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UNCONVENTIONAL NUCLEOTIDE ANALOGUES—I

N_9-PURINYL α-AMINO ACIDS

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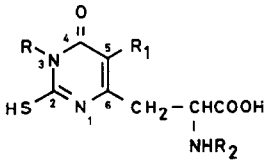
Abstract—Reaction of 6-chloropurine (**3**), 2,6-dichloropurine (**4**) and 2-amino-6-chloropurine (**5**) with t-butyl α-benzyloxycarbonylamido-γ-bromobutyrate (**6b**) gave high yields of the corresponding N_9-alkylated products (**10a, 11a, 12a**). Suitable transformations of the latter led to the formation of 4-(N_9-purinyl)-α-aminobutyric acids corresponding to adenine (**16**), hypoxanthine (**19**), guanine (**22**) and xanthine (**23**).

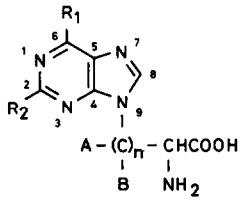
THE implication of purines and pyrimidines in nucleic acids and nucleotides imparts a position of unique biological significance to derivatives based on these heterocyclic systems. A variety of functionalized purines and pyrimidines have been consequently synthesized and examined for their possible involvement in a wide spectrum of biochemical and biological processes.[3,4] Pyrimidine and purine derivatives corresponding to bases found in the natural nucleotides and substituted at N_1 and N_9 positions, respectively, are of special interest in this connection. Since in the natural substances the heterocyclic systems are attached via these very positions to sugar-phosphate moieties, the aforementioned class of compounds may be regarded as nucleotide analogues in which unconventional structural units replace the sugar-phosphate entity.

Once departing from the natural molecular structure, the choice of potential replacements (for the sugar-phosphate moiety) which becomes available, is very extensive. However, requirement of transport-facility in biological systems suggests that the most suitable analogues would be those which are based upon *natural* carriers. In view of this and other relevant considerations* we have selected amino acids and peptides as the initial candidates for sugarphosphate replacement. Our confidence in the potentiality of such *unconventional nucleotide analogues* was supported by the known anti-cancer activity of nitrogen mustards[5] incorporating amino acids and peptides and, by the recently reported inhibition of protein synthesis by intermediacy of pyrimidyl amino acids and peptides[6] (**1**). In the latter compounds it is to be noted that the amino acid residues are attached to the C_6-position of the pyrimidine ring. Interest in the abovementioned class of analogues was also derived from the fact that systems containing the uracil base attached to an amino acid[7] or a peptide[8] are found to be of natural occurrence.

In this communication we wish to present a facile route for the synthesis of certain N_9-substituted purinyl α-amino acids (**2**). Two principle approaches for the construc-

* Coupling of such analogues via amide linkages would afford polynucleotide models in which the bases are strung along a polypeptide backbone. The synthesis and study of such models is currently under investigation in this laboratory.

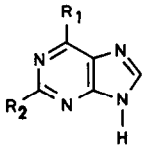




1 $R_2 = H$ or $-CCHNH_2$ (with R_3, O)

2

tion of compounds of general structure **2** may be visualized: (a) the synthesis of the purine base upon a suitable amino acid derivative and (b) coupling of an appropriate purine system with a molecular unit which can be elaborated to the desired amino acid. While the first approach has been successfully used in our laboratory for the synthesis of several N_1-substituted pyrimidyl amino acids and peptides, the latter synthetic concept has been demonstrated to provide access to both purinyl and pyrimidyl analogues.[9]



$X-(CH_2)_n-CHCOOR$ (NHZ)

$CH_2=CCOOCH_3$ (NHZ)

3: $R_1 = Cl$ $R_2 = H$

4: $R_1 = Cl$ $R_2 = Cl$

5: $R_1 = Cl$ $R_2 = NH_2$

6a: $R = CH_3$ $n = 1$ $X = OTs$ $Z =$ benzyloxycarbonyl

6b: $R = tBu$ $n = 2$ $X = Br$ $Z =$ benzyloxycarbonyl

7: Z = benzyloxycarbonyl or acetyl

In order to synthesize α-amino acid derivatives corresponding to the conventional purine bases, 6-chloro- (**3**), 2,6-dichloro- (**4**) and 2-amino-6-chloropurine (**5**) were recognized as the appropriate heterocyclic systems which could, under suitable reaction conditions, undergo alkylation at N_9.[10] Further, the chloro substituent(s) could be readily transformed into the required function(s) (OH or NH_2) at a pertinent stage of the synthetic scheme. The precise choice of the *potential* amino acid component is determined by the nature of the desired residue; an ideal system would be the polyfunctional unit **6**, which contains the amino acid in its masked form, and carries a group of capable of nucleophilic displacement. Bearing in mind the transformations that have to be eventually affected in the purine part, a careful selection of the protecting groups would have to be made.

