Racemic synthesis of carbocyclic purine nucleoside analogues with restricted glycosyl conformation

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Received (in Cambridge) 10th March 1999, Accepted 12th May 1999

Carbocyclic purine nucleoside analogues which have restricted glycosyl conformation at $\chi \approx 180°$ were designed, based on the conformational features of the L-nucleotide residue in heterochiral DNA, and synthesized. The synthesis of (±)-carbocyclic 8,6′-*O*-anhydro-8,6′-dihydroxy-2′-deoxyadenosine **3** was achieved by intramolecular cyclization of the 8-bromo-6′-*O*-tosyl-2′-deoxyadenosine derivative. (±)-Carbocyclic 8,6′-*O*-anhydro-8,6′-dihydroxy-2′-deoxyguanosine **4** was synthesized from the carbocyclic 2,6-diaminopurine nucleoside derivative *via* the carbocyclic 8-bromo-6′-*O*-mesyl-2′-deoxyguanosine derivative.

Introduction

A variety of nucleoside analogues have been synthesized to evaluate their biological activities.[1] Among them, a conformationally restricted nucleoside analogue is useful for probing oligonucleotide structures[2] and enzyme–substrate interactions.[3] However, non-covalent fixation can often lead to erroneous interpretation of results. For example, while 8-bromoadenosine adopts a *syn* conformation in the solid state as well as in solution,[4] 8-bromoadenosine 5′-diphosphoribose is forced to change from the *syn* to the *anti* conformation when it binds to horse liver alcohol dehydrogenase.[5] Therefore, for stereochemical studies of such interactions, nucleosides whose torsion angles are fixed by covalent bond should be useful.

We have investigated the structures of heterochiral oligonucleotides, which contain an unnatural L-nucleotide residue in the natural sequence, and have found that the L-nucleotide residue of the heterochiral oligonucleotide forms stable Watson–Crick base-pairing with the complementary natural residue,[6] while the overall duplex stability is slightly decreased.[7] Two-dimensional ^{1}H NMR studies suggested that the L-nucleotide residue adopts an unusual *ap* glycosyl conformation ($\chi \approx 180°$),[6] although typical double-stranded B-form DNA has the *anti* (−*ac*) conformation ($\chi = -90$ to $-135°$).[8] This unusual conformation might be critical for L-nucleotides to form Watson–Crick base-pairing in the right-handed double helix. To confirm this hypothesis, we planned to synthesize L-nucleoside analogues fixed in such a conformation.

Covalent fixation around the glycosyl linkage of nucleosides is possible in a cyclonucleoside. There have been many reports on the synthesis of O- (oxygen-bridged) and C- (carbon-bridged) cyclonucleosides fixed in the *anti*[9] and *syn*[10] regions. However, there are no reports for the synthesis of cyclonucleosides fixed in the *ap* conformation ($\chi \approx 180°$), except for our recent report for the pyrimidine nucleoside analogues **1** and **2**.[11] Indeed, the crystal structure of **2** clearly showed that the glycosyl bond angle was fixed at $\chi = 176.3°$.[12] This paper reports the synthesis of the purine nucleoside analogues **3** and **4** fixed in such conformation.

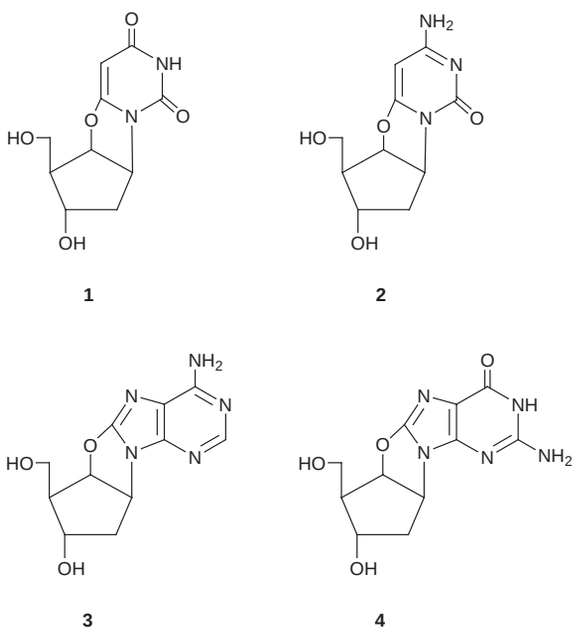


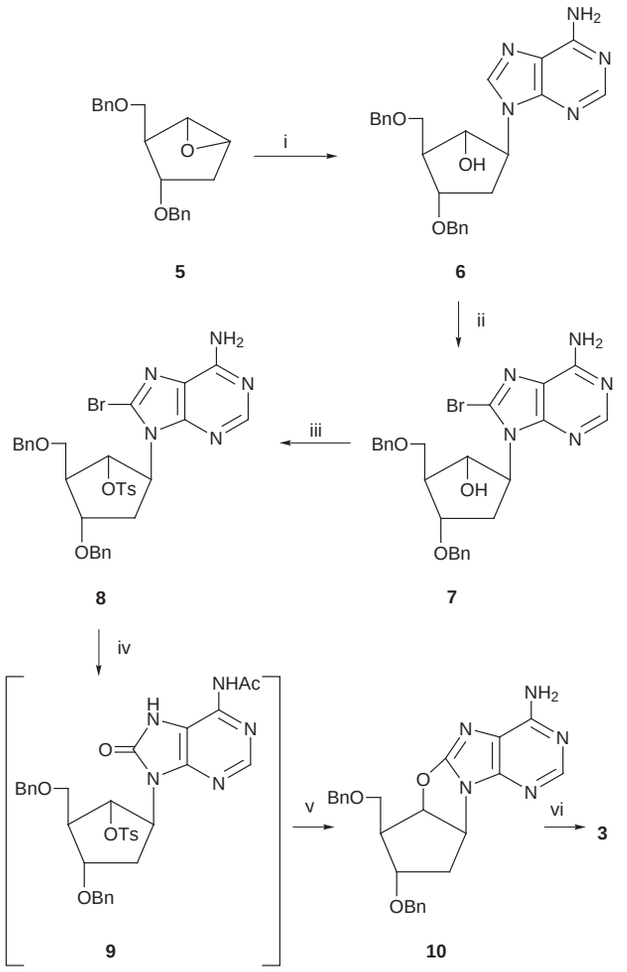
Chart 1

Results and discussion

In order to fix the glycosyl linkage in the *ap* conformation, it is necessary to cyclize between the purine C-8 position and the sugar O-4′ position. Therefore, we designed the carbocyclic nucleoside analogues (Chart 1) whose C-8 and C-6′ positions† are bridged *via* an oxygen atom.

