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Synthesis of locked pyranosyl nucleic acid (LpNA)

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ABSTRACT

A new locked pyranosyl nucleoside was synthesized by phenylsulfinyl-assisted chemistry. The novel building block was inserted into oligonucleotides and provides new insight on conformational restricted pyranosyl nucleosides on duplex formation

Conformational restriction of nucleotides is currently being used to control sugar conformation and thereby influence the function of the modified nucleic acids. The inspiration for synthesizing a locked pyranose nucleic acid (LpNA, Fig. 1a) came from the work by the groups of Imanishi[1] and Wengel,[2] who simultaneously reported the synthesis of locked nucleic acid (LNA, Fig. 1b) in 1998,[3,4] which has been shown to hybridize strongly with RNA and DNA.[5,6] Since then, numerous analogues comprising different linkages and substitution patterns have been synthesized and evaluated,[7] however, only very few of these have concerned covalently constrained nucleotides comprising a six-membered sugar-moiety. The rationale seemed to be that the locking of the pyranose nucleosides is not needed because the pyranose ring is less flexible than the furanose ring, and as a result thereof its conformation is to some degree restricted to a chair conformation. However, a completely different spatial arrangement of its substituents is achieved if the pyranose ring is locked into the corresponding boat conformation and such nucleosides seem only to have been reported by the group of Chattopadhyaya with the hydroxymethyl group transposed from the 5′ to the 4′-position.[8,9]

Huge amount of experimental knowledge is known about the chemistry of hexoses and therefore we thought it easy to develop a convergent synthesis of an LpNA monomer by coupling an appropriate carbohydrate with a nucleobase. However, during this project we ran into numerous synthetic dead ends which are reported in the Supplementary data and here we shall give an overview of the reaction sequence for the successful synthesis of a 3′-methoxy LpNA (**2**, Fig. 1a).

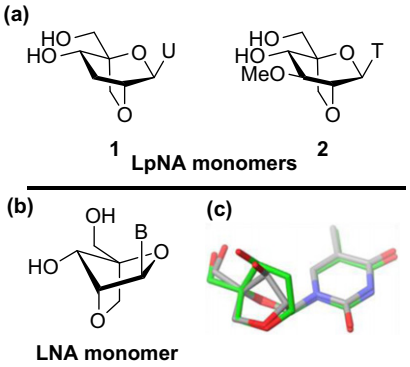

Figure 1. (a) LpNA monomers **1** and **2**. (b) LNA monomer. (c) Overlay of LNA (indicated with grey carbon atoms) and 3′-deoxy-LpNA monomer (indicated with green carbon atoms).

We did molecular modeling before starting the synthesis of an LpNA and we found a surprisingly high degree of similarity of the spatial arrangement of the substituents in an LpNA with those in an LNA monomer (Fig. 1c).

During the preparation of this paper describing the synthesis of what we thought was the first example of a pyranose sugar locked into a boat-type conformation by an O2′ to C5′ methylene linkage, a patent from ISIS Pharmaceuticals was disclosed in early 2011 describing the synthesis of the 3′-deoxy locked uridine derivative (**1**, Fig. 1a).[10] Their synthesis of **1** involved a 2,2′-anhydro

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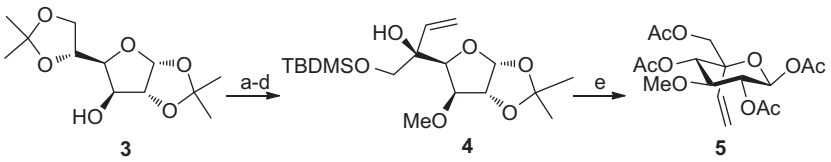
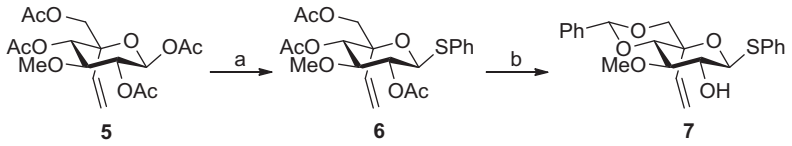
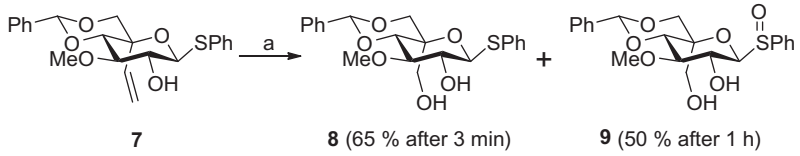


