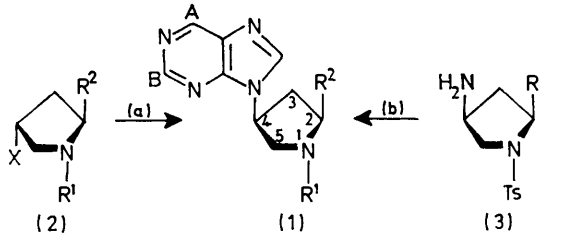
Unconventional Nucleotide Analogues. Part XIII.[1] (2*S*,4*S*)-2-Hydroxymethyl- and 2-Carboxy-4-(purin-9-yl)pyrrolidines

By **Frans M. Kaspersen** and **Upendra K. Pandit,*** Organic Chemistry Laboratory, University of Amsterdam, Nieuwe Achtergracht 129, Amsterdam, The Netherlands

The synthesis of (4S)-4-adeninyl, 4-guaninyl, and 4-hypoxanthinyl derivatives of L-proline and L-prolinol is described. Adeninyl- and hypoxanthinyl-prolinols inhibit the growth of BHK cells.

MODIFIED nucleosides and nucleotides have received widespread attention as potential substrates or inhibitors of nucleic acid biosynthesis. Several such nucleosides, both synthetic and naturally occurring, display important biological activity.[2] Molecular systems containing nucleoside bases connected to amino-acids or peptides would be useful as nucleopeptide models for biochemical studies, especially those involving interaction with proteins and nucleic acids. The synthesis of a variety of such analogues has been previously reported.[3] We now discuss two general approaches for the synthesis of purinyl-L-proline derivatives. The choice of L-proline as the amino-acid



	X	R^1	R^2		A	B	R^1	R^2	
a;	Br	Ts	CO_2Me	a;	Cl	H	Ts	CO_2Me	a; R = CH_2OH
b;	OTs	Ts	CO_2Me	b;	Cl	H	Z	CO_2Me	b; R = CO_2Bu^t
c;	Br	Z	CO_2Me	c;	NH_2	H	Ts	CO_2Me	
d;	OTs	Z	CO_2Me	d;	Cl	NH_2	Ts	CO_2Me	
e;	OTs	Z	CH_2OH	e;	Cl	NH_2	Z	CO_2Me	
f;	OTs	Z	CH_2OThp	f;	Cl	NH_2	Z	CH_2OThp	
				g;	OH	NH_2	H	CO_2H	
				h;	OH	NH_2	H	CH_2OH	
				i;	NH_2	H	H	CO_2H	

Z = benzyloxycarbonyl Thp = tetrahydropyranyl

SCHEME 1

unit was stimulated by the prospect of obtaining nucleopeptide models of known absolute configuration. Furthermore, the cyclic structure of proline bears a superficial analogy to the pentoses.

The synthesis of purine nucleoside analogues of general structure (1), from a suitable proline derivative, may be achieved by the two approaches, (a) and (b)' shown in Scheme 1. In route (a) the ' pseudo-glycoside ' linkage is formed by a coupling reaction between a purine and an appropriate pyrrolidine derivative; in route (b) the amino-group of a suitable 4-aminopyrrolidine is elaborated to form the heterocyclic base.

In view of the fact that procedure (a) leads to the nucleosides *via* a reaction between two synthons in one step, it was chosen in our initial studies. When sodium salts of 6-amino-, 6-chloro-, and 2-amino-6-chloropurines reacted with the proline derivatives (2a—d), substitution at N-9 was observed in all cases (Table 1). However, the substitution was accompanied by substantial amounts of elimination, leading to Δ^3-pyrrolines [corresponding to (2a—d)] and methyl pyrrole-2-carboxylate.[4] The ratios of substitution to elimination are presented in Table 1. In the case of the bromides

TABLE 1

Ratio of substitution to elimination in the reaction of purinyl anions with pyrrolidine derivatives (2a—d) (in dimethylformamide or dimethylacetamide for 6 days at 60 °C)

Purinyl anion	Pyrrolidine derivatives	*S* : *E* ratio	N-9 Substitution yield (%)
6-Chloro-	2a	0.14 : 1	5
6-Chloro-	2c	0.20 : 1	10
6-Chloro-	2b	1.00 : 1	7
6-Chloro-	2d	4.00 : 1	20
6-Amino-	2b	0.12 : 1	3
2-Amino-6-chloro	2b	0.33 : 1	10
2-Amino-6-chloro	2d	1.50 : 1	35

(2a and c), reaction led to C-4 epimers corresponding to the coupling products. These presumably arise from epimerization of the starting bromides, *via* a nucleophilic exchange reaction with the liberated bromide ions, prior to nucleophilic attack by the purinyl anion. The main product, in each case, was assigned the *cis*-stereochemistry (1a) in view of the presumed S_N2 nature of the substitution.[5] This was supported by the fact that the main product of the reaction of 6-chloropurinyl anion with the tosylate (7c) was identical with the purinyl

[1] Part XII, H. P. M. Thiellier, A. M. van der Burg, G. J. Koomen, and U. K. Pandit, *Heterocycles*, 1974, **2**, 457; Part XI, F. M. Kaspersen, H. Bieräugel, and U. K. Pandit, *ibid.*, p. 15.

[2] (*a*) Roy-Burman, ' Analogues of Nucleic Acid Components,' Springer-Verlag, Berlin, 1970; (*b*) R. J. Suhadolnik, ' Nucleoside Antibiotics,' Wiley–Interscience, New York, 1970.

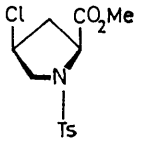
[3] (*a*) A. J. H. Nollet, C. M. Huting, and U. K. Pandit, *Tetrahedron*, 1969, **25**, 5971; (*b*) A. J. H. Nollet and U. K. Pandit, *ibid.*, pp. 5983, 5989; (*c*) H. de Koning and U. K. Pandit, *Rec. Trav. chim.*, 1971, **90**, 874; **91**, 1069.