Synthesis of the adenosine derivative **3** is outlined in Scheme 1. Ring opening of the racemic epoxide **5**, which is readily prepared from cyclopentadiene in three steps,[14] by the adenine sodium salt proceeded regioselectively to give the 6′α-hydroxy derivative **6**. Treatment of **6** with bromine in 0.5 M sodium acetate (pH 5.0)–1,4-dioxane (1:1) afforded the 8-bromo derivative **7**, which showed λ_{max} at 266 nm in EtOH. The 8-bromo derivative **7** was treated with toluene-*p*-sulfonyl chloride (TsCl) in the presence of DMAP to give the 6′-*O*-tosyl derivative **8**. Compound **8** was subjected to intramolecular cyclization into the protected 8,6′-*O*-anhydro-8,6′-dihydroxyadenosine **10** in two steps according to Ikehara's

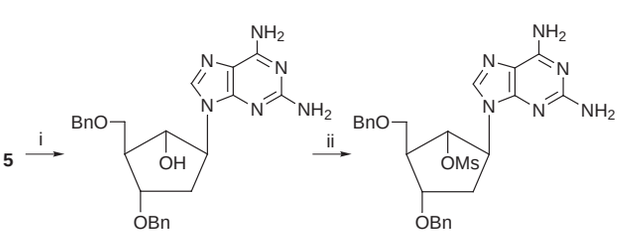
† The numbering system used for carbocyclic nucleosides in ref. 13 is employed in the text and Experimental section to facilitate comparison of the NMR spectra. In this nomenclature, the carbon atom replacing the furanose ring oxygen of natural nucleosides is designated C-6′.



Scheme 1 *Reagents and conditions* (*and yields*): i, adenine, adenine sodium salt, DMF, 140 °C, 27 h (65.7%); ii, Br_2, 1,4-dioxane–0.5 M NaOAc (pH 5.0), rt, 10 h (62.3%); iii, TsCl, DMAP, CH_2Cl_2, rt, overnight (93.4%); iv, NaOAc, Ac_2O, AcOH, reflux, 3 h, crude; v, NH_3, MeOH, 60 °C, 7 h (74.3%); vi, 20% $Pd(OH)_2/C$, cyclohexene, EtOH, reflux, 18 h, (88.6%).

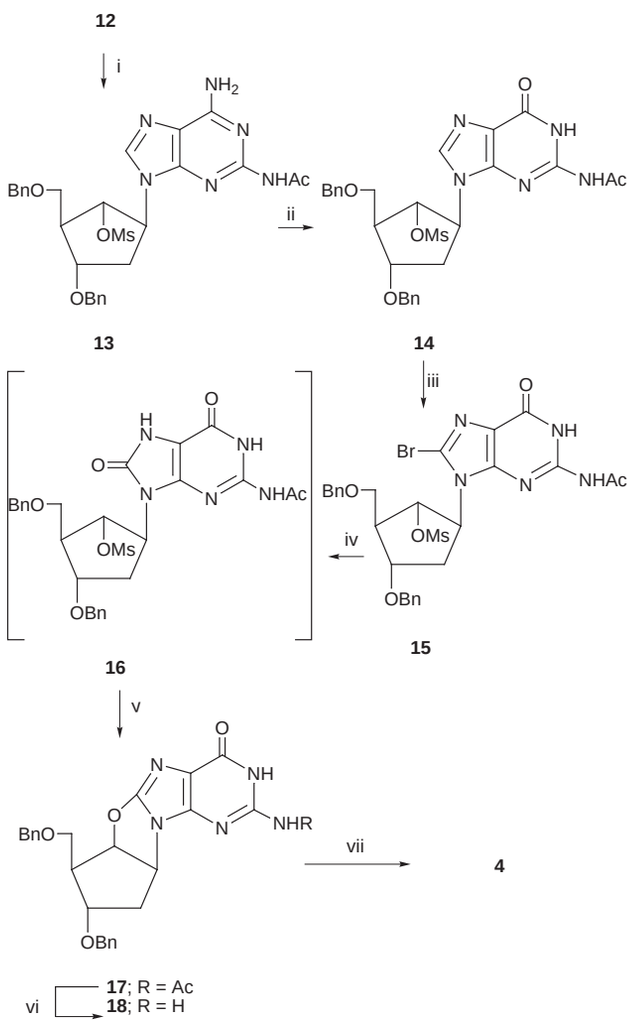
method.[15] First, acetolysis of compound **8** with sodium acetate in acetic acid–acetic anhydride at reflux temperature afforded the 8-keto derivative **9**. Secondly, resulting crude compound **9** was treated with methanolic ammonia at 60 °C to furnish the 8,6′-*O*-anhydro-8,6′-dihydroxyadenosine derivative **10** in 74.3% yield. Compound **10** was deprotected with 20% $Pd(OH)_2/C$ and cyclohexene in EtOH at reflux temperature to afford (±)-carbocyclic 8,6′-*O*-anhydro-8,6′-dihydroxy-2′-deoxyadenosine **3** in 88.6% isolated yield.

