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Synthesis of 2′-amino-LNA: a new strategy †

Christoph Rosenbohm,[a] Signe M. Christensen,[a] Mads D. Sørensen,[b] Daniel Sejer Pedersen,‡[a] Lotte-Emilie Larsen,[a,b] Jesper Wengel[c] and Troels Koch*[a]

[a] *Cureon A/S, Fruebjergvej 3, DK-2100 Copenhagen, Denmark. E-mail: tk@cureon.com; Fax: +45 7026 0097; Tel: +45 7026 0096*

[b] *Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark*

[c] *Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark*

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In this paper we present revised and significantly improved synthetic routes to 2′-amino-LNA (locked nucleic acid). The optimal route is convergent with the synthesis of LNA monomers ("2′-oxy-LNA") *via* a common intermediate obtained by a mild deacetylation for the liberation of the 2′-hydroxy group to give compound **23** without the concomitant ring closure that affords the 2′-oxy-LNA skeleton. After inversion of the stereochemistry at C2′ and triflate formation at the 2′-hydroxy group a new common intermediate **16** is obtained which gives easy access to a range of other analogues exemplified by the introduction of a sulfur nucleophile leading to the 2′-thio-LNA structure. After substitution of the triflate with azide a basic reduction affords the desired 2′-amino-LNA structure, *i.e.*, compound **18**. This new synthesis strategy towards 2′-amino-LNA improves the overall yield significantly and converges the syntheses of 2′-oxy-LNA and LNA analogues.

Introduction

The promising aspects of using modified analogues of natural nucleic acids as therapeutic agents have stimulated much interest in this field during recent years. LNA (locked nucleic acid)§ was introduced independently by Wengel[1,2] and by Imanishi[3] in 1998 as a novel class of conformationally restricted oligonucleotide analogues.

It has been shown beyond any doubt that LNA is the single nucleic acid modification that contributes the highest affinity ever obtained by Watson–Crick hydrogen bonding.[6] The 2′,4′-linked bicyclic structure brings not only unprecedented affinity/specificity of fully LNA modified oligonucleotides, but LNA monomers can be mixed and act co-operatively with DNA and RNA monomers and with most of the known nucleic acid analogues such as phosphorothioates and 2′-*O*-alkyl modified RNA.[7] These unique properties position LNA as a central player in nucleic acid chemistry.

The first LNA monomer was based on the 2′-OCH_2-4′ bicyclic structure (LNA/2′-oxy-LNA). Later similar high affinity/specificity functionalised LNA monomers such as 2′-$NHCH_2$-4′, 2′-$N(CH_3)CH_2$-4′ (2′-amino-LNA),[5,8] and 2′-SCH_2-4′ (2′-thio-LNA)[8,9] were synthesised. We anticipate that such LNA analogues will have different pharmacological, in particular pharmacokinetic, properties from the parent 2′-oxy-LNA. Having a broad range of biological properties within a high affinity/specificity structure will provide the necessary tools to design improved antisense compounds. It is therefore of great importance for the use of LNA analogues that they can be synthesised by efficient and convergent synthesis strategies.

The first synthesis of an LNA nucleoside was performed by a linear approach using uridine as starting material,[10] but by virtue of being a convergent synthesis an alternative route[1,2,7,11] became the method of choice for the synthesis of LNA nucleosides. 2′-Amino- and 2′-thio-LNA were originally synthesised using slightly different intermediates from those used for the 2′-oxy-LNA monomers, but in this paper we demonstrate that common intermediates at late stages can be used for synthesis of both 2′-oxy-LNA, 2′-amino-LNA, and 2′-thio-LNA.

Results and discussion

During our implementation of the previously described synthesis of 2′-amino-LNA monomers[5,8] (Scheme 1) to the 100 g scale synthesis we encountered several major problems. The first reaction that required attention in the scale-up work was the regioselective benzylation at C5 of compound **1**.[1] Working in the 100 g scale the reaction yielded a mixture of compound **2**, the 4-*C*-benzyloxymethyl derivative, and the fully benzylated derivative, even under optimised conditions. The maximum yield of the desired compound **2** was 59% but with a general yield of 45–50% compared to 71% on smaller scale.[1] Furthermore, compound **2** could only be isolated through tedious chromatography of closely eluting products.

The second key step in the original strategy[5,8] that caused problems during scale-up was the double nucleophilic substitution of the di-*O*-tosyl nucleoside **5** using benzylamine giving nucleoside **6**. Originally it was reported that on an 8 g scale the reaction afforded 52% of nucleoside **6** and no side reaction was reported.[5] However, in our hands the reaction on a larger scale (22 g) afforded a significant amount of a by-product identified as the 2′-oxy-LNA derivative. One can envision the formation of this by-product by substitution on the sulfur atom of the 2′-*O*-tosylate **5** resulting in a free hydroxy group that can make a nucleophilic attack on the remaining 4′-*C*-tosyloxymethyl functionality to afford the 2′-oxy-LNA structure. The desired *N*-benzylated-2′-amino-LNA product **6** was obtained in only 15% yield together with 13% of the 2′-oxy-LNA by-product.

† Electronic supplementary information (ESI) available: further experimental details and the structure of compound **S11** (.pdb file). See http://www.rsc.org/suppdata/ob/b2/b208864a/

‡ *Present address*: Cambridge University, University Chemical Laboratory, Lensfield Road, Cambridge, UK CB2 1EW.

§ LNA is defined as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl nucleotide monomers (LNA monomers).[1,2] For clarity the term 2′-oxy-LNA is used herein for the parent LNA and 2′-amino-LNA and 2′-thio-LNA refer to the analogues where the 2′-oxygen has been substituted by respectively a nitrogen atom or a sulfur atom.

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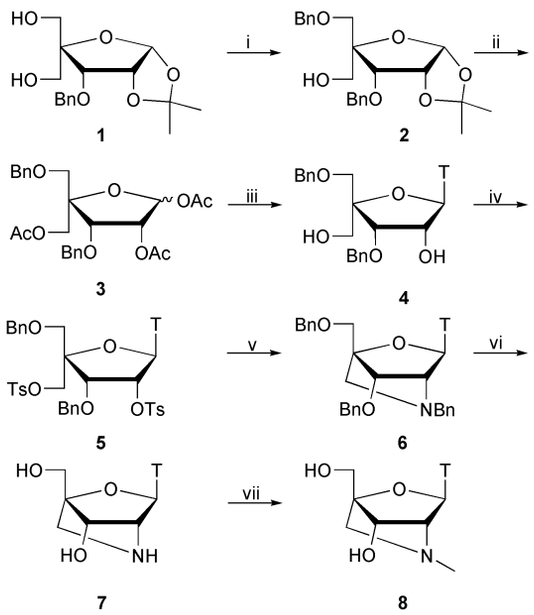
The remaining material cannot be accounted for, presumably due to decomposition by the strong base at elevated temperature.

Yet another problem encountered was the debenzylation of nucleoside **6** using ammonium formate and 10% Pd/C in methanol. We found that the reaction proceeded very slowly and literally never went to completion. Therefore, we always found two products in the isolated material: one being the desired product **7** and the other being one out of three mono-benzylated compounds (LC–MS). Furthermore it proved to be difficult to isolate product **7** from the mixture on a larger scale.

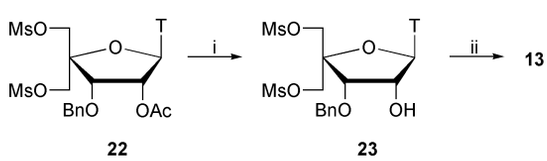


Scheme 1 The previously published synthesis of **8**. *Reagents and conditions:* i) BnBr, NaH, DMF; ii) a) Ac_2O, pyridine, b) 80% aq AcOH, c) Ac_2O, pyridine; iii) a) thymine, *N*,*O*-bis(trimethylsilyl)-acetamide, TMS triflate, MeCN; b) NaOMe, MeOH; iv) TsCl, DMAP, pyridine, CH_2Cl_2; v) $BnNH_2$, heat; vi) 10% Pd/C, $HCOONH_4$, MeOH; vii) a) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine, b) MeI, DBU, THF, CH_2Cl_2; c) TBAF, THF. **1** was prepared according to Youssefyeh *et al.*[4]; steps i–iii according to Koshkin *et al.*[1]; steps iv to vii according to Singh *et al.*[5]