[4] A. V. Robertson, J. E. Francis, and B. Witkop, *J. Amer. Chem. Soc.*, 1962, **84**, 1709.

[5] G. M. Fraser and H. M. R. Hoffmann, *J. Chem. Soc.* (*B*) 1967, 425.

derivative (10) obtained by construction of the heterocyclic base *via* the amino-group of (3b). In (3b) the amino- and the ester group are in a *cis* relationship. The C-4 epimer of (1a) derived from the reaction with (2a), was obtained as a crystalline product. The related C-4 epimer of (1b) was not isolated pure; however, it was identified from its spectral data (see Experimental section).

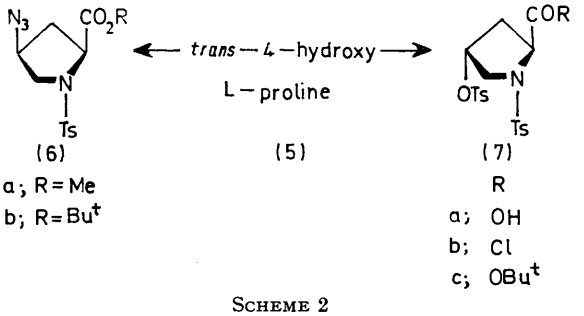
Although the 4-tosylate (2b) gave a higher degree of substitution, its reaction with 6-chloro- and 2-amino-6-chloro-purinyl anions gave, besides the expected coupling products, small amounts of the C-4 epimers of (1a and d). The C-4 epimer of (1d) was not isolated in a pure state. That these epimers were formed by reaction of the anions with the *cis*-4-chloro-L-prolinate ester (4) is



(4)

supported by the isolation of the latter compound from the reaction. Formation of (4) can be best accounted for in terms of substitution of (2b) by chloride ions, generated by an intermolecular reaction between the chloropurinyl anions. The observed influence of the leaving group upon the substitution–elimination ratio (Table 1) is similar to that reported in a recent study [5] and is consistent with a 'merged substitution–elimination mechanism.' Furthermore, subtle steric and electronic effects of the substituents in both the purine and pyrrolidine rings, upon the overall reaction pattern, are indicated by the results in Table 1.

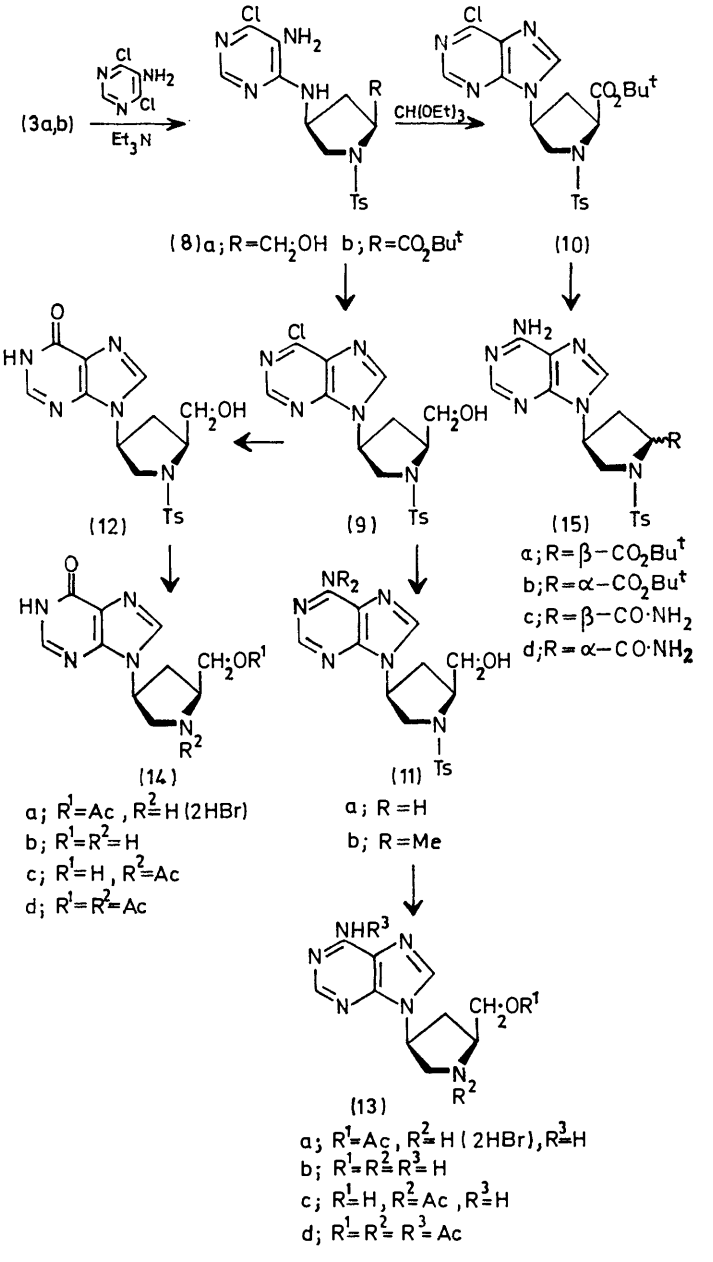
Treatment of (1e) with hydrogen bromide–acetic acid and aqueous hydrochloric acid followed by liberation of the free amino-acid resulted in the guanosine derivative (1g) (70%). For the synthesis of the corresponding



SCHEME 2

hydroxymethyl analogue (1h), the hydroxyproline derivative (2d) was subjected to the sequence (2d) ⟶ (2e) ⟶ (2f) and the tosylate (2f) was coupled with 2-amino-6-chloropurinyl anion to yield (1f). Removal of the protecting groups (HBr–HOAc and aqueous HCl) gave (1h) in 45% yield.

