Pergamon

Tetrahedron Letters 39 (1998) 4381–4384

TETRAHEDRON LETTERS

Novel Convenient Syntheses of LNA [2.2.1]Bicyclo Nucleosides

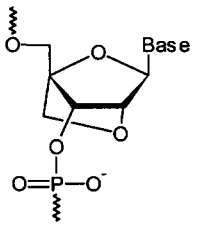
Alexei A. Koshkin, Vivek K. Rajwanshi and Jesper Wengel*

Department of Chemistry, Chemical Laboratory II, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark

Received 10 March 1998; accepted 31 March 1998

Abstract: LNA (Locked Nucleic Acids) is a novel oligonucleotide analogue containing [2.2.1]bicyclo nucleoside monomers. A novel and significantly improved method for convergent synthesis of LNA [2.2.1]bicyclo nucleosides using a 4-*C*-tosyloxymethyl-1,2-di-*O*-acetyl furanose as a key synthon is described. In addition, an alternative, robust linear approach allowing selective formation of the desired [2.2.1]bicyclo LNA nucleosides *via* a tricyclic nucleoside intermediate is introduced.

Stimulated in part by the immense potential of oligonucleotide analogues as therapeutic agents or diagnostic molecules, a vigorous search for an ideal nucleic acid mimic has been conducted during the last years.[1,2] We have recently introduced LNA (Locked Nucleic Acids) as a novel class of conformationally restricted oligonucleotide analogues showing very interesting properties as indicated below in Figure 1.[3-6]

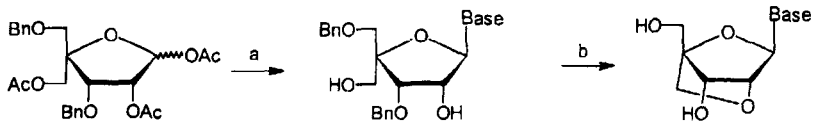


- Unprecedented thermal stabilities of duplexes towards complementary DNA and RNA (ΔT_m = +3 to +8 °C)
- Stability towards 3'-exonucleolytic degradation
- Efficient automated oligomerization
- Good aqueous solubility

Fig. 1. Structure and properties of LNA.[3-6] ΔT_m = change in duplex melting temperatures per LNA monomer incorporated. Base = pyrimidine or purine nucleobases.

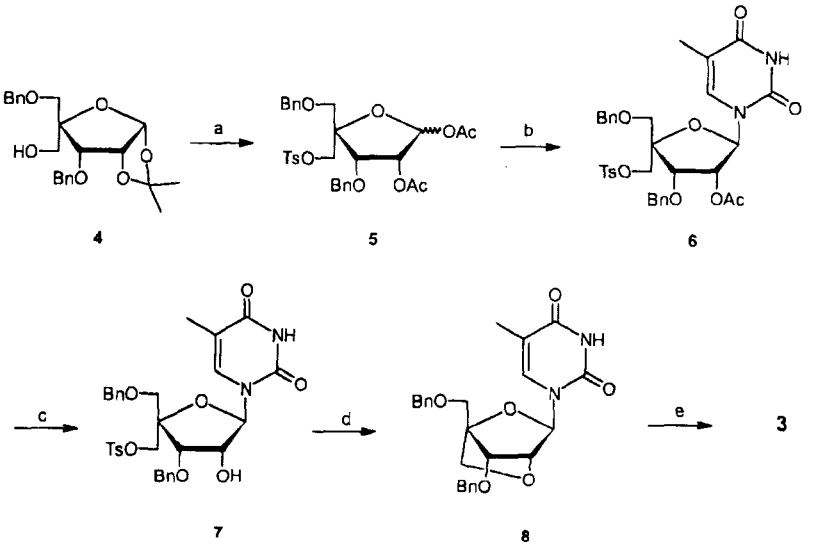
Based on these appealing features, development of efficient syntheses of LNA monomeric nucleosides is an important goal. In our initial approach (Scheme 1), the 4-*C*-acetoxymethyl-1,2-di-*O*-acetyl furanose **1** was condensed with silylated nucleobases using the Vorbrüggen methodology[7,8] to give partly deprotected nucleosides **2** after deacylation. Monotosylation, base-induced ring-closure and debenzylation afforded the parent LNA nucleosides **3** in overall yields of 7-30%.[4,9] Especially the monotosylation of nucleoside diols **2** proved troublesome resulting in conversion of **2** to 3',5'-di-*O*-benzyl[2.2.1]bicyclo nucleoside intermediates in yields of only 29-51%.[4]