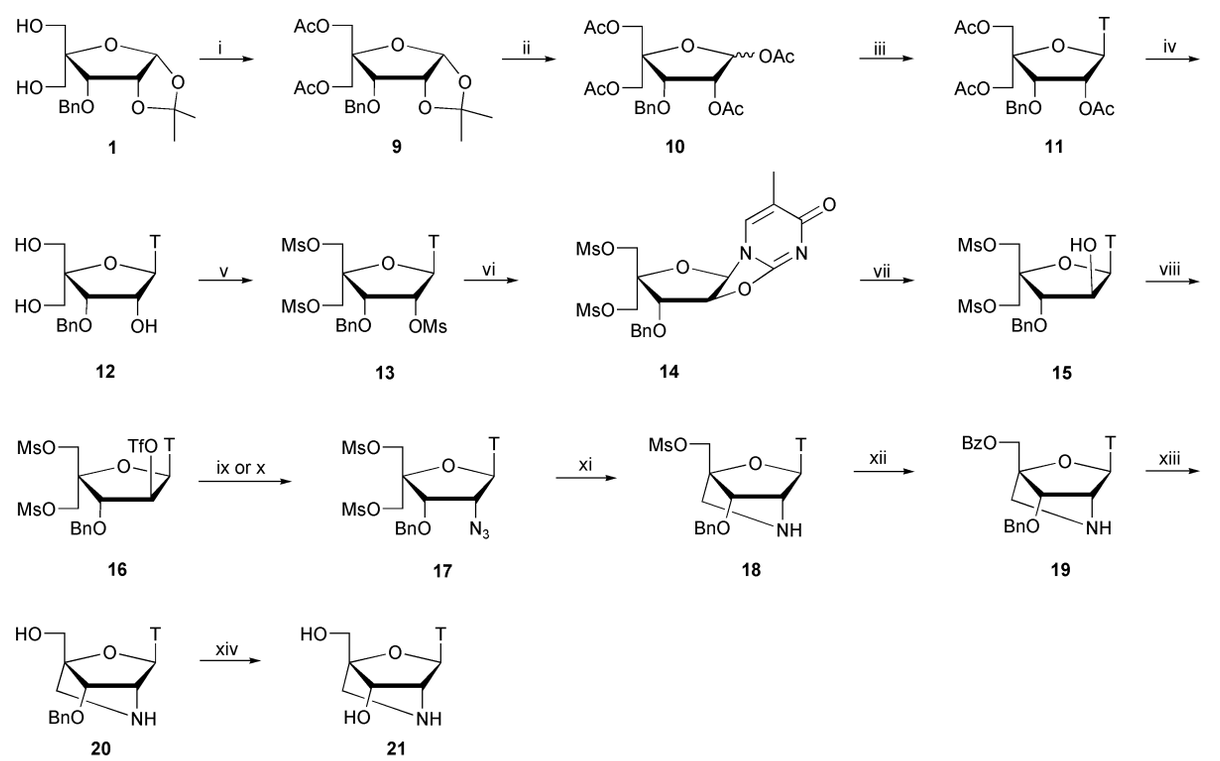
Inspired both by the improvement in the large scale synthesis of 2′-oxy-LNA[11] and the synthesis of α-L-LNA[12] we developed a new strategy for the synthesis of 2′-amino-LNA monomers avoiding the need for selectivity between the two hydroxy groups of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose **1** (Scheme 2). Compound **1** was synthesised from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose according to the procedure of Youssefyeh *et al.*[4]

Initially we chose a synthetic route where the two primary alcohols on the glucofuranose **1** were protected as benzoates. However, this approach was not successful presumably due to unfavorable interactions from the benzoyl groups in the critical triflation and azide displacement steps (see supplementary material †).

We identified the known trimesylate **13**[12] as a key compound and achieved its synthesis by two different approaches outlined in Schemes 2 and 3. On a small scale (200 mg starting material) compound **1** was converted to the tetraacetylated anomeric mixture **10** by acidic hydrolysis and *in situ* acetylation using acetic acid, acetic anhydride and sulfuric acid. The crude product was subjected to a modified Vorbrüggen reaction[13] using in situ silylation of thymine and trimethylsilyl triflate-mediated coupling to give the β-configured thymine nucleoside **11** in 91%.



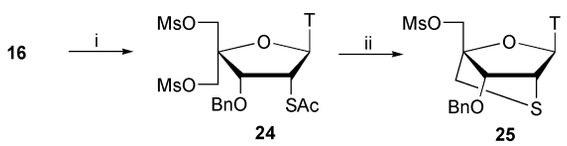
Scheme 3 *Reagents and conditions:* i) half sat. NH_3 in MeOH; ii) MsCl, pyridine.



Scheme 2 *Reagents and conditions:* i) Ac_2O, pyridine; ii) AcOH, Ac_2O, c. H_2SO_4 (100 : 10 : 0.1); iii) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS triflate, MeCN; iv) sat. NH_3 in MeOH; v) MsCl, pyridine, CH_2Cl_2; vi) DBU, MeCN; vii) acetone, 0.1 M H_2SO_4 (aq); viii) Tf_2O, DMAP, pyridine, CH_2Cl_2; ix) NaN_3, DMF; x) NaN_3, 15-crown-5, DMF, 80 °C; xi) PMe_3, NaOH (aq), THF; xii) NaOBz, DMF; xiii) sat. NH_3 in MeOH; xiv) 20% $Pd(OH)_2/C$, H_2, abs EtOH.

When the same synthetic procedure was used on a larger scale (5+ g starting material) the yield dropped and the formation of an unidentified by-product was observed, presumably due to the formation of an acetylated pyranose during the hydrolysis and *in situ* acetylation step. The formation of the pyranose can be avoided on large scale by performing the acetylation of the two primary hydroxy groups of **1** to give compound **9** before subjecting the compound to hydrolysis and *in situ* acetylation yielding the tetraacetylated anomeric mixture **10**. Deacetylation of nucleoside **11** was performed using saturated methanolic ammonia to give the nucleoside triol **12** in 84% yield. Subsequent reaction with mesyl chloride in pyridine gave the known trimesylate **13**[12] in 93% yield.

The trimesylate **13** is also readily available from the 2′-*O*-acetyl dimesylated nucleoside **22** (Scheme 3) used in the improved and recently published synthesis of 2′-oxy-LNA nucleosides.[11] This synthetic route has the great advantage of a high degree of convergence with the 2′-oxy-LNA synthesis making nucleoside **22** the common building block for both 2′-oxy-LNA and LNA nucleosides with other heteroatoms at C2′, *e.g.* nitrogen or sulfur, *via* the triflate **16** (see Scheme 4).



Scheme 4 *Reagents and conditions:* i) KSAc, DMF; ii) LiOH (aq), THF.

Deacetylation of nucleoside **22** was performed using half-saturated methanolic ammonia producing the dimesylated nucleoside **23** in quantitative yield. Subsequent reaction with mesyl chloride in pyridine gave the trimesylate **13** in 96% on a 20 g scale.

When nucleoside **13** was reacted in neat benzylamine at 130 °C in a similar manner as described for the synthesis of nucleoside **6** (see Scheme 1),[5] formation of the 2′-benzylamino-2′-deoxynucleoside with the desired 2-oxa-5-azabicyclo[2.2.1]-heptane skeleton was not observed, but instead a compound in which the nucleobase had been modified by attack of benzylamine at C2 was obtained. To test if the desired 2,2′-anhydro intermediate was formed during the reaction, nucleoside **13** was refluxed with 1.1 equivalents of benzylamine in acetonitrile. This in fact lead to the formation of 2,2′-anhydronucleoside **14** in 96% yield. However, it was not possible to achieve nucleophilic attack at C2′ of **14** with either benzylamine or NaN_3 in excess of up to 10 equivalents. Even reaction of nucleoside **14** in neat benzylamine at room temperature gave no reaction. The temperature had to be raised to 130 °C before any reaction would take place, and again only the undesired product where the nucleobase had been affected was formed.

The 2,2′-anhydro intermediate **14** could also be synthesised by treatment of **13** with 1.1 equivalents of DBU in anhydrous acetonitrile. Opening of the 2,2′-anhydro intermediate by refluxing it in a mixture of aqueous sulfuric acid (0.1 M) and acetone (1 : 1 v/v) resulted in a clean reaction giving the *threo*-configured nucleoside **15** in 98% yield.

Treatment of the *threo*-nucleoside **15** with trifluoromethanesulfonic anhydride, pyridine and DMAP in anhydrous dichloromethane at 0 °C gave the desired triflate **16** in 80% yield after chromatography. However, a better overall yield was obtained if the sensitive triflate was not purified prior to the substitution reaction with NaN_3 producing the desired 2′-azido-2′-deoxynucleoside **17** in 91% yield over the two steps.

Following the nucleophilic displacement with azide, compound **17** was converted to the amine by reduction with hydrogen and Pd/C or $Pd(OH)_2$/C. Unfortunately, these conditions also induced partial deprotection of the 3′-*O*-benzyl group. The use of Lindlar's catalyst[14,15] resulted in the exclusive reduction of the azide but the reaction was too sluggish to be of practical use (approx. 80% conversion after 7 days).

