Recl. Trav. Chim. Pays-Bas **107**, 343–346 (1988) 0165–0513/88/04343–04$1.50

Synthesis and conformation of 1-(3′-*C*-methyl-2′-deoxy-β-D-xylofuranosyl)uracil and 9-(3′-*C*-methyl-2′-deoxy-β-D-xylofuranosyl)adenine; two novel sugar-methylated nucleoside analogues

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(Received October 21st, 1987)

Abstract. The preparation and high-resolution ^{1}H NMR conformational analysis of the two novel nucleoside analogues, 1-(3′-*C*-methyl-2′-deoxy-β-D-xylofuranosyl)uracil (compound **1**) and 9-(3′-*C*-methyl-2′-deoxy-β-D-xylofuranosyl)adenine (compound **2**), are described. Compounds **1** and **2** show a pronounced preference for the north conformation of the sugar ring. The north conformation corresponds with a sterically favoured equatorial location of the methyl group. The C4′–C5′ conformation in **1** and **2** is predominantly γ^t (*trans* orientation of O5′ and C3′). The configuration at C3′ in **1** and **2** was corroborated via a one-dimensional NOE experiment. The structure of compound **1** (originally assigned as having the C3′-methyl group on the β-face of the furanosyl moiety) is revised on the basis of these data.

Structurally modified DNA or RNA constituents have received considerable attention on account of their possible utility as antitumour or antiviral agents[1-3]. Investigations in this field have mostly been focussed on base-altered nucleosides such as 5-fluoro-2′-deoxyuridine, which has been important in cancer chemotherapy for a number of years. From the current literature, it can perhaps be concluded that there is a growing interest in sugar-modified nucleosides. For instance, it has recently been found that arabino nucleosides, such as 1-(β-D-arabinofuranosyl)cytidine[4] and 9-(β-D-arabinofuranosyl)adenine[5], are potentially powerful cytostatic drugs. Generally speaking, there is, as yet, no detailed information available as to the mechanism of action of altered nucleosides. It seems to be clear, however, that the biological activity is somehow correlated with the preferred secondary structure in solution. It is known, for example, that the naturally occurring cytostatic antibiotic cordycepin (3′-deoxyadenosine) is a functional analogue of adenosine, but not of the isomeric 2′-deoxyadenosine[6]. This may be related to the fact that cordycepin and adenosine both show a preference for a north sugar pucker[7], whereas 2′-deoxyadenosine resides predominantly in a south conformation[8,9]. The use of conformationally restricted nucleosides in biochemical and biological experiments (focussed on protein synthesis or cell growth) could evidently provide further insight into any essential structure activity relationships. In this respect, it may be of interest to note that we recently reported on two novel cordycepin analogues in which the sugar ring is conformationally locked in a south form[10]. The preparation of these structures involves the introduction of a methyl group at the positions C2′-*exo* or C3′-*endo* of the furanose moiety. We now wish to report that the introduction of a methyl group at the C3′-*exo* position brings about the contrary effect, *i.e.* a strong preference for a north conformation. We made this observation with the compounds **1** and **2** (Fig. 1) which were synthesized via

Fig. 1. Structural formulas of the modified nucleosides **1** *and* **2**.

a stereospecific Grignard reaction. High-resolution ^{1}H NMR spectroscopy at 300 and 500 MHz was used for the structural assignment and conformational analysis of these compounds.

Conformation of compound 1

The preparation of compound **1** has been previously reported[11]. The structure of **1** was originally assigned as having the C3′-methyl on the β-face, by comparison of similar reactions of carbohydrates and also from the downfield shift of H4′ [11]. In the present work, we performed a homonuclear ^{1}H NMR double-resonance experiment at 300 MHz, in which we specifically saturated the methyl signal. The NOE difference spectrum (Fig. 2) shows that H2″ has a stronger NOE effect than H2′. Hence, the proton H2″ is closer to the methyl group than H2′, which is only in agreement with the location of the methyl group on the α-side of the furanose ring as shown in Fig. 1. The vicinal proton–proton coupling constants $J_{1'2'}$, $J_{1'2''}$, $J_{4'5'}$ and $J_{4'5''}$ were measured at 500 MHz in the temperature range 278–340 K (Table I). A computer simulation iteration procedure was used to extract spectral parameters from non-first-order patterns. The conformation of the sugar ring in **1** could be monitored using the couplings $J_{1'2'}$ and $J_{1'2''}$.

