Fluorocarbocyclic Nucleosides: Synthesis and Antiviral Activity of 2'- and 6'-Fluorocarbocyclic 2'-Deoxy Guanosines[1]

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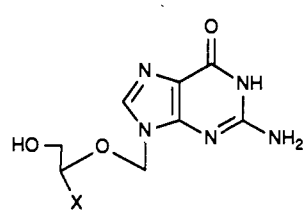
A series of four isomeric 2'- and 6'-fluorocarbocyclic guanosine analogues have been prepared and evaluated as potential anti-herpes agents. The racemic 2'β-fluoro isomer 2-amino-1,9-dihydro-9-[(1α,2α,3β,4α)-2-fluoro-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-6*H*-purin-6-one (**11a**, C-AFG) and its 2'α-fluoro epimer **11b** plus the chiral 6'β-fluoro isomer 2-amino-1,9-dihydro-9-[[1*S*-(1α,2α,3α,4β)]-2-fluoro-4-hydroxy-3-(hydroxymethyl)cyclopentyl]-6*H*-purin-6-one (**11c**) and its 6'α-fluoro epimer **11d** were prepared from their respective fluoro amino diol hydrochlorides (**6a,d**). For comparison, the furanosyl compound 9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)guanine (**17**, AFG) was prepared by coupling 2-amino-6-chloropurine with 2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide followed by base hydrolysis. The 6'α-fluoro derivative **11d** exhibited comparable activity to that of acyclovir (ACV) against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro but was >30-fold more active than ACV against HSV-1 and HSV-2 in vivo in the mouse systemic model. The 2'β-fluoro derivative (**11a**, C-AFG) was extremely potent in vitro against HSV-1 and HSV-2 (ID_{50} 0.006 and 0.05 μg/mL) and in vivo it was greater than 2 orders of magnitude more potent than ACV against HSV-1 and 70-fold more potent against HSV-2. The 2'α-fluoro **11b** and 6'β-fluoro **11c** isomers were much less active.

Nucleoside analogues have been extensively investigated in the search for effective antiviral agents with modifications being made in the heterocyclic base and/or the sugar moiety. The latter has been variously substituted and also replaced by acyclic and carbocyclic entities. From these studies it is apparent that the base guanine is unique in tolerating a wide variety of such sugar modifications while retaining potent anti-herpes activity.[2-4] The most prominent acyclic derivative is 9-[(2-hydroxyethoxy)methyl]-guanine (acyclovir, ACV, **1**)[5] which is in clinical use for the treatment of diseases caused by herpes simplex virus (HSV), while 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, **2**),[6] a derivative of ACV, has been reported to be even more effective than ACV in vivo.[6,7] Other acyclic guanine derivatives with potent anti-herpes activity include (*S*)-9-[(2,3-dihydroxy-1-propoxy)methyl]guanine (iNDG),[8] 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG),[9] and the cyclic phosphate derivative of DHPG (Cp-DHPG).[10] Carba analogues of acyclic guanine nucleosides have also been prepared, and (*R*)-9-(3,4-dihydroxybutyl)guanine (R-DHBG)[11] is reported to be active against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro and in vivo. In contrast, the carba analogues of acyclovir (HBG)[12] and DHPG (C-DHPG)[13] are 1 order of magnitude less active than ACV or DHPG against HSV-1 and HSV-2 in cell culture,[1] and HBG is inactive in vivo.[11]

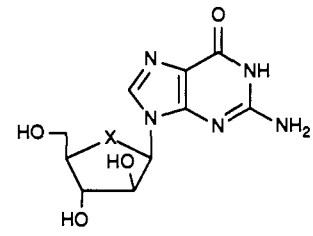
Activity in the cyclic series was first reported for 9-β-D-arabinofuranosylguanine (*ara*-G, **3**) which was claimed to have a therapeutic effect greater than *ara*-A against HSV-2 in vivo.[14] In contrast, its carbocyclic analogue **4** was reported[15] to be inactive against HSV-1.

However the carbocyclic analogue of 2'-deoxyguanosine (2'-CDG, **5**) has been reported to be highly potent against HSV-1 and HSV-2 in vitro.[3]

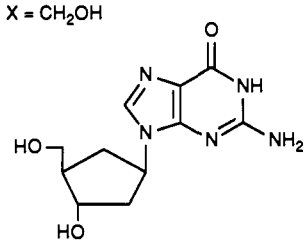
We recently prepared a series of fluorocarbocyclic pyrimidine nucleoside analogues[16] including the carbocyclic versions of the potent anti-herpetics FMAU and FIAU but found only moderate activity against HSV-1 and no activity against HSV-2. However, we thought it desirable



1. X = H
2. X = CH_2OH



3. X = O
4. X = CH_2



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to extend our synthetic program to include the preparation of guanine fluorocarbocyclic nucleosides and chose as our

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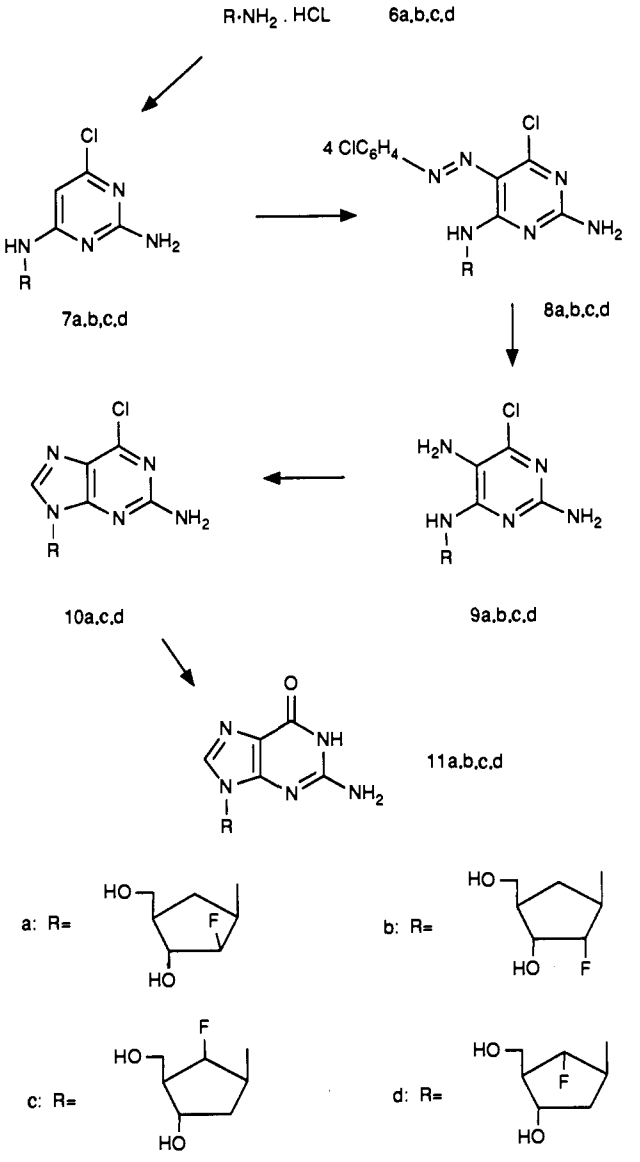
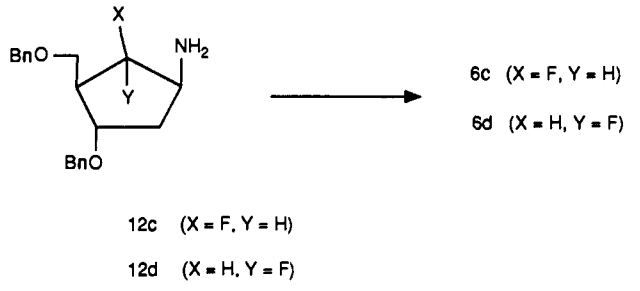
Scheme I

