Facile preparation of the oxetane-nucleosides†

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Efficient and practical large scale synthesis of suitably protected 1,2-oxetane locked pyrimidine nucleosides for incorporation in oligo-DNA or -RNA by solid-phase synthesis is reported. A high regio and stereoselectivity with preferential formation of the β-anomer in the glycosylation reaction, using the Vorbrüggen procedure, was achieved by a convergent synthetic procedure with orthogonal protection strategy using either 1,2-di-O-acetyl-3,4-O-isopropyliden-6-O-(4-toluoyl)-d-psicofuranose or 2-O-acetyl-6-O-benzyl-1,3,4-tri-O-(4-toluoyl)-d-psicofuranose as the glycosyl donor.

Introduction

Antisense oligonucleotides (AONs) incorporated with sugar modified nucleosides have been widely used as a valuable alternative for down-regulation of genes.1–3 Among these, the AONs modified with North-East conformationally (−1° < P < 34°) constrained [LNA]14–17 and oxetanes18–21 (Fig. 1) into the AONs nucleotide blocks have unique abilities to dictate conformational preorganisation of the AON–RNA duplex to the rigid RNA–RNA type duplex which results in the modulation of the target affinity as well as a loss of the RNase H cleavage efficiency.21–23 However, RNase H eliciting capability can partly or fully be regained by adopting various mixmer and gapmer strategies utilizing these modifications17,18 (a gap size of 8–10 nucleotides is necessary for the β-D-LNA modified23 AONs, whereas a gap of 4 nt is necessary for the oxetane-modified counterpart).17,18 The length of the gap in the mixmer can however potentially make the AON vulnerable to endonucleases.22

The mixmer AON–RNA hybrids incorporated with oxetane-T units (Tm drops by ~5–6°C/T unit, Fig. 1) were found to be excellent substrates for RNase H promoted cleavage, which is very comparable to that of the native hybrid.19,20 However, the incorporation of the oxetane-C moiety (Fig. 1) into the AONs imparts only ~3°C loss in Tm per modification14,24 whereas no loss in Tm is observed for the oxetane-A or -G modified AONs. The loss of thermodynamic stability in the case of oxetane-T and -C was fully or partly regained by the introduction of the non-toxic25 DPPZ (dipyridophenazine) group24,25 at the 3’ end, which gave also additional stability against exonucleases similar to that of the phosphorothioate AONs.26 Another interesting property of these oxetane-incorporated AONs was that only 4 deoxynucleotide gaps in the AON strand were needed to achieve the RNase H cleavage of the RNA in their hybrid duplexes,14,17 thereby reducing endonuclease susceptibility. Michaelis–Menten kinetics of the RNase H cleavage showed that Vmax and Km increase with increasing the number (one to three) of T/C/A/G modifications in the AONs, indicating higher catalytic activity and lower enzyme binding affinity of the oxetane-modified AON–RNA hybrids.14,18 In addition to the favorable RNase H cleavage properties of these oxetane-modified molecules, their endonuclease susceptibility was significantly reduced compared to the native counterpart, and it was proportional to the number of oxetane-modified nucleotides per AON molecule: single modification gave 2-fold protection to the cleavage and double and triple modification gave 4-fold protection compared to that of the native phosphodiester oligonucleotide.14,15

The AON constructs with the oxetane-C and 3-DPPZ were found to be non-toxic in K562 human leukemia cells and have been successfully employed to down-regulate the proto-oncogene c-myb in very efficient manner.21 QRT-PCR (Quantitative RealTime–Polymerase Chain Reaction) and Western blotting (the “gold standard” in antisense efficacy)14,27 have shown that rationally designed oxetane-C modified AONs were highly efficient both in diminishing the c-myb mRNA (85% has been reduced) and the c-myb protein (70% of its expression was found to be halted) of the targeted gene. Based on the amount of AON uptake after delivery, determined by slot blot, it was apparent that the oxetane modified AONs are 5–6 times more efficient antisense agents than those of the corresponding isosequential phosphorothioate analogue.14,28

Because of the effective down-regulation of genes by oxetane-modified AONs and their possible applications in the RNAi approach,29 we have been naturally interested in synthesizing the oxetane-modified nucleoside blocks on a large scale in a reproducible manner with a high overall yield. Here we report two convenient procedures for the preparation of building blocks covering both pyrimidine and purine derivatives of oxetane functionalized nucleosides.

† Electronic supplementary information (ESI) available: experimental procedures for compounds 23 and 24. 1H NMR spectra of compounds 13, 14a–d, 15–27, 31a–d, 32a,b, 33, 35–42. See DOI: 10.1039/b511406c

Fig. 1 Oxetane constrained nucleosides and their structural and NMR parameters.
Results and discussion

For the synthesis of oxetane-T (1) and oxetane-C (2) nucleosides, we employed the protected sugar, 6-O-(4-toluoyl)-1,2,3,4-di-O-isopropylidene-β-D-PSF (5a) which was coupled with persilylated thymine or N′-benzoylcytosine in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a Lewis acid catalyst to afford an inseparable, as well as unfavorable, anomer mixture of nucleosides 6 and 7 (α : β, 1 : 1 in 67% and 3 : 2 in 75% yield, respectively) (Fig. 2). The use of sugar 5a for direct coupling with persilylated uracil gave 8 as an even more undesirable anomer mixture with poor yield (α : β, 55 : 45 in 52% yield) of the corresponding purified PSF nucleoside, which uses the rate of sugar block 5a not only cumbersome but clearly inadequate for the large scale synthesis of the oxetane-U block in order to explore its use for the preparation of oxetane-modified small interfering RNA (siRNA).

In addition to the above problem, the coupling of the sugar block 5a with persilylated purine nucleobases was also unsuccessful, hence we employed an alternative strategy for the synthesis of 9-(1′,3′,5′-O-anhydro-β-D-PSF)-adenine/guanine [oxetane-A (3) and oxetane-G (4)] nucleosides involving the key bromosugar. The synthesis of this bromosugar itself involved a 5-step preparation from 5a giving a poor overall yield (45% after four steps).

Two common drawbacks of both of the above approaches are (i) poor anomeric α : β ratio of the coupling reaction of the functionalized sugar with the nucleobase resulting in a loss of material in the form of a redundant α-anomeric nucleoside, and (ii) unsatisfactory coupling yield.

It was therefore clear that we require a convergent synthetic procedure with an orthogonal protection strategy to make all four oxetane-nucleoside blocks available for easy preparation of oxetane-modified AONs as well as small siRNAs for further biological studies.

Due to the rather low reactivity of ketoses in comparison to aldoses the reaction yields of many of the known methods of condensation used generally for ribose or 2-deoxyribose derivatives are not always satisfactory for the coupling with the corresponding ketoses. This is especially true for the synthesis of psico-guanosine derivatives by a coupling reaction with a ketole-like sugar, which remains a challenge.

Here we report the application of two sets of acetylated sugars 13 and 30, based on an orthogonal protection strategy at 1′, 3′, 4′ and 6′ positions, for the large scale straightforward synthesis of the oxetane-locked U, A and G nucleosides, using Vorbrüggen glycosylation methods. The choice of suitable orthogonal protection strategy at positions 1′, 3′, 4′ and 6′ clearly played an important role (Schemes 1 and 2) to enable smooth completion of the synthesis of 6-O-DMT or oxetane-locked building blocks 21, 37 and 42.

