Supplementary Material for Organic & Biomolecular Chemistry

Supplementary data

Supplementary information for the manuscript:

Synthesis of 2'-Amino-LNA: A New Strategy

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An alternative synthesis of azide **17** providing orthogonal protection groups is presented below. This was the original approach for the synthesis of 2-amino-LNA. However, due to problems in the critical triflation and azide substitution steps it was abandoned in favour of the mesylate strategy presented in the manuscript.

Compound **1** was synthesised from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose according to the procedure of Youssefyeh *et al.*[1] Subsequent benzoylation of both primary hydroxy groups with benzoyl chloride and pyridine in anhydrous dichloromethane gave compound **S1** (Scheme S1) quantitatively on a 100 g scale. Acidic hydrolysis of the isopropylidene group and in situ acetylation formed the glycosyl donor **S2** according to the previously published procedure.[2] Coupling of compound **S2** with thymine by means of standard Vorbrüggen glycosylation conditions[3] yielded the *β*-nucleoside **S3** exclusively.

Chemoselective deacetylation of the 2'-*O*-acetyl group of nucleoside **S3** was accomplished by treatment with half-saturated methanolic ammonia according to the procedure described by Neilson and Werstiuk.[4] However, the reaction had to be stopped before completion in order to minimize the subsequent debenzoylation of the desired product **S4**. This was accomplished by neutralisation of the reaction mixture with 1.0 M aqueous HCl before evaporation of the solvent. The resulting reaction mixture was a 1:9:1-mixture of starting material, the desired deacetylated product and more polar products, which could easily be separated by Dry Column Vacuum Chromatography.[5] Compound **S4** was obtained in approximately 40% yield over the 7 steps outlined in scheme S1 from the commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. Formation of the 2,2'-anhydro nucleoside **S6** was achieved by mesylation of the 2'-hydroxy group of nucleoside **S4** and subsequent treatment of the mesylate with DBU in dimethylformamide.

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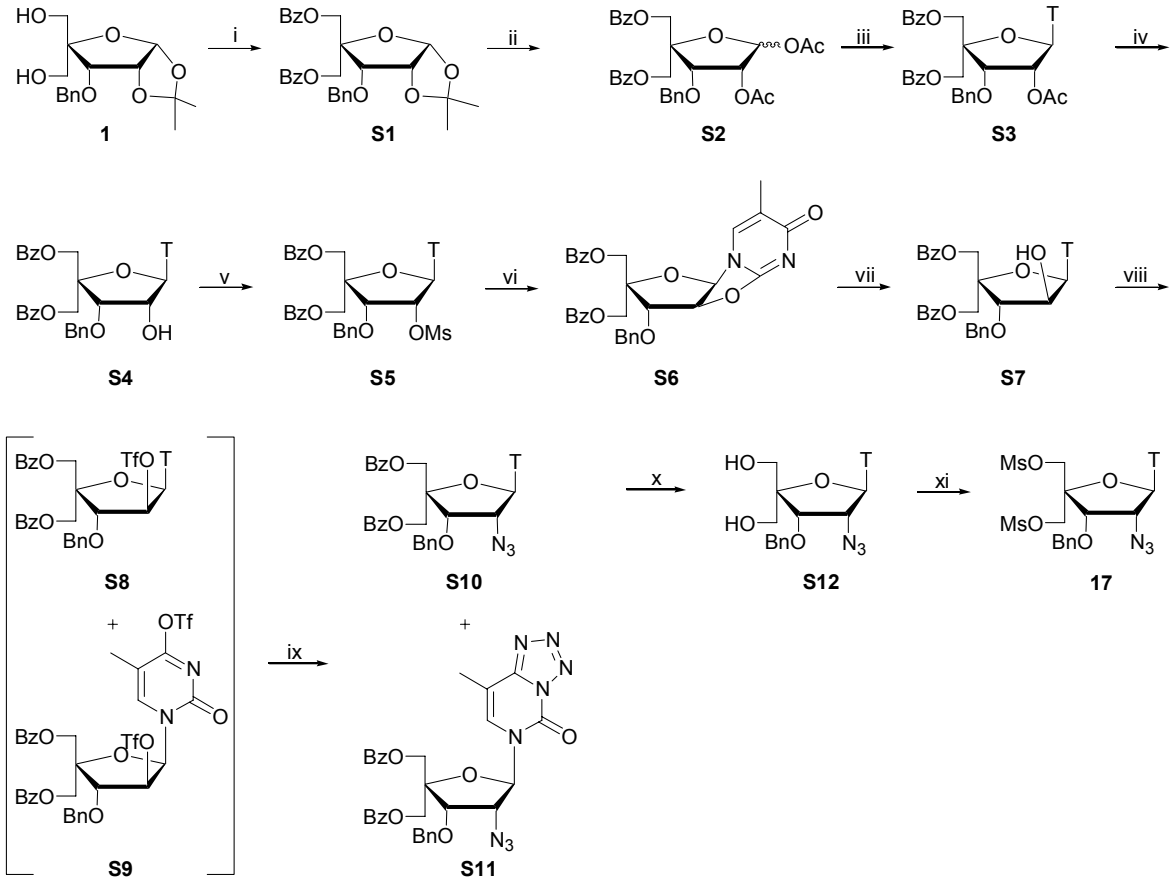