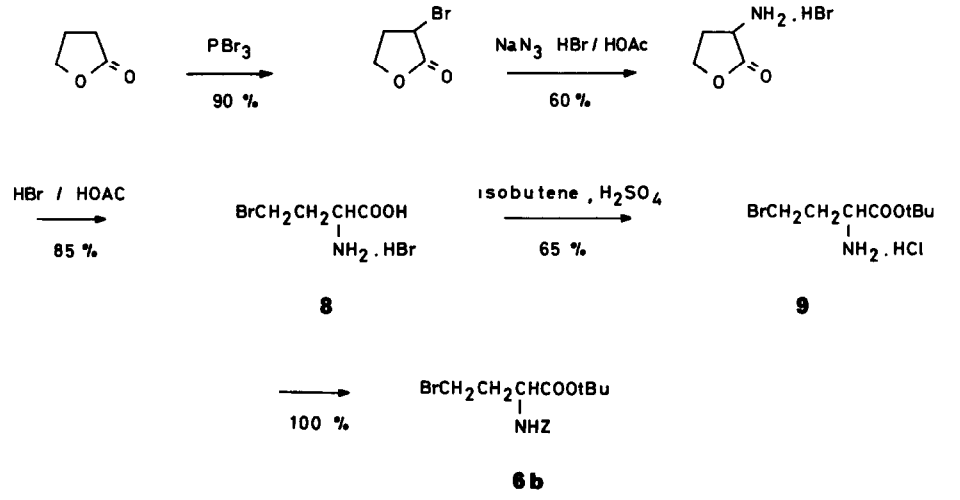
Preliminary experiments on the reaction of 6-chloropurine with **6a** indicated that the N-alkylated product constituted an insignificantly minor portion of the reaction

mixture;* instead, not unexpectedly,[11] an elimination reaction occurred, resulting in the formation of ester **7** and its polymerization products.

In view of the facile loss of tosylic acid from **6a**, its next higher homologue **6b**—which is structurally insulated against the aforementioned base-catalyzed elimination process—was chosen as a candidate for attachment of an amino acid residue to the purine system.

The synthesis of **6b** was achieved in excellent yields via the sequence of reactions starting from butyrolactone, described in Chart I. Amino acid **8** has been described earlier,[12c] however, practical procedures for obtaining the substance in large quantities were developed. The order of protecting the amino acid functions in the sequence is important, since attempts to block the amino group prior to esterification resulted in rapid lactonization via nucleophilic attack of the carboxylate ion on the γ-carbon.

CHART I

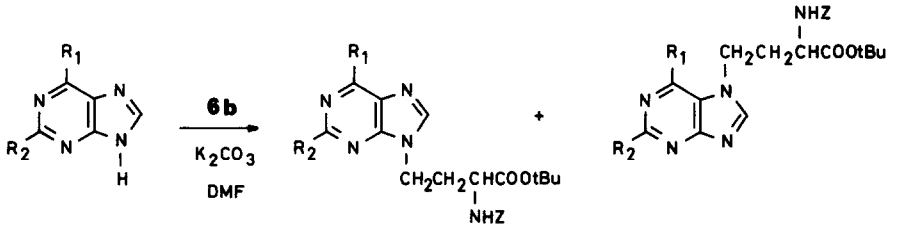


The somewhat low yield of the esterification step (**8** → **9**) is related to the intramolecular amination reaction—leading to azetidine formation[13]—which proceeds readily in the free amino ester that is produced during the isolation of the product. The yield of **9** is consequently dependent upon the manner of working-up of the reaction mixture; best results being obtained when the amino ester is rapidly trapped as its stable hydrochloride salt (Experimental). It might be pointed out that the t-butyl and the benzyloxycarbonyl groups are particularly suitable protecting groups for the projected synthetic plan. Both can be removed by treatment with HBr/HOAc under conditions where the purine system is stable. Alternately, selective unmasking of the protected functions is also possible, the benzyloxycarbonyl being removed by hydrogenolysis (H_2/Pd-C),[14] and the tertiary butyl group by treatment with trifluoroacetic acid;[15] these again being reactions which leave the heterocyclic system

* This product may represent an SN_2 alkylation of the purine or, alternately result from a Michael addition of the heterocyclic system to 7.

unaffected. Finally, the protecting groups ideally permit the transformations visualized in the functionality of the purine moiety.