(A) Preparation of the sugar block 13 and its coupling with uracil

6-O-(p-Toluoyl)-1,2,3,4-diisopropylidene-PSF (5a) was treated in a mixture of acetic acid (100 eq.), acetic anhydride (10 eq.) and triflic acid (0.02 eq.) for 2 h at room temperature providing, after work-up, one crude product (>90% pure by 1H-NMR) which was identified as 1,2-di-O-acytetyl-3,4-O-isopropylidene-6-O-(p-toluoyl)-α-D-PSF (13) (Scheme 1). The α-anomeric nature of 13 was evident from 1D diff NOE studies (see Experimental Section). This crude product was subsequently coupled with silylated uracil or N′-benzoylcytosine using a Vorbrüggen procedure to give an α : β mixture (1 : 9 by 1H-NMR) of psicouridine (76%) and psico-N′-benzoylcytidine (74%), respectively, after work-up and chromatography. The α-anomer of either of the above pyrimidine nucleosides is not chromatographically separable from the predominant β-anomer, which could be however separated by simple crystallization from methanol to give pure β-anomer 14a (54%) or 14b (33%) in two steps from 5a. Since the preparation of the oxetane derivative of cytidine blocks from 5a to 7 works very well with a favorable yield for the β-anomer (Fig. 2), we report here only the synthesis of the oxetane derivative of the uridine block, which required improvement because our earlier unpublished procedure gave an undesirable α : β mixture of 55 : 45, as well as a poor yield in the glycosylation step (52%).

Since we required a leaving group at C1′ in order to achieve a 1,3′-ring-closure to complete the oxetane synthesis, selective deprotection of 1′-acetate ester in the presence of the 6-(p-toluoyl) ester in 14a was necessary to introduce a leaving group at C1′. Selective removal of 1′-acetate over 6-(p-toluoyl) ester by 16% methanolic ammonia led to the well separable mixture of desired alcohol 15 (48%) and fully deprotected diol 16 (44%). This is a consequence of the longer reaction time required for full conversion of the starting material 14a to the desired alcohol 15 (6 h compared to 2 h usually needed for acetate deprotection in the ribonucleoside). Leading to significant concomitant deprotection of 6-(p-toluoyl) ester giving diol 16. The observed resistance of the 1′-acetate group in 14a is probably the result of steric hindrance by the nucleobase in the proximity. Alcohol 15 was mesylated to 1′-O-mesylate 17 (95%), which was then subjected to acidic hydrolysis of the 3′,4′-isopropylidene protecting group by the action of 90% aqueous trifluoroacetic acid to give 3′,4′-diol 18, which was treated with sodium bis(trimethylsilyl)amide in THF to afford 6-O-toluoyl oxetane-uridine derivative 19 in 76% yield (in 2 steps from 17). Deprotection of the 6′-(p-toluoyl) ester from the oxetane 19 with methanolic ammonia and subsequent 6-O-alkylation of 20 by DMT-Cl in pyridine completed the synthesis of 6-O-DMT oxetane-U block 21.

As the above mentioned selective deprotection of the 1′-acetate in the diester 14a was only partly successful and a significant amount (44%) of 1′,6′-diol 16 was formed, we have explored various means to convert this diol 16 to the desired
oxetane 20. This is also an attractive goal because the diol 16 can be easily synthesized by full deprotection of the diester 14a by its treatment with methanolic sodium methoxide in a quantitative yield. Although the conversion of 1′,6-diol 16 to 6-O-mesyloxetane nucleoside 24 through 1′,6′-dimesyIates 22 and 23 seemed to be the most straightforward way to produce 20, the subsequent displacement of the 6′-O-mesyI group from the nucleoside 24 by sodium benzoate in DMF at 90 °C was not successful because of significant concomitant oxetane ring opening during the reaction.

Hence we decided to find an alternative efficient method for the conversion of this easily accessible 1′,6′-bis-mesyIate (22) to the 6′-(p-toluoyl) block 15 as a key precursor for the synthesis of oxetane 21. The internal displacement of 1′-mesylate in 22 by DBU in dry CH3CN at RT gave 2′,1′-O-anhydIoxetane nucleoside 25 smoothly (83% crystalline yield) because the formation of this 5-membered spirocyclic compound is strongly thermodynamically preferred over the 7-membered 2,6-anhydIro derivative. The 6-mesyI group from 25 was then cleanly displaced by sodium 4-toluoyIate at the C6′ center exclusively to give 26 (94%) because of the high stability of the 5-membered 2′,1′-O-anhydIro ring in 25. The ring opening of the 2′,1′-O-anhydrIo system in 6′-O-mesyI 2′,1′-anhydrIo nucleoside 25 was however accomplished by the action of sodium hydroxide in aqueous dioxane at RT providing 6′-O-mesyI alcohol 27 in 92% yield. Compound 27 was subsequently reacted with sodium 4-toluoyIate in DMF at 90 °C to displace the 6-mesyI group to give the desired 6′-O-(p-toluoyI) alcohol 15 (88%), which was further transformed to oxetane-1 building block 21, as described above.

We have also performed the glycosylation of N′-benzoyladenine and O′-diphenylcarbamoylguanine with 1,2-di-O-acetyl-3,4-O-isopropylidene-6-O-toluoyl-a-D-psicofuranose (13) under the above conditions to yield pure β-anomer of psicofuranosyl nucleosides 14c and 14d (in 39% and 40% yields in 2 steps from 5a, respectively), after work-up and chromatography. Clearly, the origin of the anomeric selectivity arises from the presence of a participating acetate group at C1′, giving spirocyclic 1,3-dioxolanium ions as intermediates, which is consistent with the earlier works on glycosylation with sugar-acetates as donors in the glycosylation reaction.47,48,49 Unfortunately, during the deprotection of the 3,4-O-isopropylidene group from either 14c or 14d under acidic conditions (Dowex H⁺ in dioxane),50 considerable depurination took place, which made this approach redundant. Hence we had to adopt an alternative strategy using base-labile protecting groups in the sugar building blocks as in 30 for the psico-purine nucleoside synthesis (see Part B).

(B) Preparation of the sugar block 30 and its coupling with appropriately-protected adenine and guanine blocks

Use of perbenzoylated psicofuranoside51,52 as a glycosyl donor has severe limitations: (i) the coupling works well with persilylated N′-benzoyladenine, 6-chloropurine and uracil bases but the α : β anomer ratio (1 : 2) is very unfavorable for preparative purposes, and more importantly, (ii) the coupling does not work with the appropriately protected guaninyl base.53 In addition, we also required an orthogonal protection strategy to manipulate functionalization at C1′ vis-à-vis C6′ with
high selectivity for the oxetane synthesis, which perbenzoylated psicofuranose does not offer. Hence, we have successfully explored and developed 6-O-benzyl 1,2,4,5-bis-isopropylidene psicofuranose 5b as a starting material for the synthesis of appropriately-protected sugar donor 30 for the glycosylation step (Scheme 2). Thus compound 30 was prepared from the bisketal 5b, which upon treatment with aqueous acetic acid at 70 °C afforded 6-O-benzyl psicofuranose 28. This was p-toluoylated under mild conditions (pyridine–dichloromethane 1 : 7, v/v, 3.1 eq. of 4-toluoyl chloride, 0 °C) to give sugar 29 (75% in two-steps from 5b) with a free anomeric OH function.4,4 The subsequent acetylation with acetic anhydride (20 eq.) in pyridine presence of glycosyl donor 30 was achieved from the 3-hydroxyl group with sodium bis(trimethylsilyl)amide, DMTr = 4,4-dimethoxytrityl, Dmf = N,N-dimethylaminomethylethyl, Ts = p-toluensulfonyl, PAC = phenoxyacetyl.