0040-4039/98/$19.00
PII: S0040-4039(98)00706-0



Scheme 1. The initial strategy for synthesis of LNA nucleosides.[4] (a) i) silylated base, TMS-triflate, ii) deacetylation; (b) i) monotosylation, ii) ring-closure, iii) debenzylation. Base = thymin-1-yl, uracil-1-yl, 2-*N*-isobutyrylguanin-9-yl, 4-*N*-benzoylcytosin-1-yl (**2**), cytosin-1-yl (**3**), 6-*N*-bezoyladenin-9-yl (**2**), and adenin-9-yl (**3**).

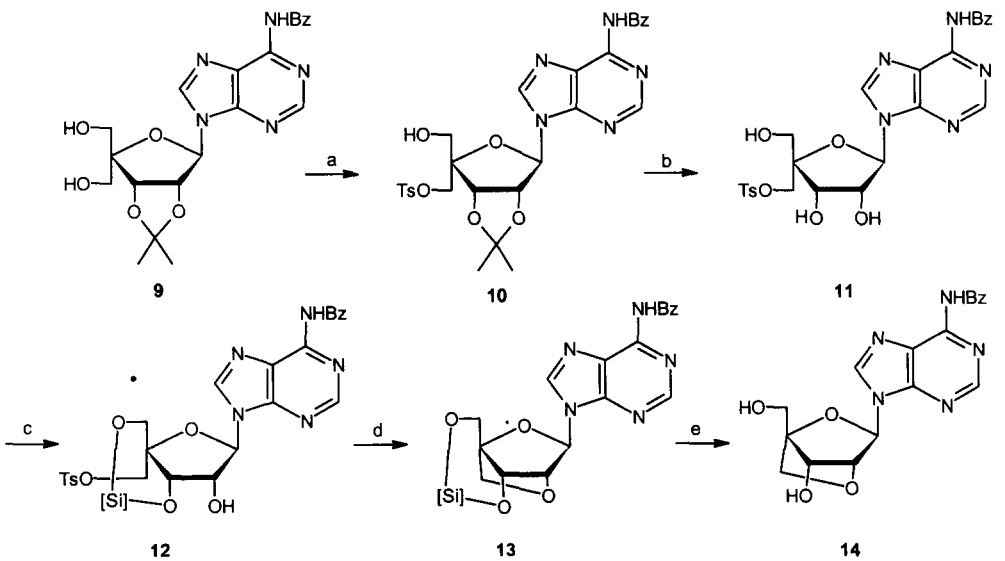
As part of our program for optimizing and upscaling the syntheses of LNA nucleosides we have developed the improved convergent approach depicted in Scheme 2. Tosylation of 4-*C*-hydroxymethyl furanose **4**[4] followed by acetolysis afforded 1,2-di-*O*-acetyl **5**[10] as an anomeric mixture in 59% yield. To evaluate the potential of furanose **5** as an intermediate for nucleoside synthesis, **5** was reacted with thymine, *N,O*-bis(trimethylsilyl)acetamide and trimethylsilyl triflate[7,8] in acetonitrile giving stereoselectively the desired β-configurated 4'-*C*-tosyloxymethyl nucleoside **6** in 92% yield. Subsequent deacetylation was accomplished using half-concentrated methanolic ammonia (4 h, room temperature, 87% yield) affording nucleoside **7**.[11] The tosyloxy group remained intact during this reaction. Treatment of **7** with sodium hydride in anhydrous DMF (37 h, 0 °C) resulted in efficient ring-closure to give (*1S,3R,4R,7S*)-7-benzyloxy-1-benzyloxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (**8**) in 92% yield. Though the universality of this procedure needs to be proven, these preliminary results (corresponding to overall 43% yield of thymine nucleoside **3** from fruanose **4**) compare well with our previously reported method.[4,9]