CHART II



3: $R_1 = Cl$ $R_2 = H$ **10a:** $R_1 = Cl$ $R_2 = H$ **10b:** $R_1 = Cl$ $R_2 = H$

4: $R_1 = Cl$ $R_2 = Cl$ **11a:** $R_1 = Cl$ $R_2 = Cl$ **11b:** $R_1 = Cl$ $R_2 = Cl$

5: $R_1 = Cl$ $R_2 = NH_2$ **12a:** $R_1 = Cl$ $R_2 = NH_2$ **12b:** $R_1 = Cl$ $R_2 = NH_2$

YIELDS AND UV MAXIMA of N_7 and N_9 ALKYLATED PURINES

	N_9-Alkylation			N_7-Alkylation	
	Yield (%)	$\lambda_{max.}^{ethanol}$(nm)		Yield (%)	$\lambda_{max}^{ethanol}$(nm)
10a	70	266	**10b**	11	270
11a	33	276	**11b**	13	278*
12a	88	311, 247, 223	**12b**	2	324

* Slightly impure.

Reaction of **3**, **4** and **5** with **6b** in DMF, in the presence of one equivalent of K_2CO_3, resulted, in each case, in a mixture of N_9 and N_7 alkylated products (**10a, b**; **11a, b**; **12a, b**). The isomeric mixtures were separated and the products identified by their analytical and spectral data. Comparison of the UV spectra with those of suitable model compounds[16] were of particular value in distinguishing and recognizing the positional isomers (Chart II). Inspection of Chart II indicates that the ratio of N_9 to N_7 products (isolated) is significantly influenced by the nature and location of substituents in the purine nucleus. The fact is clearly emphasized in the case of alkylation of **3** and **5** (under identical conditions) where the isolated products account for 80–90% of the reaction. There is obviously no simple relationship between the stereoelectronic properties of the substituents and the alkylation pattern of the various purine derivatives. An attempt to derive such a correlation, on the basis of the charge density at N_7 and N_9 (HMO calculation) in the anions of the relevant systems, also led to no success; although the data were consistent with the observed preference for electrophilic substitution at N_9.[17] Apparently, and not unexpectedly, a variety of complex and not easily distinguishable factors play an imperative role in determining the site of the reaction.

The large N_9 to N_7 substitution ratio in the case of 2-amino-6-chloropurine is sufficiently impressive to deserve comment. While no evidence bearing on this point

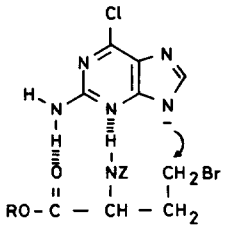


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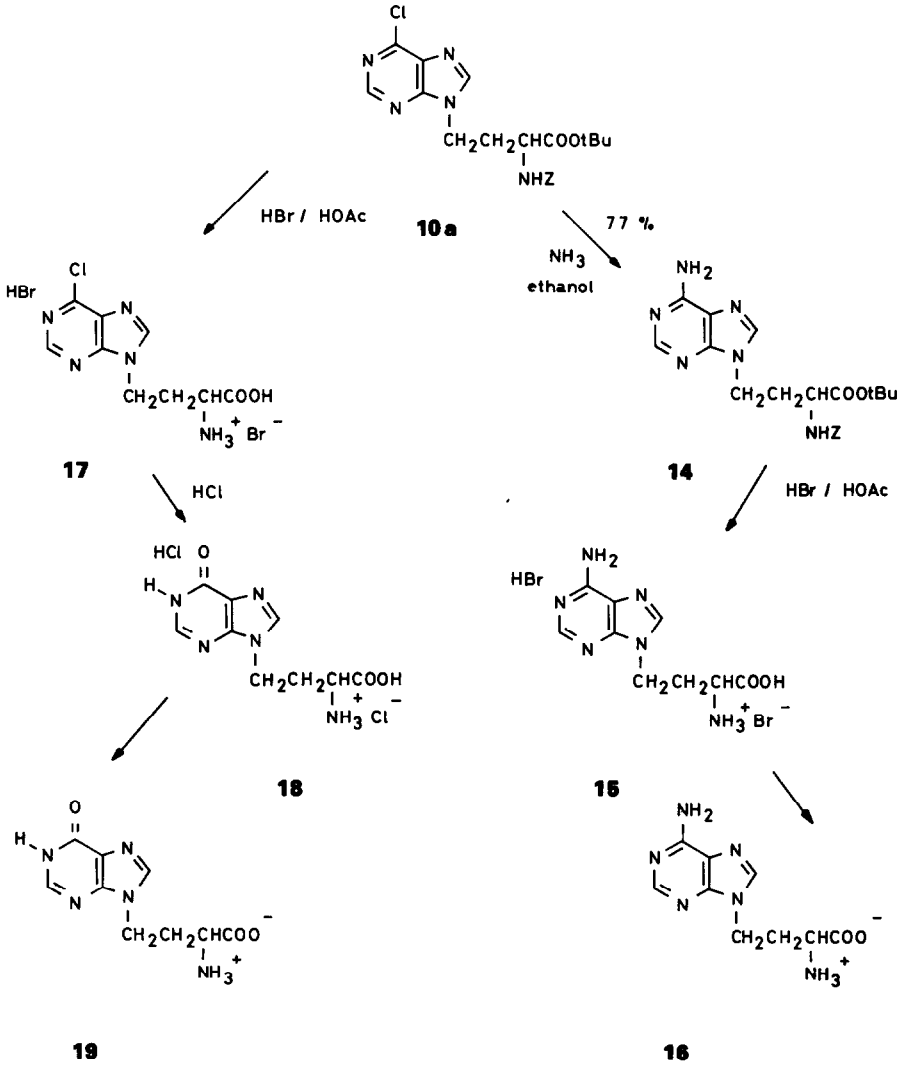
CHART III