Synthesis of the guanosine analogue **4** was more troublesome. The 2,6-diaminopurine nucleoside derivative **11** was synthesized from the racemic epoxide **5** by treatment with 2,6-diaminopurine in the presence of sodium hydride and 15-crown-5 in 72.8% yield (Scheme 2). This reaction was highly



Scheme 2 *Reagents and conditions* (*and yields*): i, 2,6-diaminopurine, NaH, 15-crown-5, DMF, reflux, 4 h (72.8%); ii, MsCl, pyridine, rt, 1.5 h (90.5%).

regioselective for N-9 as reported.[16] In order to synthesize compound **4** by a similar strategy to the case of compound **3**, selective protection of the 2-amino group of **11**, subsequent conversion of the 2,6-diaminopurine moiety into a guanine base, and introduction of the leaving group at the 6′-hydroxy group are required. Preliminary experiments showed that acetylation at the 2-amino group of **11** proceeded nonselectively and gave the N-2 monoacetyl derivative in rather low yield with difficulty in purification. Alternatively, we tried to introduce the leaving group into the 6′-hydroxy group of **11**. Tosylation of compound **11** at 0 °C afforded a complex mixture of the *N*-mono- and bis-tosylated products. On the other hand, treatment of **11** with 1 equivalent of MsCl at room temperature successfully gave the 6′-*O*-mesyl ester **12** in excellent yield. Acetylation and subsequent selective deacetylation of the mesyl derivative **12** afforded the N-2-acetyl derivative **13** in 91.8% yield (Scheme 3). After the base moiety of compound **13** had been converted into the guanine derivative **14** with sodium nitrite in aqueous acetic acid, the C-8 position of the base moiety was brominated with NBS in DMF to furnish compound **15** in good yield. In preliminary experiments, compound **15** was subjected to intramolecular cyclization into 8,6′-*O*-anhydro-8,6′-dihydroxyguanosine derivative **17** in a similar manner to compound **8**. However, compound **17** was obtained in unexpectedly low yield. Thus, we isolated

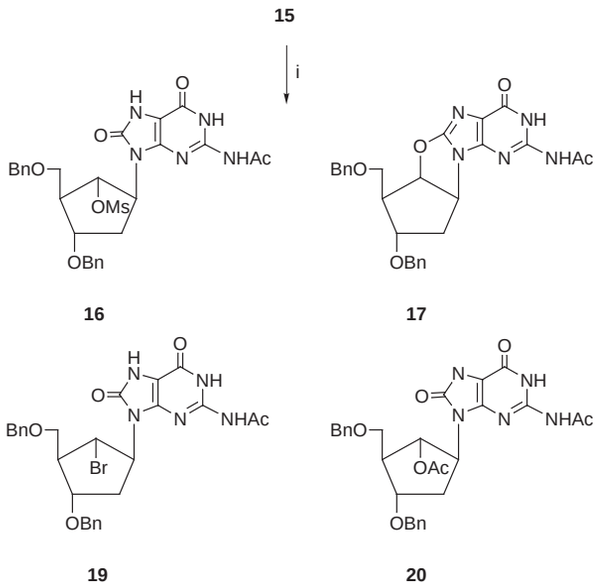


Scheme 3 *Reagents and conditions* (*and yields*): i, (a) Ac_2O, pyridine, 60 °C, overnight, (b) NH_3, MeOH, rt, 2 h (91.8%); ii, sodium nitrite, AcOH, H_2O, 60 °C, 2 h (91.8%); iii, NBS, DMF, rt, 23 h (83.7%); iv, Ag_2CO_3, Ac_2O, AcOH, 80 °C, 50 h, crude; v, sodium bicarbonate, DMF, 100 °C, 2 h (83.1%); vi, NH_3, MeOH, 60 °C, 2 h (98.3%); vii, 20% $Pd(OH)_2/C$, cyclohexene, DMF, 90 °C, 3 h (80.3%).

Table 1 Acetolysis reaction of **15** in acetic acid

Entry	Additives	Conditions	Yield (%)				
			16[a]	**19**[b]	**20**[a]	**17**[b]	Others[b,c]
1	NaOAc	reflux, 4 h	16	35	37	trace	0
2	NaOAc Ac_2O	reflux, 4 h	31	28	22	11	0
3	none	reflux, 3 h	39	45	4	0	4
4	none	80 °C, 36 h	47	21	trace	0	26
5	Ag_2CO_3 Ac_2O	80 °C, 46 h	80	14	trace	trace	trace

[a] Yields are estimated from NMR spectra of the mixture of **16** and **20** after isolation of other products. [b] Yields refer to pure isolated products. [c] Debenzylated products were obtained.



Scheme 4 *Reagents and conditions*: i, NaOAc, Ac_2O, AcOH, reflux, 3 h.

the products of the acetolysis reaction; these were characterized by ^{1}H NMR and MS spectra as **16**, **17**, **19** and **20** in 31, 11, 28 and 22% yield,‡ respectively (Scheme 4), and we investigated this reaction in detail. The results are summarized in Table 1. It is clearly demonstrated that sodium acetate increases the yield of **20** (entries 1 and 3) and acetic anhydride increases the yield of **16** (entries 1 and 2) as expected. Reaction without any additives in acetic acid at 80 °C afforded the desired product **16** as a main product, although significant amounts of the debenzylated products were observed (entry 4). This phenomenon can be explained by the absence of any base, which would capture the bromide anion liberated by acetolysis of **15** under these conditions. Then, addition of 1 equivalent of Ag^+ cation (Ag_2CO_3) in the presence of acetic anhydride minimized the formation of the 6′-bromo derivative **19** and other undesirable products, and gave an 80% yield of the desired 6′-*O*-mesylate **16** (entry 5). The resulting 6′-*O*-mesyl ester **16** was subjected to cyclization with sodium bicarbonate in DMF at 100 °C to afford compound **17**, and then treatment of **17** with methanolic ammonia at 60 °C furnished the protected 8,6′-*O*-anhydro-8,6′-dihydroxyguanosine **18**. Deprotection of **18** with 20% $Pd(OH)_2$/C and cyclohexene in DMF afforded (±)-carbocyclic 8,6′-*O*-anhydro-8,6′-dihydroxy-2′-deoxyguanosine **4** in 80.3% isolated yield.

