Analogues of LNA (Locked Nucleic Acid): Synthesis of the 2′-Thio-LNA Ribothymidine and 5-Methylcytidine Phosphoramidites

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Abstract: The bicyclic 2′-thio-LNA ribothymidine phosphoramidite (**13**) has been synthesised using a strategy making the synthesis of 2′-thio-LNA convergent with the syntheses of LNA and 2′-amino-LNA. The key step was the formation of anhydro-nucleoside **4** that prevented unwanted oxetane (**3**) formation during debenzylation of the 3′-OBn group, while concomitantly furnishing the necessary inversion of configuration at the C2′ position. No oxetane was observed even under basic conditions. We believe that this is due to the rigid tricyclic anhydro-nucleoside limiting the conformational freedom of the furanose, thereby preventing the formation of a strained tetracyclic system. After removal of the benzyl protection group the liberated 3′-OH group (**5**) was easily benzoylated (**6**) and the anhydro-nucleoside ring opened to give the *threo* configured nucleoside **7**. The 2′-thio-LNA ribothymidine phosphoramidite was synthesised in 9 steps from anhydro-nucleoside **4** in 30% yield. The 2′-thio-LNA 5-methylcytidine phosphoramidite was synthesised from nucleoside **12** using standard protocols.

Key words: nucleosides, bicyclic compounds, antisense agents, hydrogenations, bioorganic chemistry

Locked Nucleic Acid (LNA[2]) is a class of conformationally restricted oligonucleotide analogues comprising bicyclic 2′,4′-O-CH_2-furanosyl residues (Figure 1).[3–5] This class of oligonucleotides have many promising properties in the fields of diagnostics and therapeutics.[6–14] Wengel and co-workers were the first to report syntheses of LNA analogues in which the oxygen in the original 2′,4′-O-CH_2-furanosyl bicyclic structure had been substituted with sulfur (2′,4′-S-CH_2-, 2′-thio-LNA) and nitrogen (2′,4′-NH-CH_2-, 2′-amino-LNA).[15–17] Both analogues (Figure 1) showed hybridisation properties toward native nucleic acids comparable to those of the parent LNA.[15,16]

Incorporation of thiols, sulfides and disulfides in nucleosides can lead to a number of interesting biological properties of the corresponding oligonucleotides.[18–20] For example the in vivo distribution of oligonucleotides as well as resistance towards exonuclease degradation can be improved significantly.[21]

As part of our ongoing study of the individual LNA analogues we turned our attention to 2′-thio-LNA to study what properties it can confer to antisense oligonucleotides.

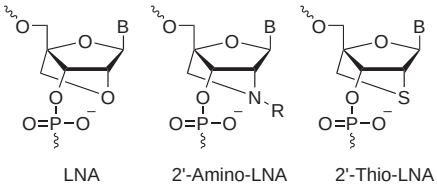

Figure 1 B = Nucleobase or nucleobase analogue

There is no convergence between the published synthesis of 2′-thio-LNA[15,17] and the synthesis of LNA.[22] In the published procedure there are problems with respect to synthesis on larger scales and several of the steps are low yielding. In this paper we describe the synthesis of the 2′-thio-LNA ribothymidine phosphoramidite building block that is convergent with the published procedure of LNA[22] and 2′-amino-LNA.[23] The key feature in the synthesis is the prevention of oxetane formation (**3**, Scheme 1) during debenzylation of the 3′-hydroxyl group by locking the carbohydrate moiety as the tricyclic anhydro-nucleoside (**4**, Scheme 2). Using this novel strategy oxetane formation was prevented during the debenzylation while obtaining inversion of configuration at C2′, producing the *threo* configured nucleoside **7**, after benzoyl protection of the 3′-OH position and opening of the anhydro-nucleoside with aqueous acid.

The strategy employed in the synthesis of 2′-amino-LNA proved to be inefficient for the preparation of 2′-thio-LNA.[23] Although a wide range of conditions were investigated it proved impossible to debenzylate the 3′-OH position after the introduction of sulfur. Clearly, it was necessary to debenzylate prior to the introduction of sulfur at the 2′-position. Initially, we attempted debenzylating the trimesylate **1** (Scheme 1). Under neutral conditions the major product was the undesired oxetane **3**. However, if the hydrogenation was performed in glacial acetic acid nucleoside **2** was the exclusive product.

