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1-Azaribofuranoside Analogues as Designed Inhibitors of Purine Nucleoside Phosphorylase. Synthesis and Biological Evaluation

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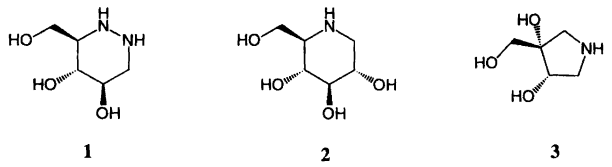
Pyrrolidine analogues of 2-deoxyribofuranose, having nitrogen in place of anomeric carbon, have been synthesised as potential transition state analogues of enzymatic nucleoside cleavage. Efficient synthetic methods were developed that allowed the synthesis of a wide range of 4-substituted 3-hydroxypyrrolidines starting from pyrroline and using opening of the pyrrolidine 3,4-epoxide with carbon nucleophiles. Among the compounds synthesised were the 4-cyano- [(±)-**16**], 4-hydroxymethyl [(±)-**22**] and 4-carboxymethyl derivatives [(±)-**18**]. From the hydroxymethyl derivative [(±)-**22**] *N*-alkylation with chloromethyluracil gave an inosine analogue [(±)-**23**]. The new compounds were tested for inhibition of human erythrocyte purine nucleoside phosphorylase. Compound (±)-**22** was found to show non-competitive inhibition of the enzyme with a K_i of 160 μM. This suggested that (±)-**22** binds to the ribofuranose portion of the active site. Furthermore, a solid-phase synthesis of 1′-azanucleoside analogues was developed.

Potent inhibitors of glycosyl-cleaving enzymes offer the opportunity to modulate selectively the crucial metabolism of carbohydrates, and hence open a large number of potential applications. While a variety of different glycosidase inhibitors are known,[1] much less is known about how to inhibit other glycosyl-cleaving enzymes such as glycosyl transferases or phosphorylases.

Previous results from our group suggest that sugar analogues with a nitrogen in place of the anomeric carbon are inhibitors of glycosyl phosphorylases. Thus recently we observed that the glucosidase inhibitor (±)-1-azafagomine (**1**),[2] inhibited glycogen phosphorylase, in contrast with the closely related glucosidase inhibitor **2** which did not inhibit this enzyme.[3] Secondly the pyrrolidine **3** was found a moderate inhibitor of purine nucleoside phosphorylase.[4]



1 2 3

Fig. 1. The azasugars 1-azafagomine (**1**), 1-deoxynojirimycin (**2**) and pentofuranose analogue **3**.

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In this paper we have tried to exploit the latter results and generate a more potent purine nucleoside phosphorylase inhibitor. Compound **3** obviously resembled the substrate (inosine or adenine) or the transition state by resembling 2-deoxyribose. It had, however, two deficiencies which could be improved: (a) it contained a hydroxy group for which no equivalent existed in the substrate and (b) it lacked the aglycone portion of the molecule. In this work we have synthesised a molecule lacking the hydroxy group and also introduced a nucleobase bioisostere. Furthermore, we present a method for the solid-phase synthesis of this kind of nucleoside analogue, which allows combinatorial chemistry to be performed on this compound.

Results and discussion

Our goal was to synthesise *trans*-3-hydroxy-4-alkylpyrrolidines (**4**), which then could be modified by substitution at nitrogen with suitable nucleobase bioisosteres. Compound **4**, which was unknown, we imagined could readily be obtained from pyrroline (**5**) through a sequence of epoxidation and epoxide-opening with a suitable carbon nucleophile (Fig. 2). A study of the different available routes to pyrroline revealed that a multistep procedure[5] starting from (*Z*)-1,4-dichloro-2-butene (**6**, Scheme 1) was much more efficient than the

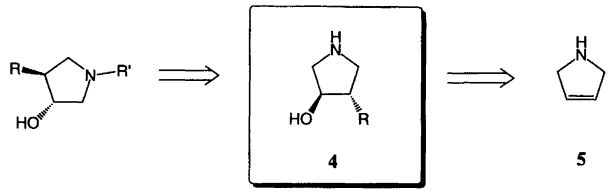


Fig. 2. Synthetic plan for synthesis of nucleoside analogues.

seemingly attractive one-step reduction of pyrrole.[6] In the latter procedure it was difficult to obtain a pure product in an acceptable yield.

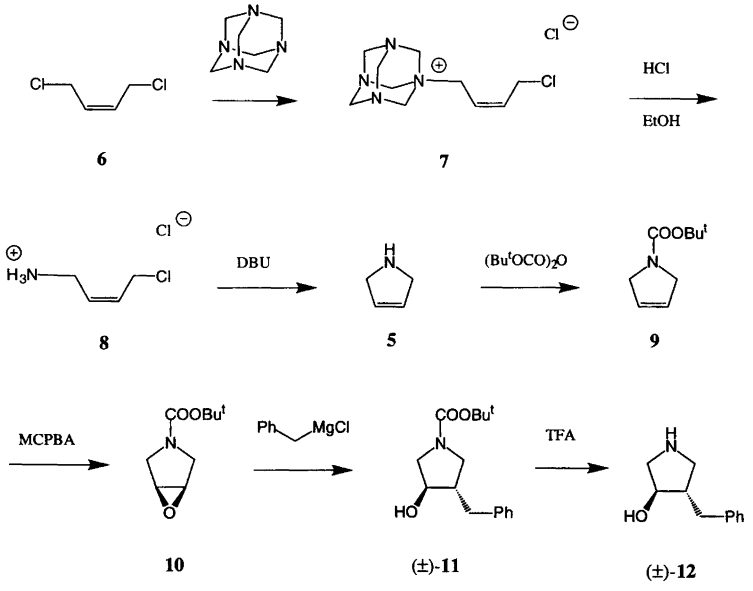
BOC-protection of **5**–**9**[7] followed by epoxidation with *m*-chloroperbenzoic acid to give the epoxide **10**[7] proceeded in 79% overall yield (Scheme 1). We were then set up to introduce the R substituent in (±)-**4** (Fig. 2). Among R-groups desired was primarily a hydroxymethyl group, so that there was a direct resemblance with ribofuranose. However, to be able to link a compound to a solid phase, for further combinatorial variation of its structure, an RCH_2COOH or COOH was desired. Thus to obtain the CH_2COOH group we carried out substitution with benzyl with the objective of obtaining an acid by ozonolysis. Direct reaction of the epoxide **10** with benzylmagnesium chloride in THF at 25 °C gave the benzyl derivative (±)-**11** in excellent yield. Instability of the BOC-group to Grignard reagents, which might be anticipated,[8] was not observed. Removal of the BOC-group using TFA–H_2O readily gave the pyrrolidine (±)-**12**. Ozonolysis of (±)-**12** was not successful as the phenyl group seemed very resistant even to prolonged treatment with ozone.

