Synthesis and Biological Activities of Some N^6- and N^9-Carbamoyladenines and Related Ribonucleosides

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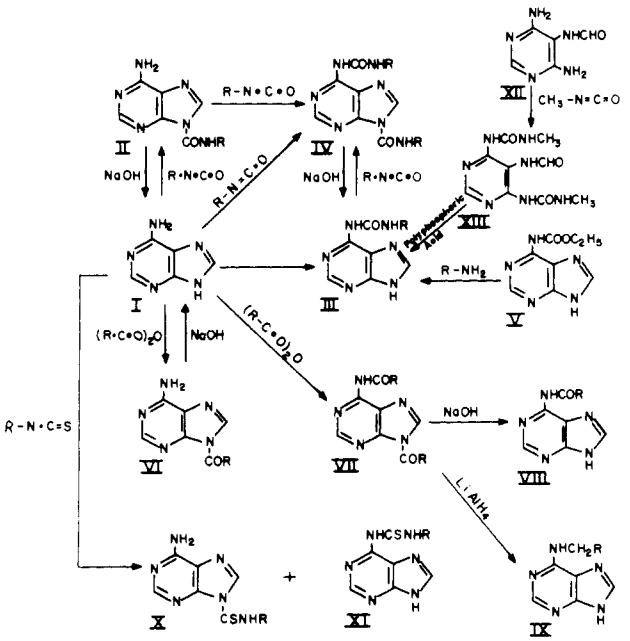
This paper describes a systematic investigation of the carbamoylation reactions of adenine, N^6-methyladenine, and adenosine. Adenine and N^6-methyladenine reacted with equimolar amounts of isocyanates to yield N^9-carbamoyl derivatives, whereas with an excess of isocyanates, N^6,N^9-dicarbamoyl derivatives were the main products. Similarly, acylation of adenine at room temperature with acid anhydrides afforded N^9-acyladenine derivatives. The structures of N^9-acyl- and N^9-carbamoyladenines were determined by comparison of their UV and NMR spectra with those of the N^9-acetyladenine and benzyl 6-aminopurine-9-carboxylate. Some 8-deuterated N^6- and N^9-carbamoyladenines were prepared from adenine-*8-d*, and from the NMR spectra of deuterated and undeuterated derivatives, the H_2 and H_8 protons in the carbamoyladenines were assigned. Significant differences were observed in the $\Delta\delta$ (H_2,H_8) values between the N^9-carbamoyladenines and N^6-carbamoyladenines. These compounds were tested for their growth-inhibitory activity in cultured mammalian cells. The N^9-carbamoyladenines, in general, showed greater growth inhibitory activity as compared to N^6-carbamoyl and N^6,N^9-dicarbamoyl derivatives. N^9-(*N*-Methylcarbamoyl)adenine (1) and N^9-(*N*-methylthiocarbamoyl)adenine (15) were two of the more potent analogues. Some of the N^6-carbamoyladenine ribonucleosides and their tri-*O*-acetyl derivatives also showed significant growth-inhibitory activity in cultured cell lines.

The 6-ureidopurine ribonucleoside derivative, *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside[1-5] (t^6A), is present in tRNA's which respond to codons beginning with A. The corresponding glycine analogue, *N*-(purin-6-ylcarbamoyl)glycine ribonucleoside (g^6A) has been isolated and characterized from total yeast tRNA.[6] The threonine analogue, t^6A, has also been isolated from human and rat urine.[1,3] The synthesis and biological activity of these naturally occurring 6-ureidopurines and some of their analogues have been reported.[7-11] The N^6-methyl derivative of t^6A (mt^6A) has been isolated and characterized from *Escherichia coli* $tRNA^{Thr}$.[12] We have recently reported the synthesis of the naturally occurring mt^6A and some of its analogues.[13] Although the reactions of purines, pyrimidines, and their ribonucleosides with isocyanates leading to the formation of the corresponding ureido derivatives have been described earlier,[14-16] no attempts have been made to study these reactions systematically under a variety of conditions. This paper describes the reactions of adenine, N^6-methyladenine, and adenosine with different types of isocyanates under diverse reaction conditions. The initial sites of carbamoylation in these reactions have been established and the final products are characterized. Also reported herein are the growth inhibitory activities of these compounds toward cultured mammalian cells. It is of interest to note that some antitumor compounds belonging to the group of the alkylnitrosoureas react with nucleosides, in vitro, leading to the formation of N-carbamoylated nucleoside derivatives.[17] Preliminary studies with N^9-carbamoyladenines suggest that these compounds are capable of carbamoylating the nucleic acid bases and amino acids.

Chemistry. The reaction of adenine I with 1 molar equiv of alkyl isocyanates in Me_2SO, both at room temperature and at 85 °C for 5 h, gave 60–75% yield of the N^9-carbamoyladenine II (Scheme I). The same reaction with 2 molar equiv of alkyl isocyanates with adenine at room temperature gave mainly the N^9-substituted product II, along with a small amount (<5%) of the N^6,N^9-disubstituted material IV. However, at an elevated temperature (90 °C) reaction of adenine with 2 molar equiv of alkyl isocyanates gave the N^6-substituted compound III as the major product along with some N^9-substituted compound II. There was also obtained a trace of the N^6,N^9-disubstituted product IV in addition to the above two compounds. The same reaction with a tenfold excess of alkyl isocyanates both at room temperature and at 80 °C gave the N^6,N^9-dicarbamoyl derivative IV as the major product.

Scheme I



Adenine reacted with 2 molar equiv of methyl and *n*-butyl isothiocyanates at 80 °C to give the N^9-thiocarbamoyl derivative X of adenine as the major product along with some N^6-thiocarbamoyladenine derivative XI (Scheme I). No significant amount of N^6,N^9-dithiocarbamoyl product could be obtained from this reaction. Reaction of adenine with 2 molar equiv of aryl isocyanates at room temperature for 6 h gave the N^6,N^9-dicarbamoyl derivative IV, whereas the same reaction at 90 °C furnished some N^6-carbamoyladenine III along with the N^6,N^9-disubstituted material IV, which was the major product. The N^9-carbamoyl compound II, however, was the only product formed in the reaction of adenine with 1 molar equiv of aryl isocyanates at room temperature. The reaction of adenine at room temperature with an excess of aryl isocyanates having an electron-withdrawing substituent on the phenyl ring yielded exclusively the N^9-carbamoyl compound II. The same reaction at 90 °C gave only the

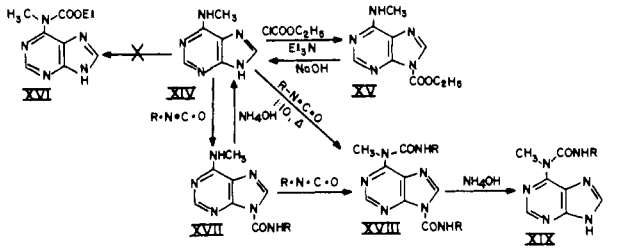
N^6-carbamoyl compound III.

Treatment of the N^9-carbamoyladenines II with either boiling water for 15 min or dilute NaOH at room temperature for 5 min gave adenine I in a quantitative yield. The N^6,N^9-dicarbamoyladenines IV, under similar conditions, gave N^6-carbamoyladenines III, indicating the lability of the N^9-carbamoyl group. In this respect, as well as in their method of synthesis, N^9-carbamoyl- and N^6,-N^9-dicarbamoyladenines resemble the corresponding acyl derivatives of adenine. Treatment of adenine with acetic anhydride at room temperature furnished N^9-acetyladenine VI (R = CH_3), the structure of which was confirmed by an alternate synthesis from adenine-9-thallium(I) salt and acetyl chloride following the method of Taylor et al.[18] and of Chheda and Hong.[7] Adenine on refluxing with acetic anhydride gave 6-acetamido-9-acetylpurine VII (R = CH_3).[19] Reaction of both N^9-acetyladenine and 6-acetamido-9-acetylpurine either with hot water or with dilute NaOH at room temperature led to a facile cleavage of the 9-acetyl function, resulting in the formation of adenine I or 6-acetamidopurine VIII (R = CH_3), respectively.