Reduction of the azide under modified Staudinger conditions[16,17] using trimethylphosphine and aqueous NaOH in THF gave the primary amine which reacted directly with the mesylate at C1″ in an intramolecular S_N2 reaction forming nucleoside **18** with the desired 2-oxa-5-azabicyclo[2.2.1]heptane skeleton.

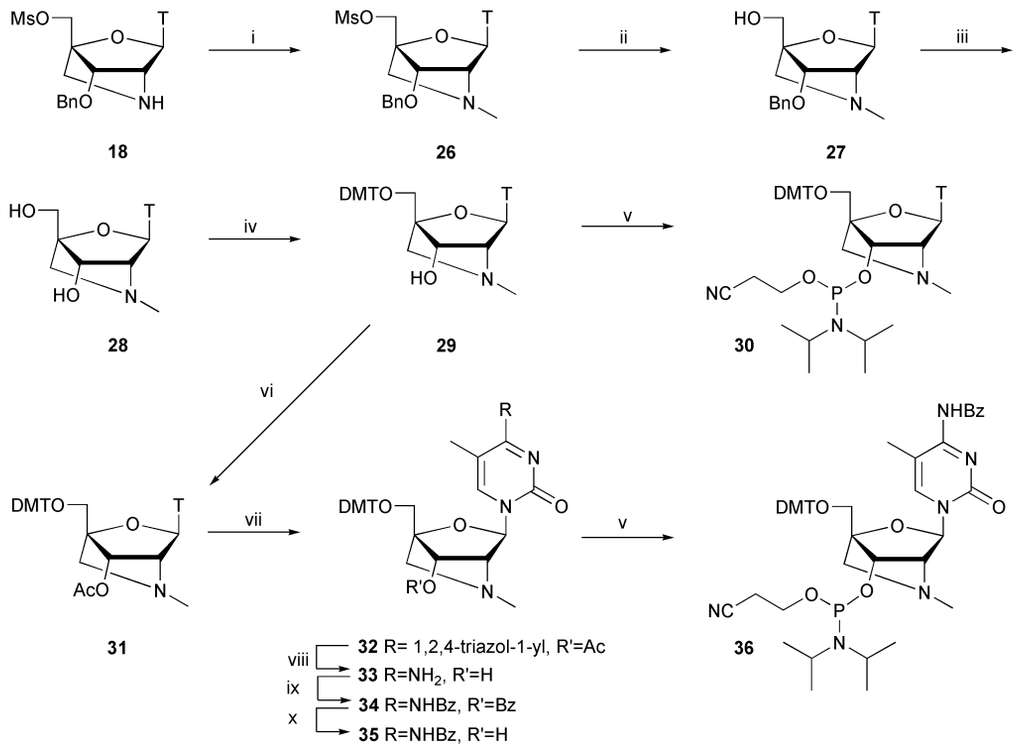
Nucleophilic replacement of the mesylate on C5′ of nucleoside **18** with benzoate gave nucleoside **19** which subsequently could be hydrolysed in saturated methanolic ammonia giving the 5′-hydroxy nucleoside **20**. Subsequent reductive debenzylation with hydrogen and 20% $Pd(OH)_2$/C in ethanol gave nucleoside **21** in an overall yield of 95% from **18** and with all analytical data identical to those previously reported.[8] After standard protection and phosphitylation nucleoside **21** can be incorporated in oligonucleotides where the free secondary amine will undoubtedly contribute with some interesting new properties.

Interestingly, compound **16** is not only a useful synthon for the synthesis of the 2′-amino-LNA skeleton but also a possible precursor for a range of LNA analogues by reaction with appropriate nucleophiles. As an example of the usefulness of compound **16** the synthesis of the 2-oxa-5-thiabicyclo[2.2.1]-heptane skeleton found in 2′-thio-LNA was accomplished by a substitution reaction with potassium thioacetate in DMF producing compound **24** (Scheme 4). Formation of the sulfur-containing bicyclic nucleoside was achieved by hydrolysis of the thioacetate with LiOH (aq) in THF to produce **25** in 47% yield from **16**. The conformation of **25** was confirmed by NOE difference NMR experiments showing an unusually high NOE enhancement between H6 of the nucleobase and H3′ (9.0%) as expected due to the extreme north conformation adopted by the nucleoside.[10,18] The synthon **16** should therefore find many useful applications for the preparation of LNA analogues in the future.

Returning to the 2′-amino-LNA monomer synthesis, compound **18** is ready for the introduction of different groups at the "handle" that the secondary nitrogen atom provides, *e.g.* different alkyl chains.

Because we are interested in studying the effects of 2′-amino-LNA monomers when incorporated into oligonucleotides we wanted to avoid bulky substituents in our initial studies. In addition we were looking for a substituent that would be stable under the standard conditions employed in oligonucleotide synthesis and purification. For these reasons we decided to develop a new strategy for the synthesis of the known amidite **30** with a *N*-methyl group.[5]

The method used for *N*-methylation (MeI, DBU) in the previously published synthesis of 2′-amino-LNA[5] gave additional methylation on the nucleobase when attempted on nucleoside **18**. Eschweiler–Clarke conditions[19,20] (formaldehyde in formic acid) proved more efficient giving exclusively the desired 2′-*N*-methyl derivative **26** in 90% yield after purification (Scheme 5). The expected extreme north conformation of the bicyclic nucleoside **26** was confirmed by NOE difference NMR experiments showing an unusually high NOE enhancement between H6 of the nucleobase and H3′ (9.0%) just as observed for nucleoside **25**. Nucleophilic displacement of the mesylate on C5′ of nucleoside **26** with sodium benzoate followed by transesterification with methoxide gave the 5′-hydroxy nucleoside **27** in 98% yield. Subsequent reductive debenzylation with hydrogen and 20% $Pd(OH)_2$/C in acetic acid yielded nucleoside **28** in 97% yield. All analytical data for nucleoside **28** were identical to those previously reported.[5] 4,4′-Dimethoxytritylation of the 5′-hydroxy group of **28** and phosphitylation of the 3′-hydroxy group[21] gave the amidite **30**[5] in 90% yield ready for the automated incorporation into oligonucleotides. The DMT protected thymine nucleoside **29** can be further converted into the 5-methylcytosine nucleoside **35** *via* the triazolation strategy[22]



Scheme 5 *Reagents and conditions:* i) CH_2O, HCO_2H; ii) a) NaOBz, b) MeONa, DMF; iii) 20% $Pd(OH)_2$/C, H_2, AcOH; iv) DMT–Cl, pyridine; v) $NC(CH_2)_2OP(N(iPr)_2)_2$, 4,5-dicyanoimidazole, MeCN, CH_2Cl_2; vi) Ac_2O, pyridine; vii) Et_3N, 1,2,4-triazole, $POCl_3$, MeCN; viii) 1 : 1 MeCN, sat. aq NH_3; ix) BzCl, pyridine; x) LiOH (aq), THF.

where thymine is initially activated by conversion into the triazole **32** and subsequently substituted with ammonia to give the 5-methylcytosine nucleoside **33**. Followed by protection in the usual manner and phosphitylation of the 3′-OH nucleoside **33** was converted to the 5-methyl cytosine phosphoramidite **36** ready for oligonucleotide synthesis.

Conclusion

We have developed a synthetic method using cheap and commercially available reagents in scalable reactions with a minimum use of chromatography. This gives access to sufficient amounts of both 2′-amino-LNA thymine and 2′-amino-LNA 5-methyl cytosine phosphoramidites as well as other LNA phosphoramidites needed for the automated synthesis of, *e.g.*, 2′-amino-LNA oligomers and in depth evaluation of their *in vitro* and *in vivo* properties.

Furthermore, by the presence of common intermediates at late stages in the synthesis of both 2′-oxy-LNA, 2′-amino-LNA, and 2′-thio-LNA monomers, the convergence between these has been increased significantly. In addition this new strategy provides a stable triflate as an intermediate that is a potential synthon for a wide variety of LNA analogues when reacted with appropriate nucleophiles.

In vitro and *in vivo* evaluations of 2′-amino-LNA containing oligonucleotides are currently in progress and will be published in due course.

Experimental

For reactions conducted under anhydrous conditions glassware was dried overnight in an oven at 150 °C and was allowed to cool in a desiccator 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 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.[23] ^{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*-substituent 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. X-Ray Crystallographic Data of nucleoside **S11** were obtained from Cambridge University Chemical Laboratory X-Ray Department. CCDC reference number 198987. See http://www.rsc.org/suppdata/ob/b2/b208864a/ for crystallographic files in .cif or other electronic format.