Unfortunately, attempts to purify nucleoside **2** by chromatography led to a substantial loss of the target molecule, due to on-column formation of **3**, giving only a low yield of **2** (ca. 25%), even when acidic chromatography conditions were employed. Thus, it was clear that another strategy had to be devised in which both oxetane formation was avoided, and where the 3′-OBn group was debenzylated prior to introduction of sulfur. We envisaged that debenzylation of the rigid tricyclic anhydro-nucleoside **4** (Scheme 2), would prevent the formation of a strained tetracyclic oxetane system.

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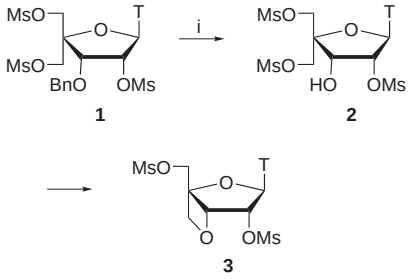


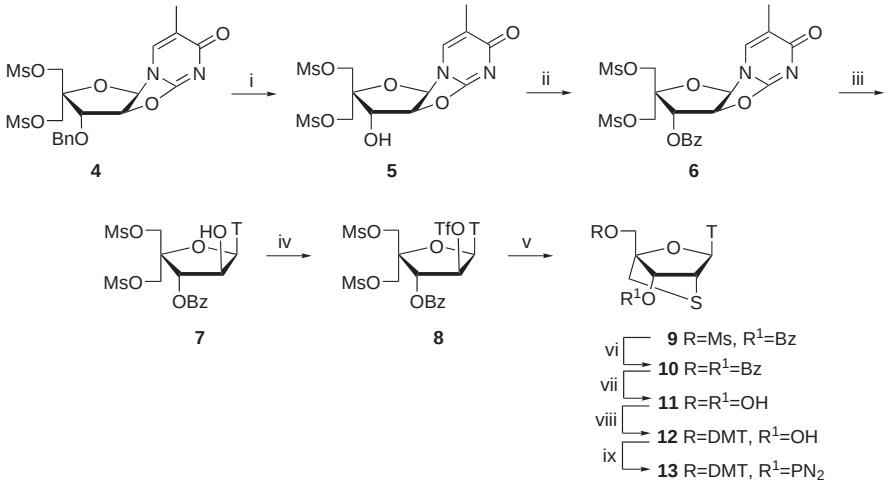
Scheme 1 Undesired oxetane formation. *Reagents and conditions:* i) $Pd(OH)_2/C$, H_2, concd HOAc (ca. 25%).

Indeed, no oxetane formation was observed when catalytic hydrogenation in glacial acetic acid was employed for debenzylation. However, the conditions resulted in a 1:1 mixture of the desired nucleoside **5** and a nucleoside where undesired reduction of the nucleobase had occurred.

To prevent reduction of the nucleobase a range of different solvent combinations under neutral conditions using either Pd/C or $Pd(OH)_2/C$ were investigated. Reduction with H_2 and 10 mol% Pd on carbon in a 1:1 mixture of acetone and MeOH was identified as the optimal conditions, yielding almost exclusively **5** (Scheme 2). However, due to the very polar nature of **5** work-up and purification was difficult. In order to obtain the product the palladium catalyst had to be refluxed in DMF and chromatography of the product was not feasible. A 'one-pot' strategy turned out to be the solution to this problem. Thus, concentration of the reaction mixture from the debenzylation produced **5** that was dissolved in DMF. Benzoyl chloride and pyridine were added and the reaction was stirred at ambient temperature overnight and quenched with MeOH. After removal of excess MeOH in vacuo, aqueous H_2SO_4 was added and the reaction mixture was heated to 80 °C overnight. Chromatography of the product mixture gave nucleoside **7** in 79% yield from nucleoside **4**. In spite of the basic conditions employed for the benzoylation of nucleoside **5**, no oxetane formation was observed. Presumably the rigid molecular framework of anhydro-nucleoside **5** makes it impossible to form a strained tetracyclic oxetane.