The structures of N^9-carbamoyladenines II were assigned by comparison of their NMR and UV spectra with those of N^9-acyladenines VI (R = CH_3). The H_2 and H_8 protons in the NMR spectra of N^9-acetyladenine appear at 8.70 and 9.02 ppm, respectively [$\Delta\delta$ (H_8–H_2) = 0.32 ppm], as compared to 8.76 and 9.01 ppm [$\Delta\delta$ (H_8–H_2) = 0.25 ppm], respectively, for N^9-(*N*-methylcarbamoyl)adenine II (R = CH_3). Further confirmation of the structures of the N^9-carbamoyladenines was obtained from a comparison of their NMR and UV spectra with those of benzyl 6-aminopurine-9-carboxylate and benzyl 6-aminopurine-7-carboxylate, prepared according to the procedure of Altman and Ben-Ishai.[20] The structures of both of these benzyl carboxylates have now been unequivocally determined in our laboratories by x-ray crystallography.[21] The UV spectra of the benzyl 6-aminopurine-7-carboxylate showed absorption maxima at 287 nm in neutral and at 272 nm in acidic media, while the 9-isomer, benzyl 6-aminopurine-9-carboxylate, showed the UV absorption maxima at 253 nm in neutral and acid pH. The UV spectra of N^9-alkylcarbamoyladenines (λ max 254–262 nm in neutral and acidic pH) show a close resemblance to the spectra of the 9-benzylcarboxylate and differ markedly from those of the 7-isomer. In the NMR spectrum of benzyl 6-aminopurine-7-carboxylate H_2 and H_8 protons appeared at 8.66 and 9.20 ppm, respectively [$\Delta\delta$ (H_8–H_2) = 0.54 ppm], whereas in the spectrum of benzyl 6-aminopurine-9-carboxylate the H_2 and H_8 protons appeared at 8.63 and 8.91 ppm [$\Delta\delta$ (H_8–H_2) = 0.28 ppm]. Comparison of these chemical shifts and the $\Delta\delta$ values with the corresponding values of N^9-carbamoyladenines II further supports the structural assignment of N^9 compounds. The structures of N^6-carbamoyladenine derivatives III were established by an alternate synthesis of N^6-(*N*-methylcarbamoyl)adenine III (R = CH_3) from ethyl purine-6-carbamate V (Scheme I).[7]

In order to establish the structures of N^6,N^9-dicarbamoyladenines IV, an alternate synthesis of a model dicarbamoyl compound IV (R = CH_3) of this type was attempted from 4,6-diamino-5-formylaminopyrimidine XII. Reaction of this compound with methyl isocyanate gave the corresponding dicarbamoyl derivative XIII. Attempted ring closure of this compound with polyphosphoric acid[22] gave N^6-(*N*-methylcarbamoyl)adenine III (R = CH_3) instead of the desired dicarbamoyl compound. However, the structures of N^6,N^9-dicarbamoyl-

Scheme II



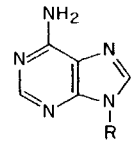
adenines IV could be assigned on the basis of their ready formation from the reactions of N^9-carbamoyladenines II as well as of the N^6-carbamoyladenines III with an alkyl or aryl isocyanate and also by their conversion to the N^6-carbamoyladenines III by treatment either with boiling water or dilute NaOH at room temperature. In the diacyl series, the attempts to reduce N^6,N^9-diacetyladenine VII (R = CH_3)[19,23] with a variety of reducing agents to the N^6,N^9-diethyladenine of known structure also ended in failure. The only product obtained from the reduction of this compound with $LiAlH_4$ was N^6-ethyladenine IX (R = CH_3). The acetyl and carbamoyl functions attached to the imidazole ring nitrogen are labile under the conditions of reduction and ring closure.

Carbamoylation reactions were also studied with the naturally occurring modified base N^6-methyladenine XIV (Scheme II). Reaction of N^6-methyladenine with ethyl chloroformate in triethylamine afforded the corresponding N^9-urethane XV in low yield. However, using a variety of reaction conditions, no 6-urethane XVI could be obtained. Reaction of N^6-methyladenine with a tenfold excess of alkyl isocyanate at room temperature afforded the corresponding N^9-carbamoyl compound XVII in about 90% yield. With a tenfold excess of alkyl or aryl isocyanates at 85 °C, it gave exclusively the N^6,N^9-dicarbamoyl derivatives XVIII. Treatment of both N^6-methyl-N^9-(*N*-methylcarbamoyl)adenine XVII (R = CH_3) and ethyl 6-methylaminopurine-9-carboxylate XV with boiling aqueous ammonia gave N^6-methyladenine in quantitative yield. The N^6,N^9-dicarbamoyl derivatives XVIII, under similar conditions, gave the N^6-methyl-N^6-carbamoyl compounds XIX.

Reaction of 2′,3′,5′-tri-*O*-acetyladenosine with alkyl and aryl isocyanates followed by removal of the protecting groups afforded N^6-(*N*-alkylcarbamoyl)- and N^6-(*N*-arylcarbamoyl)adenosines in good yield. When the required isocyanates were not readily available, these compounds were prepared from a reaction of ethyl (2′,-3′,5′-tri-*O*-acetyl-9-β-D-ribofuranosyl)-9*H*-purine-6-carbamate and the corresponding amine, followed by removal of the blocking groups.[7,8] These compounds are fairly stable in base at room temperature. However, they degrade to adenosine and the corresponding amine when heated in 0.1 N NaOH at 100 °C for 1 h.

The UV spectra of these carbamoyladenines (Tables I–VI) vary, depending upon the site of carbamoylation, and are different from the spectra of the corresponding *N*-alkyladenines. The N^6-carbamoyladenines III with an alkyl side chain show UV absorption maxima at 268–270 and 276–277 nm in neutral pH and at 277–278 nm in acidic and alkaline pH.[1,8] The N^6-methyl-N^6-(*N*-methylcarbamoyl)adenine XIX (R = CH_3) absorbs in UV at 283, 286, and 285 nm in neutral, acidic, and basic pH, respectively. This compound is an analogue of the free base of the naturally occurring modified nucleoside, mt^6A, and its UV spectra are similar to those of the mt^6A base.[13] The N^9-carbamoyladenines (II) show UV absorption maxima in the range of 254–262 nm in both neutral and acid pH,

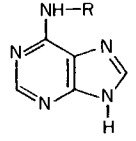
Table I. N^9-Carbamoyladenines and N^9-Acyladenines

