46 mmol) and dry dichloromethane (120 mL) under nitrogen. The reaction mixture was stirred at −50°C for 30 minutes and methyl-3-O-tert-butyldimethylsilyl 2′-deoxyribofuranoside (7.39 g, 30 mmol) in CH_2Cl_2 (50 mL) was added drop wise via syringe during a period of 30 minutes under vigorous stirring. After complete addition, the reaction mixture was further stirred at −55°C for one more hour, time after which 30 mL of dry triethylamine were added drop wise via syringe and the mixture allowed to warm to room temperature. Saturated sodium bicarbonate (200 mL) was added, the organic layer removed, subsequently washed with saturated sodium bicarbonate, brine, and later dried over Na_2SO_4 and filtered through Celite. The solvent was removed under reduced pressure and the resulting aldehyde was dissolved in 50 mL of THF and ^{2}H-AlpineBorane (18.3 mL, 60 mmol), prepared according to the Midland's procedure from (+)-α-pinene and deutero-BBN[7] was added under atmosphere of nitrogen. The reaction was stirred overnight at room temperature, quenched by addition of 6 mL acetaldehyde, stirred for an additional hour, followed by dropwise addition of 6 mL THF, 60 mL of 1M NaOH and 20 mL of 30% H_2O_2 with vigorous stirring and cooling in an ice bath. Saturated sodium bicarbonate (200 mL) and ethyl acetate (200 mL) were added, organic layer separated, washed with brine, dried over Na_2SO_4, and evaporated. The transparent viscous oil residue was dissolved in 150 mL of acetonitrile and washed with hexane (2 × 100 mL), the acetonitrile layer was separated, evaporated, co-evaporated with pyridine (2 × 50 mL), dissolved in anhydrous pyridine (100 mL) followed by addition of p-toluoyl chloride (6 mL, 45 mmol). The reaction was left 5 hours at room temperature, partitioned between sodium bicarbonate and ethyl acetate, the organic layer was separated, washed with sodium bicarbonate (3 × 200 mL), water (2 × 200) and brine, dried over Na_2SO_4, evaporated to dryness and co-evaporated with toluene. Flash chromatography of the residue (hexane–EtOAc 1:1) gave methyl-5–^{2}H-5-O-p-toluoyl-3-O-*tert*-butyldimethylsilyl-2-deoxyribofuranoside as a semitransparent viscous oil (5.1 g, 12.9 mmol, 43% from **1**). NMR showed that the desired product was still contaminated with low polar compounds that were separated out at the next step. ^{1}H-NMR, anomeric mixture (500 MHz, $CDCl_3$) δ 7.98–7.95 (m, 2H, ArH), 7.23–7.28 (m, 4H, ArH), 5.08–5.04 (m, 1H, H1 $\alpha+\beta$), 4.53 (m, 1H, H4 $\alpha+\beta$) 4.46 (m, 1H, H5 $\alpha+\beta$), 4.21 (q, 0.5H, H3 α or β), 4.14 (m, 0.5H, H3 α or β), 3.41(s, 1.5H, OCH_3 α or β), 3.32 (s, 1.5H, OCH_3 α or β), 2.48 m, 1H, 2.42 (s, 3H, $ArCH_3$ α or β), 2.24–2.08 (m, 1H, H2′$\alpha+\beta$) 1.90–1.86 (m, 1H, H2 $\alpha+\beta$) 0.89 (s, 9H, t-Bu), 0.06 (s, 6H, $SiCH_3$). ^{13}C-NMR (125.7 MHz, $CDCl_3$) δ166.7, 143.9, 130.4, 130.0, 129.9, 129.7, 129.4, 129.3, 129.2, 127.5, 105.2, 104,6, 84.0, 81.1, 72.5, 72.0, 64.8 (t, $J_{C,D} = 19.6$ Hz), 63.6 (t, $J_{C,D} = 19.6$ Hz), 55.3, 55.1, 42.3, 42.0, 33.6, 31.7, 27.4, 25.9, 25.6, 23.1, 21.9, 18.14, −4.3, −4.4, −4.6.