Opening of the epoxide with other carbon nucleophiles that could function as latent acids was studied. Consequently a nitrile was introduced as a masked carboxylic acid group. Reaction of **10** with trimethylsilyl cyanide (TMSCN) in the presence of $AlCl_3$ gave a 46% yield of the silylated derivative (±)-**14**. The moderate yield was caused by a limited stability of the BOC group under the Lewis acidic conditions. Removal of the TMS group using K_2CO_3 in methanol gave the nitrile (±)-**15** in 91% yield. The BOC-group could be removed by treating (±)-**15** with TFA to give pyrrolidine (±)-**16** in 96% yield. We tried to reduce the nitrile of (±)-**15** with DIBAL to obtain an aldehyde (which could be reduced or oxidised as desired); however, this attempt was not successful, apparently due to cleavage of the BOC-group under the reaction conditions.

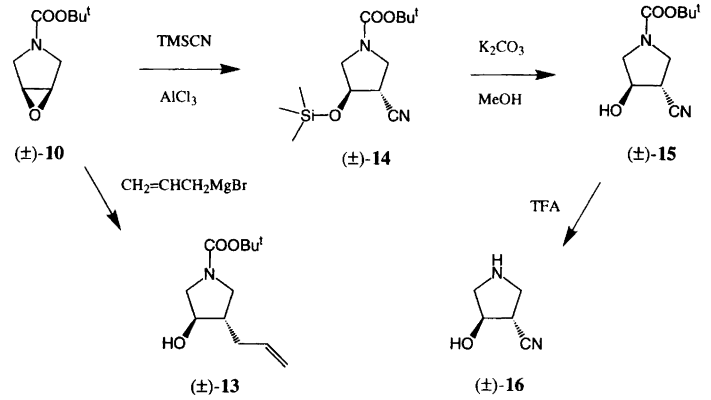
We then turned our attention to synthesising allyl derivative (±)-**13** and attempted to ozonise oxidatively this compound to an acid. The reaction of (±)-**10** with allylmagnesium bromide in ether at 0 °C gave the allyl derivative (±)-**13** in 89% yield. This reaction was much faster than the epoxide opening above using benzyl Grignard.

Ozonolysis of (±)-**13** in MeOH at −78 °C proceeded smoothly with the consumption of one mole of ozone to form the ozonide. The oxidation of the ozonide was then studied. However, known procedures of oxidative work-up of ozonides [NaOH–MeOH (present during ozonolysis),[9] H_2O–HCOOH,[10] DMF,[11] SiO_2 (present during ozonolysis)[12]] were not successful, possibly because they required strong hydrolytic conditions, which might have been incompatible with the BOC group. Thus a set of milder oxidation conditions was needed. Ag_2O is known to oxidise aldehydes to acids,[13] and this was attempted directly on the ozonide. In fact, the Ag_2O-oxidation gave the acid (±)-**17** in 67% yield. Removal of the BOC group with TFA gave the amino acid (±)-**18** in 97% yield.

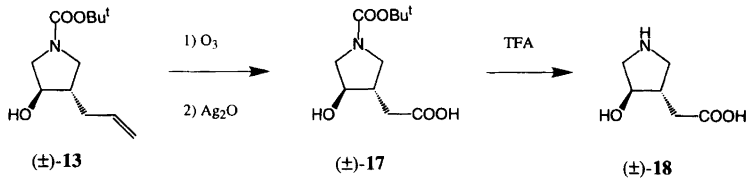
The other target, the hydroxymethyl derivative **4** (R = CH_2OH), we synthesised through the vinyl derivative.



Scheme 1. Synthesis of pyrroline (**5**), pyrrolidine epoxides **10** and **12**.



Scheme 2. Epoxide opening of **10** with carbon nucleophiles.



Scheme 3. Conversion of **13** into **18**.

Attempted introduction of a vinyl group gave some surprising results. The reaction of **10** with vinylmagnesium bromide resulted not only in the anticipated formation of (±)-**19**, but also in equimolar formation of the epimer (±)-*cis*-**19**. The yield was modest (36%). Given the stereospecific reaction of **10** with allylmagnesium bromide and benzylmagnesium chloride this was quite unexpected. However, lack of stereospecificity in the epoxide opening with vinyl Grignard reagents or cuprates is not without precedent. Henin and Muzart reported[14] that cyclohexene oxide reacted with vinylmagnesium bromide in the presence of CuI to give a mixture of *cis* and *trans* isomers. A change from CuI to CuBr–SMe_2 gave only the *trans* isomer, while complete omission of copper salts gave no reaction.[14] Use of organocuprates has also been reported to give a *cis–trans* isomeric mixture. No explanation for these findings has been offered.

We tried the reaction of **10** with vinylmagnesium bromide in the presence of CuBr–SMe_2 (Table 1). This resulted in an improved yield of the *trans*-vinyl derivative **19** given a 9:1 ratio of the diastereomers in 56% yield. Using the higher-order cuprate $(CH_2{=}CH)_2CuCNLi_2$ an even higher stereoselectivity was obtained as no formation of *cis*-**19** was observed. The yield was, however, only 36%; therefore the CH_2=CHMgBr/CuBr–SMe_2 procedure was generally preferred. Removal of the BOC group with TFA gave smoothly the amine (±)-**20** in 93% yield.

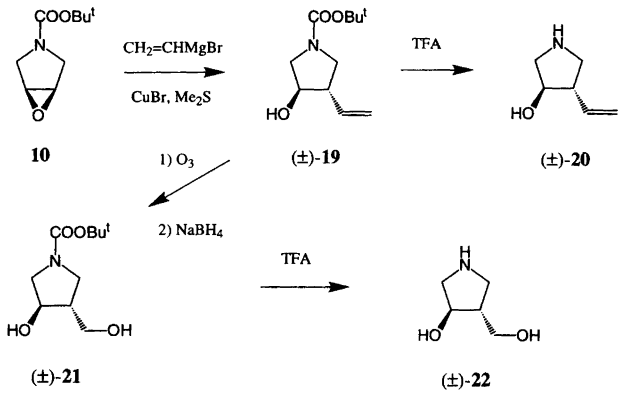
Table 1. Reactions of **10** with organometallic reagents.

