Facile synthesis and conformation of 3′-*O*,4′-*C*-methyleneribonucleosides

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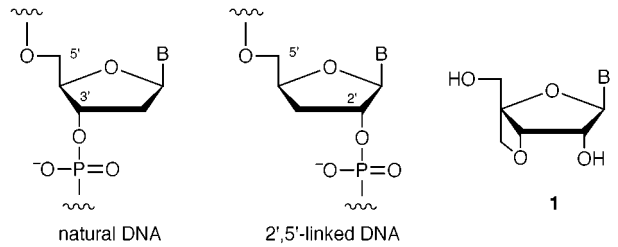
Received (in Cambridge, UK) 7th September 1999, Accepted 26th October 1999

Bicyclic nucleoside analogues, 3′-*O*,4′-*C*-methyleneribonucleosides 1, including thymine, cytosine, adenine and guanine nucleobases, were conveniently synthesized from D-glucose, and the ribofuranose ring of 1 was found to exist predominantly in a *S*-conformation by means of ^{1}H NMR and X-ray analysis.

In recent studies aimed at developing an effective antisense molecule, numerous oligonucleotide analogues have been synthesized with various chemical modifications of the phosphodiester backbone, sugar moiety and/or nucleobase region.[1] In these attempts, the oligonucleotide analogues containing 'non-genetic' 2′,5′-phosphodiester linkages (2′,5′-linked oligonucleotides) were found to be favorable as antisense molecules because of their RNA selective hybridization ability and

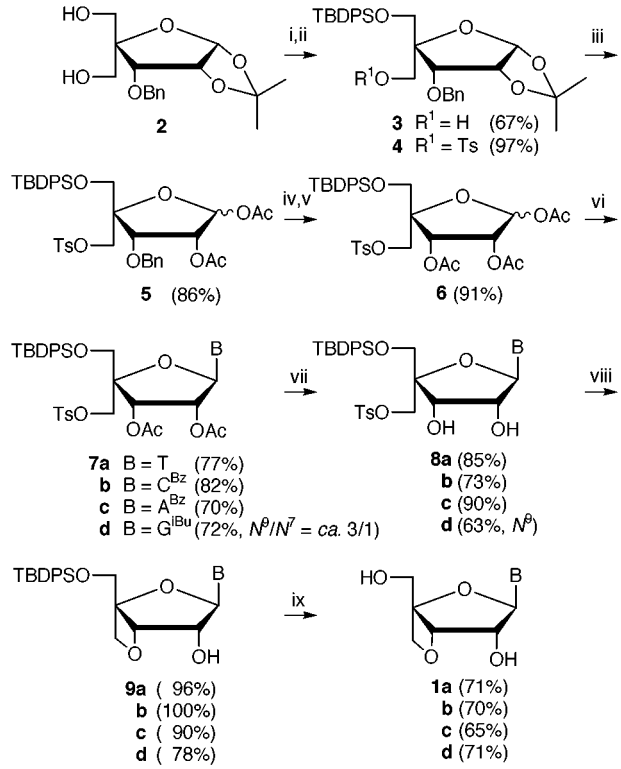


enzymatic stability.[2] Furthermore, 2-5A (2′,5′-linked oligoadenylate 5′-triphosphate) is well-known to have an important role in the interferon-mediated antiviral system in living cells,[3] and intensive studies on various 2-5A analogues have been reported.[4] However, there is only limited information available on the relationship between the sugar conformation in 2′,5′-linked oligonucleotides and their attractive properties, such as hybridization ability and antiviral activity.[5]

From the consideration that the restriction of sugar puckering in nucleosides to a proper conformation would serve as an advantageous strategy to develop a desired antisense (antigene) molecule,[6,7] we have recently accomplished the synthesis of conformationally restricted nucleoside analogues, 3′-*O*,4′-*C*-methylene-uridine and -cytidine **1** (B = U and C) by using uridine as a starting material,[8] and also demonstrated interesting properties of the 2′,5′-linked oligonucleotide analogues containing **1** (B = U and T), *e.g.* RNA selective hybridization abilities.[9]

Unfortunately, the synthetic route of **1** was not practical for purine analogues. We now report a novel and practical synthetic route to **1** bearing various nucleobases, exemplfied by synthesis of all four nucleoside analogues **1** (B = T, C^{Bz}, A^{Bz} and G^{iBu}), and also discuss their conformation.