Scheme 2. (a) i) TsCl, anhydrous pyridine, CH_2Cl_2, ii) 80% aqueous AcOH, iii) Ac_2O, anhydrous pyridine; (b) thymine, *N,O*-bis(trimethylsilyl)acetamide, trimethylsilyl triflate, anhydrous CH_3CN; (c) NH_3, MeOH; (d) NaH, anhydrous DMF; (e) reference 4. Base = thymin-1-yl.

Alternatively, we have used a linear strategy to synthesize the 6-*N*-benzoylated adenine nucleoside **14** (Scheme 3).[12] Tosylation of 4'-*C*-hydroxymethyl-2',3'-*O*-isopropylidine-6-*N*-benzoyl adenosine (**9**)[13] afforded 4'-*C*-tosyloxymethyl nucleoside **10** (1.5 eqv. TsCl, 4 h, 0 °C, 37% yield). Besides, the 5'-*O*-tosyl regioisomer was isolated in 13% yield and unreacted starting material in 19% yield. Trifluoroacetic acid (30 min, room temperature, 94% yield) was applied to remove the isopropylidene group giving nucleoside **11**. Direct base-induced ring-closure of the corresponding 5'-*O*-dimethoxytrityl protected uracil nucleoside has been reported to yield exclusively the 3'-*O*,4'-*C*-linked oxetane nucleoside.[14] Analogously, our attempts on direct ring-closure of the monotosylated uracil nucleoside corresponding to **11** resulted in predominant formation of the undesired 3'-*O*,4'-*C*-linked product.[15] Based on these observations we decided to prevent both 3',4'- and 4',5'-oxetane formation by synthesizing the 3'-*O*,5'-*O* protected nucleoside **12** (1.2 eqv. 1,3-dichlorodisiloxane reagent, 2 h, 0 °C, then room temperature, 78% yield). Subsequent cyclization (NaH, 30 min, 0 °C, 84%) yielded smoothly the tricyclic nucleoside intermediate **13** which was desilylated (1 h, room temperature) to afford the desired [2.2.1]bicyclo adenine nucleoside **14**.[4] The overall yield from nucleoside **9** to the parent LNA adenine nucleoside **14** was 21%.

Recently, an analogous linear approach to the synthesis of the corresponding uracil nucleoside, employing a selective C-O bond cleavage of a 2',3'-*O*-benzylidene intermediate as a key step, has been developed independently (overall yield was 24%).[6] Thus, the yields for the two alternative linear strategies are comparable, but the method introduced here involves only five steps. In addition, the universality of the reported selective benzylidene opening[6,12] has not yet been established, and participation of the uracil base can not be ruled out. As nucleobase participation is very unlikely (the purine adenine was employed) in the reaction sequence **9** to **14** it can be anticipated that analogous reactions for the corresponding 4'-*C*-hydroxymethyl pyrimidine nucleosides should be possible.



Scheme 3. (a) TsCl, anhydrous pyridine; (b) 90% aqueous CF_3COOH; (c) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, anhydrous pyridine; (d) NaH, anhydrous THF; (e) $Et_3N{\cdot}3HF$, anhydrous THF. [Si] = 1,1,3,3-tetraisopropyldisiloxan-1,3-diyl.

Summarizing, a novel and significantly improved method for convergent synthesis of LNA [2.2.1]bicyclo nucleosides has been developed. Thus, furanose **5** is a novel convenient key synthon for condensation with silylated nucleobases as shown by synthesis of the thymine nucleosides **6-8**. An alternative, robust linear approach allowing selective formation of the desired [2.2.1]bicyclo LNA nucleosides *via* tricyclo nucleoside **13** has been described and exemplified by synthesis of the adenine nucleoside **14**. Evaluation of these methods for synthesis of LNA nucleosides in general is under way.

