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Conformationally locked nucleosides. Synthesis and stereochemical assignments of 3′-*N*,5′-*C*-bridged 3′-amino-3′-deoxythymidines

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Abstract

3′-Amino-3′-deoxythymidine was converted, in multi-steps, to its 5′-*O*-(4,4′-dimethoxytrityl)-5′-*C*-tosyloxyethyl-3′-*N*-carbobenzoxy derivative **7** and 5′-*C*-mesyloxyethyl-3′-*N*-(9-fluorenyl)methoxycarbonyl derivatives **6** and **11**. Subsequent hydrogenolysis or treatment with DMAP afforded 3′-*N*,5′-*C*-bridged bicyclic thymidines. Stereochemical assignments were accomplished with the help of NOE. © 1999 Elsevier Science Ltd. All rights reserved.

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Modified oligonucleotides (ONs) as antisense inhibitors of gene expression have been intensively explored in the past decade.[1–4] Among a number of promising modifications is the N3′–P5′ phosphoramidate backbone, which significantly increases hybridization of the modified ONs to complementary RNA.[5,6] Recently, the ONs containing conformationally locked nucleosides have also drawn considerable attention.[7–11] It was reported that 2′,4′-bridged nucleosides (**a**, **b**) having locked 3′-*endo* sugar puckers significantly increased hybridization of the modified ONs to complementary RNA.[7,8,11] It seems that certain conformationally locked nucleosides are promising candidates as building blocks of antisense ONs. Can the effects from the locked sugar pucker and the modified backbone be synergistic if a favorable, locked, 3′-*endo* sugar moiety is introduced into N3′–P5′ phosphoramidate ONs? One way to answer this question is to introduce a new ring onto the ribose as shown by the structures below (**c**, **d**). As can be seen from a stick–ball model, only C3′-*endo*-like and O4′-*endo*-like sugar puckers are possible for the ribose ring in the rigid **c** and **d**. Geometry optimization (Alchemy 2000, PM3) predicts a C3′-*endo* sugar pucker for the ribose rings and a chair form for the six-membered rings of **c** and **d**. In these optimized conformations the torsion angle γ is 62° for **c** and 179° for **d**, which indicates that **c** can fit well into DNA–DNA and DNA–RNA double helices.[12] In addition to their potential use as monomers of ONs, bicyclonucleosides themselves may be useful as antiviral compounds. It was

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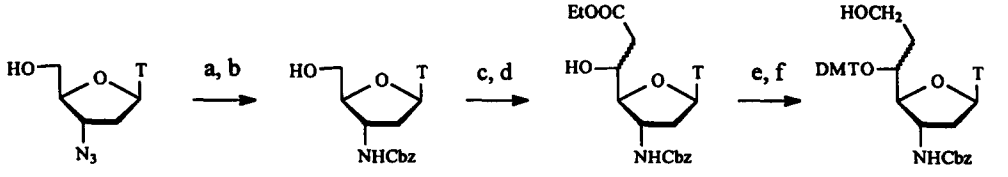
reported that 4′,6′-methanocarbocyclic nucleosides having a 3′-*endo* sugar pucker demonstrated potent activity against HSV, HCMV, and EBV.[13,14] Obviously, bicyclonucleosides merit further investigation. In this communication, synthesis and stereochemical assignments of 3′-*N*,5′-*C*-bridged 3′-amino-3′-deoxythymidines (**c**, **d**) are described.