The obtained *threo* configured nucleoside **7** was subsequently triflated, to give crude nucleoside **8**, that was treated with Na_2S in DMF producing 2′-thio-LNA nucleoside **9** in 72% yield from **7**. In the next reaction the 5′-mesylate was substituted with sodium benzoate to give the dibenzoate **10** in 81% yield, followed by hydrolysis of both benzoyl esters to yield the fully deprotected 2′-thio-LNA nucleoside **11** in a 76% yield. The 2′-thio-LNA ribothymidine phosphoramidite (**13**) was finally obtained by protection of the 5′-hydroxyl group with 4,4-dimethoxytrityl chloride, and phosphitylation of the 3′-hydroxyl group according to standard procedures[24] in an overall yield of 88% from **11**. Starting from 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose Wengel and co-workers prepared the 2′-thio-LNA ribouridine phosphoramidite in an overall yield of 3%.[3,15,17] The method for the preparation of 2′-thio-LNA ribothymidine presented herein constitutes a significant improvement when compared to the original procedure producing the 2′-thio-LNA ribothymidine phosphoramidite **13** in an overall yield of 21% starting from the same material. The 2′-thio-LNA 5-methylcytidine phosphoramidite was synthesised starting from nucleoside **12** using standard methods.[23]

An efficient procedure for the preparation of the 2′-thio-LNA ribothymidine phosphoramidite, convergent with the syntheses of LNA and 2′-amino-LNA has been presented. The key step is the formation of the 2′-anhydro-nucleoside **5** by which the configuration at the 2′-position is inverted and oxetane formation is prevented. The presented synthetic route to 2′-thio-LNA ribothymidine was



Scheme 2 Synthesis of the 2′-thio-LNA ribothymidine phosphoramidite. *Reagents and conditions:* i) Pd/C, H_2, acetone, MeOH; ii) BzCl, pyridine, DMF; iii) 0.25 M H_2SO_4 (aq), DMF, 80 °C (79% from **4**; 3 steps); iv) Tf_2O, DMAP, CH_2Cl_2, 0 °C; v) Na_2S, DMF (72% from **7**; 2 steps); vi) NaOBz, DMF, 100 °C (81%); vii) NH_3, MeOH (76%); viii) DMT-Cl, pyridine (88%); ix) $P(OCH_2CH_2CN)[N(i\text{-}Pr)_2]_2$, 4,5-dicyanoimidazole, CH_2Cl_2 (99%). DMT = 4,4′-dimethoxytrityl, PN_2 = 2-cyanoethoxy(diisopropylamino)phosphinoyl.

extended to provide the 2′-thio-LNA 5-methylcytidine phosphoramidite using standard protocols.[23] The properties of 2′-thio-LNA containing oligonucleotides are currently being evaluated and the results will be reported in due course.

For reactions conducted under anhydrous conditions glassware was dried overnight in an oven at 150 °C and was allowed to cool in a dessicator over anhyd KOH. Anhydrous reactions were carried out under an atmosphere of Ar using anhydrous solvents. Solvents were HPLC grade, of which DMF, pyridine, MeOH and CH_2Cl_2 were dried over molecular sieves (4 Å from Grace Davison) and THF was freshly distilled from Na·benzophenone to a water content below 20 ppm. TLC was run on Merck silica 60 F_{254} aluminium sheets. Dry Column Vacuum Chromatography (DCVC) was performed according to the published procedure.[25] Nucleoside **4** was synthesised as previously published.[23] ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, with solvents as internal standard (δ_H: $CDCl_3$ 7.26 ppm, DMSO-d_6 2.50; δ_C: $CDCl_3$ 77.0 ppm, DMSO-d_6 39.4 ppm). ^{31}P NMR was run at 121 MHz with 85% H_3PO_4 as external standard. Coupling constants (J) were calculated using Mestre-C 2.3a software.[26] Assignments of NMR spectra are based on 2D spectra and follow the standard carbohydrate/nucleoside nomenclature (the carbon atom of the 4′-*C*-substituent is numbered C1″) even though the systematic compound names of the bicyclic nucleoside derivatives are given according to the von Baeyer nomenclature. Elemental analyses were obtained from the University of Copenhagen, Microanalytical Department.