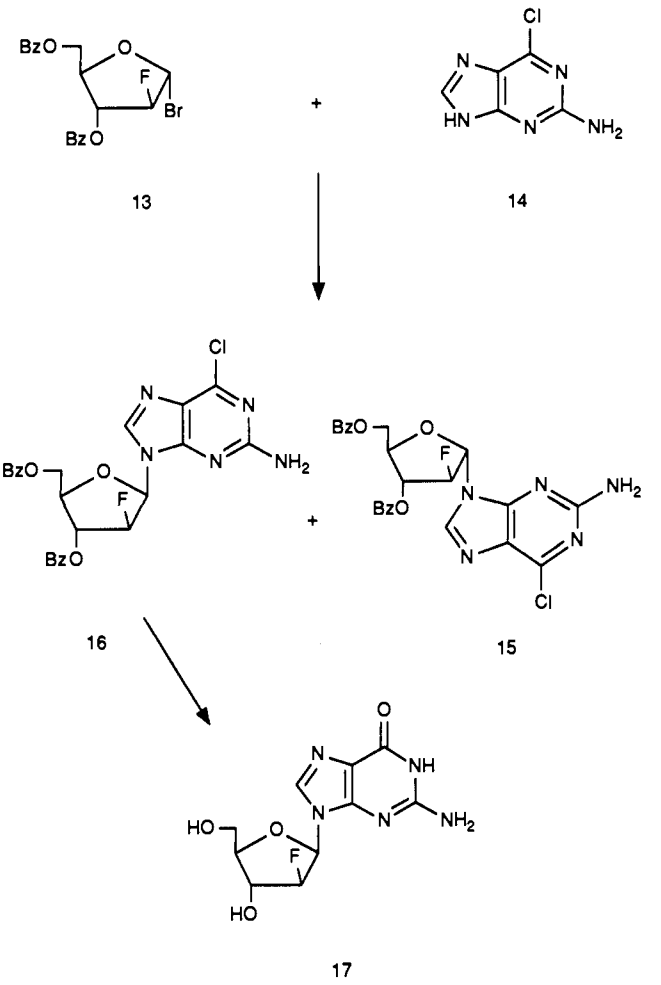


Scheme II



initial targets the 2′- and 6′-fluoro-substituted carbocyclic 2′-deoxyguanosine derivatives **11a–d**.

Scheme III



Chemistry

The synthetic route (Scheme I) to the guanine derivatives **11a–d** from the fluorocyclopentylamine hydrochlorides **6a–d** was based on the general methodology for the synthesis of carbocyclic guanine nucleosides developed earlier by Shealy et al.[3] The synthesis of the racemic fluorocyclopentylamine hydrochlorides **6a** and **6b** has been described earlier.[16] The chiral fluorocyclopentylamine hydrochlorides **6c** and **6d** were prepared in high yield from the known benzyl-protected derivatives[16] **12c** and **12d** (Scheme II), by hydrogenolysis in methanol containing 1 N hydrochloric acid in the presence of palladium on carbon. Reaction of 2-amino-4,6-dichloropyrimidine with the fluoro amino diol hydrochlorides **6a–d** in refluxing butanol or ethanol in the presence of triethylamine under a nitrogen atmosphere gave the diamines **7a–d** in high yield

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(68–88%). Coupling of 4-chlorobenzenediazonium chloride with **7a–d** in aqueous acetic acid buffered with sodium acetate afforded the azo derivatives **8a–d** in high (71–89%) yield. Reduction of the azo derivatives **8b–d** using zinc in aqueous acetic acid[3] gave the triamines **9b–d** in good yield (54–78%). However, the isolation and purification of the triamine products is laborious. We therefore modified this procedure and used zinc in methanol in the presence of ammonium chloride and removed the 4-chloroaniline byproduct by sublimation. With use of this modification **8a** was reduced to **9a** on a 30-g scale in ≥74% yield. The 2-amino-6-chloropurines (**10a,c,d**) were obtained in 67–93% yield by acid-catalyzed reaction of **9a,c,d** with triethyl orthoformate followed by treatment of the crude product with 0.6 N hydrochloric acid to liberate the derivatized hydroxyl and amino groups. However, the preferred method was found to be the reaction of the triamine derivative with formamidine acetate[17] in 1-butanol as this was easier to perform when the reaction was done on a large scale. With use of this modification **10a** was obtained from **9a** on a 15.5-g scale in 85% yield. The guanine analogue **11a** was prepared in 76% yield by refluxing the chloro analogue **10a** in 1 N hydrochloric acid for 1 h. Similar treatment of **10c,d** gave **11c,d**. The guanine derivative **11b** was prepared from the triamine **9b** by combining the cyclization and hydrolysis steps without isolation of the intermediate 2-amino-6-chloro derivative.

The synthesis of the 2′-*ara*-fluoro furanose analogue **17** (Scheme III) appeared to be best accomplished by condensation of the readily available[18] 2-deoxy-2-fluoroarabinose derivative **13** with a suitably substituted purine. In a recent preparation[19] of *ara*-G it was found that the most selective coupling method was the reaction of the trimethylsilylated derivative of 2-amino-6-chloropurine **14** with an arabinosyl chloride in the presence of molecular sieves. These conditions were reported to give only the β-anomer with substitution occurring exclusively at the N-9 position of the purine ring. However, reaction of the 2-deoxy-2-fluoroarabinosyl bromide **13** with the trimethylsilylated derivative of **14** under similar conditions gave only a low yield (<10%) of 2-amino-6-chloro-9-(2-deoxy-3,5-di-*O*-benzoyl-2-fluoro-β-D-arabinofuranosyl)purine (**16**) (Scheme III). An alternative coupling procedure[20] where highly selective N-9 couplings of purines have been achieved in high yield involves reaction of silylated purines with glycosyl halides in the presence of mercuric cyanide. With use of this procedure the trimethylsilylated derivative of **14** was reacted with the arabinosyl bromide **13** to give **16** and its α-isomer **15** in a ratio of 3.8:1 and in an overall yield of 43%. Treatment of **16** with aqueous NaOH in dioxane simultaneously displaced the 6-chloro group and deprotected the benzoate groups to give the required guanine derivative **17**. This two step preparation of **17** is more efficient than the five-step method previously reported,[21] and is similar in strategy to the recently reported method of Chu et al.[22]

Table I. ^{1}H NMR Parameters of 2′- and 6′-Fluorocarbocyclic Guanosine Analogues[a,b]

	chemical shifts, δ								coupling constants, Hz				
	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-8	others	$J_{1',F}$	$J_{2',F}$	$J_{3',F}$	$J_{4',F}$	$J_{6',F}$	$J_{6,F}$
11a (C-AGF)	ca. 4.81 dm	4.80 dm	4.00 dm	1.9–2.4 m	3.4–3.7 m	1.9–2.4 m	7.75 d	4.80 t (5′-OH), 5.43 d (3′-OH), 6.5 bs (2-NH_2), 10.63 bs (NH)	ca. 25	50	21	–	–	2
11b	4.8–5.1 m	5.13 dm	4.04 dm	2.10 m	3.4–3.7 m	1.66, 2.32 m	7.86 s	4.7 bs (5′-OH), 5.16 d (3′-OH), 6.43 bs (2-NH_2), 10.59 bs (NH)	*	52	ca. 12	–	–	–
(–)-**11c**	4.93 dm	2.63 m, 2.09 m	4.02 m	2.16 dm	3.5–3.7 m	5.16 dm	7.75 d	4.75 t (5′-OH), 5.15 d (3′-OH), 6.50 bs (NH_2), 10.63 bs (NH)	32	–	–	ca. 32	55	2
(+)-**11d**	ca. 5.09 m	2.30 m, ca. 2.04 m	4.08 bs	ca. 2.11 d	3.5–3.7 m	ca. 5.13 dm	7.90 s	4.90 (5′-OH), 5.13 s (3′-OH), 6.50 bs (NH_2), 10.69 bs (NH)	*	–		23	55	–
5 (2′-CDG)	4.81 m	ca. 1.96 m, 2.09	4.05 m	ca. 1.96 m	3.3–3.6 m	1.58 m, 2.27 m	7.80 s	4.64 t (5′-OH), 4.76 d (3′-OH), 6.41 bs (NH_2), 10.54 bs (NH)	–	–	–	–	–	–
17 (AFG)	6.41 d	5.10 dt	4.36 dm	3.81 m	3.5–3.7 m		7.78 d	5.10 m (5′-OH), 5.95 bs (3′-OH), 6.66 bs (NH_2), 10.92 bs (NH)	16	52	18	–	–	2

[a] The spectra were recorded in Me_2SO-d_6 solutions. Signals are quoted as a (singlet), d (doublet), dd (double doublet), dt (double triplet), dm (double multiplet), bs (broad singlet), t (triplet), m (multiplet), and coupling constants reported are first order. (*) Coupling not established due to signal overlap. [b] The numbering of positions on the cyclopentane ring is the same as has been used previously; ref 1 and 16.

