

# Synthesis and chemoselective activation of phenyl 3,5-di-*O*-benzyl-2-*O*,4-*C*-methylene-1-thio- $\beta$ -D-ribofuranoside: a key synthon towards $\alpha$ -LNA

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A bicyclic thiofuranoside (phenyl 3,5-di-*O*-benzyl-2-*O*,4-*C*-methylene- $\beta$ -D-ribofuranoside) was efficiently synthesized and introduced as the key synthon in a method for convergent synthesis of  $\alpha$ - and  $\beta$ -LNA nucleosides; acid-induced ring-opening reactions of the corresponding bicyclic methyl furanoside are also described.

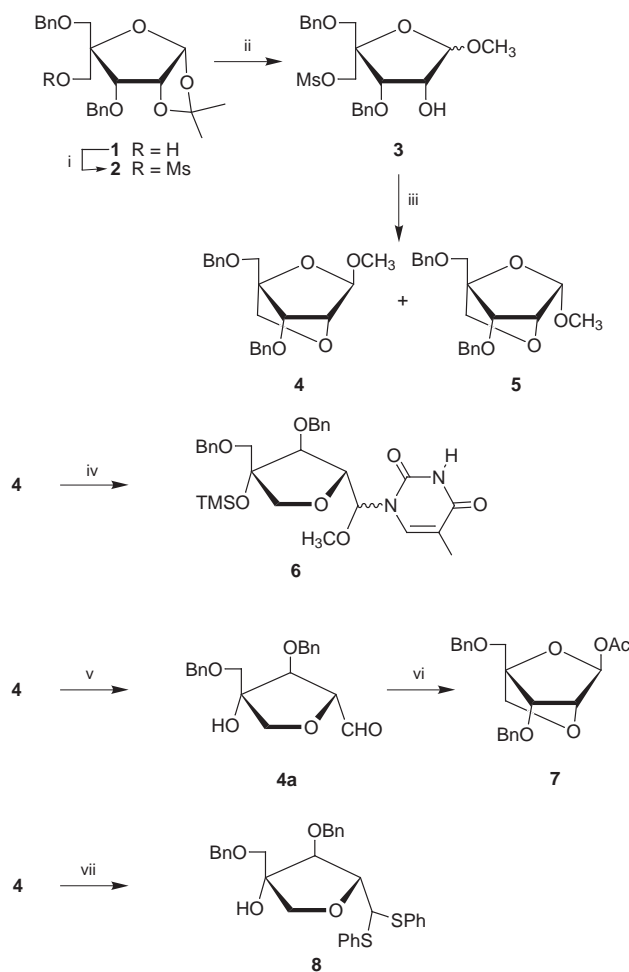
In the search for an ideal nucleic acid mimic, intensive research towards conformationally restricted oligonucleotide analogues has been carried out during the last years.<sup>1</sup> We have recently introduced LNA (Locked Nucleic Acid) as a novel class of preorganized oligonucleotide analogues showing very interesting properties.<sup>2–4†</sup> In our initial synthetic approaches, monomeric  $\beta$ -configured LNA nucleosides (e.g. the thymine derivative **13**, 5-methyl-2'-*O*,4'-*C*-methylenuridine or (1*S*,3*R*,4*R*,7*S*)-7-hydroxy-1-hydroxymethyl-3-(thymine-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, Scheme 2) were synthesized by stereoselective condensation of appropriately protected 4-*C*-hydroxymethyl-1,2-di-*O*-acetyl furanoses with silylated nucleobases and subsequent base-induced ring-closure and deprotection;<sup>2,3</sup> linear syntheses of LNA nucleosides have also been accomplished.<sup>5–7</sup> Here, a novel synthetic strategy is introduced, involving the use of a bicyclic carbohydrate precursor for nucleobase coupling reactions thus revealing the first synthesis of  $\alpha$ -configured LNA nucleosides. Our interest in these  $\alpha$ -anomers, and in  $\alpha$ -LNA, was stimulated by reports that  $\alpha$ -DNA, in comparison with  $\beta$ -DNA, forms a more stable duplex with complementary RNA.<sup>8,9</sup>

