Heterocyclic analogs of nucleosides: synthesis and biological evaluation of novel analogs of puromycin[1]

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The diastereomeric 1-(piperidin-3′-yl)uracil compounds **20***a*, *b*, and the N^6-dimethyl-9-(piperidin-3′-yl)adenine compounds **21***a*, *b*, have been prepared as analogs of the naturally occurring aminoacyl nucleoside antibiotic puromycin. The diastereomers were separated using high-pressure liquid chromatography, and the absolute configuration of the more mobile diastereomer **20***a* was assigned as (3′*S*,5′*R*) by ^{1}H and ^{13}C nuclear magnetic resonance analysis, and by molecular modelling. Therefore, the less mobile diastereomer **20***b* had the (3′*R*,5′*S*) configuration. The configurations of **21***a* and **21***b* were assigned by analogy with **20***a* and **20***b*. These puromycin analogs have been tested for anti-HIV and antitumor activity in vitro.

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On a préparé les composés 1-(pipéridin-3′-yl) uracilee **20***a*, *b*, et N^6-diméthyl-9-(pipéridin-3′-yl) adénines **21***a*, *b*, comme analogues de la puromycine, un antibiotique naturel de type aminoacyle nucléoside. On a séparé les diastéréoisomères par chromatographie liquide á haute pression et on a attribué au diastéréoisomère le plus mobile, **20***a*, la configuration absolue (3′*S*,5′*R*) en se basant sur les résultats de la RMN du ^{1}H et du ^{13}C et sur les modèles moléculaires. Par conséquent, le diastéréoisomère le moins mobile, **20***b*, a la configuration (3′*R*,5′*S*). Par analogie avec les composés **20***a* et **20***b* on attribue les configurations des composés **21***a* et **21***b*. On a testé ces analogues de la puromycine in vitro pour leur activité anti-HIV et anti-tumorale.

[Traduit par la rédaction]

Introduction

The nucleoside derivative puromycin (Fig. 1) has been the focus of consistent interest since its discovery 40 years ago in the fermentation broth of *Streptomyces alboniger* (1). This

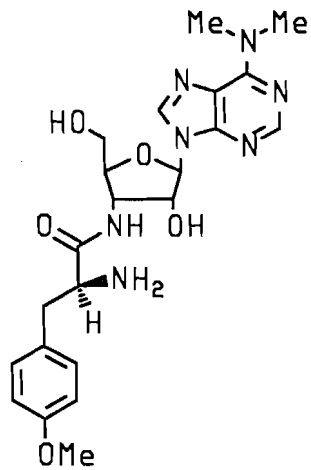

FIG. 1. Puromycin.

potent antibiotic mimics the 3′-end of aminoacyl-tRNA, interrupting protein synthesis on the ribosome (2). Puromycin has antimicrobial, antitumor (3), and antimalarial (4) activities, and is a reversible inhibitor of DNA synthesis (5). However, its therapeutic use is precluded by its extreme toxicity, particularly towards the kidney (6).

To address the problem of toxicity, many analogs of puromycin have been prepared. The ribosyl ring has been replaced by a 3-aminopentopyranose unit (7) or a daunosaminyl group (8), or modified by attachment of the N^6-dimethyladeninyl moiety at the sugar 5′-carbon rather than at the anomeric center (9); however, these modifications afforded no significant biological activities. 2′-Deoxypuromycin was found to be ineffective as an antimicrobial agent (10), as was the 2′,3′-*seco* analog (11). Carbocyclic analogs of puromycin have also been examined. The replacement of the furanose-ring oxygen by a methylene group yielded drugs whose cytotoxicities towards P-388 leukemia were found to be equal to that of puromycin itself (12). The carbocyclic 8-aza analog was identified also as an effective antibiotic (13), whereas a 7-deaza version was not (14). Hydroxycyclopentyl analogs displayed some antitumor effects in *HeLa* cells (15), while the cyclohexyl carbocyclic analog of puromycin was found to be somewhat less active than the cyclopentyl compounds (16).

As part of our ongoing investigation of the synthesis, structure, and biological activities of nucleoside analogs containing a variety of 5- and 6-membered heterocycles in place of the carbohydrate moiety (17), we wished to prepare puromycin analogs **20***a*, *b* and **21***a*, *b* incorporating a piperidine ring. Such compounds should be resistant to removal of the N^6-dimethyladeninyl group in vivo, since the base moiety is not attached by a glycosidic linkage. We wished to look for antitumor effects and activity against HIV. While this work was in progress, Peterson and Vince (18) reported the synthesis of a structurally similar pyrrolidinyl analog.

Results and discussion

Our synthetic strategy involved the construction of a pyrimidine- or purine-ring system on a *cis*-3-amino-5-(hydroxymethyl)piperidine precursor. There are few synthetic approaches available leading to such compounds;[4] however, we felt that lactam **1** (19) would offer the best starting point. Although both enantiomers of this lactam are commercially available,[5] the

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[4]A route which was examined but discarded is described in ref. 32.

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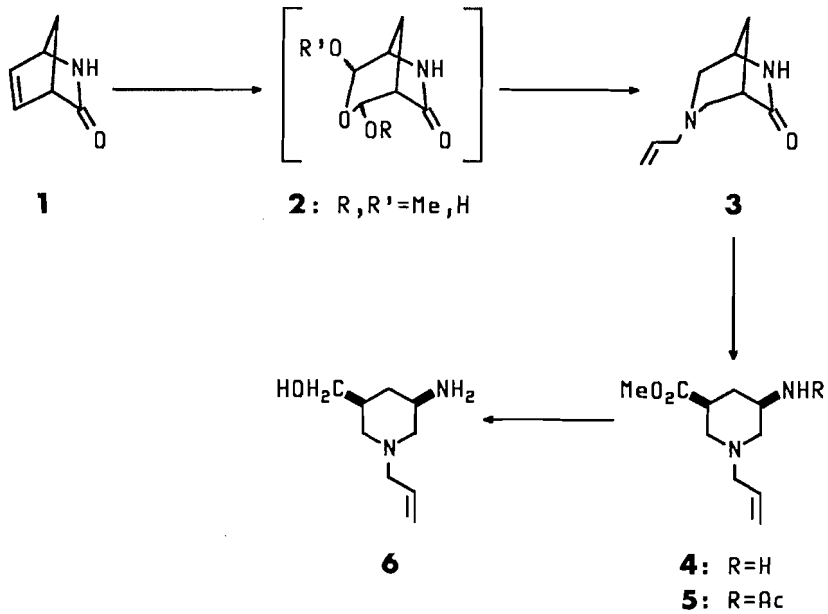


SCHEME 1

price of this material dictated that our nucleoside precursors would be racemic. The diastereomers produced by condensation with the amino acid could be separated and identified at the end of the sequence.

Initial attempts, based on our synthesis of *cis*-3-amino-5-(hydroxymethyl)thiane from **1** by nucleophilic displacement on the derived ditosylate (17), were unsuccessful; elimination products were obtained. Therefore, a reductive amination approach was tried (Scheme 1). Ozonolysis of **1** in methanol, followed by dimethyl sulfide processing, did not produce the expected dialdehyde. The product appeared to be **2**, identified by its ^{1}H and ^{13}C nmr spectra, which contained signals characteristic of the cyclic ketal structure, as well as by its positive reaction to a 2,4-dinitrophenylhydrazine test. This ketal was immediately subjected to reductive amination with allylamine hydrochloride and sodium cyanoborohydride. Standard conditions (20) failed to give the desired product; however, after considerable experimentation, **3** was obtained in 35% overall yield from **1**. No improvement in the yield could be achieved, and the remainder of the product of the reaction consisted of unidentifiable, highly polar material.

The lactam product **3** proved to be rather unstable, discoloring and decomposing on standing overnight. Accordingly, it was immediately converted into amino ester **4** (69%) by acid-promoted methanolysis. A sample of the amino ester was converted into its crystalline acetamido derivative **5** for analysis, while the bulk of the material was reduced, using lithium aluminium hydride, to afford the key amino alcohol, **6** (67%).

Synthesis of uracil-containing puromycin analogs

The construction of the uracil ring is shown in Scheme 2. Condensation of **6** with the acylisocyanate **7** (21), prepared in situ from the corresponding acid chloride, provided the acylurea derivative **8** (80%). Closure of the ring was effected with dilute sulfuric acid, yielding **9**, which was protected for easier purification as the *tert*-butyldimethylsilyl (TBDMS) ether **10** (82% from **8**).

The allyl blocking group was smoothly removed in 74% yield by a tris(triphenylphosphine)rhodium chloride-promoted hydrolysis, using the method of Laguzza and Ganem (22). The free piperidine was then acylated with *N*-(*tert*-butoxycarbonyl)-L-*p*-methoxyphenylalanine (**12**) (23), using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), to provide a 1:1 mixture of diastereomeric protected analogs **13***a*, *b* in 77% yield. It was not possible to separate these diastereomers by chromatographic means.

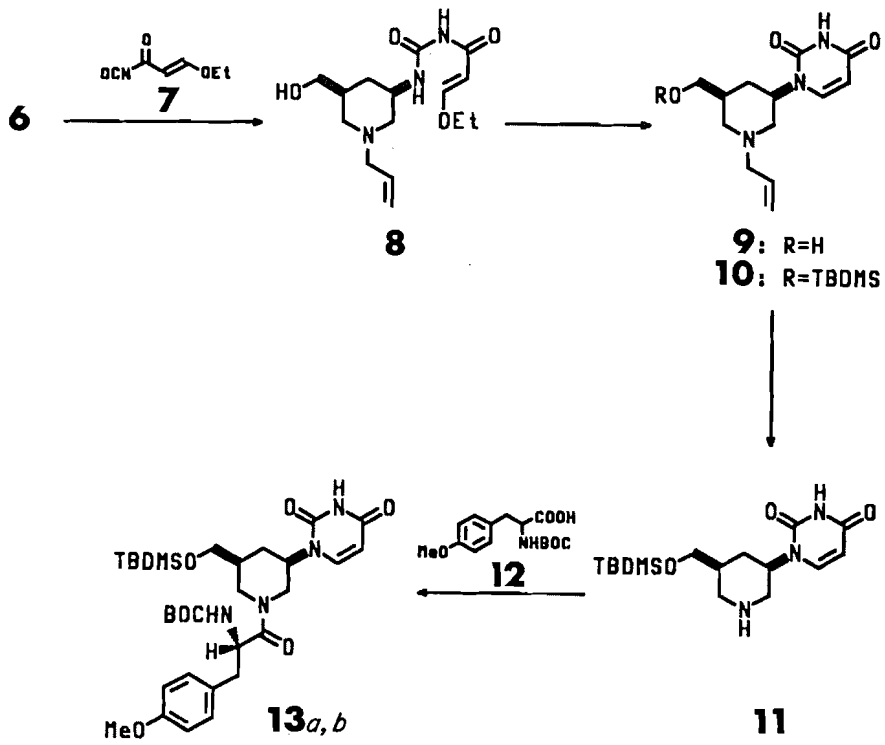
Synthesis of puromycin analogs

Reaction of **6** with 5-amino-4,6-dichloropyrimidine (Scheme 3) provided the chloropyrimidine derivative **14** in 80% yield. Closure of the purine-ring system with triethyl orthoformate and hydrochloric acid gave the chloropurine **15** (69%), which afforded the N^6-dimethyladenine derivative **16** (93%) on treatment with aqueous dimethylamine. This reaction proved difficult to monitor by tlc, as **15** and **16** co-eluted and had similar responses to visualizing agents. The alcohol group of **16** was silylated to give **17** in 87% yield, and the piperidine ring was deblocked to provide **18** (68%). A quantitative yield of diastereomeric adducts **19***a*, *b* was obtained on treatment of **18** with **12**–EDC. These also proved resistant to chromatographic separation.