Thus, we have achieved synthesis of racemic carbocyclic 8,6′-*O*-anhydro-8,6′-dihydroxy-2′-deoxynucleosides having four natural nucleobases (compounds **1**–**4**). Synthesis of an optically active form of them would be readily achieved by using optically active epoxide **5** which can be prepared from cyclopentadiene *via* asymmetric hydroboration.[14] Further investigations on these problems and their incorporation into oligonucleotides are now in progress.

‡ Complete separation of compounds **16** and **20** could not be achieved. Therefore, the yields were estimated from the ratio of signals corresponding to each compound in the ^{1}H NMR spectrum of the mixture of **16** and **20**.

Experimental

Mps were measured on a Yanagimoto apparatus and are uncorrected. UV spectra were measured with a JASCO Ubest-55 spectrophotometer. ^{1}H NMR spectra were obtained by a Varian gemini 200 or Varian mercury 300 spectrometer. Chemical shifts were measured relative to internal tetramethylsilane for $CDCl_3$ and d_6-DMSO, and are given in ppm, with coupling constants (*J*) in Hz. Mass spectra were recorded on a Hitachi M-4100 double-focusing spectrometer. TLC was carried out on Merck coated plates $60F_{254}$. Silica gel column chromatography was performed with Merck silica gel 60 or 60H. The racemic epoxide **5** was synthesized according to a literature procedure.[14] NMR assignments of nucleoside analogues are labelled according to the scheme of Biggadike.[13]

(±)-(1′β,2′α,3′β,4′α)-9-(4-Benzyloxy-3-benzyloxymethyl-2-hydroxycyclopentyl)adenine 6

To a suspension of adenine (1.02 g, 7.52 mmol) and adenine sodium salt (157 mg, 1 mmol) in dry DMF (40 ml) was added the epoxide **5** (1.55 g, 5 mmol) and the mixture was heated at 140 °C for 27 h under argon. After cooling, the solvent was evaporated and the residue was diluted with ethyl acetate (200 ml). The mixture was washed with distilled water (100 ml × 3). After the organic layer had been mixed with Na_2SO_4 and concentrated, the residue was purified by column chromatography on silica gel ($CHCl_3$–4% MeOH) to give 1.47 g (65.7%) of **6** as a colorless foam. An analytical sample was recrystallized from ethyl acetate to afford *pale yellow crystals* of **6**, mp 146–147 °C (Found: C, 67.32; H, 6.04; N, 15.65. $C_{25}H_{27}N_5O_3$ requires C, 67.40; H, 6.11; N, 15.72%) (Found: $M^+ + 1$, 446.2189. $C_{25}H_{28}N_5O_3$ requires *m*/*z*, 446.21904); λ_{max}(EtOH)/nm 261 (ε/l mol^{-1} cm^{-1} 14 900); δ_H($CDCl_3$, 200 MHz) 2.27–2.42 (1H, m, H-2′), 2.42–2.58 (2H, m, H-2′, H-4′), 3.64–3.73 (2H, m, H_2-5′), 4.07 (1H, m, H-3′), 4.42 (1H, t, *J* 9.0, H-6′), 4.50–4.64 (4H, m, Ar-CH_2 × 2), 4.77 (1H, m, H-1′), 6.07 (2H, br s, NH_2), 7.25–7.40 (10H, m, ArH), 7.68 (1H, s, H-2), 8.20 (1H, s, H-8); *m*/*z* (EI) 446 ($M^+ + 1$).

(±)-(1′β,2′α,3′β,4′α)-9-(4-Benzyloxy-3-benzyloxymethyl-2-hydroxycyclopentyl)-8-bromoadenine 7

A solution of **6** (668 mg, 1.5 mmol) in 1,4-dioxane (20 ml) and 0.5 M aqueous sodium acetate (pH 5.0) was treated with bromine (38.5 µl, 0.75 mmol), and the mixture was stirred at room temperature. After 3, 6, and 8 h, bromine (38.5 µl each) was added. After 10 h, the reaction was quenched with aqueous

sodium bisulfite. The mixture was concentrated to about one-half of the original volume, and was extracted with $CHCl_3$ (100 ml). The organic layer was washed with H_2O (50 ml × 2), dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$–3% MeOH) to give 490 mg (62.3%) of **7** (Found: $M^+ + 1$, 524.1298. $C_{25}H_{27}N_5O_3Br$ requires *m*/*z*, 524.1296); λ_{max}(EtOH)/nm 266 (ε/l mol^{-1} cm^{-1} 15 400); δ_H($CDCl_3$, 200 MHz) 2.22 (1H, m, H-2′), 2.40 (1H, m, H-4′), 2.75 (1H, m, H-2′), 3.79 (2H, m, H_2-5′), 4.10 (1H, m, H-3′), 4.54 (2H, s, Ar-CH_2), 4.60 (2H, s, Ar-CH_2), 4.93 (1H, q, *J* 9.1, H-1′), 5.07 (1H, t, *J* 9.1, H-6′), 5.91 (2H, br s, NH_2), 7.23–7.40 (10H, m, ArH), 7.96 (1H, s, H-2); *m*/*z* (EI) 524 ($M^+ + 1$).