Scheme S1 *Reagents and conditions:* i) BzCl, pyridine, CH_2Cl_2; ii) AcOH, Ac_2O, c. H_2SO_4 (100:10:0.1); iii) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-triflate, MeCN; iv) half sat. NH_3 in MeOH; v) MsCl, pyridine, CH_2Cl_2; vi) DBU, MeCN; vii) 2 M HCl (aq), DMF; viii) Tf_2O, pyridine, CH_2Cl_2; ix) NaN_3, DMF; x) 1.0 M LiOH (aq), THF; xi) MsCl, pyridine, CH_2Cl_2

In the previously reported synthesis of 2'-amino-LNA an anhydro intermediate (formed *in situ* from nucleoside **5**, see manuscript scheme 1) similar to **S6** was opened with benzylamine. We were unable to achieve satisfactory results with this particular reaction. Many different methods for the opening of the 2,2'-bond of anhydro-nucleosides have been reported. With anhydro-nucleoside **S6** we tried both

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sodium azide in DMF[6], sodium azide in aqueous DMF[7] and the in situ formation of lithium azide from lithium fluoride and azidotrimethylsilane in the presence of *N*,*N*,*N*',*N*'-tetramethylethylenediamine.[8,9] In addition to these experiments we also tried some of the different reactions reported for the azide substitutions of mesylate groups on nucleosides, e.g. sodium azide in aqueous DMF with Bu_4NBr and sodium azide in DMF with 18-crown-6.[10] All our attempts to open the 2,2'-anhydro nucleoside **S6** with these methods either failed completely, or were very low yielding.

The difficulty of opening the 2,2'-bond of nucleoside **S6** partly explains the low yield obtained in the double nucleophilic substitution of di-*O*-tosyl nucleoside **5** using benzylamine (see manuscript, scheme 1).

The published synthesis of 2'-thio-LNA[11,12] takes advantage of an anhydro-nucleoside very similar to **S6** formed in situ in the presence of potassium thioacetate. When subjecting nucleoside **S6** to the reported conditions only extensive decomposition of the starting material was observed.

The 2,2'-anhydro nuceloside **S6** could however be opened by acidic hydrolysis without concomitant debenzoylation[13] to give the *threo*-configured nucleoside **S7**. Conversion of the 2'-hydroxy group of nucleoside **S7** into a mesyloxy group went smoothly but the subsequent nucleophilic substitution of the leaving-group with azide yielded only very little of compound **S10** despite using many of the different azide substitution reactions mentioned earlier.

Instead, compound **S7** was reacted with trifluoromethanesulfonic anhydride in a mixture of pyridine and dichloromethane at 0 °C to obtain the triflate **S8**. The trifluoromethanesulfonylation was performed at 0 °C in an attempt to minimize formation of the by-product **S9** identified by mass- and ^{19}F-NMR spectroscopy to contain two triflate-groups. The second triflate is presumed (see below) to be formed at the O4 of the nucleobase. The triflate mixture of **S8** and **S9** was not purified and characterised further due to the products' instability. The crude mixture was then treated with sodium azide in anhydrous DMF at 80 °C to produce the desired nucleoside **S10** in 37% yield together with a nucleoside were both triflates had been replaced by azides (25% yield) according to Mass Spectroscopy. We were unable to establish the structure of the nucleoside conclusively by NMR techniques but had strong indications

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that it was the nucleoside **S11**. This was confirmed by X-ray crystallography (Figure 1). This class of modified nucleobases (6-substituted tetrazolo[1,5-c]pyrimidin-8-methyl-5(6H)-one) are known from the literature[14-18] but to our knowledge this is the first time that the structure has been proven by X-ray crystallography. The poor yield of nucleoside **S12** due to the lack of chemoselectivity during triflate formation was clearly not satisfactory resulting in the alternative route to 2'-amino-LNA described in the manuscript.