Scheme 1. Reagents and conditions: (a) KOH, Bu_4NBr, MeI, acetone, 0 °C to rt to reflux, 27 h, 74%; (b) 60% aq AcOH, 60 °C, 2 h, 84%; (c) TBDMS-Cl, imidazole, DMF, rt, 4 h, 69%; (d) (i) DMSO, $(COCl)_2$, NEt_3, DCM, −78 °C, 2 h, (ii) $CH_2CHMgBr$, THF, rt, 1 h, 73%; (e) (i) IR-120 H^+ resin, 1,4-dioxane/H_2O (1:1, v/v), 80 °C, 3 days, (ii) Ac_2O, pyridine, rt, 3.5 h, 83%. DCM = dichloromethane, TBDMS = *t*-butyldimethylsilyl.



Scheme 2. Reagents and conditions: (a) PhSH, BF_3OEt_2, DCM, rt, 3.5 h, 81%; (b) (i) CH_3ONa, MeOH, rt, 1 h, IR-120 H^+ resin, 1 h, (ii) $PhCH(OMe)_2$, *p*$TsOH{\cdot}H_2O$, DMF, 70 °C, 3 h, 76%. DCM = dichloromethane, Ts = toluene-4-sulfonyl.



Scheme 3. Reagents and conditions: (a) (i) O_3, DCM, −78 °C, 3–60 min, then quenched with Me_2S, (ii) $NaBH_4$, abs EtOH (**8**) or MeOH (**9**), 0 °C to rt, 2 h. DCM = dichloromethane.

nucleoside derivative in the key step, whereas we were looking for a convergent strategy for the nucleosides that normally allows all natural nucleobases to be introduced.[2]

The synthesis of compound **5** was performed according to literature procedures in five steps (Scheme 1)[11,12] with transformation of the furanose sugar **4** into the pyranose sugar **5** in the last step as the key step in achieving a suitable starting material for the pyranose nucleoside **2**.

It was possible to couple **5** with silylated thymine, but we failed to transform the synthesized nucleoside into an LpNA monomer (Scheme S7). By making the anomeric substituent orthogonal to other substituents concerning deprotection, it opens up other routes to an LpNA monomer and we therefore turned to thioglycosides as key intermediates. Fernández and Castillón have shown that C1-thiophenyl hexopyranose derivatives can be used for the synthesis of nucleosides using *N*-bromosuccinimide (NBS) as activator.[13] Consequently, we synthesized the 3-methoxy-1-thiophenylglycoside **7** (Scheme 2) in a similar procedure as reported by Blériot et al. who used 3-*O*-benzyl derivative in the synthesis of a locked glucoside.[14]

However, when we proceeded according to the procedure of Blériot et al. with ozonolysis of the vinyl group in compound **7**, we succeeded to synthesize **8** in a low scale synthesis, only (Scheme 3). It was indeed possible to synthesize the corresponding locked glycoside from **8** and to use it in a low yield coupling reaction with silylated thymine to give an anomeric mixture of LpNA monomers (Scheme S11). Alternatively, when we made the nucleoside from **8** before locking it into an LpNA monomer, then we also ran into experimental difficulties (Scheme S12). Therefore we decided to take advantage of the observation that phenylsulfinyl glycoside **9** is formed in a large scale synthesis in the ozonolysis of compound **7**. In a large scale synthesis an increased reaction time was required in order to saturate the solution with ozone. We presume that the formed ozonide oxidizes the C1-thiophenyl via an intra- or intermolecular reaction, since direct oxidation of thiophenyl by ozonolysis has not been reported in the literature (Scheme 3).