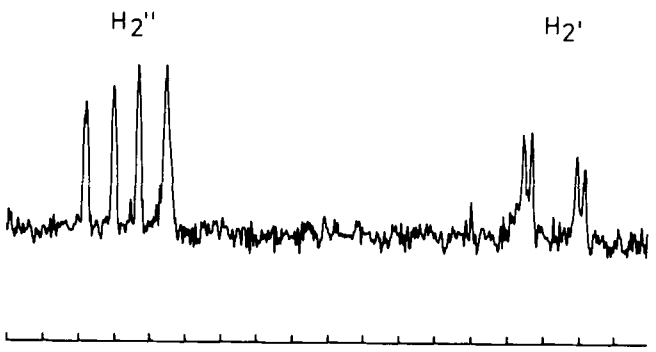


Fig. 2. NOE difference spectrum of compound **1**, *obtained upon saturation of the CH_3 resonance (128 transients, 300 MHz). Mark separation corresponds to 20 Hz.*

Using the generalized *Karplus* equation of *Altona* et al.[12,13], and assuming a maximum pucker amplitude of 39° (the averaged value for nucleosides[14,15]), we calculated the variation of $J_{1'2'}$ and $J_{1'2''}$ over the entire pseudorotation circuit. Fig. 3 depicts $J_{1'2'}$ and $J_{1'2''}$ as a function of P, the phase angle of pseudorotation. The experimental data points on **1** (black dots) are situated in the left-hand region of the graph, showing that the conformation is heavily biased towards a north form. Furthermore, it appears that increasing the sample temperature from 278 to 340 K leads to a slightly decreased preference for the north conformation. It follows that the north form (equatorial location of the methyl group) corresponds to a lower enthalpy content than the counterpart south form (axial methyl group), which is in line with our former observations on furanose methylated derivatives of cordycepin[10]. The conformation of the exocyclic C4′–C5′ bond was estimated from the coupling constants $J_{4'5'}$ and $J_{4'5''}$ (Table I). Assignment of the protons H5′ and H5″ was carried out as described by *Remin* and *Shugar*[16]. A clear preference for the γ^t conformation is observed, which appears to be typical of nucleosides which have an *endo* oriented 3′-OH group *(vide infra)*.

Table I NMR spectral data for compound **1** *at various temperatures.*

Temp.	$J_{1'2'}$	$J_{1'2''}$	$J_{4'5'}$	$J_{4'5''}$	$x(\gamma^+)$	$x(\gamma^t)$	$x(\gamma^-)$
278	2.0	7.8	2.9	8.0	0.24	0.72	0.04
288	2.2	7.9	2.9	7.8	0.26	0.70	0.04
297	2.3	7.9	2.9	7.6	0.28	0.68	0.04
320	2.6	7.9	3.0	7.4	0.30	0.64	0.05
340	2.8	8.0	3.0	7.1	0.33	0.62	0.05

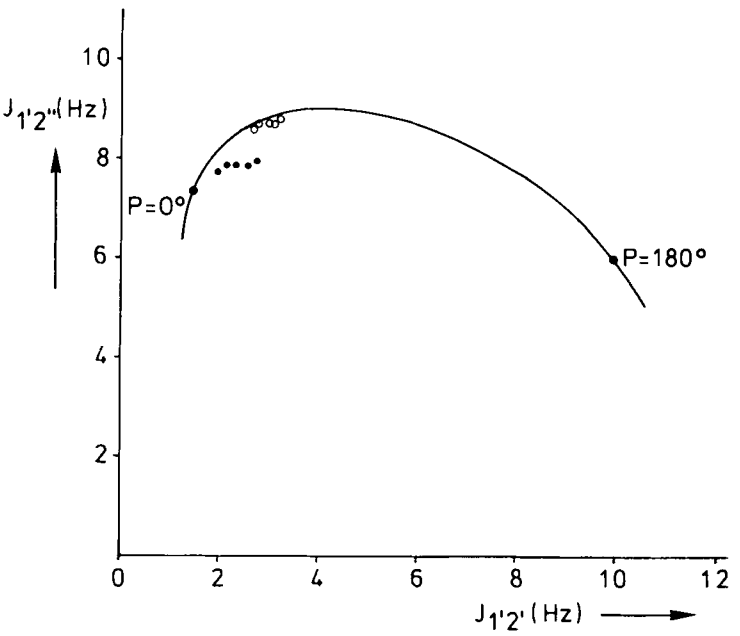
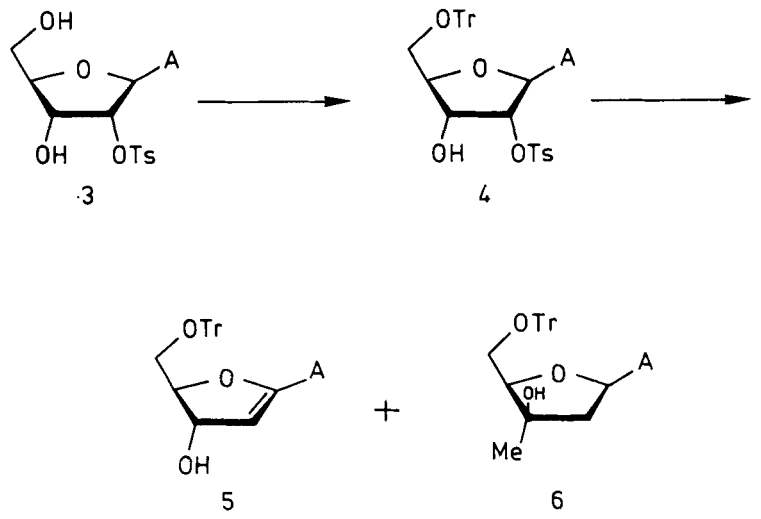