Although procedure (a) proved to be convenient for the preparation of guanosine analogues (1g and h), the



SCHEME 3

relatively low yields of the coupling products of 6-chloropurinyl anion with (2a—d) prompted us to examine route (b) for the synthesis of adenine and related purine derivatives. The required precursors (3a and b) were both obtained from *trans*-4-hydroxy-L-proline (5) (Scheme 2). The conversion of (5) into the azide (6a) is described in the literature.[6] Reduction of (6a) ($LiAlH_4$) yielded the amine (3a) in high yield. The amino-ester (3b) was obtained by the sequence (5) ⟶ (7a) ⟶

[6] R. H. Andreatta, V. Nair, A. V. Robertson, and W. R. J. Simpson, *Austral. J. Chem.*, 1967, **20**, 1493.

(7b) → (7c) → (6b) → (3b) in an overall yield of 29%.

Condensation of compounds (3a and b) with 5-amino-4,6-dichloropyrimidine gave the expected pyrimidine derivatives (8a and b), respectively. Treatment of (8a) with triethyl orthoformate–hydrochloric acid (room temperature) gave the 6-chloropurine derivative (9) in quantitative yield. Ring closure of (8b) leading to (10) was effected by heating with triethyl orthoformate (100 °C) in the presence of catalytic amounts of methanolic hydrogen chloride. Under these conditions, the t-butyl ester function was unaffected. Reaction of (9) with methanolic ammonia or dimethylamine–dioxan led to the corresponding adeninyl or 6-(dimethylamino)-purin-9-yl derivative (11a or b). Hydrolysis of (9) (HCl–H_2O) yielded the hypoxanthine derivative (12). Detosylation of (11a) and (12) (HBr–HOAc) gave the corresponding *O*-acyl dihydrobromides (13a) and (14a), which, upon basification (Dowex resin) released the free *O*-acetylated amines [which underwent a partial O → N transfer of the acyl group (13c) and (14c)] and the hydrolysis products (13b) and (14b). Acetylation of the mixtures of products, which were highly hygroscopic, gave the tri- and di-acetates (13d) and (14d) (Scheme 3).

Amination of (10) for 20 h gave the amines (15b and d) as the main products, besides the expected adeninyl derivative (15a) and the corresponding amide (15c). Presumably, racemization of the chiral centre (C-2) is effected by the deprotonation–protonation equilibrium created under the amination conditions. Under conditions of incomplete amination (2 h), isomerization to the 2α-compounds (15b and d) was suppressed. Deprotection of (15a), to yield (li), was conveniently effected (HBr–HOAc–PhOH).

Biological Activity.—Compounds (13d) and (14d) inhibited the growth of BHK cells. However, this effect was considerably less than that shown by the antibiotic puromycin. The results are described in Table 2.

TABLE 2

Inhibition of growth of BHK cells by compounds (13d) and (14d) and puromycin

Compound	Concentration (μg ml^{-1})	Inhibition (%)
(13d)	0.1	0
(13d)	10.0	35
(14d)	0.1	21
(14d)	10.0	50
Puromycin	0.1	62
Puromycin	10.0	100

EXPERIMENTAL

I.r. spectra were recorded on a Unicam SP 200 or a Perkin-Elmer 257 instrument and u.v. spectra on a Cary 14 recording spectrophotometer. N.m.r. spectra were obtained with a Varian A-60 or HA-100 spectrometer with tetramethylsilane as standard. Optical rotations were determined with a Carl-Zeiss L.E.P. polarimeter and o.r.d. curves were recorded on a Spectropol-1 instrument. Mass spectra were obtained on a Varian MAT 711 spectrometer by direct insertion.

trans-4-*Bromo*-N-*tosyl*-L-*proline Methyl Ester* (2a).—A solution of *cis*-4-hydroxy-*N*-tosyl-L-proline methyl ester (0.9 g, 0.003 mol), carbon tetrabromide (2.0 g, 0.006 mol), and triphenylphosphine (1.57 g, 0.006 mol) in tetrahydrofuran (25 ml) was stirred for 6 h, then evaporated. The residual oil was passed over a silica gel column, to provide pure bromide (0.86 g, 80%), $[\alpha]_D^{22}$ −88° (*c* 3.5 in $CHCl_3$); (lit.,[1] $[\alpha]_D$ −90.3°).

N-*Benzyloxycarbonyl*-trans-4-*bromo*-L-*proline Methyl Ester* (2c).—This compound was prepared from *N*-benzyloxycarbonyl-*cis*-4-hydroxy-L-proline methyl ester as described for (2a); yield 65% (oil); $\nu_{max.}$ ($CHCl_3$) 1 745 (ester C=O) and 1 705 cm^{-1} ($PhCH_2O{\cdot}CO$); δ ($CDCl_3$) 2.35—2.55 (m, 3-H), 3.40—4.00 (m, 5-H and Me), 4.10—4.60 (m, 2- and 4-H), 5.15br (s, $PhCH_2$), and 7.30 (s, Ph) (Found: C, 49.1; H, 4.8; Br, 23.3. $C_{14}H_{16}BrNO_4$ requires C, 49.1; H, 4.7; Br, 23.35%).