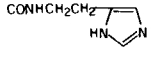


Compd no.	R	Method	Yield, %	Mp,[a] °C	Crystn solvent	Formula[b]	UV max, nm (ε × 10⁻³) pH 1-2	pH 5-7	pH 11-12[c]	NMR, δ 2-H	8-H	Biological act. at 1 × 10⁻⁴ M[d] Nc 37	RPMI 6410	L1210
1	$CONHCH_3$	A	69	345-350	$CHCl_3$-MeOH	$C_7H_8N_6O$	256 (14.3)	257 (13.1)	268	8.76	9.01	++, +[g]	++, +[g]	++
2	$CONHCH_2$-$CH=CH_2$	B	19	>320	$CHCl_3$	$C_9H_{10}N_6O$	257 (15.6)	258 (14.2)	268	8.70	9.00	++	+	>++
3	$CONHC(CH_3)_3$	A B	68 19	350-360 dec	$CHCl_3$	$C_{10}H_{14}N_6O$	254 (13.2)	256 (12.3)	268	8.72	9.00	+	+	>++
4	$CONHCH_2CH_2$-CH_2CH_3	A	65	230-232	$CHCl_3$	$C_{10}H_{14}N_6O$	258 (15.9)	259 (13.7)	268	8.67	8.90	++	++	>++
5	$CONHCH_2$-$(CH_2)_6CH_3$	A	64	188-189	$CHCl_3$	$C_{14}H_{22}N_6O$	258 (15.8)	259 (14.1)	268					
6	CONH-c-C_6H_{11}	A	62	355-360 dec	1,4-Dioxane-EtOH	$C_{12}H_{16}N_6O$	257 (14.3)	258 (13.8)	268	8.72	8.93	++	++	>++
7	$CONHC_6H_5$[e]	A	94	>350 dec	THF	$C_{12}H_{10}N_6O \cdot 0.5H_2O$	257 (15.4)	258 (16.2)	268	8.80	9.06	NA	±	++
8	$CONHC_6H_4$-2-F[e]	A	90	>350 dec	THF	$C_{12}H_9FN_6O$	260 (16.9)	262 (16.2)	268			NA	±	+
9	$CONHCH_2$-$COOC_2H_5$	A	41	282-284 dec	$CHCl_3$	$C_{10}H_{12}N_6O_3$	260 (10.6)	260 (11.6)	268	8.72	8.97	±	±	++
10	$CONHCH_2$-$COOCH_2C_6H_5$	A	44	198-200	$CHCl_3$	$C_{15}H_{14}N_6O_3$	257 (14.1)	257 (12.8)	268	8.73	9.00	+	±	>++
11	CONHCH-(COOH)-$CH_2C_6H_5$	E	74	310-315 dec	AcOH-EtOH	$C_{15}H_{14}N_6O_3 \cdot 2H_2O$	256 (12.0)	256 (12.6)	268	8.70	8.97	++	±	>++
12	CONHCH-$(COOCH_2C_6H_5)CH_2C_6H_5$	A	65	163-164	$CHCl_3$-petr ether	$C_{22}H_{20}N_6O_3$	257 (20.1)	257 (19.5)	268			+	NA	++
13	CONHCH-$(COOCH_2C_6H_5)CH(CH_3)_2$	B	34	156-158	$CHCl_3$-petr ether	$C_{18}H_{20}N_6O_3$	259 (19.2)	260 (18.7)	268	8.75	9.00	±	++	++
14	CONHCH-$(COOCH_2C_6H_5)CH(OCH_2$-$C_6H_5)CH_3$[f]	B	18	150-154	$CHCl_3$-petr ether	$C_{24}H_{24}N_6O_4$	260 (15.8)	261 (14.8)	268	8.65	8.97	±	±	>++
15	$CSNHCH_3$	I	40	344-345 dec	$CHCl_3$-MeOH	$C_7H_8N_6S$	260 (17.1), 280 (sh)	260 (17.3), 279 (sh)	258 (16.6)	8.79	9.36	++[g]	++[g]	>++[g]
16	$CSNHCH_2CH_2$-CH_2CH_3	I	20	>350 dec	$CHCl_3$-MeOH	$C_{10}H_{14}N_6S$	275 (21.1)	267 (20.0), 274 (sh)	274 (17.9)			±	+	+
17	$COCH_3$	J K	87 38	362-364	Me_2SO-pyridine	$C_7H_7N_5O$	260 (18.8)	254 (12.9), 259 (sh), 286 (sh)	268	8.70	9.02	NA	NA	++
18	$COCH_2CH_2CH_3$	J	51	219-220	Me_2SO-pyridine	$C_9H_{10}N_5O$	258 (13.3)	253 (12.7), 258 (sh), 285 (sh)	268	8.75	9.06	NA	±	+
19	$COCH_2$-$(CH_2)_5CH_3$	J	49	165-166	Me_2SO-pyridine	$C_{13}H_{19}N_5O$	258 (13.2)	253 (14.2), 258 (sh), 284 (sh)	268	8.65	8.95	+	+	++

[a] s = soften; dec = decomposition. [b] All compounds were analyzed for C, H, and N and the analytical results were within ±0.4% of the calculated values. [c] At alkaline pH all these compounds except 15 and 16 underwent rapid decomposition to adenine. [d] The notations here represent the viable cell number relative to the controls after 72 h of incubation: ++, 30-60%; +, 60-80%; ±, 80-90%; NA, 90-110%. [e] Adenine was completely dissolved in Me_2SO by warming and the solution cooled to room temperature before addition of the isocyanate. [f] Purified from the accompanying 6-ureido compound by chromatography over a silica gel column from which this compound was eluted out with $CHCl_3$-EtOH (19:1). [g] At 10^{-5} M.

Table II. N^6-Carbamoyladenines



Compd no.	R	Method	Yield, %	Mp,[a] °C	Crystn solvent	Formula[b]	UV max, nm (ε × 10^{-3}) pH 1-2	pH 5-7[c]	pH 11-12	NMR, δ 2-H	8-H	Biological act. at 1 × 10^{-4} M[d] Nc 37	RPMI 6410	L1210
20	$CONHCH_2CH=CH_2$	B	42		EtOH	$C_9H_{10}N_6O$	279 (20.5)	268 (18.2)	279 (14.9)	8.94	8.89	±	±	+
		C	59	>300 dec										
21	$CONHCH_2CH_2CH_2CH_3$	D	81	205-206 dec	$CHCl_3$	$C_{10}H_{14}N_6O$	278 (22.6)	270 (21.4)	279 (20.1)	8.92	8.79	±	±	++
22	$CONHC(CH_3)_3$	B	40	352-358 dec	MeOH	$C_{10}H_{14}N_6O \cdot 0.25H_2O$	277 (17.1)	269 (14.7)	278 (13.2)	8.93	8.80	+	+	+
23	CONH-c-C_6H_{11}	B	40	262-265	THF	$C_{12}H_{16}N_6O$	277 (21.4)	268 (18.3)	278 (15.1)	8.93	8.80	±	±	+
		D	82											
24	$CONHCH_2CH_2C_6H_4$-2-Cl	C	71	250 s	EtOH	$C_{14}H_{13}ClN_6O$	278 (20.7)	269 (18.1)	278 (16.5)			++	+	+
		D	81	>350 dec										
25		C	54	254	EtOH	$C_{11}H_{12}N_8O \cdot 0.5H_2O$	275 (20.0)	269 (19.8)	276 (15.0)					
26	$CONHC_6H_5$	B	31	378-380 dec	THF	$C_{12}H_{10}N_6O$	287 (21.7)	278 (23.8)	284 (22.2)	9.03	8.85	+	±	+
		C	40											
27	$CONHC_6H_4$-4-F	B[e]	95	338-340 dec	Me_2SO-MeOH	$C_{12}H_9FN_6O$	285 (18.6)	278 (19.8)	283 (18.2)	9.03	8.85	NA	NA	++
28	$CONHC_6H_4$-2-CF_3	B[e]	85	>350	Me_2SO-MeOH	$C_{13}H_9F_3N_6O$	279 (22.0)	275 (22.1)	280 (20.0)			++	NA	
29	$CONHC_6H_4$-4-CH_3	B[e]	44	>320 dec	THF	$C_{13}H_{12}N_6O \cdot 0.5H_2O$	287 (23.1)	278 (21.0)	284 (22.2)	9.07	8.90	NA	+	>++
30	$CONHCH_2CONHCH_2COOH$	C	81	179-180 eff	EtOH	$C_{10}H_{11}N_7O_4 \cdot 1.5H_2O$	277 (20.7)	269 (18.7)	278 (17.5)			NA	NA	NA
31	$CONHCH(COOH)CH_2CH_2CH_2NHCOOCH_2C_6H_5$	C	13	119-120	EtOH	$C_{19}H_{21}N_7O_5$	278 (14.5)	269 (12.6)	275 (14.4)					
32	$CSNHCH_3$	I	12	270-275	$CHCl_3$-MeOH	$C_7H_8N_6S$	289 (24.7)	286 (27.6)	292 (15.0)	9.06	9.00	±	±	±
33	$CSNHCH_2CH_2CH_2CH_3$	I	20	208-210	$CHCl_3$-MeOH	$C_{10}H_{14}N_6S$	289 (23.0)	287 (25.6)	294 (16.5)	8.88	8.76	++	+	++