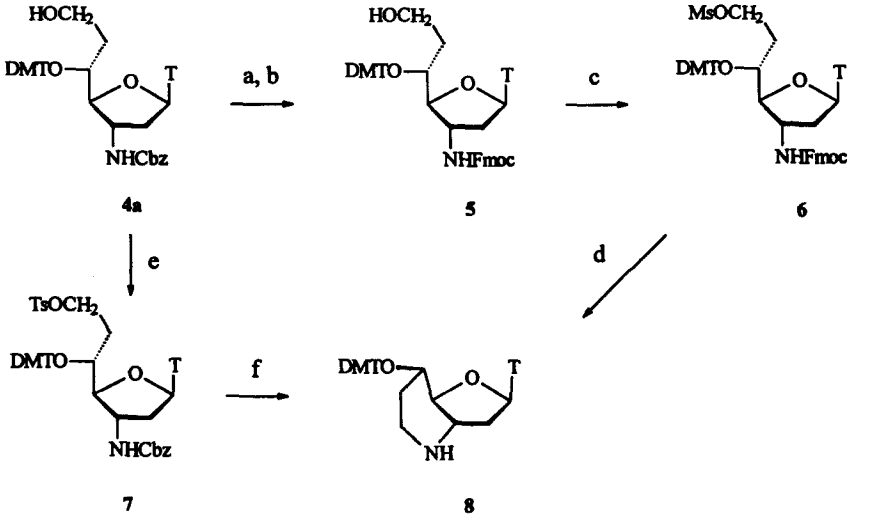
a **b** **c** **d**

Synthesis of 3′,5′-bridged thymidine derivatives is shown in Schemes 1–3. 3′-Azido-3′-deoxythymidine **1**[15] was hydrogenolyzed to a 3′-amino derivative, which was protected with carbobenzoxy to give **2**. A mild oxidation reaction converted **2** to an aldehyde, which was subjected to a Reformatsky reaction to give a mixture of **3a** and **3b** (5′(*R*)- and 5′(*S*)-isomer). Tritylation and the subsequent reduction converted **3a** and **3b** into **4a** and **4b**, respectively.



1 **2** **3a, 3b** **4a, 4b**

Scheme 1. (a) H_2, 10% Pd/C, rt, 6 h, 87%; (b) BnOCOCl, Na_2CO_3, rt, 1 h, 87%; (c) DMSO, DCC, TFA, pyridine, rt, 4 h, 79%; (d) Zn, $BrCH_2COOEt$, THF, 40°C, 20 h, 62%; (e) DMT–Cl, $AgNO_3$, pyridine, 50°C, 24 h, 92%; (f) $LiAlH_4$, THF, 0°C, 2 h, 84%



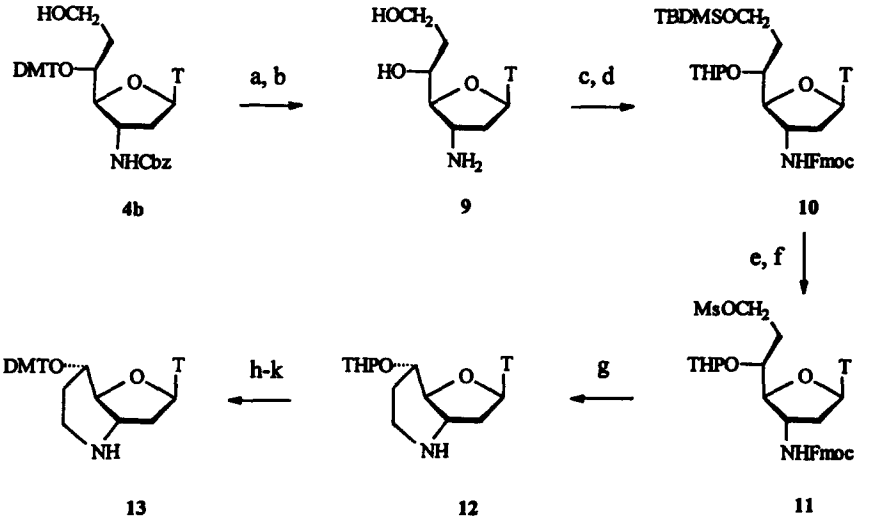
4a **5** **6** **7** **8**

Scheme 2. (a) H_2, 10% Pd/C, rt, 14 h; (b) Fmoc–OSu, Et_3N, THF, rt, 3 h; 79% (two steps); (c) Ms–Cl, pyridine, rt, 0.5 h; (d) DMAP, THF, 40°C, overnight, 70% (two steps); (e) TsCl, pyridine, rt, 4 h; (f) H_2, 10% Pd/C, rt, 14 h, 72% (two steps)

Compound **4a**, the higher R_f (5% EtOH in CH_2Cl_2) product of the reduction (step f in Scheme 1), was subjected to a controlled hydrogenolysis over Pd/C and the subsequent reaction with Fmoc–OSU to give **5**, which was converted into the mesylate **6**. Treatment of **6** with excess DMAP afforded the bicyclic thymidine **8** (5′(*R*)-isomer) in good yield. However, some by-products were formed when DBU was used