1-(3-*O*-Benzoyl-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-β-d-*threo*-pentofuranosyl)thymine (7)

Anhydro-nucleoside 4[23] (30.0 g, 58.1 mmol) was heated to 70 °C in a mixture of MeOH (1000 mL) and acetone (1000 mL) until a clear solution was obtained and the solution was allowed to reach r.t. The reaction flask was flushed with Ar and Pd/C (10 wt.% Pd on carbon, 6.2 g, 5.8 mmol) was added. The mixture was stirred vigorously under an atmosphere of hydrogen gas (balloon). After 23 h the slurry was filtered through a pad of celite. The catalyst was recovered from the celite and refluxed in DMF (1000 mL) for 1 h. The hot DMF slurry was filtered through a pad of celite and the organic layers were combined and evaporated in vacuo to give nucleoside 5 as a yellow powder. Residual solvents were removed with the help of a high vacuum pump overnight.

The crude nucleoside **5** (23 g) was heated to 70 °C in DMF (300 mL) to give a clear yellow solution that was allowed to cool to r.t. Benzoyl chloride (81.7 g, 581 mmol, 67.4 mL) was added followed by pyridine (70 mL). After 18 h the reaction was quenched with MeOH (200 mL) and excess MeOH was removed in vacuo.

To the dark brown solution of nucleoside **6** aq H_2SO_4 (0.25 M, 400 mL) was added. The solution was heated to 80 °C on an oil bath (At approx 50 °C precipitation occurs. The solution becomes clear again at 80 °C). After 22 h at 80 °C the solution was allowed to cool to r.t. The reaction mixture was transferred to a separatory funnel with EtOAc (1000 mL). The organic layer was washed with sat. aq $NaHCO_3$ (2 × 1000 mL). The combined aqueous layers were extracted with EtOAc (1000 + 500 mL). The organic layers were combined and washed with sat. aq $NaHCO_3$ (1000 mL), dried (Na_2SO_4), filtered and evaporated in vacuo to give a yellow liquid. Residual solvents were removed on a high vacuum pump overnight to give a yellow syrup. The product was purified by Dry Column Vacuum Chromatography (id = 10 cm; 100 mL fractions; 50–100% EtOAc in *n*-heptane; 10% increments; 2-24% MeOH in EtOAc; 2% increments). Fractions containing the product were combined and evaporated in vacuo giving nucleoside **7** (25.1 g, 79%) as a white foam; R_f 0.54 (5% MeOH in EtOAc).

^{1}H NMR (DMSO-d_6): δ = 11.39 (br s, 1 H, NH), 8.10–8.08 (m, 2 H, Ph), 7.74–7.70 (m, 1 H, Ph), 7.60–7.56 (m, 2 H, Ph), 7.51 (d, J = 1.1 Hz, 1 H, H6), 6.35 (d, J = 4.9 Hz, 1 H, H1′), 6.32 (d, J = 5.3 Hz, 1 H, 2′-OH), 5.61 (d, J = 4.0 Hz, 1 H, H3′), 4.69 (d, J = 10.8 Hz, 1 H), 4.59 (m, 1 H, H2′), 4.55 (d, J = 10.8 Hz, 1 H), 4.52 (d, J = 10.8 Hz, 1 H), 4.46 (d, J = 10.6 Hz, 1 H, H5′, H1″), 3.28 (s, 3 H, Ms), 3.23 (s, 3 H, Ms), 1.81 (s, 3 H, CH_3).

^{13}C NMR (DMSO-d_6): δ = 164.5, 163.6 [C4, PhC(O)], 150.3 (C2), 137.7 (C6), 133.8, 129.6, 128.7, 128.6 (Ph), 108.1 (C5), 84.8 (C1′), 81.1 (C4′), 78.0 (C3′), 73.2 (C2′), 68.0, 67.1 (C5′, C1″), 36.7, 36.6 (2 × Ms), 11.9 (CH_3).

ESI–MS: m/z $[MH]^+$ calcd: 549.1; found: 549.0.

Anal. Calcd for $C_{20}H_{24}N_2O_{12}S_2 \cdot 0.33\ H_2O$: C, 44.34; H, 4.65; N, 4.85. Found: C, 44.32; H, 4.58; N, 4.77.