Methyl-5–^{2}H-5-O-p-toluoyl-2-α,β-D-erythro-pentofuranoside (5)

A 1.0 M solution of TBAF in THF (40 mL) was added to methyl-5–^{2}H-5-O-p-toluoyl-3-O-*tert*-butyldimethylsilyl-2-deoxyribofuranoside (4.24 g, 11.64 mmol) and the homogeneous solution was left overnight at room temperature. The mixture was poured into a saturated sodium bicarbonate solution, extracted with ethyl acetate, the organic layer was washed with saturated sodium bicarbonate, dried over Na_2SO_4, and evaporated to dryness. Flash chromatograpy of resulting oily residue (methanol, 0–5% in methylene chloride) gave 2.6 g of methyl-5–^{2}H-5-O-p-toluoyl-2-deoxyribofuranoside (10.35 mmol, 89%) as a yellowish transparent oil. ^{1}H-NMR, anomeric mixture (500 MHz, $CDCl_3$) δ 7.96 (d, 1H, ArH), 7.90 (d, 1H, ArH), 7.24 (d, 2H, ArH), 5.14 (d, 0.5H, H1 β or α), 5.10 (d, 0.5H, H1 α or β), 4.55 (m, 1H, H4$\alpha+\beta$), 4.43–4.39(m, 1H, H5$\alpha+\beta$), 4.32–4.18(m, 1H, H3 $\alpha+\beta$), 1H, 3.40 (s, 1.5H, OCH_3 α or β), 3.32 s, 1.5H, OCH_3 α or β), 2.41 (s, 3H, $ArCH_3$), 2.32–2.08 (m, 2H, H2 $\alpha+\beta$). ^{13}C-NMR (125.7 MHz, $CDCl_3$) δ166.8, 166.6, 144.1, 144.0, 130.0, 129.9, 129.4, 129.3, 127.4, 127.2, 105.7, 105.3, 85.2, 83.9, 73.3, 72.6, 65.1 (t, $J_{C,D} = 19.1$ Hz), 64.3 (t, $J_{C,D} = 19.1$ Hz), 55.2, 41.8, 41.1, 21.8.

Methyl-5–^{2}H-3,5-O-bis-p-toluoyl-2-α,β-D-erythro-pentofuranoside (6)

N-methylimidazole (100 μl) were added to methyl-5–^{2}H-5-O-p-toluoyl-2-deoxyribofuranoside (2.27 g, 9.22 mmol) dissolved in 20 mL of anhydrous pyridine and p-toluoyl chloride (1.32 mL, 10 mmol). The mixture was left overnight at room temperature. Methanol (5 mL) was added and the reaction mixture evaporated, the residue dissolved in 100 mL of methylene chloride, washed with saturated sodium bicarbonate (3 × 200 mL), brine, dried over Na_2SO_4, and evaporated in vacuo. The residue (3.5 g, 8.67 mmol, 95%) was used without further purification. A sample for NMR study was additionally purified by crystallization from methanol. ^1H-NMR, anomeric mixture (500 MHz, $CDCl_3$) δ 7.98 (m, 2H, ArH), 7.94 (d, 2H, ArH), 7.23 (m, 4H, ArH), 5.60 (t, 0.53H, H1β), 5.41 (m, 0.47H, H1α), 5.19–5.24 (m, 0.5H, H4 α or β) 5.19 (m, 0.5H, H4 α or β), 4.36–4.61 (m, 2H, H3+H5 $\alpha+\beta$), 3.43 (s, 1.5H, OCH_3 α or β) 3.37 (s, 1.5H, OCH_3 β or α), 2.58–2.52 (m, 2H, H2 $\alpha+\beta$) 2.42 (s, 3H, $ArCH_3$) 2.40 (s, 3H, $ArCH_3$), 2.57–2.22 (m, 2H, H2 $\alpha+\beta$). ^{13}C-NMR (125.7 MHz, $CDCl_3$) δ 166.7, 166.5, 166.3, 144.2, 144.1, 144.0, 130.0, 129.9, 129.3, 127.3, 105.9, 105.3, 82.0, 81.1, 75.6, 74.8, 65.0 (t, $J_{C,D}$ = 19.5 Hz), 64.2 (t, $J_{C,D}$ = 19.5 Hz), 39.5, 21.9.