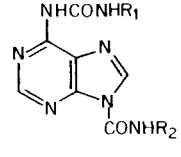
[a] eff = melts with effervescence; s = softens; dec = melts with decomposition. [b] All compounds were analyzed for C, H, and N and the analytical results were within ±0.4% of the calculated values. [c] At neutral pH, pronounced shoulder at 276-277 nm except for 26-29, 32, and 33. [d] The notations here represent the viable cell number relative to the controls after 72 h of incubation: ++, 30-60%; +, 60-80%; ±, 80-90%; NA, 90-110%. [e] In the case of these compounds, the N^6-carbamoyl derivative was the major product in the reaction.

Table III. N^6-Carbamoyladenine Ribonucleosides

Compd no.	R_1	R_2	Method	Yield, %	Mp,[a] °C	Crystn solvent	Formula[b]	UV max, nm ($\epsilon \times 10^{-3}$) pH 1-2	pH 5-7	pH 11-12	NMR, δ 2-H	8-H	Biological act. at 1×10^{-4} M[c] Nc 37	RPMI 6410	L1210
34	$CH_2C(CH_3)_3$[d]	Ribosyl	H	56	194-195	EtOH	$C_{16}H_{24}N_6O_5$	277 (25.7)	269 (25.4), 276 (21.8)	270 (17.9), 278 (17.6), 299 (12.8)			NA	NA	±
35	$CH_2CH_2C_6H_4$-2-Cl[d]	Ribosyl	H	72	130-135 s, 148-150	EtOH	$C_{19}H_{21}ClN_6O_5$	277 (23.6)	269 (23.5), 276 (20.1)	270 (16.7), 278 (16.1), 299 (11.9)			+		
36	C_6H_4-2-F[d]	Ribosyl	G	51	211-212	EtOH	$C_{17}H_{17}FN_6O_5$	280 (25.3)	277 (25.3)	305 (32.7)	9.00	9.06	+	++	++
37	C_6H_4-4-Cl	Ribosyl	G	77	191-192	MeOH	$C_{17}H_{17}ClN_6O_5 \cdot 0.5H_2O$	285 (21.1)	278 (22.2)	309 (24.9)	9.16	9.25	++	++	>++
38	C_6H_4-4-Cl	2',3',5'-Tri-O-acetylribosyl	F	64	175-176	MeOH	$C_{23}H_{23}ClN_6O_8$	285 (26.7)	278 (28.3)	310 (36.1)			++	++	>++
39	C_6H_4-2-Cl	2',3',5'-Tri-O-acetylribosyl	F	52	146-148	MeOH	$C_{23}H_{23}ClN_6O_8$	280 (16.3)	278 (19.9)	308 (26.4)			++	++	>++

[a] s = softens; dec = melts with decomposition. [b] All compounds were analyzed for C, H, and N and the analytical results were within ±0.4% of the calculated values. [c] The notations here represent the viable cell number relative to the controls after 72 h of incubation: ++, 30-60%; +, 60-80%; ±, 80-90%; NA, 90-110%. [d] In these cases the intermediate triacetate was not isolated but directly hydrolyzed to the free ribonucleoside.

Table IV. N^6,N^9-Dicarbamoyladenines

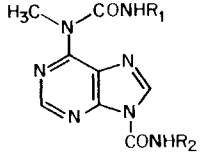


Compd no.	R_1	R_2	Method	Yield, %	Mp,[a] °C	Crystn solvent	Formula[b]	UV max, nm ($\epsilon \times 10^{-3}$) pH 1-2	pH 5-7	pH 11-12[c]	NMR, δ 2-H	8-H	Biological act. at 1×10^{-4} M[d] Nc 37	RPMI 6410	L1210
40[e]	CH_3	CH_3	N	70	306-310	DMF	$C_9H_{11}N_7O_2$	274 (21.8)	267 (22.7)	278	9.16	9.30	NA	±	±
			L	65											
41[e]	$CH_2CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_3$	L	50	216-220	$CHCl_3$	$C_{15}H_{23}N_7O_2$	275 (20.4)	268 (18.8)	276	9.05	9.15	NA	+	++

42[e]	c-C_6H_{11}	c-C_6H_{11}	L	35	189-190	$CHCl_3$–petr ether	$C_{19}H_{27}N_7O_2$	277 (16.8)	268 (16.9)	278	8.80	8.93	Insoluble at 10^{-5} M		
43	C_6H_5	C_6H_5	L	72	350-352 dec	THF	$C_{19}H_{15}N_7O_2$	284 (27.7)	281 (29.7)	287	8.83	9.03	+	±	++
44	C_6H_4-2-CH_3	C_6H_4-2-CH_3	L	84	>360 dec	Me_2SO–MeOH	$C_{21}H_{19}N_7O_2 \cdot H_2O$	282 (17.2)	277 (18.0)	284	8.80	9.05	+	NA	+
45	C_6H_4-4-OCH_3	C_6H_4-4-OCH_3	L	82	>325 dec	Dioxane	$C_{21}H_{19}N_7O_4 \cdot 0.5H_2O$	283 (22.0)	281 (24.1)	287	8.83	9.02	NA	NA	++
46	C_6H_3-2,5-Cl_2	C_6H_3-2,5-Cl_2	L	72	>350 dec	Me_2SO–EtOH	$C_{19}H_{11}Cl_4N_7O_2$	279 (32.5)	278 (34.5)	287			Very insoluble		
47			L	68	>300 dec	Me_2SO–EtOH	$C_{27}H_{19}N_7O_2$	281 (23.2)	277 (19.2)	287	8.83	9.13	++	++	>++
48	CH_3	C_6H_5	M	55	338-340	THF	$C_{14}H_{13}N_7O_2$				8.82	9.10	NA	NA	++
49	C_6H_4-4-Br	C_6H_4-4-Br	L	89	340-345 dec	Me_2SO–EtOH	$C_{19}H_{13}Br_2N_7O_2$	288 (17.6)	281 (19.4)	286	8.97	9.05	+		
50	C_6H_4-2-F	$C(CH_3)_3$	M	65	>350 dec	$CHCl_3$	$C_{17}H_{16}FN_7O_2$	279 (22.9)	278 (24.4)	284	8.93	9.03	+	+	>++
51	C_6H_4-4-OCH_3	$C(CH_3)_3$	M	63	>350 dec	$CHCl_3$	$C_{18}H_{21}N_7O_3 \cdot 0.5H_2O$	283 (21.1)	280 (24.0)	285			NA	±	±

[a] dec = melts with decomposition. [b] All compounds were analyzed for C, H, and N and the analytical results were within ±0.4% of the calculated values. [c] At basic pH these compounds degrade rapidly to the corresponding 6-ureido derivatives. [d] These notations represent the viable cell numbers relative to the controls after 72 h of incubation: ++, 30-60%; +, 60-80%; ±, 80-90%; NA, 90-110%. [e] In the case of these compounds 10 mmol of the respective isocyanates was used for each millimole of adenine.

