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Facile preparation of the oxetane-nucleosides†

Małgorzata Bogucka, Petr Nauš, Wimal Pathmasiri, Jharna Barman and Jyoti Chattopadhyaya*

Department of Bioorganic Chemistry, Box 581, Biomedical Center, University of Uppsala, S-75123, Uppsala, Sweden. E-mail: jyoti@boc.uu.se; Fax: +4618554495

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Efficient and practical large scale synthesis of suitably protected 1′,2′-oxetane locked purine and 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*-isopropylidene-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 ($-1° < P < 34°$)[4] constrained [LNA[5–13] and oxetanes[14–17] (**1–4**, Fig. 1)] nucleotide blocks have unique abilities to dictate conformational preorganisation[7,17,18] 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.[18,19] However, RNase H eliciting capability can partly or fully be regained by adopting various mixmer and gapmer strategies utilizing these modifications[17,18] (a gap size of 8–10 nucleotides is necessary for the β-D-LNA modified[20,21] AONs, whereas a gap of 4 nt is necessary for the oxetane-modified counterpart).[17,18] The length of the gap in the gapmer can however potentially make the AON vulnerable to endonucleases.[22]

The mixmer AON–RNA hybrids incorporated with oxetane-**T** units (T_m 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.[14–18,23] However, the incorporation of the oxetane-**C** moiety (Fig. 1) into the AONs imparts only ~3 °C loss in T_m per modification,[14] whereas no loss in T_m 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-toxic[23] DPPZ (dipyridophenazine) group[24,25] at the 3′ end, which gave also additional stability against exonucleases similar to that of the phosphorothioate AONs.[15,26] 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 V_{max} and K_m 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.[14,16] 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.[23] QRT-PCR (Quantitative RealTime–Polymerase Chain Reaction) and Western blotting (the "gold standard" in antisense efficacy)[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.[23]

Because of the effective down-regulation of genes by oxetane-modified AONs and their possible applications in the RNAi approach,[28] 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**. ^{13}C NMR spectra of compounds **13**, **14a–d**, **15–27**, **31a–d**, **32a,b**, **33**, **35–42**. See DOI: 10.1039/b511406c

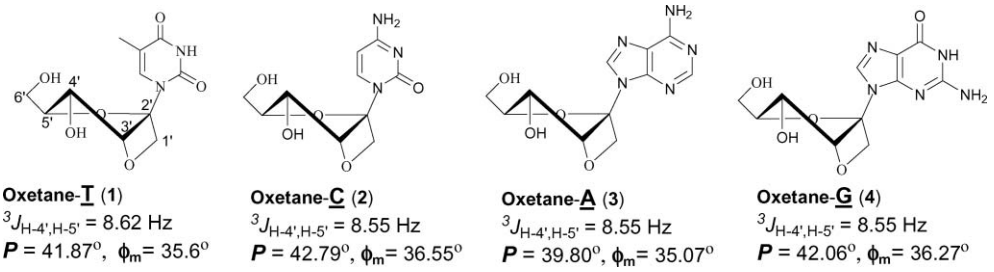

Fig. 1 Oxetane constrained nucleosides and their structural and NMR parameters.

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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-psicofuranose (**5a**)[14,18] which was coupled with persilylated thymine or N^4-benzoylcytosine in the presence of trimethylsilyl trifluromethanesulfonate (TMSOTf) as a Lewis acid catalyst to afford an inseparable, as well as unfavorable, anomeric 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 anomeric mixture with poor yield (α : β, 55 : 45 in 52% yield)[29] of the corresponding purified psicofuranose nucleoside, which makes the use 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).[30]

In addition to the above problem, the coupling of the sugar block **5a** with persilylated purine nucleobases was also unsuccessful, hence we employed[16] an alternative strategy for the synthesis of 9-(1′,3′-*O*-anhydro-β-D-psicofuranosyl)adenine/guanine [oxetane-**A** (**3**) and oxetane-**G** (**4**)] nucleosides involving the key bromosugar **12**. The synthesis of this bromosugar **12** 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[8,9,31–34] derivatives are not always satisfactory for the coupling with the corresponding ketoses.[35,36] This is especially true for the synthesis of psico-guanosine derivatives[16] by a coupling reaction with a ketose-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.[37] 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*-DMTr 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-diisopropylidenepsicofuranose (**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 ^{1}H-NMR) which was identified as 1,2-di-*O*-acetyl-3,4-*O*-isopropylidene-6-*O*-(*p*-toluoyl)-α-D-psicofuranose (**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^4-benzoylcytosine using a Vorbrüggen[37] procedure to give an α : β mixture (1 : 9 by ^{1}H-NMR) of psicouridine (76%) and psico-N^4-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** → **7** works very well with a favorable yield for the β-anomer (Fig. 2),[14] we report here only the synthesis of the oxetane derivative of the uridine block, which required improvement because our older unpublished procedure[29] 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*-toluate) ester in **14a** was necessary to introduce a leaving group at C1′. Selective removal of 1′-acetate over 6′-(*p*-toluate) ester by 16% methanolic ammonia[38] 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)[38,39] leading to significant concomitant deprotection of 6′-(*p*-toluate) 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′-*O*-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*-toluate) ester from the oxetane **19** with methanolic ammonia and subsequent 6-*O*-alkylation of **20** by DMTr-Cl in pyridine completed the synthesis of 6′-*O*-DMTr 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

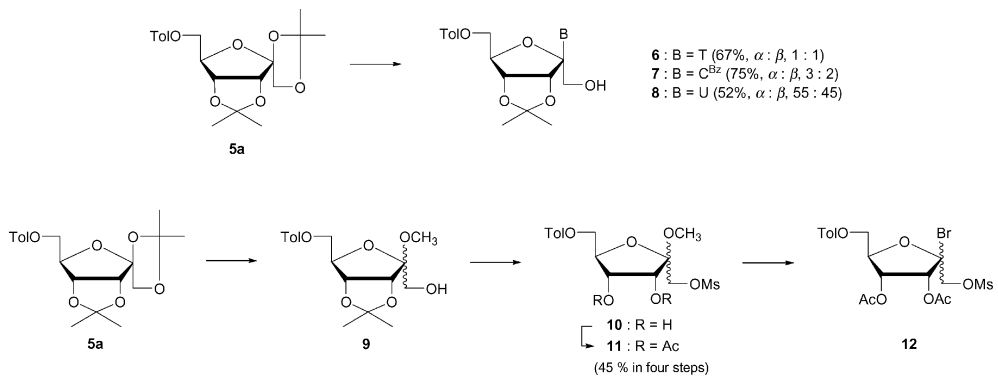
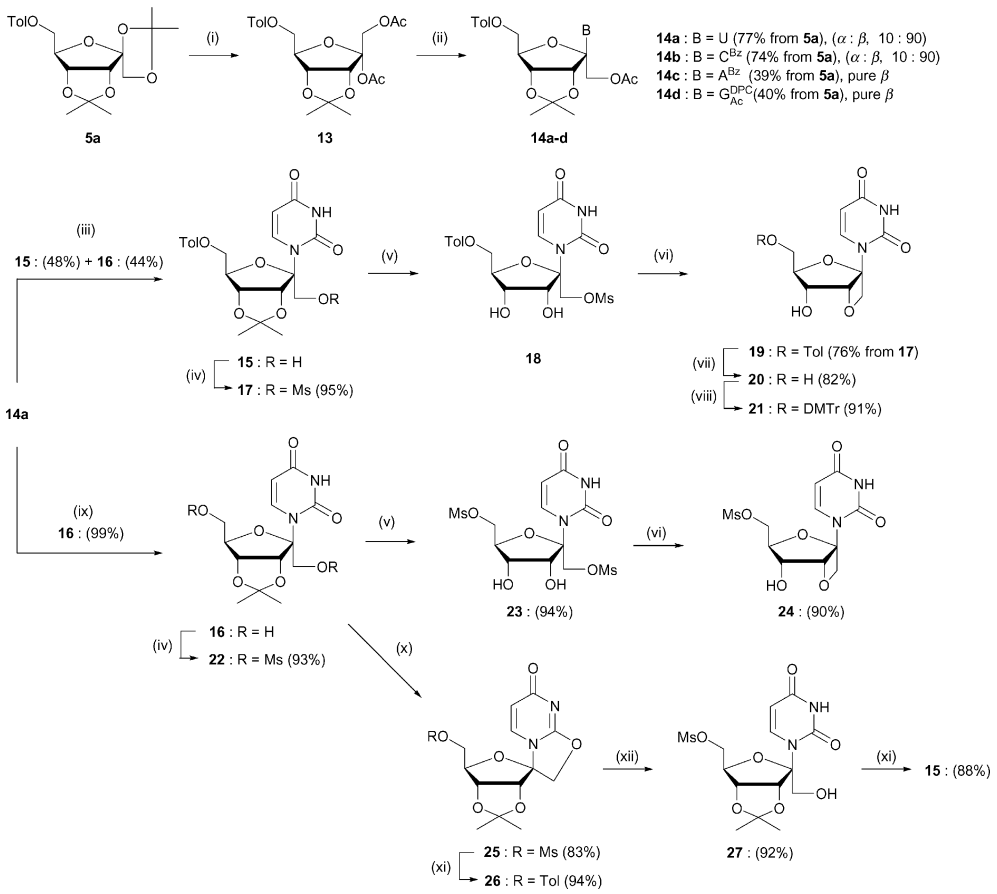


Fig. 2 Synthesis route to bromosugar **12**.



Scheme 1 *Reagents and conditions*: (i) Ac_2O, AcOH, TfOH, RT, 3 h; (ii) 1. base, BSA, MeCN, 90 °C, 1 h, 2. TMSOTf; (iii) 16% methanolic ammonia, RT, 6 h; (iv) Ms-Cl, pyridine, 0 °C, 2 h; (v) 90% TFA (aq.), RT, 40 min; (vi) NaHMDS, THF, 0 °C, 2 h; (vii) 25% methanolic ammonia, RT, 2 days; (viii) DMTr-Cl, pyridine, RT, 24 h; (ix) MeONa, MeOH, RT, 6 h; (x) DBU, MeCN, RT, 2 h; (xi) TolONa, DMF, 90 °C, 6 h for **26** or 15 h for **15**; (xii) NaOH, dioxane–H_2O, RT, 1 h. *Abbreviations*: Tol = *p*-toluoyl, Ac = acetyl, Bz = benzoyl, U = uracil, C^{Bz} = N^4-benzoylcytosin-1-yl, A^{Bz} = N^6-benzoyladenin-9-yl, G^{DPC}_{AC} = N^2-acetyl-O^6-diphenylcarbamoylguanin-9-yl, DMTr = 4,4′-dimethoxytrityl, Ms = methylsulfonyl, NaHMDS = sodium bis(trimethylsilyl)amide.