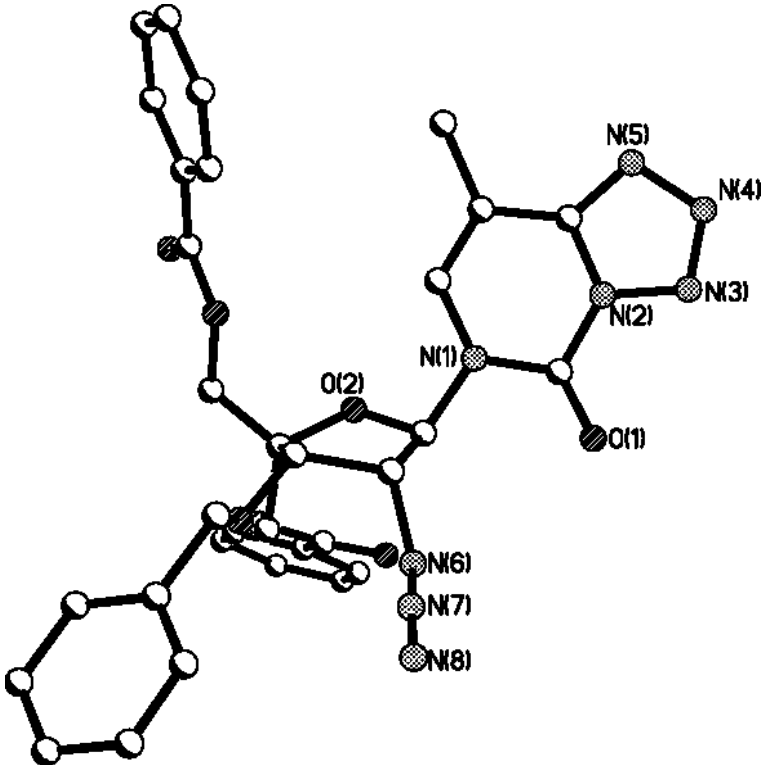

Figure 1. X-ray structure of nucleoside **S11**.[19]

Debenzoylation of nucleoside **S12** with aqueous lithium hydroxide in THF, and subsequent mesylation with mesyl chloride in anhydrous pyridine afforded the di-mesylated nucleoside **17** in 86% yield (over 2 steps) which was converted to 2-amino-LNA as described in the manuscript.

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Experimental Section

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 anhydrous KOH. Anhydrous reactions were carried out under an atmosphere of argon. Solvents were HPLC grade, of which DMF, pyridine, acetonitrile and dichloromethane were dried over molecular sieves (4 Å from Grace Davison) and THF was freshly destilled from Na·benzophenone to a water content below 20 ppm. TLC was run on Merck silica 60 F_{254} aluminum sheets. Dry Column Vacuum Chromatography (DCVC) was performed according to the published procedure.[5] ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were recorded at respectively 400 MHz, 100 MHz, 376 MHz, and 121 MHz 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 with 85% H_3PO_4 as external standard. *J* values are given in Hz. Assignments of NMR spectra are based on 2D spectra and follow the standard carbohydrate/nucleoside nomenclature (the carbon atom of the 4'-*C*-substitiuent is numbered C1'') even though the systematic compound names of the bicyclic nucleoside derivatives are given according to the von Baeyer nomenclature. Crude compounds were used without further purification if they were ≥95% pure by TLC and HPLC-MS (RP C18 column, UV detection). Elemental analyses were obtained from the University of Copenhagen, Microanalytical Department. Crystal X-ray Crystallographic Data of nucleoside **S11** was obtained from Cambridge University Chemical Laboratory X-Ray Department.

5-*O*-Benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (**S1**)

To a solution of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose **1**[1] (40 g, 129 mmol) in anhyd dichloromethane (300 mL) was added anhyd pyridine (52 mL) and the mixture was cooled to 0 °C. Benzoyl chloride (45.0 mL, 387 mmol) was added over 30 min and the reaction mixture was allowed to reach rt. After 3 h the reaction was quenched by pouring onto ice-cold sat. aq $NaHCO_3$ (300 mL). The phases were separated and the organic phase was washed with sat. aq $NaHCO_3$ (2 x 300 mL) and brine (300 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure to give crude **S1** (74 g) which was used without further

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purification. An analytical sample was obtained by DCVC (Ø 2 cm, 0-75% EtOAc in *n*-heptane v/v, 5% increments, 50 mL fractions) and **S1** was isolated as a clear viscous liquid. R_f = 0.58 (50% EtOAc in *n*-heptane, v/v); ESI-MS *m/z* found 536.2 ($[MNH_4]^+$, calcd 536.2); ^{1}H NMR ($CDCl_3$) δ 7.97 (d, *J* = 8.2, 2H, Bz), 7.84 (d, *J* = 8.4, 2H, Bz), 7.47-7.44 (m, 2H, Ph), 7.35-7.29 (m, 4H, Ph), 7.23-7.14 (m, 5H, Ph), 5.76 (d, *J* = 3.8, 1H, H1), 4.93 (d, *J* = 12.4, 1H), 4.73 (d, *J* = 11.7, 1H), 4.68-4.65 (m, 2H), 4.55 (d, *J* = 11.7, 1H), 4.48 (d, *J* = 11.9, 1H), 4.34 (d, *J* = 11.7, 1H), 4.14 (d, *J* = 5.1, 1H), 1.63 (s, 3H, CH_3), 1.29 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.1, 165.9 (C(O)), 137.0, 133.1, 132.9, 130.1, 130.0, 129.8, 129.7, 129.6, 128.5, 128.4, 128.3, 128.0, 127.8 (Ph), 113.9 ($\underline{C}(CH_3)_2$), 104.3 (C1), 84.0 (C4), 79.3, 78.4 (C3, C2), 72.5 ($\underline{C}H_2Ph$), 65.3, 64.4 (C5, C1'), 26.6, 26.1 ($C(\underline{C}H_3)_2$); Anal. calcd for $C_{30}H_{30}O_8 \cdot 0.25\ H_2O$: C, 68.9; H, 5.9; Found: C, 68.9, H, 5.8.