The α : β anomic mixture obtained at the glycosylation step for 31a or 31b was separated in the following manner: all protecting groups except the 6-O-benzyl were removed from the α : β anomic mixture of 31a or 31b by simple treatment with methanolic ammonia to give the corresponding crude 32a or 32b in quantitative yields. The selective 1′-tosylation of this crude α : β anomic mixture, after a simple chromatographic purification step, gave the pure β-anomer of 1′-O-tosyl-6-O-benzyl derivate 33 (44%) and 38 (45%). Subsequently, the conversion of 33 and 38 to their respective oxetane derivatives 35 (75% in two-steps from 33) and 39 (90% from 38) was achieved by activation of the 3-hydroxyl group with sodium bis(trimethylsilyl)amide using our earlier published procedure.46 The oxetane-nucleoside 34 was protected with the dimethylformamide group (DMf) to give compound 35 (90%) and subjected to reductive removal of the benzyl group to give the 6-hydroxy derivative of guanine nucleoside 36 (75%). N2-Dmf protection of the free amino function was rather stable under the reaction conditions used for the reductive removal of the 6-O-benzyl group (<5% of N2-Dmf deprotection). Compound 36 was subjected to 6-O-DMTr protection to afford a suitably protected oxetane-G block 37 (91%) with improved overall yield when used without isolation of intermediate 36 (yield 85% in two steps from 35). In the case of the adenosine derivative, first the 6-O-benzyl group was removed from 39 to give 40 (75%), followed by 4,4-O-dimethoxytritylation to give compound 41 (79%). For large scale synthesis we used crude compound 40 because of its high polarity which makes its purification difficult and inefficient and this gave an improved overall yield of compound 41 (85% in two steps from 39). Finally, the free amino function
of 1′,3′-O-anhydro adenosine building block 41 was protected by a phenoxyacetyl group (PAC), using a standard transient protection procedure to give 42 (75%).

Finally, the present 6′-O-DMTr protected oxetane-G 37 and oxetane-A 42 blocks were identical to the ones prepared earlier in our lab for conversion to the corresponding phosphoramidites for the solid-phase oligonucleotide synthesis, whereas the oxetane-U block 20 was identical to the one reported earlier from Mikhailopoulou’s lab.48

**Conclusions**

Antisense oligonucleotides containing oxetane-modified nucleosides which have a unique fixed sugar conformation have been found to be excellent substrates for RNase H promoted cleavage, and efficient both in diminishing the c-myc mRNA and the c-myc protein of the targeted gene. Because of the effective down-regulation of genes by oxetane-modified AONs, and their limited toxicity due to the fact that the natural phosphate backbone is retained in the AON, it is important to fully explore their biological applications.

We have therefore developed two synthetic routes for the multigram synthesis of 1′,2-oxetane locked nucleosides (derivatives of U, G and A). The synthesis consists of operationally simple steps and was optimized with respect to the number of required chromatographic purifications. All intermediates and final building blocks have been fully characterized. Yields and simplicity of the procedure were significantly improved by the introduction of few protection–deprotection steps without isolation of by-products which minimizes the number of steps required for completion of synthesis. Complete regioselectivity and high enhancement for the β-anomers in the critical step were achieved by the coupling of 1,2-di-O-acetyl-3,4-O-isopropylidene-6-O-(4-toluoyl)-D-psicofuranose (13) or 2-O-acetyl-6-O-benzyl-1,3,4-tri-O-(4-toluoyl)-D-psicofuranose (30) with persilylated bases [N′-acetyl-O-diphenylcarbamoyl]guanine, N′-benzoyladenine, N′-benzoyloxycytosine, and uracil, respectively using the Vorbrüggen procedure. Compounds 14a, 31b and 31b were used as starting materials for the synthesis of suitable protected 1′,3′-O-anhydro psicofuranosyluracil 21, 37 and 42, as precursors for solid phase synthesis required for further evaluation of the biological properties of this novel class of conformationally restricted nucleosides.

**Experimental**

**General**

Acetonitrile, pyridine and toluene were dried over calcium hydride, distilled and stored over 4 Å molecular sieves. Anhydrous THF (99.9%) was commercial (Aldrich). 1H NMR spectra were recorded on JEOL GX 270 or Bruker DRX 600 spectrometers at 270.1 MHz and 600 MHz, respectively. 13C NMR spectra were recorded on the same spectrometers at 67.9 MHz or 150.9 MHz. Chemical shifts are reported in ppm using TMS (0.0 ppm) or DMSO (2.54 ppm for 1H and 40.45 ppm for 13C) as internal standards. Assignments are based on 2D spectra. Mass spectra (MALDI-TOF) were measured on an Ultraflex ToF/ToF instrument (Bruker Daltonics, Germany). Melting points were measured on Büchi 510 capillary apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F-254 plates (Merck) using UV light detection and charring with 10% aqueous H2SO4 or anisaldehyde reagent [4-anisaldehyde–EtOH–AcOH–H2O, 10 : 340 : 4 : 12.5 (v/v/v/v)]. Flash chromatographic separations were performed on Kieselgel 60 G (Merck) columns.