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Table II. Antiherpes Activity of 2'- and 6'-Fluorocarbocyclic Guanosine Analogues[a]

compd	ID_{50}, μg/mL HSV-1 strain KOS[b]	HSV-2 strain 186[b]	cytotoxicity[c]
11a (C-AFG)	0.006	0.05	>300
11b	2.35	70	>300
(-)-11c	15	155	>300
(+)-11d	0.16	0.77	>300
17 (AFG)	5	19	>100
5 (2'-CDG)	0.06	0.44	300
ACV	0.2	0.6	>300

[a] Tested in Vero cells by a plaque reduction assay. [b] Compound concentration required to inhibit HSV cytopathic effect by 50%. [c] Assessed by microscopic examination of cofluent Vero cell monolayers incubated with test compounds for 48 h at 37 °C.

Data from the 250-MHz proton NMR spectra of the fluorocarbocyclic guanines in Me_2SO-d_6 are listed in Table I. The position of the protons are designated by the numbering system used previously[1,16] for carbocyclic nucleosides.

An interesting feature of these spectra was the observation of a small fluorine coupling (ca. 2 Hz) to the 8-proton of the purine base in the β-fluoro compounds **11a**, **11c**, and **17**. This coupling was not observed in the α-fluoro anomers **11b**, **11d** and was thought to be a through-space coupling.[23] A similar coupling has been reported for *ara*-fluoroadenosine.[24]

As in the pyrimidine series,[16] differences are observed for the vicinal fluorine–proton couplings to H-1' and H-3' for the 2'β-fluoro carbocycle **11a** and its furanose counterpart **17** that suggest different conformational equilibria for the cyclopentane and furanose rings. However, for a given fluoro-substituted carbocyclic ring the vicinal fluorine–proton couplings observed for these guanine derivatives (Table I) are essentially the same as those for the corresponding pyrimidine analogues.[16] Additionally, in the 2'-*ara*-fluoro series, inspection of the spectra also indicates that the vicinal proton–proton couplings to H-3' are independent of the heterocyclic base, suggesting that the conformation of the carbocyclic ring in the highly potent (vide infra) guanine C-AFG (**11a**) is the same as in the virtually inactive pyrimidine C-FMAU.

Biological Results

The fluorocarbocyclic guanosine analogues were tested in vitro for selective inhibition of herpes simplex virus (HSV) replication in Vero cells (Table II). A modification of the plaque reduction assay of Lopez et al.[25] was used to evaluate the activity of these analogues against the cytopathic effect of herpes simplex virus type 1 (HSV-1, strain KOS) and herpes simplex virus type 2 (HSV-2, strain 186). The potency of each compound is given by the concentration required for 50% reduction of plaque formation (ID_{50}). The results are summarized in Table II. The nucleoside analogues ACV and 2'-CDG were used as positive controls in these experiments.

All the fluorocarbocyclic guanine derivatives (Table II) were more active against HSV-1 than HSV-2, which is the usual trend seen for anti-herpes nucleoside analogues. The 2'-*ara*-fluorocarbocyclic guanine derivative (C-AFG, **11a**) was outstandingly the most potent of the isomers **11a–d** against both HSV-1 and HSV-2, with ID_{50} values of 0.006 and 0.05 μg/mL, respectively. This 2'β-fluoro isomer **11a** was 1400-fold more potent than the corresponding 2'α-isomer **11b** against HSV-2 and thus followed a similar trend seen for FIAC and its ribo isomer.[26] The opposite trend was seen with the 6'-isomers where the β-isomer **11c** was 200-fold less active against HSV-2 than its corresponding α-isomer **11d**. Although the 6'α-isomer **11d** exhibited a similar potency to ACV against both HSV-1 and HSV-2, the 2'β-isomer **11a** was 1 order of magnitude more active than ACV or 2'-CDG against HSV-2 and 30-fold more potent than ACV against HSV-1.

In contrast to FMAU, which was 80-fold more active against HSV-1 than its carbocyclic analogue (C-FMAU),[16] the 2'-fluoroarabinofuranosyl guanine nucleoside (AFG, **17**) was 800-fold less active than its carbocyclic analogue (C-AFG, **11a**) against the same virus. Usually carbocyclic versions of antiviral nucleosides have been found to be less active than their furanose equivalents.[27] However, compound **11a**, which is the carbocyclic analogue of an unnatural nucleoside, exhibits greater anti-herpes activity than its furanose parent.

These results prompted us to perform animal studies with **11a** and **11d** (Table III). The fluorocarbocyclic guanine derivatives C-AFG (**11a**) and **11d** were administered subcutaneously twice a day for 4 days, starting 1 h after infecting mice intraperitoneally with HSV-1 and HSV-2 ((5–15) × LD_{50}). The survivors were counted 21 days after infection. In comparison to acyclovir (ACV) **11a** (C-AFG) is 2 orders of magnitude more potent against HSV-1 and 70-fold more potent against HSV-2 (Table III). C-AFG (**11a**) is more potent than its 6α-isomer **11d** against HSV-1 and has comparable activity to that of the latter against HSV-2. The 6α-isomer **11d** is more potent than ACV against HSV-1 and is approximately equipotent against both HSV-1 and HSV-2.

The 2'β-fluorocarbocyclic guanosine (**11a**, C-AFG) is one of the most potent purine nucleoside analogues so far reported against HSV-1 and HSV-2 in vitro and in vivo. Further studies are in progress to assess the therapeutic potential of this outstandingly potent class of antiherpes agents.

Experimental Section

^1H and ^{19}F NMR spectra were measured on a Bruker AM250 (250 MHz) spectrometer (by Dr. R. Fletton and his staff). Proton chemical shifts are expressed as δ values with reference to Me_4Si. For ^{19}F NMR, the peak positions were determined by reference to $CFCl_3$ as an internal standard. IR spectra were recorded on Perkin-Elmer 580F, 257, and 177 spectrophotometers and UV spectra were measured on a Perkin-Elmer Lambola 5 spectrometer (by Dr. R. Fletton and his staff). Microanalyses were performed by Miss P. J. McDonough and her staff. Analytical results indicated by element symbols are within ±0.4% of the theoretical

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Table III. In Vivo Efficacy of Fluorocarbocyclic Guanine Nucleosides against HSV-1 and HSV-2 in the Mouse Systemic Model

	HSV-1 strain A133			HSV-2 strain CT		
compound	dose,[a] mg/kg	survivors[c] no./total	ED_{50}, mg/kg per dose	dose,[a] mg/kg	survivors[c] no./total	ED_{50}, mg/kg per dose
11a (C-AFG)	3.1	10/10		6.25*	9/10	
	0.8	10/10		1.6	7/10	
	0.2	4/10	0.2	0.7	2/10	0.8
	0.05	1/10		0.1	2/10	
	0.012	1/10		0.025	0/10	
ACV	100*	8/10		100*	7/10	
	25	1/10	58	25	2/10	58
	6.25	0/10		6.25	0/10	
untreated		2/10		untreated	2/10	
(+)-**11d**	3.1	4/5		6.25*	5/5	
	1.6	2/5	0.97	3.1	4/5	1.1
	0.4	2/5		1.6	4/5	
	0.1	0/5		0.8	2/5	
				0.4	0/5	
ACV	100*	3/5		100*	2/5	
	25	2/5	~50	50	1/5	NDR[b]
	6.25	0/5		25	3/5	
untreated		1/5		untreated	0/5	

[a] Mice challenged ip; (5–15) × LD_{50}, dosed twice daily subcutaneously for 4 days starting 1 h after the challenge at the maximum tolerated dose(*) and serial dilutions thereof. [b] No dose response. [c] Survivors counted on day 21.