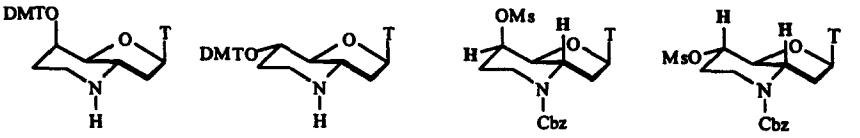
to remove Fmoc. Probably, the thymine base was converted to an anion that attacked the mesyl group to form the by-products. Similarly, compound **4b**, the lower R_f product of the reduction, was converted to the bicyclic thymidine **13**. In an unrelated work in this laboratory, it was found that the tosyl groups of 3′-*O*-*t*-butyldimethylsilyl-5′-*O*-tosyl-4′-*C*-tosyloxymethylthymidine were intact when it was subjected to the hydrogenolysis condition at which Cbz was removed. Therefore, **4a** was converted to the tosylate **7**, which was directly hydrogenolyzed to give **8**.

Because the selective removal of Cbz from **4a** and **4b** by hydrogenolysis was accompanied by a partial removal of DMT, an alternative route was explored. Compound **4b** was completely hydrogenolyzed to **9**, which can also be prepared from the reduction of **3b**. Compound **9** was protected with Fmoc at N3′, with TBDMS at the primary hydroxyl, and with THP at the 5′-hydroxyl to give **10**. After replacement of TBDMS with mesyl, the resulting compound **11** was treated with DMAP to give the bicyclic thymidine **12**, which was subjected to protection and deprotection to give **13**, the 5′(*S*)-isomer of **8**.



Scheme 3. (a) 10% Pd/C, H_2, rt; (b) Fmoc–OSu, Et_3N, DMF, rt, 3 h, 80% (two steps); (c) TBDMS–Cl, pyridine, rt, 5 h, 99%; (d) dihydropyran, PPTs, CH_2Cl_2, rt, 5 h, 90%; (e) TBAF–AcOH (pH 6), THF, rt, 15 h, 86%; (f) Ms–Cl, pyridine, rt, 0.5 h, 100%; (g) DMAP, 40°C, 20 h, 69%; (h) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, rt, 3 h, 76%; (i) 80% AcOH, rt, 5 h, 100%; (j) DMT–Cl, AgOTf, pyridine, 50°C, 24 h, 89%; (k) NH_4OH, MeOH, rt, 2 h, 95%

For stereochemical assignments through NOE experiments, compounds **8** and **13** were converted to **14** and **15**, respectively, by removal of DMT, protection with Cbz, and the subsequent mesylation. It can be seen from a ball–stick model that there should be no NOE between the H3′ and H5′ of **14** since the H5′ directs away from the rest of the molecule (equatorial), whereas a significant NOE enhancement should be observed between the H3′ and H5′ of **15** since the H5′ directs upward (axial) and is adjacent to the H3′. In consistency to the prediction, 3.0% NOE enhancement was observed on the H3′ when the H5′ of **15**, prepared from the lower R_f isomer (**4b**) of the reduction, was saturated. Similarly, 2.6% NOE enhancement was observed on the H5′ when the H3′ of **15** was saturated. In contrast, no NOE was observed in **14**, prepared from the higher R_f isomer (**4a**) of the reduction. Since all the reactions after the reduction did not alter the chirality of the C5′, it is clear that **8**, the precursor of **14**, is the 5′(*R*)-isomer and **13**, the precursor of **15**, is the 5′(*S*)-isomer.



8 13 14 15

In summary, this letter has reported synthesis and stereochemical assignments of 3′,5′-bridged 3′-amino-3′-deoxythymidines. The synthetic approaches are anticipated to be applicable to the preparation of 3′,5′-bridged nucleosides containing other pyrimidine or purine bases. Both the 5′(*R*)- and 5′(*S*)-isomers of these bicyclic nucleosides are potentially useful as building blocks of N3′–P5′ phosphoramidate and other N-backbone oligonucleotides. Synthesis and hybridization studies of oligonucleotides containing the 3′,5′-bridged nucleosides are under way and will be reported in due time.

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