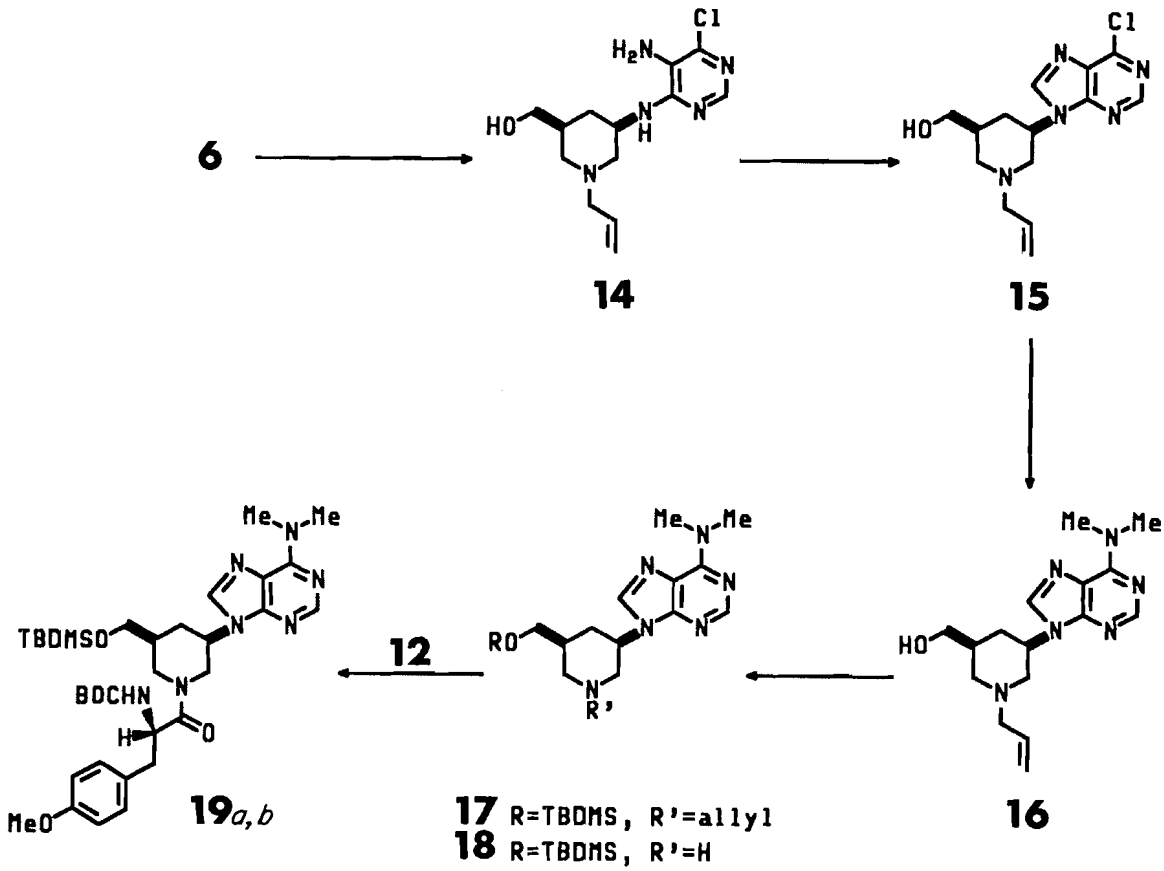
Removal of both the silyl ether and *tert*-butyl carbamate protecting groups of **13** and **19** was achieved by treatment with trifluoroacetic acid in either dichloromethane or methanol solution (Scheme 4). Anisole was added as a scavenger for *tert*-butyl cation; *tert*-butylated material was recovered from an experiment on **13** in the absence of scavenger. The resulting diastereomeric uracil-puromycin analogs **20***a*, *b* (87%) and puromycin analogs **21***a*, *b* (93%) were then separated by reverse-phase hplc.

Identification of product diastereomers

With samples of the diastereomeric products in hand, it remained to ascertain their configurations. For this purpose we turned to ^{1}H nmr spectroscopy. The problem seemed initially to be complicated by the presence of two stable rotamers in all of the spectra, as a result of hindered rotation around the amide C—N bond. Moreover, while spectra of both diastereomers of

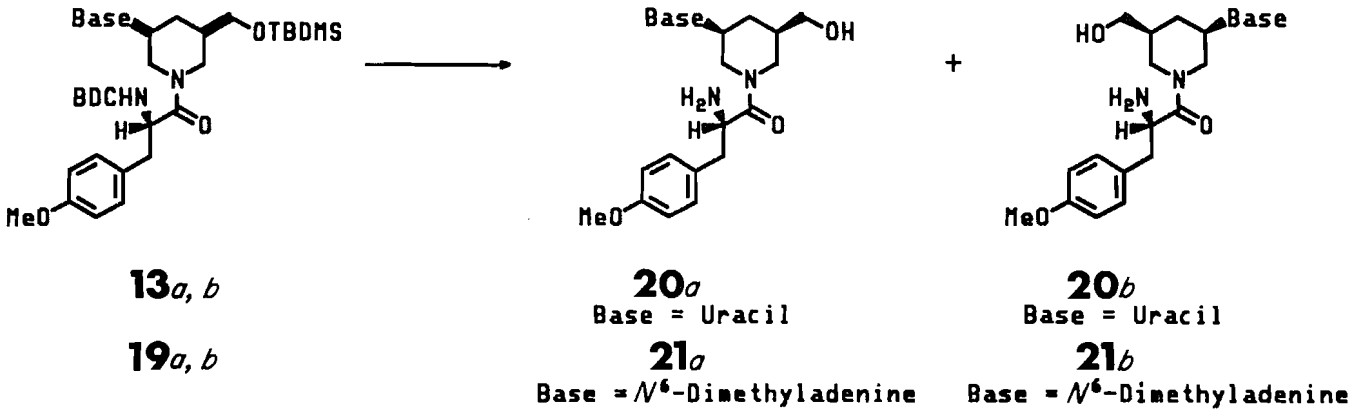


SCHEME 2



SCHEME 3

20 in D_2O solution were well resolved, the N^6-dimethyladenine compounds **21***a*, *b* were not soluble in this solvent, and gave severely broadened spectra in either $CDCl_3$ or DMSO-d_6 solutions. However, we were intrigued by the presence of the extremely high-field signal (δ 0.249 ppm) in the spectrum of the first-eluting fraction of **20** (Fig. 2(*A*)), which was lacking in



SCHEME 4

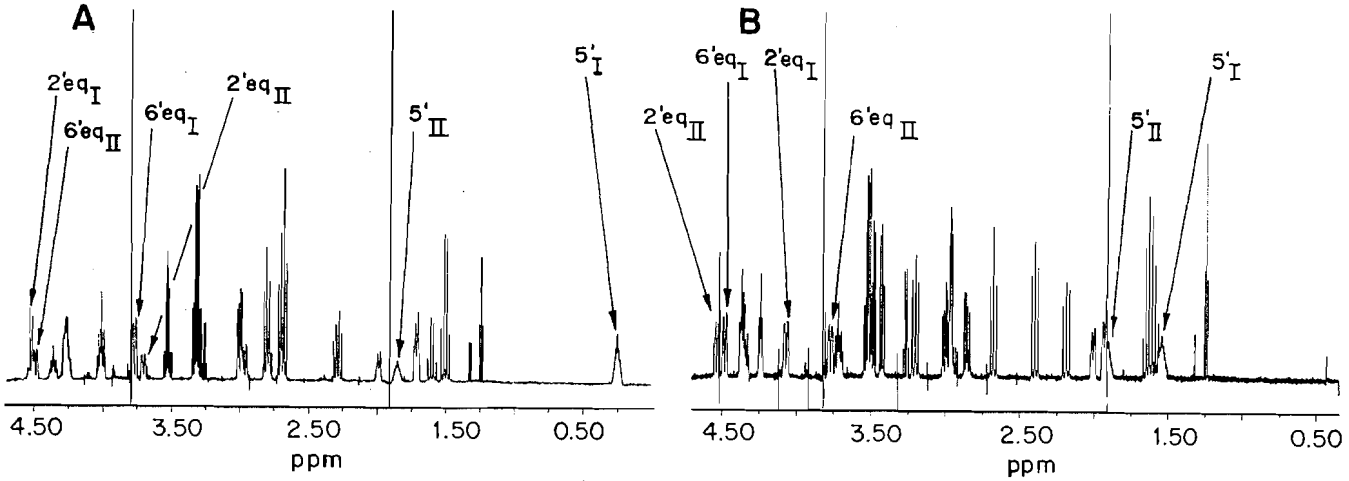


FIG. 2. Aliphatic regions of resolution-enhanced 600-MHz ^{1}H nmr spectra of (*A*) the first-eluting and (*B*) the second-eluting fractions of **20**. Peaks due to the major rotamer are labelled I, and those due to the minor rotamer are labelled II. The triplet at δ 1.142, singlet at δ 1.808, and quartet at δ 3.161 are attributable to $Et_3NH(OAc)$ from the HPLC buffer.

that of the second fraction (Fig. 2(*B*)), and we soon realized that this signal held the solution to our configurational problem.

A ^{1}H-COSY spectrum proved that this anomalously high-field signal arose from the H-5′ proton[6] of the major rotameric form (rotamer I) of the first fraction of **20**. The corresponding signal from H-5′ of the minor rotamer, II, was observed at δ 1.85 ppm. Such a high-field position for a methine proton most likely was due to shielding by the phenyl ring. Such shielding would occur if the ring were "tucked under" the piperidinyl ring. That this situation was only possible for *one* rotamer was evident from the ^{1}H nmr spectra, as was the fact that neither rotamer of the second fraction could adopt such a conformation (Fig. 2(*B*): H-5′$_I$ δ 1.50–1.58 ppm; H-5′$_{II}$ δ 1.87–1.95 ppm). It was clear from the ^3J values of the ring protons that the conformation of the piperidinyl ring was a chair having the 3′ and 5′ substituents equatorially oriented. Therefore, we needed to determine whether the major rotamer of fraction 1 had the carbonyl *syn* to the pyrimidinedione ring, or *anti*. We required also coupling constant and nOe information to set limits on possible side-chain conformations. With these data, we could construct models to ascertain which configuration could adopt the required orientation of the phenyl ring.

The chemical shifts of protons on *N*,*N*-dialkylamides depend on the orientation of the carbonyl: the signals of protons *syn* to the carbonyl are downfield of those of protons *anti* (24). In fraction 1, the signal of H-2′$_{eq}$ (I) is 0.82 ppm downfield of that of H-2′$_{eq}$ (II) and the signal of H-6′$_{eq}$ (I) is 0.72 ppm upfield of that of H-6′$_{eq}$ (II) (see Fig. 2). Carbon chemical shifts also are indicative of amide conformation; however, the *syn* carbon signal occurs *upfield* of that of the *anti* carbon (24). A heteronuclear correlation spectrum showed that the signal of C-2′ (I) is 2.83 ppm upfield of that of C-2′ (II), while the signal of C-6′ (I) is 3.27 ppm downfield from that of C-6′ (II). Further, irradiation of the side-chain H-α produced a 3.3% nOe in the signal of H-6′$_{eq}$ of rotamer I, and a 1.4% nOe in the signal of H-2′$_{eq}$ of rotamer II. These results are consistent with the carbonyl being *syn* to C-2′ and the pyrimidinedione ring in the major rotamer of fraction 1. Similar arguments allowed assignment of the rotamers of fraction 2.

The structures shown in Fig. 3 represent calculated local minima[7] relevant to our nmr observations of carbonyl orienta-

[6]The piperidine-ring system has been numbered according to IUPAC rules.

[7]Computations were performed on an IBM RS/6000 RISC 550 computer operating under AIX. Initial structures approximating the "tucked" and "extended" conformers were constructed based on our ^{1}H nmr data, and minimized using the Dreiding force field as implemented in the program BIOGRAF, from Biodesign, Inc. Energy minimization was accomplished using the conjugate-gradient method with an energy cutoff criterion of 0.001 kcal mol^{-1}. The resulting gas-phase minima were

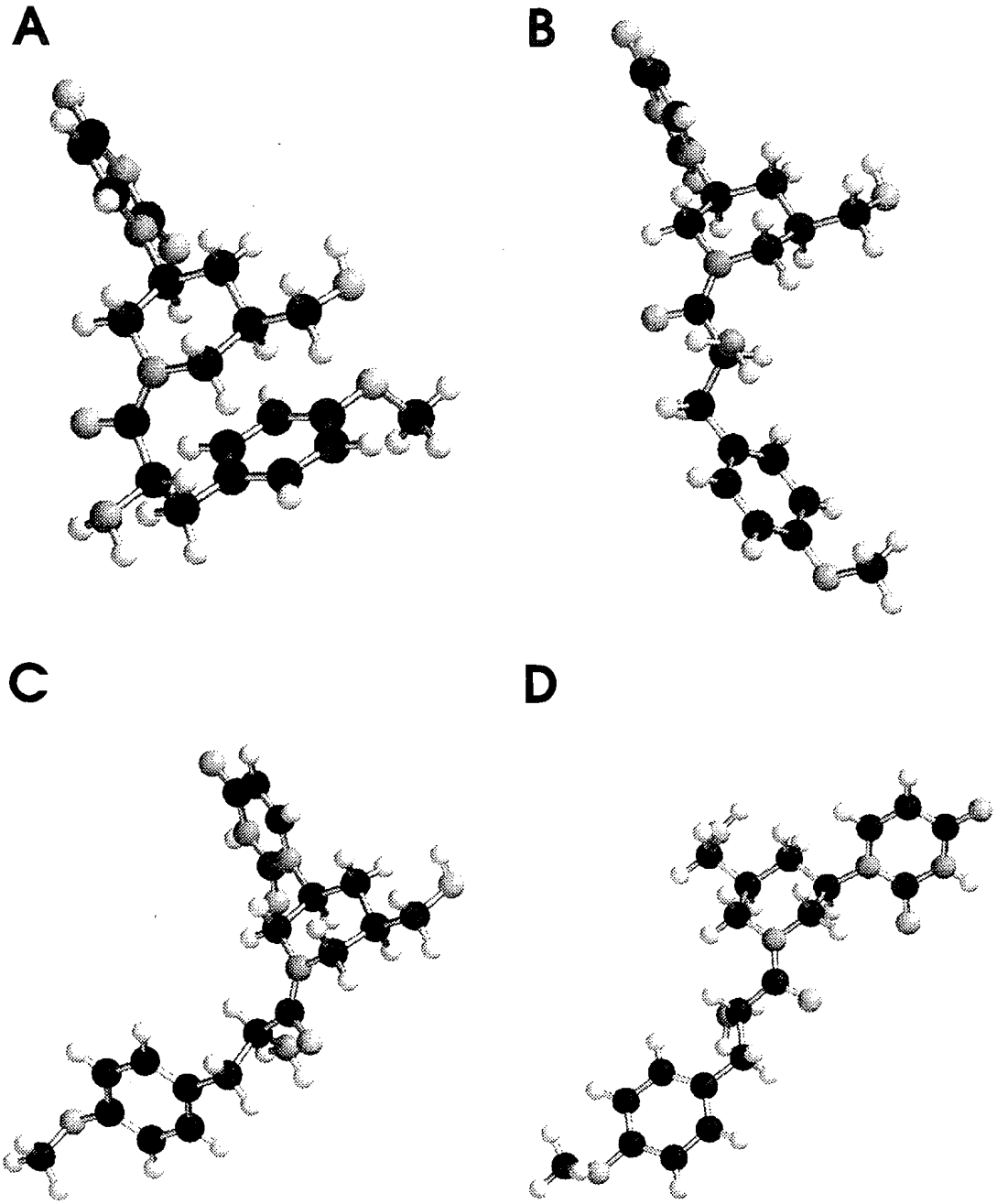


FIG. 3. Possible conformations of analogs **20**. (*A*) The "tucked" conformation of **20***a* with the carbonyl *syn* to the uracil ring. (*B*) An "extended" conformation of **20***a* with the carbonyl *syn*. (*C*) An "extended" conformation of **20***a* with the carbonyl *anti*. (*D*) An "extended" conformation of **20***b* with the carbonyl *syn*. The corresponding "tucked" conformer (not shown) would require overlap of the α-amino group with the C-6′ methylene.