(±)-(1′β,2′α,3′β,4′α)-9-[4-Benzyloxy-3-benzyloxymethyl-2-(*p*-tolylsulfonyloxy)cyclopentyl]-8-bromoadenine 8

To a solution of **7** (448 mg, 0.854 mmol) in CH_2Cl_2 (10 ml) were added TsCl (244 mg, 1.28 mmol) and DMAP (260 mg, 2.13 mmol), and the mixture was stirred overnight at room temperature. After addition of H_2O, the mixture was extracted with $CHCl_3$ (70 ml) and the organic layer was washed successively with aqueous 0.5 M KH_2PO_4 (50 ml × 3) and saturated aqueous $NaHCO_3$ (50 ml × 2). After the organic layer had been dried with Na_2SO_4 and concentrated, the residue was purified by column chromatography on silica gel ($CHCl_3$–2% MeOH) to give 542 mg (93.4%) of **8** as a *colorless foam* (Found: M^+, 677.1309. $C_{32}H_{32}N_5O_5SBr$ requires *M*, 677.1306); λ_{max}(EtOH)/nm 266 (ε/l mol^{-1} cm^{-1} 13 200); δ_H($CDCl_3$, 200 MHz) 2.08 (1H, m, H-2′), 2.29 (3H, s, Ar-CH_3), 2.66 (1H, m, H-4′), 2.80 (1H, m, H-2′), 3.73–3.88 (2H, m, H_2-5′), 4.17 (1H, m, H-3′), 4.48 (1H, d, *J* 12.1, Ar-CH_2), 4.55 (1H, d, *J* 12.1, Ar-CH_2), 4.59 (2H, s, Ar-CH_2), 5.18 (1H, m, H-1′), 5.53 (2H, br s, NH_2), 5.70 (1H, t, *J* 8.5, H-6′), 6.86 (2H, d, *J* 8.0, ArH), 7.23–7.46 (12H, m, ArH), 8.10 (1H, s, H-2); *m*/*z* (EI) 677 (M^+).

(±)-(6aα,7β,8α,9aα)-8-Benzyloxy-7-benzyloxymethyl-7,8,9,9a-tetrahydro-6a*H*-cyclopenta[4,5]oxazolo[3,2-*e*]purin-4-amine 10

A mixture of **8** (534 mg, 0.786 mmol) and sodium acetate (1.164 g, 14.2 mmol) in acetic acid–acetic anhydride (1 : 1, v/v; 19.5 ml) was refluxed for 3 h. After the solvent had been removed under reduced pressure, the residue was coevaporated with EtOH and was partitioned between $CHCl_3$ (50 ml) and saturated aqueous $NaHCO_3$ (30 ml × 2). The organic layer was dried with Na_2SO_4, and concentrated. The residue was dissolved in MeOH (20 ml), through which ammonia gas was bubbled for 20 min under cooling at −20 °C. After the reaction tube had been sealed, the mixture was heated at 60 °C for 7 h and allowed to cool. Volatile materials were evaporated off, and the residue was extracted with ethyl acetate (50 ml). The solution was washed with saturated aqueous $NaHCO_3$ (30 ml × 2). The organic layer was dried with Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$–3% MeOH) to give 259 mg (74.3%) of **10** as a pale pink solid. An analytical sample was recrystallized from $CHCl_3$–*n*-hexane to give *pale pink crystals* of **10**, mp 150–153 °C (Found: C, 67.75; H, 5.80; N, 15.53; M^+, 443.1952. $C_{25}H_{25}N_5O_3$ requires C, 67.70; H, 5.68; N, 15.79%; *M*, 443.1955); λ_{max}(EtOH)/nm 259 (ε/l mol^{-1} cm^{-1} 14 600); δ_H($CDCl_3$, 200 MHz) 2.08 (1H, m, H-2′), 2.54–2.73 (2H, m, H-2′, H-4′), 3.77–3.95 (3H, m, H-3′, H_2-5′), 4.38 (1H, d, *J* 11.3, Ar-CH_2), 4.52 (1H, d, *J* 11.3, Ar-CH_2), 4.55 (2H, s, Ar-CH_2), 5.10 (1H, m, H-1′), 5.49 (2H, br s, NH_2) 5.92 (1H, dd, *J* 7.0 and 6.0, H-6′), 7.20–7.40 (10H, m, ArH), 8.22 (1H, s, H-2); *m*/*z* (EI) 443 (M^+).

(±)-(6aα,7β,8α,9aα)-4-Amino-7-hydroxymethyl-7,8,9,9a-tetrahydro-6a*H*-cyclopenta[4,5]oxazolo[3,2-*e*]purin-8-ol 3

A mixture of **10** (80 mg, 0.18 mmol), 20% $Pd(OH)_2$/C (20 mg) and cyclohexene (1 ml) in EtOH (5 ml) was refluxed for 18 h. The catalyst was removed by filtration and was washed with hot 50% EtOH. The filtrate was concentrated to dryness and the residue was recrystallized from 50% EtOH to give pure *title product* **3** (42 mg, 88.6%), mp >240 °C (decomp.) (Found: C, 49.99; H, 5.01; N, 26.49; M^+, 263.1019. $C_{11}H_{13}N_5O_3$ requires C, 50.18; H, 4.98; N, 26.61%; *M*, requires 263.10174); λ_{max}(H_2O)/nm 262 (ε/l mol^{-1} cm^{-1} 14 300); δ_H(d_6-DMSO, 200 MHz) 1.84 (1H, m, H-2′), 2.07 (1H, m, H-4′), 2.27 (1H, dd, *J* 6.7 and 13.6, H-2′), 3.55–3.76 (3H, m, H-3′, H_2-5′), 4.73 (1H, t, *J* 5.0, 5′-OH), 5.03 (1H, t, *J* 6.7, H-1′), 5.12 (1H, d, *J* 5.2, 3′-OH), 5.83 (1H, t, *J* 6.7, H-6′), 6.74 (2H, br s, NH_2), 7.99 (1H, s, H-2); *m*/*z* (EI) 263 (M^+).