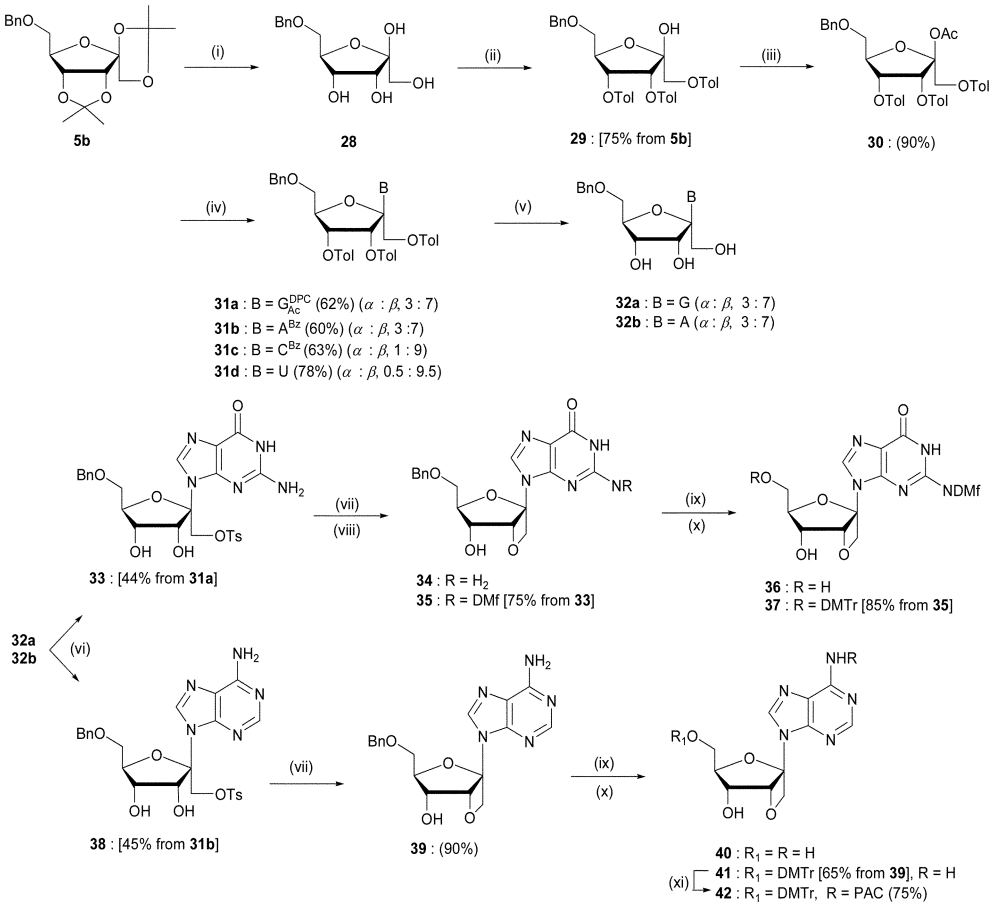
oxetane **20**. This is also an attractive goal because the diol **16** can be easily synthesized by full deprotection of the diester **14a** by its treatment with methanolic sodium methoxide in a quantitative yield. Although the conversion of 1′,6′-diol **16** to 6′-*O*-mesyl-oxetane nucleoside **24** through 1′,6′-dimesylates **22** and **23** seemed to be the most straightforward way to produce **20**, the subsequent displacement of the 6′-*O*-mesyl 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-mesylate (**22**) to the 6′-(*p*-toluate) block **15** as a key precursor for the synthesis of oxetane **21**. The internal displacement of 1′-mesylate in **22** by DBU in dry CH_3CN at RT gave 2,1′-*O*-anhydro 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′-*O*-anhydro derivative. The 6′-mesyl group from **25** was then cleanly displaced by sodium 4-toluate at the C6′ center exclusively to give **26** (94%) because of the high stability of the 5-membered 2,1′-*O*-anhydro ring in **25**. The ring opening of the 2,1′-*O*-anhydro system in 6′-*O*-mesyl 2,1′-anhydro nucleoside **25** was however accomplished by the action of sodium hydroxide in aqueous dioxane at RT providing 6′-*O*-mesyl alcohol **27** in 92% yield. Compound **27** was subsequently reacted with sodium 4-toluate in DMF at 90 °C to displace the 6′-mesylate group to give the desired 6-*O*-(*p*-toluoyl) alcohol **15** (88%), which was further transformed to oxetane-**U** building block **21**, as described above.

We have also performed the glycosylation of N^6-benzoyladenine and O^6-diphenylcarbamoylguanine with 1,2-di-*O*-acetyl-3,4-*O*-isopropylidene-6-*O*-toluoyl-α-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.[36,40,41] Unfortunately, during the deprotection of the 3,4-*O*-isopropylidene group from either **14c** or **14d** under acidic conditions (Dowex H^+ in dioxane),[40] 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 psicofuranoside[35,36] as a glycosyl donor has severe limitations: (i) the coupling works well with persilylated N^6-benzoyladenine, 6-chloropurine and uracil bases but the α : β anomeric ratio (1 : 2) is very unfavorable for preparative purposes, and more importantly, (ii) the coupling does not work with the appropriately protected guaninyl base.[16] (iii) In addition, we also required an orthogonal protection strategy to manipulate functionalization at C1′ *vis-à-vis* C6′ with



Scheme 2 *Reagents and conditions*: (i) 70% aqueous acetic acid, 70 °C, 5 h; (ii) *p*-toluoyl chloride, pyridine–dichloromethane 1 : 7, 0 °C, 4 h, then 4 °C overnight; (iii) acetic anhydride, pyridine, 0 °C–RT, 48 h; (iv) persilylated G^{DPC}_{Ac}, CH_3CN, TMSTf, 80 °C, 1.5 h for **31a** (α : β, 3 : 7), persilylated A^{Bz}, CH_3CN, TMSTf, 80 °C 1.5 h for **31b** (α : β, 3 : 7); (v) methanolic ammonia, RT, 48 h, (vi) *p*-toluenosulfonyl chloride, pyridine, 0 °C, 5 h; (vii) NaHMDS, 0 °C–RT, THF, 8 h; (viii) *N*,*N*-dimethylformamide dimethylacetal, methanol, RT, overnight; (ix) ammonium formate, MeOH, $Pd(OH)_2/C$, 75 °C, 5 h; (x) DMTr-Cl, pyridine, RT, 2 h; (xi) TMS-Cl, pyridine, RT, 2 h, then PAC-Cl, 2 h, RT. *Abbreviations*: Tol = 4-toluoyl, Ac = acetyl, Bn = benzyl, Bz = benzoyl, G^{DPC}_{AC} = N^2-acetyl-O^6-diphenylcarbamoylguanin-9-yl, A^{Bz} = N^6-benzoyladenin-9-yl, NaHMDS = sodium bis(trimethylsilyl)amide, DMTr = 4,4′-dimethoxytrityl, Dmf = *N*,*N*-dimethylaminomethylene, Ts = *p*-toluenosulfonyl, PAC = phenoxyacetyl.

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–CH_2Cl_2 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.[42,43] The subsequent acetylation with acetic anhydride (20 eq.) in pyridine was slow but led to compound **30** (90%) on standing at RT (2 days) without any detectable side reactions.

The silylation of nucleobases was carried out *in situ* in the presence of glycosyl donor **30**, followed by the addition of trimethylsilyl triflate affording adenosine **31a** (62%, α : β, 3 : 7), guanosine nucleosides **31b** (60%, α : β, 3 : 7), and pyrimidine nucleosides **31c** (63%, α : β, 1 : 9) and **31d** (78%, α : β, 5 : 95) with various ratios of the α : β anomeric mixture. The stereochemical assignment of α or β-anomeric configuration was based on 1D differential NOE experiments (see Experimental section).

Since the yield and the distribution of anomers for pyrimidine nucleosides by the above procedure (B) were not significantly improved compared to procedure (A) for the psicofuranosyl-uracil derivative or to the literature procedure[14,18] for the corresponding cytidine derivative, we focused only on the purine derivatives, which were not possible to obtain using either procedure (A) or by any other published procedures on a preparative scale.[16,35,36,44,45]

The α : β anomeric 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 anomeric 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 anomeric mixture, after a simple chromatographic purification step, gave the pure β-anomer of 1′-*O*-tosyl-6′-*O*-benzyl derivative **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.[16] 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%). N^2-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 N^2-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,[16] whereas the oxetane-**U** block **20** was identical to the one reported earlier from Mikhailopulo's lab.[45]

Conclusions

Antisense oligonucleotides containing oxetane-modified nucleosides which have a unique fixed *North-East* sugar conformation have been found to be excellent substrates for RNase H promoted cleavage, and efficient both in diminishing the c-myb mRNA and the c-myb 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^2-acetyl-O^6-diphenylcarbamoyl)guanine, N^6-benzoyladenine, N^4-benzoylcytosine, and uracil, respectively] using the Vorbrüggen procedure. Compounds **14a**, **31a** and **31b** were used as starting materials for the synthesis of suitable protected 1′,3′-*O*-anhydro psicofuranose nucleosides **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). ^{1}H NMR spectra were recorded on JEOL GX 270 or Bruker DRX 600 spectrometers at 270.1 MHz and 600 MHz, respectively. ^{13}C 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 ^{1}H and 40.45 ppm for ^{13}C) 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% aqeous H_2SO_4 or anisaldehyde reagent [4-anisaldehyde–EtOH–AcOH–H_2SO_4, 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 $NaHCO_3$ (120 ml); the aqueous phase was re-extracted with EtOAc (2 × 40 ml) and the combined organic phases were dried over $MgSO_4$ and evaporated providing crude diacetate **13** as a colorless oil (9.32 g, 104%, 90% ^{1}H 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 irradiated. R_f = 0.54 (c-hexane–EtOAc, 6 : 4). MALDI-TOF *m/z* $[M + H]^+$ 423.12 (Calcd. 423.17 for $C_{21}H_{27}O_9$). ^{1}H NMR (600 MHz, $CDCl_3$): 7.95, 7.26 (2 × d, 2 × 2H, J = 8.0 Hz, Tol), 4.96 (d, 1H, J_{3-4} = 6.0 Hz, H-3), 4.91 (dd, 1H, J_{4-5} = 1.8 Hz, H-4), 4.68 (ABq, 2H, J_{gem} = 12.0 Hz, H-1 and H-1′), 4.62 (ddd, 1H, J_{5-6} = 6.6 Hz, $J_{5-6'}$ = 6.0 Hz, H-5), 4.45 (dd, 1H, J_{gem} = 11.4 Hz, H-6), 4.38 (dd, 1H, H-6′), 2.42 (s, 3H, CH_3, Tol), 2.11, 2.00 (2 × s, 2 × 3H, 2 × CH_3, Ac), 1.53, 1.36 (2 × s, 2 × 3H, 2 × CH_3, i-Pr). ^{13}C NMR (67.9 MHz, $CDCl_3$): 170.2, 169.2, 166.3 (3 × C=O), 144.2, 129.9, 129.3, 126.9 (4 × Tol), 113.9 (*C*–Me_2), 110.8 (C-2), 85.4 (C-5), 85.3 (C-3), 82.0 (C-4), 64.4 (C-6), 62.4 (C-1), 26.5, 25.1 (2 × *C*H_3, i-Pr), 22.0 (*C*H_3, Ac), 21.8 (*C*H_3, Tol), 20.9 (*C*H_3, Ac).