tion and side-chain conformation in **20**. Only the (3′*S*,5′*R*) configuration affords a stable conformation with the carbonyl *syn* and the phenyl ring in the correct position to shield H-5′ (Fig. 3(*A*)). The *anti* rotamer cannot adopt this type of conformation, and must have the side chain extended away from the piperidine ring (Fig. 3(*C*)). The "tucked" conformer shown in Fig. 3(*A*) is the lowest in energy of the structures found for the (3′*S*,5′*R*) configuration, and the calculated torsional angles between H-α and the benzylic H's (55.8° and 171.1°) correspond well to those estimated from ^3J values of rotamer I of the first fraction of **20**, using a modified Karplus equation (25) (50° and 168°).

In the (3′*R*,5′*S*) configuration with the carbonyl *syn*, the α-amino group and the 6′-methylene group would come into contact if the side chain was "tucked under"; both rotamers must exist in conformations having the side chain extended (Fig. 3(*D*)). Therefore, we assign the (3′*S*,5′*R*) stereochemistry **20***a* to fraction 1, and the (3′*R*,5′*S*) stereochemistry **20***b* to the second fraction.

Analogs **21** were not soluble in D_2O, as noted above. The broadened ^1H nmr spectra obtained in $CDCl_3$ solution indicated the presence of an approximately 1:1 mixture of rotamers for both diastereomers; however, there was no high-field signal as

then solvated in a hexagonal lattice of water. The inner cutoff distance was 2.8 Å, the outer cutoff was 8.4 Å, and the lattice spacing was 2.8 Å to give ~3 layers of water. Re-minimization then provided the structures shown in Fig. 3.

in **20***a*. Lowering the temperature failed to "freeze-out" the rotamers above the freezing point of $CDCl_3$. Spectra in DMSO-d_6 solution were also broad; however, raising the temperature to 383 K allowed confirmation of the structure, at the cost of any conformational information. We assigned (tentatively) the (3′*S*,5′*R*) stereochemistry **21***a* to the first-eluting fraction, and the (3′*R*,5′*S*) configuration **21***b* to the second-eluting fraction, by analogy with the pyrimidinedione analogs **20**.

The absence of a "tucked-under" conformation analogous to that of Fig. 3(*A*) for **21** can be explained by the difference in solvents used for the nmr spectra. Such a conformation should be favored in water solutions since it minimizes contact of hydrophobic portions of the molecule with the solvent. In organic solvents, this effect would be unimportant, and extended conformations would result. It is interesting to note that puromycin itself does not adopt such a tucked conformation either in water solution (26), or in the crystal (27), although the phenyl ring is oriented near the 5′-hydroxymethyl group in both states.

Anti-HIV and antitumor assays

Samples of **20***a*, **20***b*, **21***a*, and **21***b* were submitted to the U.S. National Cancer Institute (N.C.I.) for anti-HIV screening by the soluble-formazan assay in human T4 lymphocytes (28). All compounds were inactive in this assay. Some toxicity was observed at high concentrations of **21***a* (1.4×10^{-4} M) but otherwise the compounds had no effect on infected or uninfected cells. Analogs **20***a*, **20***b*, **21***a*, and **21***b* were tested also by N.C.I. against a panel of human leukemia, lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, and renal cancer cell cultures. No useful activity was observed in these assays, although **20***a* displayed a weak effect against HL-60(TB) leukemia ($GI_{50} = 5.84 \times 10^{-5}$ M), and **21***a* had a similarly low activity against HL-60(TB) ($GI_{50} = 7.34 \times 10^{-5}$ M) and against non-small-cell lung cancers HOP-18 ($GI_{50} = 4.57 \times 10^{-5}$ M) and HOP-92 ($GI_{50} = 5.70 \times 10^{-5}$ M).

The pyrrolidinyl puromycin analog of Peterson and Vince (18) was reported to be inactive in an assay against P-388 leukemia cells. The authors suggested that the absence of a 2′-OH group may explain this result, as this hydroxyl has been shown to be essential in puromycin itself (10). The lack of activity of the pyrrolidinyl and our piperidinyl analogs **20**–**21** may also be due to differences in conformation. Our compounds prefer to orient the aminoacyl chain below the mean plane of the piperidine ring, while puromycin exists in a C-3′ *endo* conformation in the solid phase (27) and in solution (26), in which the aminoacyl chain lies near or above the plane of the ribosyl ring.

Experimental

The ^{1}H nmr spectra were recorded on a Bruker AMX-600, AM-400, or AC-F 200 spectrometer at 600.140, 400.136, or 200.132 MHz. The signals due to residual protons in the deuterated solvents indicated were used as internal standards except for spectra recorded in D_2O, in which case 3-(trimethylsilyl)propane sulfonic acid sodium salt was added as an internal standard. Chemical shifts are reported in ppm (δ) downfield from the position of tetramethylsilane (TMS). The symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broadened) are used to describe the multiplicity and shape of the signals. The ^{13}C nmr spectra were obtained at 100.6 MHz on the AM-400 spectrometer or at 50.323 MHz on the AC-F 200 spectrometer, using the JMOD spin-echo sequence (29) to aid in peak identification. Chemical shifts in ppm (δ) downfield from the position of TMS were measured using the solvent signals as internal standards, except for spectra measured in D_2O, where 1,4-dioxane-H_8 (δ 66.5) (30) was added as a standard.

EI mass spectra were recorded on a VG Micromass 7070F mass spectrometer at an ionizing voltage of 70 eV, or on a VG Analytical ZAB-E mass spectrometer, and CI spectra were recorded using NH_3 at ~1 Torr (133.3 Pa) as reagent gas; data are given as *m/z* (% relative intensity). The exact masses were determined under EI conditions. The high-resolution measurements were performed by peak matching using perfluorokerosene as a reference standard.

Melting points were determined using a Fisher–Johns apparatus, and are uncorrected. Flash chromatography was performed on Kieselgel 60 (230–400 mesh), and thin-layer chromatography (tlc) was performed on glass plates coated with Kieselgel 60. High-pressure liquid chromatography (hplc) was carried out using a Beckman System Gold instrument.

The homogeneity of the products was established on the basis of chromatographic, spectroscopic (^{1}H and ^{13}C nmr), and melting point determinations.

(±)-3,6-Diaza-7-oxo-3-(2-propenyl)bicyclo[3.2.1]octane (3)

A solution of freshly recrystallized lactam **1** (see ref. 19) (3.00 g, 27.49 mmol) in dry methanol (300 mL) was purged with oxygen, while being cooled to −70°C in a Dry Ice – acetone bath. Ozone was passed through the solution, maintaining a temperature of −70°C or below, until tlc analysis (0.5% methanol in ethyl acetate as eluant) and the presence of the blue color of an excess of ozone indicated completion. The solution was purged of ozone with a stream of nitrogen. Dimethyl sulfide (10 mL) was added and the mixture was stirred for 1 h. The temperature was allowed to rise to 23°C, the solvents were evaporated, and the residue was left in vacuo for several hours. The oily product (6.934 g) was identified as the mixed ketal **2** by ^{1}H and ^{13}C nmr spectroscopy, and by a positive response to a 2,4-dinitrophenylhydrazine test.

The oil **2** was dissolved in *N*,*N*-dimethylformamide (150 mL). To this solution were added 4 Å molecular sieves (15 g), allylamine hydrochloride (2.57 g, 27.5 mmol), and glacial acetic acid (1.6 mL, 27.5 mmol). The stirred mixture was cooled on ice under a nitrogen atmosphere, and sodium cyanoborohydride (3.45 g, 54.96 mmol) was added. The reaction was allowed to proceed overnight, gradually warming to 23°C. Methanol (150 mL) was added, and the mixture was filtered. The filter cake was washed thoroughly with methanol, and the combined filtrates were evaporated. The residue was dissolved in chloroform (100 mL) and the solution was washed with a 10% aqueous solution of potassium carbonate (50 mL). The aqueous layer was extracted with chloroform (2 × 50 mL), and the combined organic layers were dried ($MgSO_4$) and evaporated. The residue was evaporated twice more from toluene to remove residual *N*,*N*-dimethylformamide, before being chromatographed on silica gel (5% (v/v) methanol in chloroform as eluant) to afford **3** as an oil (1.607 g, 35%). The oil could be distilled (bp 130°C, 0.25 Torr) but even carefully purified material was found to discolor and decompose on standing overnight; ^{1}H nmr ($CDCl_3$, 200 MHz) δ: 1.59 (1H, dd, J_{gem} = 11.0 Hz, J_{vic} = 3.0 Hz, H-8_{eq}), 2.19–2.41 (4H, m, H-1, H-2_{ax}, H-4_{ax}, H-8_{ax}), 2.88 (1H, m, H-4_{eq}), 3.06–3.16 (3H, m, H-2_{eq}, =CHCH_2N), 3.71 (1H, m, H-5), 5.06–5.20 (2H, m, H_2C=), 5.69–5.95 (2H, m, =C*H*CH_2, NH); ^{13}C nmr ($CDCl_3$, 50 MHz) δ: 37.548 (C-8), 40.188 (C-1), 50.989 (C-5), 53.444 (C-2), 54.242 (C-4), 59.955 (=CHCH_2N), 117.512 (H_2C=), 134.749 (=*C*HCH_2), 179.942 (C-7).

(±)-(3β,5β)-3-Amino-5-(methoxycarbonyl)-1-(2-propenyl)piperidine (4)

To a solution of lactam **3** (2.80 g, 16.8 mmol) in methanol (50 mL) was added concentrated aqueous hydrochloric acid (3 mL), and the mixture was heated at reflux temperature for 2 h. A further portion of hydrochloric acid (1 mL) was added, and heating was continued. After 5 h, the mixture was cooled, and concentrated. The residue was dissolved in water (40 mL), and the solution was adjusted to pH 9.0 with solid potassium carbonate. The aqueous solution was extracted with chloroform (3 × 50 mL), and the organic extracts were dried ($MgSO_4$) and evaporated. The residual oil was distilled (bp 75–85°C, 0.01 Torr) using a Kugelrohr apparatus, to afford the aminoester **4** (2.29 g, 69%);

^{1}H nmr ($CDCl_3$, 400 MHz) δ: 1.13 (1H, ddd, $J_{3,4ax}$ = 11.0 Hz, $J_{4ax,5}$ = 12.5 Hz, $J_{4ax,4eq}$ = 12.5 Hz, H-4_{ax}), 1.24 (2H, br s, NH_2), 1.60 (1H, dd, $J_{2ax,3}$ = 10.0 Hz, $J_{2ax,2eq}$ = 10.5 Hz, H-2_{ax}), 1.93 (1H, dd, $J_{5,6ax}$ = 11.4 Hz, $J_{6ax,6eq}$ = 11.1 Hz, H-6_{ax}), 2.20 (1H, m, H-4_{eq}), 2.65 (1H, dddd, $J_{4ax,5}$ = 12.5 Hz, $J_{5,6ax}$ = 11.4 Hz, J_{vic} = 4.0 Hz, J_{vic} = 3.8 Hz, H-5), 2.85 (1H, dddd, $J_{2ax,3}$ = 10.0 Hz, $J_{3,4ax}$ = 11.0 Hz, J_{vic} = 4.5 Hz, J_{vic} = 4.0 Hz, H-3), 2.96 (1H, m, H-2_{eq}), 2.99–3.02 (2H, m, =CHCH_2N), 3.07 (1H, m, H-6_{eq}), 3.65 (3H, s, OCH_3), 5.11–5.19 (2H, m, H_2C=), 5.82 (1H, m, =CHCH_2); ^{13}C nmr ($CDCl_3$, 50 MHz) δ: 37.060 (C-4), 41.061 (C-5), 47.639 (C-3), 51.542 (OCH_3), 54.402 (C-6), 61.275 (=CHCH$_2$N), 62.065 (C-2), 117.940 (H_2C=), 134.618 (=CHCH$_2$), 173.791 (C=O).