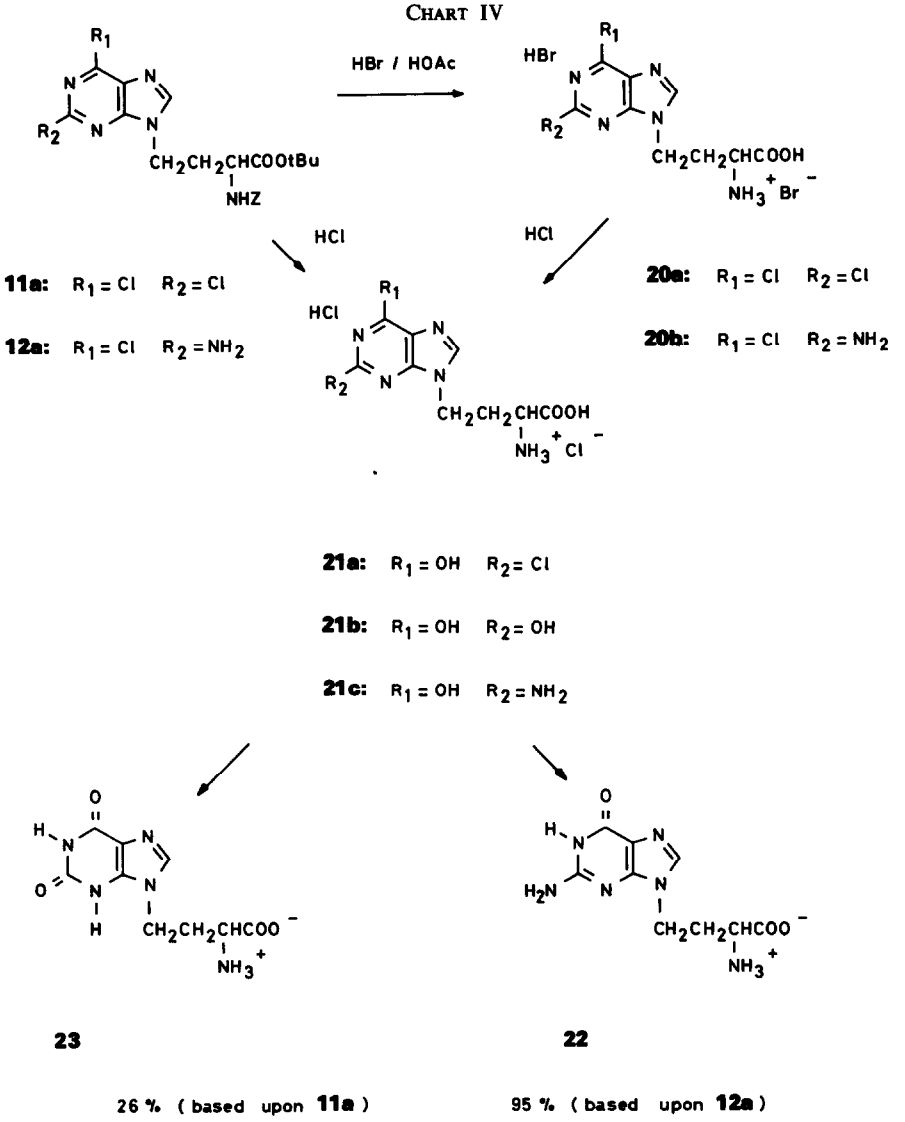

HBr / HOAc
10a
77 %
NH3
ethanol
17
14
HCl
HBr / HOAc
18
15
19
16
91 % (based upon 10a)
80 % (based upon 14)

is available at present, the facility of formation of the N_9 isomer may be ascribed to the generation of a complex such as **13** in which H-bonding between **5** and the alkylating agent stabilizes the transition-state for substitution at N_9. Acceleration in rates of certain hydrolytic reactions has been ascribed to similar hydrogen-bonding processes invoked in the corresponding transition states.[18]

The conversion of **10a**, **11a** and **12a** to purinyl amino acids containing adenine, guanine, hypoxanthine and xanthine is described in Charts III and IV.