1-(2,5-Di-*O*-acetyl-4-*C*-acetyloxymethyl-3-*O*-benzyl-β-D-*erythro*-pentofuranosyl)thymine (11)

To a stirred solution of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose **1**[4] (200 mg, 0.64 mmol) in acetic acid (3.69 mL, 64.4 mmol) at 0 °C was added acetic anhydride (0.61 mL, 6.44 mmol) and conc H_2SO_4 (0.34 μL, 6.44 μmol). After 25 min the reaction mixture was allowed to warm to rt. Stirring was continued for 2 h after which the mixture was poured into ice cooled sat. aq $NaHCO_3$ (150 mL). The solution was extracted with dichloromethane (2 × 150 mL), and the combined organic phases were washed with sat. aq $NaHCO_3$ (2 × 100 mL), dried (Na_2SO_4), filtered and evaporated to dryness *in vacuo* to give the crude anomeric mixture **10** as a colorless liquid (258 mg, 0.59 mmol). The liquid (246 mg, 0.56 mmol) was dissolved in anhydrous acetonitrile (5 mL) with stirring. Thymine (144 mg, 1.14 mmol) and

N,*O*-bis(trimethylsilyl)acetamide (0.99 mL, 4.00 mmol) were added, and the mixture was refluxed for 1.5 h and then cooled to 0 °C. Trimethylsilyl triflate (0.23 mL, 1.25 mmol) was added dropwise during 5 min and the mixture was heated to 80 °C for 3.5 h. The reaction mixture was allowed to cool to rt, and ice cooled sat. aq $NaHCO_3$ (10 mL) was added. Extraction was performed with dichloromethane (2 × 20 mL), and the combined organic phases were washed successively with sat. aq $NaHCO_3$ (2 × 20 mL) and brine (20 mL), dried (Na_2SO_4), filtered and evaporated to dryness *in vacuo*. The residue was purified by flash chromatography (0–1% MeOH in dichloromethane v/v) to give nucleoside **11** (259 mg, 91%) as a white solid material. FAB-MS *m*/*z* found 505.0 ($[MH]^+$, calcd 505.2); ^{1}H NMR ($CDCl_3$) δ 9.93 (s, 1H, NH), 7.37–7.28 (m, 5H, Ph), 7.09 (d, J = 0.9, 1H, H6), 5.79 (d, J = 3.5, 1H, H1′), 5.53 (dd, J = 6.3, 3.7, 1H, H2′), 4.64–4.08 (m, 7H, CH_2Ph, H3′, H5′a, H5′b, H1″a, H1″b), 2.11 (s, 3H, $CH_3C(O)$), 2.10 (s, 3H, $CH_3C(O)$), 2.07 (s, 3H, $CH_3C(O)$), 1.91 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.4, 169.9, 163.9, 149.9 (CH_3C(O), C2, C4), 137.1, 136.8, 128.3, 128.0, 127.8 (C6, Ph), 111.0 (C5), 90.6 (C1′), 84.2 (C4′), 77.0 (C3′), 74.2 (CH_2Ph), 73.7 (C2′), 63.6, 62.2 (C5′, C1″), 20.6, 20.5 (CH_3C(O)), 12.3 (CH_3).

1-(3-*O*-Benzyl-4-*C*-hydroxymethyl-β-D-*erythro*-pentofuranosyl)-thymine (12)

Nucleoside **11** (149 mg, 0.30 mmol) was dissolved in a sat. solution of NH_3 in MeOH (15 mL). The mixture was stirred overnight at rt in a sealed flask and evaporated to dryness under reduced pressure. The residue was dissolved in EtOAc (30 mL) and washed with water (10 mL). The aqueous phase was extracted with EtOAc (30 mL) and the combined organic phases were coevaporated to dryness with acetonitrile (2 × 10 mL) under reduced pressure. The residue was purified by DCVC (1–4% MeOH in dichloromethane v/v), affording nucleoside **12** (93 mg, 84%) as a viscous liquid. R_f = 0.32 (10% MeOH in EtOAc, v/v); FAB-MS *m*/*z* found 379.0 ($[MH]^+$, calcd 379.1); ^{1}H NMR (DMSO-d_6) δ 11.29 (br s, 1H, NH), 7.73 (d, J = 1.3, 1H, H6), 7.40–7.26 (m, 5H, Ph), 5.90 (d, J = 6.2, 1H, H1′), 5.51 (d, J = 7.5, 1H, OH), 5.18 (t, J = 5.0, 1H, OH), 4.86 (t, J = 5.49, 1H, OH), 4.81 (d, J = 11.7, 1H), 4.56 (d, J = 11.7, 1H), 4.36 (q, J = 6.3, 1H, H2′), 4.08 (d, J = 5.5, 1H, H3′), 3.60–3.50 (m, 4H) (H5′, H1″, CH_2Ph), 1.79 (d, J = 1.1, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.6 (C4), 150.7 (C2), 138.6, 136.3, 128.0, 127.2 (C6, Ph), 109.3 (C5), 87.7, 87.5 (C1′, C4′), 78.5 (C3′), 73.3 (C2′), 72.7, 62.8, 61.3 (C5′, C1″, CH_2Ph), 12.2 (CH_3); Anal. calcd for $C_{18}H_{22}N_2O_7$·0.25 H_2O: C, 56.5; H, 5.9; N, 7.3. Found: C, 56.5; H, 5.9; N, 7.0%.

1-(3-*O*-Benzyl-2,5-di-*O*-methanesulfonyl-4-*C*-(methanesulfonyl-oxymethyl)-β-D-*erythro*-pentofuranosyl)thymine (13)

Nucleoside **12** (0.83 g, 3.2 mmol) was dissolved in anhydrous pyridine (20 mL) and cooled to 0 °C with stirring. Methanesulfonyl chloride (0.85 mL, 11 mmol) was added dropwise and the temperature was allowed to reach 15 °C over 3 h. The reaction was quenched with sat. aq $NaHCO_3$ (50 mL) and transferred to a separatory funnel with brine (50 mL) and EtOAc (100 mL). The phases were separated and the aqueous phase extracted with EtOAc (2 x 50 mL). The combined organic phases were extracted with brine (100 mL), dried (Na_2SO_4), filtered and evaporated *in vacuo* to give a viscous yellow liquid. The liquid was dissolved in a mixture of dichloromethane and toluene and evaporated *in vacuo* to give nucleoside **13** (1.48 g, 93%) as a white foam. Analytical data were identical to those previously published.[12]

2,2′-Anhydro-1-(3-*O*-benzyl-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-β-D-*threo*-pentofuranosyl)thymine (14)

Nucleoside **13** (10 g, 16.3 mmol) was dissolved in anhydrous acetonitrile (100 mL) and DBU (2.69 mL, 18.0 mmol) was added. The product slowly precipitated from the reaction mixture. After 2 h the reaction was completed and concentrated *in vacuo* to facilitate precipitation. The reaction mixture was cooled to −20 °C and the product collected by filtration to afford nucleoside **14** (7.64 g, 91%) as a white solid material. FAB-MS *m*/*z* found 517.0 ($[MH]^+$, calcd 517.1); ^{1}H NMR (DMSO-d_6) δ 7.79 (d, J = 1.3, 1H, H6), 7.45–7.32 (m, 5H, Ph), 6.40 (d, J = 6.0, 1H, H1′), 5.60 (dd, J = 6.1, 2.8, 1H, H2′), 4.82 (d, J = 11.5, 1H, CH_2Ph), 4.70 (d, J = 11.5, 1H, CH_2Ph), 4.51 (d, J = 2.8, 1H, H3′), 4.43 (d, J = 10.6, 1H), 4.36 (d, J = 6.2, 1H), 4.33 (d, J = 5.9, 1H), 4.25 (d, J = 11.0, 1H) (H5′, H1″), 3.22 (s, 3H, Ms), 3.16 (s, 3H, Ms), 1.80 (s, J = 1.1, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 171.5 (C4), 159.1 (C2), 136.9, 132.1, 128.5, 128.1, 127.9 (C6, Ph), 117.1 (C5), 89.1 (C1′), 86.1 (C2′), 85.4 (C4′), 83.7 (C3′), 72.4 (CH_2Ph), 68.6, 68.0 (C5′, C1″), 36.9, 36.8 (Ms), 13.6 (CH_3); Anal. calcd for $C_{20}H_{24}N_2O_{10}S_2$: C, 46.5; H, 4.7; N, 5.4. Found: C, 46.6; H, 4.8; N, 5.3%.

