The Synthesis of 3-(2′-Deoxy-D-ribofuranosyl)adenine. Application of a New Protecting Group, Pivaloyloxymethyl (Pom)[1]

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Abstract: Alkylation of adenine with chloromethyl pivalate (**6**) gives either 7- or 9-pivaloyloxymethyladenine (**1c** and **8**, respectively) as the main product depending on the conditions employed. This pivaloyloxymethyl protecting group (Pom) may be removed under mildly basic conditions, which makes it highly suitable for the synthesis of sensitive nucleosides. 3-Substituted adenine derivatives are readily prepared by alkylating 7-pivaloyloxymethyladenine (**1c**) and then deblocking the 3,7-disubstituted intermediates with methanolic ammonia at room temperature. Using this procedure, 3-benzyladenine (**12a**), 3-benzyloxymethyladenine (**12b**), and the α and β anomers of 3-(2′-deoxy-D-ribofuranosyl)adenine (**16**α and **16**β) were readily synthesized. The sensitive 1-benzyloxymethyladenine (**15**) was made by an analogous procedure from 9-pivaloyloxymethyladenine (**8**). When 7-pivaloyloxymethyladenine derivatives were treated with ethanolic sodium hydroxide, rapid intramolecular migration of the pivaloyl group was observed and N^6-pivaloylated products could be isolated in good yield.

Among the numerous, ubiquitous purine derivatives which are naturally occurring, substituents are normally located on the imidazole ring, attached to the 7- or 9-nitrogen. 3 Substitution, exemplified by triacanthine (3-(γ,γ-dimethylallyl)adenine)[2-5] and 3-ribosyluric acid,[6] is considered exceptional. However, enzymic pathways to 3-substituted purines do exist, as shown by the formation of 3-ribosyl and 3-(2′-deoxyribosyl) derivatives of xanthine, hypoxanthine, and related purines,[7,8] and the 3 position is the preferred site of attack in the direct, chemical alkylation of adenine under approximately neutral conditions.[2,9-18] Earlier considerations of this type together with the similarity in over-all structure of the 3-substituted adenine derivatives to the corresponding 9-substituted derivatives, which might give rise to interesting biological activity, led us to the synthesis of 3-β-D-ribofuranosyladenine (3-isoadenosine)[14,15] and the corresponding 3-iso-AMP,[15] 3-iso-ADP, 3-iso-ATP, and NMN-3-iso-AMP derivatives.[19] The unusual biological activity found for these adenosine analogs[19-23] has prompted the synthesis of 3-β-(2′-deoxy-D-ribofuranosyl)adenine, the 3-iso analog of 2′-deoxyadenosine.

Several routes have been devised for the synthesis of 3-substituted adenines, including the method of Elion from alkylthioureas[24,25] and that of Denayer[3,26] involving the alkylation of a formamidopyrimidine precursor followed by cyclization under fairly vigorous (basic) conditions. Both methods are applicable to the preparation of relatively simple 3-substituted derivatives. While the direct alkylation of adenine, which in general may lead to a mixture of 1-, 9-, and 3-substituted products, with the latter predominating,[9] provided a facile method for the synthesis of 3-β-D-ribofuranosyladenine, it was not readily applicable to the corresponding 2′-deoxy derivative. The lability of 2′-deoxyribosyl halides[27a] and 2′-deoxyribonucleosides[27b] and the low stability of the 3-1′ glycosidic linkage to both basic and acidic hydrolytic conditions[15] combined to thwart this approach. The relatively mild conditions required to alkylate 7-substituted adenines and the exclusive formation of 3,7-disubstituted products in good yields[9] offered a more promising route. 7-α- and 7-β-D-ribofuranosyladenines have been synthesized by a complementary procedure from 3-benzyladenine.[12]

1-Chloro-3,5-di-O-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranoside (**2**)[28] reacted rapidly with 7-benzyladen-

