Communications

Synthesis of Novel Bicyclo[2.2.1] Ribonucleosides: 2′-Amino- and 2′-Thio-LNA Monomeric Nucleosides

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The immense potential of oligonucleotide analogues as therapeutic agents or diagnostic molecules has stimulated intensive research on nucleic acid mimics during the last years.[1] Conformationally restricted analogues have been synthesized,[2] and especially oligonucleotide 2′-fluoro N3′−P5′-phosphoramidates have shown appealing characteristics.[2a] We have recently introduced LNA (locked nucleic acids) as a novel class of preorganized oligonucleotide analogues showing very interesting properties (Figure 1).[3,4] Parent LNA monomeric nucleosides were in our initial approach synthesized as indicated in Scheme 1.[3] Condensation of 4-*C*-(acetoxymethyl)-1,2-di-*O*-acetylfuranose **1** with silylated nucleobases afforded nucleoside diols **2** after deacetylation. LNA nucleosides **3** were subsequently obtained after monotosylation, base-induced ring closure, and debenzylation.[3,5]

The characteristics of LNA appear to be related to the fixed 3′-endo conformation of the monomeric LNA nucleosides (Figure 2).[6] One possible implication is that duplex formation involving LNA is entropically favored. Stimulated by LNA, a number of novel analogues can be identified as attracting synthetic targets, among these the 2′-amino- and 2′-thio-LNA nucleosides **7** and **11**. As the first step toward the evaluation of the properties of 2′-amino- and 2′-thio-LNA oligomers, e.g., the affinities toward complementary nucleic acids, we have investigated the synthesis and conformational behavior of compounds **7** and **11**. Especially appealing seems the possibility of utilizing the 2′-amino functionality of nucleoside **7** as a structurally well-defined conjugation site in LNAs.

4′-*C*-(Hydroxymethyl) nucleoside diols **4a** and **4b**[3] were converted into the di-*O*-tosylated nucleosides **5a** and **5b** in 80% and 78% yield, respectively, by reactions with *p*-toluenesulfonyl chloride and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) in dichloromethane. Reaction of the thymine nucleoside **5a** in benzylamine afforded in 30% yield the desired bicyclo[2.2.1] 2′-(benzylamino)-2′-deoxynucleoside **6a**. Analogously, the uracil nucleoside **5b** using potassium thioacetate in DMF gave the bicyclo[2.2.1] 2′-deoxy-2′-thionucleoside **6b** in 75% yield (deacetylation proceeds during the reaction). The strategy of double nucleophilic substitution reactions to obtain heteroatom analogues of LNA nucleosides thus proved effective. Mechanistically, these reactions are expected to proceed via 2,2′-anhydro nucleoside intermediates,[2d] explaining the overall retention of the configuration at the 2′-carbon atom. As the formation of such intermediates requires intramolecular displacement by an O-2 carbonyl in the base, this mechanistic suggestion implies that the synthetic strategy will be applicable for pyrimidine nucleosides only. The concomitant debenzylation proved troublesome. Thus, catalytic hydrogenation (20% Pd-$(OH)_2$/C, H_2) of 3′,5′-di-*O*-benzyl 2′-(benzylamino)-LNA nucleoside **6a** furnished a monobenzylated intermediate even after prolonged reaction time (5 days) using a large excess of catalyst. This intermediate could eventually be debenzylated using ammonium formate and 10% palladium on carbon, giving the desired parent 2′-amino-LNA nucleoside **7** [1-(2-amino-2-deoxy-2-*N*,4-*C*-methylene-*β*-D-ribofuranosyl)-thymine] in 54% yield from **6a**. Alternatively, one-step deprotection of **6a** (affording **7** in 67% yield) was performed with ammonium formate and 10% palladium on carbon.

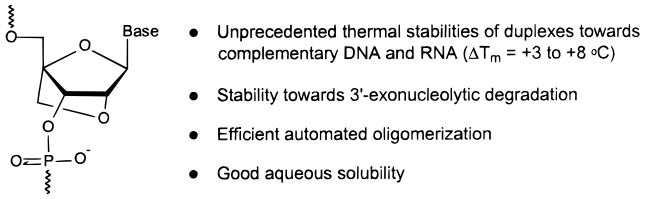
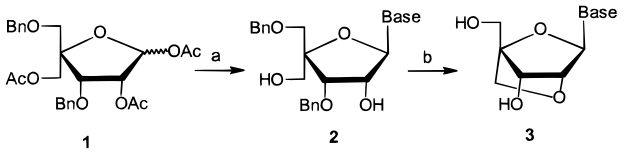


Figure 1. Structure and properties of LNA. ΔT_m = change in duplex melting temperatures per LNA monomer incorporated. Base = pyrimidine or purine nucleobases.

Scheme 1. Synthesis of LNA Nucleosides[a]



[a] Key: (a) (i) silylated base, TMS-triflate, (ii) deacetylation; (b) (i) monotosylation, (ii) ring-closure, (iii) debenzylation. Base = nucleobase.