(1*R*,3*R*,4*R*,7*R*)-7-Benzoyloxy-1-methansulfonyloxymethyl-3-(thymin-1-yl)-2-oxa-5-thiabicyclo[2:2:1]heptane (9)

1-(3-*O*-Benzoyl-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-β-D-*threo*-pentofuranosyl)thymine (**7**) (10.00 g, 18.23 mmol) was dissolved in CH_2Cl_2 (500 mL) and cooled to 0 °C. Pyridine (15 mL) and DMAP (8.91 g, 72.9 mmol) were added followed by dropwise addition of trifluoromethanesulfonic anhydride (10.30 g, 36.5 mmol, 6.0 mL). After 1 h the reaction was quenched with sat. aq $NaHCO_3$ (500 mL) and transferred to a separatory funnel. The organic layer was washed with 1.0 M aq HCl (500 mL), sat. aq $NaHCO_3$ (500 mL) and brine (500 mL). The organic layer was evaporated in vacuo with toluene (100 mL) to give 1-(3-*O*-benzoyl-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-2-*O*-trifluoromethanesulfonyl-β-D-*threo*-pentofuranosyl)thymine (**8**) as a yellow powder.

The crude nucleoside **8** was dissolved in DMF (250 mL) and Na_2S (1.57 g, 20.1 mmol) was added to give a dark green slurry. After 3 h the reaction was quenched with half sat. aq $NaHCO_3$ (500 mL) and extracted with CH_2Cl_2 (500 + 2 × 250 mL). The combined organic layers were washed with brine (500 mL), dried (Na_2SO_4), filtered and concentrated in vacuo to give a yellow liquid. Residual solvent was removed overnight on a high vacuum pump to give a yellow gum that was purified by Dry Column Vacuum Chromatography (id = 6 cm: 50 mL fractions; 50–100% EtOAc in *n*-heptane: 10% increments; 2–20% MeOH in EtOAc; 2% increments) to give nucleoside **9** (6.15 g, 72%) as a yellow foam; R_f 0.27 (20% *n*-heptane in EtOAc).

^{1}H NMR ($CDCl_3$) δ = 8.70 (br s, 1 H, NH), 8.01–7.99 (m, 2 H, Ph), 7.67 (d, J = 1.1 Hz, 1 H, H6), 7.65–7.61 (m, 1 H, Ph), 7.50–7.46 (m, 2 H, Ph), 5.98 (s, 1 H, H1′), 5.34 (d, J = 2.4 Hz, 1 H, H3′), 4.66 (d, J = 11.7 Hz, 1 H, H5′a), 4.53 (d, J = 11.5 Hz, 1 H, H5′b), 4.12 [m (overlapping with residual EtOAc), 1 H, H2′), 3.15–3.13 (m, 4 H, H1″a, Ms), 3.06 (d, J = 10.6 Hz, 1 H, H1″b), 1.98 (d, J = 1.1 Hz, 3 H, CH_3).

^{13}C NMR ($CDCl_3$): δ = 165.2, 163.5 [C4, PhC(O)], 149.9 (C2), 134.1, 133.9, 129.8, 128.7, 128.3 (C6, Ph), 110.7 (C5), 91.1 (C1′), 86.8 (C4′), 72.6 (C3′), 65.8 (C5′), 50.5 (C2′), 37.9 (Ms), 35.1 (C1″), 12.5 (CH_3).

ESI–MS: m/z $[MH]^+$ calcd: 469.1; found: 469.0.

Anal. Calcd for $C_{19}H_{20}N_2O_8S_2 \cdot 0.33$ EtOAc: C, 49.21; H, 4.72; N, 5.47. Found: C, 49.25; H, 4.64; N, 5.48.

(1*R*,3*R*,4*R*,7*R*)-7-Benzoyloxy-1-benzoyloxymethyl-3-(thymin-1-yl)-2-oxa-5-thiabicyclo[2:2:1]heptane (10)

Nucleoside **9** (1.92 g, 4.1 mmol) was dissolved in DMF (110 mL). Sodium benzoate (1.2 g, 8.2 mmol) was added and the mixture was heated to 100 °C for 24 h. The reaction mixture was transferred to a separatory funnel with half sat. brine (200 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried

(Na_2SO_4), filtered and evaporated in vacuo to give a brown liquid. The product was put on a high vacuum pump to remove residual solvent. The resulting brown gum was purified by Dry Column Vacuum Chromatography (id = 4 cm; 50 mL fractions; 0–100% EtOAc in *n*-heptane; 10% increments; 2–10% MeOH in EtOAc; 2% increments) to give nucleoside **10** (1.64 g, 81%) as a slightly yellow foam; R_f 0.57 (20% *n*-heptane in EtOAc).