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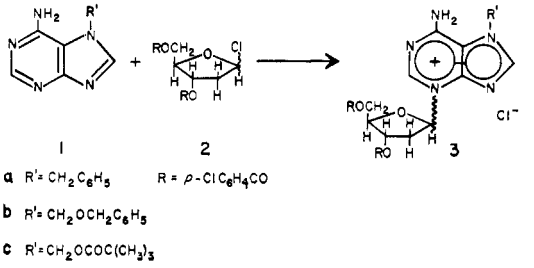
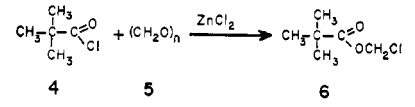


a R' = $CH_2C_6H_5$ R = p-ClC_6H_4CO

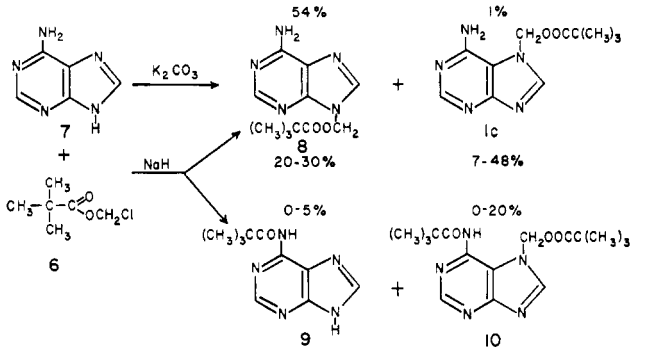
b R' = $CH_2OCH_2C_6H_5$

c R' = $CH_2OCOC(CH_3)_3$

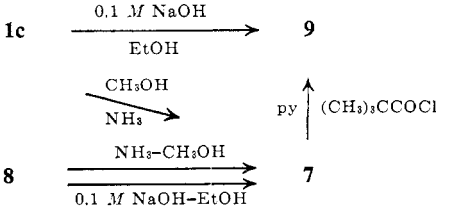
ine (**1a**)[3,29] in anhydrous acetonitrile at 60° to give up to 42% of pure 3-(3′,5′-di-O-(*p*-chlorobenzoyl)-2′-deoxy-D-ribofuranosyl)-7-benzyladenine hydrochloride (**3a**), which precipitated directly from the reaction medium within 12–15 min. If the reaction was allowed to proceed for longer periods, increasing contamination of **3a** by 7-benzyladenine hydrochloride was observed. This decomposition of **3a** to 7-benzyladenine hydrochloride and a dihydrofuran derivative, and further to a furan (not isolated) and *p*-chlorobenzoic acid, was complete within about 1.75 hr at 60°. Similar eliminations have been observed[27a,30] with other 2-deoxyribosyl derivatives. Attempts to remove the 7-benzyl substituent from the protected nucleoside (**3a**) or the corresponding partially deblocked nucleoside (**3**, R′ = CH_2-C_6H_5; R = H) by hydrogenolytic (H_2–Pd in ethanol or acetic acid),[9,12,31,32] reductive (Na–NH_3[32] or K–dimethoxyethane), or oxidative ($Pb(OAc)_4$, SeO_2, I_2) processes were unsuccessful. Rupture of the 3–1′ glycosidic bond occurred more readily than removal of the benzyl group. Difficulty in removing N-benzyl substituents from other purine nucleoside derivatives has been encountered previously.[33a] The preparation and use of the more readily cleaved 7-benzyloxymethyladenine (**1b**) also gave discouraging results. Accordingly, a search was made for a protecting group that could be removed under mildly basic conditions; 3-(2′-deoxyribofuranosyl)-7-alkyladenine derivatives (**3a** and **3b**) were found to be particularly sensitive to acidic conditions. 7-(N,N-Diphenylcarbamoyl)adenine was prepared, in low yield, by a modification of the general Denayer–Montgomery synthesis of 7-substituted[3,33b] adenines. The carbamoyl group was found to be readily removed by methanolic ammonia at room temperature, but alkylation of this carbamoyladenine derivative was difficult owing to deactivation of the ring system by the acyl group. Attention was then directed to the possibility of utilizing an acyloxymethyl group, $RCOOCH_2$–, which should have the activity and directive properties of an alkyl substituent but should be readily cleaved under mildly basic conditions. Chloromethyl esters such as chloromethyl benzoate are well known, but attempts to prepare 7-benzoyloxymethyladenine by reaction of chloromethyl benzoate with the sodium salt of 4,6-diamino-5-formamidopyrimidine[3] resulted in attack at the carbonyl carbon, *i.e.*, acylation, rather than attack at the chloromethyl carbon. To bypass this difficulty, chloromethyl pivalate (**6**) was synthesized from pivaloyl chloride (**4**) and paraformaldehyde (**5**). Since this reagent has a sterically hindered ester group, reaction with bulky nucleophiles should occur at the chloromethyl