After several attempts,† the synthesis of the target compounds **1** was performed by a coupling reaction of a 1-*O*-acetylribofuranose derivative with silylated nucleobases and a subsequent oxetane ring formation, as shown in Scheme 1. A stereoselective silylation of the diastereotopic hydroxy groups in 3-*O*-benzyl-4-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose **2**[10] gave the desired compound **3** (67%). The stereochemistry at C4 in **3** was confirmed by means of NOE measurements. A *p*-tolylsulfonylation of **3** afforded the tosylate **4** (97%), which was converted to diacetate **5** (86%) by treatment with AcOH and Ac_2O in the presence of a catalytic amount of H_2SO_4. Debenzylation and subsequant acetylation of the 3-hydroxy group in **5** gave the triacetate **6** (91%). The reaction of **6** with *O*,*O*′-bis(trimethylsilyl)thymine (T·2TMS) under Vorbrüggen's conditions[11] afforded only the β-anomer of



Scheme 1 *Reagents and conditions*: i, TBDPSCl, Et_3N, CH_2Cl_2, room temp., 14 h; ii, TsCl, Et_3N, DMAP, CH_2Cl_2, room temp., 16 h; iii, AcOH, Ac_2O, conc. H_2SO_4, room temp., 30 min; iv, 10% Pd-C/H_2, Et-OAc–$CHCl_3$, room temp., 17 h; v, Ac_2O, Py, room temp., 20 h; vi, sililated base, TMSOTf, $CH_2CH_2Cl_2$, reflux, 8–18 h; vii, K_2CO_3, room temp., 15 min; viii, NaHMDS, THF, room temp., 1h; ix, TBAF, THF, room temp., 15 min.

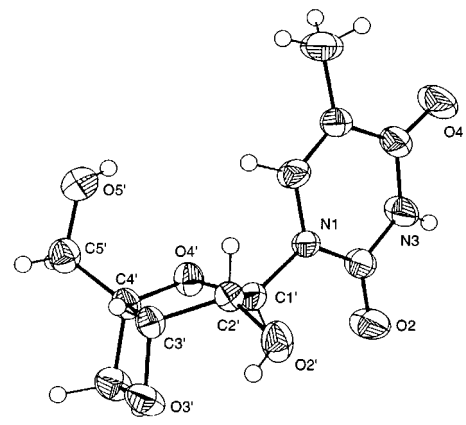


Fig. 1 ORTEP drawing of **1a**.

thymidine derivative **7a** (77%). The triacetate **6** was also coupled with silylated N^4-benzoylcytosine (C^{Bz}·2TMS), N^6-benzoyladenine (A^{Bz}·2TMS) and N^2-isobutyrylguanine (G^{iBu}·3TMS) to give the corresponding β-nucleoside derivatives **7b** (82%), **7c** (70%) and **7d** (72%),‡ respectively. Methanolysis of **7** gave diols **8** (63–90%) and then oxetane ring formation from **8** was accomplished on treatment with sodium hexamethyldisilazide in THF at room temperature, yielding only the corresponding 3′-*O*,4′-*C*-methyleneribonucleoside derivatives **9** (78–100%). The desired products **1** were obtained (65–71%) by removal of a TBDPS group in **9**. We have, thus, achieved a facile synthesis of 3′-*O*,4′-*C*-methyleneribonucleosides **1** in good yield.§