Reagent	Yield (%)	*trans/cis*
Vinylmagnesium bromide	36	1:1
Vinylmagnesium bromide + $CuBrMe_2S$	56	9:1
$(CH_2{=}CH)_2CuCNLi_2$	36	1:0

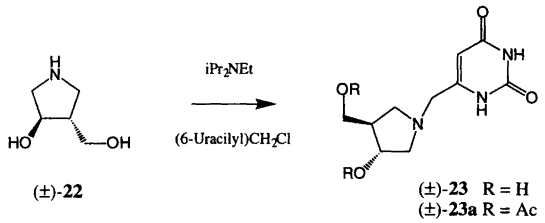
Ozonolysis of (±)-**19** in MeOH at −78 °C followed by reduction of the ozonide with $NaBH_4$ gave the hydroxymethyl derivative (±)-**21** in 68% yield. Deprotection of (±)-**21** using TFA gave the amine (±)-**22**.

Next we attempted to introduce a nucleobase bioisostere into (±)-**22**. Such a group should mimic a purine as much as possible but still allow the nitrogen to accept a proton. Therefore alkylation of (±)-**22** with 6-chloromethyl-2,4-dioxopyridimine was chosen as it would give as product a tertiary amine that could mimic the pyrimidine ring of the purine. (±)-**22** was allowed to react with 6-chloromethyl-2,4-dioxopyridimine and Hünig's base in MeOH. Since the product (±)-**23** was quite polar and difficult to purify, it was subjected to acetylative work-up prior to isolation to give the diacetate (±)-**23a** in 66% yield. Subsequently, (±)-**23a** was deacetylated with a catalytic amount of NaOMe in MeOH to give (±)-**23** in 68% yield.

Enzymatic resolution of (±)-**21** was investigated to see whether optically active pyrrolidines could be obtained in this way (Scheme 6). Thus (±)-**21** was reacted with vinyl acetate and three different lipases (Table 2). This gave the 5-*O*-acetate **24** with varying stereoselectivity. Lipase PS (from Amano) was not very selective, converting both enantiomers. *Candida antarctica* Lipase (Lipozym) gave preferentially one enantiomer with a selectivity of 2, while *Mucor mihei* Lipase (Novozym) esterified preferentially the other stereoisomer with a selectivity of 2.1. The enantiomeric ratio of **24** was determined by removal of the BOC group with TFA



Scheme 4. Synthesis of *trans*-3-hydroxy-4-hydroxymethylpyrrolidine (**22**).



Scheme 5. Synthesis of nucleoside analogue **23**.

Table 2. Enantioselectivity of enzymatic acylation of **21**.

Enzyme	*t*/h	Conversion	e.e. (%)	*trans*
Candida antarctica lipase (immobilised)	2.3	0.45	24.7	2
Lipase PS	20	1.0	0	1
Mucor mihei lipase (immobilised)	4	0.46	28[a]	2.1[a]

[a]Reverse selectivity.

followed by reaction with Sanger's reagent to give **25**. Chiral HPLC of **25** on an amylose acetate column efficiently separated the enantiomers.

The relatively low selectivity in these acylations was probably due to the fact that the reacting alcohol and the stereocentre were too far from each other. However, enzymatic acylation of the secondary hydroxy group in **24** was not possible. No reaction took place on prolonged treatment of (±)-**24** with enzymes and vinyl acetate or vinyl butyrate.

To enable a combinatorial approach to inhibitors of this kind a solid-phase synthesis of alkylated pyrrolidines was developed. Thus, acid (±)-**17** was reacted with a 4-methylbenzhydrylamine resin (MBHA) in DMF using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as the coupling reagent. Consecutive deprotection with TFA and treatment of the resin with 5 equiv. of BnCl in DMF followed by deprotection with trifluoromethanesulfonic acid (TfOH) gave compound (±)-**26** in an overall yield of 50%.

Compounds (±)-**16**, (±)-**17**, (±)-**22** and (±)-**23** were tested for inhibition of purine nucleoside phosphorylase from human erythrocytes. Only (±)-**22** showed considerable inhibition, while the other compounds showed negligible inhibition even at millimolar concentrations. Compound (±)-**22** was found to be a non-competitive (mixed) inhibitor of the enzyme with a K_i of 1.6×10^{-4} M (Fig. 3). Purine nucleoside phosphorylase has an ordered

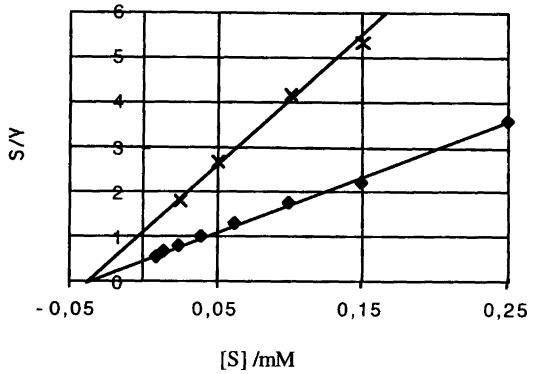
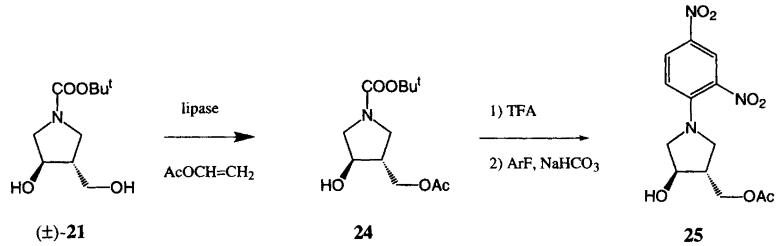
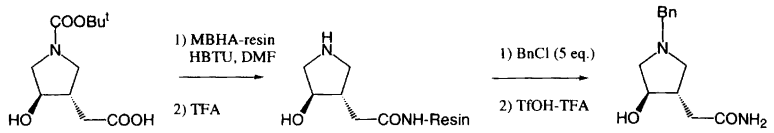


Fig. 3. Hanes plot of the PNPase-catalysed phosphorylysis of inosine: ◆, without inhibitor; ×, inhibitor (**22**).



Scheme 6. Enzyme-catalysed acylation of **21**.