(±)-(1′β,2′α,3′β,4′α)-9-(4-Benzyloxy-3-benzyloxymethyl-2-hydroxycyclopentyl)-9*H*-purine-2,6-diamine 11

A mixture of 60% sodium hydride (200 mg, 5 mmol) and 2,6-diaminopurine (1.06 g, 7.26 mmol) in dry DMF (25 ml) was stirred at 85 °C for 20 min under argon. After cooling of the mixture to room temperature, the epoxide **5** (1.55 g, 5 mmol) and 15-crown-5 (0.14 ml, 0.7 mmol) was added to the mixture which was then refluxed for 4 h and allowed to cool. After the solvent had been evaporated off, the residue was diluted with ethyl acetate (100 ml) and was washed with distilled water (70 ml × 2). The organic layer was dried with Na_2SO_4 and concentrated, then the residue was purified by column chromatography on silica gel ($CHCl_3$–4% MeOH) to give 1.72 g (72.8%) of **11** as a pale yellow crystalline solid. An analytical sample was recrystallized from MeOH to afford *colorless needles* of **11**, mp 173–175 °C (Found: C, 64.99; H, 6.08; N, 18.44; M^+, 460.2222. $C_{25}H_{28}N_6O_3$ requires C, 65.20; H, 6.13; N, 18.25%; *M*, 460.2221); λ_{max}(EtOH)/nm 283 (ε/l mol^{-1} cm^{-1} 10 800), 257 (9200) and 253sh (8600); δ_H(d_6-DMSO, 300 MHz) 2.08–2.18 (1H, m, H-4′), 2.19-2.28 (2H, m, H_2-2′), 3.53 (1H, dd, *J* 9.4 and 7.3, H-5′), 3.66 (1H, dd, *J* 9.4 and 4.0, H-5′), 3.86–3.92 (1H, m, H-3′), 4.13 (1H, m, H-6′), 4.49–4.61 (5H, m, Ar-CH_2 × 2, H-1′), 5.59 (1H, d, *J* 5.5, OH), 5.73 (2H, br, NH_2), 6.63 (2H, br, NH_2), 7.24–7.38 (10H, m, ArH), 7.77 (1H, s, H-8); *m*/*z* (EI) 460 (M^+).

(±)-(1′β,2′α,3′β,4′α)-9-[4-Benzyloxy-3-benzyloxymethyl-2-(methylsulfonyloxy)cyclopentyl]-9*H*-purine-2,6-diamine 12

MsCl (0.762 ml, 9.84 mmol) was added dropwise to a solution of **11** (4.53 g, 9.84 mmol) in dry pyridine (60 ml) at 0 °C, and the mixture was kept at room temperature for 1.5 h. After addition of EtOH, the mixture was evaporated under reduced pressure, and the residue was extracted with $CHCl_3$ (200 ml). The solution was washed successively with saturated aqueous $NaHCO_3$ (150 ml × 2) and H_2O (100 ml). The separated organic phase was dried with Na_2SO_4, and the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$–4% AcOH to remove a bis-Ms derivative, then $CHCl_3$–4% MeOH) to give 4.8 g (90.5%) of **12** as a *pale yellow foam* (Found: M^+, 538.1993. $C_{26}H_{30}N_6O_5S$ requires *M*, 583.1996); λ_{max}(EtOH)/nm 282 (ε/l mol^{-1} cm^{-1} 9500), 258 (8600) and 253sh (8100); δ_H($CDCl_3$, 300 MHz) 2.29 (1H, dd, *J* 13.7 and 8.0, H-2′), 2.58 (3H, s, SO_2CH_3), 2.59–2.80 (2H, m, H-2′, H-4′), 3.77 (2H, d, *J* 4.4, H_2-5′), 4.09–4.15 (1H, m, H-3′), 4.53 (2H, s, Ar-CH_2), 4.57 (2H, s, Ar-CH_2), 4.58 (2H, br s, NH_2), 5.02 (1H, m, H-1′), 5.47 (2H, br, NH_2), 5.57 (1H, dd, *J* 8.0 and 6.7, H-6′), 7.26–7.40 (10H, m, ArH), 7.51 (1H, s, H-8); *m*/*z* (EI) 538 (M^+).

(±)-(1″β,2″α,3″β,4″α)-*N*-{6-Amino-9-[4-benzyloxy-3-benzyloxymethyl-2-(methylsulfonyloxy)cyclopentyl]-9*H*-purin-2-yl}-acetamide 13

Acetic anhydride (2.13 ml, 22.5 mmol) was added to a solution of **12** (1.21 g, 2.25 mmol) in dry pyridine (20 ml), and the mixture was kept at 60 °C overnight. After addition of EtOH, the

mixture was concentrated, and the residue was extracted $CHCl_3$ (200 ml). The solution was washed successively with saturated aqueous $NaHCO_3$ (150 ml × 2), and H_2O (100 ml). The separated organic phase was dried with Na_2SO_4, and the solvent was removed under reduced pressure. The residue was dissolved in MeOH (30 ml), through which ammonia gas was bubbled for 30 min under cooling at −20 °C. The reaction tube was sealed and the mixture was kept at room temperature for 2 h before being concentrated under reduced pressure. The residue was extracted with $CHCl_3$ (200 ml), and the solution was washed with H_2O (150 ml × 2). After the organic layer had been dried with Na_2SO_4, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$–60% acetone) to give 1.2 g (91.8%) of **13** as a *pale yellow foam* (Found: $M^+ + 1$, 581.2195. $C_{28}H_{33}N_6O_6S_1$ requires *m*/*z*, 581.2180); λ_{max}(EtOH)/nm 273 (ε/l mol^{-1} cm^{-1} 16 600) and 227 (28100); δ_H($CDCl_3$, 300 MHz) 2.34 (1H, dd, *J* 13.6 and 8.1, H-2′), 2.49–2.62 (7H, m, SO_2CH_3, $COCH_3$, H-4′), 2.70–2.84 (1H, m, H-2′), 3.68 (2H, d, *J* 4.4, H_2-5′), 4.04–4.12 (1H, m, H-3′), 4.44–4.60 (4H, m, Ar-CH_2 × 2), 5.01 (1H, m, H-1′), 5.57 (1H, t, *J* 8.1, H-6′), 6.98 (2H, br, NH_2), 7.21–7.40 (10H, m, ArH), 7.68 (1H, s, H-8), 10.03 (1H, br, NH); *m*/*z* (EI) 581 ($M^+ + 1$).