1,2-Di-*O*-acetyl-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-D-*erythro*-pentofuranose (**S2**)
Compound **S1** (73 g, 141 mmol, crude) was dissolved in acetic acid (600 mL) and acetic anhydride (85.2 mL, 0.90 mol) was added followed by dropwise addition of concd sulfuric acid. The reaction mixture was stirred at rt for 18 h and the acetic acid was removed in vacuo. The residue was diluted with dichloromethane (300 mL) and washed with sat. aq $NaHCO_3$ (3 x 300 mL) and brine (300 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure to give crude **S10** (74 g) which was used without further purification. An analytical sample was obtained by DCVC (Ø 2 cm, 0-60% EtOAc in *n*-heptane v/v, 5% increments, 50 mL fractions) and **S2** was isolated as a clear viscous liquid. R_f = 0.54 (50% EtOAc in *n*-heptane, v/v); ESI-MS *m/z* found 580.0 ($[MNH_4]^+$, calcd 580.2); ^{1}H NMR ($CDCl_3$) δ 7.96 (d, *J* = 8.2, 2H, Bz), 7.90 (d, *J* = 8.0, 2H, Bz), 7.49-7.45 (m, 2H, Ph), 7.36-7.30 (m, 4H, Ph), 7.18-7.14 (m, 5H, Ph), 6.38 (d, *J* = 4.8, 1H, H1α), 6.19 (s, 1H, H1β), 5.37 (d, *J* = 4.9, 1H, H2), 4.62-4.56 (m, 4H, H1', H5, $C\underline{H}_2Ph$), 4.46 (d, *J* = 4.7, 1H, H3), 4.40-4.36 (m, 2H, H1', H5), 2.09 (s, 3H, CH_3), 1.85 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.6, 168.8, 166.1, 165.8 ($CH_3\underline{C}(O)$, $Ph\underline{C}(O)$), 136.6, 133.5, 133.3, 133.1, 133.0, 129.7, 129.6, 129.5, 128.5, 128.4, 128.3, 128.1,

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127.8, 127.5 (Ph), 97.8 (C1β), 94.1 (C1α), 84.2 (C4), 78.5 (C3), 73.9, 73.3 ($\underline{C}H_2$Ph, C2), 64.9, 64.6 (C5, C1'), 20.9, 20.8 (CH_3); Anal. calcd for $C_{31}H_{30}O_{10}$: C, 66.2; H, 5.4; Found: C, 66.4; H, 5.4.

1-(2-*O*-Acetyl-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-β-D-*erythro*-pentofuranosyl)thymine (**S3**)

To a solution of **S2** (70 g, 124 mmol, crude) in anhyd acetonitrile (700 mL) was added thymine (20.3 g, 161.2 mmol) followed by *N*,*O*-bis(trimethylsilyl)acetamide (71.1 mL, 290.2 mmol). The reaction mixture was refluxed for 1 h and cooled to 0 °C. Trimethylsilyl triflate was added over 30 min at 0 °C and the reaction mixture was heated to 65 °C for 2 h. The reaction was quenched by the addition of sat. aq $NaHCO_3$ (700 mL). The aq phase was extracted with dichloromethane (3 x 500 mL). The combined organic phases were washed with sat. aq $NaHCO_3$ (2 x 500 mL) and brine (500 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure to give crude **S3** (72 g) which was used without further purification. An analytical sample was obtained by DCVC (Ø 2 cm, 0-100% EtOAc in *n*-heptane v/v, 5% increments, 50 mL fractions) and **S3** was isolated as a white solid. R_f = 0.27 (65% EtOAc in *n*-heptane, v/v); ESI-MS *m/z* found 646.2 ($[MNH_4]^+$, calcd 646.2); ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1H, NH), 7.97 (d, J = 8.4, 2H, Bz), 7.90 (d, J = 8.4, 2H, Bz), 7.52-7.47 (m, 2H, Ph), 7.39-7.33 (m, 4H, Ph), 7.19-7.16 (m, 5H, Ph), 6.96 (d, J = 1.1, 1H, H6), 5.84 (d, J = 3.6, 1H, H1'), 5.57 (dd, J = 6.2, 3.7, 1H, H2'), 4.93 (d, J = 12.4, 1H), 4.65–4.33 (m, 6H) ($C\underline{H}_2$Ph, H3', H5'a, H5'b, H1''a, H1''b), 2.06 (s, 3H, $CH_3C(O)$), 1.61 (d, J = 0.7, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.1, 166.2, 165.8, 163.3 ($CH_3\underline{C}(O)$, Ph$\underline{C}(O)$, C4), 149.7 (C2), 137.0, 136.8, 133.5, 133.3, 129.8, 129.7, 129.6, 129.3, 128.5, 128.4, 128.3, 128.2, 128.0 (C6, Ph), 111.3 (C5), 90.7 (C1'), 84.8 (C4'), 77.1 (C3'), 74.4, 73.9 ($\underline{C}H_2$Ph, C2'), 64.3, 62.8 (C5', C1''), 20.8 ($\underline{C}H_3C(O)$), 12.1 (CH_3); Anal. calcd for $C_{34}H_{32}N_2O_{10}\cdot 0.25\ H_2O$: C, 64.5; H, 5.2; N, 4.4; Found: C, 64.5; H, 5.2, N, 4.7.