N-*Tosyl*-trans-4-*tosyloxy*-L-*proline Methyl Ester* (2b).—This was synthesized as described by Andreatta *et al.*[6]

N-*Benzyloxycarbonyl*-trans-4-*tosyloxy*-L-*prolinol* (2e).—This compound was prepared from *N*-benzyloxycarbonyl-*trans*-4-tosyloxy-L-proline methyl ester [7] (2d) by reduction with lithium borohydride as described by Fujita *et al.*;[8] yield 93% (oil); $\nu_{max.}$ ($CHCl_3$) 1 685 (C=O) and 1 175 cm^{-1} (SO_2); δ ($CDCl_3$) 1.75—2.35 (m, 3-H), 2.41 (s, $ArCH_3$), 3.25—4.30 (m, 2-H, 5-H, and $CH_2{\cdot}O$), 5.00 (m, 4-H), 5.10 (s, $PhCH_2$), and 7.32 (s) and 7.56 (2d) (Ph); $[\alpha]_D^{22}$ −22.7° (*c* 5.5 in $CHCl_3$) (Found: C, 59.4; H, 5.8; N, 3.4. $C_{20}H_{23}NO_6S$ requires C, 59.25; H, 5.7; N, 3.45%). This compound was converted into its tetrahydropyranyl ether (2f) by reaction with 2,3-dihydro-4*H*-pyran;[8] yield 75% (oil); the ether slowly decomposed at 20 °C; δ ($CDCl_3$) 1.30—1.70 and 3.20—4.65 (m, pyran).

Reactions of Pyrrolidine Derivatives (2a—d *and* f) *with Various Purinyl Anions.*—A solution of the pyrrolidine and the sodium salt of the purine (1.1 equiv.) in dimethylacetamide (5 ml per mmol) was heated to 60 °C with stirring for 144 h. The solvent was distilled off *in vacuo* and the residue in chloroform was placed on a silica gel column and eluted with chloroform. After elution of elimination products, unconverted pyrrolidine, and chlorides, the column was eluted with ethyl acetate to give the coupling products.

cis-4-(6-*Chloropurin*-9-*yl*)-N-*tosyl*-L-*proline methyl ester* (1a) had m.p. 166—168°; $\nu_{max.}$ (KBr) 1 740 (C=O), 1 590 and 1 555 (purine), and 1 155 cm^{-1} (SO_2); $\lambda_{max.}$ (EtOH) 265.5 nm (ε 10 000); δ ($CDCl_3$) 2.35—2.65 and 2.75—3.10 (m, 3-H), 2.45 (s, Me), 3.70 (s, Me), 3.84 (m, 5-H), 4.47 (m, 2-H), 5.20 (m, 4-H), 7.60 (2d, Ph), and 8.43 and 8.69 (2s, purine); $[\alpha]_D^{22}$ +12° (*c* 1.5 in $CHCl_3$) (Found: C, 49.7; H, 4.2; Cl, 8.1; N, 16.2. $C_{18}H_{18}ClN_4O_5S$ requires C, 49.6; H, 4.15; Cl, 8.15; N, 16.05%).

trans-4-(6-*Chloropurin*-9-*yl*)-N-*tosyl*-L-*proline methyl ester* had m.p. 163—167°; $\nu_{max.}$ (KBr) 1 740 (C=O), 1 590 and 1 555 (purine), and 1 160 cm^{-1} (SO_2); $\lambda_{max.}$ (EtOH) 266 (ε 7 500) and 233 nm (10 000); δ ($CDCl_3$) 2.4—3.1 (m, 3-H), 2.42 (s, Me), 3.76 (s, CO_2Me), 4.00 (m, 5-H), 5.32 (m, 4-H), 7.46 (2d, Ph), and 8.04 and 8.62 (2s, purine); $[\alpha]_D^{21}$ ($CHCl_3$) −40° (*c* 1.1%).

N-*Benzyloxycarbonyl*-cis-4-(6-*chloropurin*-9-*yl*)-L-*proline methyl ester* (1b) was an oil, $\nu_{max.}$ ($CHCl_3$) 1 745 (C=O),

[7] A. A. Patchett and B. Witkop, *J. Amer. Chem. Soc.*, 1957, **79**, 185.

[8] A. Fujita, A. Gottlieb, B. Peterkofsky, S. Udenfriend, and B. Witkop, *J. Amer. Chem. Soc.*, 1964, **86**, 4709.

1 705 ($PhCH_2$·O·CO), and 1 590 and 1 560 cm^{-1} (purine), $\lambda_{max.}$ (EtOH) 265 nm; δ ($CDCl_3$; at 56 °C) 2.40—2.75 and 2.80—3.20 (m, 3-H), 3.62 (s, Me), 3.90—4.40 (m, 5-H), 4.58 (m, 2-H), 5.00—5.40 (m, 4-H, $PhCH_2$), 7.32 (s, Ph), and 8.26 and 8.70 (2s, purine).

N-Benzyloxycarbonyl-*trans*-4-(6-chloropurin-9-yl)-L-proline methyl ester was contaminated with the *cis*-epimer; $\nu_{max.}$ ($CHCl_3$) 1 740 (C=O), 1 705 ($PhCH_2$·O·CO), and 1 590 and 1 560 cm^{-1} (purine); $\lambda_{max.}$ (EtOH) 265 nm; δ ($CDCl_3$; at 56 °C), 2.45—2.75 and 2.80—3.20 (m, 3-H), 3.70 (s, Me), 3.90—4.35 (m, 5-H), 4.69 (m, 2-H), 5.15 (AB system, $PhCH_2$), 5.30 (m, 4-H), 7.30 (s, Ph), and 8.10 and 8.66 (2s, purine).

cis-4-(*Adenin*-9-*yl*)-N-*tosyl*-L-*proline methyl ester* (1c) was a crystalline compound, $\nu_{max.}$ (KBr) 1 740 (C=O), 1 635 and 1 595 (purine), and 1 160 cm^{-1} (SO_2), $\lambda_{max.}$ (EtOH) 262 nm (ε 17 000); δ ($CDCl_3$) 2.30—3.10 (m, 3-H), 2.43 (s, Me), 3.73 (s, Me), 3.82 (m, 5-H), 4.44 (m, 2-H), 5.10 (m, 4-H), 5.55br (s, NH_2), 7.61 (2d, Ph), and 8.11 and 8.32 (2s, purine).