values. Where organic solvents are noted as part of the elemental analysis, they were seen in the ^1H NMR spectrum in proper amounts. Preparative and analytical HPLC were performed on a Gilson HPLC instrument using a Spherisorb ODS-2 reverse-phase silica column (by Dr. S. Laing and his staff). Mass spectral data MS were obtained (by Mr. R. Dennis and his staff) with either a Finnigan MAT 4600 single quadrupole instrument or a Finnigan MAT 8400 double-focusing sector instrument. The peaks listed are those arising from the molecular ion M^+, those attributable to loss of certain fragments (M^+ minus a fragment), and some other prominant peaks. Fragments containing the complete pyrimidine or purine moiety may be designated P plus an atom or group. Column chromatography was perfomred on Merck Kieselgel 60 (Art. 7734). Thin-layer chromatography TLC was performed on Merck silica gel 60F254 and developed plates were examined by UV light (254 nm). Optical rotations were measured with a Optical Activity AA-10 polarimeter. Solvents were dried and purified according to standard procedures.[29]

[1*R*-(1α,2β,3α,5β)]-3-Amino-2-fluoro-5-hydroxycyclopentanemethanol, Hydrochloride Salt (6d). To a solution of dibenzyl fluoroamine **12d**[16] (2.30 g, 6.98 mmol) in methanol (50 mL) and 1 N hydrochloric acid (8.7 mL, 8.7 mmol) was added 10% palladium on charcoal (0.95 g). The mixture was stirred vigorously under an atmosphere of hydrogen at room temperature for 3 h. The mixture was filtered and the catalyst washed with methanol (2 × 10 mL) and water (2 × 10 mL). The combined filtrate and washings was evaporated, ethanol (50 mL) was added, and the mixture was evaporated. The residue was left under vacuum for 16 h, taken up in ethanol (50 mL), and evaporated to give the title compound **6d** (1.35 g, 94%) as cream crystals: mp 155–158 °C; $[\alpha]^{22}_D$ = +12° (*c* 0.53, MeOH); IR (Nujol) ν_{max} 3700–2300, 2040, 1615, 1520 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.75–2.15 (3 H, m, 2H-2′, H-4′), 3.4–3.6 (3 H, m, 2H-5′, 5′-OH), 3.81 (1 H, m, $J_{HF} \simeq$ 20 Hz, H-1′), 3.96 (1 H, m, H-3′), 4.9 (1 H, dt, $J_{HF} \simeq$ 55 Hz, H-6′), 5.15 (1 H, d, 3′-OH), 8.45 (≃3 H, bs, 1′-NH_2·HCl). Anal. ($C_6H_{12}FNO_2$·HCl·0.3H_2O·0.1EtOH) C, H, N.

[1*R*-(1α,2α,3α,5β)]-3-Amino-2-fluoro-5-hydroxycyclopentanemethanol, Hydrochloride Salt (6c). Compound **12c**[16] was deprotected (for 2 h) as described for compound **12d** to give the title compound **6c** (96%) as a white crystalline solid: mp 147–150 °C; $[\alpha]^{22}_D$ = +65° (*c* 0.52, MeOH); IR (Nujol) ν_{max} 3680–2300, 1615 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.82–2.23 (3 H, m, 2H-2′,H-4′), 3.4–4.0 (4 H, m, 2H-5′,H-3′,H-1′), 4.82 (1 H, broad, 5′-OH), 5.04 ($^1/_2$H, m, $^1/_2$H-6′), 5.26 ($^1/_2$H, m, $^1/_2$H-6′, $J_{HF} \simeq$ 55), 5.14 (1 H, d, 3′-OH), 8.45 (≃3 H, broad, 1′-NH_2·HCl). Anal. ($C_6H_{12}FNO_2$·HCl·0.2H_2O) C, H, N, F.

(29) Perrin, D. D.; Armareg, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd ed.; Pergammon Press: New York, 1980.

(±)-(1α,2β,3α,4α)-4-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-3-fluoro-2-hydroxycyclopentanemethanol (7a). A solution of (±)-(1α,2β,3α,4α)-4-amino-3-fluoro-2-hydroxycyclopentanemethanol, hydrochloride salt[16] (**6a**) (25.83 g, 139.2 mmol), 2-amino-4,6-dichloropyrimidine (33.42 g, 203 mmol, 1.46 equiv) and triethylamine (133 mL) in 1-butanol (770 mL) was heated under reflux in an atmosphere of nitrogen for 24 h. The mixture was cooled, filtered, and concentrated in vacuo to give, after azeotroping with ethanol (2 × 100 mL), a sticky brown solid residue. The crude product was partitioned between water (250 mL) and dichloromethane (200 mL). The aqueous phase was separated and washed with dichloromethane (3 × 50 mL) and the combined organic extract was then back-extracted with water (3 × 50 mL). The combined aqueous phase was charcoaled, saturated with sodium chloride, and then repeatedly extracted with ethyl acetate (9 × 100 mL). The combined ethyl acetate extract was charcoaled, dried (Na_2SO_4), and evaporated to afford the title compound **7a** as an off-white solid (39 g, 88%, contains ca. 0.45 mol of ethyl acetate), mp 102–105 °C. Recrystallization of a sample from ethyl acetate afforded colorless prisms: mp 109–110 °C; IR (Nujol) ν_{max} 3620–3030, 1733, 1650, 1590 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.38, 2.06 (2 H, 2 m, 2H-6′), 1.83 (1 H, m, H-4′), 3.30–3.55 (2 H, m, 2H-5′), 3.86 (1 H, dt, J_{HF} = 22 Hz, H-3′), 4.68 (1 H, t, 5′-OH), 4.67 (1 H, dd, J_{HF} = 52 Hz, H-2′), 4.34–4.74 (1 H, m, H-1′), 5.23 (1 H, d, 3′-OH), 5.86 (1 H, s, H-5), 6.48 (2 H, bs, NH_2), 7.25 (1 H, bd, NH). Anal. ($C_{10}H_{14}ClFN_4O_2$·0.5EtOAc) C, H, N.

(±)-(1α,2β,3β,4α)-4-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-3-fluoro-2-hydroxycyclopentanemethanol (7b). Compound **6b** was reacted with 2-amino-4,6-dichloropyrimidine as described for **6a** but in refluxing ethanol for 24 h to give the title compound **7b** (67.7%) as a white hygroscopic foam, which was homogeneous by TLC (EtOAc, R_f = 0.21). This was used directly in the next stage. ^1H NMR (Me_2SO-d_6) δ 1.1–1.3, 2.21 (2 H, 2 m, 2H-6′), 1.95 (1 H, m, H-4′), 3.35–3.55 (2 H, m, 2H-5′), 3.88 (1 H, dm, J_{HF} = 17 Hz, H-3′), 4.2–4.5 (1 H, m, H-1′), 4.54 (1 H, dm, J_{HF} = 53 Hz, H-2′), 4.65 (1 H, t, 5′-OH), 4.98 (1 H, d, 3′-OH), 5.75 (1 H, s, H-5), 6.49 (2 H, bs, NH_2), 7.24 (1 H, d, NH); MS (EI, 70 eV), *m/e* 276 (M^+), 259 (M^+ - OH), 239 (M^+ - OH - HF), 225 (M^+ - CH_2OH - HF), 199, 182, 169, 157 (PNH + CH_2), 144 (PNH + H).

[1*R*-(1α,2α,3α,5β)]-3-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-2-fluoro-5-hydroxycyclopentanemethanol (7c). Compound **6c** was reacted with 2-amino-4,6-dichloropyrimidine as described for **6a** but for 51 h to give after crystallization from ethyl acetate the title compound **7c** (76%) as white prisms: mp 81–83 °C; $[\alpha]^{22}_D$ = −38° (*c* 0.55, MeOH); IR (Nujol) ν_{max} 3600–2540, 1658, 1632, 1582 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.78–2.14 (3 H, m, 2H-2′,H-4′), 3.44–3.66 (2 H, m, 2H-5′), 3.86 (1 H, m, H-3′), 4.56 (1 H, m, J_{HF} = 30 Hz, H-1′), 4.66 (1 H, t, 5′-OH), 4.92 (1 H, d, 3′-OH), 5.02 (1 H, m, H-6′), 5.85 (1 H, s, H-5), (2 H, bs, NH_2),

7.25 (1 H, d, NH). Anal. ($C_{10}H_{14}ClFN_4O_2 \cdot 0.5H_2O \cdot 0.5EtOAc$) C, H, N.