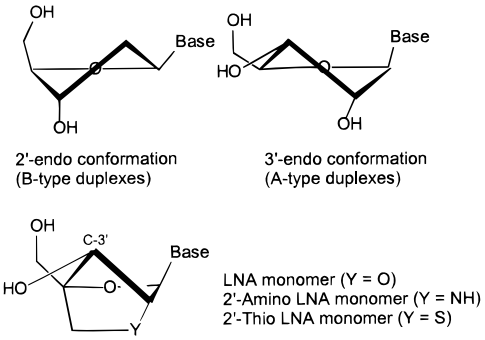


Figure 2. Conformations of nucleic acid monomers in B-type (generally DNA/DNA) duplexes and in A-type (generally DNA/RNA and RNA/RNA) duplexes and of LNA nucleosides.

(1) (a) Herdewijn, P. *Liebigs Ann.* **1996**, 1337. (b) Freier, S. M.; Altmann, K.-H. *Nucleic Acids Res.* **1997**, *25*, 4429.

(2) (a) Schultz, D. G.; Gryaznov, S. M. *Nucleic Acids Res.* **1996**, *24*, 2966. (b) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739. (c) Bolli, M.,; Litten, J. C.; Schütz, R.; Leumann, C. J. *Chem. Biol.* **1996**, *3*, 197. (d) Nielsen, P.; Pfundheller, H. M.; Olsen, C. E.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3423. (e) Steffens, R.; Leumann, C. J. *J. Am. Chem. Soc.* **1997**, *119*, 11548.

(3) Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455. (b) Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.

(4) LNA is defined as an oligonucleotide (analogue) containing one or more LNA nucleoside monomers.

(5) The LNA nucleosides containing uracil and cytosine nucleobases have been synthesized independently by a linear approach. See: Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735.

(6) Preorganization of LNA nucleosides into a 3′-endo-type conformation has been shown by X-ray crystallography, see ref 5, and by NMR studies, see ref 3.

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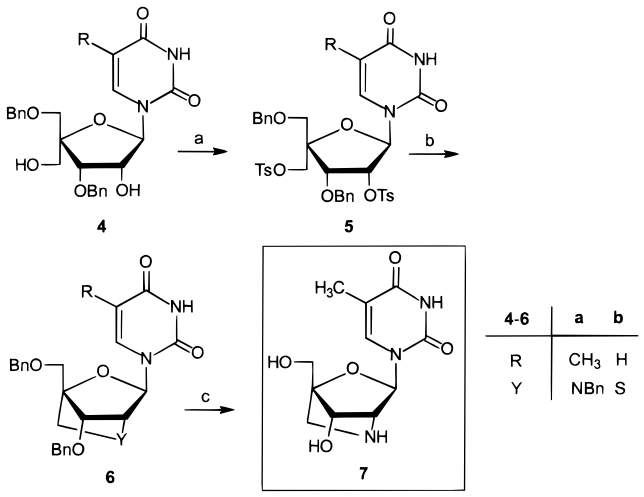
Scheme 2[a]



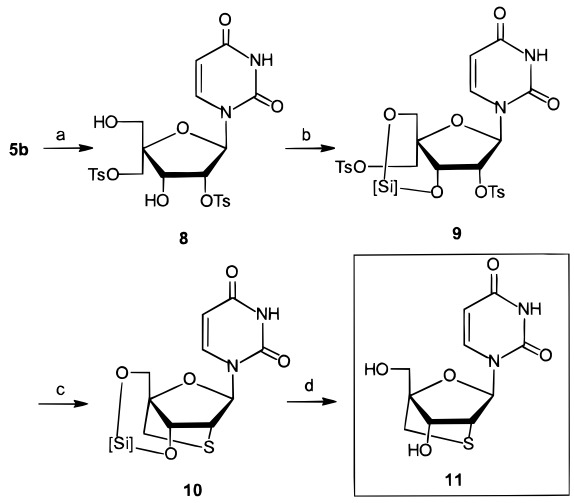
[a] Key: (a) TsCl, DMAP, CH_2Cl_2; (b) $BnNH_2$ (to give **6a**); KSAc, DMF (to give **6b**); (c) (i) 20% $Pd(OH)_2/C$, H_2, ethanol, (ii) 10% Pd/C, ammonium formate, methanol (alternatively 10% Pd/C, ammonium formate, methanol, one step) (to give **7**).

Debenzylation of 2′-thio nucleoside **6b** proved impossible in our hands. Thus, probably because of catalyst poisoning, catalytical hydrogenations (20% $Pd(OH)_2/C$, H_2, ethanol; 10% Pd/C, H_2, methanol; 10% Pd/C, 1,4-cyclohexadiene, methanol) failed. Likewise, when a variety of other methods (BCl_3, dichloromethane, hexane; 20% $Pd(OH)_2/C$, ammonium formate, methanol; BBr_3, dichloromethane; sodium, ethanol; CrO_3/CH_3COOH; iodotrimethylsilane) were employed,[7] either no reaction or cleavage of the glycosidic bond was observed (Scheme 2).