**1,2-Di-O-acetyl-3,4-O-isopropylidene-6-O-(4-toluoyl)-α- D-psicofuranose (13).** Triflic acid (40 μl, 0.45 mmol) was added dropwise to a stirred solution of 6-O-p-toluoyl-1,2,3,4-di-O-isopropylidene-D-psicofuranose 5a (8 g, 21.2 mmol) in AcOH (120 ml, 2.1 mol) and acetic anhydride (20 ml, 212 mmol). The mixture was stirred 3 h at RT and then triethylamine (60 μl) was added and volatiles were evaporated in vacuo. The residue was co-evaporated 3 times with toluene and dissolved in EtOAc (120 ml). This solution was washed with saturated aqueous NaHCO3 (120 ml); the aqueous phase was re-extracted with EtOAc (2 × 40 ml) and the combined organic phases were dried over MgSO4 and evaporated providing crude diacetate 13a as a colorless oil (9.32 g, 104%, 90% 1H NMR purity). This crude product was directly used in the next step after co-evaporation 3 times with dry toluene. A 1D differential NOE experiment showed a 3.7% enhancement of the H-1 signal while H-3 was not irradiated. R1 = 0.54 (c-hexane-EtOAc, 6 : 4). MALDI-TOF m/z [M + H]+ 423.12 (Calcd. 423.17 for C21H27O9). 1HNMR (600 MHz, CDCl3); δ 7.95, 7.26 (2 × d, 2 × H, J = 8.0 Hz, Tol), 4.96 (d, 1H, Jα = 6.0 Hz, H-3), 4.91 (dd, 1H, Jα = 1.8 Hz, H-3), 4.68 (ABq, 2H, J = 12.0 Hz, H-1 and H-1′), 4.62 (ddd, 1H, Jα = 6.6 Hz, Jα′ = 6.0 Hz, H-5), 4.45 (ddd, 1H, Jα = 11.4 Hz, H-6), 4.38 (dd, 1H, H-6′), 2.42 (s, 3H, CH3), Tol, 2.11, 2.00 (2 × s, 2 × 3H, 2 × CH3, Ac), 1.53, 1.36 (2 × s, 2 × 3H, 2 × CH3, i-Pr). 13C NMR (67.9 MHz, CDCl3): 170.2, 169.2, 144.2, 129.9, 129.3, 126.9 (4 × Tol), 131.9 (C–Me), 110.8 (C–5), 85.4 (C–5), 85.3 (C–5), 82.0 (C–5), 64.4 (C–6), 64.2 (C–5), 26.5, 25.1 (2 × CH3, i-Pr), 22.0 (CH3, Ac), 21.8 (CH3, Tol), 20.9 (CH3, Ac).
CH₃CN (10 ml) was stirred at 90°C and chromatographed on silica (petroleum ether–EtOAc, 1:8). 8.9 (s, 1H, H-2), 8.86 (br s, 1H, N-), 4.93 (dd, 1H, J=1.0 Hz, H-4), 4.90–4.87 (m, 2H, H-1 and H-5), 4.68 (dd, 1H, J=12.6 Hz, J′=3.1 Hz, H-6), 4.57 (d, 1H, J=12.1 Hz, H-1′), 4.29 (dd, 1H, J′=3.1 Hz, H-6′), 2.16 (s, 3H, CH₃, Tol), 1.96 (s, 3H, CH₃, Ac), 1.67, 1.44 (2×2×3H, 2×CH₃, i-Pr).

1″-O-Acetyl-3′,4″-O-isopropylidene-6′-O-(4-toluoyl)-β-D-psicofuranosyluracil (14c). A mixture of diacetate 13 [prepared from compound 5a (378 mg, 1 mmol)], N″-benzoyladenine (287 mg, 1.2 mmol) and N,O-bistrimethylsilyl)acetamide (0.59 ml, 2.4 mmol) in dry CH₂CN (10 ml) was stirred at 90°C for 1 h under a nitrogen atmosphere. After cooling, TMSOTf (0.2 ml, 1.1 mmol) was added and the mixture was stirred for 30 min at 90°C. After cooling, saturated aqueous NaHCO₃ (10 ml) was added and the mixture was extracted with CH₂Cl₂ (40 ml, 2×10 ml). The combined organic extracts were dried over MgSO₄, evaporated and chromatographed on silica (petroleum ether–EtOAc, 1:1) providing pure β-anomer 14c (234 mg, 39% in 2 steps from 5a). A 1D differential NOE experiment showed a 2.2% enhancement of the H-8′ signal while H-3′ was irradiated. Rf = 0.70 (CH₃Cl–MeOH, 10 : 1). MALDI-TOF m/z [M + H]+ 602.2 (Caled. 602.2 for C₂₁H₂₂N₂O₈). ¹H NMR (600 MHz, CDCl₃): 8.93 (s, 1H, H-1′), 8.86 (br s, 1H, N-), 8.15 (s, 1H, H-8), 7.99–6.68 (m, 9H, Tol and Bz), 6.14 (d, 1H, J=6.0 Hz, H-3′), 5.05 (d, 1H, J=12.2 Hz, H-4′), 4.93 (dd, 1H, J=2.7 Hz, J′=3.5 Hz, H-5′), 4.79 (d, 1H, J=12.7 Hz, H-6′), 4.63 (ABq, 2H, J=12.1 Hz, H-1′ and H-1″), 4.29 (dd, 1H, J=6.2 Hz, H-3′), 4.90 (dd, 1H, J=1.2 Hz, H-4′), 4.82 (dd, 1H, J=2.7 Hz, J′=3.4 Hz, H-5′), 4.59 (dd, 1H, J=12.6 Hz, H-6′), 4.38 (dd, 1H, J=6.2 Hz, J′=3.4 Hz, H-5′), 4.16 (m, d after 1′-OH decoupling, 1H, J=12.4 Hz, H-1′), 3.89 (m, d after 1′-OH decoupling, 1H, H-1″′), 2.41 (s, 3H, CH₃, Tol), 2.01 (br s, 1H, OH-1′). ¹³C NMR (67.9 MHz, CDCl₃): 165.9 (C=O), 164.4 (C=O), 150.4 (C-2), 144.8 (Tol), 141.8 (C-6), 129.5, 126.1, 121.0 (3×Tol), 113.6 (C-2), 101.6 (C-1), 100.7 (C-5), 86.3 (C-6), 83.6 (C-5), 81.9 (C-4), 64.5 (C-6′), 64.2 (C-1′), 25.8, 24.5 (2×CH₃, i-Pr), 21.8 (CH₃, Tol).

N″-Acylation of 1″-O-acetyl-3′,4″-O-isopropylidene-6′-O-(4-toluoyl)-β-D-psicofuranosyluracil (14d). A mixture of 13 [prepared from compound 5a (378 mg, 1 mmol)] N″-O-bis(trimethylsilyl)acetamide (0.59 ml, 2.4 mmol) in dry CH₂CN (10 ml) was stirred at 90°C for 1 h under a nitrogen atmosphere. After cooling, TMSOTf (0.2 ml, 1.1 mmol) was added and the mixture was stirred for 30 min at 90°C. After cooling, saturated aqueous NaHCO₃ (20 ml) was added and the mixture was extracted with CH₂Cl₂ (40 ml, 2×10 ml). The combined organic extracts were dried over MgSO₄, evaporated and chromatographed on silica (petroleum ether–EtOAc, 1:1) providing pure β-anomer 14d (299 mg, 40% in 2 steps from 5a). A 1D differential NOE experiment showed a 10% enhancement of the H-8′ signal while H-3′ was irradiated. Rf = 0.90 (CH₃Cl–MeOH, 10 : 1). MALDI-TOF m/z [M + H]+ 751.24 (Caled. 751.27 for C₂₁H₂₂N₂O₈). ¹H NMR (600 MHz, CDCl₃): 8.39 (br s, 1H, NH), 8.17 (s, 1H, H-8), 7.51–7.19 (m, 12H, Tol and DPC), 6.87–6.83 (m, 2H, DPC), 5.85 (d, 1H, J=5.5 Hz, H-3), 5.00 (dd, 1H, J=1.8 Hz, H-4), 4.86 (m, 1H, H-5), 4.60–4.52 (m, 3H, H-1′ and H-1″), 4.38 (dd, 1H, J=12.2 Hz, J′=3.1 Hz, H-6′), 2.58 (s, 3H, CH₃, AcNH), 2.19 (s, 3H, CH₃, Tol), 1.87 (s, 3H, CH₃, Ac), 1.64 (1H, 2×CH₃, i-Pr), 1.41 (2×CH₃, 2×CH₃, i-Pr).