[1*R*-(1α,2β,3α,5β)]-3-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-2-fluoro-5-hydroxycyclopentanemethanol (7d). Compound **6d** was reacted with 2-amino-4,6-dichloropyrimidine as described for **6a** but in ethanol for 48 h to give the title compound **7d** (80%) as a white hygroscopic foam which was homogeneous by TLC (EtOAc–HOAc–H_2O, 3:2:2, R_f = 0.76). This was used directly in the next stage. $[\alpha]^{22}_D$ = +35° (*c* 0.52, MeOH); ^{1}H NMR (Me_2SO-d_6) δ 1.55–2.05 (3 H, 2 m, 2H-2′,H-4′), 3.4–3.6 (2 H, m, 2H-5′), 3.95 (1 H, m, H-3′), 4.68 (1 H, m, J_{HF} = 55 Hz, H-6′), 4.82 (1 H, bs, 5′-OH), 4.5–4.9 (1 H, m, H-1′), 4.94 (1 H, d, 3′-OH), 5.74 (1 H, s, H-5), 6.48 (2 H, bs, NH_2), 7.31 (1 H, bd, NH); MS (EI, 70 eV) *m/e* 276 (M^+), 259 (M^+ – OH), 239 (M^+ – OH – HF), 225 (M^+ – CH_2OH – HF), 171 (PNH + C_2H_4), 157 (PNH + CH_2), 144 (PNH + H).

(±)-(1α,2β,3α,4α)-4-[[2-Amino-6-chloro-5-[(4-chlorophenyl)azo]-4-pyrimidinyl]amino]-3-fluoro-2-hydroxycyclopentanemethanol (8a). A solution of sodium nitrite (17.93 g, 260 mmol, 1.02 equiv) in water (105 mL) was added dropwise over 20 min to a stirred solution of 4-chloroaniline (32.35 g, 254 mmol) in a mixture of water (325 mL) and concentrated hydrochloric acid (105 mL), while the internal temperature was kept below 5 °C. The resulting solution of 4-chlorobenzenediazonium chloride (254 mmol, 1.63 equiv) was immediately transferred to an ice-jacketed dropping funnel and added over 20 min to a stirred and cooled (8 °C) solution of (±)-(1α,2β,3α,4α)-4-[(2-amino-6-chloro-4-pyrimidinyl)amino]-3-fluoro-2-hydroxycyclopentanemethanol (**7a**) (49.3 g, containing ca. 0.45 mol of ethyl acetate, 156 mmol) and sodium acetate trihydrate (335 g) in a mixture of water and acetic acid (750 mL of each). The mixture was allowed to warm to room temperature, stirred mechanically overnight, and then cooled in ice. The yellow precipitate was collected and washed with ice–water (500 mL). The filtrate was set aside to yield a second crop while the first crop was washed again with ice–water (3 × 500 mL) and dried in vacuo over P_2O_5 to give a crystalline product (44.5 g, 69%). After 3 days at room temperature a second crop of slightly darker yellow crystals was collected, washed with ice-water (6 × 250 mL), and dried in vacuo over P_2O_5 to give a crystalline product (17.7 g, 27%). The two crops of crystals were combined and stirred with methanol (350 mL) at room temperature for 15 min. After cooling in ice, the crystals were collected, washed with ice-cold methanol (250 mL), and dried in vacuo at 70 °C to yield the title compound **8a** (57.8 g, 89%, purity ca. 95%, by microanalysis (CHN)). This material was homogeneous by TLC (EtOAc–EtOH, 10:1, R_f = 0.6), showed no evidence of impurities by NMR, and was used in the next step without further purification. Recrystallization of a portion (2 g) of the product from methanol (200 mL) afforded an analytical sample as fluffy yellow needles (0.95 g): mp 265–267 °C; IR (Nujol) ν_{max} 3500–3100, 1642, 1570 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.42, 2.29 (2 H, 2 m, 2H-6′), 1.88 (1 H, m, H-4′), 3.35–3.6 (2 H, m, 2H-5′), 3.95 (1 H, dt, J_{HF} = 22 Hz, H-3′), 4.58–4.82 (1 H, m, H-1′), 4.72 (1 H, t, 5′-OH), 4.82 (1 H, dd, J_{HF} = ca. 50 Hz, H-2′), 5.36 (1 H, d, 3′-OH), 7.65–7.8 (6 H, m, NH_2 and chlorophenyl protons), 10.61 (1 H, d, NH). Anal. ($C_{16}H_{17}Cl_2FN_6O_2$) C, H, N.

(±)-(1α,2β,3β,4α)-4-[[2-Amino-6-chloro-5-[(4-chlorophenyl)azo]-4-pyrimidinyl]amino]-3-fluoro-2-hydroxycyclopentanemethanol (8b). Compound **7b** was reacted with 4-chlorobenzenediazonium chloride as described for **7a** to give the title compound **8b** (71%) as a yellow hygroscopic solid, mp 236–239 °C, which was homogeneous by TLC (EtOAc–EtOH, 10:1, R_f = 0.74). This was used directly in the next stage. ^{1}H NMR (Me_2SO-d_6) δ 1.43, 2.38 (2 H, 2 m, 2H-6′), 2.03 (1 H, m, H-4′), 3.45–3.65 (2 H, 2 m, 2H-5′), 3.95 (1 H, dm, J_{HF} = 20 Hz, H-3′), 4.6–5.2 (4 H, m, H-1′, H-2′, 3′-OH, 5′-OH), 7.59 (2 H, d, chlorophenyl H-2, H-6), 7.70 (2 H, d, NH_2), 7.83 (2 H, d, chlorophenyl H-3, H-5), 10.29 (1 H, d, NH); MS (EI, 70 eV) *m/e* 414 (M^+), 288 (M^+ – NHC_6H_4Cl), 139 ($^+N_2C_6H_4Cl$), 127 ($NH_2C_6H_4Cl$), 111 (C_6H_4Cl).

[1*R*-(1α,2α,3α,5β)]-3-[[2-Amino-6-chloro-5-[(4-chlorophenyl)azo]-4-pyrimidinyl]amino]-2-fluoro-5-hydroxycyclopentanemethanol (8c). Compound **7c** was reacted with 4-chlorobenzenediazonium chloride as described for **7a** to give the title compound **8c** (76%) as a yellow crystalline solid: mp 257–260 °C; $[\alpha]^{22}_D$ = −38° (*c* 0.52, Me_2SO); IR (Nujol) ν_{max} 3355, 3200, 1662, 1575 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.88–2.28 (3 H, m, 2H-2′,H-4′), 3.53–3.73 (2 H, m, 2H-5′), 3.97 (1 H, m, H-3′), 4.74 (1 H, bs, 5′-OH), 4.67–4.97 (1 H, m, H-1′), 5.03 (1 H, bs, 3′-OH), 5.18 (1H, m, H-6′), 7.62 (2 H, s, chlorophenyl H-2,H-6), 7.5–7.9 (2 H, bs, NH_2), 7.70 (2 H, s, chlorophenyl H-3,H-5), 10.66 (1 H, bd, NH). Anal. ($C_{16}H_{17}FN_6O_2$) C, H, N.

[1*R*-(1α,2β,3α,5β)]-3-[[2-Amino-6-chloro-5-[(4-chlorophenyl)azo]-4-pyrimidinyl]amino]-2-fluoro-5-hydroxycyclopentanemethanol (8d). Compound **7d** was reacted with 4-chlorobenzenediazonium chloride as described for **7a** to give the title compound **8d** (77%) as yellow crystals: mp 238–240 °C; $[\alpha]^{22}_D$ = +41° (*c* 0.54, Me_2SO); IR (Nujol) 3540–3000, 1659, 1637, 1578 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.82–2.14 (3 H, 2 m, 2H-2′,H-4′), 3.45–3.70 (2 H, m, 2H-5′), 4.04 (1 H, m, H-3′), 4.7–5.05 (2 H, 2 m, H-1′,H-6′), 4.9 (1 H, t, 5′-OH), 5.08 (1 H, d, 3′-OH), 7.61 (2 H, d, chlorophenyl, H-2,H-6), 7.66–7.74 (2 H, bs, NH_2), 7.8 (2 H, d, chlorophenyl, H-3, H-5), 10.38 (1 H, bd, NH). Anal. ($C_{16}H_{17}Cl_2FN_6O_2 \cdot 0.2H_2O \cdot 0.7MeOH$) C, H, N.