1-(3-*O*-Benzyl-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxy-methyl-β-D-*threo*-pentofuranosyl)thymine (15)

Nucleoside **14** (3.70 g, 7.16 mmol) was suspended in a mixture of acetone (160 mL) and aq H_2SO_4 (0.1 M, 160 mL). The mixture was refluxed overnight with stirring. After cooling to rt a white solid precipitated. The volume was reduced to approx. half *in vacuo* and a white solid was isolated by filtration. The solid was washed thoroughly with water and dried *in vacuo* to give nucleoside **15** (3.77 g, 98%) as a white solid. FAB-MS *m*/*z* found 535.0 ($[MH]^+$, calcd 535.1); ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H, NH), 7.41–7.32 (m, 6H, H6, Ph), 6.20 (d, J = 5.0, 1H, H1′), 6.10 (d, J = 4.8, 1H, 2′-OH), 4.77 (d, J = 11.9, 1H, CH_2Ph), 4.67 (d, J = 11.9, 1H, CH_2Ph), 4.56 (d, J = 10.6, 1H), 4.50–4.41 (m, 3H), 4.32 (d, J = 10.6, 1H), 4.16 (d, J = 3.7, 1H, H3′), 3.25 (s, 3H, Ms), 3.20 (s, 3H, Ms), 1.79 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.9 (C4), 150.6 (C2), 137.8, 137.6, 128.4, 127.9, 127.7 (C6, Ph), 108.2 (C5), 84.8 (C1′), 84.3 (C3′), 81.7 (C4′), 73.3 (C2′), 72.3 (CH_2Ph), 68.1, 67.6 (C5′, C1″), 37.0, 36.8 (Ms), 12.2 (CH_3); Anal. calcd for $C_{20}H_{26}N_2O_{11}S_2$: C, 44.9; H, 4.9; N, 5.2. Found: C, 44.5; H, 4.8; N, 5.1%.

1-(3-*O*-Benzyl-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxy-methyl-2-*O*-trifluoromethanesulfonyl-β-D-*threo*-pentofuranosyl)-thymine (16)

Nucleoside **15** (300 mg, 0.56 mmol) was dissolved in anhydrous pyridine (2 × 5 mL) and concentrated *in vacuo* to remove water traces. The compound was dissolved in a mixture of anhydrous dichloromethane (20 mL) and anhydrous pyridine (0.45 mL, 5.60 mmol) followed by the addition of DMAP (274 mg, 2.24 mmol). After cooling to 0 °C trifluoromethanesulfonic anhydride (0.19 mL, 1.12 mmol) was added dropwise during 30 min. The reaction mixture was stirred for an additional 1.5 h and poured into ice cooled sat. aq $NaHCO_3$ (20 mL). The organic phase was separated and washed successively with aq HCl (1 M, 2 × 20 mL) and sat. aq $NaHCO_3$ (2 × 20 mL), dried (Na_2SO_4), filtered and evaporated *in vacuo*. The residue was purified by DCVC (0–100% EtOAc in *n*-heptane v/v) yielding nucleoside **16** (302 mg, 80%) as a white foam. FAB-MS *m*/*z* found 667.0 ($[MH]^+$, calcd 667.0); ^{1}H NMR (DMSO-d_6) δ 11.62 (br s, 1H, NH), 7.51 (s, 1H, H6), 7.40–7.33 (m, 5H, Ph), 6.45 (br s, 1H, H1′), 5.91 (t, J = 6.0, 1H, H2′), 4.97 (d, J = 5.7, 1H, H3′), 4.82–4.36 (m, 6H, CH_2Ph, H5′a, H5′b, H1″a, H1″b), 3.30 (s, 3H, Ms), 3.24 (s, 3H, Ms), 1.81 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.3 (C4), 150.0 (C2), 136.5, 128.3, 128.0, 127.8 (C6, Ph), 117.6 (q, J = 320, CF_3), 110.1 (C5), 88.0 (C1′), 81.7, 81.0 (C3′, C4′), 73.1 (CH_2Ph), 68.0, 67.6 (C5′, C1″), 36.7, 36.6 (Ms), 11.8 (CH_3); Anal. calcd for $C_{21}H_{25}F_3N_2O_{13}S_3$: C, 37.8; H, 3.8; N, 4.2. Found: C, 38.1; H, 3.8; N, 4.1%.

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

Method A. To a solution of nucleoside **16** (215 mg, 0.32 mmol) in anhydrous DMF (10 mL), NaN_3 (23 mg, 0.35 mmol) and 15-crown-5 (64 μL, 0.32 mmol) were added. The mixture was stirred at 80 °C for 1 h and then cooled to rt whereupon water (20 mL) was added. The solution was extracted with EtOAc (50 mL) and the organic phase was washed with sat. aq $NaHCO_3$ (2 × 20 mL), dried (Na_2SO_4), filtered and evaporated to dryness *in vacuo*. The residue was purified by DCVC (50–100% EtOAc in *n*-heptane v/v) yielding nucleoside **17** (164 mg, 91% from **16**) as a white foam. Analytical data were identical to those reported below.

Method B. A solution of nucleoside **15** (5.35 g, 10 mmol) in anhydrous dichloromethane (300 mL) was cooled to 0 °C. Anhydrous pyridine (8.08 mL, 100 mmol) and DMAP (4.89 g, 40 mmol) were added followed by the dropwise addition of trifluoromethanesulfonic anhydride (3.3 mL, 20 mmol). After 2 h at 0 °C the reaction was quenched by the addition of ice cold sat. aq $NaHCO_3$ (200 mL) and the reaction mixture was transferred to a separatory funnel. The phases were separated and the aq phase was extracted with dichloromethane (200 mL). The combined organic phases were washed with aq HCl (1.0 M, 2 × 300 mL) and sat. aq $NaHCO_3$ (300 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a white solid. The solid was dissolved in anhydrous DMF (300 mL) and NaN_3 (1.86 g, 30 mmol) was added. After stirring at rt for 4 h brine (300 mL) was added and the mixture was transferred to a separatory funnel. The aqueous phase was extracted with dichloromethane (3 × 200 mL) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo* yielding a yellow residue that was purified by DCVC (id 5 cm, 25–100% EtOAc in *n*-heptane v/v, 5% increments, 100 mL fractions) affording nucleoside **17** (5.1 g, 91% from **15**) as a white solid. ESI-MS *m*/*z* found 560.0 ($[MH]^+$, calcd 560.1); ^{1}H NMR ($CDCl_3$) δ 9.30 (br s, 1H, NH), 7.40–7.34 (m, 5H, Ph), 7.15 (d, J = 1.3, 1H, H6), 5.69 (d, J = 4.8, 1H, H1′), 4.80 (d, J = 11.2, 1H), 4.66–4.63 (m, 2H), 4.56 (d, J = 11.7, 1H), 4.47 (dd, J = 6.6, 4.8, 1H, H2′), 4.40 (d, J = 10.8, 1H), 4.32 (d, J = 7.7, 1H, H3′), 4.29 (d, J = 8.6, 1H) (CH_2Ph, H5′, H1″), 3.03 (s, 3H, Ms), 2.99 (s, 3H, Ms), 1.92 (d, J = 1.1, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.4 (C4), 150.0 (C2), 136.9, 136.0, 128.7, 128.5, 128.2 (C6, Ph), 112.5 (C5), 90.8 (C1′), 83.7 (C4′), 78.8 (C3′), 74.9 (CH_2Ph), 68.1, 67.5 (C5′, C1″), 60.3 (C2′), 37.5 (Ms), 12.1 (CH_3); Anal. calcd for $C_{20}H_{25}N_5O_{10}S_2$·0.33 EtOAc: C, 43.5; H, 4.7; N, 11.9. Found: C, 43.7; H, 4.5; N, 12.2%.

(1*R*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-methanesulfonyloxymethyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (18)

To a solution of **17** (5.83 g, 10.4 mmol) in THF (300 mL) at rt, aq NaOH (2.0 M, 104 mL, 208 mmol) and PMe_3 in THF (1.0 M, 20.8 mL, 20.8 mmol) were added with stirring. After 8 h the THF was partly removed under reduced pressure. Brine (200 mL) and EtOAc (300 mL) was added and the phases were separated. The aqueous phase was extracted with EtOAc (2 × 300 mL) and dichloromethane (2 × 300 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give nucleoside **18** (4.22 g, 93%) as a white solid. R_f = 0.15 (10% MeOH in EtOAc, v/v); ESI-MS *m*/*z* found 438.0 ($[MH]^+$, calcd 438.1); ^{1}H NMR (DMSO-d_6) δ 11.33 (br s, 1H, NH), 7.46 (s, 1H, H6), 7.36–7.27 (m, 5H, Ph), 5.44 (s, 1H, H1′), 4.67 (d, J = 11.7, 1H), 4.59 (d, J = 11.5, 1H), 4.56 (d, J = 11.9, 1H), 4.52 (d, J = 11.7, 1H) (H5′, CH_2Ph), 3.84 (s, 1H, H3′), 3.65 (s, 1H, H2′), 3.26 (s, 3H, Ms), 3.06 (d, J = 10.1, 1H, H1″a), 2.78 (d, J = 9.9, 1H, H1″b), 1.77 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.9 (C4), 150.1 (C2), 137.9, 134.7, 128.2, 127.7, 127.6 (C6, Ph), 108.3 (C5), 88.4 (C1′), 85.6 (C4′), 76.3 (C3′), 70.9, 66.6 (CH_2Ph, C5′), 59.4 (C2′), 50.1 (C1″), 36.9 (Ms), 12.3 (CH_3); Anal. calcd for $C_{19}H_{23}N_3O_7S$: C, 52.1; H, 5.3; N, 9.6. Found: C, 52.0; H, 5.2; N, 9.2%.