Fig. 3. Calculated dependence of $J_{1'2'}$ and $J_{1'2''}$ upon the phase angle of pseudorotation (P). The maximum pucker amplitude was fixed at 39°. Black dots refer to compound **1**; *open circles correspond with compound* **2**. *See text.*

Preparation and conformation of compound 2

The synthesis of compound **2** is outlined in Scheme 1. The first step comprises tritylation at the 5′-site of 2′-O-(*p*-toluenesulfonyl)adenosine[17] (**3**) by reaction with triphenylmethyl chloride and 4-(dimethylamino)pyridine in dry pyridine. Compound **4** was subsequently reacted with methylmagnesium iodide in dry THF/ether, after which a minor quantity of compound **5** (2.7%), and compound **6** (34%) could be isolated. Lewis acid detritylation of **6** ($ZnBr_2$ in dry nitromethane) finally yielded compound **2**. In order to obtain a corroboration of the assigned structure of **2**, we performed a one-dimensional NOE experiment at 300 MHz. Specific saturation of the methyl resonance was found to cause a slightly stronger NOE effect for H2″ than for H2′ (Fig. 4), indicating that the methyl group is indeed on the α-face of the molecule *(vide supra)*. The conformational analysis of **2** was again based on 500-MHz ^{1}H NMR spectra in the temperature range 278–340 K. In some cases, non-first-order patterns were obtained, from which we extracted the spectral parameters by means of an iterative computer simulation–iteration procedure. The relevant data are summarized in Table II. Conformational analysis of the C4′–C5′ bond shows a clear preference for γ^t, which is in agreement with the data on compound **1**. With respect to the furanose conformation of **2**, it is clear that a definite preference for a north type conformation exists (open



Scheme 1. Essential steps in the synthesis of compound **2**, *see text. A = adenine, Ts =* p-*toluenesulfonyl, Tr = triphenylmethyl.*

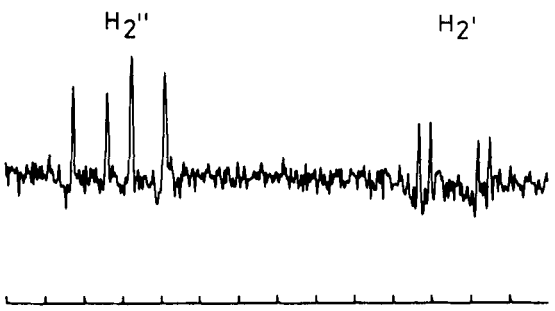


Fig. 4. NOE difference spectrum of compound **2**, *obtained upon saturation of the CH_3 resonance (256 transients, 300 MHz). Mark separation corresponds to 20 Hz.*

circles in Fig. 3). Comparison with the data on compound **1** reveals that the phase angle of pseudorotation in the preferred north form is substantially greater for **2**. This difference may be due to steric repulsion between the 3'-OH group and the adenine base in **2**, which are both in an axial position on the α-face of the furanose ring. A slight distortion of the sugar ring may then facilitate the *syn* ⇄ *anti* rotation of the adenine around the glycosidic (C1'–N9) bond[18]. It seems plausible that this ring distortion is not found for compound **1**, since pyrimidine structures are generally less flexible on account of a substantial preference for the *anti* conformation of the base[19].