(±)-(1α,2β,3α,4α)-4-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]-3-fluoro-2-hydroxycyclopentanemethanol (9a). Zinc powder (50 g) was added in one portion to a vigorously stirred mixture of (±)-(1α,2β,3α,4α)-4-[[(2-amino-6-chloro-5-[(4-chlorophenyl)azo]-4-pyrimidinyl]amino]-3-fluoro-2-hydroxycyclopentanemethanol (**8a**) (32.5 g, 78.3 mmol) and ammonium chloride (50 g) in a mixture of alumina-dried tetrahydrofuran (400 mL) and methanol (100 mL) under an atmosphere of nitrogen. The slightly exothermic reaction that ensued after ca. 10 min was controlled by brief immersion in an ice bath. After 30 min the mixture was filtered and the filtrate was evaporated to a syrup which was azeotroped with ethanol to give a brown solid. This crude product was transferred to a sublimation apparatus and most of the 4-chloroaniline was thereby removed in vacuo at 60 °C overnight. The residual brown solid was triturated with ice-cold ethanol (100 mL) and the beige solid was collected, washed successively with ice-cold ethanol (50 mL) and ice-cold ether (2 × 50 mL), and dried in vacuo to yield **9a** (17 g, 74.5%). This material was homogeneous by TLC (EtOAc–EtOH, 10:1, R_f = 0.3) and showed no evidence of impurities by NMR and was used in the next step without further purification. An analytical sample was obtained from a similar experiment as light brown crystals: mp 165–166 °C; IR (Nujol) ν_{max} 3440, 3380, 3300, 3190, 1620, 1585 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.52 (1 H, q, H-6′), 1.87 (1 H, m, H-4′), 2.10 (1 H, m, H-6′), 3.43, 3.53 (2 H, 2 m, 2H-5′), 3.89 (1 H, dd, J_{HF} = 22 Hz, H-3′), 4.54 (1 H, m, J_{HF} = 25 Hz, H-1), 4.73 (1 H, dd, J_{HF} = 52 Hz, H-2′), 4.02, 5.63 (4 H, 2 bs, 2-NH_2, 5-NH_2), 6.35 (1 H, d, NH). Anal. ($C_{10}H_{15}ClFN_5O_2$) C, H, N.

(±)-(1α,2β,3β,4α)-4-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]-3-fluoro-2-hydroxycyclopentanemethanol (9b). Zinc powder (300 mg) was added portionwise over 10 min to a stirred suspension of **8b** (415 mg, 1 mmol) in a mixture of water (10 mL), ethanol (10 mL), and acetic acid (0.78 mL) at 70 °C under an atmosphere of nitrogen. The mixture was stirred at 70 °C for 1 h, cooled and filtered and the filtrate evaporated to a brown syrup. This product was azeotroped with ethanol (3 × 20 mL), ethanol (5 mL) was added, and the zinc acetate (230 mg) was filtered off and washed with ethanol (5 mL). The combined filtrate and washing was evaporated, the residue was dissolved in water (10 mL), and the solution was washed with dichloromethane (3 × 5 mL) to remove the 4-chloroaniline. The aqueous phase was evaporated to a brown foam which was purified by column chromatography on silica gel. Elution with ethyl acetate/ethanol (10:1) afforded a pink crystalline residue (185 mg). Trituration with cold ethanol (3 mL) removed the color, and the fawn crystals were collected, washed with cold ethanol (2 mL) and ether (2 mL), and dried in vacuo to give **9b** (154 mg, 58%): mp 198–202 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.4, 2.24 (2 H, m, 2H-6′), 1.97 (1 H, m, H-4′), 3.35–3.7 (2 H, m, 2H-5′), 3.75–4.0 (3 H, m, 5-NH_2, H-3′), 4.35–4.8 (3 H, m, 5′-OH, H-2′, H-1′), 4.99 (1 H, d, 3′-OH), 5.70 (2 H, s, 2-NH_2), 6.52 (1 H, d, NH). This material was homogeneous by TLC (EtOAc–EtOH, 10:1, R_f = 0.36) and was used directly in the next stage.

[1*R*-(1α,2α,3α,5β)]-3-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]-2-fluoro-5-hydroxycyclopentanemethanol (9c). Compound **8c** was reduced as described for **8b** to give the title compound **9c** (54%) as an off-white solid: mp 192–195 °C; $[\alpha]^{22}_D$ = +45° (*c* 0.54, MeOH); IR (Nujol) ν_{max} 3040–3600, 1580 cm^{-1};

^{1}H NMR (Me_2SO-d_6) δ 1.8–2.2 (3 H, 2 m, 2H-2′, H-4′), 3.45–3.70 (2 H, m, 2H-5′), 3.89 (1 H, m, H-3′), 4.07 (2 H, bs, 5-NH_2), 4.60 (1 H, m, H-1′), 4.68 (1 H, bs, 5′-OH), 4.94 (1 H, bd, 3′-OH), 5.11 (1 H, m, J_{HF} = 55 Hz, H-6′), 5.71 (2 H, bs, 2-NH_2), 6.39 (1 H, bd, NH). Anal. ($C_{10}H_{15}ClFN_5O_2$) C, H, N.

[1*R*-(1α,2β,3α,5β)]-3-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]-2-fluoro-5-hydroxycyclopentanemethanol (9d). Compound **8d** was reduced as described for **8b** to give the title compound **9d** (78%) as a brown foam, which was homogeneous by TLC ($CHCl_3$–MeOH, 3:1, R_f = 0.45). This was used directly in the next stage. IR (Nujol) ν_{max} 3680–2300, 1615, 1573 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.7 (1 H, m, H-2′), 1.85–2.1 (2 H, m, H-2′, H-4′), 3.43–3.63 (2 H, m, 2H-5′), 3.93 (2 H, bs, 5-NH_2), 3.98 (1 H, m, H-3′), 4.65–5.0 (4 H, m, H-1′, H-6′, 3′-OH, 5′-OH), 5.67 (2 H, bs, 2-NH_2), 6.61 (1 H, bd, NH); MS (EI, 70 eV) *m/e* 291 (M^+), 271 (M^+ – HF), 240 (M^+ – CH_2OH – HF), 212, 226, 186 (PNH + C_2H_4), 170, 159 (PNH + H).

(±)-(1α,2β,3α,4α)-4-(2-Amino-6-chloro-9*H*-purin-9-yl)-3-fluoro-2-hydroxycyclopentanemethanol (10a). Method A. Concentrated hydrochloric acid (6.9 mL) was added dropwise to a stirred solution of (±)-(1α,2β,3α,4α)-4-[(2,5-diamino-6-chloro-4-pyrimidinyl)amino]-3-fluoro-2-hydroxycyclopentanemethanol (**9a**) (11 g, 37.7 mmol) in a mixture of dimethylformamide (100 mL) and redistilled triethyl orthoformate (200 mL). After 3.5 h the solvents were removed in vacuo at 30 °C, and the residual sticky foam was immediately dissolved in 0.6 N hydrochloric acid (220 mL). After 30 min at room temperature the mixture was filtered to remove a trace of insoluble material and the red filtrate was concentrated on a rotary evaporator at 30 °C for a further 45 min during which time ca. 75 mL of water was removed. The residual suspension was then adjusted to pH 7 with 6 N sodium hydroxide solution and cooled in ice. The product was collected, washed with ice–water (2 × 25 mL), and dried in vacuo over P_2O_5 to give **10a** as a cream solid (9.69 g, 85%). This material was homogeneous by TLC (EtOAc–EtOH, 10:1, R_f = 0.25), showed no evidence of impurities by NMR, and was used in the next step without further purification. An analytical sample was obtained from methanol as pale yellow crystals: mp 200–202 °C; UV (EtOH) λ_{max} 223.5 nm (ε 28900), 248.5 (6700), 310 (8300); IR (Nujol) ν_{max} 3600–3000, 1650, 1620, 1570 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.95–2.54 (3 H, 2 m, H-4′, 2H-6′), 3.45–3.65 (2 H, m, 2H-5′), 4.02 (1 H, dm, J_{HF} = 22 Hz, H-3′), 4.75–5.05 (3 H, m, H-1′, H-2′, 5′-OH), 5.48 (1 H, d, 3′-OH), 6.98 (2 H, s, NH_2), 8.21 (1 H, s, H-8). Anal. ($C_{11}H_{13}ClFN_5O_2$) C, H, N, Cl, F.