The protected 4-*C*-hydroxymethyl furanose **1** was synthesized according to the known method<sup>3,10</sup> and converted to the methanesulfonate **2** in 99% yield (Scheme 1). This compound was treated with HCl in MeOH–H<sub>2</sub>O (7:1 v/v) to give the anomeric mixture of methyl furanosides **3** in 95% yield. Treatment with NaH gave the two isomeric bicyclic methyl furanosides **4** and **5** in 60 and 30% yield, respectively. The structures of the two products were verified using NMR experiments. Thus, mutual NOE contacts between H-1, H-2 and H-3 verified the  $\alpha$ -configuration of **5** and the absence of NOE contacts between H-1 and H-3 verified the  $\beta$ -configuration of **4**. The coupling constants <sup>3</sup>*J*<sub>H1,H2</sub> and <sup>3</sup>*J*<sub>H2,H3</sub> were in both cases extremely small (~0 Hz) confirming the bicyclo[2.2.1]heptane structures. An attempt to use these bicyclic methyl furanosides as precursors for synthesis of LNA nucleosides failed. Thus, coupling of thymine to furanoside **4** using a modified Vorbrüggen methodology<sup>11</sup> [*N,O*-bis(trimethylsilyl)acetamide (BSA) and Me<sub>3</sub>SiOTf in MeCN] afforded in 59% yield one major product which was assigned as the ring-opened derivative **6** existing as a mixture of diastereoisomers.<sup>12</sup> The considerable ring strain in the bicyclic structure is a plausible explanation for the favouring of the Lewis acid mediated ring-opening reaction over the cleavage of the anomeric bond.

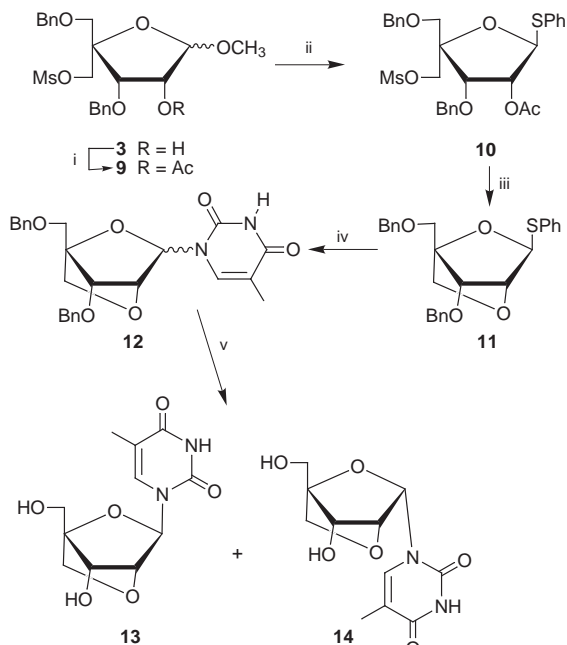
As an attempt to overcome this problem, better leaving groups were introduced at the anomeric position (Scheme 1 and 2). In order to obtain a mixture of 1'-*O*-acetyl derivatives, methyl furanoside **4** (and/or **5**) was treated with 80% aq. AcOH

to give a deprotected intermediate, which was subsequently acetylated. The latter reaction was slow and problematic giving a major product in only 23% yield, which was assigned as the pure  $\beta$ -anomer **7**. NMR spectra of the deprotected intermediate showed distinctive aldehyde signals suggesting ring strain and the predominance of the monocyclic intermediate **4a** to be responsible for the slow and low-yielding conversion to furanose **7**. Summarizing, the strategies depicted in Scheme 1 are not convenient for synthesis of the bicyclic nucleosides.

Thioglycosides have been intensively investigated for glycosylation reactions due to their ability to react with sulfur-specific electrophiles, thereby creating sulfonium cations readily displaced as leaving groups.<sup>13,14</sup> In the case of phenyl thioglycosides, there have been reports of nucleobase coupling reactions yielding natural as well as modified nucleosides.<sup>15,16</sup>



**Scheme 1** Reagents and conditions: i, MsCl, pyridine; ii, 20% HCl in MeOH, H<sub>2</sub>O; iii, NaH, DMF; iv, thymine, BSA, Me<sub>3</sub>SiOTf, MeCN; v, 80% AcOH; vi, Ac<sub>2</sub>O, pyridine; vii, Me<sub>3</sub>SiPh, Me<sub>3</sub>SiOTf, CH<sub>2</sub>Cl<sub>2</sub>.