1″-O-Methanesulfonyl-6′-O-(4-toluoyl)-β-D-psicofuranosyluracil (18). The mesylate 17 (2.6 g, 5.9 mmol) was treated with 90% (v/v) aqueous trifluoroacetic acid (15 ml) for 40 min at RT. The volatiles were removed in vacuo and the residue was dissolved in CH₂Cl₂ (70 ml) and washed with saturated aqueous NaHCO₃ (20 ml). The aqueous phase was re-extracted with CH₂Cl₂ (2×15 ml). Combined organic phases were dried over MgSO₄ and evaporated. This material was dried by co-evaporation with dry toluene (3 times) and used in the next step without further purification. Colorless foam. Rf = 0.4 (CH₃Cl–MeOH, 10 : 1). MALDI-TOF m/z [M + H]+ 741.1 (Caled. 741.1 for C₂₁H₂₂N₂O₈). ¹H NMR (600 MHz, CDCl₃): 142.7 (C-8), 141.8, 129.2, 129.1, 129.0, 127.0 (br), 125.6, 121.7, 114.4 (C-3), 98.4 (C-2′), 85.1 (C-3′), 84.5 (C-5′), 82.0 (C-4′), 64.9 (C-1′), 64.2 (C-6′), 26.0 (CH₃, i-Pr), 25.2 (CH₃, AcNH), 24.5 (CH₃, i-Pr), 21.5 (CH₃, Tol), 20.5 (CH₃, Ac).
10.2 (br s, 1H, NH), 7.79—7.75 (m, 3H, Tol and H-6), 7.19 (d, 2H, J = 8.1 Hz, Tol), 5.61 (d, 1H, J, χ-s = 8.3 Hz, H-5), 5.50—5.00 (br m, 1H, OMe), 4.88 (d, 1H, J, χ-s = 11.7 Hz, H-1), 4.84 (d, 1H, J, χ-s = 4.4 Hz, H-3), 4.65—4.55 (m, 3H, H-6, H-1′ and H-4′), 4.32—4.35 (m, 2H, H-2′ and H-5′), 4.10—3.10 (br m, 1H, OMe), 2.96 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), Tol). ¹³C NMR (67.9 MHz, CDCl₃): 163.3 (C-10), 160.4 (C-4, C-5), 151.6 (C-2′), 144.9 (Tol), 140.9 (C-6′), 129.5 (2C, 2 × Tol), 126.3 (Tol), 102.0 (C-5′), 97.3 (C-2), 83.9 (C-4′), 77.0 (C-3′), 71.8 (C-5′), 69.5 (C-1′), 63.2 (C-6′), 37.6 (CH₃), 21.7 (CH₃, Tol).

1-[3′,4′-O-Isopropylidene-β-D-psicofuranosyl]uracil (16). To the suspension of compound 14a (5.43 g, 11.44 mmol) in MeOH (60 ml) was added 1 M methanolic MeONa (11.5 ml, 11.5 mmol). The solution was stirred for 6 h at RT and then neutralized by addition of Dowex 50 (pyridinium form). The resin was filtered off, washed with MeOH and the filtrate was evaporated in vacuo. Crude product was dissolved in acetonitrile and precipitated with hexane. The separated gel-like precipitate was collected by filtration and washed carefully with hexane providing diol 16 (3.56 g, 99%) as a white amorphous powder after drying. The compound is well soluble in MeOH or acetone but with other common solvents (e.g. CHCl₃, EtOAc, diethyl ether) forms gels. Rf = 0.20 (CHCl₃-MeOH, 10:1).

MALDI-TOF m/z [M-H]⁻ 313.1 (Calcd. 313.1 for C₉H₇N₂O₇). ¹H NMR (600 MHz, d₅-DMSO): 11.15 (br s, 1H, NH), 7.78 (d, 1H, J, χ-s = 8.2 Hz, H-6), 5.49 (d, 1H, J, χ-s = 6.1 Hz, H-5), 5.04 (t, 1H, J, χ-s = 4.7 Hz, 6-OH), 4.94 (t, 1H, J, χ-s = 6.4 Hz, 1-OH), 4.72 (dd, 1H, J, χ-s = 1.1 Hz, H-4′), 4.35 (m, 1H, H-5′), 4.04 (dd, 1H, J, χ-s = 12.0 Hz, H-1′), 3.51 (dd, 1H, J, χ-s = 10.4 Hz, 4-OH), 2.41 (2H, CH₂, i-Pr). ¹³C NMR (67.9 MHz, d₅-DMSO): 164.9 (C-4'), 151.5 (C-2), 143.6 (C-6'), 112.3 (C-Me), 101.6 (C-2′), 99.8 (C-5′), 86.7 (C-5), 86.1 (C-3′), 82.2 (C-4′), 63.1 (C-1′), 61.9 (C-6′), 26.6, 25.2 (2 × CH₃, i-Pr).

1-[3′,4′-O-Isopropylidene-[6′-di-o-methanesulfonyl-β-D-psicofuranosyl]uracil (22). Diol 16 (4.95 g, 15.75 mmol) was transformed to co-evaporated with dry pyridine (50 ml) and methanesulfonyl chloride (3.7 ml, 47.50 mmol) was added dropwise at 0.5 °C and the mixture was stirred for 2 h at 0 °C. Then saturated aqueous NaHCO₃ (100 ml) was added and the mixture was extracted with CHCl₃ (100 ml, 2 × 25 ml). The combined organic extracts were dried over MgSO₄, evaporated, and co-evaporated 3 times with toluene. The product was purified by passing through a short column of silica (CHCl₃, with gradient of MeOH: 0-8%) afforded 22 (1769 mg, 82%) as an amorphous glassy solid. Rf = 0.1 (CHCl₃-MeOH, 10:1).

MALDI-TOF m/z [M-H]⁻ 331.0 (Calcd. 331.0 for C₉H₇N₂O₇). ¹H NMR (600 MHz, d₅-DMSO): 11.15 (br s, 1H, NH), 7.78 (d, 1H, J, χ-s = 8.2 Hz, H-6), 5.49 (d, 1H, J, χ-s = 6.1 Hz, H-5), 5.04 (t, 1H, J, χ-s = 4.7 Hz, 6-OH), 4.94 (t, 1H, J, χ-s = 6.4 Hz, 1-OH), 4.72 (dd, 1H, J, χ-s = 1.1 Hz, H-4′), 4.35 (m, 1H, H-5′), 4.04 (dd, 1H, J, χ-s = 12.0 Hz, H-1′), 3.51 (dd, 1H, J, χ-s = 10.4 Hz, 4-OH), 2.41 (2H, CH₂, i-Pr). ¹³C NMR (67.9 MHz, d₅-DMSO): 164.9 (C-4′), 151.5 (C-2), 143.6 (C-6′), 112.3 (C-Me), 101.6 (C-2′), 99.8 (C-5′), 86.7 (C-5), 86.1 (C-3′), 82.2 (C-4′), 63.1 (C-1′), 61.9 (C-6′), 26.6, 25.2 (2 × CH₃, i-Pr).