(±)-**17** (±)-**26**

Scheme 7. Solid-phase alkylation of **17**.

bimolecular–bimolecular reaction mechanism[15] with the nucleoside substrate entering the enzyme's active site, followed by phosphate, while ribose phosphate product leaves the active site before purine.[16] The non-competitive inhibition may be explained by the inhibitor acting as an analogue of ribose phosphate.[15] The inhibitor binds to the enzyme–purine complex (EQ-complex) giving a non-competitive inhibition pattern. The inhibitory potency was similar in magnitude to that of the 3-hydroxy analogue **3**. Thus apparently the influence of the 3-hydroxy group on inhibition was negligible.

In summary, an interesting new type of nucleoside analogue has been synthesized consisting of a 1-aza-analogue of 2-deoxyribofuranose. Particularly interesting is the finding that the monosaccharide analogue does inhibit purine nucleoside phosphorylase, and that solution and solid-phase methods were developed that allow efficient substitution of the 1-nitrogen. This may allow future synthesis of potent selective inhibitors of the enzyme. In particular, combinatorial approaches are under investigation.

Experimental

General. ^{13}C NMR and ^1H NMR spectra were recorded on a Varian Gemini 200 instrument. D_2O was used as the solvent with DHO (^1H NMR: δ 4.7) and acetone (^1H NMR: δ 2.05; ^{13}C NMR: δ 29.8) as the reference. With $CHCl_3$ as solvent Me_4Si and $CHCl_3$ (^{13}C NMR: δ 76.93) were used as references. Mass spectra were obtained on a VG TRIO-2 instrument. Melting points are uncorrected. Solutions were concentrated on a rotary evaporator at a temperature below 40 °C. Dry tetrahydrofuran and diethyl ether were prepared by distillation from sodium and benzophenone.

N-tert-*Butoxycarbonyl-3-pyrroline* (**9**).[7] In a two-necked round-bottomed flask was dissolved 7.7 g (0.111 mol) of **5** in 70 ml of ice-cooled MeOH and the flask was equipped with a condenser, drying tube and an addition funnel. The addition funnel was charged with 27.8 g (0.127 mol) of di-*tert*-butyl dicarbonate dissolved in 30 ml of MeOH. After 30 min the addition was complete, and stirring was continued for 20 h. The solvent was evaporated off leaving 21.1 g of the crude product **9**, which was used in the next step without purification. ^1H NMR ($CDCl_3$): δ 5.72 (m, 2 H, H-3,4), 4.06 (m, 4 H, H-2,5), 1.43 (s, 9 H, H-1′). ^{13}C NMR ($CDCl_3$): δ 154.8 (C-1′), 126.28 (C-3 or C-4), 126.22 (C-3 or C-4), 79.7 (C-1″), 53.5 (C-2 or C-5), 53.3 (C-2 or C-5), 29.0 (C-1‴).

N-tert-*Butoxycarbonyl-3,4-epoxypyrrolidine* (**10**).[7] In a round-bottomed flask with stirring, 21.1 g of **9** were dissolved in 120 ml of CH_2Cl_2. An addition funnel containing 37 g of MCPBA (Aldrich, 57–86%) dissolved in 120 ml of CH_2Cl_2 was fitted, and the reagent was added within 2 h. After 20 h a white precipitate had appeared and the reaction mixture was filtered. The filtrate was washed with $NaHSO_3$ (100 ml, 10%), $NaHCO_3$ (100 ml, saturated) and water (100 ml). After drying with $MgSO_4$, filtration and evaporation, the resulting residue was column chromatographed in EtOAc–petroleum ether (1:4) to give 16.29 g (79% based on **5**) of **10** as an oil. ^1H NMR ($CDCl_3$): δ 3.66 (m, 4 H, H-2,5), 3.23 (dd, 2 H, *J* 13 Hz, 2 Hz, H-3,4), 1.37 (s, 9 H, H-1′). ^{13}C NMR ($CDCl_3$): δ 155.5 (C-1′), 80.2 (C-1″), 56.0 (C-2 or C-5), 55.5 (C-2 or C-5), 47.7 (C-3 or C-4), 47.3 (C-3 or C-4), 28.8 (C-1‴). MS (EI): *m/z* 185 (M^+), 130 ($M-55$), 85 ($M-100$).

N - tert - *Butoxycarbonyl* - trans - *3 - hydroxy - 4 - benzyl - pyrrolidine* (**11**). In a predried flask with a septum, a 2 M solution of benzylmagnesium chloride (3.5 ml, 7 mmol) in THF was diluted with 15 ml of dry THF. Using a syringe 0.245 g (1.32 mmol) of **10** dissolved in 5 ml of dry THF was added to the stirred solution. After 12 h the reaction was quenched by the careful addition of dilute HCl (1 M). The mixture was extracted with 2 × 50 ml of ether and the organic phases dried ($MgSO_4$). The solvent was evaporated off and the residue column chromatographed in CH_2Cl_2–EtOAc (4:1). The residue could be crystallized from EtOAc–petroleum ether yielding 0.300 g (82%) of **11** as a white crystalline powder. M.p. 66–68 °C, ^1H NMR ($CDCl_3$): δ 7.23 (m, 5 H, H-4″), 4.07 (q, 1 H, *J* 5 Hz, H-3), 3.65 (dd, 1 H, *J* 11.5 Hz, 5.5 Hz, H-2a), 3.52 (dd, 1 H, *J* 11 Hz, H-2b), 3.24 (dd, 1 H, *J* 11.5 Hz, 4.5 Hz, H-5a), 3.12 (dd, 1 H, *J* 11 Hz, 5.5 Hz, H-5b), 2.77 (dd, 1 H, *J* 13.5 Hz, 6.5 Hz, H-4′a), 2.60 (br s, 1 H, H-3′), 2.53 (dd, 1 H, *J* 13.5 Hz, 8.5 Hz, H-4′b), 2.35 (sextet, 1 H, *J* 6.5 Hz, H-4), 1.44 (s, 9 H, H-1′). ^{13}C NMR ($CDCl_3$): δ 155.5 (C-1′), 140.0 (C-4″), 128.5 (C-4″), 128.2 (C-4″), 126.9 (C-4″), 80.0 (C-1″), 74.6 (C-3), 52.8 (C-2), 49.6 (C-5), 47.9 (C-4), 38.0 (C-4′), 28.8 (C-1‴). MS (EI): *m/z* 277 (M^+), 221 ($M-56$), 177 ($M-100$).

trans-*3-Hydroxy-4-benzylpyrrolidine* (**12**). To 64 mg (0.23 mmol) of **11** were added 2 ml of trifluoroacetic acid (TFA). The mixture was stirred for 30 min after which TFA was evaporated off leaving 65 mg (97%) of the trifluoroacetic acid salt of **12**. ^{1}H NMR (D_2O): δ 7.23 (m, 5 H, H-4″), 4.22 (q, 1 H, *J* 2 Hz, H-3), 3.43 (m, 2 H, H-2), 3.17 (dd, 1 H, *J* 12.5 Hz, 2 Hz, H-5a), 3.01 (dd, 1 H, 12 Hz, 5 Hz, H-5b), 2.5–2.8 (m, 3 H, H-4,4′). ^{13}C NMR (D_2O): δ 141.6 (C-4″), 131.8 (C-4″), 131.7 (C-4″), 129.6 (C-4″), 75.9 (C-3), 53.7 (C-5), 51.0 (C-2), 49.7 (C-4), 38.8 (C-4′). MS (EI): *m/z* 177 (M^+).