**Scheme 2** Reagents and conditions: i, Ac<sub>2</sub>O, pyridine; ii, Me<sub>3</sub>SiSPh, Me<sub>3</sub>SiOTf, CH<sub>2</sub>Cl<sub>2</sub>; iii, NH<sub>3</sub>, MeOH, then NaH, DMF; iv, thymine, HMDS, then NBS, **11**, 4 Å molecular sieves, CH<sub>2</sub>Cl<sub>2</sub>; v, H<sub>2</sub>, Pd(OH)<sub>2</sub>/C, EtOH, CH<sub>2</sub>Cl<sub>2</sub>.

Furthermore, oxidized phenylsulfenyl glycosides have been used in glycosylations<sup>17</sup> and in nucleobase coupling reactions.<sup>18,19</sup> Thioglycosides have been obtained from *O*-glycosides,<sup>13</sup> but treatment of the bicyclic methyl furanoside **4** with Me<sub>3</sub>SiSPh and Me<sub>3</sub>SiOTf<sup>13</sup> gave the ring-opened dithioacetal derivative **8** in 61% yield (Scheme 1). However, after protection of the methyl furanoside **3** to give 2'-*O*-acetyl derivative **9** in 97% yield (Scheme 2), the β-thiofuranoside **10** was obtained in 66% yield using Me<sub>3</sub>SiSPh and Me<sub>3</sub>SiOTf (25% of starting material **9** was recovered). Only trace of the α-anomer of **10** was detected due to the expected anchimeric assistance from the 2'-*O*-acetyl group. The acetyl group was removed with methanolic ammonia and direct ring-closure was very efficiently performed using NaH affording phenyl 3,5-di-*O*-benzyl-2-*O*,4-*C*-methylene-β-D-ribofuranoside **11**† in 95% yield.

Condensation of the bicyclic phenyl thiofuranoside **11** with silylated thymine<sup>20</sup> using NBS as a thiophilic activator<sup>13,16</sup> gave an inseparable mixture of anomeric nucleosides **12** (α:β ~ 2:1) in 61% yield (or 100% yield based on the recovery of 39% starting material). This mixture was directly deprotected by hydrogenation to give the known β-LNA nucleoside **13** and its α-LNA nucleoside analogue **14**§ (in preliminary yields of 12 and 25%, respectively). The expected bicyclic structure of **14** was verified by mass spectrometry and NMR spectroscopy which revealed, as for **13**,<sup>2,3,5</sup> negligible <sup>3</sup>J<sub>H1',H2'}</sub> and <sup>3</sup>J<sub>H2',H3'}</sub> coupling constants (~0 Hz). Importantly, no ring-opening reactions were detected using this nucleobase coupling method taking advantage of the chemoselective cleavage of the anomeric bond by NBS.

A general bicyclic thioglycoside synthon **11** for nucleobase coupling reactions has been efficiently synthesized. The applicability of thiofuranoside **11** has been demonstrated by the synthesis of the known β-LNA nucleoside **13** and the first α-LNA nucleoside **14**, and analogous thioglycosides may prove

useful for convergent syntheses of other constrained bicyclic nucleoside derivatives. The general use of **11** as a precursor for synthesis of α- and β-LNA nucleosides is currently under investigation.

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## Notes and references

† LNA is defined as an oligonucleotide (analogue) containing one or more monomeric LNA nucleosides. These LNA monomers are preorganized in a 3'-*endo* conformation as shown by X-ray crystallography (see ref. 5) and NMR studies (see ref. 2 and 3).

‡ Selected data for **11**: δ<sub>H</sub>(CDCl<sub>3</sub>) 7.46–7.26 (15 H, m, Bn, SPh), 5.35 (1 H, s, H-1), 4.68–4.56 (4 H, m, Bn), 4.31 (1 H, s, H-2), 4.10 (1 H, s, H-3), 4.09 (1 H, d, *J* 7.3, H-5'), 3.93 (1 H, d, *J* 7.8, H-5'), 3.79 (2 H, m, H-5); δ<sub>C</sub>(CDCl<sub>3</sub>) 138.03, 137.45, 133.42, 132.36, 129.19, 128.55, 128.46, 128.05, 127.84, 127.83, 127.76 (Bn, SPh), 89.96 (C-1), 87.18 (C-4), 79.71 (C-2), 79.40 (C-3), 73.64 (Bn), 73.23 (C-5'), 72.30 (Bn), 66.31 (C-5); *m/z* (FAB) 435 (M + H), 457 (M + Na) (Found: C, 71.76; H, 6.18; C<sub>26</sub>H<sub>26</sub>O<sub>4</sub>S requires C, 71.86; H, 6.03%).