Amination of **10a** was conveniently achieved by heating a solution of the compound in ethanol saturated with ammonia, at 100° for 2 hr. The amino acid functions in **14** were then unmasked simultaneously by treatment with HBr in acetic acid, where-

CHART IV



upon the dihydrobromide **15** was obtained. The adenine derivative (**16**) was set free by passing the salt over a Dowex (50 W-X 8) ion-exchange column and elution with ammonia. Conversion of **10a** to hypoxanthine derivative **19** was achieved by removal of the protecting groups (HBr/HOAc) in the initial step, followed by acid hydrolysis of the chloro compound **17**. Attempts to hydrolyse **10a** under basic conditions led to destruction of the heterocyclic nucleus. Also, while it was possible to achieve a direct conversion of **10a** to **18**, by refluxing the former with dilute HCl, the reaction was accompanied by significant decomposition.

Transformations of the 2,6-disubstituted purinyl derivatives **11a** and **12a** were initiated with the removal of the protecting functions by treatment with HBr/HOAc. Refluxing of the dihydrobromide **20b** with 1N HCl for 3 hr resulted in formation of the guanine derivative **21c**, which was converted into the free amino acid **22** by standard treatment over a Dowex column. The relative inertness of the Cl atom at 2-position was clearly indicated by its retention during the treatment of **20a** with 1N HCl. Concentrated HCl, however, was able to hydrolyse both Cl atoms. The difference in rates of the reaction at the two positions can be conveniently shown by recognition of the intermediate **21a**—in the process **20a** → **21a** → **21b**—by following the overall reaction with thin layer chromatography.

The structure of the compounds described in the aforementioned transformations was attested by their analytical and spectral data. The UV spectra of the N_9-purinyl amino acids is highly characteristic of the substitution site[19] and the maxima exhibit the typical pH dependency. These are described in Table 1.

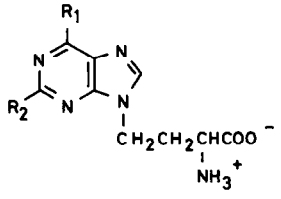


TABLE 1

			0·1N HCl		pH 7·0		0·1N NaOH	
	R_1	R_2	λ_{max}(nm)	$\varepsilon \times 10^{-3}$	λ_{max}(nm)	$\varepsilon \times 10^{-3}$	λ_{max}(nm)	$\varepsilon \times 10^{-3}$
19	OH	H	250	(10·4)	250	(11·0)	255	(11·7)
23	OH	OH	234	(7·6)	247	(9·9)	247	(9·8)
			262	(9·6)	277	(9·5)	279	(10·0)
16	NH_2	H	259	(14·4)	262	(13·9)	262	(13·7)
22	OH	NH_2	254	(11·9)			257	(10·0)
			277	(8·2)			269	(10·7)

EXPERIMENTAL

All m.p.s are uncorrected. Analyses were carried out by Messrs. H. Pieters, W. J. Buis and W. van Duyl of the Microanalytical Department of this laboratory. IR, UV and mass spectra were recorded on Unicam SP 200, Cary-14 Recording and AE MS-9 spectrometers, respectively. NMR spectra (concentration 100 mg/0·5 ml unless otherwise stated) were measured on a Varian Associates Model A-60 instrument.

t-Butyl-α-amino-γ-bromo-butyrate* HCl *salt* (**9**). To a suspension of 31·56 g (0·12 mol) of DL-α-amino-γ-bromobutyric acid HBr salt in 300 ml dioxan were added, 23·40 ml conc H_2SO_4 (0·42 mol) and under continuous stirring—with the aid of a refluxcondensor maintained at −20° with EtOH—300 ml of isobutene. The mixture was stirred for 65 hr, after which time the original yellow oil disappeared and a white ppt was formed. The isobutene was allowed to evaporate partially at room temp following which 500 ml 2N NaOH were added and the soln extracted with ether. The ether layer was washed to neutrality with a sat NaCl aq dried quickly over anhyd $MgSO_4$ and evaporated at room temp under reduced press. The resulting oil was dissolved in 250 ml ether and dry HCl gas passed into this soln. The precipitated salt was filtered off and dried to give directly an analytically pure sample of **9**, m.p. 118–121°, yield 21·4 g (65%); IR (KBr) 1735 cm^{-1} (ester C=O); NMR (D_2O) δ 1·64, s (9H, t-butyl), 2·4–2·7, m (2H, C_3-methylene), 3·75, t (2H, C_4-methylene, J = 7 c/s) and 4·31, t (1H, C_2-proton). (Found: C, 35·0; H, 6·4; N, 5·1. $C_8H_{17}O_2NBrCl$ requires: C, 34·99; H, 6·24; N, 5·10%).