1-(5-*O*-Benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-β-D-*erythro*-pentofuranosyl)thymine (**S4**)

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Nucleoside **S3** (54 g, 86 mmol) was dissolved in MeOH (500 ml), cooled to 0 °C and sat. methanolic ammonia (500 ml) was added. The mixture was stirred at 0 °C for 30 min and then allowed to reach rt. After 3 h at rt the reaction was quenched by neutralisation with aq HCl (1.0 M) followed by the removal of the solvents in vacuo. The residue was dissolved in EtOAc (300 mL) and washed with aq HCl (1.0 M, 250 mL) followed by brine (250 mL). The organic phase was dried (Na_2SO_4), filtered and the solvent removed in vacuo to afford a yellow liquid. Purification by DCVC (Ø 10 cm, 50-100% EtOAc in *n*-heptane v/v, 5% increments, 100 mL fractions) afforded **S4** (43 g, 84%) as a white solid. R_f = 0.27 (35% EtOAc in *n*-pentane, v/v); ESI-MS *m/z* found 587.2 ($[MH]^+$, calcd 587.2); ^{1}H NMR ($CDCl_3$) δ 9.07 (br s, 1H, NH), 8.06-8.03 (m, 2H, Bz), 7.99-7.96 (m, 2H, Bz), 7.61-7.54 (m, 3H, Ph), 7.46-7.41 (m, 4H, Ph, H6), 7.34-7.23 (m, 5H, Ph), 5.86 (d, *J* = 3.8, 1H, H1'), 4.98 (d, *J* = 12.3, 1H, C$\underline{H}_2$Ph), 4.79 (d, *J* = 11.4, 1H, C$\underline{H}_2$Ph), 4.68 (dd, *J* = 11.5, 5.3, 2H), 4.60-4.49 (m, 4H) (H2', H3', H5', H1''), 3.58 (br s, 1H, OH), 1.67 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.9, 165.8, 163.4 (Ph$\underline{C}$(O), C4), 150.3 (C2), 136.4, 133.5, 133.2, 129.6, 129.5, 129.1, 128.6, 128.4, 128.1 (C6, Ph), 111.1 (C5), 91.9 (C1'), 84.7 (C4'), 78.6 (C3'), 74.6, 73.9 (C2', $\underline{C}H_2$Ph), 65.0, 63.4 (C5', C1''), 12.0 (CH_3); Anal. calcd for $C_{32}H_{30}N_2O_9$: C, 65.5; H, 5.2; N, 4.8. Found: C, 65.2; H, 5.2; N, 4.7.

2,2'-Anhydro-1-(5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-β-D-*threo*-pentofuranosyl)thymine (**S6**)

Nucleoside **S4** (23 g, 39 mmol) was dissolved in anhyd dichloromethane (230 mL) and anhyd pyridine (9.5 mL, 117 mmol) was added. The reaction mixture was cooled to 0 °C and methanesulfonyl chloride (4.6 mL, 58.5 mmol) was added. The reaction was stirred at 0 °C for 30 min and then allowed to reach rt. After 24 h the reaction had not gone to completion. Additional methanesulfonyl chloride (2 × 1.5 mL) was added until the reaction was completed (1 h). The reaction was quenched by addition of sat. aq $NaHCO_3$ (250 mL). The phases were separated and the organic phase was washed with water (250 mL) and brine (250 mL), dried (Na_2SO_4), filtered and the solvent removed in vacuo to afford a white foam. The product was dissolved in anhyd DMF (150 mL) and DBU (6 mL, 39 mmol) was added. The