^{1}H NMR ($CDCl_3$): δ = 9.02 (br s, 1 H, NH), 8.07–7.99 (m, 4 H, Ph), 7.62–7.58 (m, 2 H, Ph), 7.47–7.42 (m, 5 H, Ph, H6), 5.95 (s, 1 H, H1′), 5.46 (d, *J* = 2.2 Hz, 1 H, H3′), 4.93 (d, *J* = 12.8 Hz, 1 H, H5′a), 4.60 (d, *J* = 12.8 Hz, 1 H, H5′b), 4.17 (d, *J* = 2.2 Hz, 1 H, H2′), 3.27 (d, *J* = 10.6 Hz, 1 H, H1′′a), 3.16 (d, *J* = 10.6 Hz, 1 H, H1′′b), 1.55 (d, *J* = 1.1 Hz, 3 H, CH_3).

^{13}C NMR ($CDCl_3$): δ = 165.8, 165.1, 163.7 [C4, 2 × PhC(O)], 150.0 (C2), 133.9, 133.7, 133.6, 129.8, 129.6, 129.0, 128.8, 128.6, 128.5 (C6, 2 × Ph), 110.3 (C5), 91.3 (C1′), 87.5 (C4′), 72.9 (C3′), 61.3 (C5′), 50.6 (C2′), 35.6 (C1′′), 12.3 (CH_3).

ESI–MS: *m*/*z* $[MH]^+$ calcd: 495.1; found: 495.1.

Anal. Calcd for $C_{25}H_{22}N_2O_7S$: C, 60.72; H, 4.48; N, 5.66. Found: C, 60.34; H, 4.49; N, 5.35.

(1*R*,3*R*,4*R*,7*R*)-7-Hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2-oxa-5-thiabicyclo[2:2:1]heptane (11)

Nucleoside **10** (1.50 g, 3.0 mmol) was dissolved in MeOH saturated with NH_3 (50 mL). The reaction flask was sealed and stirred at ambient temperature for 20 h. The reaction mixture was concentrated in vacuo to give a yellow gum that was purified by Dry Column Vacuum Chromatography (id = 4 cm; 50 mL fractions; 0–16% MeOH in EtOAc; 1% increments) giving nucleoside **11** (0.65 g, 76%) as clear needles; R_f 0.31 (10% MeOH in EtOAc).

^{1}H NMR (DMSO-d_6) δ = 11.32 (br s, 1 H, NH), 7.96 (d, *J* = 1.1 Hz, 1 H, H6), 5.95 (s, 1 H, H6), 5.70 (d, *J* = 4.2 Hz, 1 H, 3′-OH), 5.62 (s, 1 H, H1′), 4.49 (t, *J* = 5.3 Hz, 1 H, 5′-OH), 4.20 (dd, *J* = 4.1, 2.1 Hz, 1 H, H3′), 3.77–3.67 (m, 2 H, H5′), 3.42 (d, *J* = 2.0 Hz, 1 H, H2′), 2.83 (d, *J* = 10.1 Hz, 1 H, H1′′a), 2.64 (d, *J* = 10.1 Hz, 1 H, H1′′b), 1.75 (d, *J* = 1.1 Hz, 3 H, CH_3).

^{13}C NMR (DMSO-d_6): δ = 163.8 (C4), 150.0 (C2), 135.3 (C6), 107.5 (C5), 90.2, 89.6 (C1′, C4′), 69.4 (C3′), 58.0 (C5′), 52.1 (C2′), 34.6 (C1′′), 12.4 (CH_3).

ESI–MS: *m*/*z* $[MH]^+$ calcd: 287.1; found: 287.1.

Anal. Calcd for $C_{11}H_{14}N_2O_5S$: C, 46.15; H, 4.93; N, 9.78. Found: C, 46.35; H, 4.91; N, 9.54.