1-[1′-*O*-Acetyl-3′,4′-*O*-isopropylidene-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]uracil (14a). A mixture of diacetate **13** [prepared from **5a** (8 g, 21.2 mmol)], uracil (3.08 g, 27.5 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (13.7 ml, 55.2 mmol) in dry CH_3CN (80 ml) was stirred at 90 °C for 1 h under a nitrogen atmosphere. After cooling to 0 °C, TMSOTf (4.96 ml, 27.5 mmol) was added dropwise over 5 min. The mixture was stirred for 15 min at 0 °C and then 4 h at RT. Then saturated aqueous $NaHCO_3$ (200 ml) and CH_2Cl_2 (150 ml) were added and the precipitated unreacted uracil was removed by filtration of the mixture through a dense sintered filtration funnel. The organic phase was separated and the aqueous phase was re-extracted with CH_2Cl_2 (2 × 50 ml). The combined organic extracts were dried over $MgSO_4$, evaporated and chromatographed on silica (petroleum ether–EtOAc, 1 : 1, sample was loaded on silica as concentrated CH_2Cl_2 solution) providing product **14a** as a colorless solid (7.7 g, 77% in 2 steps from **5a**, product contains 9% of α-anomer). Crystallization from hot methanol (45 ml) afforded pure β-anomer **14a** as colorless needles (5.43 g, 54% in 2 steps from **5a**). Mp 193–195 °C (MeOH). R_f = 0.67 (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* $[M–H]^-$ 473.09 (Calcd. 473.16 for $C_{23}H_{25}N_2O_9$). ^{1}H NMR (600 MHz, $CDCl_3$): 9.06 (br s, 1H, N*H*), 7.72 (d, 2H, J = 8.1 Hz, Tol), 7.61 (d, 1H, J_{5-6} = 8.3 Hz, H-6), 7.22 (d, 2H, Tol), 5.48 (d, 1H, H-5), 5.43 (d, 1H, $J_{3'-4'}$ = 6.2 Hz, H-3′), 4.90 (dd, 1H, $J_{4'-5'}$ = 1.4 Hz, H-4′), 4.84 (ddd, 1H, $J_{5'-6'}$ = 2.7 Hz, $J_{5'-6''}$ = 3.4 Hz, H-5′), 4.83 (d, 1H, J_{gem} = 12.2 Hz, H-1′), 4.58 (dd, 1H, J_{gem} = 12.6 Hz, H-6′), 4.39 (d, 1H, H-1″), 4.37 (dd, 1H, H-6″), 2.38 (s, 3H, CH_3, Tol), 1.99 (s, 3H, CH_3, Ac), 1.63, 1.41 (2 × s, 2 × 3H, 2 × CH_3, i-Pr). ^{13}C NMR (67.9 MHz, $CDCl_3$): 170.0, 165.9 (2 × C=O), 163.2 (C-4), 150.2 (C-2), 144.9 (Tol), 140.4 (C-6), 129.5, 129.2, 126.1 (3 × Tol), 113.9 (*C*–Me_2), 101.1 (C-5), 99.6 (C-2′), 86.6 (C-3′), 84.0 (C-5′), 81.7 (C-4′), 64.7 (C-1′), 64.4 (C-6′), 25.9, 24.5 (2 × *C*H_3, i-Pr), 21.7 (*C*H_3, Tol), 20.7 (*C*H_3, Ac).

1-[1′-*O*-Acetyl-3′,4′-*O*-isopropylidene-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]-N^4-benzoyl cytosine (14b). This was prepared from diacetate **13** according to the procedure for the preparation of compound **14a**. The reaction of diacetate **13** [prepared from compound **5a** (500 mg, 1.32 mmol)] and N^4-benzoylcytosine (370 mg, 1.72 mmol) afforded psicocytidine **14b** (562 mg, 74% in 2 steps from **5a**, product contains 10% of α-anomer). Crystallization from hot MeOH provided pure β-anomer **14b** (250 mg, 33% in 2 steps from **5a**). Mp 200–201 °C. R_f = 0.80

(CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* $[M + H]^+$ 578.19 (Calcd. 578.21 for $C_{30}H_{32}N_3O_9$). ^1H NMR (600 MHz, $CDCl_3$): 8.50 (br s, 1H, N*H*), 8.04–7.96 (m, 1H, H-6), 7.87–7.78 (m, 2H, Bz), 7.64–7.49 (m, 5H, Bz and Tol), 7.30–7.22 (m, 1H, H-5), 7.10 (d, 2H, $J = 8.0$ Hz, Tol), 5.51 (d, 1H, $J_{3'-4'} = 6.2$ Hz, H-3′), 4.93 (dd, 1H, $J_{4'-5'} = 1.2$ Hz, H-4′), 4.90–4.87 (m, 2H, H-1′ and H-5′), 4.68 (dd, 1H, $J_{gem} = 12.6$ Hz, $J_{5'-6'} = 2.5$ Hz, H-6′), 4.57 (d, 1H, $J_{gem} = 12.1$ Hz, H-1′), 4.29 (dd, 1H, $J_{5'-6''} = 3.1$ Hz, H-6″), 2.16 (s, 3H, C*H*$_3$, Tol), 1.96 (s, 3H, C*H*$_3$, Ac), 1.67, 1.44 (2 × s, 2 × 3H, 2 × C*H*$_3$, i-Pr). ^{13}C NMR (67.9 MHz, $CDCl_3$): 169.9, 165.7, 162.2 (3 × C=O), 145.3 (br, C-6), 144.3 (Tol), 133.2 (Bz), 129.25, 129.21, 129.0 (1 × Bz and 2 × Tol), 127.5 (Bz), 126.1 (Tol), 113.8 (*C*–Me$_2$), 100.4 (C-2′), 95.8 (br, C-5), 86.4 (C-3′), 84.0 (C-5′), 81.7 (C-4′), 64.7 (C-6′), 64.4 (C-1′), 25.9, 24.5 (2 × *C*H$_3$, i-Pr), 21.5 (*C*H$_3$, Tol), 20.7 (*C*H$_3$, Ac).

9-[1′-*O*-Acetyl-3′,4′-*O*-isopropylidene-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]-N^6-benzoyladenine (14c). A mixture of diacetate **13** [prepared from compound **5a** (378 mg, 1 mmol)], N^6-benzoyladenine (287 mg, 1.2 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (0.59 ml, 2.4 mmol) in dry CH_3CN (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 30 min at 90 °C. After cooling, saturated aqueous $NaHCO_3$ (20 ml) was added and the mixture was extracted with CH_2Cl_2 (20 ml, 2 × 10 ml). The combined organic extracts were dried over $MgSO_4$, 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 H-8 signal while H-3′ was irradiated. $R_f = 0.70$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* $[M + H]^+$ 602.2 (Calcd. 602.2 for $C_{31}H_{32}N_5O_8$). ^1H NMR (600 MHz, $CDCl_3$): 8.89 (s, 1H, H-2), 8.86 (br s, 1H, N*H*), 8.15 (s, 1H, H-8), 7.99–6.88 (m, 9H, Tol and Bz), 6.14 (d, 1H, $J_{3'-4'} = 6.0$ Hz, H-3′), 5.05 (d, 1H, $J_{4'-5'} = 1.2$ Hz, H-4′), 4.93 (ddd, 1H, $J_{5'-6'} = 2.7$ Hz, $J_{5'-6''} = 3.5$ Hz, H-5′), 4.67 (dd, 1H, $J_{gem} = 12.7$ Hz, H-6′), 4.63 (ABq, 2H, $J_{gem} = 12.1$ Hz, H-1′ and H-1″), 4.29 (dd, 1H, H-6″), 2.26 (s, 3H, C*H*$_3$, Tol), 1.86 (s, 3H, C*H*$_3$, Ac), 1.70, 1.51 (2 × s, 2 × 3H, 2 × C*H*$_3$, i-Pr). ^{13}C NMR (150.9 MHz, $CDCl_3$): 169.7, 165.5, 164.0 (3 × C=O), 152.8 (C-2), 149.3 (C-4), 144.7 (Tol), 141.7 (C-8), 133.7 (Bz), 132.8 (Bz), 128.94, 128.91, 128.7, 127.7, 125.4, 123.2, 114.4 (*C*–Me$_2$), 98.9 (C-2′), 85.2 (C-3′), 84.6 (C-5′), 82.3 (C-4′), 65.5 (C-1′), 64.6 (C-6′), 26.1, 24.7 (2 × *C*H$_3$, i-Pr), 21.6 (*C*H$_3$, Tol), 20.5 (*C*H$_3$, Ac).