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reaction was stirred at rt for 24 h during which time more DBU (10.5 mL, 68 mmol) was added in portions. Most of the solvent was removed in vacuo and the residue was diluted with dichloromethane (250 mL) and washed with water (250 mL). The aq phase was extracted with dichloromethane (2 × 250 mL) and the combined organic phases were dried (Na_2SO_4), filtered and the solvent removed in vacuo to afford an yellow liquid that was precipitated from boiling EtOAc affording **S6** (22 g, 98%) as an off-white compound. Further purification can be achieved by recrystalisation from methanol. R_f = 0.22 (100% EtOAc); ESI-MS *m/z* found 569.2 ($[MH]^+$, calcd 569.2); ^{1}H NMR ($CDCl_3$) δ 8.00 (d, *J* = 11.4, 2H, Bz), 7.70 (d, *J* = 11.4, 2H, Bz), 7.64-7.24 (m, 11H, Ph), 7.07 (d, *J* = 1.3, 1H, H6), 6.23 (d, *J* = 6.2, 1H, H1'), 5.44 (dd, *J* = 6.2, 3.8, 1H, H2'), 4.85 (d, *J* = 12.0, 1H), 4.75 (d, *J* = 12.0, 1H), 4.67 (d, *J* = 12.0, 1H) (H1'a, H5'), 4.52 (d, *J* = 3.7, 1H, H3'), 4.50 (d, *J* = 12.0, 1H, C$\underline{H}_2$Ph), 4.41 (d, *J* = 12.0, 1H, H1'b), 4.15 (d, *J* = 12.0, 1H, C$\underline{H}_2$Ph), 1.80 (d, *J* = 1.3, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 171.6, 165.5, 165.1, 158.6 (Ph$\underline{C}$(O), C2, C4), 135.5, 133.5, 129.4, 129.3, 129.1, 128.6, 128.5, 128.2, 127.9 (C6, Ph), 119.4 (C5), 88.6 (C1'), 86.6, 86.3 (C2', C4'), 82.9 (C3'), 73.2 ($\underline{C}H_2$Ph), 63.4, 62.2 (C5', C1''), 13.7 (CH_3); Anal. calcd for $C_{32}H_{28}N_2O_8 \cdot 0.25\ H_2O$: C, 67.1; H, 5.0; N, 4.9. Found: C, 67.1; H, 4.9; N, 4.9.

1-(3-*O*-Benzyl-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-*β*-D-*threo*-pentofuranosyl)thymine (**S7**)

Nucleoside **S6** (22 g, 38.7 mmol) was dissolved in DMF (220 mL) and aq HCl (2 M, 73 mL) was added. The reaction mixture was heated to 80 °C for 5 h during which time more aq HCl (2 M, 100 mL) was added. The reaction was quenched by the addition of EtOAc (250 mL) and brine (250 mL). The phases were separated and the aq phases was extracted with EtOAc (250 mL). The combined organic phases were washed with sat. aq $NaHCO_3$ (2 × 250 mL), dried (Na_2SO_4), filtered and the solvent removed in vacuo to afford a yellow residue that was purified by DCVC (Ø 10 cm, 30-100% EtOAc in *n*-heptane v/v, 5% increments, 100 mL fractions, followed by 0-5% MeOH in EtOAc v/v, 1% increments, 100 mL fractions) and **S7** (18 g, 80%) was isolated as a white solid. R_f = 0.52 (100% EtOAc); ESI-MS *m/z* found 587.0 ($[MH]^+$, calcd 587.2); ^{1}H NMR ($CDCl_3$) δ 11.20 (s, 1H, NH), 7.99 (dd, *J* = 8.3, 1.3, 2H, Bz), 7.93 (dd, *J* = 8.3, 1.3, 2H, Bz), 7.59-7.54 (m, 3H, Ph), 7.46-7.40 (m, 4H, Ph,

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H6), 7.27-7.19 (m, 5H, Ph), 6.33 (d, *J* = 3.1, 1H, H1'), 5.33 (s, 1H, 2'-OH), 5.05 (d, *J* = 11.4, 1H, C$\underline{H}_2$Ph), 4.79 (d, *J* = 11.7, 1H, C$\underline{H}_2$Ph), 4.70-4.38 (m, 5H), 4.20 (s, 1H) (H2', H3',H5', H1''), 1.70 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.9, 165.8, 163.9, 150.6 (Ph$\underline{C}$(O), C2 C4), 136.7, 133.2, 133.0, 129.7, 129.6, 129.4, 128.4, 128.3, 128.1, 127.8 (C6, Ph), 107.8 (C5), 87.7 (C1'), 84.9, 83.3 (C3', C4'), 73.0 (C2'), 71.6 ($\underline{C}H_2$Ph), 63.3, 62.2 (C5', C1''), 12.0 (CH_3); Anal. calcd for $C_{32}H_{30}N_2O_9 \cdot 0.5\ H_2O$: C, 65.0; H, 5.2; N, 4.7. Found: C, 65.2; H, 5.0; N, 4.8.