t-Butyl-α-benzyloxycarbonylamido-γ-bromo-butyrate (**6b**). A suspension of 14·00 g (0·051 mol) of **9** and 9·41 g (0·112 mol) of $NaHCO_3$ in 60 ml water was cooled to 0°, after which 9·70 ml (10·4 g, 0·061 mol) of benzyloxycarbonylchloride was added dropwise in 10 min. A clear soln resulted. The reaction mixture was brought to 20° and stirred at that temp for 30 min, whereupon an oily ppt was formed. The reaction mixture was extracted repeatedly with ether and the combined extracts were washed with dil $NaHCO_3$ aq and dried over $MgSO_4$. After evaporation of the solvent a clear oil resulted. This was triturated with light petroleum (b.p. 40–60°) and cooled to −60°, after which the ether was discarded. This procedure was repeated several times. After removal of traces of the solvent at 0·1 mm/60° the resulting oil gave one spot on TLC (silicagel; $CHCl_3$ R_f = 0·55) and was used as such in the following experiments, yield 18·9 g, (100%,) IR (neat) 1700 (amide C=O) and 1720 cm^{-1} (ester C=O); NMR ($CDCl_3$) δ 1·43, s (9H, t-butyl), 5·12, s (2H, benzylic protons), 5·53, d (1H, NH, J = 8·5 c/s) and 7·35, s (5H, aromatic protons).

6-Chloro-9-(γ-benzyloxycarbonylamido-γ-t-butoxycarbonylpropyl) purine (**10a**). A suspension consisting of 7·66 g (49·6 mmol) of 6-chloropurine and 6·93 g (50·1 mmol) of anhyd K_2CO_3, in 150 ml DMF, was stirred for 15 min, after which 18·54 g (49·6 mmol) of **6b** were added. The mixture was stirred for 21 hr at 25°. The salts formed (KBr and $KHCO_3$) were filtered off by suction and the solvent was evaporated on the rotatory evaporator (0·2 mm/80°). The residue was taken up in 450 ml $CHCl_3$ and 120 ml water. After shaking the water layer was discarded. The organic layer was washed to neutrality with a sat NaCl aq, dried over anhyd $MgSO_4$ and evaporated to give an oil which—according to NMR—contained some DMF and a mixture of **10a** and **10b** in the ratio 7:1. The mixture was taken up in $CHCl_3$ and passed over an Al_2O_3 column with EtOAc. After the initial appearance of a small quantity of the starting bromo-ester, **10a** came off as a single component. The EtOAc was evaporated and the residual oil crystallized by trituration with ether, yield of pure **10a**, m p 132–133°, yield 15·45 g (70%); IR (KBr) 1725 (ester C=O), 1675 (amide C=O) and 940 cm^{-1} (characteristic band for N_9 substitution); UV (EtOH) 266 nm (9,300); NMR ($CDCl_3$) δ 1·41, s (9H, t-butyl), 5·12, s (2H, benzylic protons), 5·74, d (1H, NH, J = 8 c/s), 7·34, s (5H, aromatic protons), 8·20, s (1H, C_2-H) and 8·70, s (1H, C_8-H). (Found: C, 56·6; H, 5·5; N, 15·4; Cl, 7·9. $C_{21}H_{24}O_4N_5Cl$ requires: C, 56·58; H, 5·42; N, 15·71; Cl, 7·95; TLC silicagel/EtOAc, R_f: 0·47.

6-Chloro-7-(γ-benzyloxycarbonylamido-γ-t-butoxycarbonylpropyl) purine (**10b**). After elution of the column with EtOAc till no more **10a** came over, an EtOAc-EtOH (9:1) mixture was used as eluent. This provided, after evaporation of the solvent and trituration with ether, 2·38 g of a crystalline **10b**, m.p. 91–96°, yield 2·38 g (10·8%). IR (KBr) 1715 (ester C=O), 1690 (amide C=O) and 980 cm^{-1} (characteristic band for N_7 substitution); UV (EtOH) 270 nm (7,400); NMR ($CDCl_3$) δ 8·40, s (1H, C_2-H) and 8·80, s (1H, C_8-H). (Found: C, 56·7; H, 5·5; N, 15·4; Cl, 7·9. $C_{21}H_{24}O_4N_5Cl$ requires: C, 56·58; H, 5·42; N, 15·71; Cl, 7·95%); TLC silicagel/EtOAc, R_f: 0·34.

6-Amino-9-(γ-benzyloxycarbonylamido-γ-t-butoxycarbonylpropyl) purine (**14**). Compound **10a** (1 g) was suspended in 35 ml abs EtOH saturated with NH_3, and the mixture heated at 105° for 2·5 hr in a Carius tube, whereupon a clear soln resulted. The solvent was removed and the residue taken up in $CHCl_3$ and filtered. The mixture showed the following characteristics. TLC (silicagel: EtAOc–EtOH 3:1) indicated the presence of three compounds: (i) R_f 0·24 (very weak): (ii) product **14** R_f 0·52 (strong) and (iii) starting material, R_f 0·62 (very weak). Separation via column chromatography (silicagel: EtOAc–EtOH 3:1) provided, after crystallization with ether, pure white material, m.p 149·5–151·5°, yield 0·74 g, (77·4%); IR (KBr) 940 cm^{-1} absent; UV (EtOH) 262 nm (13,800); NMR (HA 100, $CDCl_3$) δ 1·38, s (9H, t-butyl), 2·0–2·6, m (2H, C_β-methylene), 4·1–4·5, m (3H, C_α-methylene, C_γ-proton), 5·14, s (2H, benzylic protons),

* All amino acid derivatives are racemic mixtures.

