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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.



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.



Scheme 1. (a) H_2 , 10% Pd/C, rt, 6 h, 87%; (b) BnOCOCl, Na₂CO₃, rt, 1 h, 87%; (c) DMSO, DCC, TFA, pyridine, rt, 4 h, 79%; (d) Zn, BrCH₂COOEt, THF, 40°C, 20 h, 62%; (e) DMT-Cl, AgNO₃, pyridine, 50°C, 24 h, 92%; (f) LiAlH₄, THF, 0°C, 2 h, 84%



Scheme 2. (a) H_2 , 10% Pd/C, rt, 14 h; (b) Fmoc-OSu, Et₃N, 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 CH₂Cl₂) 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, H₂, rt; (b) Fmoc-OSu, Et₃N, 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) (CF₃CO)₂O, Et₃N, 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) NH₄OH, 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.



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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