1-(2-Azido-3-*O*-benzyl-2-deoxy-5-*O*-benzoyl-4-*C*-(benzoyloxymethyl)-β-D-*erythro*-pentofuranosyl)thymine (**S10**)

Compound **S7** (3.78 g, 6.4 mmol) was dissolved in anhyd dichloromethane (150 mL) and cooled to 0 °C with stirring. Anhyd pyridine (1.6 mL, 19.8 mmol) was added followed by dropwise addition of trifluoroacetic anhydride (1.6 mL, 11.3 mmol). The reaction mixture was stirred at 0 °C for 1 h and was transferred to a refrigerator (7 °C) where it was stored for 72 h without stirring. The reaction was quenched by addition of water (150 mL) and the phases were separated. The organic phase was dried (Na_2SO_4), filtered and the solvent removed in vacuo to afford a reddish foam. The product was dissolved in anhyd DMF (100 mL) and NaN_3 (2.23 g, 34.3 mmol) was added. The reaction mixture was stirred at 80 °C for 30 min and then at rt for 18 h. The reaction was quenched by addition of water (100 mL) and extracted with EtOAc (2 × 250 mL). The combined organic phases were dried (Na_2SO_4), filtered and the solvent removed in vacuo to afford a clear yellow liquid. The product was purified by DCVC (Ø 6 cm, 30-50% EtOAc in *n*-heptane v/v, 2% increments, 100 mL fractions) and afforded **S10** as a white foam (1.44 g, 37% from **S7**) together with **S11** (1.0 g, 25% from S7).

Analytical data for **S10**: R_f = 0.36 (50% EtOAc in *n*-heptane, v/v); ESI-MS *m/z* found 634.0 ($[MNa]^+$, calcd 634.2); ^{1}H NMR ($CDCl_3$) δ 8.60 (s, 1H, NH), 7.96 (dd, *J* = 8.1, 1.1, 2H, Bz), 7.84 (dd, *J* = 8.1, 0.9, 2H, Bz), 7.53-7.47 (m, 3H, Ph), 7.38-7.33 (m, 4H, Ph, H6), 7.27-7.16 (m, 5H, Ph), 5.75 (d, *J* = 4.6, 1H, H1'), 4.93 (d, *J* = 12.5, 1H), 4.77 (d, *J* = 11.5, 1H), 4.62-4.54 (m, 3H), 4.44-4.34 (m, 3H) (H2', H3', H5', H1'', C$\underline{H}_2$Ph), 1.70 (d, *J* = 1.1, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.1, 165.8, 163.3, 149.8

Supplementary data

(PhC(O), C2, C4), 136.5, 133.6, 133.3, 129.7, 129.6, 129.1, 128.6, 128.5, 128.2 (C6, Ph), 111.5 (C5), 90.1 (C1'), 84.9 (C4'), 78.4 (C3'), 74.5 (CH_2Ph), 64.2, 64.0, 63.0 (C2', C5', C1''), 12.1 (CH_3); Anal. calcd for $C_{32}H_{29}N_5O_8 \cdot 0.5\ H_2O$: C, 61.9; H, 4.9; N, 11.3. Found: C, 61.9; H, 4.9; N, 11.2.

6-(2-Azido-3-*O*-benzyl-2-deoxy-5-*O*-benzoyl-4-*C*-(benzoyloxymethyl)-*β*-D-*erythro*-pentofuranosyl)-tetrazolo[1,5-c]pyrimidin-8-methyl-5(6H)-one **S11** was crystallised from EtOAc, *n*-hexane affording white needles. R_f = 0.49 (50% EtOAc in *n*-heptane, v/v); ESI-MS *m/z* found 659.2 ($[MNa]^+$, calcd 659.2); ^{1}H NMR ($CDCl_3$) δ 7.97 (d, *J* = 8.1, 2H), 7.84 (d, *J* = 8.1, 2H), 7.55-7.48 (m, 3H), 7.39-7.16 (m, 9H), 6.02 (d, *J* = 4.2, 1H), 5.06 (d, *J* = 12.4, 1H), 4.82 (d, *J* = 11.5, 1H), 4.70-4.67 (m, 3H), 4.57 (d, *J* = 11.5, 1H), 4.50 (dd, *J* = 6.2, 4.2, 1H), 4.42 (d, *J* = 12.3, 1H), 4.36 (d, *J* = 12.6, 1H), 2.07 (d, *J* = 0.7, 3H); ^{13}C NMR ($CDCl_3$) δ 165.9, 165.6, 151.4, 141.9, 135.8, 133.6, 133.2, 131.1, 129.5, 129.4, 129.2, 128.7, 128.3, 104.6, 91.1, 85.7, 77.5, 74.4, 64.1, 63.7, 62.7, 12.6.