6·47, s (2H, NH_2; collapses upon exchange with D_2O), 7·35, s (5H, aromatic protons), 7·83, broadened s (1H, C_2–H) and 8·31, s (1H, C_8-H). (Found: C, 59·1; H, 6·2; O, 15·0; N, 19·7. $C_{21}H_{26}O_4N_6$ requires: C, 59·13; H, 6·32; O, 15·00; N, 19·76%).

9-(γ-*Amino*-γ-*carboxypropyl*) *adenine* (**16**). 0·500 g (1·17 mmol) of the protected amino acid was dissolved with stirring in 2·3 ml anhyd HOAc and to this soln 1·5 ml HBr/HOAc (sat) was added. Almost immediately a ppt appeared. After stirring for 2 hr a few ml abs ether were introduced into the flask and stirring continued for some min. The ppt was filtered off and thoroughly washed with dry ether, taking care not to suck the ppt dry, which is very hygroscopic. The HBr salt was taken up in water and the amino acid freed from its salt with a cation exchanger [Dowex 50 W-X 8, H^+ form]. The column was washed with water until the eluate was free from Br^-—according to $AgNO_3$ test—and eluted with 10% ammonia. Evaporation of the ammonia soln and recrystallization from water provided 221 mg of **16** as the analytically pure sample, yield 0·211 g (80·0%), m.p. 250° (dec); IR (KBr) no absorption above 1660 cm^{-1}: typical amino acid bands; UV: see Table 1; NMR (D_2O) δ 7·82, s (1H, C_2–H) and 7·88, s (1H, C_8–H), Mol. wt (M.S.) 236. 10218; Calc: 236. 10217. (Found: C, 43·5; H, 5·7; N, 33·4. $C_9H_{12}O_2N_6$. 0·75 H_2O requires: C, 43·29; H, 5·46; N, 33·66%).

9-(γ-*Amino*-γ-*carboxypropyl*) *hypoxanthine* (**19**). 3·00 g (6·70 mmol) of **10a** were dissolved in 15 ml HOAc. To this soln, 15 ml of HBr/HOAc (40%) were added. After stirring at room temp for 2·5 hr abs ether (40 ml) was added and the stirring continued for a further ½ hr. The precipitated 6-chloro-9-(γ-amino-γ-carboxypropyl)purinedihydrobromide was isolated by suction and washed thoroughly with dry ether. After drying over $CaCl_2$/KOH in the desiccator the weight amounted to 3 g. The di-HBr salt was dissolved in 225 ml 1N HCl and refluxed for 3 hr. TLC [isopropanol:water:conc NH_4OH 7:2:1] showed one ninhydrin positive spot and a spot corresponding to the base. The soln was evaporated on the rotatory-evaporator. The so formed HCl salt of the amino acid was dissolved in 40 ml water and put on the cation exchanger [Dowex 50 W-X8 (70 meq) in the H^+ form]. After washing with distilled water until the eluate showed a negative test for Cl^- with $AgNO_3$, elution was carried out with 2N NH_4OH, until the eluate was basic. Upon further elution with 10% NH_4OH (about 4 L was employed) and evaporation, 1·45 g of **19** was obtained as a colourless product, overall yield 1·45 g (91%); m.p. dec above 310°; IR (KBr) no absorption above 1690 cm^{-1}; amino acid bands; UV: see Table 1. (Found: C, 45·1; H, 4·9; O, 20·3; $C_9H_{11}O_3N_5$. 0·05 H_2O requires: C, 45·38; H, 4·70; O, 20·52%).