cis-4-(2-*Amino*-6-*chloropurin*-9-*yl*)-N-*tosyl*-L-*proline methyl ester* (1d) had m.p. 165.5—168.5°; $\nu_{max.}$ (KBr) 1 755 (C=O), 1 615, 1 560, and 1 505 (purine), and 1 160 cm^{-1} (SO_2); $\lambda_{max.}$ (MeOH) 312 nm (ε 7 800); δ ($CDCl_3$) 2.35—3.00 (m, 3-H), 2.44 (s, Me), 3.55—4.05 (m, 5-H), 3.71 (s, Me), 4.49 (m, 2-H), 4.93 (m, 4-H), 5.22br (s, NH_2), 7.60 (2d, Ph), and 7.95 (s, purine) (Found: C, 47.8; H, 4.4; Cl, 8.0; N, 18.6. $C_{18}H_{19}ClN_6O_4S$ requires C, 47.95; H, 4.25; Cl, 7.85; N, 18.65%).

cis-4-(2-*Amino*-6-*chloropurin*-9-*yl*)-N-*benzoyloxycarbonyl*-L-*proline methyl ester* (1e) had m.p. 175—177°; $\nu_{max.}$ (KBr) 1 735 (C=O), 1 700 ($PhCH_2$·O·CO), and 1 605, 1 555, and 1 505 cm^{-1} (purine); $\lambda_{max.}$ (EtOH) 313 (ε 8 350) and 248 nm (7 050); δ ($CDCl_3$) 2.35—3.10 (m, 3-H), 3.45—4.40 (m, Me and 5-H), 4.54 (m, 2-H), 5.00 (m, 4-H), 5.2 (m, $PhCH_2$ and NH_2), 7.33 (s, Ph), and 7.87 (s, purine); $[\alpha]_D^{22}$ +6° (*c* 1.5 in $CHCl_3$) (Found: C, 53.1; H, 4.4; Cl, 8.3; N, 19.4. $C_{19}H_{19}ClN_6O_4$ requires C, 52.95; H, 4.45; Cl, 8.25; N, 19.5%).

cis-4-(2-*Amino*-6-*chloropurin*-9-*yl*)-N-*benzyloxycarbonyl*-L-*prolinoltetrahydropyranyl ether* (1f) (yield 35%) had m.p. 75—105° (from ethyl acetate); $\nu_{max.}$ (KBr) 1 685 ($PhCH_2$·O·CO), 1 605, 1 555, and 1 505 cm^{-1} (purine); δ ($CDCl_3$) 1.35—1.85 (m, pyran), 2.35—2.85 (m, 3-H), 3.30—4.70 (m, pyran, 2-H, 5-H, and CH_2O), 4.97 (m, 4-H), 5.18 (s, $PhCH_2$), 5.23br (s, NH_2), 7.37 (s, Ph), and 7.75, 7.82, 7.93, and 7.97 (4s, purine) (Found: C, 56.6; H, 5.5; Cl, 7.4; N, 17.3. $C_{13}H_{27}ClN_6O_4$ requires C, 56.75; H, 5.6; Cl, 7.3; N, 17.25%).

cis-4-(*Guanin*-9-*yl*)-L-*proline* (1g).—*cis*-4-(2-Amino-6-chloropurin-9-yl)-*N*-benzyloxycarbonyl-L-proline methyl ester (1e) (180 mg) and phenol (5 mg) were dissolved in acetic acid (5 ml) and hydrogen bromide in acetic acid (5 ml) was added. After stirring for 3 h, dry ether (100 ml) was added and the precipitate was washed with ether. After drying the yellow crystals were dissolved in N-hydrochloric acid (10 ml) and refluxed for 3 h. The mixture was evaporated and the residue was put on a Dowex-50 (H^+) cation exchanger; this was washed with distilled water until the eluate was no longer acidic. Elution with 2% ammonia gave (1g), obtained as *needles* (80 mg, 70%), m.p. >345°; $\nu_{max.}$ (KBr) 1 685 (C=O purine) and 1 610 cm^{-1} (CO_2^-); $\lambda_{max.}$ (0.1N-HCl), 255 (ε 8 200) and 276 nm (11 500); δ (D_2O–HCl) 2.55—3.00 (m, 3-H), 4.05 (m, 5-H), 5.64 (m, 4-H), and 8.94 (s, purine); o.r.d. (0.1N-HCl) ϕ_{287} −2 300 (max.), ϕ_{280} 0 (Found: C, 45.3; H, 4.7; N, 32.0. $C_{10}H_{12}N_6O_3$ requires C, 45.45; H, 4.6; N, 31.8%).

cis-4-(*Guanin*-9-*yl*)-L-*prolinol* (1h).—This was synthesized from (1f) as described for (1g). Because after treatment with hydrogen bromide a black tarry mass was obtained, crystallization from methanol was carried out before reaction with hydrochloric acid; yield 45%, m.p. 165—175°; $\nu_{max.}$ (KBr) 1 675 (C=O purine), and 1 650 and 1 600 cm^{-1} (purine); $\lambda_{max.}$ (H_2O) 253 nm (ε 11 000), δ (D_2O–HCl) 2.10—2.60 and 2.80—3.15 (m, 3-H), 3.35—3.65 (m, 2-H, 5-H, and CH_2O), 5.45—5.75 (m, 4-H), and 8.99 (s, purine); o.r.d. (H_2O) ϕ_{249} 0; *m/e* 250 (M^+, 2%) and 152 (guanine — H^+, 100%).

cis-4-*Amino*-N-*tosyl*-L-*prolinol* (3a).—This *compound* was synthesized from *cis*-4-azido-*N*-tosyl-L-proline methyl ester [8] (6a) by reduction with lithium aluminium hydride; yield 2.85 g (95%), m.p. 119—120°; $\nu_{max.}$ (KBr) 3 355 and 3 260 (NH_2), and 1 155 cm^{-1} (SO_2); δ ($CDCl_3$) 1.50—1.80 and 1.85—2.25 (m, 3-H), 2.42 (s, Me), 2.72 (s, NH_2 and OH), 3.10—4.10 (m, 2-H, 4-H, 5-H, and CH_2O), and 7.53 (2d, Ph), $[\alpha]_D^{23}$ −49° (*c* 2.9 in $CHCl_3$) (Found: C, 53.5; H, 6.6; N, 10.4. $C_{12}H_{18}N_2O_3S$ requires C, 53.3; H, 6.7; N, 10.35%).