(±)-(1″β,2″α,3″β,4″α)-*N*-{9-[4-Benzyloxy-3-benzyloxymethyl-2-(methylsulfonyloxy)cyclopentyl]-6,9-dihydro-6-oxo-1*H*-purin-2-yl}acetamide 14

Sodium nitrite (1.07 g, 15.5 mmol) was added to a solution of **13** (1.13 g, 1.94 mmol) in acetic acid (20 ml), and H_2O (≈5 ml) was added to the mixture until the suspension became a clear solution. The solution was heated at 60 °C for 2 h, and allowed to cool. After the reaction had been quenched with ammonium sulfamate, the solvent was removed under reduced pressure, the residue was extracted with $CHCl_3$ (100 ml), and the solution was washed successively with saturated aqueous $NaHCO_3$ (70 ml × 2), and H_2O (70 ml). After the organic layer had been dried with Na_2SO_4, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$–50% acetone) to give 1.04 g (91.8%) of **14** as a *colorless foam* (Found: M^+, 581.1924. $C_{28}H_{31}N_5O_7S$ requires *M*, 581.1942); λ_{max}(EtOH)/nm 278sh (ε/l mol^{-1} cm^{-1} 11 300) and 259 (16 500); δ_H($CDCl_3$, 300 MHz) 2.12 (3H, s, $COCH_3$), 2.35 (1H, dd, *J* 13.5 and 8.0, H-2′), 2.48–2.62 (2H, m, H-2′, H-4′), 2.63 (3H, s, -SO_2CH_3), 3.65–3.76 (2H, m, H_2-5′), 4.04–4.12 (1H, m, H-3′), 4.46–4.59 (4H, m, Ar-CH_2 × 2), 5.03 (1H, m, H-1′), 5.48 (1H, dd, *J* 8.0 and 7.1, H-6′), 7.26–7.39 (10H, m, ArH), 7.68 (1H, s, H-8), 9.23 (1H, br s, NH), 12.01 (1H, br s, NH); *m*/*z* (EI) 581 (M^+).

(±)-(1″β,2″α,3″β,4″α)-*N*-{9-[4-Benzyloxy-3-benzyloxymethyl-2-(methylsulfonyloxy)cyclopentyl]-8-bromo-6,9-dihydro-6-oxo-1*H*-purin-2-yl}acetamide 15

A solution of **14** (1.01 g, 1.73 mmol) in dry DMF was treated with NBS (340 mg, 1.91 mmol), and the mixture was stirred at room temperature in the dark. After 14 and 20 h, further NBS (170 mg each) was added to the solution. After 23 h, the reaction was quenched with aqueous sodium hydrosulfite at 0 °C. After the solvent had been removed under reduced pressure, the residue was extracted with $CHCl_3$ (100 ml), and the solution was washed successively with saturated aqueous $NaHCO_3$ (70 ml × 2) and H_2O (70 ml). After the organic layer was dried with Na_2SO_4, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$–2% MeOH) to give 960 mg (83.7%) of **15** as a *colorless foam* (Found: $M^+ + 1$, 660.1144. $C_{28}H_{31}N_5O_7SBr$ requires *m*/*z*, 660.1128); λ_{max}(EtOH)/nm 282sh (ε/l mol^{-1} cm^{-1} 13 300) and 264 (17 800); δ_H($CDCl_3$, 300 MHz) 1.97 (3H, s, $COCH_3$), 2.24–2.35 (1H, m, H-2′), 2.51–2.56 (1H, m, H-4′), 2.69–2.80 (4H, m, H-2′, -SO_2CH_3), 3.72–3.84 (2H, m, H_2-5′), 4.17–4.22 (1H, m, H-3′), 4.45–4.65 (4H, m, Ar-CH_2 × 2), 5.20 (1H, m, H-1′), 5.87 (1H, dd, *J* 8.4 and 8.0, H-6′), 7.26–7.40 (10H, m, ArH), 8.54 (1H, br s, NH), 11.96 (1H, br s, NH); *m*/*z* (SIMS) 660 and 662 ($M^+ + 1$).

(±)-(6aα,7β,8α,9aα)-*N*-(8-Benzyloxy-7-benzyloxymethyl-3,4,7,8,9,9a-hexahydro-4-oxo-6a*H*-cyclopenta[4,5]oxazolo[3,2-*e*]-purin-2-yl)acetamide 17

15 (200 mg, 0.3 mmol) was treated with acetic anhydride (3 ml) and silver carbonate (41 mg, 0.15 mmol) in acetic acid (30 ml) at 80 °C for 50 h. After cooling to room temperature, the solvent was evaporated under reduced pressure, and $CHCl_3$ (50 ml) was added to the residue. After removal of silver bromide by filtration, the filtrate was washed successively with saturated aqueous $NaHCO_3$ (30 ml × 2), and H_2O (30 ml). The organic layer was dried with Na_2SO_4, and the solvent was removed under reduced pressure to give crude **16** as a *colorless solid* (Found: $M^+ + 1$, 598.1970. $C_{28}H_{32}N_5O_8S$ requires *m*/*z*, 598.1969); λ_{max}(EtOH)/nm 303 (ε/l mol^{-1} cm^{-1} 5600) and 267 (14 200); δ_H($CDCl_3$, 300 MHz) 2.07 (3H, s, $COCH_3$), 2.17–2.26 (1H, m, H-2′), 2.46–2.65 (2H, m, H-2′, H-4′), 2.85 (3H, s, SO_2CH_3), 3.70 (1H, dd, *J* 9.6 and 4.3, H-5′), 3.78 (1H, dd, *J* 9.6 and 4.7, H-5′), 4.14 (1H, dd, *J* 9.6 and 6.0, H-3′), 4.43–4.60 (4H, m, Ar-CH_2 × 2), 5.08 (1H, m, H-1′), 5.69 (1H, dd, *J* 8.0 and 7.7, H-6′), 7.22–7.38 (10H, m, ArH), 8.96 (1H, br s, NH) 9.60 (1H, br s, NH), 12.02 (1H, br s, NH); *m*/*z* (SIMS) 598 ($M^+ + 1$).