2-*Amino*-6-*chloro*-9-(γ-*benzyloxycarbonylamido*-γ-*t*-*butyloxycarbonylpropyl*) *purine* (**12a**). A mixture of 2·90 g **5** (17·1 mmol) and 2·38 g (17·2 mmol) anhyd K_2CO_3 was suspended in 20 ml DMF and 6·36 g (17·1 mmol) bromine ester was added. After stirring for 68 hr at room temp the precipitated $KHCO_3$ and KBr (2·0 g) were filtered off by suction and the DMF removed by evaporation. The residue was dissolved in $CHCl_3$ and washed with water. After drying over anhyd $MgSO_4$, evaporation of the solvent gave a yellow oil TLC (silicagel; EtOAc) revealed 4 spots. These corresponded to the starting ester, N_7 and N_9 substituted products and purine **5**. After trituration with $CHCl_3$ and cooling to 0° the 9-substituted product (**12a**) crystallized; m.p. 87–92°; yield 6·97 g (88%); IR (KBr) 1720 (ester C=O), 1710 (amide C=O) and 915 cm^{-1} (N_9-substitution of 2-amino-6-chloropurine); UV (EtOH) 311 (7200), 247 nm (6000), 223 (25900); NMR (DMSO-d_6) δ 1·37, s (9H, t-butyl), 5·11, s (2H, benzylic methylene), 6·93, broadened s (2H, NH_2), 8·08, s (1H, C_8-H). (Found: C, 54·8; H, 5·4; N, 18·3; Cl, 7·7. $C_{21}H_{25}O_4N_6Cl$ requires: C, 54·72; H, 5·47; N, 18·23; Cl, 7·53%).

2-*Amino*-6-*chloro*-7-(γ-*benzyloxycarbonylamido*-γ-t-*butyloxycarbonylpropyl*) *purine* (**12b**). The $CHCl_3$ filtrate from the previous experiment was evaporated. The oil was taken up in EtOAc and passed over a silicagel column. Elution with EtOAc provided some 7-substituted product, yield 0·16 g (2%); UV (EtOH) λ_{max}: 324.

9-(γ-*Amino*-γ-*carboxypropyl*)*guanine* (**22**). By the procedure described for the conversion of **10a** to the hypoxanthine derivative, **12a** was converted into **22**: m.p. dec above 360°; overall yield: 1·55 g (95%); IR (KBr) 1670 and 1630 cm^{-1} (amino acid bands); UV: see Table 1; NMR (18% DCl/D_2O) δ 2·6–3·0, m (2H, C_β-methylene), 4·2–4·9, m (3H, C_α-methylene and C_γ-proton), 9·17, s (1H, C_8-H). (Found: C, 41·1; H, 4·8; N, 31·7. $C_9H_{12}O_3N_6$. 066H_2O requires: C, 40·95; H, 5·04; N, 31·84%).

2,6-*Dichloro*-9-(γ-*benzyloxycarbonylamido*-γ-t-*butyloxycarbonylpropyl*) *purine* (**11a**). To a stirred suspension of 2·00 g, (10·6 mmol) 2,6-dichloropurine (**4**) and 1·48 g (10·7 mmol) anhyd K_2CO_3, in 50 ml DMF, was added 3·94 g (10·6 mmol) of **6b**. The mixture was stirred for 4 days at 60°. According to TLC, minor quantities of starting material were present at that time. The inorganic salts were filtered off by suction and the DMF evaporated. The residue was taken up in $CHCl_3$ and washed with water. The organic layer was dried over $MgSO_4$. After removal of the solvent an oil remained which was passed over a silicagel

column with ether–EtOH (99:1) as eluent After the isolation of some starting bromo ester, **11a** came off the column. Evaporation of the solvent gave a residue which slowly crystallized from light petroleum (b.p. 40–60°), yield: 1·70 g (33·3%); m.p. 79–89°; IR (KBr) 1710–1720 (ester and amide C=O) and 960 cm^{-1} (N_9-substitution of 2,6-dichloropurine); NMR ($CDCl_3$) δ 1·43, s (9H, t-butyl); 2–2·6, m (2H, C_β-methylene), 4–4·5, m (3H, C_α-methylene and C_γ-proton), 5·14, s (2H, benzylic protons) 5·70, d (1H, J = 8 c/s, NH), 7·36, s (5H, aromatic protons) and 8·23 (1H, C_8-H); UV (EtOH) 276 (10,700). Mol. wt. (MS) 479.

9-(*γ-Amino-γ-carboxypropyl*)*xanthine* (**23**). 3·00 g (6·25 mmol) of **11a** were dissolved in 30 ml HOAc and 30 ml HBr/HOAc 40% was added with stirring. After further stirring for 3 hr at room temp—when, in contrary to the experiments described above, no ppt was formed—abs ether was added whereupon **20a** precipitated out. This was filtered off, washed thoroughly with ether, dissolved in 150 ml 36% HCl and refluxed for 4½ hr. The free amino acid was isolated in the usual way. The product was yellow and recrystallized from water (norite). This caused an appreciable loss of the product since the amino acid was firmly absorbed on the norite, yield: 0·47 g (26·4%); m.p. 309° (dec); IR (KBr) no absorption above 1700 cm^{-1}; typical amino acid bands; UV: see Table 1. (Found: C, 39·8; H, 5·1; N, 25·7. $C_9H_{12}N_5O_4 . 1 . 1H_2O$ requires: C, 39·59; H, 4·87; N, 25·65%).

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