N-*Tosyl*-trans-4-*tosyloxy*-L-*proline t-Butyl Ester* (7c).—To oxalyl chloride (50 ml), *N*-tosyl-*trans*-4-tosyloxy-L-proline [6] (7a) (14 g) was added in 1 g portions. After stirring for 1 h the excess of oxalyl chloride was distilled off. The residue was suspended in dry benzene (250 ml) and slowly added to a solution of t-butyl alcohol (30 g) and pyridine (20 ml) in benzene (50 ml). After stirring for 20 h the mixture was evaporated and the residue passed over a silica gel column, which provided an *oil* (13 g, 82%); $\nu_{max.}$ ($CHCl_3$) 1 740 (C=O) and 1 160 cm^{-1} (SO_2); δ ($CDCl_3$) 1.42 (s, Bu^t), 2.05—2.50 (m, 3-H), 2.40 and 2.43 (2s, 2 × Me), 3.60 (m, 5-H), 4.14 (m, 2-H), 4.80—5.05 (m, 4-H), and 7.45 and 7.51 (4d, 2 × Ph), $[\alpha]_D^{24}$ −51° (*c* 5 in $CHCl_3$) (Found: C, 55.6; H, 6.0; N, 2.8; S, 13.2. $C_{23}H_{29}NO_7S_2$ requires C, 55.75; H, 5.9; N, 2.85; S, 12.95%).

cis-4-*Azido*-N-L-*proline t-Butyl Ester* (6b).—This *compound* was synthesized from (7c) with sodium azide as described by Andreatta *et al.*; [6] yield 78%, m.p. 125—126°; $\nu_{max.}$ (KBr) 2 100 (N_3), 1 750 (C=O), and 1 155 cm^{-1} (SO_2); δ ($CDCl_3$) 1.47 (s, Bu^t), 2.00—2.50 (m, 3-H), 2.42 (s, Me), 3.20—3.40 and 3.50—3.75 (m, 5-H), 4.05 (m, 4-H), 4.38 (m, 2-H), and 7.56 (2d, Ph); $[\alpha]_D^{25}$ −29.5° (*c* 2.8 in $CHCl_3$) (Found: C, 52.4; H, 5.9; N, 15.1; S, 8.8. $C_{16}H_{22}N_4O_4S$ requires C, 52.45; H, 6.05; N, 15.3; S, 8.75%).

cis-4-*Amino*-N-*tosyl*-L-*proline t-Butyl Ester* (3b).—To palladium–charcoal (5%; 250 mg) (pre-hydrogenated), suspended in methanol (10 ml), was added the azide (6b) (2.0 g). Catalytic reduction was carried out at room temperature and atmospheric pressure for 2 h. After filtration and evaporation the resulting oil was crystallized from benzene–light petroleum (b.p. 40—60°); yield 1.5 g (81%), m.p. 100—102°; $\nu_{max.}$ (KBr) 3 370 and 3 315 (NH_2), 1 740 (C=O), and 1 150 cm^{-1} (SO_2); δ ($CDCl_3$) 1.49 (s, Bu^t), 1.64 (s, NH_2), 1.65—1.95 and 2.05—2.45 (m, 3-H), 2.42 (s, Me), 3.10—3.55 (m, 2-H and 5-H), 4.12 (m, 4-H), and 7.54 (2d, Ph); $[\alpha]_D^{23}$ −69° (*c* 2 in $CHCl_3$) (Found: C, 56.6; H, 7.0; N, 8.3. $C_{16}H_{24}N_2O_2S$ requires C, 56.45; H, 7.1; N, 8.25%).

cis-4-(5-*Amino*-4-*chloropyrimidin*-6-*ylamino*)-N-*tosyl*-L-*prolinol* (8a).—A solution of the amine (3a) (0.6 g), 5-amino-4,6-dichloropyrimidine (0.4 g, 1.1 equiv.), and triethylamine (10 ml) in butan-1-ol (10 ml) was refluxed for 72 h, then evaporated. The residue was treated with boiling ethyl

acetate and the precipitate was filtered off. The filtrate was put on a silica gel column; elution with ethyl acetate gave the *pyrimidine* (8a) (0.7 g, 80%), m.p. 139—142.5° (from ethyl acetate); $\nu_{max.}$ (KBr) 1 625 and 1 580 (pyrimidine) and 1 155 cm^{-1} (SO_2); $\lambda_{max.}$ (MeOH) 297 (ε 10 000) and 264 nm (9 700); δ ($CDCl_3$) 1.75—2.50 (m, 3-H), 2.44 (s, Me), 3.08br (s, NH_2 and OH), 3.10—3.75 (m, 5-H and CH_2O), 4.15—4.60 (m, 2-H and 4-H), 6.85 (s, NH), 7.53 (2d, Ph), and 7.97 (s, pyrimidine), $[\alpha]_D^{23}$ +79.5° (*c* 3.3 in $CHCl_3$) (Found: C, 48.2; H, 5.0; Cl, 9.0; N, 17.15. $C_{16}H_{20}ClN_5O_3S$ requires C, 48.3; H, 5.05; Cl, 8.9; N, 17.6%).