(±)-(3β,5β)-3-Acetamido-5-(methoxycarbonyl)-1-(2-propenyl)piperidine (5)

Aminoester **4** (85 mg, 0.43 mmol) was dissolved in dry pyridine (1 mL) and the solution was cooled on ice as acetic anhydride (4 drops, excess) was added. The mixture was stirred at 23°C for 1 h, at which point tlc analysis (15% (v/v) methanol in chloroform as eluant) indicated complete reaction. The pyridine was evaporated, and the residue was dissolved in chloroform (10 mL). The solution was washed with a 10% aqueous solution of sodium hydrogen carbonate (2 × 2 mL) and then with brine (2 mL). The organic layer was dried ($MgSO_4$) and concentrated to yield a solid product, 110 mg (quant.). A sample recrystallized from diisopropyl ether had mp 98–100°C; ^{1}H nmr ($CDCl_3$, 400 MHz) δ: 1.53 (1H, br m, H-4_{ax}), 1.95 (3H, s, C(O)CH_3), 1.95–2.13 (2H, br m, H-4_{eq} and H-6_{ax}), 2.34 (1H, br m, H-2_{ax}), 2.70 (1H, m, H-5), 2.79–2.86 (2H, br m, H-2_{eq} and H-6_{eq}), 2.99 (1H, dddd, J_{gem} = 13.5 Hz, J_{vic} = 6.5 Hz, 2 × allylic J's ~1 Hz each, =CHCH_2N), 3.05 (1H, dddd, J_{gem} = 13.5 Hz, J_{vic} = 6.35 Hz, 2 × allylic J's ~1 Hz each, =CHCH_2N), 3.678 (3H, s, OCH_3), 4.02 (1H, m, H-3), 5.13–5.20 (2H, m, H_2C=), 5.81 (1H, m, =CHCH$_2$), 5.88 (1H, br s, NH); ^{13}C nmr ($CDCl_3$, 100 MHz) δ: 23.441 (C(O)CH$_3$), 31.812 (C-4), 40.035 (C-5), 45.124 (C-3), 51.892 (OCH_3), 54.264 (C-6), 57.858 (C-2), 61.194 (=CHCH$_2$N), 118.179 (H_2C=), 134.487 (=CHCH$_2$), 169.376 (amide C=O), 174.38 (ester C=O); ms (EI): 241 (M + H)$^+$ (3.3%), 209 ($M^{+\cdot}$ − $\cdot OCH_3)^+$ (5.5%), 181 ($M^{+\cdot}$ − $CH_3CONH_2)^{+\cdot}$ and (or) ($M^{+\cdot}$ − $\cdot COOCH_3)^+$ (100%), 140 ($C_7H_{12}N_2O$: $M^{+\cdot}$ − H_2C=$CHCH_2\cdot$ − $\cdot COOCH_3)^{+\cdot}$ (89.3%), 122 ($C_8H_{12}N$: 181 − CH_3CONH_2 or $\cdot COOCH_3)^+$ (36.9%). Exact Mass calcd. for ($C_{12}H_{20}N_2O_3$ + H): 241.1553; found (hrms): 241.15419. Anal. calcd. for $C_{12}H_{20}N_2O_3$: C 59.98, H 8.39, N 11.66; found: C 59.89, H 8.29, N 11.43.

(±)-(3β,5β)-3-Amino-5-(hydroxymethyl)-1-(2-propenyl)piperidine (6)

A solution of aminoester **4** (2.20 g, 11.1 mmol) in dry oxolane (15 mL) was added dropwise to a suspension of lithium aluminium hydride (0.50 g, 13.2 mmol) in oxolane (10 mL) at 0°C. The reaction mixture was stirred under a nitrogen atmosphere for 30 min. A further portion of lithium aluminium hydride (255 mg, 6.7 mmol) was added, and stirring was continued for 15 min; tlc analysis (plate pretreated with NH_3 vapor, 15% (v/v) methanol in chloroform as eluant) indicated complete reaction had occurred. The reaction was quenched by cautious addition of water (1 mL), then a 15% (w/v) aqueous solution of sodium hydroxide (1 mL), followed by more water (3 mL), was added. The mixture was warmed to 23°C and stirred for 40 min. Magnesium sulfate was added, and the mixture was filtered. The filter cake was washed with oxolane (25 mL), and the combined filtrates were concentrated to afford an oil. Chromatography on silica gel using the organic layer of a 12:6:1 (v/v/v) chloroform–isopropanol – aqueous ammonium hydroxide mixture as eluant afforded the amino alcohol **6** as a solid (1.251 g, 67%). A sample recrystallized from diisopropyl ether had mp 74–75°C; ^{1}H nmr ($CDCl_3$, 200 MHz) δ: 0.73 (1H, apparent q, J_{app} = 12.0 Hz, H-4_{ax}), 1.47–2.00 (7H, m, H-2_{ax}, H-4_{eq}, H-5, H-6_{ax}, OH, NH_2), 2.83–3.13 (5H, m, H-2_{eq}, H-3, H-6_{eq}, =CHCH_2N), 3.44 (1H, dd, J_{gem} = 10.7 Hz, J_{vic} = 6.6 Hz, CHOH), 3.53 (1H, dd, J_{gem} = 10.7 Hz, J_{vic} = 5.5 Hz, CHOH), 5.06–5.24 (2H, m, H_2C=), 5.86 (1H, m, =CHCH$_2$); ^{13}C nmr ($CDCl_3$, 50 MHz) δ: 37.599 (C-4), 38.361 (C-5), 48.076 (C-3), 56.355 (C-6), 61.761 (C-2), 62.834 (=CHCH$_2$N), 65.895 (CH_2OH), 117.968 (H_2C=), 134.946 (=CHCH$_2$); ms (EI): 170 (M)$^{+\cdot}$ (12.8%), 152 ($M^{+\cdot}$ − $H_2O)^{+\cdot}$ (7.1%), 127 ($M^{+\cdot}$ − H_2C=$CHNH_2)^{+\cdot}$ (24.0%), 122 ($M^{+\cdot}$ − H_2O − $\cdot CH_2NH_2)^+$ (9.8%), 96 (15.2%), 84 ($C_5H_{10}N)^+$ (100%). Exact Mass calcd. for $C_9H_{18}N_2O$: 170.14204; found (hrms): 170.14316. Anal. calcd. for $C_9H_{18}N_2O$: C 63.49, H 10.66, N 16.45; found: C 63.45, H 10.55, N 16.50.

*(±)-*trans*-3-Ethoxy-N-{N′-[(3′β,5′β)-5′-(hydroxymethyl)-1′-(2-propenyl)piperidin-3′-yl]carbamoyl}propenamide (8)*

Following the procedure of Shealy and O'Dell (21*a*), silver cyanate was dried at 60°C and <1 Torr in the dark for 12 h. Distilled *N,N*-dimethylformamide was dried over 4 Å molecular sieves. All glassware was dried overnight at 110°C, and assembled while hot, under a nitrogen atmosphere.

A suspension of silver cyanate (1.055 g, 7.04 mmol) in dry benzene (5 mL) was heated to reflux temperature under a nitrogen atmosphere. After 30 min, a solution of *trans*-3-ethoxypropenoyl chloride (21*b*) (475 mg, 3.52 mmol) in benzene (5 mL) was added. The mixture was boiled for 45 min more. Heating and stirring were stopped, and the suspended solids were allowed to settle from the solution of acylisocyanate **7** over 2 h.

A solution of amino alcohol **6** (400 mg, 2.35 mmol) in *N,N*-dimethylformamide (15 mL) was cooled to −75°C under a nitrogen atmosphere. An 8-mL portion of the solution of **7** (~2.82 mmol) was removed with a syringe, and was added dropwise to the amine solution. The mixture was rapidly warmed to −30°C, and was then stirred for 6 h as the temperature slowly rose to 23°C. The solvents were evaporated under high vacuum, and the residue was chromatographed on silica gel using a gradient of 5% → 7% → 10% (v/v) methanol in chloroform as eluant. Acylurea derivative **8** was obtained as a glassy foam (588 mg, 80%). A sample obtained by recrystallization from diisopropyl ether had mp 113°C; ^{1}H nmr ($CDCl_3$, 400 MHz) δ: 0.97 (1H, ddd, $J_{3',4'ax}$ = 11.8 Hz, $J_{4'ax,5'}$ = 12.8 Hz, J_{gem} = 10.9 Hz, H-$4'_{ax}$), 1.35 (3H, t, J = 7.1 Hz, CH_3), 1.71 (1H, dd, $J_{5',6'ax}$ = 11.1 Hz, J_{gem} = 10.7 Hz, H-$6'_{ax}$), 1.78 (1H, dd, $J_{2'ax,3'}$ = 10.4 Hz, J_{gem} = 10.6 Hz, H-$2'_{ax}$), 1.66–1.82 (1H, br s, OH), 1.88–1.99 (1H, m, H-5′), 2.07 (1H, m, H-$4'_{eq}$), 2.99 (1H, br dd, J_{gem} = 10.7 Hz, $J_{5',6'eq}$ = 3.5 Hz, H-$6'_{eq}$), 2.99–3.08 (2H, m, =CHCH_2N), 3.13 (1H, br dd, J_{gem} = 10.6 Hz, $J_{2'eq,3'}$ = 4.2 Hz, H-$2'_{eq}$), 3.46 (1H, dd, J_{gem} = 10.5 Hz, J_{vic} = 7.1 Hz, CHOH), 3.58 (1H, dd, J_{gem} = 10.5 Hz, J_{vic} = 5.6 Hz, CHOH), 3.92–4.01 (1H, m, H-3′), 3.96 (2H, q, J = 7.1 Hz, OCH_2CH$_3$), 5.11–5.20 (2H, m, H_2C=), 5.34 (1H, d, J = 12.2 Hz, H-2), 5.84 (1H, m, =CHCH$_2$), 7.61 (1H, d, J = 12.2 Hz, H-3), 8.59 (1H, d, J = 7.9 Hz, CHNH), 9.45 (1H, s, C(O)NHC(O)); ^{13}C nmr ($CDCl_3$, 100 MHz) δ: 14.440 (CH_3), 33.928 (C-4′), 37.977 (C-5′), 46.570 (C-3′), 56.057 (C-6′), 58.496 (C-2′), 61.532 (=CHCH$_2$N), 65.593 (CH_2OH), 67.453 (OCH$_2$CH$_3$), 98.030 (C-2), 118.160 (H_2C=), 134.609 (=CHCH$_2$), 154.696 (urea C=O), 162.635 (C-3), 168.376 (C-1); ms (EI): 311 (M)$^{+\cdot}$ (3.1%), 153 ($C_9H_{15}NO$: $M^{+\cdot}$ − EtOCH=CHCONHCONH$_2)^{+\cdot}$ (100%), 122 (153 − $\cdot CH_2OH)^+$ (18.5%), 99 (EtOCH=CHC≡O)$^+$ (10.6%); ms (CI): 312 (M + H)$^+$ (100%), 272 (M − H_2C=$CHCH_2\cdot$ + 2H)$^+$ (3.6%), 214 ($C_{10}H_{20}N_3O_2)^+$ (2.0%), 197 (214 − $NH_3)^+$ (8.8%), 171 ($M^{+\cdot}$ − EtOCH=CHCONHCO$\cdot)^+$ (35.0%), 116 (EtOCH=CHCONH$_2$ + H)$^+$ (21.4%). Exact Mass calcd. for $C_{15}H_{25}N_3O_4$: 311.1845; found (hrms): 311.18593. Anal. calcd. for $C_{15}H_{25}N_3O_4$: C 57.86, H 8.09, N 13.49; found: C 57.89, H 8.03, N 13.52.

*(±)-1-{(3′β,5′β)-1′-(2-Propenyl)-5′-(*tert*-butyldimethylsilyloxymethyl)piperidin-3′-yl}-2,4(1H,3H) pyrimidinedione (10)*

Enol ether **8** (311 mg, 1 mmol) was dissolved in water (10 mL). The solution was adjusted to pH 1.0 with 1 M sulfuric acid, and then heated at reflux temperature for 20 min. The cooled reaction mixture was stirred at 23°C for 1.5 h more, before being neutralized with solid potassium carbonate. The solvent was removed by freeze-drying, and the solid residues were extracted with 20% (v/v) methanol in chloroform until no further product could be detected in the extracts. The solvent was evaporated to afford pyrimidinedione **9** as its H_2SO_4 salt (337 mg, 93%).