(1*R*,3*R*,4*R*,7*R*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-5-methyl-3-(thymin-1-yl)-2-oxa-5-thiabicyclo[2:2:1]heptane (12)

Nucleoside **11** (0.60 g, 2.1 mmol) was dissolved in pyridine (10 mL). 4,4′-Dimethoxytrityl chloride (0.88 g, 2.6 mmol) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was transferred to a separatory funnel with water (100 mL) and extracted with EtOAc (100 + 2 × 50 mL). The combined organic layers were washed with sat. aq $NaHCO_3$ (100 mL), brine (100 mL) and evaporated to dryness in vacuo to give a viscous yellow liquid. The product was redissolved in toluene (50 mL) and concentrated in vacuo to give a yellow foam. The foam was dried on a high vacuum pump overnight and purified by Dry Column Vacuum Chromatography (id = 4 cm; 50 mL fractions; 10–100% EtOAc in *n*-heptane; 10% increments) giving nucleoside **12** (1.08 g, 88%) as a white foam; R_f 0.24 (20% *n*-heptane in EtOAc).

^{1}H NMR ($CDCl_3$): δ = 8.96 (br s, 1 H, NH), 7.74 (d, *J* = 1.1 Hz, 1 H, H6), 7.46–7.44 (m, 2 H, Ph), 7.35–7.22 (m, 9 H, Ph), 7.19–7.15 (m, 2 H, Ph), 6.86–6.80 (m, 2 H, Ph), 5.82 (s, 1 H, H1′), 4.55 (dd, *J* = 9.3, 2.1 Hz, 1 H, H3′), 3.79 (s, 6 H, OCH_3), 3.71 (d, *J* = 2.0 Hz, 1 H, H2′), 3.50 (s, 2 H, H5′), 2.81 (d, *J* = 10.8 Hz, 1 H, H1′′a), 2.77 (d, *J* = 10.8 Hz, 1 H, H1′′b), 2.69 (d, *J* = 9.2 Hz, 1 H, 3′-OH), 1.42 (s, 3 H, CH_3).

^{13}C NMR ($CDCl_3$): δ = 158.7 (C4), 150.1 (C2), 144.1, 135.2, 135.1, 130.1, 129.1, 128.1, 128.0, 127.1, 127.0, 113.3 (C6, 3 × Ph), 110.0 (C5), 90.2 [C(Ph)$_3$], 89.6 (C1′), 87.0 (C4′), 71.7 (C3′), 60.9 (C5′), 55.2 (C2′), 34.7 (C1′′), 12.2 (CH_3).

ESI-MS: *m*/*z* $[M – H]^+$ calcd: 587.2; found 587.1.

Anal. Calcd for $C_{32}H_{32}N_2O_7S·0.5\ H_2O$: C, 64.31; H, 5.57; N, 4.69. Found: C, 64.22; H, 5.67; N, 4.47.

(1*R*,3*R*,4*R*,7*R*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4’-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2-oxa-5-thiabicyclo[2.2.1]heptane (13)

According to the published method[24] nucleoside **12** (0.78 g, 1.33 mmol) was dissolved in CH_2Cl_2 (5 cm^3) and a 1.0 M solution of 4,5-dicyanoimidazole in MeCN (0.93 mL, 0.93 mmol) was added followed by dropwise addition of 2-cyanoethyl-*N*,*N*,*N*’,*N*’-tetraisopropylphosphorodiamidite (0.44 mL, 1.33 mmol). After 2 h the reaction was transferred to a separatory funnel with CH_2Cl_2 (40 mL) and washed with sat. aq $NaHCO_3$ (2 × 25 mL) and brine (25 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated in vacuo to give nucleoside **13** (1.04 g, 99%) as a white foam; R_f 0.29, 0.37, two diastereoisomers (20% *n*-heptane in EtOAc).

^{31}P NMR (DMSO-d_6): δ = 150.39, 150.26.

ESI–MS: *m*/*z* $[MH]^+$ calcd:789.3; found: 789.3.

References

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(2) LNA is defined as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl nucleotide monomers (LNA monomers).[3,5] For clarity the terms 2′-amino-LNA and 2′-thio-LNA are used herein for the LNA analogues, where the 2′-oxygen has been substituted by a nitrogen atom or a sulfur atom.

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