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Synthesis of a conformationally locked AZT analogue, 3'-azido-3'-deoxy-2'-*O*,4'-*C*-methylene-5-methyluridine

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Abstract

A bicyclic 3'-azido-3'-deoxythymidine (AZT) analogue with a locked N-conformation, 3'-azido-3'-deoxy-2'-*O*,4'-*C*-methylene-5-methyluridine (**1a**), and its 3'-amino derivative, 3'-amino-3'-deoxy-2'-*O*,4'-*C*-methylene-5-methyluridine (**1b**), were successfully synthesized from D-glucose. The conformation of **1a** was also discussed by means of ^{1}H NMR measurements and a molecular modeling (PM3) study.

Keywords: Nucleic acid analogues; Azides; NMR; Molecular modeling

3'-Azido-3'-deoxythymidine (AZT, zidovudine) is widely recognized for its importance in therapy of the acquired immunodeficiency syndrome (AIDS). AZT and other nucleoside analogues, such as ddC (zalcitabine), ddI (didanosine), d4T (stavudine), and 3TC (lamivudine) represented in Fig. 1, are targeting HIV reverse transcriptase (RT) [1, 2]. On the other hand, some 4'-substituted nucleosides, such as 4'-azidothymidine [3], 4'-cyanothymidine [4], 4'-*C*,3'-*O*-methylenethymidine [5], and 4'-ethynylthymidine [6], 2'-deoxy-4'methylcytidine [7] were also found to act as an inhibitor of HIV RT.

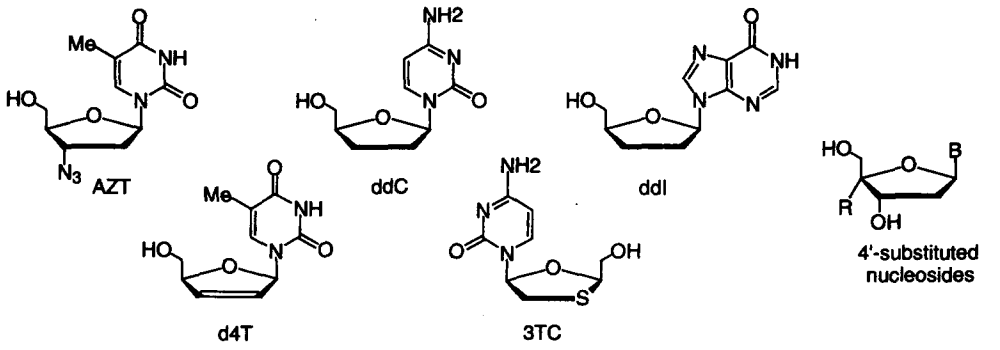

Fig. 1 Structures of AZT and other nucleoside HIV RT inhibitors

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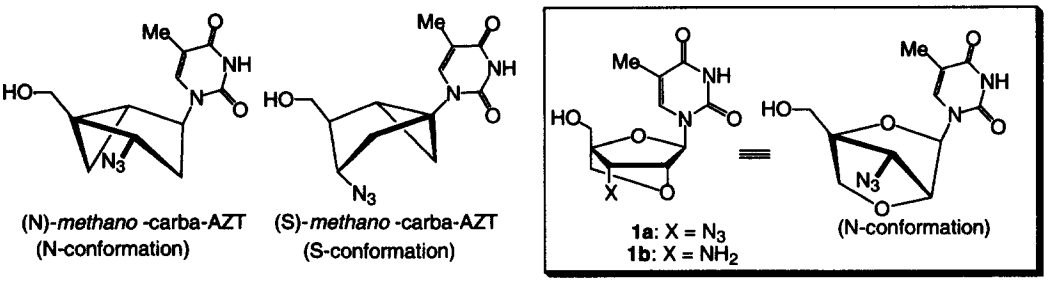

Fig. 2 Structures of conformationally locked AZT analogues, (N)- and (S)-*methano*-carba-AZT and **1**