N^2-Acetyl-9-[1′-*O*-acetyl-3′,4′-*O*-isopropylidene-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]-O^6-diphenylcarbamoylguanine (14d). A mixture of diacetate **13** [prepared from compound **5a** (378 mg, 1 mmol)], N^2-acetyl-O^6-diphenylcarbamoylguanine (410 mg, 1.2 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (0.59 ml, 2.4 mmol) in dry CH_3CN (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 $NaHCO_3$ (20 ml) was added and the mixture was extracted with CH_2Cl_2 (20 ml, 2 × 10 ml). Combined organic extracts were dried over $MgSO_4$, evaporated and chromatographed on silica (c-hexane–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. $R_f = 0.90$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* $[M + H]^+$ 751.24 (Calcd. 751.27 for $C_{39}H_{39}N_6O_{10}$). ^1H NMR (600 MHz, $CDCl_3$): 8.39 (br s, 1H, N*H*), 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, $J_{3'-4'} = 5.5$ Hz, H-3′), 5.00 (dd, 1H, $J_{4'-5'} = 1.8$ Hz, H-4′), 4.86 (m, 1H, H-5′), 4.60–4.52 (m, 3H, H-1′, H-1″ and H-6′), 4.38 (dd, 1H, $J_{gem} = 12.2$ Hz, $J_{5'-6''} = 3.1$ Hz, H-6″), 2.58 (s, 3H, C*H*$_3$, AcNH), 2.19 (s, 3H, C*H*$_3$, Tol), 1.87 (s, 3H, C*H*$_3$, Ac), 1.64, 1.41 (2 × s, 2 × 3H, 2 × C*H*$_3$, i-Pr). ^{13}C NMR (67.9 MHz, $CDCl_3$): 171.4, 169.8, 165.6, 156.1 (3 × CO), 153.5, 151.9, 150.2, 144.4, 142.7 (C-8), 141.8, 129.2, 129.1, 129.0, 127.0 (br), 125.6, 121.7, 114.4 (*C*–Me$_2$), 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 (*C*H$_3$, i-Pr), 25.2 (*C*H$_3$, AcNH), 24.5 (*C*H$_3$, i-Pr), 21.5 (*C*H$_3$, Tol), 20.5 (*C*H$_3$, Ac).

1-[3′,4′-*O*-Isopropylidene-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]-uracil (15). (*Method A*) Nucleoside **14a** (1.5 g, 3.16 mmol) in 16% methanolic ammonia (50 ml) was stirred at ambient temperature for 6 h (after that time TLC indicated nearly complete conversion of the starting material). The mixture was evaporated with silica and chromatographed on silica (CH_2Cl_2 with gradient of MeOH: 0–5%) providing the partially deprotected nucleoside **15** (656 mg, 48%) and diol **16** (437 mg, 44%). Compound **15** is a colorless amorphous solid. $R_f = 0.5$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* $[M–H]^-$ 431.1 (Calcd. 431.1 for $C_{21}H_{23}N_2O_8$). ^1H NMR (600 MHz, $CDCl_3$): 7.90 (br s, 1H, N*H*), 7.72 (d, 2H, $J = 8.0$ Hz, Tol), 7.63 (d, 1H, $J_{5-6} = 8.3$ Hz, H-6), 7.22 (d, 2H, Tol), 5.48 (d, 1H, H-5), 5.44 (d, 1H, $J_{3'-4'} = 6.2$ Hz, H-3′), 4.90 (dd, 1H, $J_{4'-5'} = 1.2$ Hz, H-4′), 4.82 (ddd, 1H, $J_{5'-6'} = 2.7$ Hz, $J_{5'-6''} = 3.4$ Hz, H-5′), 4.59 (dd, 1H, $J_{gem} = 12.6$ Hz, H-6′), 4.38 (dd, 1H, H-6″), 4.16 (m, d after 1′-O*H* decoupling, 1H, $J_{gem} = 12.4$ Hz, H-1′), 3.89 (m, d after 1′-O*H* decoupling, 1H, H-1″), 2.41 (s, 3H, C*H*$_3$, Tol), 2.01 (br s, 1H, O*H*), 1.61, 1.41 (2 × s, 2 × 3H, 2 × C*H*$_3$, i-Pr). ^{13}C NMR (67.9 MHz, $CDCl_3$): 165.9 (C=O), 164.4 (C-4), 150.4 (C-2), 144.8 (Tol), 141.8 (C-6), 129.5, 129.3, 126.1 (3 × Tol), 113.6 (*C*–Me$_2$), 101.6 (C-2′), 100.7 (C-5), 86.0 (C-3′), 83.6 (C-5′), 81.9 (C-4′), 64.5 (C-6′), 64.2 (C-1′), 25.8, 24.5 (2 × *C*H$_3$, i-Pr), 21.8 (*C*H$_3$, Tol).

1-[3′,4′-*O*-Isopropylidene-1′-*O*-methanesulfonyl-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]uracil (17). Compound **15** (2.16 g, 5 mmol) was twice co-evaporated with dry pyridine and dissolved in dry pyridine (30 ml). Methanesulfonyl chloride (0.584 ml, 7.5 mmol) was added dropwise at 0 °C and the mixture was stirred at 0 °C for 2 h. Then saturated aqueous $NaHCO_3$ (20 ml) was added and the mixture was extracted with CH_2Cl_2 (40 ml, 2 × 20 ml). The organic extracts were dried over $MgSO_4$, evaporated and co-evaporated three times with toluene. Crystallization from MeOH–diethyl ether provided **16** as a white crystalline solid (2.31 g, 90%). Purification of the mother liquors by passing through a short column of silica (CH_2Cl_2 with gradient of MeOH: 0–1%) provided additional 130 mg (5%) of product **17**. Total yield is 2.44 g (95%). Mp 156–158 °C (dec.). $R_f = 0.61$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* $[M–H]^-$ 509.1 (Calcd. 509.1 for $C_{22}H_{25}N_2O_{10}S$). ^1H NMR (600 MHz, $CDCl_3$): 8.82 (br s, 1H, N*H*), 7.70 (d, 2H, $J = 8.0$ Hz, Tol), 7.62 (d, 1H, $J_{5-6} = 8.3$ Hz, H-6), 7.22 (d, 2H, Tol), 5.51 (d, 1H, H-5), 5.39 (d, 1H, $J_{3'-4'} = 5.9$ Hz, H-3′), 4.98 (d, 1H, $J_{gem} = 11.5$ Hz, H-1′), 4.91 (dd, 1H, $J_{4'-5'} = 1.2$ Hz, H-4′), 4.90 (ddd, 1H, $J_{5'-6'} = 2.6$ Hz, $J_{5'-6''} = 3.9$ Hz, H-5′), 4.58 (dd, 1H, $J_{gem} = 12.7$ Hz, H-6′), 4.42–4.38 (m, 2H, H-1″ and H-6″), 2.97 (s, 3H, C*H*$_3$, Ms), 2.39 (s, 3H, C*H*$_3$, Tol), 1.62, 1.40 (2 × s, 2 × 3H, 2 × C*H*$_3$, i-Pr). ^{13}C NMR (67.9 MHz, $CDCl_3$): 165.8 (C=O), 162.8 (C-4), 150.3 (C-2), 145.0 (Tol), 140.1 (C-6), 129.5, 129.2, 126.0 (3 × Tol), 114.0 (*C*–Me$_2$), 101.7 (C-5), 98.8 (C-2′), 86.8 (C-3′), 84.3 (C-5′), 81.7 (C-4′), 69.6 (C-1′), 64.3 (C-6′), 37.7 (*C*H$_3$, Ms), 25.9, 24.3 (2 × *C*H$_3$, i-Pr), 21.7 (*C*H$_3$, Tol).

1-[1′-*O*-Methanesulfonyl-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]-uracil (18). The mesylate **17** (2.60 g, 5.09 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 CH_2Cl_2 (70 ml) and washed with saturated aqueous $NaHCO_3$ (20 ml). The aqueous phase was re-extracted with CH_2Cl_2 (2 × 15 ml). Combined organic phases were dried over $MgSO_4$ 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. $R_f = 0.4$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* $[M + H]^+$ 471.1 (Calcd. 471.1 for $C_{19}H_{23}N_2O_{10}S$). ^1H NMR (600 MHz, $CDCl_3$):

10.2 (br s, 1H, N*H*), 7.79–7.75 (m, 3H, Tol and H-6), 7.19 (d, 2H, $J = 8.1$ Hz, Tol), 5.61 (d, 1H, $J_{5\text{–}6} = 8.3$ Hz, H-5), 5.50–5.00 (br m, 1H, O*H*), 4.88 (d, 1H, $J_{gem} = 11.7$ Hz, H-1′), 4.84 (d, 1H, $J_{3'\text{–}4'} = 4.4$ Hz, H-3′), 4.65–4.55 (m, 3H, H-6′, H-1″ and H4′), 4.32–4.35 (m, 2H, H-6″ and H-5′), 4.10–3.10 (br m, 1H, O*H*), 2.96 (s, 3H, CH_3, Ms), 2.35 (s, 3H, CH_3, Tol). ^{13}C NMR (67.9 MHz, $CDCl_3$ + CD_3OD): 166.3 (C=O), 164.0 (C-4), 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 (*C*H_3, Ms), 21.7 (*C*H_3, Tol).

1-[1′,3′-*O*-Anhydro-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]uracil (19). Sodium bis(trimethylsilyl)amide (10.2 ml, 10.2 mmol, 1 M THF sol.) was added dropwise to a stirred solution of dry diol **18** [prepared from compound **17** (2.60 g, 5.09 mmol)] in dry THF (50 ml) at −15 °C. The mixture was stirred for 2 h at 0 °C and then CH_2Cl_2 (100 ml) and a solution of AcOH (1 ml) in water (50 ml) were added. The organic phase was separated and the aqueous phase was re-extracted with CH_2Cl_2 (2 × 25 ml). Collected organic phases were dried over $MgSO_4$, evaporated and purified by chromatography on a short column of silica (CH_2Cl_2 with gradient of MeOH: 0–2%) affording oxetane **19** as a white solid (1.44 g, 76% in 2 steps from **17**). Mp 184–185 °C. $R_f = 0.4$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M–H]$^-$ 373.1 (Calcd. 373.1 for $C_{18}H_{17}N_2O_7$). ^{1}H NMR (600 MHz, d_6-DMSO): 7.91 (d, 2H, $J = 7.9$ Hz, Tol), 7.56 (d, 1H, $J_{5\text{–}6} = 7.9$ Hz, H-6), 7.37 (d, 2H, Tol), 5.66 (d, 1H, H-5), 5.53 (d, 1H, $J_{4'\text{–}OH} = 6.6$ Hz, 4′-O*H*), 5.37 (d, 1H, $J_{3'\text{–}4'} = 3.7$ Hz, H-3′), 5.08 (d, 1H, $J_{gem} = 8.2$ Hz, H-1′), 4.70–4.64 (m, 2H, H-1″ and H-6′), 4.47–4.43 (m, 1H, H-5′), 4.39 (dd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'\text{–}6''} = 5.0$ Hz, H-6″), 4.44–4.34 (m, 1H, H-4′), 2.42 (s, 3H, CH_3, Tol). ^{13}C NMR (67.9 MHz, d_6-DMSO): 166.5 (C=O), 164.2 (C-4), 150.2 (C-2), 144.7 (Tol), 142.5 (C-6), 130.3, 130.2, 127.6 (3 × Tol), 103.1 (C-5), 92.2 (C-2′), 87.6 (C-3′), 81.1 (C-5′), 78.7 (C-1′), 70.8 (C-4′), 64.1 (C-6′), 22.1 (*C*H_3, Tol).