ACKNOWLEDGEMENTS

The Danish Natural Science Research Council, The Danish International Development Agency and Exiqon A/S, Denmark are thanked for financial support.

REFERENCES AND NOTES

1. Herdewijn, P. Liebigs Ann. 1996, 1337-1348.
2. Freier, S. M.; Altmann, K.-H. Nucleic Acids Res. 1997, 25, 4429-4443.
3. Singh, S. K.; Nielsen, P,; Koshkin, A. A.; Wengel, J. Chem. Commun. 1998, 455-456.
4. Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. Tetrahedron 1998, 54, 3607-3630.
5. LNA is defined as an oligonucleotide (analogue) containing one or more LNA monomers.
6. Preorganization of LNA nucleosides into a 3'-endo type conformation has been shown by X-ray crystallography, see Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. Tetrahedron Lett. 1997, 38, 8735-8738, and by NMR studies, see references 3 and 4.
7. Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. Chem. Ber. 1981, 114, 1234-1255.
8. Vorbrüggen, H.; Höfle, G. Chem. Ber. 1981, 114, 1256-1268.
9. Overall yields obtained for the individual LNA nucleosides (reference 4): thymin-1-yl (30%), uracil-1-yl (17%), 2-*N*-isobutyrylguanin-9-yl (21%), cytosin-1-yl (7%), and adenin-9-yl (14%).
10. 1,2-Di-*O*-acetyl-3,5-di-*O*-benzyl-4-*C*-(*p*-toluenesulphonyloxymethyl)-α,β-D-ribofuranose (**5**). δ_C ($CDCl_3$) 169.8, 169.6, 169.4, 168.8 (C=O), 144.7, 137.7, 137.6, 137.5, 132.8, 129.7, 129.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6 (Ar), 97.4, 94.2 (C-1), 86.4, 84.2 (C-4), 78.9, 77.5, 74.5, 74.1, 73.7, 73.5, 71.8, 70.6, 70.5, 69.6, 69.5 (Bn, C-2, C-3, C-5, C-1'), 21.6, 21.0, 20.8, 20.6, 20.4 (4 x $COCH_3$, 2 x CH_3). FAB-MS *m/z* 599 $[M+H]^+$ (Found C, 61.8; H, 5.6; $C_{31}H_{34}O_{10}S$ requires C, 62.2; H, 5.7).
11. 1-(3,5-Di-*O*-benzyl-4-*C*-(*p*-toluenesulphonyloxymethyl)-β-D-ribofuranosyl)thymine (**7**). δ_C ($CDCl_3$) 163.8 (C-4), 150.9 (C-2), 145.0, 137.0, 136.9, 135.9, 132.3, 129.8, 128.7, 128.6, 128.2, 128.1, 128.0, 127.6 (Ar, C-6), 111.0 (C-5), 89.6, 85.3, 78.4, 74.5, 73.8, 71.1, 69.7 (Bn, C-1', C-2', C-3', C-4', C-5', C-1"), 21.6 (CH_3), 12.0 (CH_3). FAB-MS *m/z* 623 $[M+H]^+$ (Found C, 61.5; H, 5.2; N, 4.4; S, 5.2, $C_{32}H_{34}O_9N_2S$ requires C, 61.7; H, 5.4; N, 4.5; S, 5.1).
12. Protection of the exocyclic amino groups of cytosine, adenine and guanine nucleobases is needed for automated oligonucleotide synthesis. In the original convergent strategy (reference 4), debenzylation resulted in concomitant debenzoylation in the adenine moiety.
13. Jones, G. H.; Taniguchi, M.; Tegg, D.; Moffatt, J. G. J. Org. Chem. 1979, 44, 1309-1317.
14. Obika, S.; Morio, K., Nanbu, D.; Imanishi, T. Chem. Commun. 1997, 1643-1644.
15. Meldgaard, M.; Wengel, J. Unpublished results.