Despite intensive studies on the mechanistic aspect of RT inhibition of these drugs, there has so far been limited information available on their active conformation [3, 8]. Very recently, Marquez *et al.* demonstrated that among the two isomeric conformationally locked carbocyclic AZT analogues, (N)- and (S)-*methano*-carba-AZT 5'-triphosphates, only the N-form analogue had inhibitory activity against HIV-1 RT (Fig. 2) [9]. These results encouraged us to study the development of an ideal anti-HIV nucleoside, an AZT analogue conformationally locked in N-form. We have already reported the first synthesis of the nucleoside analogue, 2'-*O*,4'-*C*-methyleneribonucleosides,[1] which have a 2,5-dioxabicyclo[2.2.1]heptane ring system, and also found by means of ^{1}H NMR measurements and X-ray crystallographic analysis that their conformation was locked in N-form [10]. Here we report the synthesis of its 3'-azido and 3'-amino congeners **1**, which have a locked N-conformation by formation of a methylenebridge between 4'-carbon and 2'-oxygen atoms.

The synthetic route to the target compound **1** is shown in Scheme 1. The starting material, 3-azido-5-*O*-benzoyl-4-benzoyloxymethyl-3-deoxy-1,2-*O*-isopropyliden-α-D-ribofuranose (**2**), was prepared from D-glucose by a several-step sequence [16]. The diol **3** was obtained by the hydrolysis of the dibenzoate **2** in 86% yield. A stereoselective silylation of the diastereotopic hydroxy groups in the diol **3** afforded the monosilyl ether **4** in 62% yield. Treatment of **4** with *p*-toluenesulfonyl chloride gave the tosylate **5** in 98% yield. An acetolysis of **5** with acetic acid, acetic anhydride and sulfuric acid afforded an anomeric mixture (α : β = *ca.* 3 : 7) of diacetate **6** in 94% yield. The diacetate **6** was employed for the coupling reaction with *O*,*O*'-bis(trimethylsilyl)thymine [17] (T•2TMS) in the presence of $SnCl_4$ to give only the β-anomer of 3'-azido-3'-deoxythymidine derivative **7** in 91% yield. Treatment of **7** with potassium carbonate in methanol caused deacetylation and ring-closure reaction to afford the AZT analogue **8** with 2,5-dioxabicyclo[2.2.1]heptane ring. The desired compound **1a** was obtained (85%) by the desilylation of **8** with tetrabutylammonium fluoride (TBAF) in THF.[2] Furthermore, an azido group in **1a** was readily reduced by using Pd-catalyzed reduction to

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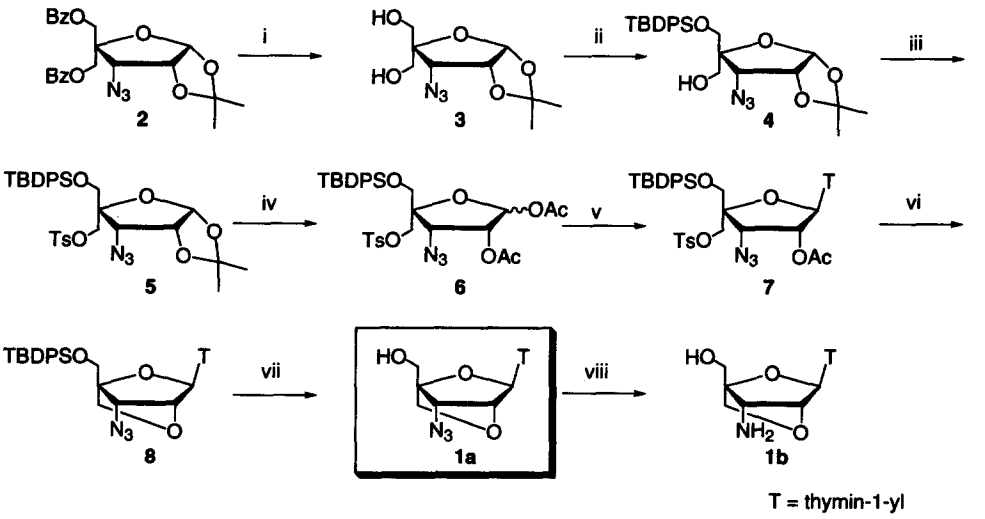
[1] Just after our report of the synthesis of 2'-*O*,4'-*C*-methyleneribonucleosides [9], Wengel and co-workers reported the synthesis of the same compounds [11,12]. It was also reported that the oligonucleotides containing these nucleoside analogues have greatly favorable properties as an antisense molecule, e.g. great thermal stability towards complementary RNA and potent RNA recognition ability [12-15].