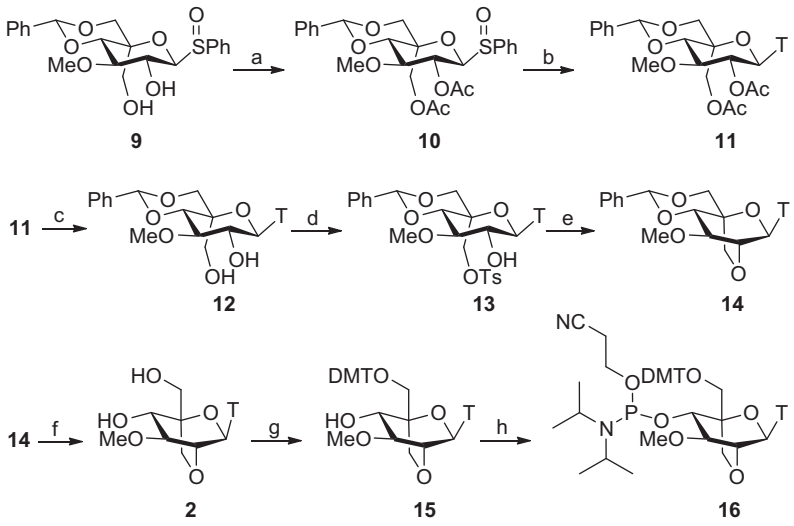
Chanteloup and Beau have previously shown that phenylsulfinyl ribofuranosides are highly suitable for nucleoside coupling reactions.[15] Scheme 4 summarizes the eight-step route to the DMT-protected phosphoramidite **16** starting from compound **9**.

The diacetylated sulfinyl derivative **10** was used in a stereoselective Vorbrüggen coupling reaction using in situ silylation of thymine by *N*,*O*-bistrimethylsilyl acetamide (BSA) before addition of the acetylated compound **10** and trimethylsilyl trifluoro-methanesulfonate (TMSOTf) as a Lewis acid catalyst. The β-nucleoside **11** was isolated in 81% yield after 4 h at room temperature. To our knowledge, this is the first example of a nucleoside coupling reaction using a phenylsulfinyl-hexopyranoside derivative. The cyclization reaction was successfully performed on the tosyl derivative **13** using NaH in DMF affording the corresponding locked β-nucleoside **14** in 74% yield. The DMT protected derivative **15** was also converted into the corresponding 5-methylcytosine nucleoside as previously performed using DMT-protected nucleosides (Scheme S13).[16]

It was also attempted to synthesize the corresponding 3′-deoxy nucleosides by starting out from the 3′-deoxy derivative of **3**, but in the step analogous to formation of the branched glucoside **5** from **4**, a mixture of the corresponding furanose derivative and an 1,6-anhydro pyranose derivative was obtained (Scheme S2).

Although structural resemblance of LpNA to LNA was expected as mentioned above, incorporation of the thymine monomer once or twice into a 21mer DNA or RNA resulted for the corresponding DNA/DNA or DNA/RNA duplex in a 6–7 °C lower melting temperature per modification (Table 1). Further lowering of melting temperature in mismatch studies (Tables S3 and S4) confirmed the base-pairing capability of LpNA.

Molecular modeling of the modified B-type duplexes shows that the axial position of the C3′-OMe group cause a distortion of the backbone. Moreover, the position of the neighbouring nucleobase



Scheme 4. Reagents and conditions: (a) Ac_2O, pyridine, rt, 23 h; 98%; (b) thymine, BSA, TMSOTf, DCE, rt, 4 h, 81%; (c) satd methanolic NH_3, rt, 7 h, 89%; (d) TsCl, pyridine, 70 °C, 29 h, 70%; (e) NaH, DMF, rt, 20 h, 74%; (f) H_2, Pd/C 10%, EtOAc, rt, 2 h, 100%; (g) DMTCl, pyridine, rt, 14 h, 87%; (h) DIPEA, 2-cyanoethyl *N*,*N*-diisopropyl-chlorophosphordiamidite, DCE, rt, 4 h, 85%. BSA = *N*,*O*-bistrimethylsilyl acetamide, TMSOTf = trimethylsilyl trifluoro-methanesulfonate, DCE = 1,2-dicloroethane, Ts = toluene-4-sulfonyl, DMT = dimethoxytrityl, DIPEA = *N*,*N*-diisopropylethylamine, T = thymin-1-yl.