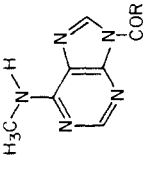
Table V. N^6-Methyl-N^6,N^9-dicarbamoyladenines



Compd no.	R_1	R_2	Method	Yield, %	Mp,[a] °C	Crystn solvent	Formula[b]	UV max, nm (ε × 10^{-3}) pH 1-2	pH 5-7	pH 11-12[c]	NMR, δ 2-H	8-H	Biological act. at 1 × 10^{-4} M[d] Nc 37	RPMI 6410	L1210
52	CH_3	CH_3	N	93	304-306 dec	DMF	$C_{10}H_{13}N_7O_2$	282 (15.6)	280 (15.6)	285	9.13	9.23	++	+	++
53	c-C_6H_{11}	c-C_6H_{11}	N	84	156-157	Petr ether	$C_{20}H_{29}N_7O_2$								
54[e]	C_6H_5	C_6H_5	N	92	315-316 dec	Toluene	$C_{20}H_{17}N_7O_2$	258 (19.8)	259 (20.8)	270			NA	±	++
55[e]	C_6H_4-2-Cl	C_6H_4-2-Cl	N	85	296-305 dec	Toluene	$C_{20}H_{15}Cl_2N_7O_2$	272 (18.5)	272 (19.2)	273			NA	+	++
56	$CH_2COOC_2H_5$	$CH_2COOC_2H_5$	N	55	140-141	Toluene	$C_{16}H_{21}N_7O_2$	281 (16.6)	278 (18.0)	284			NA	NA	+

[a] dec = melts with decomposition. [b] All compounds were analyzed for C, H, and N and the analytical results were within ±0.4% of the calculated values. [c] At basic pH all these compounds are rapidly degraded to the corresponding 6-ureido derivatives. [d] The notations represent the viable cell number relative to the controls after 72 h of incubation: ++, 30-60%; +, 60-80%, ±, 80-90%; NA, 90-110%. [e] In the case of aromatic isocyanates the reaction time was significantly less (about 4 h).

Table VI. N^6-Methyl-N^9-carbamoyladenines



Compd no.	R	Method	Yield, %	Mp,[a] °C	Crystn solvent	Formula[b]	UV max, nm ($\epsilon \times 10^{-3}$) pH 1-2	pH 5-7	pH 11-12[c]	NMR, δ 2-H	8-H	Biological act. at 1×10^{-4} M[d] Nc 37	RPMI 6410	L1210
57	OC_2H_5	e	32	150-151	Ether	$C_9H_{11}N_5O_2$	261 (14.7)	262 (13.5)	272	8.80	8.95	++	++	NA
58	$NHCH_3$	O	89	317-318	Me_2SO	$C_8H_{10}N_6O$	266 (14.8)	266 (15.8)	272			++	+	NA
59	$NHC(CH_3)_3$	O	82	316-319 dec	Cyclohexane	$C_{11}H_{16}N_6O$	260 (18.1)	262 (15.4)	272			+	+	+

[a] dec = melts with decomposition. [b] All compounds were analyzed for C, H, and N and the analytical results were within ±0.4% of the calculated values. [c] At basic pH all these compounds are rapidly degraded to 6-methylaminopurine. [d] The notations here represent the viable cell number relative to the controls after 72 h of incubation: ++, 30-60%; +, 60-80%; ±, 80-90%; NA, 90-110%. [e] See Experimental Section.

Table VII. Chemical Shifts (δ) of the H_2 and H_8 Protons in *N*-Carbamoyladenines

Type of compd	H_2, ppm	H_8, ppm	$\Delta\delta$ (H_8,H_2), ppm
N^9-Carbamoyladenines II	8.65-8.80	8.90-9.06	0.21-0.32
N^9-Acyladenines VI	8.65-8.75	8.95-9.06	0.30-0.32
N^6-Carbamoyladenines III	8.88-9.07	8.76-9.00	0.06-0.18
N^6,N^9-Dicarbamoyladenines IV	8.80-9.16	9.02-9.30	0.08-0.30
N^6-Carbamoyladenine ribonucleosides	9.00-9.16	9.06-9.25	0.06-0.09

while in basic pH (>10.5) these compounds undergo a rapid degradation to adenine. The compound N^6-methyl-N^9-(*N*-methylcarbamoyl)adenine XVII (R = CH_3) exhibits UV absorption maxima at 266 nm both in neutral and acid pH. In alkaline pH, these compounds undergo rapid degradation to N^6-methyladenine. The N^6,N^9-dicarbamoyladenines IV have λ max in the range of 274–288 nm in acidic pH and 267–281 nm in neutral pH depending on the nature of the side chain while in base (pH >10.5) they rapidly degrade to the corresponding N^6-carbamoyladenine derivatives. The N^6-methyl-N^6,N^9-dicarbamoyladenines XVIII show absorption maxima in UV at 281–282 nm in acid and 278–280 nm in neutral pH. In alkaline solution (pH >10.5) these compounds undergo rapid hydrolysis to the corresponding N^6-methyl-N^6-carbamoyladenine derivative.

In order to make the unequivocal assignment of the H_2 and H_8 protons of the purine ring in the NMR spectra of the carbamoyladenines, N^6-(*N*-methylcarbamoyl)adenine III (R = CH_3), N^9-(*N*-methylcarbamoyl)adenine II (R = CH_3), and N^6,N^9-di(*N*-methylcarbamoyl)adenine IV (R = CH_3) were prepared from 8-deuterated adenine[24] and their NMR spectra were recorded. By comparison with the NMR spectra of the corresponding undeuterated compounds, it has been possible to correctly assign H_2 and H_8 protons of the purine ring in these compounds and in their analogues.

In the 8-deuterated N^9-(*N*-methylcarbamoyl)adenine II (R = CH_3), the H_2 proton appeared as a sharp singlet at 8.76 ppm and, therefore, the peak at 9.01 ppm in the proton analogue was assigned to H_8. Similarly in the NMR spectrum of 8-deuterated N^6-(*N*-methylcarbamoyl)adenine III (R = CH_3) the peak of H_2 was observed at 9.01 ppm and thus the peak at 8.88 ppm in the proton analogue was assigned to the H_8. In the case of the 8-deuterated N^6,-N^9-di(*N*-methylcarbamoyl)adenine IV (R = CH_3), H_2 was observed at 9.16 ppm and, therefore, H_8 was assigned at 9.30 ppm from the NMR spectrum of undeuterated compound. Table VII shows the ranges in the values for chemical shifts for the H_2 and H_8 protons and the $\Delta\delta$ values in the *N*-carbamoyladenine derivatives. From the data in Table VII, it appears that the chemical shifts of H_2,H_8 and the ranges of the $\Delta\delta$ values may be employed to distinguish between N^9- and N^6-carbamoyladenine derivatives. The utility of $\Delta\delta$ values in the structural assignments of *N*-alkylpurine derivatives has recently been discussed in a review by Townsend.[25]

Biological Activity. These compounds were tested for their growth-inhibitory activity against the following cultured mammalian cells: mouse leukemia L1210 and leukocytes derived from a normal human buffy coat (Nc 37) and from a leukemic human buffy coat (RPMI 6410). In general, the N^9-carbamoyladenines II showed promising growth-inhibitory activity in the above three cell lines