Method B.[30] A mixture of diamine **9a** (15.53 g, 53 mmol) and formamidine acetate (6.5 g, 62 mmol) in 1-butanol (350 mL) was stirred at reflux under an atmosphere of nitrogen for 2.5 h. The mixture was evaporated under reduced pressure and the residue dissolved in ethanol (100 mL) and evaporated to a tan solid. This solid was treated with 1 N hydrochloric acid (176 mL), the residual brown solution stirred at room temperature for 10 min and then cooled to 5 °C, and the pH adjusted to 7 with 6 N sodium hydroxide solution (44 mL). The aqueous suspension was left at 5 °C overnight and the solid filtered off, washed with ice–water (2 × 36 mL), and dried in vacuo over P_2O_5 at 40 °C for 24 h to give **10a** (13.73 g, 85%) as a cream solid, identical with the material prepared in method A.

[1*R*-(1α,2α,3α,5β)]-3-(2-Amino-6-chloro-9*H*-purin-9-yl)-2-fluoro-5-hydroxycyclopentanemethanol (10c). Compound **9c** was reacted with triethyl orthoformate as described for **9a** and gave after trituation with ethyl acetate the title compound **10c** (93%) as a pale yellow solid: mp 202–204 °C; $[\alpha]^{22}_D$ = −28° (*c* 0.56, Me_2SO); ^{1}H NMR (Me_2SO-d_6) δ 2.05–2.34 (2 H, m, H-2′, H-4′), 2.72 (1 H, m, H-2′), 3.50–3.72 (2 H, m, 2H-5′), 4.05 (1 H, m, H-3′), 4.77 (1 H, bs, 5′-OH), 5.05 (1 H, m, J_{HF} = 30 Hz, H-1′), 5.26 (1 H, m, J_{HF} = 55 Hz, H-6′), 5.15 (1 H, bs, 3′-OH), 6.99 (2 H, bs, 2-NH_2), 8.21 (1 H, s, H-8). Anal. ($C_{11}H_{13}ClFN_5O_2$·0.2H_2O·0.1EtOAc) C, H, N.

[1*R*-(1α,2β,3α,5β)]-3-(2-Amino-6-chloro-9*H*-purin-9-yl)-2-fluoro-5-hydroxycyclopentanemethanol (10d). Compound **9d** was reacted with triethyl orthoformate as described for **9a** and gave after trituation with ethyl acetate tht title compound **10d** (67%) as pale brown solid: mp 134–144 °C; $[\alpha]^{22}_D$ = +25° (*c* 0.51, Me_2SO); ^{1}H NMR (Me_2SO-d_6) δ 2.0–2.25 (2 H, 2 m, H-2′, H-4′), 2.4 (1 H, m, H-2′), 3.64 (2 H, m, 2H-5′), 4.10 (1 H, m, H-3′), 4.92 (1 H, m, 5′-OH), 5.04–5.36 (3 H, 3 m, H-1′, H-6′, 3′-OH), 6.98 (2 H, bs, 2-NH_2), 8.37 (1 H, s, H-8). Anal. ($C_{11}H_{13}ClFN_5O_2$·0.6H_2O·0.15EtOAc) C, H, N.

(±)-2-Amino-1,9-dihydro-9-[(1α,2α,3β,4α)-2-fluoro-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-6*H*-purin-6-one (11a). (±)-(1α,2β,3α,4α)-4-(2-Amino-6-chloro-9*H*-purin-9-yl)-3-fluoro-2-hydroxycyclopentanemethanol (**10a**) (2 g, 6.63 mmol) was heated under reflux in 1 N hydrochloric acid (150 mL) for 1 h. The solution was concentrated, under reduced pressure at 40 °C, to an orange syrup which was dissolved in ethanol (30 mL) and evaporated once more to give the crude hydrochloride salt as a yellow crystalline solid (2.1 g). This material was transferred to a sinter funnel and washed with ice-cold ethanol (1 × 10 mL, 2 × 5 mL) to leave a cream solid (1.86 g). This solid was suspended in water (20 mL) and the pH adjusted to 7 with 6 N sodium hydroxide solution. The resulting suspension was heated on a steam bath to give a yellow solution which on cooling deposited the title compound **11a** as fine needles. The crystals were collected, washed with ice–water (2 × 5 mL), and dried in vacuo over P_2O_5 at 100 °C for 4 h (1.48 g, 76%): HPLC (purity 97.6% H_2O–CH_3CN, 95:5, with flow rate 10 mL/min gave a retention time of 3.47 min). An analytical sample obtained after recrystallization from water: mp 273–275 °C; UV (H_2O) λ_{max} 252.5 nm (ε 12800), 267.5 (sh 9400). Anal. ($C_{11}H_{14}FN_5O_3$·H_2O) C, H, N.

2-Amino-1,9-dihydro-9-[[1*S*-(1α,2α,3α,4β)]-2-fluoro-4-hydroxy-3-(hydroxymethyl)cyclopentyl]-6*H*-purin-6-one (11c). Compound **10c** was hydrolyzed for 2 h as described for **10a** to give the title compound **11c** (58%) as a white solid: mp >300 °C; $[\alpha]^{22}_D$ = −35° (*c* 0.54, Me_2SO); UV (H_2O) λ_{max} 251.5 nm (ε 13200), 270.5 (sh 9400); IR (Nujol) ν_{max} 3700–2300, 1725, 1690, 1628 cm^{-1}. Anal. ($C_{11}H_{14}FN_5O_3$·0.5H_2O) C, H, N.

2-Amino-1,9-dihydro-9-[[1*S*-(1α,2β,3α,4β)]-2-fluoro-4-hydroxy-3-(hydroxymethyl)cyclopentyl]-6*H*-purin-6-one (11d). Compound **10d** was hydrolyzed for 2 h as described for **10a** to give the title compound **11d** (42%) as a pale fawn solid: mp 271–276 °C; $[\alpha]^{20}_D$ = +28° (*c* 0.089, Me_2SO); UV (H_2O) λ_{max} 254 nm (ε 13400), 272 (sh 9600); IR (Nujol) ν_{max} 3680–2200, 1740, 1698, 1643, 1585 cm^{-1}. Anal. ($C_{11}H_{14}FN_5O_3$·0.5H_2O) C, H, N.

(±)-2-Amino-1,9-dihydro-[(1α,2β,3β,4α)-2-fluoro-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-6*H*-purin-6-one (11b). Concentrated hydrochloric acid (0.1 mL) was added to a stirred solution of (±)-(1α,2β,3β,4α)-4-[(2,5-diamino-6-chloro-4-pyrimidinyl)amino]-3-fluoro-2-hydroxycyclopentanemethanol (**9b**) (150 mg, 0.51 mmol) in dimethylformamide (1.88 mL) and triethyl orthoformate (3.75 mL). After 3 h the mixture was evaporated, the residue was dissolved in 0.6 N hydrochloric acid (5 mL), and then after a further 1.5 h at room temperature the mixture was refluxed for 2 h. The solution was cooled to room temperature and the pH adjusted to 7 with 2 N sodium hydroxide. The mixture was evaporated and the residue purified by HPLC (elution with 4:1, MeCN–H_2O, 10 mL/min). The combined fractions containing the product was evaporated and the off-white residue was triturated with ethanol (3 mL), filtered, washed with ethanol (1 mL) and ether (2 mL), and dried in vacuo (at 90 °C over P_2O_5 for 0.5 h) to give the title compound **11b** (57 mg, 40%): mp 236–9 °C; UV (H_2O) λ_{max} 253 nm (ε 13400), 271 (sh 9600); IR (Nujol) ν_{max} 3660–2200, 1724, 1680, 1630, 1599 cm^{-1}. Anal. ($C_{11}H_{14}FN_5O_3$·0.4H_2O) C, H, N.