1-[3′,4′-O-Isopropylidene-6′-dio-methanesulfonyl-β-D-psicofuranosyl]uracil (25). To a solution of dimesylate 22 (3.76 g, 8 mmol) in dry CH₂CN (15 ml) was added DBU (1.31 ml, 8.8 mmol) and the mixture was left to stand at RT for 2 h, during that time the majority of the product separated off on the walls of the flask in the form of big plate-like colorless crystals. The mixture was stored overnight in refrigerator at −15 °C and then the separated crystals were filtered off and washed with CHCl₃ (3 × 5 ml) affording pure 2,1-anhydroazosbacilose 25 (2.49 g, 83%). Mp 194—195 °C (dec.). Rf = 0.22 (CHCl₃-MeOH, 10:1).

MALDI-TOF m/z [M-H]⁻ 373.1 (Calcd. 373.1 for C₉H₇N₂O₇). ¹H NMR (600 MHz, d₅-DMSO): 7.88 (d, 1H, J, χ-s = 7.6 Hz, H-5), 5.09 (d, 1H, J, χ-s = 6.3 Hz, H-3), 5.08 (dd, 1H, J, χ-s = 6.2 Hz, H-5), 3.74 (s, 6H, OCH₃, DMTr), 3.49 (dd, 1H, J, χ-s = 10.6 Hz, H-6), 3.40 (dd, 1H, H-5′), 1.13 (C-2′, C-2), 149.3 (2C), 144.8 (DMTr), 139.8 (C-6), 135.9, 130.2, 128.3, 127.8, 113.6 (6 × DMTr), 130.3 (C-5′, 91.2 (C-2′), 87.7 (C-3′), 86.3 (Ar′, C′-DMTr), 84.1 (C-5′), 78.5 (C-1′), 71.4 (C-4′), 63.2 (C-6′), 55.2 (OCH₃, DMTr).

1H, J_{x,y} = 3.9 Hz, H-4'), 4.91 (d, 1H, Jgem = 10.6 Hz, H-1'), 4.77 (d, 1H, H-1'), 4.47–4.37 (m, 3H, H-6'H-6' and H-5'), 3.27 (s, 3H, CH_3, Ms), 1.55, 1.59 (2 x s, 2 x 3H, 2 x CH_3, i-Pr). 13C NMR (150.9 MHz, d_6-DMSO): 171.5 (C-4), 160.7 (C-2), 135.7 (C-6), 115.2 (C-Me), 110.3 (C-5), 99.6 (C-2'), 83.0 (C-5'), 82.6 (C-6'), 80.6 (C-4'), 73.7 (C-1'), 69.7 (C-6'), 37.7 (CH_3, Ms), 27.1, 25.9 (2 x CH_3, i-Pr).

1-[2,1'-Anhydro-3',4'-O-isopropylidene-6'-O-(4-toluoyl)]-β-D-psicofuranosyluracil (26). A mixture of 2,1-anhydro-6'-O-mesylate 25 (0.25 g, 0.67 mmol) and sodium 4-toluate (211 mg, 1.34 mmol) in DMF (3.5 ml) was stirred at 90 °C for 6 h. After cooling, DMF was removed in vacuo and the residue was washed with water (5 times) to give compound 28 which after drying (co-evaporation twice with dry pyridine) was dissolved in CH_2Cl_2–pyridine (7 : 1, v/v, 100 ml) at 0 °C, under a nitrogen atmosphere. 4-Toloyl chloride (8.2 ml, 62 mmol) was added dropwise and the reaction was stirred for 4 h at this temperature, and overnight at 4 °C. The reaction mixture was poured into saturated NaHCO_3 solution and extracted with CH_2Cl_2. The organic phase was washed over MgSO_4, filtered and then co-evaporated with toluene. Column chromatography (hexane with gradient of EtOAc: 0–30%) afforded 29 (9.4 g, 15 mmol, 75% after two steps).

MALDI-TOF m/z [M + Na]^- 647.1 (Calcd. 647.2 for C_{37}H_{36}O_{9}Na). 13C NMR (anomeric mixture, 150.9 MHz, CDCl_3): 171.20, 166.44, 166.13, 165.53, 165.46, 164.97, 164.89, 148.75, 144.31, 144.24, 144.17, 144.11, 144.02, 143.69, 143.67, 136.79, 136.84, 130.14, 129.84, 129.79, 129.66, 129.18, 129.13, 129.10, 129.05, 128.99, 128.94, 128.92, 128.57, 128.41, 128.04, 127.89, 127.61, 127.47, 127.66, 127.73, 126.41, 126.24, 126.09, 124.03, 104.28, 102.00, 81.84, 81.59, 76.55, 73.88, 73.50, 72.68, 72.45, 71.96, 69.85, 69.49, 65.97, 64.89, 61.28, 61.22, 61.21, 57.57.

2-O-Acetyl-6-O-benzyl-1,3,4-tri-O-(4-toluoyl)-β-D-psicofuranose (30). The sugar 29 (9.4 g, 15 mmol) was dried by co-evaporation with pyridine and dissolved in dry pyridine (75 ml) after which acetic anhydride (28 ml, 300 mmol) was added. The reaction mixture was stirred at RT for 48 h and was then poured into saturated NaHCO_3 solution and extracted with CH_2Cl_2. The organic phase was dried over MgSO_4, filtered and then co-evaporated with toluene. The crude product was then purified by column chromatography (hexane with gradient of EtOAc: 0–30%) afforded 30 (9 g, 13.5 mmol, 90%). MALDI-TOF m/z [M + Na]^- 689.1 (Calcd. 689.2 for C_{37}H_{36}O_{9}Na). 13C NMR (anomeric mixture, 150.9 MHz, CDCl_3): 171.40, 168.80, 167.80, 167.65, 165.61, 165.50, 164.50, 164.60, 149.60, 144.40, 144.30, 144.10, 144.00, 143.80, 143.60, 137.80, 136.60, 136.90, 130.20, 129.89, 129.74, 129.72, 129.66, 129.64, 130.93, 129.24, 129.23, 129.20, 129.12, 129.11, 129.04, 129.03, 128.94, 128.93, 128.46, 128.43, 129.70, 127.70, 126.70, 125.70, 125.76, 126.72, 126.60, 126.30, 126.20, 108.60, 105.60, 83.80, 82.80, 74.90, 73.70, 73.50, 72.90, 71.40, 70.90, 68.95, 68.93, 63.40, 61.40, 61.28, 21.70, 21.60, 21.50.

N^-Acetyl-9'-6'-O-benzyl-1,3,4-tri-O-(4-toluoyl)-β-D-psicofuranosyl-D-phenylcarbamoylglucamine (31). Sugar 30 (6 g, 10 mmol) was dried by co-evaporation with dry CH,CN, dissolved in CH,CN (100 ml) and N^-acetyl-D-phenylcarbamoylglucamine (4.1 g, 12 mmol) and N-O-bismethyleneилis(acetamide (4.95 ml, 20 mmol) were added and the reaction was heated at 90 °C for 1.5 h under a nitrogen atmosphere. The reaction mixture was then purified by column chromatography (hexane with gradient of EtOAc: 0–50%) afforded 31a (β : α : 7 : 3 : 6.2 g, 6.62 mmol, 62%). A 1D differential NOE experiment showed a 1.67% enhancement of the H-3 signal, while H-8 was irradiated. R_f = 0.45 (hexane–EtOAc, 6 : 4). MALDI-TOF m/z [M + Na]^- 1017.3 (Calcd. 1017.3 for C_{66}H_{60}N_{15}O_{14}Na). Anomeric mixture 3 : 1, NMR data is given only for β-anomer. H NMR (600 MHz, CDCl_3): 8.35 (s, 1H, H-8), 7.93–7.02 (m, 27H, Tol), 3.29 (d, 3H, i-Pr).
and the mixture was stirred at 90°C for 1.5 h under a nitrogen atmosphere. After cooling, TMSOTf (0.28 ml, 1.4 mmol) was added at RT and the reaction mixture was again heated at 40°C for 2.5 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into saturated NaHCO₃ solution and extracted with CH₂Cl₂. The organic phase was dried over MgSO₄, filtered and evaporated. Column chromatography (c-hexane with gradient of EtOAc: 0–40%) afforded 31d (β : α, 95 : 5; 1.62 g, 2.25 mmol, 78%). A 1D differential NOE experiment showed a 1.5% enhancement of the H-6 signal, while H-3 was irradiated. Rf = 0.57 (c-hexane–EtOAc 65 : 35). MALDI-TOF m/z [M + Na]+ 741.2. (Calcd. 741.2 for C₃₃H₅₃N₊O₁₉Na).