5′-^{2}H-3′,5′-bis-O-toluolyl-thymidine (8a) and 5′-^{2}H-3′,5′-bis-O-toluolyl-2′-deoxyuridine (8b)

Methyl 5-;^{2}H -3,5-bis-O-toluolyl-2-deoxyriboside (**6**) (1.91 g, 5.18 mmol) was dissolved in 15 mL of glacial acetic acid—CH_2Cl_2 (1:1), the mixture was cooled at 0°C and a stream of dry HCl was passed through the reaction mixture. After 1 hour the white precipitate was filtered off, washed with small amount of ice cold ether, dried in vacuo over KOH and immediately converted into corresponding nucleosides. Yield 1.45 g, (3.88 mmol, 75% from **6**).

Thymine (0.38 g, 3 mmol) was refluxed overnight in a mixture of chlorotrimethylsilane (5 mL) and hexamethyldisilazane (15 mL). The solution was allowed to cool and the volatile materials were evaporated in vacuo. The residue was dissolved in 20 mL of anhydrous CH_2Cl_2, the chloro sugar **7** (0.84 g, 2.25 mmol) added, and the reaction mixture was stirred for 6 hours, time after which MeOH (1 mL) was added and the excess thymine removed by filtration. The filtrate was evaporated, and the residue was recrystallized from EtOAc, yielding 0.81 g (1.69 mmol) of 5′-^{2}H -3′,5′-bis-O-toluolylthymidine (**8a**, 75% from **7**). ^1H-NMR (500 MHz, $CDCl_3$) δ 8.55 (1H, br s, NH), 7.97–7.93 (4H, m, ArH), 7.30–7.27 (5H, m, ArH+6H), 6.49–6.45 (1H, dd, J = 5.3Hz, 8.1 Hz, H1′), 5.64 (1H, m, H3′), 4.78 (1H, d, J = 2.8 Hz, H5′), 4.53 (1H, m, H4′), 2.73–2.69 (1H, ddd, J = 14.3Hz, 5.3 Hz, 1.3 Hz, H2′ or H2″), 2.50 (3H, s, $ArCH_3$) 2.44 (3H, s, $ArCH_3$), 2.33–2.29 (1H, m, H2″ or H2′), 1.64 (3H, s, CH_3).

^{13}C-NMR (125.7 MHz, $CDCl_3$) δ 166.3, 163.5, 150.4, 144.8, 130.1, 129.8, 129.7, 129.5, 126.8, 126.5, 111.9, 85.2, 83.0, 75.1, 64.1 (br. t), 38.3, 21.9, 12.3.

Uracyl (0.28 g, 2.5 mmol) was refluxed overnight in a mixture of chlorotrimethylsilane (5 mL) and hexamethyldisilazane (15 mL). The solution was allowed to cool and the volatile materials were evaporated in vacuo. The residue was dissolved in 20 mL of anhydrous CH_2Cl_2, the chloro sugar **7** (0.59 g, 1.57 mmol) was added, and the reaction mixturestirred for 6 hours, time after which MeOH (1 mL) was added and the excess of uracil was removed by filtration. The filtrate was evaporated and the residue was recrystallized from EtOAc, yielding 0.57 g (1.22 mmol) of 5′-^{2}H-3′,5′-bis-O-toluolyl-2′-deoxyuridine (**8b**, 78% from **6**). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.5 (1H, br s, NH), 7.97 (4H, m, ArH), 7.91 d, 4H, 7.54 d, 1H, 7.29–7.25 m, 4H, 6.4 t, 1H, 5.6 m, 2H, 4.7 m, 1H, 4.5 m, 1H, 2.78–2.74 m, 1H, 2.44 s, 6H, 2.34–2.30 m, 1H. ^{13}C-NMR (125.7 MHz, $CDCl_3$) δ 166.3, 162.7, 150.2, 144.9, 139.0, 130.0, 129.9, 129.7, 129.6, 129.5, 126.7, 126.5, 103.1, 85.6, 83.2, 74.8, 63.6 (br. t), 38.6.