N-tert-*Butoxycarbonyl*-trans-*3-hydroxy-4-allylpyrrolidine* (**13**). Using glass equipment dried in the oven, a two-necked flask equipped with septum, stirring bar and an addition funnel was charged with 35 ml of a 1.0 M ether solution of allylmagnesium bromide (35 mmol) via a syringe. Dry ether (70 ml) was added and the solution cooled to 0 °C using an ice bath. From the addition funnel were added 2.91 g (15.6 mmol) of **10** dissolved in 30 ml of dry ether. The product was immediately observed as a white precipitate. After the addition the solution was stirred for 15 min and quenched by careful addition of dilute aqueous HCl (1 M). The organic phase was separated and the water phase extracted with 2 × 100 ml of ether. The organic phases were dried ($MgSO_4$), filtered and evaporated leaving a yellow oil. This was purified by filtration through a 2 cm layer of silica gel with CH_2Cl_2 to remove impurities and then eluting the product with EtOAc. This left 3.17 g (89%) of **13** as an oil. ^{1}H NMR ($CDCl_3$): δ 5.75 (m, 1 H, H-4″), 5.02 (m, 2 H, H-4‴), 3.99 (q, 1 H, *J* 7 Hz, H-3), 3.55 (dd, 2 H, *J* 5 Hz, 11 Hz, H-2), 3.11 (m, 3 H, H-5,3′), 2.05 (m, 3 H, H-4,4′), 1.41 (s, 9 H, H-1′). ^{13}C NMR ($CDCl_3$): δ 155.3 (C-1′), 136.3 (C-4″), 117.2 (C-4‴), 79.9 (C-1″), 74.7 (C-3), 53.0 (C-2), 49.3 (C-5), 45.7 (C-4), 36.1 (C-4′), 29.0 (C-1‴).

N-tert-*Butoxycarbonyl*-trans-*3-trimethylsilyloxy-4-cyanopyrrolidine* (**14**). 20 mg of $AlCl_3$ were placed in an oven-dried flask, which was then flushed with nitrogen and a septum was inserted. 2.07 g (11.2 mmol) of **10** and 1.5 ml (11.3 mmol) of trimethylsilyl cyanide (TMSCN) were added via syringe and the solution was heated to 50 °C with stirring for 30 h. When cooled the product crystallized. The crystals were dissolved in acetone to remove any remaining TMSCN. The residue was recrystallized from ether to give 1.47 g (46%) of **14** as white needles. M.p. 84–85 °C. ^{1}H NMR ($CDCl_3$): δ 4.48 (q, 1 H, *J* 5.5 Hz, H-3), 3.69 (m, 3 H, H-2,5a), 3.20 (dd, 1 H, *J* 11.5 Hz, 5 Hz, H-5b), 2.93 (m, 1 H, H-4), 1.45 (s, 9 H, H-1′), 0.18 (s, 9 H, H-3′). ^{13}C NMR ($CDCl_3$): δ 119.2 (C-4′), 80.8 (C-1″), 74.1 and 73.3 (rotamer C-3), 53.1 and 52.6 (rotamer C-2), 47.2 and 46.9 (rotamer C-5), 37.5 and 36.8 (rotamer C-4), 28.9 (C-1‴), 0.3 (C-3′). MS (EI): *m/z* 284 (M^+), 229 ($M-55$), 211 ($M-73$), ($M-100$).

N-tert-*Butoxycarbonyl*-trans-*3-hydroxy-4-cyanopyrrolidine* (**15**). To a solution of 570 mg (2.0 mmol) of **14** in 10 ml of MeOH were added 30 mg of K_2CO_3. After 10 min the solvent was evaporated off, 5 ml of HCl (0.1 M) added and the solution extracted with EtOAc, 2 × 10 ml. Drying ($MgSO_4$), filtration and evaporation left 385 mg (91%) of **15** as a sticky solid. ^{1}H NMR ($CDCl_3$): δ 4.58 (q, 1 H, *J* 5 Hz, H-3), 3.72 (m, 3 H, H-5a,2), 3.35 (m, 1 H, H-5b), 3.04 (m, 1 H, H-4), 1.43 (s, 9 H, H-1′). ^{13}C NMR ($CDCl_3$): δ 154.7 (C-1′), 119.3 (C-4′), 81.3 (C-1″), 73.6 and 72.7 (rotamer C-3), 52.7 and 52.6 (rotamer C-2), 47.5 and 47.2 (rotamer C-5), 37.0 and 36.5 (rotamer C-4), 28.9 (C-1‴).

trans-*3-Hydroxy-4-cyanopyrrolidine* (**16**). To 45 mg (0.21 mmol) of **15** were added 2 ml of trifluoroacetic acid. The solution was stirred for 30 min after which TFA was evaporated off leaving 46 mg (96%) of the trifluoroacetic acid salt of **16**. ^{1}H NMR (D_2O): δ 4.82 (dq, 1 H, *J* 2.5 Hz, 2 Hz, H-3), 3.79 (dd, 1 H, *J* 12.5 Hz, 8 Hz, H-2a), 3.67 (dd, 1 H, *J* 12.5 Hz, 4.5 Hz, H-2b), 3.56 (dd, 1 H, 13 Hz, 4.5 Hz, H-5a), 3.46 (m, 1 H, H-4), 3.37 (ddd, 1 H, *J* 13 Hz, 2.5 Hz, 1 Hz, H-5b). ^{13}C NMR (D_2O): δ 120.9 (C-4′), 75.1 (C-3), 54.2 (C-2), 49.1 (C-5), 38.8 (C-4), MS (EI): *m/z* 112 (M^+).