**N⁵-Benzyl-9-[(6-O-benzyl-1,3,4-tri-O-(4-toluoyl)-d-psicofuranosyl)adenine (31b).** Sugar 30 (13.2 g, 20 mmol) was dried by co-evaporation with dry CH₂CN and N⁵-benzoyladenine (5.7 g, 24 mmol), N,O-bis(trimethylsilyl)acetamide (10 ml, 40 mmol) and CH₂CN (200 ml) were added and the reaction mixture was stirred at 90°C for 1.5 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into saturated NaHCO₃ solution and extracted with CH₂Cl₂. The organic phase was dried over MgSO₄, filtered, and evaporated. Column chromatography (c-hexane with gradient of EtOAc: 0–40%) afforded 31b (β : α, 7 : 3; 10.1 g, 12 mmol, 60%). A 1D differential NOE experiment showed a 1.6% enhancement of the H-3′ signal, while H-8 was irradiated. Rf = 0.25 (c-hexane–EtOAc : 6 : 4). MALDI-TOF- MS [M + H]+ 846.3 (Calcd. 846.3 for C₃₆H₄₃N₇O₉). Anomeric mixture 3 : 7, NMR data is given only for the β-anomer. 1H NMR (600 MHz, CDCl₃): 8.59 (s, 1H, H-2), 8.44 (s, 1H, H-8), 7.96–7.11 (m, 22H, Tol, Bn and Bz), 6.82 (d, 1H, J₂₋₃ = 5.4 Hz, H-3), 5.91 (d, 1H, J₁₋₂ = 3.6 Hz, H-4), 4.80 (s, 1H, H-5), 5.33 (d, 1H, J₅₋₆ = 12.0 Hz, H-1), 5.16 (d, 1H, H-8), 4.54 (d, 1H, J₃₋₄ = 12.0 Hz, CH₂Ph), 4.43 (d, 1H, H-6), 3.86 (dd, 1H, J₂₋₃ = 5.4 Hz, 3.1 Hz, H₁₃), 3.64 (m, 3x, 3 x CH₃, Tol). 13C NMR (150.9 MHz, CDCl₃): 165.7, 165.4, 164.6 (3 x C=O), 147.9, 147.6, 131.2, 129.7, 129.6, 128.9, 128.5, 128.3, 128.7, 96.2 (C-2), 83.8 (C-3), 76.0 (C-3′), 73.7 (CH₂Ph), 72.6 (C-4′), 68.8 (C-6′), 64.3 (C-1′), 24.9, 21.7, 21.6 (3 x CH₃, Tol) and 1 x CH₃, Ac.).

**9-[6-O-Benzyl-1,3,4-tri-O-(4-toluoyl)-d-psicofuranosyl]uracil (31d).** Sugar 30 (1.9 g, 2.9 mmol) was dried by co-evaporation with dry CH₂CN, dissolved in 28 ml of this same solvent and uracil (0.38 g, 3.5 mmol) was added. N,O-Bis(trimethylsilyl)acetamide (0.6 ml, 2.4 mmol) was added and the mixture was stirred at 90°C for 1.5 h under a nitrogen atmosphere. After cooling, TMSOTf (0.28 ml, 1.4 mmol) was added at RT and the reaction mixture was again heated at 40°C for 2.5 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into saturated NaHCO₃ solution and extracted with CH₂Cl₂. The organic phase was dried over MgSO₄, filtered and evaporated. Column chromatography (c-hexane with gradient of EtOAc: 0–40%) afforded 31d (β : α, 95 : 5; 1.62 g, 2.25 mmol, 78%). A 1D differential NOE experiment showed a 1.5% enhancement of the H-6 signal, while H-3 was irradiated. Rf = 0.57 (c-hexane–EtOAc 65 : 35). MALDI-TOF- m/z [M + Na]+ 741.2. (Calcd. 741.2 for C₃₃H₅₃N₇O₁₉Na).