(1*R*,3*R*,4*R*,7*S*)-1-Benzoyloxymethyl-7-benzyloxy-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (19)

Compound **18** (950 mg, 2.17 mmol) was dissolved in anhydrous DMF (20 mL) and sodium benzoate (626 mg, 4.34 mmol) was added. The reaction mixture was heated to 80 °C for 5.5 h and then cooled to rt. The reaction was diluted with dichloromethane (50 mL) and washed with brine (2 × 50 mL). The combined aqueous phases were extracted with dichloromethane (50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by DCVC (id 2.2 cm, 0–10% MeOH in dichloromethane v/v, 0.5% increments, 10 mL fractions) afforded nucleoside **19** (980 mg, 97%) as a white solid. R_f = 0.34 (10% MeOH in dichloromethane, v/v); FAB-MS *m*/*z* found 464.3 ($[MH]^+$, calcd 464.5); ^{1}H-NMR (DMSO-d_6) δ 11.39 (br s, 1H, NH), 7.91 (dd, J = 8.2, 1.3, 2H, Bz), 7.66 (m, 1H, Bz), 7.51 (m, 2H, Bz), 7.33–7.22 (m, 6H, Ph, H6), 5.46 (s, 1H, H1′), 4.78 (d, J = 12.6, 1H, H5′a), 4.65 (d, J = 11.9, 1H, CH_2Ph), 4.59 (d, J = 12.6, 1H, H5′b), 4.52 (d, J = 11.7, 1H, CH_2Ph), 3.92 (s, 1H, H3′), 3.74 (s, 1H, H2′), 3.16 (d, J = 10.1, 1H, H1″a), 2.87 (d, J = 10.1, 1H, H1″b), 1.44 (d, J = 0.9, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 165.1 (PhC(O)), 163.7 (C4), 149.9 (C2), 137.7, 134.0, 133.5, 129.1, 128.8, 128.0, 127.6, 127.5 (C6, Ph), 108.1 (C5), 88.4 (C1′), 86.1 (C4′), 76.1 (C3′), 70.7 (CH_2Ph), 60.6 (C5′), 59.4 (C2′), 50.3 (C1″), 11.9 (CH_3); Anal. calcd for $C_{25}H_{25}N_3O_6$·0.33H_2O: C, 63.9; H, 5.5; N, 9.0. Found: C, 63.8; H, 5.3; N, 9.0%.

(1*R*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-hydroxymethyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (20)

Nucleoside **19** (1.12 g, 2.41 mmol) was dissolved in a saturated solution of NH_3 in MeOH (200 mL). The mixture was stirred for 30 h at rt in a sealed flask and evaporated to dryness under reduced pressure. Purification by DCVC (id 3.5 cm, 0–10% MeOH in dichloromethane v/v, 0.5% increments, 30 mL fractions) afforded nucleoside **20** (862 mg, 99%) as a white solid. R_f = 0.20 (10% MeOH in dichloromethane, v/v); FAB-MS *m*/*z* found 360.1 ($[MH]^+$, calcd 360.4); ^{1}H-NMR (DMSO-d_6) δ 11.30 (br s, 1H, NH), 7.70 (d, J = 1.1, 1H, H6), 7.33–7.24 (m, 5H, Ph), 5.39 (s, 1H, H1′), 4.56 (d, J = 11.7, 1H, CH_2Ph), 4.50 (d, J = 11.7, 1H, CH_2Ph), 3.83 (s, 1H, H3′), 3.76 (d, J = 12.8, 1H, H5′a), 3.71 (d, J = 12.8, 1H, H5′b), 3.57 (s, 1H, H2′), 2.96 (d, J = 9.7, 1H, H1″a), 2.64 (d, J = 9.9, 1H, H1″b), 1.76 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.9 (C4), 150.0 (C2), 138.1, 135.3, 128.1, 127.5, 127.4 (C6, Ph), 107.7 (C5), 88.9 (C4′), 88.0 (C1′), 75.9 (C3′), 70.8 (CH_2Ph), 59.3, (C2′), 57.1 (C5′), 50.0 (C1″), 12.5 (CH_3); Anal. calcd for $C_{18}H_{21}N_3O_5$·0.5H_2O: C, 58.7; H, 6.0; N, 11.4. Found: C, 58.1; H, 6.0; N, 11.9%.

(1*R*,3*R*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (21)

Nucleoside **20** (675 mg, 1.87 mmol) was dissolved in absolute ethanol (30 mL). $Pd(OH)_2$ and charcoal (20% moist, 200 mg) was added and the reaction flask was evacuated and filled with hydrogen gas several times. The reaction was stirred vigorously under an atmosphere of hydrogen gas overnight. The catalyst was removed by filtration through a plug of Celite. The Celite was washed thoroughly with hot methanol (250 mL). The solvents were removed *in vacuo* yielding nucleoside **21** (500 mg, 99%) as a white solid. R_f = 0.08 (20% MeOH in dichloromethane, v/v). All analytical data were identical to those previously reported.[8]

1-(3-*O*-Benzyl-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-β-D-*erythro*-pentofuranosyl)thymine (23)

Nucleoside **22**[11] (30 g, 52 mmol) was dissolved in MeOH (600 mL), and the solution was cooled to 0 °C. Freshly prepared sat. methanolic ammonia (600 mL) was added, and the temperature was allowed to reach rt. After 5 h at rt the reaction was quenched with glacial acetic acid (50 mL) and transferred to a beaker, where it was neutralised with sat. aq $NaHCO_3$. EtOAc (900 mL) and brine (500 mL) was added and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 500 mL) and the combined organic phases were washed with sat. aq $NaHCO_3$ (500 mL) and brine (500 mL). The organic phase was dried (Na_2SO_4), filtered and the solvent removed *in vacuo* to afford **23** (27 g, 97%) as a white foam. R_f = 0.33 (100% EtOAc); ESI-MS *m*/*z* found 557.0 ($[MNa]^+$, calcd 557.1); ^{1}H NMR ($CDCl_3$) δ 10.21 (br s, 1H, NH), 7.33–7.25 (m, 6H, Ph, H6), 5.77 (d, J = 3.9, 1H, H1′), 4.84 (d, J = 11.4, 1H, H3′), 4.59–4.57 (m, 3H), 4.42–4.37 (m, 3H), 4.26–4.19 (m, 2H) (H2′, H2″, H5″, CH_2Ph, OH), 2.98 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 1.80 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 162.5 (C4), 151.0 (C2), 136.7 (Ph), 136.2 (C6), 128.5, 128.3, 128.2 (Ph), 111.3 (C5), 92.1 (C1′), 84.0 (C4′), 77.7 (C3′), 74.1, 73.5 (C2′, *C*H$_2$Ph), 68.6, 68.3 (C5′, C1″), 37.2, 37.1 (Ms), 12.0 (CH_3); Anal. calcd for $C_{20}H_{26}N_2O_{11}S_2$: C, 44.9; H, 4.9; N, 5.2. Found: C, 45.0; H, 4.7; N, 5.1%.