1-(2-Azido-3-*O*-benzyl-2-deoxy-5-*O*-methanesulfonyl-4-*C*-(methanesulfonyloxymethyl)-*β*-D-*erythro*-pentofuranosyl)thymine (**17**)

Nucleoside **S10** (3.70 g, 6.0 mmol) was dissolved in THF (400 mL) and aq LiOH (1.0 M, 50 mL) was added. After 9 h the reaction volume was reduced to approx. ¼ in vacuo and water (50 mL) and EtOAc (200 mL) was added. The phases were separated and the aq phase was extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with brine (2 x 200 mL), dried (Na_2SO_4), filtered and the solvent removed in vacuo to afford a clear liquid (**S12**). Water traces were removed by azeotropic destillation with anhyd pyridine (2 x 50 mL). The liquid was dissolved in anhyd pyridine (250 mL) and cooled to 0 °C with stirring. Methanesulfonyl chloride (1.40 mL, 18.0 mmol) was added and the temperature was allowed to reach rt. After 3 h the reaction volume was reduced to approx. ¼ in vacuo, diluted with EtOAc (200 mL) and washed with aq HCl (0.1 M, 200 mL), sat. aq $NaHCO_3$ (2 x 200 mL), and brine (200 mL). The organic phase was dried (Na_2SO_4), filtered and the solvent was removed

Supplementary data

in vacuo to afford a brown residue that was purified by DCVC (Ø 5 cm, 0-100% EtOAc in *n*-heptane v/v, 5% increments, 50 mL fractions). Nucleoside **17** (2.91 g, 86% from **S10**) was isolated as a white foam.

References

1. R. D. Youssefyeh, J. P. H. Verheyden, and J. G. Moffatt, *J.Org.Chem.*, 1979, **44**, 1301-1309.
2. V. K. Rajwanshi, R. Kumar, M. Kofod-Hansen, and J. Wengel, *J.Chem.Soc., Perkin Trans.1*, 1999, 1407-1414.
3. H. Vorbrüggen and G. Höfle, *Chem.Ber.*, 1981, **114**, 1256-1268.
4. T. Neilson and E. S. Werstiuk, *Can.J.Chem.*, 1971, **49**, 493-499.
5. D. S. Pedersen and C. Rosenbohm, *Synthesis*, 2001, 2431-2434.
6. I. Lavandera, S. Fernandez, M. Ferrero, and V. Gotor, *J.Org.Chem.*, 2001, **66**, 4079-4082.
7. R. P. Glinski, M. S. Khan, R. L. Kalamas, and M. B. Sporn, *J.Org.Chem.*, 1973, **38**, 4299-4305.
8. G. P. Kirschenheuter, Y. Zhai, and W. A. Pieken, *Tet.Lett.*, 1994, **35**, 8517-8520.
9. C. Mathe and G. Gosselin, *Nucleosides, Nucleotides & Nucleic Acids*, 2000, **19**, 1517-1530.
10. J. G. Moffatt, *Chemical Transformation of the Sugar Moiety of Nucleosides*, Plenum Press, New York, 1979,71-164.
11. R. Kumar, S. K. Singh, A. A. Koshkin, V. K. Rajwanshi, M. Meldgaard, and J. Wengel, *Bioorg.Med.Chem Lett.*, 18-8-1998, **8**, 2219-2222.
12. S. K. Singh, R. Kumar, and J. Wengel, *J.Org.Chem.*, 1998, **63**, 6078-6079.
13. J. F. Codington, R. Fecher, and J. J. Fox, *J.Am.Chem.Soc.*, 1960, **82**, 2794-2803.
14. K. Ciszewski, L. Celewicz, and K. Golankiewicz, *Synthesis*, 1995, 777-779.
15. K. Ciszewski, L. Celewicz, and K. Golankiewicz, *Biochem.Biophys.Res.Com.*, 1992, **187** , 1545-1550.
16. L. De Napoli, A. Messere, D. Montesarchio, G. Piccialli, and C. Santacroce, *Nucleosides & Nucleotides*, 1991, **10**, 1719-1728.

Supplementary data

17. P. Herdewijn and A. Van Aerschot, *Bull.Soc.Chim.Belg.*, 1989, **98**, 943-947.

18. L. De Napoli and L. Mayol, *J.Heterocyclic.Chem.*, 1986, **23**, 1401-1403.

19. Crystallographic data for nucleoside **S11**: $C_{32}H_{28}N_8O_7$, M = 636.62, orthorhombic, space group $P2_12_12_1$, a = 7.6880(2) Å, b = 13.2934(3) Å, c = 31.1120(9) Å, V = 3179.64(14) $Å^3$, Z = 4, D_c = 1.330 Mg/m^3, μ = 0.097 mm^{-1}, F(000) = 1328, Data (7601 reflections collected, 3480 independent reflections R_{int} = 0.0450) were measured on a Nonius Kappa CCD diffractometer at 180(2) K. Refinement method Full-matrix least-squares on F^2, [R1 = 0.0427($I>2\sigma I$), wR_2 = 0.0892]. Goodness-of-fit on F^2 1.10.