5′-^{2}H-5′-dimethoxytritylthymidine (10a)

5′-^{2}H-3′,5′-O-bis-*p*-toluoylthymidine (**8a**, 0.66 g, 1.38 mmol) was added to 100 mL of saturated methanolic ammonia solution and the mixture was stirred overnight at room temperature. When the nucleotide was fully deprotected (TLC, chloroform-methanol 10:1), the mixture was evaporated in vacuo. The residue was co-evaporated with pyridine (2 × 10 mL) and dissolved in 10 mL of dry pyridine. Dimetoxytrityl chloride (0.68 g, 2 mmol) was added and the reaction mixture left overnight at room temperature. After disappearance of the starting nucleoside (TLC, chloroform-methanol 9:1), 150 mL of saturated sodium bicarbonate was added and the reaction mixture extracted with EtOAc (2 × 100). Combined organic extracts were washed with sodium bicarbonate, brine, dried over over Na_2SO_4, evaporated in vacuo and co-evaporated with toluene (2 × 10 mL). Flash-chromatography of the resulting residue (metanolic gradient, 0–5% in CH_2Cl_2 + 1% triethylamine) gave the final product, 5′-^{2}H-5′-dimethoxytritylthymidine (**10a**) as yellowish foam (0.61 g, 1.12 mmol, 81% from **8a**). ^{1}H-NMR (500 MHz, $CDCl_3$ + triethylamine) δ 7.61 (1H, s, H6), 7.40 (1H, d, J = 7.2 Hz, ArH), 7.30 (6H, m, ArH), 7.20 (7H, m, ArH) 6.84 (4H, d, J = 9.1 Hz, ArH), 6.43 (1H, ψt, J = 6.1 Hz, H1′), 4.56 (1H, m, H3′), 4.07 (1H, m, H4′), 3.78 (6H, s, OCH_3), 3.50 (H1, d, J = 3.0 Hz, H5′), 2.45–2.41 (1H, m, H2′ or H2″), 2.33–2.75 (1H, m, H2″ or H2′), 1.43 (3H, s, CH_3). ^{13}C-NMR (125.7 MHz, $CDCl_3$ + triethylamine) δ 164.3, 158.9, 150.8, 144.6, 135.9, 135.7, 135.6, 130.3, 128.3, 128.2, 127.3, 113.5, 111.4, 86.4, 85.0, 72.39, 64.5 (br. t.), 55.4, 46.2, 41.2, 12.0, 11.4. HRMS [M-Na]$^+$ Calc. m/z 568.2164, obs. m/z 568.2159 (mass tolerance 10 ppm).

5′-^{2}H-5′-dimethoxytrityl-2′-deoxyuridine (10b)

