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3′,4′-*trans*-Linked bicyclic nucleosides locked in *S*-type conformations

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A novel class of 3′,4′-*trans*-linked bicyclic nucleosides with locked S-type furanose conformations is introduced by synthesis of two model derivatives; one was obtained by cyclic ether formation and the other by ring-closing metathesis methodology.

Conformationally restricted oligonucleotides have enabled high affinity recognition of DNA and RNA.[1,2] Thus, LNA (locked nucleic acid) is a prime example with the monomers (*e.g.* **1**) locked in an *N*-type conformation (Fig. 1).[3–5] We have earlier presented oligonucleotides containing the bicyclic nucleosides **2**[6] and **3**[7] modeling natural nucleosides locked in *S*-type conformations,[6,7] and *S*-type mimics without the natural ribofuranose skeleton have also been presented, *e.g.* **4**.[8] However, oligomers containing **2**, **3** or **4** display decreased affinities towards natural nucleic acid complements, probably due to steric problems or unfavorable duplex hydration. Additional structurally related analogues include *arabino*-configured 2′-ethynyl,[9] 2′-methoxy[10] and 2′-fluoro[11] oligonucleotides, none of which, however, is an ideal *S*-type mimic.[9–11] Recently, we introduced a tricyclic nucleoside derivative also being strongly restricted in an *S*-type conformation.[12] However, for this nucleoside, and the bi- and tricyclic nucleoside analogues of Leumann and coworkers,[13,14] the C4′–C5′ bond is involved in the conformational restricted skeleton imposing unfavorable positioning of the 5′-oxygen atom for formation of native Watson–Crick type double helices.[12–14] Herein we report the synthesis of two new bicyclic nucleoside derivatives in which the furanose rings are locked in typical *S*-type conformations and the C4′–C5′ bonds retain their natural flexibility. In addition, the introduction of C3′–C4′ *trans*-fused six-membered rings is expected to be sterically well tolerated in the major groove of B-type nucleic acid duplexes.

Diacetone-D-glucose was converted in three steps to the 3′-*C*-allyl derivative **5** (Scheme 1).[15] *In situ* regioselective cleavage of the primary acetonide and subsequent diol cleavage[16] was followed by an aldol condensation of the resulting aldehyde with formaldehyde and a Cannizzaro reaction affording the diol **6**. Acetylation to give **7** and subsequent acetolysis followed by another acetylation afforded the glycosyl donor **8**. Coupling with silylated thymine in a modified Vorbrüggen coupling reaction gave exclusively the β-nucleoside **9** due to anchimeric assistance from the 2-*O*-acetyl group.[17,18] The β-configuration of nucleoside **9** and of nucleosides **10**–**15** was confirmed by the large values of $^3J_{H1',H2'}$ (between 7.1 and 8.3 Hz). Deacetylation of **9** to give nucleoside **10** was followed by regioselective monotosylation at the 4′-*C*-hydroxymethyl functionality affording **11**. The site of tosylation was confirmed chemically, as it would be possible to cyclize **11** to give a 3′-*C*-branched-2′-*O*,4′-*C*-methylene linked LNA-type nucleoside if the tosylation had occurred at the assigned position (**11**, R_3 = Ts).[3,4,6] Actually, derivative **11** upon treatment with NaH in DMF was efficiently

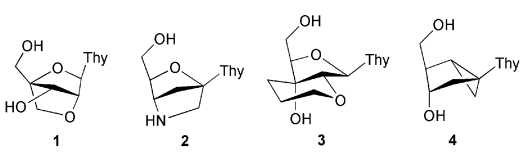
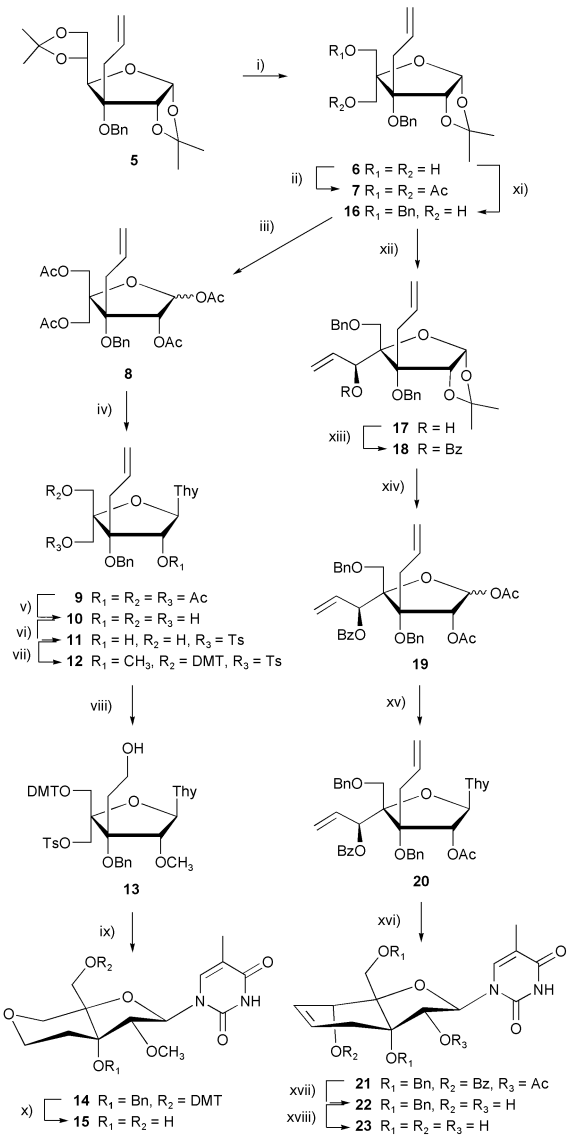