2-Amino-6-chloro-9-(2-deoxy-3,5-di-*O*-benzoyl-2-fluoro-β-D-arabinofuranosyl)purine (16) and Its α-Anomer (15). To a suspension of 2-amino-6-chloropurine (**14**) (603 mg, 3.52 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (15 mL) was added ammonium sulfate (165 mg) and the mixture refluxed for 1.5 h under an atmosphere of nitrogen. The clear solution was evaporated, to the white residue were added benzene (15 mL) and mercuric cyanide (1.76 g, 6.9 mmol), and the mixture was stirred and refluxed under an atmosphere of nitrogen for 30 min. To this mixture was added a solution of 2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide[18] (**13**) (1.36 g, 3.21 mmol) in benzene (15 mL) and refluxing continued for 3.4 h. The mixture was cooled and filtered and the precipitate washed well with dichloromethane. The combined filtrate and washings was evaporated, the oily residue was dissolved in dichloromethane (20 mL), and the solution was stirred with 1 M tetrabutylammonium fluoride in tetrahydrofuran (3.2 mL) for 15 min. The solution was evaporated and the residue was dissolved in ethyl acetate (200 mL), washed with water (2 × 100 mL), dried (Na_2SO_4), and

evaporated. The residue was purified by column chromatography on silica gel. Elution with ethyl acetate–light petroleum (40–60 °C) (1:1) gave first a colorless oil. Attempted crystallization of this compound from ethanol was unsuccessful and evaporation of the solvent afforded the α-anomer **15** (0.143 g, 87%) as a white foam: UV (EtOH) λ_{max} 224.4 nm (ε 45 300), 230 (sh 36 000), 244.8 (sh 14 900), 282.4 (4400), 309.6 (6900); IR ($CHBr_3$) 3525, 3425, 1730, 1265 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.71 (2 H, d, 2H-5′), 4.93 (1 H, q, H-4′), 5.08 (2 H, bs, NH_2), 5.77 (1 H, dd, J_{HF} = 17 Hz, H-3′), 6.10 (1 H, d, J_{HF} = 48 Hz, H-2′), 6.41 (1 H, d, J_{HF} = 14 Hz, H-1′), 7.3–7.7 (6 H, m, 6 phenyl protons), 7.72 (2 H, d, 2 ortho phenyl protons), 8.02 (1 H, s, H-8), 8.12 (2 H, d, 2 ortho phenyl protons). Anal. ($C_{24}H_{19}ClFN_5O_5$·0.25H_2O·EtOH) C, H, N; Cl: calcd, 6.31; found, 6.91.

This was followed by the β-anomer **16**, which was crystallized from dichloromethane–petroleum (40–60 °C) and dried in vacuo over P_2O_5 at room temperature for 48 h to give white fluffy hygroscopic crystals (543 mg, 35%): mp 84–87 °C; UV (EtOH) λ_{max} 222.4 nm (ε 43 700), 228 (sh 33 900), 244 (sh 11 100), 277.8 (3200), 309 (7900); IR ($CHBr_3$) ν_{max} 3520, 3415, 1720, 1286 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.58 (1 H, q, H-4′), 4.7–4.9 (2 H, m, 2H-5′), 5.13 (2 H, bs, NH_2), 5.34 (1 H, dd, J_{HF} = 50 Hz, H-2′), 5.77 (1 H, dd, J_{HF} = 17 Hz, H-3′), 6.46 (1 H, dd, J_{HF} = 22 Hz, H-1′), 7.40–7.80 (6 H, m, 6 phenyl protons), 8.04 (1 H, d, J_{HF} = 4 Hz, H-8), 8.09 (4 H, d, 4 ortho phenyl protons). Anal. ($C_{24}H_{19}ClFN_3O_5$·0.25CH_2Cl_2) C, H, N.

2-Amino-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1,9-dihydro-6*H*-purin-6-one (17). To a solution of **16** (0.458 g, 0.89 mmol) in dioxane (8 mL) was added 0.5 N sodium hydroxide solution (13.5 mL, 6.75 mmol) and the mixture heated at 100 °C for 1 h. The mixture was cooled to room temperature and 1 N hydrochloric acid added to adjust the solution to pH 7. The mixture was evaporated and the residue purified by preparative HPLC (elution 4:1 MeCN–H_2O, 10 mL/min) to give **17** (51 mg, 20%), which was crystallized from water–ethanol (9:1): mp 245–247 °C; $[\alpha]^{22}_D$ = +41.6° (c 0.3, MeOH); UV (MeOH) λ_{max} 253 nm (ε 14 300), 262.4 (sh, 11 400); IR (Nujol) ν_{max} 3510–3000, 1720, 1675, 1633, 1602 cm^{-1}. Anal. ($C_{10}H_{12}FN_5O_4$·0.2EtOH·H_2O) C, H, N. This data and the NMR parameters (Table I) are consistent with those previously reported for **17**.[21,22]

Antiviral Activity. (A) Anti-Herpes Activity. Anti-herpes activity was measured in a plaque-reduction assay.[25] Confluent monolayers of Vero cells in 24-well plates (NUNC) were infected with 30–40 plaque forming units of either HSV-1 (strain KOS) or HSV-2 (strain 186). The infected monolayers were incubated at 37 °C for 1 h and then overlaid with maintainence medium containing 0.75% (carboxymethyl)cellulose and various concentrations of test compound. The monolayers were incubated for a further 2 days at 37 °C, after which the cells were fixed and stained, the plaques were counted, and the concentration of compound causing 50% inhibition of plaque formation was calculated.

(B) Cytotoxicity. The cytotoxic effects of the test compounds on Vero cells were determined by examination of mock infected cell monolayers incubated with the compounds. Gross changes in cell staining, number, or morphology were noted and scored. Cytotoxic doses quoted were those that caused 50% of the cell monolayer to be affected.

(C) Animal Studies. Efficacy Tests. Female albino mice weighing 15–18 g (Charles River UK Ltd.) were inoculated intraperitoneally with 0.2 mL of virus suspension equivalent to (5–15) × LD_{50} dose. One hour after the challenge the mice were treated (0.2 mL sc) with solutions of test compounds in saline. Mice were treated 2× daily for 4 days, in groups of 5–10 animals, for a range of doses. The experiment was terminated on day 21 and the number of mice survivors in the various treatment groups was used to calculate the ED_{50} dose. The median effective dose (ED_{50}, mg/kg per dose) was calculated by logit transformation from the numbers of animals surviving at each dose level on day 21 as described by Litchfield and Wilcoxon.[31]

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Registry No. **6a**·HCl, 110312-76-0; **6b**·HCl, 111001-52-6; **6c**·HCl, 131043-35-1; **6d**·HCl, 131101-17-2; **7a**, 110289-10-6; **7b**, 131101-18-3; **7c**, 131043-36-2; **7d**, 131101-19-4; **8a**, 110289-09-3; **8b**, 131101-20-7; **8c**, 131043-37-3; **8d**, 131101-21-8; **9a**, 110289-11-7; **9b**, 131101-22-9; **9c**, 131043-38-4; **9d**, 131101-23-0; **10a**, 110289-21-9; **10c**, 131043-39-5; **10d**, 131101-24-1; **11a**, 110289-24-2; **11b**, 131101-25-2; **11c**, 131043-40-8; **11d**, 131101-26-3; **12c**, 123238-61-9; **12d**, 110567-26-5; **13**, 97614-44-3; **15**, 118373-60-7; **16**, 118373-61-8; **17**, 103884-98-6; 2-amino-6-chloropurine, 10310-21-1; 2-amino-4,6-dichloropyrimidine, 56-05-3; 4-chlorobenzenediazonium chloride, 2028-74-2.

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N-(5-Fluorobenzothiazol-2-yl)-2-guanidinothiazole-4-carboxamide. A Novel, Systemically Active Antitumor Agent Effective against 3LL Lewis Lung Carcinoma

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N-(5-Fluorobenzothiazol-2-yl)-2-guanidinothiazole-4-carboxamide (1) is a member of a series of amides found to substantially increase lifespan in mice bearing established micrometastatic 3LL Lewis lung carcinoma. Amide 1 is effective after either oral or intraperitoneal dosing in acute, subacute, or chronic regimens. 1 is well tolerated in this model with an excellent therapeutic index relative to the cytotoxic anticancer drug adriamycin.

The control of disseminated tumor growth by systemically active chemotherapeutants remains a major challenge for cancer chemotherapy despite decades of focused effort. Although there are some notable successes with certain forms of cancer, drug therapy has had only limited impact against the three major killers: carcinoma of the lung, breast, and colorectal system.

Considerable data support the usefulness of transplantable tumor systems for discovery of clinically active cancer therapeutants.[1] Among these certain tumor models have been shown to have higher predictivity for clinical success.[2] In our analysis of this literature we concluded that it was important to employ a model system for discovering anticancer drugs that embraced systemic drug treatment, i.e., administration of drug at a site distal to

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