cis-4-(5-*Amino*-4-*chloro*-*pyrimidin*-6-*ylamino*)-N-*tosyl*-L-*proline t-Butyl Ester* (8b).—This *compound* was synthesized from the amine (3b) and isolated as described for (8a); yield 50%, m.p. 186—188° (from ethyl acetate); $\nu_{max.}$ (KBr) 1 715 (C=O), 1 640 and 1 575 (pyrimidine), and 1 155 cm^{-1} (SO_2); $\lambda_{max.}$ (MeOH) 298 (ε 9 300) and 265 nm (7 200); δ ($CDCl_3$) 1.47 (s, Bu^t), 1.85—2.45 (m, 3-H), 2.43 (s, Me), 3.20—3.70 (m, 4-H, 5-H, and NH), 4.13 (m, 2H), 4.80br (s, NH), 6.35 (d, *J* 9 Hz, NH), 7.55 (2d, Ph), and 7.96 (s, pyrimidine); $[\alpha]_D^{23}$ +32° (*c* 2.7 in $CHCl_3$) (Found: C, 51.4; H, 5.7; Cl, 7.6; N, 14.9. $C_{20}H_{26}ClN_5O_4S$ requires C, 51.35; H, 5.6; Cl, 7.6; N, 14.95%).

cis-4-(6-*Chloropurin*-9-*yl*)-N-*tosyl*-L-*prolinol* (9).—A solution of the pyrimidine (8a) in triethyl orthoformate (5 ml), to which some hydrochloric acid (0.125 ml) was added, was stirred for 3 days. After removal of the triethyl orthoformate, the *purine* was isolated by chromatography over silica gel and aluminium oxide; yield 275 mg (75%), m.p. 174—175°, $\nu_{max.}$ ($CHCl_3$) 1 590 and 1 565 (purine) and 1 155 cm^{-1} (SO_2); $\lambda_{max.}$ (EtOH) 265 (ε 10 500) and 230 nm (16 000); δ ($CDCl_3$) 2.40—2.80 (m, 3-H), 2.46 (s, Me), 3.05br (s, OH), 3.65—4.35 (m, 2-H, 5-H, and CH_2O), 4.68 (m, 4-H), 7.59 (2d, Ph), and 8.41 and 8.69 (purine), $[\alpha]_D^{22}$ +91° (*c* 3.5 in $CHCl_3$) (Found: C, 50.1; H, 4.4; Cl, 8.8; N, 17.2. $C_{17}H_{18}ClN_5O_3S$ requires C, 50.05; H, 4.45; Cl, 8.7; N, 17.15%).

cis-4-(6-*Chloropurin*-9-*yl*)-N-*tosyl*-L-*proline t-Butyl Ester* (10).—A solution of the pyrimidine derivative (8b) (670 mg) in triethyl orthoformate (10 ml) was stirred for 6 h at 100 °C while at intervals of 20 min methanol saturated with hydrogen chloride (0.1 ml) was added. The mixture was evaporated and the residue crystallized from ethyl acetate to yield the *product* (10) (600 mg, 88%), m.p. 165—167°, $\nu_{max.}$ (KBr) 1 730 (C=O), 1 590 and 1 555 (purine), and 1 155 cm^{-1} (SO_2); $\lambda_{max.}$ (EtOH) 266 (ε 8 800) and 231 nm (12 800); δ ($CDCl_3$) 1.41 (s, Bu^t), 2.30—2.65 and 2.70—3.05 (m, 3-H), 2.44 (s, Me), 3.79 (m, 5-H), 4.29 (m, 2-H), 5.19 (m, 4-H), 7.60 (2d, Ph), and 8.49 and 8.70 (2s, purine); $[\alpha]_D^{25}$ +33.5° (*c* 2.0 in $CHCl_3$) (Found: C, 52.7; H, 5.2; Cl, 7.5; N, 14.7. $C_{21}H_{24}ClN_5O_4S$ requires C, 52.75; H, 5.05; Cl, 7.4; N, 14.65%).

Reaction of 6-chloropurinyl anion with (1c) according to the general procedure used for the coupling of purinyl anions with led to the formation of a product which was identical with (10).

cis-4-(*Adenin*-9-*yl*)-N-*tosyl*-L-*prolinol* (11a).—A solution of the purine (9) (190 mg) in methanol, saturated with ammonia at 0 °C (10 ml), was placed in a Carius tube. After 3 h at 100 °C the mixture was evaporated and the residue was put on a silica gel column and eluted with ethyl acetate–propan-2-ol (9 : 1) to give the *product* (105 mg, 60%), m.p. 155—165° (from acetone); $\nu_{max.}$ (KBr) 3 400 and 3 200 (NH_2), 1 635, 1 595, and 1 575 (purine), and 1 155 cm^{-1} (SO_2); $\lambda_{max.}$ (MeOH) 262 (ε 15 600) and 233 nm (13 800); δ [$(CD_3)_2SO$] 2.30—2.60 (m, 3-H), 2.43 (s, Me), 3.40—4.15 (m, 2-H, 5-H, and CH_2O), 4.20—4.55 (m, 4-H), 4.97 (OH), 7.20br (s, NH_2), 7.65 (2d, Ph), and 8.11 and 8.14 (s, purine); $[\alpha]_D^{23}$ +70° (*c* 1 in $CHCl_3$) (Found: 52.4; H, 5.1; N, 21.5. $C_{17}H_{20}N_6O_3S$ requires C, 52.55; H, 5.2; N, 21.65%).

cis-4-(6-*Dimethylaminopurin*-9-*yl*)-N-*tosyl*-L-*prolinol* (11b).—A solution of the purine derivative (9) (1.85 g) in dioxan (25 ml) was saturated with dimethylamine. After being stirred for 2 h the mixture was evaporated and the residue put on a silica gel column and eluted with ethyl acetate to give the *product* (1.65 g, 87%), m.p. 75—85° (from ethyl acetate ether); $\nu_{max.}$ (KBr) 1 595 and 1 555 (purine) and 1 155 cm (SO_2); $\lambda_{max.}$ (EtOH) 276 (ε 16 000) and 218 nm (19 500); δ ($CDCl_3$) 2.30—2.65 (m, 3-H), 2.45 (s, Me), 3.50 (s, NMe), 3.60—4.30 (m, 2-H, 5-H, and CH_2O), 4.63 (m, 4-H), 7.59 (2d, Ph), and 7.81 and 8.26 (s, purine); $[\alpha]_D^{23}$ +81.5° (*c* 2.5 in $CHCl_3$) (Found: C, 54.8; H, 5.9; N, 20.1. $C_{19}H_{24}N_6O_3S$ requires C, 54.8; H, 5.8; N, 20.2%).