Fig. 1 Structures of bicylic nucleosides; Thy = thymin-1-yl.



Scheme 1 *Reagents and conditions*: i) a, H_5IO_6, EtOAc; b, H_2CO, NaOH, THF, H_2O then $NaBH_4$ (82%); ii) Ac_2O, DMAP, pyridine (85%); iii) a, 80% aq. AcOH, 90 °C; b, Ac_2O, DMAP, pyridine (88%); iv) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-triflate, MeCN, 60 °C (67%); v) NaOMe, MeOH (81%); vi) TsCl, pyridine (70%); vii) a, DMTCl, pyridine; b, NaH, CH_3I, THF, 0°C (65%); viii) a, OsO_4, $NaIO_4$, H_2O, dioxane; b, $NaBH_4$, H_2O, dioxane (48%); ix) NaH, DMF (89%); x) H_2, Pd/C, EtOH (67%); xi) NaH, BnBr, DMF (55%); xii) a, PCC, CH_2Cl_2; b, vinylMgBr, THF (63%); xiii) BzCl, pyridine (98%); xiv) a, 80% aq. AcOH, 90 °C; b, Ac_2O, pyridine (92%); xv) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-OTf, MeCN (93%); xvi) 2 mol-% Grubbs' catalyst,[21] $ClCH_2CH_2Cl$ (90%); xvi) NaOMe, MeOH, reflux (69%); xviii) BCl_3, hexane, CH_2Cl_2 (69%). Thy = thymin-1-yl.

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converted into a compound for which the ^{1}H NMR spectral data were in accordance with data expected for an LNA-type nucleoside (data not shown). As the next step selective protection of the 5′-OH group as its DMT ether was accomplished followed by chemoselective 2′-O-methylation. In order to limit the competing methylation at N3, we applied conditions which have earlier been used for chemoselective O-methylation of nucleosides,[19] and obtained **12** in reasonable yield.† Oxidative cleavage of the C3′-allyl moiety of **12** followed by reduction afforded the 2-hydroxyethyl substituted nucleoside **13**. Subsequent efficient cyclization to give **14** and hydrogenolysis furnished the conformationally locked bicyclic nucleoside **15**.‡§

In order to obtain the related bicyclic nucleoside **23** likewise conformationally locked in an *S*-type conformation due to the additional C3′–C4′-*trans*-fused six-membered ring, a ring-closing metathesis-based synthesis starting from the diol **6** was accomplished. Differentiation between the two primary alcohols of **6** was possible probably because of steric shielding of the α-face of the bicyclic system, and benzylation afforded a 4 : 1 ratio of bisbenzylic ethers of which **16** was obtained in 55% yield as the major isomer after chromatographic separation. The full assignments of **16** and its 4-epimer were performed by ^{1}H NMR spectroscopy. A similar ratio between 4-epimers has earlier been obtained on a similar substrate without the 3′-*C*-allyl group.¶ When exploring the ^{1}H NMR data given for that case,[20] the H1′ signals‖ of both isomers were seen to be shifted downfield compared to the H5 signals.¶[20] We ascribe this phenomenon to deshielding by the electronegative 3-O atom. For **16**, the highest chemical shifts were observed for the signal coupling to an OH-signal hereby confirming the 5-O-benzylation, whereas in the 4-epimer the situation is opposite. Subsequently, **16** was oxidized to an aldehyde followed by another Grignard addition to give two epimers in a 1 : 3 ratio from which **17** was isolated as the major isomer after chromatographic separation.** Protection as the benzoic ester **18** was followed by hydrolysis and acetylation to give the anomeric mixture **19**. A Vorbrüggen-type coupling gave exclusively the β-nucleoside **20**. The RCM reaction was performed using Grubbs' commercially available carbene precatalyst[21] affording smoothly the bicyclic nucleoside **21** in a high yield. The structure of this compound was confirmed by NMR and MS verifying the loss of ethylene, and by the large coupling constant $^3J_{H1'H2'} = 7.4$ Hz confirming this nucleoside to be β-configured and locked in an *S*-type conformation (*vide infra*). A basic treatment of **21** afforded cleavage of both the ester moieties to give **22** and finally, a Lewis acid mediated cleavage of the benzylic ethers gave the target bicyclic nucleoside **23**.§