N-tert-*Butoxycarbonyl*-trans-*3-hydroxy-4-carboxymethylpyrrolidine* (**17**). In a two-necked flask were dissolved 3.17 g (13.9 mmol) of **13** in 60 ml MeOH and the solution was cooled to −78 °C using a dry ice–acetone bath. The flask was connected to the ozonizer through a trap and with a sintered inlet tube. To the exhaust was connected a destruction trap containing KI and acetic acid. Ozone was bubbled through the solution until it acquired a blue colour. Remaining ozone was removed by blowing a stream of nitrogen through the system. The ozonide was oxidized without work-up. A solution of Ag_2O was prepared *in situ* by mixing 7.8 g (45.9 mmol) of $AgNO_3$ in 100 ml water with 13.3 ml of NaOH (27.65%) (91.9 mmol) (a brown precipitate was formed). The cold ozonide solution was added in one portion. A black precipitate was formed and after 30 min the solution was filtered and washed with hot water. MeOH was evaporated off leaving a yellow, basic water solution. This was made acidic with aqueous HCl (1 M), which caused the colour to disappear, and extracted with EtOAc 3 × 100 ml. After drying ($MgSO_4$), filtration and evaporation, a sticky yellow crude product was isolated. This was crystallized from EtOAc giving 2.69 g (67%) of **17** as a white crystalline powder. M.p. 116–117 °C. ^{1}H NMR ($CDCl_3$): δ 5.87 (br s, 1 H, H-4″), 4.05 (q, 1 H, *J* 5 Hz, H-3), 3.64 (m, 2 H, H-2), 3.20 (dd, 1 H, *J* 11.5 Hz, 5.5 Hz, H-5a), 3.04 (m, 1 H, H-5b), 2.42 (m, 3 H, H-4,4′), 1.42 (s, 9 H, H-1′). ^{13}C NMR ($CDCl_3$): δ 176.0 (C-4″), 155.6 (C-1′), 80.7 (C-1″), 74.7 and 74.0 (rotamer C-3), 52.6 and 52.1 (rotamer C-2), 49.9 and 49.3 (rotamer C-5), 42.4 and 42.0 (rotamer C-4), 36.1 (C-4′), 28.9 (C-1‴). MS (EI): *m/z* 190 (M^+-55).

trans-*3-Hydroxy-4-carboxymethylpyrrolidine* (**18**). To 103 mg (0.42 mmol) of **17** were added 3 ml of trifluoroacetic acid. The reaction was stirred for 30 min after which TFA was evaporated off leaving 106 mg (97%) of the trifluoroacetic acid salt of **18**. ^{1}H NMR (D_2O): δ 4.27 (q, 1 H, *J* 4.5 Hz, H-3), 3.66 (dd, 1 H, *J* 12.5 Hz, 7 Hz, H-2a), 3.44 (dd, 1 H, *J* 13 Hz, 5.5 Hz, H-2b), 3.19 (dd, 1 H, *J* 12.5 Hz, 3.5 Hz, H-5a), 3.08 (dd, 1 H, *J* 12 Hz, 6 Hz, H-5b), 2.58 (m, 2 H, H-4'a,4), 2.42 (dd, 1 H, *J* 18 Hz, 10 Hz, H-4'b). ^{13}C NMR (D_2O): δ 178.0 (C-4''), 75.8 (C-3), 53.2 (C-2), 50.9 (C-5), 44.1 (C-4), 37.3 (C-4').

N-tert-*Butoxycarbonyl*-trans-*3-hydroxy-4-vinylpyrrolidine* (**19**). (A) Compound **10** (1.76 g, 9.5 mmol) dissolved in 10 ml Me_2S was placed in an oven-dried flask. The solution was cooled to −30 °C using dry ice–EtOH and 500 mg of the $CuBr \cdot Me_2S$ complex were added. Using a syringe, 20 ml of 1 M vinylmagnesium bromide (20 mmol) were added through a septum, maintaining the temperature between −30 and −20 °C. Stirring was continued for 90 min keeping the temperature below −10 °C. The reaction mixture was quenched with 30 ml NH_4Cl (10%) and stirred until the water phase was blue and the organic phase was yellow (5 h). The organic phase was separated and the water phase extracted with ether, 2 × 30 ml. The collected organic phases were dried ($MgSO_4$), filtered, evaporated and column chromatographed with CH_2Cl_2–EtOAc (4:1) giving 1.13 g (56%) of **19** as a 9:1 *trans/cis* mixture.

(B) CuCN (200 mg, 2.23 mmol) was placed in an oven-dried flask, which was flushed with nitrogen and equipped with a septum. Addition of 2 ml of dry THF and cooling to 0 °C was followed by the addition of 2 ml of 2 M vinyllithium (4 mmol) via syringe. To the dark brown solution obtained, 342 mg (1.85 mmol) of **10** dissolved in 2 ml THF, were added slowly via syringe. Stirring was continued for 4 h at 0 °C and the mixture left overnight at room temperature. Quenching with 3 ml saturated NH_4Cl and 2 ml $NH_2OH \cdot HCl$ (10%) was followed by extraction with ether, 2 × 30 ml. Drying ($MgSO_4$), filtration, evaporation and column chromatography with CH_2Cl_2–EtOAc (4:1) left 140 mg (36%) of **19** as a yellow oil, which darkened on standing. ^{1}H NMR ($CDCl_3$): δ 5.69 (m, 1 H, H-4'), 5.12 (m, 2 H, H-4''), 4.06 (q, 1 H, *J* 5.5 Hz, H-3), 3.61 (dd, 2 H, *J* 11 Hz, 6.5 Hz, H-2), 3.22 (dd, 1 H, *J* 11 Hz, 3 Hz, H-5a), 3.19 (dd, 1 H, *J* 11 Hz, 1 Hz, H-5b), 2.91 (br s, 1 H, H-3'), 2.68 (quintet, 1 H, *J* 6 Hz, H-4), 1.43 (s, 9 H, H-1'). ^{13}C NMR ($CDCl_3$): δ 155.2 (C-1'), 136.8 (C-4'), 117.6 (C-4''), 80.1 (C-1''), 74.8 (C-3), 52.6 (C-2), 50.5 (C-5), 49.0 (C-4), 29.0 (C-1'''). MS (EI): *m/z* 158 ($M^+ - 55$).

trans-*3-Hydroxy-4-vinylpyrrolidine* (**20**). To 15 mg (0.07 mmol) of **19** was added trifluoroacetic acid (1.5 ml). The reaction was stirred for 30 min after which TFA was evaporated off leaving 15 mg (93%) of the trifluoroacetic acid salt of **20**. ^{1}H NMR (D_2O): δ 5.71 (m, 1 H, H-4'), 5.22 (m, 2 H, H-4''), 4.33 (q, 1 H, *J* 5 Hz, H-3), 3.57 (dd, 1 H, *J* 12.5 Hz, 8 Hz, H-2a), 3.46 (dd, 1 H, *J* 12.5 Hz, 5.5 Hz, H-2b), 3.24 (dd, 1 H, *J* 12 Hz, 6.5 Hz, H-5a), 3.16 (dd, 1 H, *J* 12.5 Hz, 4 Hz, H-5b), 2.89 (quintet, 1 H, *J* 6.5 Hz, H-4). ^{13}C NMR (D_2O): δ 136.9 (C-4'), 121.1 (C-4''), 76.3 (C-3), 53.2 (C-2), 51.6 (C-5), 50.4 (C-4).