Table II NMR spectral data for compound **2** *at various temperatures.*

Temp.	$J_{1'2'}$	$J_{1'2''}$	$J_{4'5'}$	$J_{4'5''}$	$x(\gamma^+)$	$x(\gamma^t)$	$x(\gamma^-)$
278	2.7	8.6	2.6	7.8	0.28	0.72	0.00
288	2.8	8.7	2.7	7.8	0.28	0.71	0.01
297	3.0	8.7	2.7	7.8	0.28	0.71	0.01
320	3.1	8.7	2.8	7.6	0.29	0.68	0.03
340	3.2	8.8	3.0	7.3	0.31	0.64	0.05

Concluding remarks

The conformational analysis of **1** and **2** shows that the introduction of a methyl group on the C3'-*exo* position results in a pronounced preference for a north conformation of the sugar ring. The methyl group occupies an equatorial position, in which steric interactions with the other substituents on the sugar ring are minimized. A preference for equatorial location was also observed for C3'-*endo*- and C2'-*exo*-methylated derivatives of cordycepin[10]. It seems to us that the availability of synthetic procedures for regio- and stereo-specific introduction of a methyl group on the furanose ring in nucleosides could be of importance in the design of nucleotide analogues with predictable and well-defined structural properties.

Experimental

High-resolution ^{1}H NMR spectra were recorded in the FT mode at 300 MHz on a Bruker CXP 300 spectrometer, or at 500 MHz on a Bruker WM 500 spectrometer[20]. Tetramethylsilane was used as the internal standard, and chemical shifts are reported in ppm (δ scale). For the NMR experiments in D_2O, a trace of dry acetonitrile (δ 2.00 ppm) was added for δ-calibration. Melting points are uncorrected.

2'-O-(p-Toluenesulfonyl)-5'-O-(triphenylmethyl)adenosine (**4**)

2'-*O*-(*p*-Toluenesulfonyl)adenosine (**3**)[17] (662 mg, 1.57 mmol), dried by azeotropic removal of moisture with pyridine (twice), was dissolved in dry pyridine (4.5 ml). To the resulting solution were added triphenylmethyl chloride (506 mg, 1.73 mmol) and 4-(dimethylamino)pyridine (213 mg, 1.73 mmol). The reaction mixture was heated overnight at 90°C. After cooling, it was poured into a saturated solution of sodium hydrogen carbonate and then extracted with chloroform. The organic phase was washed with water, dried and evaporated. The crude crystalline product was chromatographed over a short column of silica gel with dichloromethane as the first eluent, and then with 2% ethanol/chloroform mixture to afford compound **4** (561 mg, 55%); m.p. 185–186°C; R_f (eluent chloroform/methanol 9:1) 0.40. IR (KBr): ν_{max} 3060 cm^{-1} (aromatic C–H), 1640/1600 cm^{-1} (C=O amide), 1380/1180 cm^{-1} (tosylate). UV: λ_{max} 261 nm (at pH 4, 7 and 12). ^{1}H NMR (80 MHz, $CDCl_3$): δ 8.0 (s, 1H, H8), 7.75 (s, 1H, H2), 7.88 (d, 2H, tosyl), 7.25 (m, 15H, trityl), 6.98 (d, 2H, tosyl), 5.96 (d and s, 3H, H1' and OH, $J_{1'2'}$ 6 Hz), 5.72 (t, 1H, H2', $J_{2'3'}$ 6 Hz), 4.55–4.40 (m, 1H, H3'), 4.32–4.15 (m, 1H, H4'), 4.05 (bs, 2H, NH_2), 2.25 (s, 3H, CH_3 tosylate). MS: $C_{36}H_{33}N_4O_6S$, M = 649.18. Chemical ionization (isobutane): (M – trityl)$^+$ 420, 0.4%; (M – trityl – tosyl)$^+$ 249, 0.4%; (base)$^+$ 134, 0.4%; (sugar)$^+$ 115, 22.5%.

9-(3'-C-Methyl-5'-O-(triphenylmethyl)-2'-deoxy-β-D-xylofuranosyl)adenine (**6**)

A diethyl ether solution (10 ml) of methylmagnesium iodide (20 eq.) was added dropwise to a dry tetrahydrofuran solution

(25 ml) of compound **4** (516 mg, 0.77 mmol) at 0°C over 30 min under a nitrogen atmosphere. The reaction mixture was then stirred at room temperature or 90 min and heated at 65°C for 2 h. After cooling at 0°C, 10% aqueous ammonium chloride (9 ml) was added. After extraction with chloroform and evaporation of the dried organic phase, the residue was chromatographed on a silica gel column using 9:1 ethyl acetate/methanol as eluent. Elution of the more polar compound gave **5** (10 mg, 2.7%). Continued elution with the same solvent gave the Grignard reaction product which was finally purified by chromatography on a short silica gel column, using dichloromethane/2% ethanol as eluent. Yield of compound **6** 134 mg (34%).