(Table I). The L1210 cells were more sensitive as compared to the other two cell lines. Many of the analogues exhibited 50% growth inhibition at about 10^{-5} M concentration or below in L1210 leukemia. At the 10^{-4} M concentration several N^9-carbamoyladenines (**1–4**, **6**, **10**, **11**, **14**, **15**) exhibited 70% or greater inhibition of growth of L1210 cells. Two compounds in this series, N^9-(*N*-methylcarbamoyl)adenine (**1**) and N^9-(*N*-methylthiocarbamoyl)adenine (**15**), were the most potent of all the compounds studied here. Lengthening of the side chain and the introduction of branching or unsaturation in the side chain did not enhance the activity. N^9-Carbamoyladenines II with an aromatic side chain (**7** and **8**) were less active as compared to those with an alkyl side chain. N^9-Acyladenines (**17–19**) also showed some activity against the L1210 cell line (Table I). In contrast, the N^9-carbamoyl-N^6-methyladenines XVII were inactive against L1210 cells but had some activity against the leukemic cell line (RPMI 6410) (Table VI). It is possible that these N^9-substituted compounds exhibit their growth inhibitory activity by transcarbamoylation of nucleic acids or proteins in the cultured cells. Preliminary in vitro studies indicate that nucleic acid components such as cytosine and adenine and amino acids such as glycine get carbamoylated by the N^9-carbamoyladenines.[26]

In general, N^6-carbamoyladenines III (Table II) were less active than the corresponding N^9-carbamoyladenines II. In several instances, a given substituent at the N^9 position of adenine was more potent than at N^6 (**2** vs. **20**, **4** vs. **21**, **6** vs. **23**). In this group 6-*n*-butylureidopurine (**21**), 6-*p*-fluorophenylureidopurine (**27**), 6-*p*-tolyureidopurine (**29**), and 6-*n*-butylthioureidopurine (**33**) were significantly effective against the growth of L1210 cells. Concentrations required for 50% inhibition of the growth were in the order of 10^{-5} M or lower. In this class also, the introduction of branching or unsaturation in the side chain did not enhance the activity. Some of the N^6-carbamoyladenosines (**36–39**) showed good inhibitory activity against these cell lines (Table III). In some compounds of this series acetylation of the ribose moiety (**37** and **38**) led to an enhancement of the growth inhibitory activity. N^6,N^9-Dicarbamoyladenines were also less active (Table IV) than the N^9-carbamoyladenines II. In many instances the same substituent in both 6 and 9 positions reduced the activity as compared to what was found for the corresponding N^9-monosubstituted adenines. Some of the disubstituted compounds were, however, significantly active against L1210 cells. The most active compound in this series was the N^6,N^9-di(*N*-α-naphthylcarbamoyl)adenine (**47**). A mixed dicarbamoyladenine (**50**) carrying a *tert*-butylcarbamoyl group in the 9 position and an *o*-fluorophenylureido group in the 6 position retained the activity of the parent 9-substituted compound **3**. The N^6,N^9-dicarbamoyl compounds **52–56** derived from N^6-methyladenine were also significantly active toward the L1210 leukemia cells in culture (Table V). Compounds **1**, **15**, **37**, and **47** were evaluated in mice bearing L1210 leukemia. These compounds did not extend significantly the life of the mice bearing L1210 leukemia. In vivo evaluation of other compounds which were potent in tissue culture is in progress.

Experimental Section

General. Melting points were determined in a Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 457 and 137B spectrophotometer in KBr disks. UV spectra were determined on a Cary Model 14 and a Beckman Acta V spectrophotometer. NMR spectra were determined in Me_2SO-d_6 on a Varian A-60A spectrometer using Me_4Si as an external reference unless otherwise specified. Mass spectra were determined by a Dupont/CEC 21-491 double-focusing mass spectrometer. TLC was carried out on silica gel PF_{254} (E. Merck AG) coated glass plates in the following solvent systems: (A) EtOAc–*n*-PrOH–H_2O (4:1:2, upper phase); (B) EtOAc–2-ethoxyethanol–16% HCOOH (4:1:2, upper phase); (C) *i*-PrOH–H_2O–concentrated NH_4OH (7:2:1); (D) EtOAc–EtOH (49:1). C, H, and N analyses were carried out by Galbraith Laboratories, Knoxville, Tenn., and by Heterocyclic Chemical Corp., Harrisonville, Mo.

Isocyanates from Amino Acid Esters. Isocyanates of *O*-benzyl-L-threonine benzyl ester, glycine benzyl ester, L-phenylalanine benzyl ester, and L-valine benzyl ester were prepared in good yields by bubbling anhydrous $COCl_2$ into a stirred suspension of amino acid benzyl esters in anhydrous toluene at 90 °C for 4 h, as reported earlier.[7] The isocyanates obtained after evaporation of the solvent were used in the next step. These compounds exhibited absorption at 2273–2300 cm^{-1} (N=C=O) in IR (film).

N^9-(*N*-Methylcarbamoyl)adenine (1). Method A. A mixture of 1.35 g of adenine (10 mmol) and 0.63 g of methyl isocyanate (11 mmol) in anhydrous Me_2SO (20 mL) was stirred in a glass bomb at room temperature for 6 h. The precipitated product was collected on a filter and washed with toluene. The solid residue was then crystallized twice from $CHCl_3$–MeOH (19:1): yield 1.33 g (69%); mp 345–350 °C dec; NMR δ 3.02 (d, 3, *J* = 5 Hz, –NHCH_3), 7.75 (br, 2, –NH_2), 8.76 (s, 1, 2-H), and 9.01 (s, 1, 8-H).

N^9-(*N*-*tert*-Butylcarbamoyl)adenine (3) and N^6-(*N*-*tert*-Butylcarbamoyl)adenine (22). Method B. A stirred mixture of 1.35 g of adenine (10 mmol) and 2.18 g of *tert*-butyl isocyanate (22 mmol) in 20 mL of anhydrous Me_2SO was heated with stirring in a glass bomb at 90 °C for 5 h. After cooling to room temperature overnight, the precipitated product was collected on a filter and washed with toluene. The product was crystallized from 200 mL of warm $CHCl_3$ to give compound **3**: yield 445 mg (19%); mp 350–360 °C dec. The combined filtrates were evaporated to dryness. The residue was triturated with hot $CHCl_3$ (75 mL) and the insoluble material was collected on a filter. It was crystallized twice from MeOH to give compound **22**: yield 936 mg (40%); mp 352–358 °C dec.

N^6-(*N*-Allylcarbamoyl)adenine (20). Method C. A stirred mixture of 1.04 g (5.0 mmol) of urethane V[7] and 0.75 mL (10 mmol) of allylamine in 50 mL of anhydrous pyridine was heated in a glass bomb at 120 °C for 6 h. After evaporating to dryness, the residue was azeotroped with toluene (10 mL) and then crystallized from 100 mL of hot EtOH (yield 414 mg). An additional crop (222 mg) was obtained from the filtrate. The total yield was 636 mg (58.9%): mp >300 °C dec; IR max (cm^{-1}) 1680 (ureido C=O), 1620 and 1550 (C=C, C=N); NMR (CF_3COOD, external Me_4Si) δ 4.0 (d, 2, *J* = 5 Hz, =CH_2), 5.15 (m, 1, –CH=), 5.38 (q, 2, *J* = 4 Hz, *J* = 1.5 Hz, –NHCH_2–), 8.89 (s, 1, 8-H), and 8.94 (s, 1, 2-H).

N^6-Carbamoyladenines (6-Ureidopurines). Method D. These compounds (III) were also prepared by hydrolyzing the disubstituted compounds IV (Scheme I) in 1 N NaOH at room temperature overnight or by refluxing in H_2O for 15 min. The yields were excellent (~85%).

6-Amino-*N*-L-phenylalanyl-9*H*-purine-9-carboxamide (11). Method E. A solution of compound **12** (600 mg) in a mixture of EtOH and AcOH (1:1, 50 mL) containing 250 mg of Pd/C (10%, 250 mg) was hydrogenated at room temperature in a Parr apparatus at 50 psi for a period of 24 h. The catalyst was removed and the filtrate was diluted with 200 mL of absolute ethanol and cooled at 4 °C for 4 h. The product that separated out was collected on a filter and washed with ethanol and then with ether: yield 350 mg (74%); mp 310–315 °C dec; NMR δ 3.28 (d, 2, *J* = 7 Hz, –C$H_2C_6H_5$), 4.80 (m, 1, –CH), 7.33 (s, 5, C_6H_5), 7.73 (s, 2, –NH_2), 8.70 (s, 1, 2-H), 8.97 (s, 1, 8-H), and 9.45 (d, 1, *J* = 3 Hz, –NH).