N-tert-*Butoxycarbonyl*-trans-*3-hydroxy-4-hydroxymethyl)pyrrolidine* (**21**). 0.76 g (3.56 mmol) of **19** in 15 ml MeOH was placed in a two-necked flask and the solution was cooled to −78 °C using a dry ice–acetone bath. The flask was connected to the ozonizer through a trap and with a sintered inlet tube. To the outlet was connected a destruction trap containing KI and acetic acid. Ozone was bubbled through the solution until it acquired a blue colour. Remaining ozone was removed by blowing a stream of nitrogen through the system. To the stirred cold solution were added 340 mg $NaBH_4$ (9 mmol) in small portions. After 60 min the reaction was quenched with 15 ml HCl (1 M) and MeOH was removed by evaporation. The solution was extracted with EtOAc, 3 × 25 ml, dried ($MgSO_4$), filtered and evaporated, and the residue was dissolved in MeOH and evaporated thus removing boric acid esters. Column chromatography using EtOAc gave 523 mg (68%) of **21** as a syrup. ^{1}H NMR ($CDCl_3$): δ 4.23 (q, 1 H, *J* 5 Hz, H-3), 3.62 (m, 5 H, H-2,4'), 3.22 (dd, 1 H, *J* 11 Hz, 5 Hz, H-5a), 3.10 (dd, 1 H, *J* 11 Hz, 5.5 Hz, H-5b), 2.29 (sextet, 1 H, *J* 6 Hz, H-4), 1.43 (s, 9 H, H-1'). ^{13}C NMR ($CDCl_3$): δ 155.4 (C-1'), 80.4 (C-1''), 72.7 (C-3), 63.0 (C-4'), 52.9 (C-2), 48.4 (C-5), 46.9 (C-4), 29.0 (C-1''').

trans-*3-Hydroxy-4-hydroxymethylpyrrolidine* (**22**). To 25 mg (0.12 mmol) of **21** were added 2 ml of trifluoroacetic acid. The reaction was stirred for 30 min after which TFA was evaporated off leaving 25 mg (94%) of the trifluoroacetic acid salt of **22**. ^{1}H NMR (D_2O): δ 4.39 (dq, 1 H, *J* 3.5 Hz, 3 Hz, H-3), 3.59 (m, 3 H, H-2a,4'), 3.42 (dd, 1 H, *J* 12 Hz, 5 Hz, H-2b), 3.24 (dd, 1 H, *J* 10 Hz, 2.5 Hz, H-5a), 3.14 (dd, 1 H, *J* 6 Hz, 6 Hz, H-5b), 2.44 (d sextet, 1 H, *J* 5 Hz, 2 Hz, H-4). ^{13}C NMR (D_2O): δ 74.3 (C-3), 63.3 (C-4'), 54.5 (C-2), 50.4 (C-5), 49.0 (C-4). MS (EI): *m/z* 117 (M^+).

trans-*3-Acetoxy-4-acetoxymethyl*-N-*(6-uracilylmethyl)-pyrrolidine* (**23a**). To 86 mg (0.37 mmol) of the trifluoroacetic acid salt of **22** dissolved in 2 ml MeOH and 1 ml *N,N*-diisopropylethylamine were added 72 mg (0.45 mmol) of 6-(chloromethyl)uracil. The mixture was stirred for 12 h after which the solvents were removed. The residue was treated with 1 ml pyridine and 1 ml acetic anhydride for 1 h and the solvents removed by evaporation with toluene, 3 × 10 ml. Column chromatography using CH_2Cl_2–MeOH (98:2) gave 80 mg (66%) of **23a**. ^{1}H NMR ($CDCl_3$): δ 10.05 [br s, 1 H-u(= uracil)1 or u3], 9.74 (br s, 1 H, H-u1 or u3), 5.51 (s, 1 H, H-u5), 4.94 (m, 1 H, H-3), 4.17 (m, 2 H, H-4'), 3.42

(m, 2 H, H-1′), 2.3–3.0 (m, 5 H, H-2,5,4), 2.08 (s, 6 H, H-3′,4″). ^{13}C NMR ($CDCl_3$): δ 171.6 (C-3′ or C-4″), 171.3 (C-3′ or C-4″), 165.1 (C-u4 or u2 or u6), 153.1 (C-u4 or u2 or u6), 152.6 (C-u4 or u2 or u6), 99.9 (C-u5), 75.9 (C-3), 64.7 (C-4′), 59.7 (C-1′), 56.1 (C-2), 55.5 (C-5), 44.8 (C-4), 21.5 (C-3″ or C-4‴), 21.4 (C-3″ or C-4‴). MS (EI): *m/z* 325 (M^+-4).

trans - *3 - Hydroxy - 4 - hydroxymethyl* - N - *(6 - uracilyl - methylpyrrolidine* (**23**). To 2 ml of dry MeOH were added 2 mg Na. When sodium had reacted, 80 mg of **23a** was added. The reaction was stirred for 1 h after which the solvent was removed and the residue ion-exchange chromatographed with Amberlite IR-120+ leaving 40 mg (68%) of **23**. ^{1}H NMR (D_2O): δ 5.68 (s, 1 H, H-u5), 4.05 (q, 1 H, *J* 4.5 Hz, H-3), 3.54 (m, 2 H, H-2), 3.43 (d, 2 H, *J* 2.5 Hz, H-4), 3.01 (dd, 1 H, *J* 8.5 Hz, 7.5 Hz, H-5a), 2.70 (d, 2 H, *J* 5 Hz, H-1′), 2.1–2.3 (m, 2 H, H-5b,4). ^{13}C NMR (D_2O): δ 170.0 (C-u4 or u2 or u6), 157.5 (C-u4 or u2 or u6), 156.0 (C-u4 or u2 or u6), 102.5 (C-u5), 75.5 (C-3), 65.1 (C-4′), 63.7 (C-1′), 58.3 (C-2), 58.0 (C-5), 51.7 (C-4), MS (EI): *m/z* 205 ($M-36$), 192 ($M-49$).