The salt (297 mg, 0.817 mmol) was suspended in dry *N,N*-dimethylformamide (5 mL). Imidazole (218 mg, 3.2 mmol) and *tert*-

butyldimethylsilyl chloride (180 mg, 1.2 mmol) were added, followed by a catalytic amount of 4-(dimethylamino)pyridine. After stirring under a nitrogen atmosphere for 1.5 h, a further portion of *tert*-butyldimethylsilyl chloride (100 mg, 0.66 mmol) was added, and the reaction was allowed to proceed overnight. Water (10 mL) was added, followed by dichloromethane (10 mL), and the phases were separated. The aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layers were washed with a 10% (w/v) aqueous solution of sodium hydrogen carbonate (2 × 10 mL), and with brine (10 mL). The dried ($MgSO_4$) organic solution was evaporated, and the residue was evaporated once more from toluene to remove traces of *N,N*-dimethylformamide. Chromatography of the residue on silica gel (5% (v/v) methanol in chloroform as eluant) afforded **10** as a foam (314 mg, 82% overall), which became a crystalline solid on a further evaporation from chloroform. A sample recrystallized from water–acetonitrile formed needles; mp 142–143°C; ^{1}H nmr ($CDCl_3$, 200 MHz) δ: 0.03 (6H, s, $Si(CH_3)_2$), 0.87 (9H, s, $SiC(CH_3)_3$), 1.29 (1H, apparent q, J_{app} = 12.2 Hz, H-4′$_{ax}$), 1.69 (1H, dd, $J_{5',6'ax}$ = 11.0 Hz, J_{gem} = 11.1 Hz, H-6′$_{ax}$), 1.87–2.10 (3H, m, H-2′$_{ax}$, H-4′$_{eq}$, H-5′), 2.93–3.16 (4H, m, H-2′$_{eq}$, H-6′$_{eq}$, =CHC*H*$_2$N), 3.49 (2H, apparent d, J_{app} = 5.6 Hz, CH_2OSi), 4.69 (1H, m, H-3′), 5.10–5.23 (2H, m, H_2C=), 5.70 (1H, d, *J* = 8.0 Hz, H-5), 5.82 (1H, m, =C*H*CH$_2$), 7.21 (1H, d, *J* = 8.0 Hz, H-6), 9.49–9.71 (1H, br, NH); ^{13}C nmr ($CDCl_3$, 50 MHz) δ: −5.47 ($Si(CH_3)_2$), 18.252 ($SiC(CH_3)_3$), 25.861 ($SiC(CH_3)_3$), 32.116 (C-4′), 38.278 (C-5′), 52.529 (C-3′), 55.882 (C-6′), 56.912 (C-2′), 61.331 (=CH*C*H$_2$N), 65.522 (CH_2OSi), 102.178 (C-5), 118.207 (H_2C=), 134.520 (=*C*HCH$_2$), 140.846 (C-6), 150.818 (C-2), 163.189 (C-4); ms (EI): 380 (M + H)$^+$ (3.8%), 379 (M)$^{+\cdot}$ (4.0%), 364 (M$^{+\cdot}$ − ·CH$_3$)$^+$ (5.2%), 322 (M$^{+\cdot}$ − *tert*-Bu·)$^+$ (5.5%), 268 (M − pyrimidinedione + H)$^+$ (26.0%), 267 (M$^{+\cdot}$ − pyrimidinedione)$^{+\cdot}$ (100%), 226 (267 − H_2C=CHCH_2·)$^+$ (34.5%), 210 (267 − *tert*-Bu·)$^+$ (17.3%), 170 (13.9%), 134 (19.7%), 122 (267 − *tert*-BuSiMe$_2$OCH$_2$·)$^+$ (26.2%). Exact Mass calcd. for $C_{19}H_{33}N_3O_3Si$: 379.2293; found (hrms): 379.2281. Anal. calcd. for $C_{19}H_{33}N_3O_3Si$: C 60.12, H 8.76, N 11.07; found: C 60.15, H 8.67, N 11.05.

*(±)-1-[(3′*β*,5′*β*)-5′-(*tert*-Butyldimethylsilyloxymethyl)piperidin-3′-yl]-2,4(1*H*,3*H*)pyrimidinedione (**11**)*

Using the procedure and apparatus described by Laguzza and Ganem (22), a solution of allyl amine derivative **10** (310 mg, 0.82 mmol) in 84:16 (v/v) acetonitrile–water (15 mL) was heated at reflux temperature under a stream of nitrogn, in the presence of tris(triphenylphosphine)rhodium chloride (20 mg). Fresh solvent was added during the reaction to maintain a constant volume. After 2.5 h, tlc analysis (10% (v/v) methanol in chloroform as eluant) showed that the reaction was essentially complete. The mixture was cooled, and the solvents were evaporated. The residue was evaporated several times from acetonitrile before being purified by preparative tlc on a 2 mm × 20 cm × 20 cm silica gel plate (E. Merck no. 5717) using 10% (v/v) methanol in chloroform as eluant. The free amine **11** was obtained as a yellowish solid (205 mg, 74%); ^{1}H nmr (CD_3OD, 200 MHz)[8] δ: 0.06 (6H, s, $Si(CH_3)_2$), 0.89 (9H, s, $SiC(CH_3)_3$), 1.55 (1H, apparent q, J_{app} = 12.4 Hz, H-4′$_{ax}$), 1.80–2.01 (2H, m, H-4′$_{eq}$ and H-5′), 2.28 (1H, br dd, Σ*J* = 23.9 Hz, H-6′$_{ax}$), 2.66 (1H, br dd, Σ*J* = 23.4 Hz, H-2′$_{ax}$), 3.01–3.16 (2H, m, H-2′$_{eq}$ and H-6′$_{eq}$), 3.51 (1H, dd, J_{gem} = 10.2 Hz, J_{vic} = 6.2 Hz, CHOSi), 3.55 (1H, dd, J_{gem} = 10.2 Hz, J_{vic} = 5.3 Hz, CHOSi), 4.50 (1H, m, H-3′), 5.67 (1H, d, *J* = 8.0 Hz, H-5), 7.64 (1H, d, *J* = 8.0 Hz, H-6); ^{13}C nmr (CD_3OD, 50 MHz)[8] δ: −5.337 ($Si(CH_3)_2$), 19.334 ($SiC(CH_3)_3$), 26.352 ($SiC(CH_3)_3$), 33.004 (C-4′), 41.400 (C-5′), 49.136 (C-6′), 49.940 (C-2′), 54.474 (C-3′), 66.780 (CH_2OSi), 102.550 (C-5), 143.525 (C-6), 152.699 (C-2), 166.177 (C-4); ms (EI): 340 (M + H)$^+$ (4.0%), 324 (M$^{+\cdot}$ − ·CH$_3$)$^+$ (4.2%), 282 (M$^{+\cdot}$ − *tert*-Bu·)$^+$ (7.1%), 227 (M$^{+\cdot}$ − pyrimidinedione)$^{+\cdot}$ (65.3%), 170 (227 − *tert*-Bu·)$^+$ (100%), 95 (60.1%); ms (CI): 340 (M + H)$^+$ (100%), 228 (7.5%), 227 (9.6%), 170 (8.6%), 113 (pyrimidinedione + H)$^+$ (4.7%), 95 (10.5%). Exact Mass calcd. for ($C_{16}H_{29}N_3O_3Si$ + H): 340.2058; found (hrms): 340.2029.

[8]In $CDCl_3$, all of the nmr signals were observed to be very broad.

*1-[(3′*RS*,5′*SR*)-1′-(2*S*-[*tert*-Butoxycarbamoyl]-3-(4-methoxyphenyl)propanoyl)-5′-(*tert*-butyldimethylsilyloxymethyl)-piperidin-3′-yl]-2,4(1*H*,3*H*)pyrimidinedione (**13**a, b)*

The amine **11** (190 mg, 0.56 mmol) and amino acid derivative **12** (23) (185 mg, 0.63 mmol) were dissolved in dry dichloromethane (15 mL) and the solution was cooled to 0°C. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (140 mg, 0.73 mmol) was added, with a further portion of dichloromethane (2 mL). The reaction was stirred under nitrogen for 1.5 h at 0°C. Water (5 mL) was added to quench the reaction. The phases were separated, and the organic layer was washed successively with water (10 mL), a 10% (w/v) aqueous sodium hydrogen carbonate solution (10 mL), and with brine (10 mL) before being dried ($MgSO_4$) and evaporated. The resulting thick yellow oil was chromatographed on silica gel using a slow gradient of 0% → 5% (v/v) methanol in chloroform as eluant. No separation of the product diastereomers was observed, and a 1:1 mixture of **13***a* and **13***b* was obtained as a glassy solid (265 mg, 77%); ^{1}H nmr ($CDCl_3$, 200 MHz)[9] δ: 0.01 (6H, br s, $Si(CH_3)_2$), 0.86 (9H, br s, $SiC(CH_3)_3$), 1.34–1.37 (9H, 2 × s, $OC(CH_3)_3$), 3.73 (3H, 2 × s, $ArOCH_3$), 5.64–5.72 (1H, 2 × d, H-5), 6.74–7.16 (5H, m, phenyl H's and H-6); ^{13}C nmr ($CDCl_3$, 50 MHz)[9] δ: −5.482 ($Si(CH_3)_2$), 18.259 ($SiC(CH_3)_3$), 25.885 ($SiC(CH_3)_3$), 28.329 ($OC(CH_3)_3$), 32.167 (C-4′), 38.060 (C-5′), 45.051 (CH_2Ar), 51.14–51.60 (C-3′), 55.19–55.37 ($ArOCH_3$), 64.41–64.70 (CH_2OSi), 79.66 ($OC(CH_3)_3$), 102.5–102.67 (C-5), 113.48–114.23 (phenyl C-3), 123.24–128.48 (phenyl C-1), 130.44–130.76 (phenyl C-2), 140.34–140.89 (C-6), 150.49–150.62 (C-2), 155.11 (phenyl C-4), 158.51–158.82 (NHC(O)O), 163.24 (C-4), 170.70–171.12 (amide C=O); ms (EI): 617 (M + H)$^+$ (4.9%), 559 (M$^{+\cdot}$ − *tert*-Bu·)$^+$ (2.9%), 543 (M$^{+\cdot}$ − *tert*-BuO·)$^+$ (3.9%), 517 (M − CO_2 − $(CH_3)_2C$=CH_2 + H)$^+$ (11.0%), 499 (M$^{+\cdot}$ − *tert*-BuOC(O)NH$_2$)$^{+\cdot}$ (10.5%), 442 (499 − *tert*-Bu·)$^+$ (100%), 395 ($C_{18}H_{31}N_4O_4Si$)$^+$ (15.7%), 387 (499 − pyrimidinedione)$^{+\cdot}$ (17.1%), 330 (442 − pyrimidinedione)$^+$ (34.7%), 282 (18.8%), 226 (15.6%), 194 (15.0%), 170 (26.9%), 161 (4-OMeArCH=CHC≡O)$^+$ (37.5%), 150 (4-OMeArCH$_2$CH=NH$_2$)$^+$ (50.2%), 121 (4-OMeArCH$_2$)$^+$ (85.4%). Exact Mass calcd. for ($C_{31}H_{48}N_4O_7Si$ + H): 617.3373; found (hrms): 617.3353.

*(±)-5-Amino-6-chloro-4-[[(3′*β*,5′*β*)-5′-(hydroxymethyl)-1′-(2-propenyl)piperidin-3′yl]amino]pyrimidine (**14**)*

Following the method of Daluge and Vince (19), a solution of amino alcohol **6** (500 mg, 2.93 mmol), 5-amino-4,6-dichloropyrimidine (971 mg, 5.92 mmol), and triethylamine (2 mL) in *n*-butanol (15 mL) was heated at reflux temperature under a calcium chloride drying tube for 20 h. The solvent was evaporated from the cooled mixture, and a solution of the residue in chloroform was adsorbed onto a small amount of silica gel. This material was applied to a short column of silica gel and eluted with a gradient of ethanol in ethyl acetate (0% → 2% → 5% → 10% → 20% (v/v)). The product-containing fractions were dissolved in a small amount of ethyl acetate, filtered, and the filtrate was evaporated. This process was repeated, to afford **14** (569 mg). The combined insoluble material was rechromatographed on silica gel using 5% (v/v) methanol in chloroform as eluant, to give a further 132 mg of **14**, for a total yield of 701 mg (80%). Recrystallization from water, after treatment with decolorizing carbon, provided very hygroscopic white needles, mp 88–93°C; ^{1}H nmr (CD_3OD, 200 MHz) δ: 1.04 (1H, apparent q, J_{app} = 12.0 Hz, H-4′$_{ax}$), 1.72 (1H, dd, J_{gem} = 11.0 Hz, $J_{5',6'ax}$ = 11.0 Hz, H-6′$_{ax}$), 1.79 (1H, dd, J_{gem} = 10.5 Hz, $J_{2'ax,3'}$ = 10.5 Hz, H-2′$_{ax}$), 1.80–2.17 (2H, m, H-4′$_{eq}$ and H-5′), 3.04–3.15 (3H, m, H-6′$_{eq}$ and =CHC*H*$_2$N), 3.27 (1H, m, H-2′$_{eq}$), 3.40 (1H, dd, J_{gem} = 11.0 Hz, J_{vic} = 6.5 Hz, C*H*OH), 3.50 (1H, dd, J_{gem} = 11.0 Hz, J_{vic} = 6.0 Hz, C*H*OH), 4.25 (1H, dddd, $J_{2'ax,3'}$ = 11.0 Hz, $J_{3',4'ax}$ = 12.0 Hz, $J_{2'eq,3'}$ = 4.0 Hz, $J_{3',4'eq}$ = 4.0 Hz, H-3′), 5.15–5.29 (2H, m, H_2C=), 5.91 (1H, m, =C*H*CH$_2$), 7.77 (1H, s, H-2); ^{13}C nmr (CD_3OD, 50 MHz) δ: 34.671 (C-4′), 39.140 (C-5′), 48.817 (C-3′), 57.355 (C-6′), 59.204 (C-

[9]The ^{1}H and ^{13}C nmr spectra were complicated by hindered rotation in each of the two diastereomers present. Only key diagnostic signals could be distinguished.