1-[1′,3′-*O*-Anhydro-β-D-psicofuranosyl]uracil (20). The mixture of 6′-*O*-toluoyl oxetane **19** (1.37 g, 3.66 mmol) was stirred with 25% methanolic ammonia (60 ml) at RT for 48 h. The volatiles were removed under reduced pressure and the rest was evaporated with silica and final chromatography on a column of silica (CH_2Cl_2 with gradient of MeOH: 0–8%) afforded oxetane **20** (769 mg, 82%) as an amorphous glassy solid. $R_f = 0.1$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M–H]$^-$ 255.1 (Calcd. for 255.1 $C_{10}H_{11}N_2O_6$). ^{1}H NMR (600 MHz, d_6-DMSO): 7.46 (d, 1H, $J_{5\text{–}6} = 7.9$ Hz, H-6), 5.64 (d, 1H, H-5), 5.28 (d, 1H, $J_{3'\text{–}4'} = 3.9$ Hz, H-3′), 5.03 (d, 1H, $J_{gem} = 8.1$ Hz, H-1′), 4.60 (d, 1H, H-1″), 4.12–4.08 (m, 1H, H-5′), 4.06 (dd, 1H, $J_{4'\text{–}5'} = 8.6$ Hz, H-4′), 3.77 (dd, 1H, $J_{gem} = 12.2$ Hz, $J_{5'\text{–}6'} = 1.5$ Hz, H-6′), 3.52 (dd, 1H, $J_{5'\text{–}6''} = 5.6$ Hz, H-6″). ^{13}C NMR (67.9 MHz, d_6-DMSO): 164.9 (C-4), 150.7 (C-2), 142.4 (C-6), 103.0 (C-5), 91.9 (C-2′), 87.9 (C-3′), 84.3 (C-5′), 78.9 (C-1′), 70.7 (C-4′), 61.6 (C-6′).

1-[1′,3′-*O*-Anhydro-6′-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]uracil (21). The oxetane **20** (700 mg, 2.73 mmol) was co-evaporated with dry pyridine (3 times) and dissolved in dry pyridine (10 ml) and DMTr-Cl (972 mg, 2.87) was added. The mixture was stirred at RT for 24 h. Saturated aqueous $NaHCO_3$ (40 ml) was added and the product was extracted with CH_2Cl_2 (40 ml, then 3 × 10 ml). Combined organic extracts were dried under $MgSO_4$, evaporated and co-evaporated with toluene (3 times). Column chromatography on silica (0.5% triethylamine in CH_2Cl_2 with gradient of MeOH: 0–0.75%) provided product **21** (1.39 g, 91%) as a white solid. $R_f = 0.46$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M–H]$^-$ 557.2 (Calcd. 557.2 for $C_{31}H_{29}N_2O_8$). ^{1}H NMR (600 MHz, $CDCl_3$): 7.45–7.14 (m, 10H, DMTr and N*H*), 6.90 (d, 1H, $J_{5\text{–}6} = 7.8$ Hz, H-6), 6.81–6.76 (m, 4H, DMTr), 5.90 (br s, 1H, 4′-O*H*), 5.72 (d, 1H, H-5), 5.40 (d, 1H, $J_{3'\text{–}4'} = 4.0$ Hz, H-3′), 5.10 (d, 1H, $J_{gem} = 7.9$ Hz, H-1′), 4.73 (d, 1H, H-1″), 4.37 (dd, 1H, $J_{4'\text{–}5'} = 8.0$ Hz, H-4′), 4.31 (ddd, 1H, $J_{5'\text{–}6'} = 2.7$ Hz, $J_{5'\text{–}6''} = 5.5$ Hz H-5′), 3.74 (s, 6H, OCH_3, DMTr), 3.49 (dd, 1H, $J_{gem} = 10.6$ Hz, H-6′), 3.40 (dd, 1H, H-6″). ^{13}C NMR (67.9 MHz, $CDCl_3$): 163.7 (C-4), 158.5 (DMTr), 149.3 (C-2), 144.8 (DMTr), 139.8 (C-6), 135.9, 130.2, 128.3, 127.8, 126.8, 113.1 (6 × DMTr), 103.3 (C-5), 91.2 (C-2′), 87.7 (C-3′), 86.3 (Ar_3–*C*, DMTr), 83.6 (C-5′), 78.5 (C-1′), 71.4 (C-4′), 63.2 (C-6′), 55.2 (O*C*H_3, DMTr).

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 acetone 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.* CH_2Cl_2, EtOAc, diethyl ether) forms gels. $R_f = 0.20$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M–H]$^-$ 313.1 (Calcd. 313.1 for $C_{13}H_{17}N_2O_7$). ^{1}H NMR (600 MHz, d_6-DMSO): 11.15 (br s, 1H, N*H*), 7.78 (d, 1H, $J_{5\text{–}6} = 8.2$ Hz, H-6), 5.49 (d, 1H, H-5), 5.24 (d, 1H, $J_{3'\text{–}4'} = 6.1$ Hz, H-3′), 5.04 (t, 1H, $J = 4.7$ Hz, 6′-O*H*), 4.94 (t, 1H, $J = 6.4$ Hz, 1′-O*H*), 4.72 (dd, 1H, $J_{4'\text{–}5'} = 1.1$ Hz, H-4′), 4.35 (m, 1H, H-5′), 4.04 (dd, 1H, $J_{gem} = 12.0$ Hz, H-1′), 3.51 (dd, 1H, H-1″), 3.46–3.42 (m, 2H, H-6′ and H-6″), 1.47, 1.30 (2 × s, 2 × 3H, 2 × CH_3, i-Pr). ^{13}C NMR (67.9 MHz, d_6-DMSO): 164.9 (C-4), 151.5 (C-2), 143.6 (C-6), 112.3 (*C*–Me_2), 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 × *C*H_3, i-Pr).

1-[3′,4′-*O*-Isopropylidene-1′,6′-di-*O*-methanesulfonyl-β-D-psicofuranosyl]uracil (22). Diol **16** (4.95 g, 15.75 mmol) was twice co-evaporated with dry pyridine, dissolved in dry pyridine (50 ml) and methanesulfonyl chloride (3.7 ml, 47.50 mmol) was added dropwise at 0 °C and the mixture was stirred for 2 h at 0 °C. Then saturated aqueous $NaHCO_3$ (100 ml) was added and the mixture was extracted with CH_2Cl_2 (100 ml, 2 × 25 ml). The combined organic extracts were dried over $MgSO_4$, evaporated and co-evaporated 3 times with toluene. The product was purified by passing through a short column of silica (CH_2Cl_2 with gradient of MeOH: 0–2%). Product **22** was obtained as an amorphous yellowish solid (6.9 g, 93%). $R_f = 0.54$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M–H]$^-$ 469.0 (Calcd. 469.1 for $C_{15}H_{21}N_2O_{11}S_2$). ^{1}H NMR (600 MHz, $CDCl_3$): 8.18 (br s, 1H, N*H*), 7.70 (d, 1H, $J_{5\text{–}6} = 8.4$ Hz, H-6), 5.75 (d, 1H, H-5), 5.26 (d, 1H, $J_{3'\text{–}4'} = 6.4$ Hz, H-3′), 4.97 (d, 1H, $J_{gem} = 11.6$ Hz, H-1′), 4.82 (dd, 1H, $J_{4'\text{–}5'} = 1.7$ Hz, H-4′), 4.80–4.73 (m, 1H, H-5′), 4.50–4.30 (m, 3H, H-1″, H-6′ and H-6″), 3.01, 3.00 (2 × s, 2 × 3H, 2 × CH_3, Ms), 1.60, 1.38 (2 × s, 2 × 3H, 2 × *C*H_3, i-Pr). ^{13}C NMR (67.9 MHz, $CDCl_3$): 163.9 (C-4), 150.5 (C-2), 140.9 (C-6), 114.3 (*C*–Me_2), 101.5 (C-5), 98.5 (C-2′), 86.3 (C-3′), 83.8 (C-5′), 81.0 (C-4′), 69.3 (C-1′), 67.7 (C-6′), 37.7, 37.6 (2 × *C*H_3, Ms), 25.9, 24.3 (2 × *C*H_3, i-Pr).

1-[2,1′-Anhydro-3′,4′-*O*-isopropylidene-6′-*O*-methanesulfonyl-β-D-psicofuranosyl]uracil (25). To a solution of dimesylate **22** (3.76 g, 8 mmol) in dry CH_3CN (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 CH_2Cl_2 (3 × 5 ml) affording pure 2,1′-anhydronucleoside **25** (2.49 g, 83%). Mp 194–195 °C (dec.). $R_f = 0.42$ (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M–H]$^-$ 373.1 (Calcd. 373.1 for $C_{14}H_{17}N_2O_8S$). ^{1}H NMR (600 MHz, d_6-DMSO): 7.88 (d, 1H, $J_{5\text{–}6} = 7.6$ Hz, H-6), 5.99 (d, 1H, H-5), 5.31 (d, 1H, $J_{3'\text{–}4'} = 6.3$ Hz, H-3′), 5.08 (dd,

1H, $J_{4'-5'}$ = 3.9 Hz, H-4′), 4.91 (d, 1H, J_{gem} = 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, 1.39 (2 × s, 2 × 3H, 2 × CH_3, i-Pr). ^{13}C NMR (150.9 MHz, d_6-DMSO): 171.5 (C-4), 160.7 (C-2), 135.7 (C-6), 115.2 (*C*–Me_2), 110.3 (C-5), 99.6 (C-2′), 83.0 (C-5′), 82.6 (C-3′), 80.6 (C-4′), 73.7 (C-1′), 69.7 (C-6′), 37.7 (*C*H_3, Ms), 27.1, 25.9 (2 × *C*H_3, i-Pr).