N - tert - *Butoxycarbonyl* - trans - *3 - hydroxy - 4 -acetoxy-methylpyrrolidine* (**24**). To a solution of 46 mg (0.21 mmol) of **21** in 4 ml 1,4-dioxane were added 94 mg of immobilized *Candida antarctica* lipase and 168 µl vinyl acetate. After being stirred for 1 h the solution was filtered and the solvent removed from the filtrate yielding 51 mg (92%) of **24**. ^{1}H NMR ($CDCl_3$): δ 4.18 (q, 1 H, *J* 5.5 Hz, H-3), 4.07 (d, 2 H, *J* 6.5 Hz, H-4′), 3.62 (m, 2 H, H-2), 3.26 (dd, 1 H, *J* 11 Hz, 4.5 Hz, H-5a), 3.17 (dd, 1 H, *J* 11 Hz, 5.5 Hz, H-5b), 2.41 (sextet, 1 H, 6.5 Hz, H-4), 2.21 (br s, 1 H, H-3′), 2.07 (s, 3 H, H-4″), 1.44 (s, 9 H, H-1′). ^{13}C NMR ($CDCl_3$): δ 171.7 (C-4″), 155.0 (C-1′), 80.2 (C-1″), 72.2 (C-3), 64.0 (C-4′), 52.9 (C-2), 47.0 (C-5), 45.8 (C-4), 29.0 (C-1‴), 21.4 (C-4‴).

N - *(2,4 - Dinitrophenyl)* - trans - *3 - hydroxy - 4 - acetoxy-methylpyrrolidine* (**25**). To a solution of 18 mg (69 µmol) of **24** was added 1 ml TFA. The reaction was stirred for 30 min after which the solvent was removed and the residues dissolved in 1 ml EtOH (99.9%). To the stirred solution were added 1 ml saturated $NaHCO_3$ and 30 µl 1-fluoro-2,4-dinitrobenzene. After a few seconds the solution became yellow. After 1 h of stirring, 5 ml water was added and the mixture was extracted with EtOAc, 2 × 10 ml, dried ($MgSO_4$), filtered, evaporated and column chromatographed using pentane–EtOAc (1 : 1) to give 16 mg (71%) of **25** as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 8.66 [d, 1 H, *J* 2.5 Hz, H-p(=phenyl)3], 8.23 (dd, 1 H, *J* 9.5 Hz, 2.5 Hz, H-p5), 6.91 (d, 1 H, *J* 9.5 Hz, H-p6), 4.39 (q, 1 H, *J* 5 Hz, H-3), 4.17 (d, 2 H, *J* 6 Hz, H-4′), 3.65 (m, 2 H, H-2), 3.32 (dd, 1 H, *J* 11 Hz, 6 Hz, H-5a), 3.23 (dd, 1 H, *J* 12 Hz, 6.5 Hz, H-5b), 2.62 (sextet, 1 H, *J* 5.5 Hz, H-4), 2.10 (s, 3 H, H-4″). ^{13}C NMR ($CDCl_3$): δ 171.6 (C-4″), 145.9 (C-1′), 128.2 (C-1′), 124-3 (C-1′), 116.1 (C-1′), 71.9 (C-3), 63.1 (C-4′), 57.4 (C-2), 51.8 (C-5), 45.6 (C-4), 21.3 (C-4‴). MS (EI): *m/z* 327 (M^+).

N - *Benzyl* - trans - *3 - hydroxy - 4 - carbamoylmethylpyrrolidine* (**26**). To a solution of 185 mg (0.75 mmol) of **17** in 1 ml DMF were added 266 mg (0.70 mmol) HBTU and 0.262 ml of *N,N*-diisopropylethylamine (DIEA). After 2 min the mixture was added to a reactor containing 239 mg of MBHA-resin and 2 ml of DMF. The reactor was shaken for 1 h. A negative Kaiser test indicated that the resin was fully loaded (capacity: 1.05 mmol g^{-1}). The resin was washed with pyridine, 4 × 2 ml, and DMF–CH_2Cl_2 (1 : 1), 4 × 2 ml and dried in a desiccator.

To 75 mg of the loaded resin (the resin swelled with CH_2Cl_2 over 1 h) was added 1.5 ml TFA and the reactor was left to stand for 10 min. This procedure was repeated once more leaving a brown resin. The resin was washed with DMF–CH_2Cl_2 (1 : 1), 3 × 2 ml, and pyridine, 3 × 2 ml. To this resin were added 3 ml DMF, 140 µl (0.80 mmol) DIEA and 45 µl (0.39 mmol) benzyl chloride. The reactor was shaken for 3 days. The resin was washed consecutively with DMF–CH_2Cl_2 (1 : 1), 2 × 2 ml, pyridine, 3 × 2 ml, and DMF–CH_2Cl_2 (1 : 1) 2 × 2 ml and dried in a desiccator. To the dry resin was added 0.5 ml TFA–TfOH (3 : 1) and the mixture was left standing for 1 h. This procedure was repeated once more, collecting the filtrates. The dark resin was washed with TFA 2 × 1 ml. The filtrates were diluted with water 5 ml and ion-exchange chromatographed with Amberlite IR-120+ leaving 7.5 mg (51%) of **26**. ^{1}H NMR (D_2O): δ 7.38 (m, 5 H, H-1″), 4.12 (m, 1 H, H-3), 3.95 (d, 2 H, *J* = 5.5 Hz, H-1′), 3.30 (dd, 1 H, *J* = 7 Hz, H-2a), 3.13 (dd, 1 H, *J* = 6 Hz, 11.5 Hz, H-2b), 2.90 (dd, 1 H, *J* = 4.5 Hz, 12 Hz, H-5a), 2.2–2.7 (m, 4 H, H-5b,4,4′). ^{13}C NMR (D_2O): δ 179.8 (C-4″), 136.2 (C-1″), 132.9 (C-1″), 131.8 (C-1″), 131.7 (C-1″), 76.7 (C-3), 62.3 (C-1′), 62.1 (C-2), 59.6 (C-5), 45.5 (C-4), 39.7 (C-4′). MS: (*m/z*) 234 (M^+).

Measurements of nucleoside phosphorylase inhibition. This was carried out as described previously.[16]

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