2′), 62.610 (=CHCH$_2$N), 65.932 (CH$_2$OH), 119.223 (H$_2$C=), 124.773 (C-5), 135.320 (=CHCH$_2$), 147.651 (C-2), 153.576 (C-4), pyrimidine C-6 not observed; ms (EI): 298 (M + H)$^+$ (1.6%), 169 (4.9%), 153 (C$_9$H$_{15}$NO: M$^{+\cdot}$ − chlorodiaminopyrimidine)$^{+\cdot}$ (100%), 124 (14.4%), 122 (153 − $\cdot$CH$_2$OH)$^+$ (23.4%), 112 (153 − H$_2$C=CHCH$_2\cdot$)$^+$ (10.6%), 96 (12.1%); ms (CI): 298 (M + H)$^+$ (100%), 145 (chlorodiaminopyrimidine + H)$^+$ (15.5%), 111 (19.7%), 94 (12.0%). Exact Mass calcd. for (C$_{13}$H$_{20}$^{35}ClN$_5$O + H): 298.1435; found (hrms): 298.1434. Anal. calcd. for C$_{13}$H$_{20}$ClN$_5$O: C 52.43, H 6.77, N 23.52; found: C 52.31, H 6.83, N 23.70.

*(±)-6-Chloro-9-[(3′β,5′β)-5′-(hydroxymethyl)-1′-(2-propenyl)-piperidin-3′-yl]purine (**15**)*

A solution of chloropyrimidine **14** (546 mg, 1.8 mmol), triethyl orthoformate (10 mL), and concentrated aqueous hydrochloric acid (0.5 mL) was stirred at 23°C for 2 h (31). The excess of the orthoformate was removed by evaporation in vacuo, and the remaining solid was stirred in the presence of 0.5 M aqueous hydrochloric acid (5 mL) for 1 h. The solution was then adjusted to pH 11 with a 15% (w/v) aqueous solution of sodium hydroxide, and extracted with chloroform (3 × 10 mL). The combined organic extracts were washed with brine (5 mL) and dried (MgSO$_4$). Evaporation of the solvent afforded a greasy foam that could not be induced to crystallize. Addition of an excess of aqueous hydrochloric acid followed by evaporation of the solvent provided the hydrochloride salt, which likewise resisted crystallization. The desired chloropurine **15** was obtained by chromatography on silica gel using the organic layer of a 12:6:1 (v/v/v) chloroform–isopropanol – concentrated aqueous ammonia mixture (385 mg, 69%); ^{1}H nmr (CDCl$_3$, 200 MHz) δ: 1.82 (1H, apparent q, J_{app} = 11.7 Hz, H-4′$_{ax}$), 1.93 (1H, dd, ΣJ = 35.09 Hz, H-6′$_{ax}$), 2.03–2.33 (2H, m, H-4′$_{eq}$ and H-5′), 2.43 (1H, dd, J_{gem} = 10.5 Hz, $J_{2'ax,3'}$ = 11.1 Hz, H-2′$_{ax}$), 2.88–3.18 (4H, m, H-6′$_{eq}$, =CHCH_2N, OH), 3.26 (1H, m, H-2′$_{eq}$), 3.62 (2H, apparent d, J_{app} = 5.7 Hz, CH_2OH), 4.79 (1H, dddd, $J_{2'ax,3'}$ = 11.1 Hz, $J_{3',4'ax}$ = 12.0 Hz, $J_{2'eq,3'}$ = 4.0 Hz, $J_{3',4'eq}$ = 4.0 Hz, H-3′), 5.11–5.24 (2H, m, H$_2$C=), 5.84 (1H, m, =CHCH$_2$), 8.16 (1H, s, H-2), 8.71 (1H, s, H-8); ^{13}C nmr (CDCl$_3$, 50 MHz) δ: 33.183 (C-4′), 38.312 (C-5′), 53.136 (C-3′), 55.697 (C-6′), 57.492 (C-2′), 61.249 (=CHCH$_2$N), 65.016 (CH$_2$OH), 118.716 (H$_2$C=), 131.761 (C-5), 134.051 (=CHCH$_2$), 143.45 (C-8), 151.091 (C-4 or C-6), 151.576 (C-6 or C-4), 151.697 (C-2); ms (EI): 308 (M + H)$^+$ (4.7%), 155 (chloropurine + H)$^+$ (3.4%), 153 (C$_9$H$_{15}$NO: M$^{+\cdot}$ − chloropurine)$^{+\cdot}$ (100%), 124 (12.0%), 122 (153 − $\cdot$CH$_2$OH)$^+$ (21.6%), 112 (153 − H$_2$C=CHCH$_2\cdot$)$^+$ (8.7%); ms (CI): 310 (^{37}Cl or ^{18}O M + H)$^+$ (34.4%), 309 (^{13}C or ^{2}H or ^{15}N or ^{17}O M + H)$^+$ (17.7%), 308 (M + H)$^+$ (100%), 157 (^{37}Cl chloropurine + H)$^+$ (3.6%), 155 (^{35}Cl chloropurine + H)$^+$ (10.6%), 154 (C$_9$H$_{15}$NO + H: M − chloropurine + H)$^+$ (19.9%), 153 (M$^{+\cdot}$ − chloropurine)$^{+\cdot}$ (27.1%). Exact Mass calcd. for (C$_{14}$H$_{18}$^{35}ClN$_5$O + H): 308.1278; found (hrms): 308.1264.

*(±)-6-(Dimethylamino)-9-[(3′β,5′β)-5′-(hydroxymethyl)-1′-(2-propenyl)piperidin-3′-yl]purine (**16**)*

Chloropurine **15** (301 mg, 0.98 mmol) was dissolved in 40% (w/v) aqueous dimethylamine (10 mL) and heated at 80°C in a sealed Pyrex tube for 28 h. At this point, tlc analysis (15% (v/v) methanol in chloroform as eluant) apparently indicated no reaction had occurred. The reaction mixture was then heated at reflux temperature for 2 h more, and left at 23°C for 24 h. Again tlc indicated no visible change. The aqueous solution was extracted with chloroform (3 × 10 mL), and the organic extracts were dried (MgSO$_4$) and concentrated. The residue was chromatographed on silica gel (using 10% (v/v) ethanol in ethyl acetate as eluant) to afford a brown oil (290 mg) that proved on ^{1}H nmr analysis to be the desired derivative **16** (93%); ^{1}H nmr (CDCl$_3$, 200 MHz) δ: 1.66 (1H, apparent q, J_{app} = 11.4 Hz, H-4′$_{ax}$), 1.86 (1H, dd, ΣJ = 21.6 Hz, H-6′$_{ax}$), 1.96–2.44 (4H, m, H-2′$_{ax}$, H-4′$_{eq}$, H-5′, OH), 3.00–3.25 (4H, m, H-2′$_{eq}$, H-6′$_{eq}$, =CHCH_2N), 3.50 (6H, br s, N(CH$_3$)$_2$), 3.55 (2H, apparent d, J_{app} = 5.7 Hz, CH_2OH), 4.67 (1H, m, H-3′), 5.06–5.20 (2H, m, H$_2$C=), 5.83 (1H, m, =CHCH$_2$), 7.72 (1H, s, H-2), 8.30 (1H, s, H-8); ^{13}C nmr (CDCl$_3$, 50 MHz) δ: 33.522 (C-4′), 38.515 (N(CH$_3$)$_2$ and C-5′), 51.788 (C-3′), 55.886 (C-6′), 57.949 (C-2′), 61.314 (=CHCH$_2$N), 65.136 (CH$_2$OH), 118.226 (H$_2$C=), 120.272 (C-5), 134.521 (=CHCH$_2$), 136.176 (C-8), 150.213 (C-6), 152.111 (C-2), 154.933 (C-4); ms (EI): 317 (M + H)$^+$ (4.3%), 164 (N^6-dimethyladenine + H)$^+$ (25.5%), 153 (C$_9$H$_{15}$NO: M$^{+\cdot}$ − N^6-dimethyladenine)$^{+\cdot}$ (100%), 124 (14.2%), 122 (153 − $\cdot$CH$_2$OH)$^+$ (24.6%), 112 (153 − H$_2$C=CHCH$_2\cdot$)$^+$ (11.0%). Exact Mass calcd. for (C$_{16}$H$_{24}$N$_6$O + H): 317.2090; found (hrms): 317.2081.

*(±)-6-(Dimethylamino)-9-[(3′β,5′β)-5′-(*tert*-butyldimethylsilyloxymethyl)piperidin-3′-yl]purine (**18**)*

A mixture of **16** (249 mg, 0.79 mmol) and imidazole (107 mg, 1.57 mmol) in dry dichloromethane (5 mL) was treated with *tert*-butyldimethylsilyl chloride (132 mg, 0.87 mmol). After 1 h of stirring at 23°C, a second portion of *tert*-butyldimethylsilyl chloride (50 mg, 0.33 mmol) was added. After a further 1 h, the reaction was quenched by the addition of water (2 mL). The phases were separated, and the organic layer was washed with water (2 mL). The combined aqueous layers were extracted with dichloromethane (5 mL) and the combined organic layers were dried (MgSO$_4$) and evaporated. The oily residue was applied to a short column of silica gel and eluted with a gradient of methanol in chloroform (0% → 10% (v/v)). The product was allowed to stand in vacuo until all traces of silicon-containing impurities had evaporated. Silyl ether **17** was obtained as an oil (295 mg, 87%); ^{1}H nmr (CDCl$_3$, 200 MHz) δ: 1.65 (1H, apparent q, J_{app} = 11.7 Hz, H-4′$_{ax}$), 1.82 (1H, dd, ΣJ = 21.6 Hz, H-6′$_{ax}$), 1.95–2.23 (2H, m, H-4′$_{eq}$ and H-5′), 2.29 (1H, dd, J_{gem} = 11.5 Hz, $J_{2'ax,3'}$ = 11.0 Hz, H-2′$_{ax}$), 2.97–3.15 (3H, m, H-6′$_{eq}$ and =CHCH_2N), 3.21 (1H, m, H-2′$_{eq}$), 4.69 (1H, dddd, $J_{2'ax,3'}$ = 11.0 Hz, $J_{3',4'ax}$ = 12.0 Hz, $J_{2'eq,3'}$ = 4.0 Hz, $J_{3',4'eq}$ = 4.0 Hz, H-3′), 5.08–5.23 (2H, m, H$_2$C=), 5.85 (1H, m, =CHCH$_2$), 7.74 (1H, s, H-2), 8.34 (1H, s, H-8).

Without further purification, the silyl ether **17** was treated with tris(triphenylphosphine)rhodium chloride (50 mg) in an 84:16 acetonitrile–water solvent mixture (22) (20 mL) as described above for the preparation of **11**. Complete reaction, as judged by tlc analysis (15% (v/v) methanol in chloroform eluant), was obtained after 7 h of heating at reflux temperature. After removal of the solvents in vacuo, the residue was purified by preparative tlc on a 2 mm × 20 cm × 20 cm silica gel plate (E. Merck no. 5717) using 10% (v/v) methanol in toluene as eluant. It was necessary to extract the product from the plate by Soxhlet extraction using an 87:13 (v/v) chloroform–methanol mixture, to obtain **18** as an oil (181 mg, 68%); ^{1}H nmr (CDCl$_3$, 200 MHz) δ: 0.034 (6H, s, Si(CH$_3$)$_2$), 0.88 (9H, s, SiC(CH$_3$)$_3$), 1.78 (1H, apparent q, J_{app} = 11.5 Hz, H-4′$_{ax}$), 1.84–2.08 (2H, m, NH and H-5′), 2.24 (1H, m, H-4′$_{eq}$), 2.44 (1H, dd, J_{gem} = 12.6 Hz, $J_{5',6'ax}$ = 10.8 Hz, H-6′$_{ax}$), 2.87 (1H, dd, J_{gem} = 11.6 Hz, $J_{2'ax,3'}$ = 11.6 Hz, H-2′$_{ax}$), 3.23 (1H, m, H-6′$_{eq}$), 3.36–3.60 (9H, m, H-2′$_{eq}$, N(CH$_3$)$_2$, CH$_2$OSi), 4.55 (1H, m, H-3′), 7.74 (1H, s, H-2), 8.33 (1H, s, H-8); ^{13}C nmr (CDCl$_3$, 50 MHz) δ: −5.672 (SiCH$_3$), −5.701 (SiCH$_3$), 18.018 (SiC(CH$_3$)$_3$), 25.638 (SiC(CH$_3$)$_3$), 33.564 (C-4′), 38.237 (br, N(CH$_3$)$_2$), 40.138 (C-5′), 48.572 (C-6′), 51.248 (C-2′), 52.322 (C-3′), 65.275 (CH$_2$OSi), 120.087 (C-5), 135.585 (C-8), 150.056 (C-4), 151.880 (C-2), 154.716 (C-6); ms (EI): 391 (M + H)$^+$ (8.5%), 375 (M$^{+\cdot}$ − $\cdot$CH$_3$)$^+$ (3.4%), 333 (M$^{+\cdot}$ − *tert*-Bu$\cdot$)$^+$ (3.2%), 227 (C$_{12}$H$_{25}$NOSi: M$^{+\cdot}$ − N^6-dimethyladenine)$^{+\cdot}$ (25.3%), 190 (6.3%), 170 (227 − *tert*-Bu$\cdot$)$^+$ (19.0%), 164 (N^6-dimethyladenine + H)$^+$ (100%), 134 (6.9%). Exact Mass calcd. for (C$_{19}$H$_{34}$N$_6$OSi + H): 391.2642; found (hrms): 391.2629.