5′-^{2}H -3′,5′-O-bis-*p*-toluoyl-2′-deoxyuridine (**8b**, 0.54 g, 1.16 mmol) was added to 100 mL of saturated methanolic ammonia solution and the mixture was stirred at room temperature overnight. When the nucleotide was fully deprotected (TLC, chloroform-methanol 10:1), the mixture was evaporated in vacuo, the residue co-evaporated with pyridine (2 × 10 mL) and dissolved in 10 mL of dry pyridine. Dimetoxytrityl chloride (0.64 g, 1.9 mmol) was added and the reaction mixture was left overnight at room temperature. After disappearance of the starting nucleoside (TLC, chloroform-methanol 9:1), 150 mL of saturated sodium bicarbonate was added and the reaction mixture extracted with EtOAc (2 × 100). Combined organic extracts were washed with sodium bicarbonate, brine, dried over Na_2SO_4, evaporated in vacuo, and co-evaporated with toluene (2 × 10 mL). Flash-chromatography of the resulting residue (metanolic gradient, 0–5% in CH_2Cl_2 + 1% triethylamine) gave the final product, 5′-^{2}H-5′-dimethoxytrityl-2′-deoxyuridine (**10b**) as yellowish foam. (0.48 g, 0.9 mmol, 78% from **8b**) ^{1}H-NMR (500 MHz, $CDCl_3$ + triethylamine) δ 7.81 (1H, d, J = 8.1 Hz, H6), 7.41 (1H, d, J = 7.2Hz, ArH), 7.28–7.22 (m, 8H, ArH) 6.84 (4H, d, J = 8.71 Hz, ArH), 6.31 (1H, ψt, J = 6.1 Hz, H1′), 5.39 (1H, d, J = 8.1 Hz, H5), 4.54 (1H, m, H3′), 4.01 (1H, m, H4′), 3.75 (6H, s, OCH_3), 3,45 (1H, d, J = 3.1 Hz, H5′), 2.46–2.41 (1H, m, H2′ or H2″), 2.27–2.22 (1H, m, H2″ or H2′). ^{13}C-NMR (125.7 MHz, $CDCl_3$+ triethylamine) δ 163.4, 159.0, 150.5, 144.5, 140.4, 135.6, 135.5, 130.3, 130.2, 128.4, 128.2, 127.4, 113.5, 102.4, 87.2, 86.2, 85.2, 71.5, 62.5 (br. t), 55.5, 46.3, 41.5, 11.5. HRMS $[M\text{-}Na]^+$ Calc. m/z 554.2008, obs. m/z 554.2004 (mass tolerance 10 ppm)

Preparation of Phosphoramidite and Oligonucleotide Synthesis

Conversion of **10a** into corresponding phosphoramidite and subsequent solid phase synthesis of the deuterated oligonucleotide (the bottom strand of duplex in Figure 3) were performed following standard protocols without modifications.[12] 5′-^{2}H-5′-dimethoxytritylthymidine (**10a**, 65 mg, 0.119 mmol) was co-evaporated with dry toluene. Diisopropylammonium tetrazolide (10mg, 0.059 mmol) was added, followed by addition of 2 ml of dry dichloromethane under argon atmosphere. *Bis*-(N-diisopropylamino)-2-cyanoethoxy phosphine (27 ul, 0.13 mmol) was added and the mixture stirred overnight under argon atmosphere. After addition of dichloromethane (5 mL), and the mixture was extracted with 2% potassium carbonate and brine, dried over Na_2SO_4, evaporated in vacuo, co-evaporated with dry acetonitrile and used immediately for the oligonucleotide synthesis. Solid phase synthesis was performed on ABI 380 DNA synthesizer at 10 umol scale. Incorporation of the deuterated residue was done via manual injection of the amidite-activator mixture directly into the column. Coupling time was increased to 2 minutes, coupling efficiency was 92%. The oligonucleotide was deprotected and purified according to standard protocols.[14] ESI [M-H^+] Cald m/z 3372.3, obs. 3373.27 ± 1.0. Synthesis of the AAII modified oligonucleotide (top) strand has been reported elsewhere.[15] Details of the two-dimensional NMR experiments, as well as full assignment of the duplex' proton resonances will be reported elsewhere.[16]