**9'-6'-O-Benzyl-1'-3',4'-tri-O-(4-toluensulfonyl)-d-psicofuranosyluracil (33).** Compound 31a (12.4 g, 12.5 mmol) was dissolved in methanolic ammonia and kept at RT for 2 days to give compound 32a (27°C NMR in ESI†). The solvent was evaporated and the residue dried by co-evaporation with dry pyridine, after which it was dissolved in this same solvent and uracil (0.38 g, 3.5 mmol) was added. The mixture was cooled to 0°C (ice-bath), and 4-toluensulfonyl chloride was added (5.2 g, 27.4 mmol in two portions). The reaction was stirred at 0°C for 5 h, poured into saturated NaHCO₃ solution and extracted with CH₂Cl₂ (three times). The organic phase was dried over MgSO₄, filtered, and evaporated. Column chromatography (CH₂Cl₂ with gradient of MeOH: 0–7%) afforded 33 (only β-anomer; 3.1 g, 5.5 mmol, 44%). Rf = 0.61 (CH₂Cl₂-MeOH, 10 : 1). MALDI-TOF-MS [M + H]+ 558.1 (Calcd. 558.1 for C₃₃H₅₀N₇O₁₉S). 1H NMR (600 MHz, CD₂OD): 7.69 (s, 1H, H-8), 7.47 (d, 2H, J₃₋₄ = 8.4 Hz, Tol), 7.25–7.21 (m, 3H, Bz), 7.19 (d, 2H, J₃₋₄ = 8.4 Hz, Tol), 7.05–7.04 (m, 2H, Bz), 4.69 (d, 1H, J₅₋₆ = 5.1 Hz, H-3′), 4.63 (d, 1H, J₅₋₆ = 11.7 Hz, CH₂Ph), 4.46 (d, 1H, CH₂Ph), 4.38 (d, 1H, J₅₋₆ = 11.7 Hz, H-1′), 4.36 (d, 1H, H-1′), 4.27 (d, 1H, J₅₋₆ = 2.6 Hz, H-4′), 3.59 (d, 1H, J₅₋₆ = 10.7 Hz, J₃₋₄ = 3.1 Hz, H-6′), 3.54 (dd, 1H, J₅₋₆ = 3.1 Hz, H-6′), 2.39 (s, 3H, CH₃, Ts). 13C NMR (150.9 MHz, CD₂OD): 157.5 (C-2′), 156.7 (C-2′), 148.9 (C-4′), 144.7, 137.0, 135.9 (C-8), 131.2, 129.7, 127.6, 127.5, 127.4, 95.0 (C-5′), 85.5 (C-5′), 77.1 (C-3′), 73.1 (CH₃Ph), 71.4 (C-6′), 69.1 (C-6′), 69.4 (C-6′), 20.9 (CH₃, Ts).
J 4-toluensulfonyl chloride (4.6 g, 24 mmol) was added in two
portions with dry pyridine, and the crude product was
then washed (2 × 10 ml) with petroleum ether (b.p. 60–70°C),
extracted with 2 ml CHCl3, evaporated and co-evaporated with dry pyridine.
A mixture of compound 31 (1.6 g, 3.7 mmol), PD(OH)2/C (0.88 g, 20% w/t) and ammonium formate (3.8 g, 60 mmol) in MeOH (100 ml) was refluxed for 5 h, cooled to RT and filtered through a 1 cm thick Celite pad which was washed with hot methanol–water (1: 1, v/v/v).
The filtrate was evaporated, co-evaporated with dry pyridine, and crude 36 (NMR data in ES1) was
then dissolved in dry pyridine (40 ml), 4,4′-dimethoxytrityl chloride (2 g, 6 mmol) was added and stirring was continued for 2 h, after which the mixture was poured into saturated NaHCO3 solution and extracted with CH2Cl2 (three times). The organic phase was dried over MgSO4, filtered, and evaporated. Column chromatography (CH2Cl2, with gradient of MeOH: 0–5%) afforded nucleoside 37 (2 g, 3.2 mmol, 85% after two steps). Rf = 0.70 (CH2Cl2–MeOH: 10 : 1), MALDI-TOF m/z [M + H]+ 653.17 (calcd. 653.27 for C33H31N7O7). 1H NMR (600 MHz, CDCl3); 8.41 (s, 1H, CH(NMe2)), 7.65 (s, 1H, H-8), 7.40–7.17 (m, 14H, DMTr and PAC), 4.52–4.49 (m, 1H, H-5), 4.39 (m, 1H, H-5), 3.47 (s, 3H, OCH3), 2.04 (s, 2CH3, Dmf). 13C NMR (150.9 MHz, CDCl3); 158.0 (DMTr), 158.0 (CH(NMe2)), 157.5, 156.8 (C-6), 149.9 (C-4), 144.5, 135.7, 135.6 (DMTr), 135.2 (C-8), 130.0, 128.1, 127.9, 126.9 (DMTr), 120.9 (C-5), 113.1 (DMTr), 88.7 (C-3), 86.4 (C-2), 83.2 (C-5), 80.1 (C-1), 71.7 (C-4), 62.5 (C-6), 55.2 (OCH3, Dmf), 41.5, 35.2 (2 CH3, Dmf).

9-[l'-3-O-Amylhydro-6'-O-(4,4'-dimethoxytrityl)-β-D-psicofuranosyl-N2'-N,N-dimethylaminomethylene]guanine (37). A mixture of compound 36 (1.6 g, 3.7 mmol), PD(OH)2/C (0.88 g, 20% w/t) and ammonium formate (3.65 g, 58 mmol) in MeOH (110 ml) was refluxed for 5 h, cooled to RT and filtered through a 1 cm thick Celite pad, which was washed with hot methanol–water (1: 1, v/v/v). The filtrate was evaporated, co-evaporated with dry pyridine, and crude 38 (NMR data in ES1) was then dissolved in dry pyridine (50 ml). 4,4′-Dimethoxytrityl chloride (2 g, 5.83 mmol) was added and stirring was continued for 2 h, after which the mixture was poured into saturated NaHCO3 solution and extracted with CH2Cl2 (three times). The organic phase was dried over MgSO4, filtered, and evaporated. Column chromatography (CH2Cl2, with gradient of MeOH: 0–5%) afforded nucleoside 39 (1.83 g, 3.16 mmol, 65% after two steps). Rf = 0.70 (CH2Cl2–MeOH: 10 : 1), MALDI-TOF m/z [M + H]+ 582.13 (calcd. 582.23 for C33H31N7O7). 1H NMR (600 MHz, CDCl3); 8.36 (s, 1H, H-2), 7.87 (s, 1H, H-8), 7.41–7.17 (m, 9H, DMTr), 6.80–6.75 (m, 4H, DMTr), 5.78 (d, 1H, Jex = 4.8 Hz, H-3), 5.65 (d, 1H, Jex = 9.6 Hz, H-1), 4.96 (d, 1H, H-1′), 4.48 (d, 1H, Jex = 7.4 Hz, H-4), 4.44 (m, 1H, H-5), 3.77 (s, 6H, OCH3), 3.57–3.46 (m, 2H, H-6 and H-6′). 13C NMR (150.9 MHz, CDCl3); 158.5 (DMTr), 155.4 (C-6), 153.5 (C-2), 149.5 (C-4), 144.4 (DMTr), 137.6 (C-8), 135.7, 130.0, 128.1, 127.8, 126.8 (5 × DMTr), 119.8 (C-5), 113.1 (DMTr), 89.2 (C-2), 88.3 (C-3), 86.3 (Ar=C), 83.8 (C-5), 80.0, 71.7 (C-4), 62.7 (C-6), 55.2 (OCH3, Dmf).

9-[l'-3-O-Amylhydro-6'-O-(4,4'-dimethoxytrityl)-β-D-psicofuranosyl-N2'-N-phenoxyacetylcytidine (42). A solution of compound 41 (1.83 g, 3.16 mmol) was dried by co-evaporation with pyridine, dissolved in pyridine (30 ml) and trimethylsilyl chloride (1.2 ml, 9.5 mmol) was added dropwise at 0°C and the reaction mixture was stirred at RT for 2.9 h. 4,4′-Dimethoxytrityl chloride (0.87 mmol, 6.3 mmol) was added and stirring was continued at 0°C. The reaction mixture was stirred for 2 h at RT, poured into saturated NaHCO3 solution and extracted with CH2Cl2 (three times). The organic phase was dried over MgSO4, filtered, and evaporated. The crude mixture was dissolved in pyridine–water (2 : 1 v/v/v) and stirred overnight. After evaporation of the solvent, column chromatography (CH2Cl2, with gradient of MeOH: 0–5%) afforded 42 (1.7 g, 2.4 mmol, 75%). Rf = 0.43 (CH2Cl2–MeOH: 95 : 5). MALDI-TOF m/z [M + H]+ 716.22 (calcd. 716.27 for C33H31N7O8). 1H NMR (600 MHz, CDCl3); 8.80 (s, 1H, H-2), 8.08 (s, 1H, H-8), 7.40–7.10 (m, 14H, DMTr and PAC), 6.76–6.75 (m, 4H, DMTr), 5.78 (d, 1H, Jex = 4.3 Hz, H-3), 5.65 (d, 1H, Jex = 9.6 Hz, H-1), 4.96 (d, 1H, H-1′), 4.87 (d, 1H, Jex = 7.4 Hz, H-4), 4.44 (m, 1H, H-5), 3.77 (s, 6H, OCH3), 3.57–3.46 (m, 2H, H-6 and H-6′).
References