1-[2,1′-Anhydro-3′,4′-*O*-isopropylidene-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]uracil (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 to the residue CH_2Cl_2 (10 ml) and saturated aqueous $NaHCO_3$ (10 ml) were added. The organic phase was separated and the aqueous phase was re-extracted with CH_2Cl_2 (2 × 5 ml). The combined organic extracts were dried over $MgSO_4$and evaporated and final chromatography on a short column of silica (CH_2Cl_2 with gradient of MeOH: 0–2%) afforded nucleoside **26** (260 mg, 94%) as a colorless amorphous glassy solid. R_f = 0.49 (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M + H]$^+$ 415.14 (Calcd. 415.15 for $C_{21}H_{23}N_2O_7$). ^{1}H NMR (600 MHz, $CDCl_3$): 7.90 (d, 2H, *J* = 8.3 Hz, Tol), 7.31 (d, 1H, J_{5-6} = 7.6 Hz, H-6), 7.28 (d, 2H, Tol), 5.83 (d, 1H, H-5), 5.12–5.07 (m, 2H, H-3′ and H-4′), 5.03 (d, 1H, J_{gem} = 10.6 Hz, H-1′), 4.63 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5'-6'}$ = 3.6 Hz, H-6′), 4.54 (d, 1H, H-1″), 4.49 (dd, 1H, $J_{5'-6''}$ = 4.4 Hz, H-6″), 4.43 (ddd, 1H, H-5′), 2.43 (s, 3H, CH_3, Tol), 1.59, 1.42 (2 × s, 2 × 3H, 2 × CH_3, i-Pr). ^{13}C NMR (67.9 MHz, $CDCl_3$): 171.6 (C-4), 166.1 (C=O), 160.0 (C-2), 144.7 (Tol), 132.8 (C-6), 129.7, 129.5, 126.5 (3 × Tol), 115.7 (*C*–Me_2), 110.9 (C-5), 98.9 (C-2′), 83.2 (C-5′), 82.9 (C-3′), 80.4 (C-4′), 72.9 (C-1′), 63.3 (C-6′), 26.6, 25.1 (2 × *C*H_3, i-Pr), 21.8 (*C*H_3, Tol).

1-[3′,4′-*O*-Isopropylidene-6′-*O*-methanesulfonyl-β-D-psicofuranosyl]uracil (27). To a stirred mixture of 2,1′-anhydro-nucleoside **25** (4.24 g, 11.32 mmol) in dioxane, (60 ml) 1 M aq. NaOH (11.4 ml, 11.4 mmol) was added and the mixture was stirred for 1 h at ambient temperature. The resulting solution was neutralized by the addition of Dowex 50 (pyridinium form), the resin was filtered off, washed by methanol and the filtrate was evaporated *in vacuo* to dryness and co-evaporated twice with toluene. The residual white foam crystallized after addition of MeOH (*ca.* 15 ml) and the separated white crystalline solid was collected by suction filtration, washed with methanol (2 × 3 ml) and dried in a vacuum oven at 60 °C. Yield 4.09 g (92%). Mp 150–151 °C (dec.). R_f = 0.35 (CH_2Cl_2–MeOH, 10:1). MALDI-TOF *m/z* [M–H]$^-$ 391.07 (Calcd. 391.08 for $C_{14}H_{19}N_2O_9S$). ^{1}H NMR (600 MHz, d_6-DMSO): 7.67 (d, 1H, J_{5-6} = 8.3 Hz, H-6), 5.53 (dd, 1H, $J_{5\text{-}NH}$ = 2.4 Hz, H-5), 5.33 (d, 1H, $J_{3'-4'}$ = 6.1 Hz, H-3′), 5.03 (br s, 1H, O*H* or N*H*), 4.78 (d, 1H, $J_{4'-5'}$ = 1.1 Hz, H-4′), 4.65 (ddd, 1H, $J_{5'-6'}$ = 4.0 Hz, $J_{5'-6''}$ = 6.1 Hz, H-5′), 4.34 (dd, 1H, J_{gem} = 10.9 Hz, H-6′), 4.28 (dd, 1H, H-6″), 4.13 (br s, 1H, O*H* or N*H*), 4.02 (d, 1H, J_{gem} = 11.9 Hz, H-1′), 3.53 (d, 1H, H-1″), 3.24 (s, 3H, CH_3, Ms), 1.49, 1.32 (2 × s, 2 × 3H, 2 × CH_3, i-Pr). ^{13}C NMR (67.9 MHz, d_6-DMSO): 164.8 (C-4), 151.5 (C-2), 142.9 (C-6), 112.8 (*C*–Me_2), 101.9 (C-2′), 100.5 (C-5), 85.9 (C-3′), 83.2 (C-5′), 81.8 (C-4′), 69.5 (C-6′), 63.1 (C-1′), 37.5 (*C*H_3, Ms) 26.6, 25.3 (2 × *C*H_3, i-Pr).

1-[3′,4′-*O*-Isopropylidene-6′-*O*-(4-toluoyl)-β-D-psicofuranosyl]-uracil (15). (*Method B*) A mixture of 6′-*O*-mesylate **27** (4.09 g, 10.42 mmol) and sodium 4-toluate (3.29 g, 20.84 mmol) in DMF (60 ml) was stirred at 90 °C for 15 h. After cooling to RT the DMF was removed *in vacuo* and the residue dissolved in CH_2Cl_2 (100 ml) and saturated aqueous $NaHCO_3$ (100 ml). The organic phase was separated and the aqueous phase was re-extracted with CH_2Cl_2 (2 × 25 ml). The combined organic extracts were dried over $MgSO_4$, evaporated and final chromatography on a short column of silica (CH_2Cl_2 with gradient of MeOH: 0–2%) afforded nucleoside **15** (3.97 g, 88%). The analytical data of this product are in agreement with those reported for compound **15** prepared by method A (see above).

6-*O*-Benzyl-1,3,4-tri-*O*-(4-toluoyl)-D-psicofuranose (29). The sugar **5b** (7 g, 20 mmol) in 70% aqueous AcOH (100 ml) was stirred at 70 °C for 5 h. After cooling, the reaction mixture was evaporated *in vacuo* and the residue was co-evaporated 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-Toluoylchloride (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 dried over $MgSO_4$, filtered, evaporated and co-evaporated with toluene. Column chromatography (c-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 $C_{37}H_{36}O_9Na$). ^{13}C 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, 137.69, 136.90, 136.84, 130.14, 129.84, 129.82, 129.78, 129.69, 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, 126.76, 126.73, 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, 65.57, 64.89, 21.68, 21.62, 21.57.

2-*O*-Acetyl-6-*O*-benzyl-1,3,4-tri-*O*-(4-toluoyl)-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, evaporated and co-evaporated with toluene. Column chromatography (c-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 $C_{39}H_{38}O_{10}Na$). ^{13}C NMR (anomeric mixture, 150.9 MHz, $CDCl_3$): 171.40, 168.80, 168.70, 165.63, 165.61, 165.50, 165,40, 164.70, 164.60, 149.00, 144.40, 144.30, 144.10, 144.00, 143.80, 143.60, 137.80, 137.60, 136.90, 130.20, 129.80, 129.74, 129.72, 129.66, 129.64, 129.30, 129.24, 129.23, 129.20, 129.12, 129.11, 129.04, 129.03, 128.94, 128,93, 128.46, 128.43, 127.90, 127.70, 127.60, 127.50, 126.75, 126.72, 126.60, 126.30, 126.20, 108.60, 106.50, 83.80, 82.40, 74.90, 73.70, 73.50, 72.90, 71.40, 70.90, 68.95, 68.93, 63.40, 61.40, 21.80, 21.70, 21.60, 21.50.

***N*^{2}-Acetyl-9-[6′-*O*-benzyl-1′,3′,4′-tri-*O*-(4-toluoyl)-D-psicofuranosyl]-*O*^{6}-diphenylcarbamoylguanine (31a).** Sugar **30** (6.6 g, 10 mmol) was dried by co-evaporation with dry CH_3CN, dissolved in CH_3CN (100 ml) and *N*^{2}-acetyl-*O*^{6}-diphenylcarbamoylguanine (4.1 g, 12 mmol) and *N*,*O*-bis(trimethylsilyl)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 cooled and TMSOTf (2 ml, 11 mmol) was added at RT and again heated at 80 °C for 1.5 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, filtered, and evaporated. Column chromatography (c-hexane with gradient of EtOAc: 0–40%) afforded **31a** (β : α, 7 : 3; 6.2 g, 6.2 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 (c-hexane–EtOAc, 6 : 4). MALDI-TOF *m/z* [M + Na]$^+$ 1017.3 (Calcd. 1017.3 for $C_{57}H_{50}N_6O_{11}Na$). Anomeric mixture 3 : 7, NMR data is given only for β-anomer. ^{1}H NMR (600 MHz, $CDCl_3$): 8.35 (s, 1H, H-8), 7.93–7.02 (m, 27H, Tol, DPC and Bn), 6.68 (d, 1H, $J_{3'-4'}$ = 5.4 Hz, H-3′), 5.86 (dd, 1H, $J_{4'-5'}$ = 3.8 Hz, H-4′), 5.27 (d, 1H, J_{gem} = 12.0 Hz,

H-1′), 5.00 (d, 1H, H-1″), 4.77 (m, 1H, H-5′), 4.48 (d, 1H, J_{gem} = 11.6 Hz, CH_2Ph), 4.40 (d, 1H, CH_2Ph), 3.82 (dd, 1H, J_{gem} = 10.8 Hz, $J_{6'-5'}$ = 2.1 Hz, H-6′), 3.70 (dd, 1H, $J_{6'-5'}$ = 2.8 Hz, H-6″), 2.44–2.19 (4 × s, 4 × 3H, 3 × CH_3, Tol and 1 × CH_3, Ac). ^{13}C NMR (150.9 MHz, $CDCl_3$): 165.3, 164.5, 165.3, 164.5, 156.0 (C-2), 153.5, 151.7, 144.9, 150.2, 144.9, 144.4, 144.1, 136.6, 142.7 (C-2), 129.8, 129.8, 129.2, 129.2, 129.1, 128.3, 127.8, 96.2 (C-2′), 83.9 (C-5′), 76.0 (C-3′), 73.7 (*C*H_2Ph), 72.6 (C-4′), 68.8 (C-6′), 64.3 (C-1′), 24.9, 21.7, 21.6 (3 × *C*H_3, Tol and 1 × *C*H_3, Ac).