***N*-[9-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosylpurin-6-yl)-carbamoyl]-*p*-chloroaniline (38). Method F.** A stirred mixture of 0.982 g (2.5 mmol) of 2′,3′,5′-tri-*O*-acetyladenosine and 0.768 g (5.0 mmol) of *p*-chlorophenyl isocyanate in 20 mL of anhydrous pyridine was heated in a glass bomb at 90 °C for 6 h. The reaction mixture was evaporated to dryness and the last traces of pyridine were azeotroped with anhydrous toluene (25 mL). The residue

was dissolved by warming in 50 mL of methanol and decolorized with activated charcoal, and the clear solution was concentrated to a small volume and chilled at 0 °C overnight. The crystalline product was collected on a filter, washed with methanol, and recrystallized once again from methanol: yield 0.870 g (64%); mp 175–176 °C.

N-[9-(β-D-Ribofuranosylpurin-6-yl)carbamoyl]-p-chloroaniline (37). Method G. Compound **38** (500 mg) was dissolved in 20 mL of 4 N NH_3–MeOH and the solution was stirred at 4 °C for 16 h. The precipitate was collected on a filter, washed with methanol, and recrystallized twice from anhydrous methanol: yield 296 mg (76.9%); mp 191–192 °C; NMR δ 6.20 (d, 1, J = 6 Hz, 1′-H), pair of doublets centered at 7.47 and 7.80 (4, J = 9 Hz in each case, phenyl protons), 9.16 (s, 1, 2-H), 9.25 (s, 1, 8-H), 10.30 (s, 1, -NH), and 12.10 (s, 1, -NH).

N-[9-(β-D-Ribofuranosylpurin-6-yl)carbamoyl]neopentylamine (34). Method H. A stirred mixture of 930 mg (2 mmol) of the ethyl 9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-purine-6-carbamate[7] and 1 mL (excess) of neopentylamine in 50 mL of anhydrous pyridine was heated in a glass bomb at 120 °C for 6 h. After evaporating to dryness, the residue was azeotroped with toluene (10 mL) and stirred in 4 N NH_3–MeOH (40 mL) at room temperature for 5 h. The mixture was evaporated to dryness and the residue was crystallized from minimal amount of EtOH: yield 430 mg (56%); mp 194–195 °C; IR max (cm^{-1}) 2950 (CH), 1690 (ureido C=O), 1610 and 1570 (C=C, C=N).

N^9-(N-Methylthiocarbamoyl)adenine (15) and N^6-(N-Methylthiocarbamoyl)adenine (32). Method I. These two compounds were prepared employing a variation of method B. A solution of 30 mmol of adenine in 40 mL of anhydrous Me_2SO was allowed to react with 60 mmol of methyl isothiocyanate in the same way as in method B. Compound **15** crystallized out on cooling. Compound **32** was recovered after decomposing the residual compound **15** in the mother liquor by boiling with water.

N^9-Acetyladenine (17). Method J. To a solution of 1.35 g (10 mmol) of adenine in anhydrous Me_2SO (50 mL) was added 20 mL of anhydrous pyridine and 5 mL of Ac_2O, and the mixture was stirred at room temperature for 16 h. The precipitated product was filtered and washed with cold pyridine and ether: yield 1.54 g (87.0%); mp 362–364 °C dec; IR max (cm^{-1}) 3500 (NH_2), 1730 (C=O), 1660 and 1610 (C=C, C=N); NMR δ 3.23 (s, 3, $-COCH_3$), 7.87 (br, 2, $-NH_2$), 8.70 (s, 1, 2-H), and 9.02 (s, 1, 8-H).

N^9-Acetyladenine (17). Method K. To a solution of 1.0 g of adenine in 100 mL of hot dimethylacetamide was added a solution of thallium(I) ethoxide in ethanol (1.0 mL), and the mixture was stirred at room temperature for 5 h. The precipitated thallium(I) salt of adenine was filtered, washed with EtOH, and dried. To a suspension of the thallium(I) salt of adenine (1.0 g) in 25 mL of anhydrous DMF was added acetyl chloride (0.5 mL), and the mixture was stirred at room temperature for 8 h. The precipitated thallium(I) chloride was removed by filtration and the filtrate was concentrated to a small volume under 45 °C and kept at 0 °C overnight. The crystalline product was filtered and washed with ether: yield 250 mg (38%); mp 362–364 °C; the IR and UV spectra of this product were identical with those of the compound **17**, prepared by method J as described above.

N^6,N^9-(Di-N-phenylcarbamoyl)adenine (43). Method L. Adenine (1.35 g, 10 mmol) was dissolved in hot anhydrous Me_2SO (30 mL). The solution was brought to room temperature, phenyl isocyanate (2.62 g, 22 mmol) was added, and the mixture was stirred at room temperature overnight. The precipitated product was collected on a filter and washed with toluene. Recrystallization of this material from THF gave the compound **43**: yield 2.70 g (72%); mp 350–352 °C; IR max (cm^{-1}) 1740, 1690 (C=O), 1610, 1595, 1550 (C=C, C=N), and 750 (C_6H_5); NMR δ 7.34–8.10 (m, 10, C_6H_5), 9.03 (s, 1, 8-H), 8.83 (s, 1, 2-H), 10.30 (m, 1, -NH), and 11.58 (m, 1, -NH).

N^6-(N-o-Fluorophenylcarbamoyl)-N^9-(N-tert-butylcarbamoyl)adenine (50). Method M. To a solution of 1.17 g (5 mmol) of N^9-(N-*tert*-butylcarbamoyl)adenine (**3**) in 20 mL of anhydrous Me_2SO was added 0.82 g of *o*-fluorophenyl isocyanate (6 mmol) and the mixture was stirred at room temperature for 4 h. The precipitated product was filtered and recrystallized from 200 mL of $CHCl_3$: yield 1.21 g (65%); mp >350 °C dec; NMR δ 1.75 [s, 9, $-C(CH_3)_3$], 8.03 (m, 3, phenyl protons), 8.66 (m, 1, phenyl proton adjacent to fluorine), 8.93 (s, 1, 2-H), 9.05 (s, 1, 8-H), 10.79 (br, 1, -NH), and 12.16 (s, 1, -NH).

In an alternate procedure, the same material was obtained in 68.5% yield by reacting N^6-(N-*o*-fluorophenylcarbamoyl)adenine[8] with *tert*-butyl isocyanate in anhydrous Me_2SO at room temperature for 6 h.

N^6-Methyl-N^6,N^9-di(N-methylcarbamoyl)adenine (52). Method N. A mixture of 0.298 g (2 mmol) of 6-methylaminopurine and 1.14 g (20 mmol) of methyl isocyanate in 60 mL of anhydrous toluene was heated with stirring at 90 °C in a bomb for 30 h. The reaction mixture was cooled to room temperature and the precipitated crystalline product was collected on a filter. Additional product was obtained after evaporation of the solvent. The combined material was recrystallized from hot DMF: yield 0.492 g (93%); mp 304–306 °C dec; IR max (cm^{-1}) 3300–3200 (NH), 1750, 1700 (C=O), 1540 (C=N, C=C); NMR δ 3.23 (d, 3, J = 5 Hz, $-NHCH_3$) 3.43 (d, 3, J = 5 Hz, $-NHCH_3$), 4.13 (s, 3, $-NCH_3$), 9.13 (s, 1, 2-H), and 9.23 (s, 1, 8-H).