REFERENCES

1. Földesi, A.; Trifonova, A.; Kundu, M.K.; Chattopadhyaya, J. The synthesis of deuterionucleosides. nucleosides. *Nucleotides and Nucleic Acids*, **2000**, 19, 10, 1615.
2. Cromsigt, J.; Schleucher, J.; Gustafsson, T.; Kihlberg, J.; Wijmenga, S. Preparation of partially 2H/13C-labelled RNA for NMR studies. Stereo-specific deuteration of the H5″ in nucleotides. *Nucl. Acids Res.* **2002**, 30, 1639.
3. Kawashima, E.; Toyama, K.; Ohshima, K.; Kainosho, M.; Kyogoku, Y.; Ishido, Y. Novel approach to diastereoselective synthesis of 2′-deoxy[5′-$^2H_1$]ribonucleoside derivatives by reduction of the corresponding 5′-*O*-acetyl-2′-deoxy-5′-phenylselenoribonucleoside derivatives with a Bu_3Sn^2H-Et_3B system. *Chirality* **1997**, 9, 435.
4. Ono, A.(M); Ono, A.; Kainosho, M. Thymidine via stereoselective deuteration of a 5-oxoribose derivative *Tetr. Lett.* **1997**, 38, 395.
5. Kundu, M.K.; Földesi, A.; Chattopadhyaya, J. Studies on the stereoselective synthesis of deuterated _-ribose derivatives. *Helv. Chim. Acta* **2003**, 86, 633.
6. Xu, L.; Price, N.P.J. Stereoselective synthesis of chirally deuterated (S)-D-(6–$^2H_1$)glucose. *Carbohydrate Res.* **2004**, 1173.
7. Midland, M.M.; Asirwatham, G.; Cheng, J.C.; Miller, J.A.; Morell, L. Synthesis and conformational analysis of(6R)-[6–2H1]-1,2:3,4-Di-O-isopropylidene-.alpha.-D-galactopyranose. NMR and molecular modeling studies. *J. Org. Chem.* **1994**, 59, **4438**.
8. Lukin, M.A.; Bushuev, V.N. Stereoselective 5′-monodeuterated ribonucleoside phosphoramidites as tools for conformational studies of RNA by the NMR approach. *Nucleosides & Nucleotides* **1999**, 18, 1255.
9. Ichikawa, Y.; Kubota, H.; Fujita, K.; Okauchi, T.; Narasaka, K. Stereoselective beta-C-glycosylation and beta-S-glycosylation of 2-deoxyfuranose derivatives controlled by the 3-hydroxy group. *Bull. Chem. Soc. Japan* **1989**, 62, 845.
10. Hoffer, M.; Dushinsky, R.; Fox, J.J. Simple synthesis of pyrimidine 2′-deoxy-ribonucleotides. *J. Am. Chem. Soc.* **1959**, 81, 4112.
11. Hodge, R.P.; Brush, C.K.; Harris, C.M.; Harris, T.M. Synthesis of 1- and 1,2,2′-deuteriated deoxyribose and incorporation into deoxyribonucleosides. *J. Org. Chem.* **1991**, 56, 1553.
12. Beaucage, S. *Protocols for Oligonucleotides and Analogs: Synthesis and Properties*; Agrawal, S., ed.; Humana Press Inc., Totowa, NJ, 1993.
13. Schmeiser, H.H.; Stiborova, M.; Arldt, V.M. Chemical and molecular basis of the carcinogenicity of Aristolochia plants. *Curr. Opin. Drug. Discov. Devel.* **2009**, 12, 141.
14. The preliminary structure was obtained by nOe based restrained minimization using semi-qualitative distance restrains obtained from 300 ms NOESY spectra.
15. Attaluri, S.; Bonala, R.R.; Yang, I.Y.; Lukin, M.A.; Wen, Y.; Grollman, A.P.; Moriya, M.; Iden, C.R.; Johnson, F. DNA adducts of aristolochic acid II: total synthesis and site-specific mutagenesis studies in mammalian cells. *Nucleic Acids Res.* **2010**, 38, 339.
16. Lukin, M.; Zaliznyak, T.; de los Santos, C. in preparation.