*6-(Dimethylamino)-9-[(3′RS,5′SR)-1′-(2S-[tert-butoxycarbamoyl]-3-[4-methoxyphenyl]propanoyl)-5′-(tert-butyldimethylsilyloxymethyl)piperidin-3′-yl]purine (**19**a, b)*

As described above for the preparation of **13**, purine derivative **18** (180 mg, 0.46 mmol) and *N*-(*tert*-butoxycarbonyl)-L-*p*-methoxyphenylalanine (**12**) (23) (152 mg, 0.51 mmol) were dissolved in dry dichloromethane (5 mL) and treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (108 mg, 0.56 mmol) at 0°C. Processing as previously described afforded, after chromatography on silica gel (0% → 2% (v/v) methanol in chloroform as eluant), a 1:1 mixture of the title compounds **19***a*, *b* (309 mg, quant.) as an oil; ^{1}H nmr (CDCl$_3$, 200 MHz)[9] δ: 0.032, 0.045 (6H, 2 × s, Si(CH$_3$)$_2$), 0.886,

0.893 (9H, 2 × s, $SiC(CH_3)_3$), 1.36, 1.40, 1.42 (9H, 3 × s, $OC(CH_3)_3$), 3.33–3.60 (9H, m + br s, $N(CH_3)_2$, CH_2OSi, H-2′$_{eq}$ or H-6′$_{eq}$), 3.75, 3.78, 3.79 (3H, 3 × s, OCH_3), 5.29–5.41 (1H, m, NH), 6.73–6.91 (2H, m, phenyl H-3), 7.02–7.25 (2H, m, phenyl H-2), 7.53, 7.62, 7.68 (1H, 3 × s, H-2), 8.28, 8.31, 8.32 (1H, 3 × s, H-8); ^{13}C nmr ($CDCl_3$, 50 MHz)[9] δ: −5.76 ($Si(CH_3)_2$), 17.91 ($SiC(CH_3)_3$), 25.57 ($SiC(CH_3)_3$), 28.03 ($OC(CH_3)_3$), 32.2, 33.1 (C-4′), 37.6, 37.7 (C-5′), 38.14 (br, $N(CH_3)_2$), 38.6, 39.0 ($ArCH_2$), 44.1, 44.3, 45.5, 45.6, 47.7, 48.0, 48.5, 49.0 (C-2′ and C-6′), 50.8, 51.1, 52.0 (C-α and C-3′), 54.82, 54.96 (OCH_3), 64.11, 64.25, 64.45 (CH_2OSi), 79.17 ($OC(CH_3)_3$), 113.49, 113.78, 113.8 (phenyl C-3), 120.1, 120.27, 120.37 (C-6), 128.07, 128.20, 128.48 (phenyl C-1), 130.09, 130.30, 130.58 (phenyl C-2), 135.7, 136.0 (C-8), 150.05, 150.15 (C-4), 151.80 (C-2), 154.64, 154.65 (phenyl C-4 and C-5), 158.20, 158.30, 158.35, 158.46 (carbamate C═O), 170.20, 170.56 (amide C═O); ms (EI): 667 $(M)^{+\cdot}$ (4.2%), 446 ($C_{21}H_{36}N_7O_2Si$: $M^{+\cdot} - CO_2 - (CH_3)_2C{=}CH_2$ − 4-OMeArCH$_2^{\cdot}$)$^+$ (40%), 387 ($C_{22}H_{33}NO_3Si$: $M^{+\cdot} - CO_2 - (CH_3)_2C{=}CH_2 - NH_3 - N^6$-dimethyladenine)$^{+\cdot}$ (8.3%), 330 (387 − *tert*-Bu$^{\cdot}$)$^+$ (21.2%), 283 ($C_{14}H_{27}N_2O_2Si$: 446 − N^6-dimethyladenine)$^+$ (8.1%), 227 (27.9%), 164 (N^6-dimethyladenine + H)$^+$ (100%), 121 (4-OMeArCH$_2$)$^+$ (62.2%). Exact Mass calcd. for $C_{34}H_{53}N_7O_5Si$: 667.3877; found (hrms): 667.3849. Exact Mass calcd. for $C_{21}H_{36}N_7O_2Si$: 446.2700; found (hrms): 446.2700. Exact Mass calcd. for $C_{22}H_{33}NO_3Si$: 387.2230; found (hrms): 387.2242. Exact Mass calcd. for $C_{18}H_{24}NO_3Si$: 330.1525; found (hrms): 330.1506. Exact Mass calcd. for $C_{14}H_{27}N_2O_2Si$: 283.1842; found (hrms): 283.1833.

*1-{(3′S,5′R)-1′-(2S-Amino-3-(4-methoxyphenyl)propanoyl)-5′-(hydroxymethyl)piperidin-3′-yl}-2,4(1H,3H)pyrimidinedione (**20**a) and 1-{(3′R,5′S)-1′-(2S-amino-3-(4-methoxyphenyl)propanoyl)-5′-(hydroxymethyl)piperidin-3′-yl}-2,4(1H,3H)pyrimidinedione (**20**b)*

The *N*-BOC *O*-TBDMS precursors **19***a,b* (252 mg, 0.41 mmol) were dissolved in dichloromethane (1 mL) and the solution was cooled in an ice bath. A mixture of trifluoroacetic acid (3 mL), anisole (1 mL), and water (0.5 mL) was added, and the mixture was stirred at 0°C for 30 min. It was then allowed to warm to 23°C over 2.5 h. Monitoring by tlc using a 2-butanone–water – acetic acid eluant (40:1:1 (v/v/v)) showed rapid conversion into an intermediate product followed by slow conversion to a ninhydrin-positive, highly polar material. The solvents were evaporated, and the residue was allowed to stand in vacuo to remove traces of anisole. The residual material was then dissolved in a 1:1 (v/v) water–ethanol mixture and applied to a short column of Dowex 50-X2 400 (H^+) ion-exchange resin (~5 mL of resin). The column was thoroughly washed with 1:1 (v/v) water–ethanol, and then eluted with 1:1 (v/v) 1 M aqueous ammonium hydroxide – ethanol. The product-containing fractions were pooled and the solvents were evaporated to give an amorphous mixture of diastereomers **20***a, b* (144 mg, 87%).

The diastereomeric mixture was resolved by reverse-phase hplc on a 300 mm × 7.0 mm Hamilton PRP-1 column. Small portions of the sample were injected onto the column and eluted with a linear gradient of 0.33 M aqueous triethylammonium hydrogen carbonate buffer (pH ~8) and acetonitrile (1.5 mL min^{-1}, 90:10 → 80:20 (v/v) over 20 min, then isocratic for 5 min), monitoring at 254 nm. Fractions were collected and the process was repeated until all of the mixture had been resolved. The fractions containing each product were combined, concentrated on a rotary evaporator to remove the organic solvent, and finally freeze-dried. To remove traces of buffer salts, it was necessary to pass each sample down a Dowex 50 column once more, as previously described, followed by freeze-drying. Samples of leading fraction **20***a* (34 mg, 41%, ~100% diastereomeric excess), and second fraction **20***b* (26 mg, 31%, ~100% diastereomeric excess) were obtained as fluffy solids.

First fraction (**20***a*); $[\alpha]_D^{25}$ +68.04 (*c* 1.02, H_2O); ^{1}H nmr spectra indicated the presence of two rotamers in a 2.26:1.00 ratio. The spectral data for the rotamers are listed separately for clarity.

Major rotamer (amide carbonyl *syn* with respect to the pyrimidinedione ring): ^{1}H nmr (D_2O, 600 MHz, 300 K) δ: 0.249 (1H, m, H-5′), 1.502 (1H, apparent q, J_{app} = 12.2 Hz, H-4′$_{ax}$), 1.711 (1H, m, H-4′$_{eq}$), 2.681 (1H, dd, J_{gem} = 12.2 Hz, $J_{2'ax,3'}$ = 9.1 Hz, H-2′$_{ax}$), 2.70 (1H, dd, J_{gem} = 12.0 Hz, $J_{6'ax,5'}$ = 10.5 Hz, H-6′$_{ax}$), 2.807 (1H, dd, J_{gem} = 12.9 Hz, J_{vic} = 10.4 Hz, $ArCH_2$), 3.003 (1H, dd, J_{gem} = 12.9 Hz, J_{vic} = 5.1 Hz, $ArCH_2$), 3.301 (1H, dd, J_{gem} = 11.5 Hz, J_{vic} = 6.07 Hz, C*H*OH), 3.331 (1H, dd, J_{gem} = 11.5 Hz, J_{vic} = 5.5 Hz, C*H*OH), 3.768 (1H, m, H-6′$_{eq}$), 3.801 (3H, s, OCH_3), 4.011 (1H, m, H-3′), 4.23–4.29 (1H, m, H-α), 4.524 (1H, m, H-2′$_{eq}$), 5.808 (1H, d, *J* = 8.01 Hz, H-5), 7.006 (2H, d, *J* = 8.61 Hz, 2 × phenyl H-3), 7.227 (2H, d, *J* = 8.61 Hz, 2 × phenyl H-2), 7.598 (1H, d, *J* = 8.01 Hz, H-6); ^{13}C nmr (D_2O, 100 MHz, 300 K) δ: 30.516 (C-4′), 37.272 (C-5′), 40.213 ($ArCH_2$), 44.833 (C-2′), 47.765 (C-6′), 51.267 (C-α), 51.419 (C-3′), 55.464 (OCH_3), 62.732 (CH_2OH), 101.850 (C-5), 114.540 (2 × phenyl C-3), 129.132 (phenyl C-1), 130.687 (2 × phenyl C-2), 142.894 (C-6), 152.169 (C-2), 158.065 (phenyl C-4), 166.492 (C-4), 174.157 (amide C═O).

Minor rotamer (amide carbonyl *anti* with respect to pyrimidinedione ring): ^{1}H nmr (D_2O, 600 MHz, 300 K) δ: 1.599 (1H, apparent q, J_{app} = 12.2 Hz, H-4′$_{ax}$), 1.85 (1H, m, H-5′), 1.99 (1H, m, H-4′$_{eq}$), 2.275 (1H, dd, J_{gem} = 13.0 Hz, $J_{5',6'ax}$ = 10.0 Hz, H-6′$_{ax}$), 2.296 (1H, dd, J_{gem} = 12.6 Hz, $J_{2'ax,3'}$ = 9.5 Hz, H-2′$_{ax}$), 2.77–2.83 (1H, dd, J_{gem} = 13.5 Hz, J_{vic} obscured by overlap, $ArCH_2$), 2.964 (1H, dd, J_{gem} = 13.5 Hz, J_{vic} = 5.6 Hz, $ArCH_2$), 3.52 (1H, dd, J_{gem} = 11.7 Hz, J_{vic} = 7.2 Hz, C*H*OH), 3.54 (1H, dd, J_{gem} = 11.7 Hz, J_{vic} = 6.0 Hz, C*H*OH), 3.70 (1H, m, H-2′$_{eq}$), 3.794 (3H, s, OCH_3), 4.23–4.29 (1H, m, H-α), 4.36 (1H, m, H-3′), 4.49 (1H, m, H-2′$_{eq}$), 5.808 (1H, d, *J* = 8.01 Hz, H-5), 6.911 (2H, d, *J* = 8.59 Hz, 2 × phenyl H-3), 7.113 (2H, d, *J* = 8.59 Hz, 2 × phenyl H-2), 7.514 (1H, d, *J* = 8.01 Hz, H-6); ^{13}C nmr (D_2O, 100 MHz, 300 K) δ: 30.392 (C-4′), 37.272 (C-5′), 39.421 ($ArCH_2$), 44.491 (C-6′), 47.661 (C-2′), 51.671 (C-α), 52.600 (C-3′), 55.261 (OCH_3), 63.280 (CH_2OH), 101.850 (C-5), 114.032 (2 × phenyl C-3), 128.475 (phenyl C-1), 130.586 (2 × phenyl C-2), 142.697 (C-6), 152.169 (C-2), 157.966 (phenyl C-4), 166.762 (C-4), 173.586 (amide C═O); ms (EI): 403 (M + H)$^+$ (3.8%), 385 (M + H − H_2O)$^+$ (8.8%), 332 (M + H − HNCO − CO)$^+$ (14.2%), 281 ($M^{+\cdot}$ − 4-OMeArCH$_2^{\cdot}$)$^+$ (84.8%), 150 (4-OMeArCH$_2$CH═NH$_2$)$^+$ (100%), 121 (4-OMeArCH$_2$)$^+$ (51.5%); ms (CI): 403 (M + H)$^+$ (100%), 226 ($C_{10}H_{16}N_3O_3$: M − aminoacyl + 2H)$^+$ (32.8%), 150 (4-OMeArCH$_2$CH═NH$_2$)$^+$ (35.3%). Exact Mass calcd. for ($C_{20}H_{26}N_4O_5$ + H): 403.1981; found (hrms): 403.1979.

Second fraction (**20***b*); $[\alpha]_D^{25}$ +51.51 (*c* 1.06, H_2O); ^{1}H nmr spectra indicated the presence of two rotamers in a 1.00:0.91 ratio. The spectral data for the two rotamers are listed separately for clarity.