1-(3-*O*-Benzyl-2,5-di-*O*-methanesulfonyl-4-*C*-(methanesulfonyloxymethyl)-β-D-*erythro*-pentofuranosyl)thymine (13)

Nucleoside **23** (20 g, 37 mmol) was dissolved in anhydrous dichloromethane (100 mL) and anhydrous pyridine (100 mL) was added. The solution was cooled to 0 °C and methanesulfonyl chloride (4.4 mL, 56 mmol) was added dropwise. After 2 h the reaction was quenched with sat. aq $NaHCO_3$ (200 mL), and the phases were separated. The aq phase was extracted with dichloromethane (2 × 150 mL), and the combined organic phases were washed with aq HCl (1 M, 2 × 200 mL), sat. aq $NaHCO_3$ (2 × 250 mL) and brine (250 mL). The organic phase was dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The crude product was co-evaporated with toluene affording **13** (22 g, 96%) as a white foam. R_f = 0.41 (100% EtOAc); ESI-MS *m*/*z* found 635.0 ($[MNa]^+$, calcd 635.1). All analytical data were identical to those previously reported.[12]

(1*R*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-methanesulfonyloxymethyl-3-(thymin-1-yl)-2-oxa-5-thiabicyclo[2.2.1]heptane (25)

Nucleoside **16** (0.10 g, 0.17 mmol) was dissolved in anhydrous DMF (1 mL) and potassium thioacetate (25 mg, 0.22 mmol) was added. The reaction was stirred at ambient temperature for 5 h and transferred to a separatory funnel with brine (10 mL). The aq phase was extracted with dichloromethane (3 × 10 mL) and the combined organic phases were dried (Na_2SO_4), filtered and evaporated *in vacuo* to give a yellow liquid. The crude product **24** was dissolved in THF (2 mL) and LiOH·H_2O (35 mg in 1 mL water, 0.84 mmol) was added. After 20 min the reaction was complete and quenched by the addition of glacial acetic acid (0.5 mL). The THF was removed *in vacuo* and the residue dissolved in dichloromethane (10 mL) and extracted with sat. aq $NaHCO_3$ (2 × 10 mL). The aqueous phases were extracted with dichloromethane (10 mL). The combined organic phases were dried (Na_2SO_4), filtered and evaporated *in vacuo* to give a yellow liquid that was purified by DCVC (id 1 cm, 0–80% EtOAc in *n*-heptane v/v, 2.5% increments, 10 mL fractions). Fractions containing nucleoside **25** were combined and evaporated *in vacuo* to afford a white powder (36 mg, 47% from **16**). R_f = 0.38 (80% EtOAc in *n*-heptane, v/v); ESI-MS *m*/*z* found 455.0 ($[MH]^+$, calcd 455.1); ^{1}H NMR (DMSO-d_6) δ 11.38 (br s, 1H, NH), 7.50 (d, J = 1.1, 1H, H6), 7.36–7.27 (m, 5H, Ph), 5.77 (s, 1H, H1′), 4.68 (d, J = 11.7, 1H), 4.61 (d, J = 11.7, 1H), 4.60 (d, J = 11.7, 1H), 4.56 (d, J = 11.5, 1H) (H5′, CH_2Ph), 4.20 (d, J = 1.8, 1H, H3′), 4.00 (d, J = 2.0, 1H, H2′), 3.29 (s, 3H, Ms), 3.02 (d, J = 10.6, 1H, H1″a), 2.90 (d, J = 10.4, 1H, H1″b), 1.78 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.9 (C4), 150.1 (C2), 137.5, 134.1, 128.3, 127.7 (C6, Ph), 108.3 (C5), 90.5 (C1′), 86.6 (C4′), 76.9 (C3′), 70.9, 66.8 (C5′, *C*H$_2$Ph), 49.5 (C2′), 36.8 (Ms), 35.1 (C1″), 12.3 (CH_3); Anal. calcd for $C_{19}H_{22}N_2O_7S_2$·0.33 EtOAc: C, 50.5; H, 5.1; N, 5.8. Found: C, 50.8; H, 5.1; N, 5.8%.

(1*R*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-methanesulfonyloxymethyl-5-methyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (26)

To a solution of **18** (4.22 g, 9.64 mmol) in formic acid (20 mL) formaldehyde (37% aq solution, 20 mL) was added with stirring and the reaction mixture was heated to 80 °C. After 1 h the reaction was diluted with EtOAc (150 mL) and quenched by carefully pouring it into sat. aq $NaHCO_3$ (100 mL). The phases were separated and the organic phase was washed with sat. aq $NaHCO_3$ (4 × 100 mL). The combined aqueous phases were extracted with dichloromethane (2 × 200 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by DCVC (id 6 cm, 0–15% MeOH in EtOAc v/v, 1% increments, 100 mL fractions) afforded nucleoside **26** (3.89 g, 90%) as an off-white solid. R_f = 0.30 (10% MeOH in EtOAc, v/v); ESI-MS *m*/*z* found 452.1 ($[MH]^+$, calcd 452.1); ^{1}H NMR (DMSO-d_6) δ 11.34 (br s, 1H, NH), 7.43 (s, 1H, H6), 7.34–7.28 (m, 5H, Ph), 5.58 (s, 1H, H1′), 4.67 (m, 4H, H5′, CH_2Ph), 3.88 (s, 1H, H3′), 3.58 (s, 1H, H2′), 3.27 (s, 3H, Ms), 2.98 (d, J = 9.7, 1H, H1″a), 2.76 (d, J = 9.7, 1H, H1″b), 2.57 (s, 3H, NCH_3), 1.76 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.9 (C4), 149.9 (C2), 137.6 (Ph), 134.6 (C6), 128.3, 127.7 (Ph), 108.4 (C5), 86.1 (C1′), 85.3 (C4′), 77.3 (C3′), 71.0, 66.3 (*C*H$_2$Ph, C5′), 64.9 (C2′), 58.7 (C1″), 40.8 (NCH_3), 36.9 (Ms), 12.3 (CH_3); Anal. calcd for $C_{20}H_{25}N_3O_7S$·0.25 H_2O: C, 52.7; H, 5.6; N, 9.1. Found: C, 52.9; H, 5.6; N, 8.9%.

(1*R*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-hydroxymethyl-5-methyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (27)

Compound **26** (3.00 g, 6.64 mmol) was dissolved in anhydrous DMF (30 mL) and sodium benzoate (1.93 g, 13.3 mmol) was added. The reaction mixture was heated to 100 °C for 7 h and then cooled to rt. Sodium methoxide (1.44 g, 26.6 mmol) was added and after 1 h the reaction was diluted with dichloromethane (100 mL) and washed with brine (2 × 100 mL). The combined aq phases were extracted with dichloromethane (3 × 100 mL) and diethyl ether (3 × 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by DCVC (id 4 cm, 0–10% MeOH in dichloromethane v/v, 0.5% increments, 50 mL fractions) afforded nucleoside **27** (2.42 g, 98%) as a white solid. R_f = 0.19 (7% MeOH in dichloromethane, v/v); ESI-MS *m*/*z* found 374.1 ($[MH]^+$, calcd 374.2), 408.1, 410.1 ($[MCl]^-$, calcd 408.1, 410.1); ^{1}H-NMR (DMSO-d_6) δ 11.31 (br s, 1H, NH), 7.67 (d, J = 1.1, 1H, H6), 7.33–7.24 (m, 5H, Ph), 5.52 (s, 1H, H1′), 5.24 (br s, 1H, 5′-OH), 4.56 (q, J = 11.3, 2H, CH_2Ph), 3.87 (s, 1H, H3′), 3.72 (br s, 2H, H5′), 3.47 (s, 1H, H2′), 2.85 (d, J = 9.5, 1H, H1″a), 2.61 (d, J = 9.5, 1H, H1″b), 2.55 (s, 3H, NCH_3), 1.75 (d, J = 1.1, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.8 (C4), 149.8 (C2), 137.8 (Ph), 135.1 (C6), 128.0, 127.3 (Ph), 107.8 (C5), 89.4 (C1′), 84.9 (C4′), 76.8 (C3′), 70.8 (*C*H$_2$Ph), 64.7 (C5′), 58.8, 56.9 (C2′, C1″), 40.9 (NCH_3), 12.4 (CH_3); Anal. calcd for $C_{19}H_{23}N_3O_5$ ·0.25H_2O: C, 60.4; H, 6.3; N, 11.1. Found: C, 60.3; H, 6.5; N, 10.8%.