The conformational analysis of the obtained 3′-*O*,4′-*C*-methyleneribonucleosides **1** was carried out by means of ^{1}H NMR and X-ray crystallographic data. Namely, all of the bicyclic nucleoside analogues **1** show a relatively large $J_{1'2'}$ value (7.3–7.6 Hz in CD_3OD), which means that these nucleoside analogues have predominantly the *S*-conformation (*S*% = 91–96%),¶ regardless of the type of the nucleobase. Furthermore, an X-ray crystallographic analysis of **1a** shows that the sugar pucker pseudorotation phase angle (*P*) is 136.2° and the maximum out-of-plane pucker (ν_{max}) is 32.3°, characteristic of the C1′-*exo*-C2′-*endo* form (*S*-conformation) of sugar puckering.‖

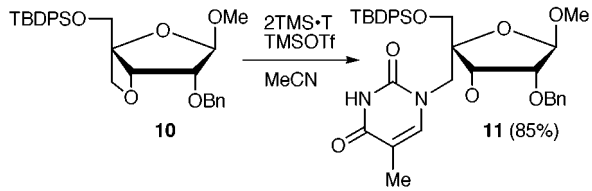
It is noteworthy and very interesting that the expressed *S*-preference of the nucleoside analogues **1** is vastly different from conformational analysis of other nucleosides possessing a 2′-OH group, *e.g.* uridine (*S*% = 52%),[8] cytidine (*S*% = 26%),[8] 3′-deoxyuridine (*S*% = 3%)[8,12] and 3′-deoxycytidine (*S*% = 0%).[8,13]

Further studies on these bicyclic nucleosides are now in progress.

A part of this work was supported by a Grant-in-Aid for Scientific Research (B), No. 09557201, from the Japan Society for the Promotion of Science. We are also grateful to the Takeda Science Foundation for financial support.

Notes and references

† As an altanative route for the synthesis of **1**, we briefly tried a coupling reaction of the oxetane derivative **10** with silylated thymine, resulting in exclusive C–O bond fission of the oxetane ring to afford only **11**.



‡ Guanosine derivative **7d** was obtained as a mixture of N^9 and N^7 regioisomers (72%, N^9/N^7 = *ca.* 1/3) which was directly converted to **8d** without separation. The N^9 and N^7 isomers of **8d** were obtained in 63 and 18% yield, respectively, after silica gel chromatography. The stereochemistry of each isomer **8d** was determined by comparison of their ^{1}H and ^{13}C NMR data.

§ *Selected data* for **1a**: mp 119–120 °C (AcOEt); $[\alpha]_D^{25}$ −63.1 (*c* 0.44, MeOH); ν_{max}(KBr)/cm^{-1} 4016, 3451, 1723; $\delta_H(CD_3OD)$ 1.89 (3H, s), 3.75, 3.83 (2H, AB, *J* 12), 4.14 (1H, d, *J* 8), 4.51, 4.83 (2H, AB, *J* 8), 5.05 (1H, d, *J* 5), 6.42 (1H, d, *J* 8), 7.52 (1H, s); *m/z* (FAB) 277 (M+Li$^+$) (calc. for $C_{11}H_{14}N_2O_6$·H_2O: C, 45.83; H, 5.59; N, 9.72. *Found*: C, 45.81; H, 5.51; N, 9.71%).

¶ The percentage of *S*-conformation (*S*%) is calculated from the equation: *S*% = 100($J_{1'2'}$ − 1)/6.9. See ref. 8 and 14.

‖ *Crystal data* for **1a**: $C_{11}H_{14}N_2O_6$·H_2O, *M* = 288.26, colourless plate, 0.30 × 0.20 × 0.10 mm, orthorhombic, $P2_12_12_1$, *a* = 8.6242(10), *b* = 20.6008(8), *c* = 7.2767(11) Å, *V* = 1292.8(3) Å^3, *T* = 283 K, *Z* = 4, μ (Cu–Kα) = 1.54 mm^{-1}, 1177 reflections measured, 1155 independent reflections, 1037 reflections observed, *R* = 0.0397, R_w = 0.0984. CCDC 182/1463. See http://www.rsc.org/suppdata/cc/1999/2423/ for crystallographic data in .cif format.

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Communication 9/07218G