Table 1
T_m (°C) data for matched DNA/DNA and DNA/RNA melting, taken from UV melting curves (λ = 260 nm)[a]

Entry	Sequence	DNA[b] 3′-ACGTGACATACAGACATGGTA **ON4**		RNA[b] 3′-ACGUGACAUACAGACAUGGUA **ON5**	
		T_m (°C)	ΔT_m	T_m (°C)	ΔT_m
ON1	5′-TGCACTGTATGTCTGTACCAT	62.5	Ref.	63.0	Ref.
ON2	5′-TGCACTGTA**T^p**GTCTGTACCAT	56.5	−6.0	56.5	−6.5
ON3	5′-TGCACTGTA**T^p**G**T^p**CTGTACCAT	48.0	−14.5	48.5	−14.5

[a] 1 μM of each ON in 10 mM NaH_2PO_4/Na_2HPO_4, 100 mM NaCl, 0.1 mM EDTA at pH 7.0.
[b] Target for monomer **T^p** (**ON2**) is underlined.

is affected resulting in less intrastrand nucleobase overlap at the 4-end and thereby loss of stabilizing π–π stacking (Fig. S4). Unfortunately, it is difficult to make conclusions about the effect of the C3′-OMe group as melting was not reported for the wild type oligo in the patent about the corresponding 2-deoxy oligonucleotides.[10]

In conclusion, we have described the synthesis and incorporation of a novel six-membered bicyclic nucleic acid (LpNA) into oligonucleotides using standard automated DNA synthesis. The 3′-methoxy-LpNA was synthesized in 2% overall yield over 16 steps starting from commercially available diacetone-D-glucose.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.005.

References and notes

1. Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735.
2. Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.
3. Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455.
4. Obika, S.; Morio, K.; Nanbu, D.; Imanishi, T. *Chem. Commun.* **1997**, 1643.
5. Vester, B.; Wengel, J. *Biochemistry* **2004**, *43*, 13233.
6. Singh, S. K.; Kumar, R.; Wengel, J. *J. Org. Chem.* **1998**, *63*, 10035.
7. Zhou, C. Z.; Chattopadhyaya, J. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 876.
8. Zhou, C. Z.; Plashkevych, O.; Liu, Y.; Badgujar, N.; Chattopadhyaya, J. *Heterocycles* **2009**, *78*, 1715.
9. Li, Q.; Yuan, F.; Zhou, C.; Plashkevych, O.; Chattopadhaya, J. *J. Org. Chem.* **2010**, *75*, 6122.
10. Migawa, M. T.; Punit, P. P.; Swayze, E. E.; Ross, B. S.; Song, Q.; Han, M. (ISIS Pharmaceuticals), WO2011/017521A2, 2011.
11. Das, S. K.; Mallet, J. M.; Esnault, J.; Driguez, P. A.; Duchaussoy, P.; Sizun, P.; Herault, J. P.; Herbert, J. M.; Petitou, M.; Sinaÿ, P. *Chem. Eur. J.* **2001**, *7*, 4821.
12. Yan, S. H.; Klemm, D. *Tetrahedron* **2002**, *58*, 10065.
13. Fernández, R.; Castillón, S. *Tetrahedron* **1999**, *55*, 8497.
14. Blériot, Y.; Vadivel, S. K.; Herrera, A. J.; Greig, I. R.; Kirby, A. J.; Sinaÿ, P. *Tetrahedron* **2004**, *60*, 6813.
15. Chanteloup, L.; Beau, J. M. *Tetrahedron Lett.* **1992**, *33*, 5347.
16. Ross, B. S.; Han, M. M.; Ravikumar, V. T. *Nucleosides Nucleotides Nucleic Acids* **2006**, *25*, 765.