(1*R*,3*R*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-5-methyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (28)

Compound **27** (2.60 g, 6.64 mmol) was dissolved in glacial acetic acid (50 mL) and the reaction flask was evacuated and

filled with argon several times. $Pd(OH)_2$ on charcoal (20% moist, 200 mg) was added and the reaction flask was evacuated and filled with hydrogen gas several times. The reaction was stirred vigorously under an atmosphere of hydrogen gas for 8 h. The catalyst was removed by filtration through a plug of Celite. The Celite was washed thoroughly with hot methanol (200 mL). The solvents were removed *in vacuo*. The residue was dissolved in water (10 mL) and lyophilised yielding the acetate salt of nucleoside **28** (2.10 g, 97%) as off-white flakes. R_f = 0.11 (0.5% Et_3N, 10% MeOH, 89.5% EtOAc, v/v/v); ESI-MS *m/z* found 284.1 ($[MH]^+$, calcd 284.1). All analytical data were identical to those previously reported.[5]

(1*R*,3*R*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-5-methyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (29)

Compound **28** (2.00 g, 5.83 mmol) was dissolved in anhydrous pyridine (2 × 50 mL) and concentrated *in vacuo*. The nucleoside was dissolved in anhydrous pyridine (50 mL) and 4,4′-dimethoxytrityl chloride (2.96 g, 8.74 mmol) was added and the reaction was stirred at rt for 9 h. The reaction was concentrated to half volume *in vacuo* and the residue was diluted with EtOAc (100 mL). The organic phase was washed with sat. aq $NaHCO_3$ (3 × 100 mL) and brine (100 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by DCVC (id 4 cm, 0–10% MeOH in EtOAc + 0.5% Et_3N v/v, 0.5% increments, 50 mL fractions) afforded nucleoside **29** (3.13 g, 92%) as an off-white solid. R_f = 0.38 (0.5% Et_3N, 10% MeOH, 89.5% EtOAc, v/v/v); ESI-MS *m/z* found 586.2 ($[MH]^+$, calcd 586.2). All analytical data were identical to those previously reported.[5]

(1*R*,3*R*,4*R*,7*S*)-7-(2-Cyanoethoxy(diisopropylamino)-phosphinoxy)-1-(4,4′-dimethoxytrityloxymethyl)-5-methyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (30)

Compound **29** (500 mg, 0.85 mmol) was dissolved in anhydrous dichloromethane (4 mL) and 4,5-dicyanoimidazole in MeCN (1.0 M, 0.59 mL, 0.59 mmol) was added at ambient temperature with stirring. 2-Cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.27 mL, 0.85 mmol) was added dropwise to the reaction mixture. After 2 h the reaction was diluted with dichloromethane (10 mL) and transferred to a separatory funnel and extracted with sat. aq $NaHCO_3$ (2 × 15 mL) and brine (15 mL). The combined aq phases were extracted with dichloromethane (10 mL). The organic phases were pooled and dried (Na_2SO_4). After filtration the organic phase was evaporated *in vacuo* to give nucleoside **30** as a slightly yellow foam (660 mg, 98% yield). R_f = 0.56 (0.5% Et_3N, 10% MeOH, 89.5% EtOAc, v/v/v); ESI-MS *m/z* found 786.3 ($[MH]^+$, calcd 786.4). ^{31}P NMR ($CDCl_3$) δ 149.8, 149.6.[5]

(1*R*,3*R*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-5-methyl-3-(4-benzoyl-5-methylcytosin-1-yl)-2-oxa-5-aza-bicyclo[2.2.1]heptane (35)

Compound **29** (1.5 g, 2.5 mmol) was dissolved in anhydrous pyridine (25 mL). Acetic anhydride (2.4 mL, 25 mmol) was added and the reaction stirred for 24 h at ambient temperature. The reaction was quenched with water (25 mL) and extracted with EtOAc (2 × 25 ml). The combined organic phases were washed with sat. aq $NaHCO_3$ (2 × 50 ml), brine (50 ml), and dried (Na_2SO_4). The organic phase was filtered and evaporated *in vacuo* to give compound **31** as a white foam. Residual water was removed from the crude product by evaporation from anhydrous MeCN. The product was then dissolved in anhydrous MeCN (50 ml) and Et_3N (3.5 mL, 25.3 mmol) was added followed by 1,2,4-triazole (1.75 g, 25 mmol). The reaction mixture was cooled on an ice bath and $POCl_3$ (0.48 mL, 5.0 mmol) was added dropwise to give a white slurry. After 15 min the reaction mixture was allowed to reach room temperature. The resulting yellow slurry was stirred under argon at ambient temperature. After 4.5 h the reaction mixture was poured into a slurry of sat. aq $NaHCO_3$ (50 mL) and ice and extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with brine (100 ml) and dried (Na_2SO_4). Filtration and evaporation *in vacuo* afforded the triazole derivative **32** as a pink foam which was immidiately dissolved in anhydrous MeCN (50 ml) and sat. aq NH_4OH (50 mL) was added. After stirring for 16 h solid NaCl was added until the phases separated. The aqueous phase was extrated with EtOAc (3 × 50 mL) and the combined organic phases dried (Na_2SO_4), filtered and evaporated to give nucleoside **33** as an off-white solid. The product was dissolved in anhydrous pyridine (50 mL) and benzoyl chloride (0.87 mL, 7.5 mmol) was added. The reaction was stirred for 3 h under argon and then concentrated *in vacuo*. The residue was diluted with EtOAc (100 mL) and extracted with sat. aq $NaHCO_3$ (100 mL). The phases were separated and the aqueous phase extracted with EtOAc (2 × 100 ml). The combined organic phases were washed with brine (200 ml) and dried (Na_2SO_4). Filtration and evaporation of the organic phase produced a clear oil that was dissolved in THF (100 mL). LiOH (aq, 1.0 M, 25 mL) was added and the reaction was stirred for 2 h. The reaction mixture was transferred to a separatory funnel with EtOAc (100 mL) and brine (100 mL) and extracted with EtOAc (2 × 100 ml). The combined organic phases were washed with brine (200 ml) and dried (Na_2SO_4). Filtration and evaporation *in vacuo* gave a yellow foam that was purified by DCVC (id 4 cm, 50–100% EtOAc, *n*-heptane v/v (the column was pretreated with 1% Et_3N in heptane v/v), 5% increments, 100 mL fractions)) affording nucleoside **35** (1.12 g, 65%) as a white solid. R_f = 0.56 (EtOAc); ESI-MS *m/z* found 689.3 ($[MH]^+$, calcd. 689.3); ^1H NMR (DMSO-d_6) δ 8.16 (s, 2H, Bz), 7.86 (s, 1H, H6), 7.61–7.44 (m, 5H, Bz, DMT), 7.36–7.24 (m, 7H, Bz, DMT), 6.92 (dd, 4H, J = 9.0, 2.4, DMT), 5.64 (s, 1H, H1′), 5.41 (d, J = 5.3, 1H, H3′), 4.14 (d, J = 5.3, 1H, H2′), 5.64 (s, 1H, H1′), 3.75 (s, 6H, OCH_3), 3.39 (d, J = 10.8, 1H, H5′), 3.28 (d, J = 10.8 Hz, 1H, H5′), 2.89 (d, J = 9.5, 1H, H1″), 2.59 (s, 3H, NCH_3), 2.58 (d, J = 9.2, 1H, H1″), 1.73 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 178.2 (Ph*C*(O)), 160.3 (C4), 158.2 (Ph), 147.0 (C2), 144.8 (Ph), 137.4 (C6), 135.4, 135.2, 132.5, 129.9, 129.3, 128.4, 128.0, 127.7, 126.9, 113.3 (Ph), 108.6 (C5), 88.9 (C1′), 85.7 (C4′), 85.0 (Ph), 70.5 (C3′), 67.0 (C5′), 59.6, 58.6 (C2′, C1″), 55.1 (OCH_3), 40.1 (NCH_3), 14.1 (CH_3); Anal. calcd. for $C_{40}H_{40}N_4O_7$: C, 69.7; H, 5.9; N, 8.1. Found: C, 69.5; H, 5.9; N, 7.7%.

(1*R*,3*R*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-(2-cyano-ethoxy(diisopropylamino) phosphinoxy)-5-methyl-3-(4-benzoyl-5-methylcytosin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (36)

Compound **35** (0.50 g, 0.73 mmol) was dissolved in anhydrous dichloromethane (10 mL) and 4,5-dicyanoimidazole in MeCN (1.0 M, 0.51 mL, 0.51 mmol) was added at ambient temperature with stirring. 2-Cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.23 mL, 0.74 mmol) was added dropwise to the reaction mixture. After 2 h the reaction was diluted with dichloromethane (20 mL) and transferred to a separatory funnel and extracted with sat. aq $NaHCO_3$ (2 × 30 mL) and brine (30 mL). The combined aqueous phases were extracted with dichloromethane (30 mL). The organic phases were pooled and dried (Na_2SO_4). After filtration the organic phase was evaporated *in vacuo* to give a yellow foam. Purification by DCVC (id 4 cm, 0–100% EtOAc, *n*-heptane + 0.5% Et_3N v/v/v (the column was pretreated with 1% Et_3N in heptane v/v), 5% increments, 50 mL fractions) afforded nucleoside **36** (0.58 g, 92%) as a white solid. R_f = 0.67 (20% heptane, 79.5% EtOAc, 0.5% Et_3N, v/v/v); ESI-MS *m/z* found 889.2 ($[MH]^+$, calcd 889.4); ^{31}P NMR (DMSO-d_6) δ 148.4, 147.4

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