N^6-Benzoyl-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 CH_3CN and N^6-benzoyladenine (5.7 g, 24 mmol), *N*,*O*-bis(trimethylsilyl)acetamide (10 ml, 40 mmol) and CH_3CN (200 ml) were added and the reaction mixture was heated at 90 °C for 1.5 h under a nitrogen atmosphere. The reaction mixture was cooled to RT and TMSOTf (2 ml, 11 mmol) was added at RT, and again heated at 80 °C for 1.5 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, 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. R_f = 0.25 (c-hexane–EtOAc, 6 : 4). MALDI-TOF $[M + H]^+$ *m*/*z* 846.3 (Calcd. 846.3 for $C_{49}H_{44}N_5O_9$). Anomeric mixture 3 : 7, NMR data is given only for the β-anomer. ^{1}H NMR (600 MHz, $CDCl_3$): 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, $J_{3'-4'}$ = 5.4 Hz, H-3′), 5.91 (dd, 1H, $J_{4'-5'}$ = 3.6, H-4′), 4.80 (m, 1H, H-5′), 5.33 (d, 1H, J_{gem} = 12.0 Hz, H-1′), 5.16 (d, 1H, H-1″), 4.54 (d, 1H, J_{gem} = 12.0 Hz, CH_2Ph), 4.43 (d, 1H, CH_2Ph), 3.86 (dd, 1H, J_{gem} = 11.0 Hz, $J_{5'-6'}$ = 2.5 Hz, H-6′), 3.75 (dd, 1H, H-6″), 2.43, 2.39, 2.34 (3 × s, 3 × CH_3, Tol). ^{13}C NMR (150.9 MHz, $CDCl_3$): 165.7, 165.4, 164.6, 164.3, 152.5 (C-2), 150.7, 149.1 (C-4), 144.4, 144.1, 141.9 (C-8), 136.8, 133.9, 132.7, 132.6, 129.9, 129.8, 129.7, 129.3, 129.2, 129.1, 128.9, 128.6, 128.3, 127.9, 127.8, 127.6, 96.3 (C-2′), 84.0 (C-5′), 76.2 (C-3′), 73.7, 72.7 (C-4′), 68.9 (*C*H_2Ph), 64.6 (C-1′), 21.75, 21.74, 21.62 (3 × *C*H_3, Tol).

N^4-Benzoyl-9-[6′-*O*-benzyl-1′,3′,4′-tri-*O*-(4-toluoyl)-D-psicofuranosyl]cytosine (31c). Sugar **30** (1.9 g, 2.9 mmol) was dried by co-evaporation with dry CH_3CN, dissolved in CH_3CN (28 ml), after which N^4-benzoylcytosine (0.75 g, 3.5 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (0.6 ml, 2.4 mmol) were 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 mixture was stirred at 40 °C for 2.5 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, filtered and evaporated. Column chromatography (c-hexane with gradient of EtOAc: 0–40%) afforded **31c** (β : α, 9 : 1; 1.5 g, 1.82 mmol, 64%). A 1D differential NOE experiment showed a 1.9% enhancement of the H-6 signal, while H-3′ was irradiated. R_f = 0.55 (CH_2Cl_2–MeOH, 95 : 5). MALDI-TOF *m*/*z* $[M + H]^+$ 822.4 (Calcd. 822.3 for $C_{48}H_{44}N_3O_{10}$). ^{1}H NMR (600 MHz, $CDCl_3$): 8.59–6.79 (m, 24H, C-5, C-6, Bn, Bz and 3 × Tol), 6.53 (d, 1H, $J_{3'-4'}$ = 5.6 Hz, H-3′), 5.88 (dd, 1H $J_{4'-5'}$ = 4.6 Hz, H-4′), 5.34 (d, 1H, J_{gem} = 11.7 Hz, H-1′), 5.23 (d, 1H, H-1″), 4.78 (m, 1H, H-5′), 4.55 (ABq, 2H, J_{gem} = 11.4 Hz, CH_2Ph), 3.82 (m, 2H, H-6′ and H-6″), 2.47, 2.44, 2.43 (3 × s, 3 × 3H, 3 × CH_3, Tol). ^{13}C NMR (150.9 MHz, $CDCl_3$): 165.7, 165.4, 164.6 (3 × C=O, Tol), 144.4, 144.1, 141.9, 136.8, 132.7, 129.9, 129.8, 129.7, 129.3, 129.1, 128.3, 127.6, 96.3 (C-2′), 84.0 (C-5′), 76.6 (C-3′), 73.7 (*C*H_2Ph), 72.7 (C-4′), 68.9 (C-6′), 64.6 (C-1′), 21.7 (*C*H_3, Tol).

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 CH_3CN, 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 $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, 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. R_f = 0.57 (c-hexane–EtOAc 65 : 35). MALDI-TOF *m*/*z* $[M + Na]^+$ 741.2. (Calcd. 741.2 for $C_{41}H_{38}N_2O_{10}Na$). ^{1}H NMR (600 MHz, $CDCl_3$): 8.67 (br s, 1H, N*H*), 7.91 (d, 1H, J_{5-6} = 8.16 Hz, H-6), 7.87–7.76 (m, 6H, Tol), 7.37–7.25 (m, 5H, Bn), 7.19–7.12 (m, 6H, Tol), 6.42 (d, 1H, $J_{3'-4'}$ = 5.4 Hz, H-3′), 5.83 (dd, 1H, $J_{4'-5'}$ = 2.9 Hz, H-4′), 5.49 (d, H-5), 5.14 (d, 1H, J_{gem} = 11.8 Hz, H-1′), 4.99 (d, 1H, H-1″), 4.71 (m, 1H, H-5′), 4.55 (d, 1H, J_{gem} = 11.3 Hz, CH_2Ph), 4.40 (d, 1H, CH_2Ph), 3.80–3.70 (m, 2H, H-6′ and H-6″), 2.37 (s, 6H, 2 × CH_3, Tol), 2.36 (s, 3H, CH_3, Tol). ^{13}C NMR (150.9 MHz, $CDCl_3$): 165.8, 165.5, 164.4 (3 × C=O), 163.6 (C-4), 149.8 (C-2), 144.4, 144.2, 141.2 (C-6), 136.8, 129.8, 129.3, 128.7, 127.9, 100.4 (C-5), 97.6 (C-2′), 84.4 (C-5′), 76.7 (C-3′), 74.0 (*C*H_2Ph), 73.7 (C-4′), 69.1 (C-6′), 64.5 (C-1′), 21.8 (*C*H_3, Tol).

9-[6′-*O*-Benzyl-1′-*O*-(4-toluenosulfonyl)-β-D-psicofuranosyl]-guanine (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** (^{13}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 2,6-lutidine was added (5.8 ml, 50 mmol). 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 $NaHCO_3$ solution and extracted with CH_2Cl_2 (three times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. Column chromatography (CH_2Cl_2 with gradient of MeOH: 0–7%) afforded **33** (only β-anomer; 3.1 g, 5.5 mmol, 44%). R_f = 0.61 (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m*/*z* $[M + H]^+$ 558.1 (Calcd. 558.1 for $C_{25}H_{28}N_5O_8S$). ^{1}H NMR (600 MHz, CH_3OD), 7.69 (s, 1H, H-8), 7.47 (d, 2H, *J* = 8.4 Hz, Tol), 7.25–7.21 (m, 3H, Bn), 7.19 (d, 2H, *J* = 8.4 Hz, Tol), 7.05–7.04 (m, 2H, Bn), 4.69 (d, 1H, $J_{3'-4'}$ = 5.1 Hz, H-3′), 4.63 (d, 1H, J_{gem} = 11.2 Hz, CH_2Ph), 4.48 (d, 1H, CH_2Ph), 4.38 (d, 1H, J_{gem} = 11.7 Hz, H-1′), 4.36 (d, 1H, H-1″), 4.27 (dd, 1H, $J_{4'-5'}$ = 2.6 Hz, H-4′), 3.59 (dd, 1H, J_{gem} = 10.7 Hz, $J_{5'-6'}$ = 3.1 Hz, H-6′), 3.54 (dd, 1H, $J_{5'-6''}$ = 3.1 Hz, H-6″), 2.39 (s, 3H, CH_3, Ts). ^{13}C NMR (150.9 MHz, CH_3OD): 157.5 (C-6), 152.7 (C-2), 148.9 (C-4), 144.7, 137.0, 135.9 (C-8), 131.2, 129.2, 127.9, 127.6, 127.5, 127.4, 95.0 (C-2′), 85.5 (C-5′), 77.1 (C-3′), 73.1 (*C*H_2Ph), 71.6 (C-4′), 69.8 (C-1′), 69.4 (C-6′), 20.9 (*C*H_3, Ts).

9-[6′-*O*-Benzyl-1′,3′-*O*-anhydro-β-D-psicofuranosyl]-N^2-(*N*,*N*-dimethylaminomethylene) guanine (35). Compound **33** (2.7 g, 4.9 mmol) was dried by co-evaporation with dry THF, and dissolved in this same solvent (50 ml). Sodium bis(trimethylsilylamide) (14.7 ml, 14.7 mmol; 1 M solution in THF) was added and stirring was continued for 8 h. The solution was neutralized with 10% (v/v) AcOH in MeOH and evaporated. Crude product **34** (NMR data in ESI†) was dissolved in dry MeOH (50 ml), *N*,*N*-dimethylformamide dimethylacetal (4 ml, 30 mmol) was added and stirring was continued overnight. The solution was evaporated and co-evaporated with toluene. Column chromatography (CH_2Cl_2 with gradient of MeOH: 0–5%) afforded **35** (1.6 g, 3.7 mmol,

75% after two steps). R_f = 0.60 (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M + Na]$^+$ 463.2 (Calcd. 463.2 for $C_{21}H_{24}N_6O_5Na$). ^{1}H NMR (600 MHz, $CDCl_3$): 8.47 (s, 1H, C*H*NMe$_2$), 7.67 (s, 1H, H-8), 7.25–7.23 (m, 5H, Bn), 5.73 (d, 1H, $J_{3'-4'}$ = 3.6 Hz, H-3′), 5.44 (d, 1H, J_{gem} = 7.8 Hz, H-1′), 4.97 (d, 1H, H-1″), 4.67–4.48 (m, 4H, H-4′, H-5′ and C*H*$_2$Ph), 3.93 (br d, 1H, J_{gem} = 10.6 Hz, H-6′), 3.82 (dd, 1H, $J_{5'-6''}$ = 5.1 Hz, H-6″), 3.02, 2.98 (2 × s, 2 × 3H, 2 × C*H*$_3$, Dmf). ^{13}C NMR (150.9 MHz, $CDCl_3$): 158.4 (*C*HNMe$_2$), 158.2 (C-2), 157.0 (C-6), 150.1 (C-4), 137.8 (Bn), 135.6 (C-8), 128.1, 127.4 (2 × Bn), 119.9 (C-5), 88.9 (C-3′), 88.4, 82.9 (C-5′), 79.4 (C-1′), 73.5 (*C*H$_2$Ph), 70.9 (C-4′), 69.3 (C-6′), 41.6, 35.0 (2 × *C*H$_3$, Dmf).