[2] Selected data for **1a**: mp 94-96 °C. IR ν_{max} (KBr) 3163, 3046, 2118, 1692, 1468, 1273, 1062 cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.89 (3H, s), 3.76, 3.86 (2H, ABq, J = 8 Hz), 3.85, 3.95 (2H, ABq, J = 13 Hz), 4.03 (1H, s), 4.58 (1H, s), 5.58 (1H, s), 7.70 (1H, s).

afford the 3'-amino derivative **1b**.[3] Since the oligonucleotides having a N3'→P5' phosphoramidate linkage are well known to show very high binding affinity for ssDNA and ssRNA, as well as for dsDNA [18-20], the 3'-amino derivative **1b** would be a potential synthon for ideal antisense and/or antigene molecules.



Scheme 1 *Reagents and Conditions*: i) K_2CO_3 aq., MeOH, 0 °C, 86%; ii) TBDPSCl, Et_3N, CH_2Cl_2, rt, 62%; iii) TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 98%; iv) cat. H_2SO_4, Ac_2O, AcOH, rt, 94%; v) T•2TMS, $SnCl_4$, $ClCH_2CH_2Cl$, rt, 91%; vi) K_2CO_3, MeOH, rt, 100%; vii) TBAF, THF, rt, 85%; viii) H_2, 10% Pd-C, EtOH, rt, 100%.

In the ^1H NMR spectra, **1a** shows all singlet signals for C1'-, C2'- and C3'-protons; furthermore, the NOEs are observed between C3'- and C6-protons (10%/11%), which clearly indicate that the conformation of AZT analogue **1a** is locked in N-form in analogy with 2'-*O*,4'-*C*-methyleneribonucleosides [10-12]. As depicted in Fig. 3, a molecular modeling study (PM3)[4] of **1a** also shows that the phase angle of pseudorotation *P* is 19.6°, which is characteristic of the typical C3'-*endo* (^3E) conformation, and that the maximum

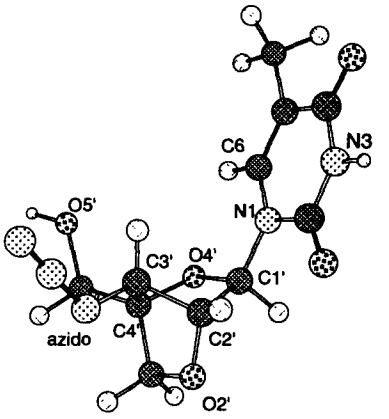


Fig. 3 Computer-generated (PM3) representation of **1a**

[3] Selected data for **1b**: mp 243-246 °C. IR ν_{max} (KBr) 3459, 3365, 1699, 1447, 1273, 1054 cm^{-1}. ^1H NMR (pyridine-d_5) δ: 1.83 (3H, s), 3.62 (1H, s), 3.92, 4.14 (2H, ABq, J = 8 Hz), 4.24 (2H, s), 4.54 (1H, s), 5.97 (1H, s), 7.90 (1H, s).

[4] The molecular modeling was performed using the MOPAC97 molecular orbital package (CS MOPAC Pro™, Cambridge Soft Corporation) with the PM3 Hamiltonian. Numerical calculations were performed on a Power Macintosh computer.

out-of-plane pucker ν_{max} is remarkably large (ν_{max} = 58.3°), compared with natural and unnatural nucleosides [21, 22].

Thus, we have successfully accomplished the synthesis of conformationally locked AZT analogue, 3'-azido-3'-deoxy-2'-*O*,4'-*C*-methylene-5-methyluridine (**1a**) and its 3'-amino derivative, 3'-amino-3'-deoxy-2'-*O*,4'-*C*-methylene-5-methyluridine (**1b**). Further studies on the biological activity of these compounds are now in progress.

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