Compound **5**: m.p. 174–176°C; R_f (eluent chloroform/methanol 9:1) 0.32. IR (KBr): ν_{max} 3060 cm^{-1} (aromatic C–H), 1650/1600 (C=O amide). UV: λ_{max} 255 nm (pH 4), 254 nm (pH 7), 256 nm (pH 12). ^1H NMR (80 MHz, $(CD_3)_2SO$): δ 8.3 (s, 1H, H8), 7.5–7.1 (m, 17H, trityl and NH_2), 5.73 (d, 1H, H2', $J_{2'3'}$ 2.2 Hz), 5.36 (d, 1H, OH, $J_{OH\text{-}3'}$ 7 Hz), 4.9–4.7 (m, 1H, H4'), 4.35–4.2 (m, 1H, H4'), 3.25 (s, 2H, H5'/5''). MS: $C_{29}H_{25}N_5O_3$, M = 491.195. Chemical ionization: (M – base)$^+$ 356, 15%; (M – base – sugar) 243, 41.63%.

Compound **6**: m.p. 153–156°C; R_f (eluent ethyl acetate/methanol 9:1) 0.50; UV: λ_{max} 261 nm (pH 4 and 7), 260 nm (pH 12). ^1H NMR (80 MHz, $CDCl_3$): δ 8.18 (s, 1H, H8), 7.85 (s, 1H, H2), 7.5–7.05 (m, 15H, trityl), 6.01 (dd, 1H, H1', $J_{1'2'}$ 2 Hz, $J_{1'2''}$ 6 Hz), 5.88 (bs, 2H, NH_2), 3.79 (t, 1H, H4', $J_{4'5'} = J_{4'5''}$ 5 Hz), 3.52 (d, 2H, H5'/5''), 2.3 (d, 2H, H2'/2''), 1.3 (s, 3H, CH_3). MS: $C_{30}H_{25}N_5O_3$, M 507.228. FAB: (M – H)$^-$ 506.2, 28.8%; (M – trityl)$^-$ 264.1, 18%; (M – trityl – sugar) 134.0, 10%.

9-(3'-C-Methyl-2'-deoxy-β-D-xylofuranosyl)adenine (**2**)

To a stirred suspension of anhydrous zinc bromide (417 mg, 1.85 mmol) in dry nitromethane (5 ml) were successively added compound **6** (1.04 mg, 0.206 mmol) and anthranilic acid (508 mg, 3.708 mmol). The reaction mixture was stirred overnight at room temperature and then filtered. The filtrate was evaporated and the residue was purified by chromatography over a short column of silica gel using chloroform/methanol (8:2) as eluent, followed by the washing of the eluate with chloroform to eliminate the last traces of anthranilic acid, yielding 39 mg (71%) of compound **2**; m.p. 119–120°C; R_f eluent chloroform/methanol 4:1) 0.4. UV: λ_{max} 260 nm (pH 4, 7 and 12). ^1H NMR (200 MHz, D_2O): δ 8.16 (s, 1H, H8), 8.06 (s, 1H, H2), 6.18 (dd, 1H, H1', $J_{1'2'}$ 3.0 Hz, $J_{1'2''}$ 8.7 Hz), 3.88 (dd, 1H, H4', $J_{4'5'}$ 2.7 Hz, $J_{4'5''}$ 7.8 Hz), 3.85 (dd, 1H, H5', $J_{5'5''}$ – 12.5 Hz), 3.73 (dd, 1H, H5''), 2.78 (dd, 1H, H2''), 2.43 (dd, 1H, H2'), 1.34 (s, 3H, CH_3). MS: $C_{11}H_{15}N_5O_3$, M 265.116. FAB: (M + H)$^+$ 266.1, 25.9%; (M – sugar + 2H)$^+$ 136, 100%; (M – sugar)$^+$ 134, 1.1%.

Acknowledgements

J. Chattopadhyaya thanks the Swedish Board for Technical Development (STU) and the Swedish Science Research Council (NFR) for financial support for this research. *H. M. Buck* thanks the Netherlands Foundation for Chemical Research (SON) and the Netherlands Organization for the Advancement of Pure Research (ZWO) for financial aid.

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