Predominant rotamer (amide carbonyl *anti* with respect to base): ^{1}H nmr (D_2O, 600 MHz, 300 K) δ: 1.50–1.58 (1H, br m, H-5′), 1.64 (1H, apparent q, J_{app} = 12.2 Hz, H-4′$_{ax}$), 1.93 (1H, m, H-4′$_{eq}$), 2.399 (1H, dd, J_{gem} = 13.5 Hz, $J_{5',6'ax}$ = 11.6 Hz, H-6′$_{ax}$), 2.856 (1H, dd, J_{gem} = 13.6 Hz, J_{vic} = 8.18 Hz, $ArCH_2$), 3.001 (1H, dd, J_{gem} = 13.6 Hz, J_{vic} = 5.95 Hz, $ArCH_2$), 3.200 (1H, dd, J_{gem} = 13.7 Hz, $J_{2'ax,3'}$ = 11.1 Hz, H-2′$_{ax}$), 3.504 (1H, dd, J_{gem} = 11.5 Hz, J_{vic} = 6.6 Hz, C*H*OH), 3.531 (1H, dd, J_{gem} = 11.5 Hz, J_{vic} = 5.46 Hz, C*H*OH), 3.716 (1H, m, H-3′), 3.815 (3H, s, OCH_3), 4.169 (1H, m, H-2′$_{eq}$), 4.367 (1H, dd, J_{vic} = 5.95 Hz, $J_{vic'}$ = 8.18 Hz, H-α), 4.481 (1H, m, H-6′$_{eq}$), 5.820 (1H, d, *J* = 8.07 Hz, H-5), 6.972 (2H, d, *J* = 8.64 Hz, 2 × phenyl H-3), 7.211 (2H, d, *J* = 8.64 Hz, 2 × phenyl H-2), 7.561 (1H, d, *J* = 8.07 Hz, H-6); ^{13}C nmr (D_2O, 100 MHz, 300 K) δ: 30.416 (C-4′), 37.442 (C-5′), 39.101 ($ArCH_2$), 44.577 (C-6′), 47.934 (C-2′), 51.50 (C-α), 53.120 (C-3′), 55.289 (OCH_3), 63.244 (CH_2OH), 101.889 (C-5), 114.287 (2 × phenyl C-3), 128.477 (phenyl C-1), 130.70 (2 × phenyl C-2), 143.02 (C-6), 153.28 (C-2), 157.89 (phenyl C-4), 167.79 (C-4), 174.69 (br, amide C═O).

Second rotamer (amide carbonyl *syn* with respect to pyrimidinedione ring): ^{1}H nmr (D_2O, 600 MHz, 300 K) δ: 1.60 (1H, apparent q, J_{app} = 12.3 Hz, H-4′$_{ax}$), 1.87–1.95 (1H, m, H-5′), 2.01 (1H, m, H-4′$_{eq}$), 2.185 (1H, dd, J_{gem} = 13.3 Hz, $J_{5',6'ax}$ = 12.4 Hz, H-6′$_{ax}$), 2.676 (1H, dd, J_{gem} = 13.7 Hz, $J_{2'ax,3'}$ = 10.5 Hz, H-2′$_{ax}$), 2.95 (1H, dd, J_{gem} = 13.2 Hz, J_{vic} = 7.7 Hz, $ArCH_2$), 2.97 (1H, dd, J_{gem} = 13.2 Hz, J_{vic} = 6.0 Hz, $ArCH_2$), 3.424 (1H, dd, J_{gem} = 11.3 Hz, J_{vic} = 6.87 Hz, C*H*OH), 3.485 (1H, dd, J_{gem} = 11.3 Hz, J_{vic} = 5.48 Hz, C*H*OH), 3.771 (1H, m, H-6′$_{eq}$), 3.820 (3H, s, OCH_3), 4.239 (1H, dd, J_{vic} = 7.7 Hz, $J_{vic'}$ = 6.0 Hz, H-α), 4.347 (1H, m, H-3′), 4.534 (1H, m, H-2′$_{eq}$), 5.835 (1H, d, *J* = 8.06 Hz, H-5), 6.960 (2H, d, *J* = 8.64 Hz, 2 × phenyl H-3), 7.156 (2H, d, *J* = 8.64 Hz, 2 × phenyl H-2), 7.624 (1H, d, *J* = 8.06 Hz, H-6); ^{13}C nmr (D_2O,

100 MHz, 300 K) δ: 30.742 (C-4′), 38.207 (C-5′), 38.899 (ArCH_2), 44.760 (C-2′), 47.544 (C-6′), 51.47 (C-α), 51.992 (C-3′), 55.289 (OCH_3), 62.926 (CH_2OH), 101.600 (C-5), 114.108 (2 × phenyl C-3), 128.124 (phenyl C-1), 130.62 (2 × phenyl C-2), 142.98 (C-6), 152.98 (C-2), 157.915 (phenyl C-4), 167.79 (C-4), 174.69 (br, amide C═O); ms (EI): identical to that obtained for **20***a*; ms (CI): identical to that obtained for **20***a*. Exact Mass calcd. for ($C_{20}H_{26}N_4O_5$ + H): 403.1981; found (hrms): 403.1971.

*6-(Dimethylamino)-9-[(3′S,5′R)-(2S-amino-3-[4-methoxyphenyl]propanoyl)-5′-(hydroxymethyl)piperidin-3′-yl]purine (**21**a) and 6-(dimethylamino)-9-[(3′R,5′S)-(2S-amino-3-[4-methoxyphenyl]propanoyl)-5′-(hydroxymethyl)piperidin-3′-yl]purine (**21**b)*

The diprotected precursors **19***a*, *b* (250 mg, 0.37 mmol) and anisole (1 mL) were dissolved in methanol (1 mL), and the solution was cooled in an ice bath. Trifluoroacetic acid (2 mL) was added, and the mixture was stirred at 0°C for 30 min. The reaction was then allowed to warm to 23°C over 2 h, at which point tlc analysis (10% (v/v) methanol in chloroform as eluant, plate pretreated with ammonia vapor) indicated only about 10% conversion. A further portion of trifluoroacetic acid (1 mL) was added, and the reaction was allowed to proceed overnight. The solvents were evaporated, and the residue was processed as described above to give a mixture of diastereomers **21***a*, *b* (158 mg, 93%).

Reverse-phase hplc separation of the diastereomers was carried out by isocratic elution at 2 mL min^{-1} with 20% (v/v) acetonitrile in 0.3 M aqueous triethylammonium hydrogen carbonate buffer (pH ~8). Further processing of the product fractions as previously described gave samples of leading fraction **21***a* (27 mg, 31%, 95% diastereomeric excess) and second fraction **21***b* (23 mg, 27%, 95% diastereomeric excess) as fluffy solids. These products were only minimally soluble in water but dissolved readily in alcohol, chloroform or DMSO.

First fraction (**21***a*); $[\alpha]_D^{25}$ +60.3 (*c* 0.58, $CHCl_3$); ^{1}H nmr (DMSO-d_6, 400 MHz, 383 K)[10] δ: 1.52–1.69 (2H, br m, H-5′ and H-6′$_{ax}$), 1.980 (1H, apparent, q, J_{app} = 11.1 Hz, H-4′$_{ax}$), 2.12 (1H, m, H-4′$_{eq}$), 2.62 (1H, dd, J_{gem} = 13.7 Hz, J_{vic} = 7.2 Hz, ArCH_2), 2.834 (1H, dd, J_{gem} = 13.6 Hz, J_{vic} = 6.1 Hz, ArCH_2), 3.117 (1H, dd, ΣJ = 23.7 Hz, H-2′$_{ax}$) 3.354 (1H, dd, J_{gem} = 10.8 Hz, J_{vic} = 7.4 Hz, C*H*OH), 3.422 (1H, dd, J_{gem} = 10.8 Hz, J_{vic} = 5.5 Hz, C*H*OH), 3.468, (6H, s, $N(CH_3)_2$), 3.735 (3H, s, OCH_3), 3.936 (1H, dd, J_{vic} = 6.1 Hz, $J_{vic'}$ = 7.2 Hz, H-α), 4.25–4.55 (3H, br m, H-2′$_{eq}$, H-3′, H-6′$_{eq}$), 6.822 (2H, d, J = 8.5 Hz, 2 × phenyl H-3), 7.132 (2H, d, J = 8.5 Hz, 2 × phenyl H-2), 8.074 (1H, s, H-2), 8.213 (1H, s, H-8); ^{13}C nmr (DMSO-d_6, 100 MHz, 298 K)[11] δ: 32.464, 32.880 (C-4′), 38.107, 38.805 (C-5′), 40.696, 41.101 (ArCH_2), 44.503, 45.289, 47.620, 48.419 (C-2′ and C-6′), 50.263, 51.587 (C-3′), 52.120, 52.290 (C-α), 54.917, 55.002 (OCH_3), 62.805, 63.288 (CH_2OH), 113.352, 113.645 (2 × phenyl C-3), 119.336, 119.537 (C-6), 130.255, 130.372 (2 × phenyl C-2), 130.503 (phenyl C-1), 149.872, 150.035 (C-4), 151.7 (C-2), 154.280 (phenyl C-4), 157.655, 157.824 (C-5), 173.058, 173.373 (amide C═O); ms (EI): 454 $(M + H)^+$ (3.7%), 453 $(M)^{+\cdot}$ (3.6%), 436 $(M^{+\cdot} - NH_3)^{+\cdot}$ or $(M + H - H_2O)^+$ (1.3%), 332 ($C_{15}H_{22}N_7O_2$: $M^{+\cdot}$ − 4-OMeArC$H_2^{\cdot}$)$^+$ (100%), 304 (14.4%), 164 (N^6-dimethyladenine + H)$^+$ (67.7%), 150 (4-OMeArCH_2CH═NH_2)$^+$ (7.8%), 134 (9.0%), 121 (4-OMeArCH_2)$^+$ (12.6%). Exact Mass calcd. for $C_{23}H_{31}N_7O_3$: 453.2488; found (hrms): 453.2484.

Second fraction (**21***b*); $[\alpha]_D^{25}$ +13.0 (*c* 0.23, $CHCl_3$); ^{1}H nmr (DMSO-d_6, 400 MHz, 383 K) δ: 1.80 (1H, m, H-5′), 1.987 (1H, apparent q, J_{app} = 12.0 Hz, H-4′$_{ax}$), 2.16 (1H, m, H-4′$_{eq}$), 2.519 (1H, dd, partly obscured by solvent, H-6′$_{ax}$), 2.645 (1H, dd, J_{gem} = 13.6 Hz, J_{vic} = 7.4 Hz, ArCH_2), 2.900 (1H, dd, J_{gem} = 13.6 Hz, J_{vic} = 5.9 Hz, ArCH_2), 3.208 (1H, dd, ΣJ = 24.04 Hz, H-2′$_{ax}$), 3.373 (1H, dd, J_{gem} = 10.8 Hz, J_{vic} = 6.3 Hz, C*H*OH), 3.446 (1H, dd, J_{gem} = 10.8 Hz, J_{vic} = 5.3 Hz, C*H*OH), 3.470 (6H, s, $N(CH_3)_2$), 3.744 (3H, s, OCH_3), 3.937 (1H, dd, J_{vic} = 5.9 Hz, $J_{vic'}$ = 7.4 Hz, H-α), 4.26–4.40 (2H, m, H-3′ and H-2′$_{eq}$ or H-6′$_{eq}$), 4.52 (1H, m, H-6′$_{eq}$ or H-2′$_{eq}$), 6.831 (2H, d, J = 8.6 Hz, 2 × phenyl H-3), 7.165 (2H, d, J = 8.6 Hz, 2 × phenyl H-2), 8.058 (1H, s, H-2), 8.202 (1H, s, H-8); ^{13}C nmr (DMSO-d_6, 100 MHz, 300 K)[12] δ: 32.330, 33.018 (C-4′), 38.128 (C-5′), 40.745, 41.004 (ArCH_2), 44.354, 45.425, 47.493, 48.728 (C-2′ and C-6′), 50.282, 51.425 (C-3′), 51.801, 52.102 (C-α), 54.958 (OCH_3), 62.870, 63.286 (CH_2OH), 113.446, 113.491 (2 × phenyl C-3), 119.323, 119.525 (C-6), 130.289, 130.533 (phenyl C-1 and 2 × phenyl C-2), 137.537, 137.737 (C-8), 149.877, 150.042 (C-4), 151.625 (C-2), 154.297 (phenyl C-4), 157.708 (C-5), 173.244 (amide C═O); ms (EI): identical to that obtained for the diastereomer **21***a*. Exact Mass calcd. for $C_{23}H_{31}N_7O_3$: 453.2488; found (hrms): 453.2479.

[10]The product **21***a* was insoluble in D_2O. Its ^{1}H nmr spectra in $CDCl_3$ or DMSO-d_6 indicated the presence of an approximately 1:1 rotameric mixture, but all resonances were severely broadened. Lowering the temperature to the freezing point of $CDCl_3$ did not allow the rotamers to be resolved. The ^{1}H nmr spectra were therefore acquired in DMSO-d_6 solution at 383 K, at which temperature most but not all signals were well resolved at the expense of any conformational information. Over 1–2 h at this elevated temperature, however, the samples discolored. Two-dimensional spectra were therefore not obtained. The ^{13}C nmr spectra could be obtained at room temperature; however, it was not possible to assign signals to individual rotamers.

[11]The signals due to the $N(CH_3)_2$ group were obscured by the DMSO solvent signal. The signal of the purine C-8 (δ 135–137 ppm) was not observed due to slow relaxation.

[12]The signals attributable to the NCH_3 groups are obscured by the solvent signal.

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