The furanose conformations of nucleosides **15** and **23** were analyzed using the theory of Altona and coworkers.[22,23] The possible H1′H2′ torsion angles derived from the vicinal $^3J_{H1'H2'}$ coupling constants were 148 and 152° for **15** and **23**, respectively. The exocyclic H1′H2′ torsion angle is a function of the pseudorotation angle, *P*, and the puckering amplitude, Φ_{max}, and considering Φ_{max} in the range from 32 to 46°, we found possible ranges of *P* of 190–205° for **15** and 180–200° for **23**. This corresponds perfectly with **15** and **23** being the first nucleosides (despite **3**) with a natural ribofuranose skeleton locked in *S*-type conformations and with preserved flexibility of the C4′–C5′ bond.

In summary, the bicyclic nucleosides **15** and **23** have been synthesized in 11 steps from **5** in overall yields of 4.5 and 10%, respectively. In a very satisfying 22% overall yield from **5**, the RCM strategy afforded smoothly a highly constrained bicyclic nucleoside derivative **21** as a key intermediate towards the construction of other bicyclic nucleosides, *e.g.*, saturated, hydroxylated or 2′-deoxygenated derivatives. In short, 3′,4′-*trans*-linked bicyclic nucleosides have been introduced herein as a novel class of locked *S*-type nucleoside mimics exemplified by the synthesis of ribonucleoside analogues.

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Note added in proof. After submission of this manuscript, a slightly different preparation of **15** was published: S. Obika, M. Sekiguchi, T. Osaki, N. Shibata, M. Masaki, Y. Hari and T. Imanishi, *Tetrahedron Lett.*, 2002, **43**, 4365.

Notes and references

† Methylation at O2′ and not N3 was verified by NMR spectroscopy. The N3-proton of **12** appeared at 8.5 ppm together with a signal at 3.5 ppm diagnostic of an OCH_3 substituent (corresponding to a signal at 60 ppm in the ^{13}C NMR spectrum).

‡ Synthesis of compound **15** was included in the Ph.D. thesis of Dr M. Meldgaard, Dept. of Chemistry, University of Copenhagen, June 2000.

§ Selected data for (1*S*,6*R*,8*R*,9*R*)-1-hydroxy-6-hydroxymethyl-9-methoxy-8-(thymin-1-yl)-4,7-dioxabicyclo[4.3.0]nonane (**15**); ^{1}H NMR (CD_3OD) δ 8.11 (d, *J* 1.4 Hz, 1H, 6-H), 6.17 (d, *J* 7.1 Hz, 1H, 1′-H), 4.63 (d, *J* 7.1 Hz, 1H, 2′-H), 4.02 (m, 4H, CH_2), 3.75 (dd, *J* 5.0, 11.0 Hz, 1H, CH_2), 3.48 (d, *J* 9.6 Hz, 1H, CH_2), 3.42 (s, 1H, OCH_3), 2.08 (m, 1H, CH_2), 1.90 (d, *J* 1.4 Hz, 3H, CH_3), 1.84 (dd, *J* 3.0, 13.2 Hz, 1H, CH_2); selected data for (1*S*,5*S*,6*S*,8*R*,9*R*)-1,5,9-trihydroxy-6-hydroxymethyl-8-(thymin-1-yl)-7-oxabicyclo[4.3.0]non-3-ene (**23**); ^{1}H NMR (DMSO-d_6) δ 11.31 (br s, 1H, N-H), 8.17 (br s, 1H, 6-H), 6.10 (d, *J* 7.4 Hz, 1H, 1′-H), 5.81–5.71 (m, 3H, 2″-H, 3″-H, 3′-OH), 5.48 (br s, 1H, 5′-OH), 5.29 (d, *J* 6.3 Hz, 1H, 2′-OH), 5.10 (d, *J* 8.9 Hz, 1H, 1″-OH), 4.64 (dd, *J* 6.3, 7.4 Hz, 1H, 2′-H), 3.76 (m, 1H, 1″-H), 3.56–3.35 (m, 2H, 5′-H), 2.32-2.26 (m, 2H, 4″-H), 1.79 (s, 3H, CH_3).

¶ The O-benzylation of 4′-*C*-hydroxymethyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose with NMR assignments of the products given from NOE-difference spectra; ref. 20.

‖ We define the first carbon of the C4 substituent as C1′ *i.e.* defined as C1″ in corresponding nucleosides.

** Determination of the C1″-configuration was accomplished by NMR spectroscopy on the tricyclic RCM products of **17** and its C1″-epimer; manuscript in preparation.

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