9-[1′,3′-*O*-Anhydro-6′-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]-*N*^{2}-(*N*,*N*-dimethylaminomethylene)guanine (37). A mixture of compound **35** (1.6 g, 3.7 mmol), $Pd(OH)_2/C$ (0.88 g, 20% wt%) 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). The filtrate was evaporated, co-evaporated with dry pyridine, and crude **36** (NMR data in ESI†) was 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 $NaHCO_3$ solution and extracted with CH_2Cl_2 (three times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. Column chromatography (CH_2Cl_2 with gradient of MeOH: 0–5%) afforded nucleoside **37** (2 g, 3.2 mmol, 85% after two steps). R_f = 0.70 (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M + H]$^+$ 653.17 (Calcd. 653.27 for $C_{35}H_{37}N_6O_7$). ^{1}H NMR (600 MHz, $CDCl_3$): 8.41 (s, 1H, C*H*NMe$_2$), 7.63 (s, 1H, H-8), 7.40–7.17 (m, 9H, DMTr), 6.78–6.75 (m, 4H, DMTr), 5.71 (d, 1H, $J_{3'-4'}$ = 3.6 Hz, H-3′), 5.55 (d, 1H, J_{gem} = 7.8 Hz, H1′), 4.94 (d, 1H, H1″), 4.42–4.38 (m, 2H, H-4′ and H-5′), 4.07 (dd, 1H, J_{gem} = 12.7 Hz, $J_{5'-6'}$ = 1.8 Hz, H-6′), 3.87 (dd, 1H, $J_{5'-6''}$ = 3.1 Hz, H-6″), 3.77 (s, 6H, OC*H*$_3$, DMTr), 3.05, 3.02 (2 × *C*H$_3$, Dmf). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 158.5 (DMTr), 158.0 (*C*HNMe$_2$), 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 (O*C*H$_3$, DMTr), 41.5, 35.2 (2 × *C*H$_3$, Dmf).

9-[6′-*O*-Benzyl-1′-*O*-(4-toluenesulfonyl)-β-D-psicofuranosyl]-adenine (38). Compound **31b** (10.14 g, 12 mmol) was dissolved in 25% methanolic ammonia (100 ml) and kept at RT for 2 days. The solution was evaporated and dried by co-evaporation with dry pyridine (twice). Crude compound **32b** (NMR data in ESI†) was dissolved in pyridine (120 ml) and 2,6-lutidine (2.8 ml, 24 mmol) was added. The mixture was cooled to 0 °C and 4-toluensulfonyl chloride (4.6 g, 24 mmol) was added in two portions. The reaction was stirred at 0 °C for 5 h, poured into saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (three times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. Column chromatography (CH_2Cl_2 with gradient of MeOH: 0–7%) afforded **38** (only β-anomer; 2.9 g, 5.4 mmol, 45%). A 1D differential NOE experiment showed a 4.1% enhancement of the H-3′ signal, while H-8 was irradiated. R_f = 0.30 (CH_2Cl_2–MeOH, 95 : 5). MALDI-TOF *m/z* [M + H]$^+$ 542.12 (Calcd. 542.17 for $C_{25}H_{28}N_5O_7S$). ^{1}H NMR (600 MHz, CD_3OD): 8.01 (s, 1H, H-2), 8.01 (s, 1H, H-8), 7.39 (d, 2H, *J* = 8.4 Hz, Ts), 7.30–7.17 (m, 3H, Bn), 7.08 (d, 2H, Ts), 6.96–6.95 (m, 2H, Bn), 4.77 (d, 1H, $J_{3'-4'}$ = 5.0 Hz, H-3′), 4.73 (d, 1H, J_{gem} = 10.8 Hz, H-1′), 4.48 (d, 1H, H-1″), 4.38 (Abq, 2H, J_{gem} = 12.0 Hz, C*H*$_2$Ph), 4.39 (m, 1H, H-5′), 4.31 (dd, 1H, $J_{4'-5'}$ = 2.8 Hz, H-4′), 3.67 (dd, 1H, J_{gem} = 10.8 Hz, $J_{5'-6'}$ = 2.8 Hz, H-6′), 3.59 (dd, 1H, $J_{5'-6''}$ = 2.8 Hz, H-6″), 2.36 (C*H*$_3$,Ts). ^{13}C NMR (150.9 MHz, CD_3OD): 155.2 (C-6), 151.7 (C2), 147.5 (C-4), 144.9 (Ts), 139.2 (C-8), 137.0 (Ts), 131.3 (Bn), 129.3 (Ts), 128.0 (Bn), 127.6 (Bn), 127.2 (Ts), 127.1 (Bn), 119.0 (C-5), 95.5 (C-2′), 85.9 (C-4′), 77.3 (C-3′), 73.3 (*C*H$_2$Ph), 71.8 (C-5′), 70.1 (C-1′), 69.4 (C-6′), 21.2 (*C*H$_3$, Ts).

9-[6′-*O*-Benzyl-1′,3′-*O*-anhydro-β-D-psicofuranosyl]adenine (39). Compound **38** (2.9 g, 5.4 mmol) was dried by co-evaporation with dry THF, and dissolved in dry THF (90 ml). Sodium bis(trimethylsilylamide) (16.2 ml, 16.2 mmol; 1 M solution in THF) was added dropwise and stirring was continued for 4 h. The solution was neutralized with 10% (v/v) AcOH in MeOH and evaporated. Column chromatography (CH_2Cl_2 with gradient of MeOH: 0–20%) afforded **39** (1.79 g, 4.86 mmol, 90%). R_f = 0.63 (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M + H]$^+$ 370.0 (Calcd. 370.1 for $C_{18}H_{20}N_5O_4$). ^{1}H NMR (600 MHz, $CDCl_3/CD_3OD$): 8.31 (s, 1H, H-2), 7.86 (s, 1H, H-8), 7.33–7.26 (m, 5H, Bn), 5.74 (d, 1H, $J_{3'-4'}$ = 4.7 Hz, H-3′), 5.61 (d, 1H, J_{gem} = 7.2 Hz, H-1′), 4.97 (d, 1H, H-1″), 4.55 (ABq, 2H, J_{gem} = 12.0 Hz, C*H*$_2$Ph), 4.52–4.49 (m, 1H, H-5′), 4.43 (dd, 1H, $J_{4'-5'}$ = 7.3 Hz, H-4′), 3.91–3.85 (m, 2H, H-6′ and H-6″). ^{13}C NMR (150.9 MHz, $CDCl_3$): 155.4 (C-6), 153.2 (C-2), 149.6 (C-4), 137.6 (C-8), 129.0 (Bn), 128.3 (Bn), 127.7 (Bn), 127.6 (Bn), 119.0 (C-5), 89.3 (C-2′), 88.7 (C-3′), 83.6 (C-5′), 79.7 (C-1′), 73.6 (*C*H$_2$Ph), 71.2 (C-4′), 69.3 (C-6′).

9-[1′,3′-*O*-Anhydro-6′-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]-adenine (41). A mixture of oxetane **39** (1.79 g, 4.86 mmol), $Pd(OH)_2/C$ (0.87 g, 20% wt%) 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). The filtrate was evaporated and co-evaporated with dry pyridine, the crude compound **40** (NMR data was identical with published earlier)[16] was dissolved in dry pyridine (50 ml). 4,4′-Dimethoxytrityl chloride (2 g, 5.83 mmol) was added and stirring was continued for 2 h, poured into saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (three times). The organic phase was dried over $MgSO_4$, filtered and evaporated. Column chromatography (CH_2Cl_2 with gradient of MeOH: 0–5%) afforded **41** (1.83 g, 3.16 mmol, 65% after two steps). R_f = 0.70 (CH_2Cl_2–MeOH, 10 : 1). MALDI-TOF *m/z* [M + H]$^+$ 582.13 (Calcd. 582.23 for $C_{32}H_{32}N_5O_6$). ^{1}H NMR (600 MHz, $CDCl_3$): 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, $J_{3'-4'}$ = 4.8 Hz, H-3′), 5.65 (d, 1H, J_{gem} = 9.6 Hz, H-1′), 4.96 (d, 1H, H-1″), 4.48 (dd, 1H, $J_{4'-5'}$ = 7.4 Hz, H-4′), 4.44 (m, 1H, H-5′), 3.77 (s, 6H, OC*H*$_3$, DMTr), 3.57–3.46 (m, 2H, H-6′ and H-6′). ^{13}C NMR (150.9 MHz, $CDCl_3$): 158.5 (DMTr), 155.4 (C-6), 153.5 (C-2), 149.5 (C-4), 144.6 (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′), 88.8 (C-3′), 86.3 (Ar$_3$–*C*, DMTr), 83.8 (C-5′), 80.0, 71.7 (C-4′), 62.7 (C-6′), 55.2 (O*C*H$_3$, DMTr).

9-[1′,3′-*O*-Anhydro-6′-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]-*N*^{6}-phenoxyacetyladenine (42). 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 h. Then phenoxyacetyl chloride (0.87 ml, 6.3 mmol) was added dropwise at 0 °C. The reaction mixture was stirred for 2 h at RT, poured into saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (three times). The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude mixture was dissolved in pyridine–water (2 : 1 v/v) and stirred overnight. After evaporation of the solvent, column chromatography (CH_2Cl_2 with gradient of MeOH: 0–3%) afforded **42** (1.7 g, 2.4 mmol, 75%). R_f = 0.43 (CH_2Cl_2–MeOH, 95 : 5). MALDI-TOF *m/z* [M + H]$^+$ 716.22 (Calcd. 716.27 for $C_{40}H_{38}N_5O_8$). ^{1}H NMR (600 MHz, $CDCl_3$): 8.80 (s, 1H, H-2), 8.08 (s, 1H, H-8), 7.40–7.10 (m, 14H, DMTr and PAC), 6.78–6.75 (m, 4H, DMTr), 5.78 (d, 1H, $J_{3'-4'}$ = 4.33 Hz, H-3′), 5.65 (d, 1H, J_{gem} = 7.9 Hz, H-1′), 4.96 (d, 1H, H-1″), 4.87 (s, 2H, C*H*$_2$, PAC), 4.52 (dd, 1H, $J_{4'-5'}$ = 8.3 Hz, H-4′), 4.47–4.43

(m, 1H, H-5′), 3.76 (s, 6H, OCH_3, DMTr), 3.55 (dd, 1H, J_{gem} = 10.7 Hz, $J_{5'-6'}$ = 2.6 Hz, H-6′), 3.47 (dd, 1H, $J_{5'-6''}$ = 4.8 Hz, H-6″). ^{13}C NMR (150.9 MHz, $CDCl_3$): 166.6 (C=O), 158.5, 153.0 (C-2), 151.3 (C-4), 148.5 (C-6), 144.5 (DMTr), 140.4 (C-8), 135.6, 130.0, 129.9, 129.8, 128.0, 127.8, 126.8, 122.8, 122.5 (DMTr and PAC), 114.9 (C-5), 113.1 (DMTr), 89.3, 88.7 (C-3′), 86.3 (C-2′), 84.0 (C-5′), 79.8 (C-1′), 71.5 (C-4′), 68.1 (CH_2, PAC), 62.5 (C-6′), 55.2 (OCH_3, DMTr).

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