Ethyl 6-Methylaminopurine-9-carboxylate (57). To a stirred suspension of 0.149 g (1.0 mmol) of 6-methylaminopurine in 4 mL of freshly distilled anhydrous triethylamine, 0.1 mL (~1 mmol) of ethyl chloroformate was added dropwise at a temperature of −60 °C. After the addition, the mixture was slowly allowed to warm to −5 °C and was stirred at that temperature for 70 min. The reaction mixture was then stirred at room temperature overnight. The precipitated material was collected on a filter and washed with triethylamine and acetone. The product was crystallized from 10 mL of an EtOAc extract of this precipitate. Concentration of the combined filtrates furnished another crop of this product. The two crops were combined and recrystallized from anhydrous ether to give compound **57**: yield 0.071 g (32%); mp 150–151 °C; IR max (cm^{-1}) 3350 (NH), 1770 (C=O), 1630, 1580, 1530 (C=N, C=C); NMR δ 1.63 (t, 3, J = 7 Hz, $-CH_2CH_3$), 3.33 (d, 3, J = 6 Hz, $NHCH_3$), 4.83 (q, 2, J = 7 Hz, $-CH_2CH_3$), 8.43 (m, 1, -NH), 8.83 (s, 1, 2-H), and 8.98 (s, 1, 8-H).

N^6-Methyl-N^9-(N-methylcarbamoyl)adenine (58). Method O. To a suspension of 0.149 g (1.0 mmol) of 6-methylaminopurine in 20 mL of anhydrous toluene was added 0.57 g (10 mmol) of methyl isocyanate, and the mixture was stirred at room temperature in a sealed tube for 70 h. The thick white precipitate was collected on a filter and washed first with 40 mL of toluene and then with 6 mL each of acetone and anhydrous ether: yield 0.183 g (89.0%); mp 317–318 °C; IR max (cm^{-1}) 3300–3200 (NH), 1720 (C=O), 1640, 1550, 1490 (C=N, C=C).

N^6-Methyl-N^6-(N-methylcarbamoyl)adenine (60). Compound **52** (50 mg) was suspended in 25 mL of concentrated NH_4OH solution and the mixture was refluxed in an oil bath for 5 min. An insoluble white residue was filtered and the filtrate was evaporated to dryness to yield 34 mg (87.0%) of compound **60**: mp 320–321 °C; IR max (cm^{-1}) 3500–3300 (NH), 1670 (C=O), 1550 (C=N, C=C); UV λ max (nm) 283 (water), 286 (0.1 N HCl), and 285 (0.1 N NaOH); NMR δ 3.13 (d, 3, J = 5 Hz, $-NHCH_3$), 4.13 (s, 3, $-NCH_3$), 8.83 (s, 1, 8-H), and 9.03 (s, 1, 2-H).

Attempted Reduction of N^6,N^9-Diacetyladenine (VII, R = CH_3) to N^6,N^9-Diethyladenine. A solution of 500 mg of VII (R = CH_3) in 75 mL of anhydrous THF was slowly added at room temperature to a suspension of 800 mg of $LiAlH_4$ in 50 mL of anhydrous THF with stirring. After the addition the reaction mixture was refluxed for 5 h. The excess $LiAlH_4$ was decomposed by careful addition of water, the precipitated inorganic materials were removed by filtration, and the filtrate was evaporated to dryness to yield 343 mg (92.1%) of a product which by TLC in solvents A, B, and C was found to be identical with 6-ethylaminopurine. The UV spectrum of this product with maxima at 267 (H_2O), 268 (0.1 N HCl), and 274 nm (0.1 N NaOH) was also identical with that of 6-ethylaminopurine.

4,6-Dimethylureido-5-formylaminopyrimidine (XIII, 61). To a solution of 500 mg (3.2 mmol) of 4,6-diamino-5-formylaminopyrimidine (XII) in 10 mL of anhydrous Me_2SO was added 798 mg (14 mmol) of methyl isocyanate and the mixture was stirred overnight at room temperature. The white crystalline product was collected on a filter, washed with toluene, and recrystallized from a mixture of Me_2SO–EtOH to give XIII: yield 512 mg (58.7%); mp >380 °C; UV λ max (nm) 268 (ε 8200) in water, 274 (ε 18 240) in 0.1 N HCl, and 272 (ε 7670) in 0.1 N NaOH;

NMR δ 2.93 (d, 3, J = 5 Hz, -NHCH_3), 3.06 (d, 3, J = 5 Hz, -NHCH_3), 7.12 (br, 2, -NH), 7.34 (d, 1, J = 6 Hz, -NHCHO), 8.43 (s, 1, 2-H), 9.22 (br, 1, -NH), 9.40 (s, 1, -NH), and 9.73 (d, 1, J = 5 Hz, -NHCHO).

Attempted Ring Closure of 4,6-Dimethylureido-5-formylaminopyrimidine (XIII) to Give N^6-Di(N-methylcarbamoyl)adenine (40). To a mixture of 269 mg (1 mmol) of XIII and 852 mg (6 mmol) of phosphorous pentoxide cooled in an ice bath was added 1.20 mg of 85% phosphoric acid, and the mixture was stirred at 25 °C for 3 h and then at 150 °C for 3.5 h. The reaction mixture was cooled to room temperature and poured into 100 mL of ice water. The brown solution was carefully neutralized to pH 6.5, when a off-white solid slowly precipitated. This material was filtered, washed with water, and dried under vacuum: yield 85.0 mg; mp >350 °C dec. The product was identified as N^6-(N-methylcarbamoyl)adenine by comparison with the authentic material[8] in solvents A, B, and C in TLC and in UV spectra: λ max (nm) 269, 276 (water), 277 (0.1 N HCl), and 277 (0.1 N NaOH). TLC of the filtrate showed more of this material; however, no desired disubstituted product could be detected. A lower reaction temperature or time did not lead to the formation of the desired disubstituted product.

Growth Inhibition. These compounds were evaluated for their growth-inhibitory activity in cultured cells derived from the buffy coats of a normal individual (Nc 37) and a patient with myeloblastic leukemia (RPMI 6410) and also in cultures of L1210 mouse leukemia.[8] Compounds were dissolved in 0.5% Me_2SO in growth medium (RPMI 1640 + 10% fetal calf serum) at 10^{-4} M and the results are expressed as percent of viable cell number relative to controls containing 0.5% Me_2SO after 72 h of incubation. At the concentration used, Me_2SO did not affect cell growth. Results are shown in Tables I-VI.

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Synthetic Models of Deoxyribonucleic Acid Complexes with Antimalarial Compounds. 3. Forces Involved in the Stacking Interaction between Aminoquinoline and the Nucleotide Bases

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As an approach to the problem of the nature of the forces responsible for the stacking interactions between the aminoquinoline ring of the antimalarial chloroquine and the monomeric nucleotide bases, we have examined models in which the aromatic nucleus of the drug is linked to the nucleotide bases by a trimethylene chain. The degree of stacking of the models was determined in different conditions of solvent, pH, and temperature by hypochromism measurement in the UV. The results show that forces of the donor-acceptor type, due to the presence of a positive charge on the quinoline ring at neutral pH, do not bring an important contribution to the stacking interaction between the aminoquinoline and the nucleotide bases, while the influence of the solvent water is fundamental.

The antimalarial chloroquine (1) is one of a series of small, positively charged molecules which interact with nucleic acids.[1] Binding models have been proposed in which ionic interactions between the diethylamino nitrogen of the drug and the phosphate groups of DNA add to ring-ring stacking interactions between the aminoquinoline and the nucleotide bases to account for the complexation.[2]

In vitro the binding of chloroquine with nucleic acids is observed in water and the species which interacts is a diprotonated one in which the 4-aminoquinoline moiety

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