To obtain the desired 2′-thio nucleoside **11**, the synthetic strategy depicted in Scheme 3 was followed. Debenzylation (49% yield, 20% $Pd(OH)_2/C$, H_2) of the di-*O*-tosylated uracil nucleoside **5b** to give **8** followed by 3′,5′-di-*O*-silylation in 97% yield using the bidentate reagent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) afforded the bicyclic intermediate **9**. Conversion of **9** into the tricyclic intermediate **10** was accomplished in 77% yield by reaction with potassium thioacetate in DMF. Eventually, desilylation using tetrabutylammonium fluoride (TBAF) in THF afforded the desired 2′-thio-LNA nucleoside **11** [1-(2-deoxy-2-mercapto-2-*S*,4-*C*-methylene-*β*-D-ribofuranosyl)uracil] in 69% yield.

The conformation of 2′-amino-LNA nucleoside **7** and 2′-thio-LNA nucleoside **11** was evaluated from the coupling constants of the ^{1}H NMR spectra as described earlier for other nucleosides.[8] Importantly, the $J_{1',2'}$ values for the bicyclo[2.2.1] nucleosides **7** and **11** were 0 Hz,[9] indicating

Scheme 3[a]



[a] Key: (a) 20% $Pd(OH)_2/C$, H_2, ethanol; (b) $TIPDSCl_2$, anhydrous pyridine; (c) KSAc, DMF; (d) TBAF, THF. [Si] = 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl.

that **7** (2′-amino-LNA nucleoside, Figure 2, Y = NH) and **11** (2′-thio-LNA nucleoside, Figure 2, Y = S), like the parent LNA nucleosides (Figure 2, Y = O), exist in 3′-endo type conformations. This conclusion is substantiated by the strong and unusual NOEs observed between H-3′ and H-6 for compounds **6b** and **7**.[10]

On the basis of the results reported herein and earlier,[3,5] the bicyclo[2.2.1] nucleoside skeleton appears very attractive as a structural element of nucleic acid mimics containing monomeric nucleosides in conformationally restricted 3′-endo-type configurations. This has been clearly demonstrated by the superior recognition of complementary nucleic acids by parent LNA,[3] and the conformational analyses presented here for 2′-amino- and 2′-thio-LNA monomeric nucleosides indicate that the corresponding 2′-amino- and 2′-thio-LNA oligonucleotides deserve further attention. In our design of novel oligonucleotide analogues, we have been rather conservative, focusing on pentofuranose analogues containing phosphate esters at the natural 3′- and 5′-positions. This strategy has been successful in the case of LNA, and incorporation of the 2′-amino-LNA nucleoside **7** into oligonucleotides could represent a significant step forward offering a potentially convenient conjugation site in a preorganized nucleic acid mimic structurally resembling RNA. The synthetic route devised in this report gives convenient access to 2′-heteroatom substituted LNA pyrimidine nucleosides and should in addition also be applicable for synthesis of other bicyclic pyrimidine nucleoside analogues.

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Supporting Information Available: Supporting Information Available: Experimental procedures, spectroscopic data for compounds **4**−**11** and a copy of the ^{13}C NMR spectrum of compound **11** (8 pages).

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(8) (a) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333. (b) Obika, S.; Morio, K., Nanbu, D.; Imanishi, T. *Chem. Commun.* **1997**, 1643.

(9) ^{1}H NMR data for **7**: δ_H (DMSO-d_6) 11.29 (br s, 1H, NH), 7.73 (d, 1H, J = 1.1 Hz, 6-H), 5.31 (s, 1H, 1′-H), 5.29 (d, 1H, J = 3.7 Hz, 3′-OH), 5.13 (t, 1H, J = 5.3 Hz, 5′-OH), 3.81 (br s, 1H, 3′-H), 3.69 (m, 2H, 5′-H), 3.23 (s, 1H, 2′-H), 2.88 (d, 1H, J = 9.8 Hz, 1″-H_a), 2.55 (d, 1H, J = 9.8 Hz, 1″-H_b), 1.77 (d, 3H, J = 0.8 Hz, CH_3). ^{1}H NMR data for **11**: δ_H (CD_3OD) 8.19 (1H, d, J = 8.1 Hz, 6-H), 5.77 (1H, s, 1′-H), 5.65 (1H, d, J = 8.1 Hz, 5-H), 4.31 (1H, d, J = 2.1 Hz, 3′-H), 3.86 (2H, s, 5′-H), 3.53 (1H, d, J = 2.2 Hz, 2′-H), 2.93 (1H, d, J = 10.3 Hz, 1″-H_a), 2.73 (1H, d, J = 10.3 Hz, 1″-H_b).

(10) For **6b**: 9% NOE in H-6 (irradiation of H-3′). For **7**: 11% NOE in H-6 (irradiation of H-3′) and 6% NOE in H-3′ (irradiation of H-6).