cis-4-(*Hypoxanthin*-9-*yl*)-N-*tosyl*-L-*prolinol* (12).—A solution of the purine derivative (9) (500 mg) in N-hydrochloric acid (35 ml) was refluxed for 3 h. After neutralisation with sodium hydrogen carbonate the *precipitate* was filtered off, washed with water, and dried *in vacuo* (P_2O_5); yield 340 mg (74%), m.p. 275—280° (from methanol); $\nu_{max.}$ (KBr) 3 380 (NH), 1 700 (C=O), 1 600 and 1 545 (purine), and 1 155 cm^{-1} (SO_2); $\lambda_{max.}$ (MeOH) 235 (ε 18 500), 250, and 270sh nm, δ [$(CD_3)_2SO$] 2.25—2.60 (m, 3-H), 2.44 (s, Me), 3.10—4.10 (m, 2-H, 5-H, and CH_2O), 4.32 (m, 4-H), 7.66 (2d, Ph), and 8.04 and 8.12 (2s, purine) (Found: C, 52.6; H, 4.8; N, 17.9. $C_{17}H_{19}N_5O_4S$ requires C, 52.45; H, 4.9; N, 18.0%).

cis-4-(*Adenin*-9-*yl*)-N-*tosyl*-L-*proline t-Butyl Ester* (15a).—This *compound* was prepared from the chloropurine (10) by reaction with ammonia at 100 °C for 2 h as described for (11a); yield 95 mg (50%), m.p. 120—140°; $\nu_{max.}$ (KBr) 3 320 and 3 160 (NH_2), 1 730 (C=O), 1 635, 1 590, and 1 570 (purine), and 1 155 cm^{-1} (SO_2); $\lambda_{max.}$ (MeOH) 262 (ε 15 200) and 233 nm (14 000); δ [$(CD_3)_2SO$] 1.35 (s, Bu^t), 2.30—3.00 (m, 3-H), 2.42 (s, Me), 3.60—4.00 (m, 5-H), 4.20 (m, 2-H), 4.83 (m, 4-H), 7.20br (s, NH_2), 7.63 (2d, Ph), and 8.09 and 8.13 (s, purine) (Found: C, 55.0; H, 5.8; N, 18.2. $C_{21}H_{26}N_6O_4S$ requires C, 55.0; H, 5.7; N, 18.35%).

Detosylation.—Detosylation of the purines (9), (12), and (15) was carried out according Weisblat [9] as described by Andreatta.[6] In the case of the L-prolinol derivatives, the products were treated with an excess of acetic anhydride and potassium carbonate in 1,2-dimethoxyethane for 4 h and, after evaporation, chromatographed on a silica gel column.

cis-4-(6-*Acetamidopurin*-9-*yl*)-N-*acetyl*-L-*prolinol acetate* (13d) showed $\nu_{max.}$ (KBr) 1 730 (OAc), 1 695 (NHAc), 1 630 (NAc), and 1 605 and 1 580 cm^{-1} (purine); $\lambda_{max.}$ (EtOH) 274 nm (ε 13 500); δ [$(CD_3)_2SO$] 2.05br (s, 2 × Me), 2.29 (s, Me), 2.45—2.95 (m, 3-H), 3.30—4.50 (m, 2-H, 5-H, and CH_2O), 4.90—5.30 (m, 4-H), and 8.59 and 8.67 (2s, purine).

N-*Acetyl*-cis-4-(*hypoxanthin*-9-*yl*)-L-*prolinol acetate* (14d) had m.p. 105—110° (from ethanol–ether); $\nu_{max.}$ (KBr) 1 730 (OAc), 1 685 (purine C=O), 1 635 (NAc), and 1 580, 1 540, and 1 510 cm^{-1} (purine); $\lambda_{max.}$ (EtOH) 2.51 (ε

[9] D. I. Weisblat, B. J. Magerlein, and D. R. Meyers, *J. Amer. Chem. Soc.*, 1953, **75**, 3630.

10 000), 2.47 (10 200), and 2.70sh nm; δ [$(CD_3)_2SO$] 2.02br (s, 2 × Me), 2.10—2.70 (m, 3-H), 3.70—4.40 (m, 2-H, 5-H, and CH_2O), 4.80—5.15 (m, 4-H), and 8.05 and 8.21 (2s, purine); $[\alpha]_D^{18}$ *ca.* 0° (*c* 2.5 in EtOH); o.r.d. (EtOH) ϕ_{240} +4 700 (max.), ϕ_{220} 0 (Found: C, 52.5; H, 5.4; N, 21.7. $C_{14}H_{17}N_5O_4$ requires C, 52.65; H, 5.35; N, 21.95%).

cis-4-(*Adenin-9-yl*)-L-*proline* (li) was hygroscopic material, $\nu_{max.}$ (KBr) 2 000—2 750 (salt bands) and 1 595—1 560 cm^{-1} (purine and CO_2^-); $\lambda_{max.}$ (H_2O) 2.59 nm (ε 12 000); δ (D_2O) 2.25—2.65 and 2.90—3.25 (m, 3-H), 3.87 (m, 5-H), 4.37 (m, 2-H), 5.20—5.40 (m, 4-H), and 8.01 and 8.04 (2s, purine); o.r.d. (H_2O) ϕ_{273} −2 100 (max.), ϕ_{261} 0.

We thank Dr. L. A. Smets, Cancer Institute of The Netherlands, Amsterdam, for the biological tests.

[5/045 *Received*, 8*th January*, 1975]