§ Selected data for **14**: δ<sub>H</sub>(CD<sub>3</sub>OD) 7.78 (1 H, d, *J* 1.3, H-6), 5.88 (1 H, s, H-1'), 4.38 (1 H, s, H-2'), 4.34 (1 H, s, H-3'), 4.08–3.69 (4 H, m, H-5', H-5''), 1.92 (3 H, d, *J* 1.2, CH<sub>3</sub>); δ<sub>C</sub>(CD<sub>3</sub>OD) 138.00 (C-6), 110.08 (C-5), 92.49, 89.01 (C-4', C-1'), 80.89, 74.27, 73.33 (C-2', C-3', C-5'), 59.29 (C-5''), 12.53 (CH<sub>3</sub>); *m/z* (EI) 270 (M<sup>+</sup>, 100%).

- P. Herdewijn, *Liebigs Ann.*, 1996, 1337.
- S. K. Singh, P. Nielsen, A. A. Koshkin and J. Wengel, *Chem. Commun.*, 1998, 455.
- A. A. Koshkin, S. K. Singh, P. Nielsen, V. K. Rajwanshi, R. Kumar, M. Meldgaard, C. E. Olsen and J. Wengel, *Tetrahedron*, 1998, **54**, 3607.
- S. K. Singh and J. Wengel, *Chem. Commun.*, 1998, 1247.
- S. Obika, D. Nanbu, Y. Hari, K. Morio, Y. In, T. Ishida and T. Imanishi, *Tetrahedron Lett.*, 1997, **38**, 8735.
- A. A. Koshkin, V. K. Rajwanshi and J. Wengel, *Tetrahedron Lett.*, 1998, **39**, 4381.
- S. Obika, D. Nanbu, Y. Hari, J. Andoh, K. Morio, T. Doi and T. Imanishi, *Tetrahedron Lett.*, 1998, **39**, 5401.
- N. T. Thuong, U. Asseline, V. Roig, M. Takasugi and C. Hélène, *Proc. Natl. Acad. Sci. U.S.A.*, 1987, **84**, 5129.
- C. Gagnor, J.-R. Bertrand, S. Thenet, M. Lemaitre, F. Morvan, B. Rayner, C. Malvy, B. Lebleu, J.-L. Imbach and C. Paoletti, *Nucleic Acids Res.*, 1987, **15**, 10419.
- T. Waga, T. Nishizaki, I. Miyakawa, H. Ohruai and H. Meguro, *Biosci. Biotechnol. Biochem.*, 1993, **57**, 1433.
- H. Vorbrüggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, 1981, **114**, 1234.
- A similar ring-opening has been observed when coupling monocyclic methyl furanosides: P. T. Jørgensen, E. B. Pedersen and C. Nielsen, *Synthesis*, 1992, 1299.
- K. C. Nicolaou, S. P. Seitz and D. P. Papahatjis, *J. Am. Chem. Soc.*, 1983, **105**, 2430.
- P. Fügedi, P. J. Garegg, H. Lönn and T. Norberg, *Glycoconjugate J.*, 1987, **4**, 97.
- L. J. Wilson, M. W. Hager, Y. A. El-Kattan and D. C. Liotta, *Synthesis*, 1995, 1465.
- H. Sugimura, K. Osumi, T. Yamazaki and T. Yamaya, *Tetrahedron Lett.*, 1991, **32**, 1813.
- D. Kahne, S. Walker, Y. Cheng and D. J. Van Engen, *J. Am. Chem. Soc.*, 1989, **111**, 6881.
- L. Chanteloup and J.-M. Beau, *Tetrahedron Lett.*, 1992, **33**, 5347.
- A. De Mesmaeker, C. Lesueur, M.-O. Bévière, A. Waldner, V. Fritsch and R. M. Wolf, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 2790.
- E. Wittenburg, *Chem. Ber.*, 1966, **99**, 2380.

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