The residue was dissolved in dry DMF and was treated with $NaHCO_3$ (252 mg, 3 mmol) at 100 °C for 2 h. After cooling to room temperature, crystalline $NaHCO_3$ was removed by filtration, and the filtrate was concentrated to dryness. The residue was purified by column chromatography on silica gel ($CHCl_3$–6% MeOH) to give 125 mg (83.1%) of **17** as a *colorless solid* (Found: $M^+ + 1$, 502.2091. $C_{27}H_{28}N_5O_5$ requires *m*/*z*, 502.2089); λ_{max}(EtOH)/nm 298 (ε/l mol^{-1} cm^{-1} 8800) and 260 (14 900); δ_H(d_6-DMSO, 300 MHz) 1.88–1.98 (1H, m, H-2′), 2.16 (3H, s, $COCH_3$), 2.42–2.56 (2H, m, H-2′, H-4′), 3.70 (2H, d, *J* 6.9, H_2-5′), 3.85–3.94 (1H, m, H-3′), 4.41 (1H, d, *J* 12.1, Ar-CH_2), 4.49 (1H, d, *J* 12.1, Ar-CH_2), 4.53 (2H, s, Ar-CH_2), 5.06 (1H, t, *J* 6.8, H-1′), 5.86 (1H, t, *J* 6.8, H-6′), 7.22–7.38 (10H, m, ArH), 11.76 (1H, br s, NH), 11.93 (1H, br s, NH); *m*/*z* (SIMS) 502 ($M^+ + 1$).

(±)-(6aα,7β,8α,9aα)-2-Amino-8-benzyloxy-7-benzyloxymethyl-7,8,9,9a-tetrahydro-6a*H*-cyclopenta[4,5]oxazolo[3,2-*e*]purin-4(3*H*)-one 18

17 (192 mg, 0.383 mmol) was suspended in MeOH (30 ml), through which ammonia gas was bubbled for 30 min under cooling at −20 °C. After the reaction tube had been sealed, the mixture was kept at 60 °C for 2 h. Volatile materials were evaporated off, the residue was treated with H_2O (10 ml), and the resulting precipitated solid was collected by filtration, and was washed by H_2O to give 173 mg (98.3%) of **18** as a colorless solid. An analytical sample was recrystallized from $CHCl_3$–MeOH to afford *colorless needles* of **18**, mp 267–268 °C (Found: C, 64.81; H, 5.42; N, 15.12. $C_{25}H_{25}N_5O_4 \cdot 1/4H_2O$ requires C, 64.71; H, 5.54; N, 15.09%) (Found: $M^+ + 1$, 460.1984. $C_{25}H_{26}N_5O_4$ requires *m*/*z*, 460.1983); λ_{max}(EtOH)/nm 287 (ε/l mol^{-1} cm^{-1} 8800) and 248 (13400); δ_H(d_6-DMSO, 300 MHz) 1.82–1.93 (1H, m, H-2′), 2.39–2.52 (2H, m, H-2′, H-4′), 3.68 (2H, d, *J* 6.9, H_2-5′), 3.82 (1H, m, H-3′), 4.41 (1H, d, *J* 12.1, Ar-CH_2), 4.49 (1H, d, *J* 12.1, Ar-CH_2), 4.53 (2H, s, Ar-CH_2), 4.94 (1H, t, *J* 6.6, H-1′), 5.76 (1H, t, *J* 6.6, H-6′), 6.38 (2H, br s, NH_2), 7.22–7.38 (10H, m, ArH), 10.48 (1H, s, NH); *m*/*z* (SIMS) 460 ($M^+ + 1$).

(±)-(6aα,7β,8α,9aα)-2-Amino-8-hydroxy-7-hydroxymethyl-7,8,9,9a-tetrahydro-6a*H*-cyclopenta[4,5]oxazolo[3,2-*e*]purin-4(3*H*)-one 4

A mixture of **18** (123 mg, 0.267 mmol), 20% $Pd(OH)_2$/C (3 mg)

and cyclohexene (5 ml) in DMF (10 ml) was heated at 90 °C for 3 h. The catalyst was removed by filtration and washed with hot aqueous EtOH. The filtrate was evaporated under reduced pressure and the residue was recrystallized with EtOH–H_2O (1 : 1) to give pale grey crystals of **4** (60 mg, 80.3%), mp 283 °C (decomp.). An analytical sample was recrystallized from H_2O to afford *slightly hygroscopic colorless needles* of **4**, mp 286 °C (decomp.) (Found: C, 43.45; H, 5.18; N, 22.70. $C_{11}H_{13}N_5O_4 \cdot 10/7H_2O$ requires C, 43.31; H, 5.24; N, 22.96%) (Found: $M^+ + 1$, 280.1040. $C_{11}H_{14}N_5O_4$ requires *m*/*z*, 280.1045); $\lambda_{max}(H_2O)$/nm 286 (ε/l mol^{-1} cm^{-1} 9100) and 248 (12 000); δ_H(d_6-DMSO, 300 MHz) 1.72–1.83 (1H, m, H-2′), 1.96–2.06 (1H, m, H-4′), 2.20 (1H, dd, *J* 13.5 and 6.6, H-2′), 3.54–3.74 (3H, m, H-3′, H_2-5′), 4.69 (1H, t, *J* 4.9, 5′-OH), 4.87 (1H, t, *J* 6.6, H-1′), 5.10 (1H, d, *J* 5.2, 3′-OH), 5.71 (1H, t, *J* 6.6, H-6′), 6.39 (2H, br s, NH_2), 10.48 (1H, br s, NH); *m*/*z* (SIMS) 280 ($M^+ + 1$).

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

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