group, while small nucleophiles such as water, hydroxide ion, and ammonia could attack the ester function. Alkylation of 4,6-diamino-5-formamidopyrimidine to give the N-pivaloyloxymethylformamido derivative proceeded readily, but attempts to effect cyclization of the intermediate under a wide variety of conditions failed to give the desired 7-pivaloyloxymethyladenine (**1c**) owing to the base sensitivity of the pivaloyloxymethyl group.



The alkylation of various purine derivatives under basic conditions has been reported to give both 7- and 9-substituted products,[2,29] with the 7 isomer predominating under some conditions. The reaction of adenine with chloromethyl pivalate in the presence of anhydrous potassium carbonate gave 9-pivaloyloxymethyladenine (**8**) (54%) along with a small amount (1%) of the 7 isomer **1c**; the structures of these isomeric products were established by ultraviolet and nmr spectroscopy.[34] These conditions provide a facile route to **8**. A more complex mixture was obtained from the addition of chloromethyl pivalate to sodium adenide, comprising four products: 9-pivaloyloxymethyladenine (**8**) (20–40%), 7-pivaloyloxymethyladenine (**1c**) (7–18%), N^6-pivaloyl-7-pivaloyloxymethyladenine (**10**) (10–20%), and N^6-pivaloyladenine (**9**) (0–10%). The structures of the latter two compounds, **9** and **10**, were confirmed by independent synthesis from adenine (**7**) and 7-pivaloyloxymethyladenine (**1c**), respectively.



The pivaloyloxymethyl derivatives were tested for cleavage under basic conditions. Methanolic ammonia at room temperature converted both 7- and 9-pivaloyloxymethyladenine to adenine, and times for half-

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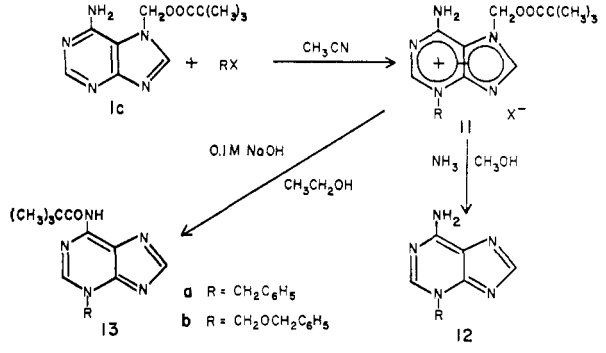
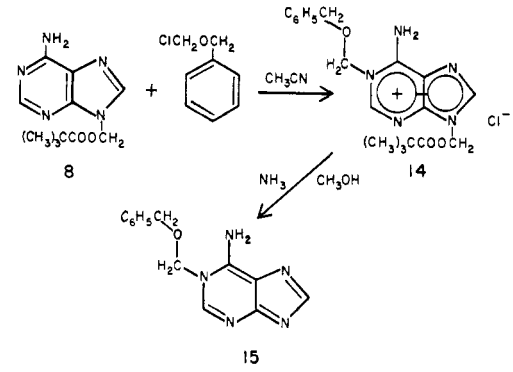
completion were about 3 min and 1 hr, respectively. 9-Pivaloyloxymethyladenine (**8**) reacted very rapidly (half-complete in *ca.* 30 sec) with 0.1 *M* ethanolic sodium hydroxide to give adenine; 7-pivaloyloxymethyladenine (**1c**) reacted even more rapidly (half-complete in <10 sec) giving N^6-pivaloyladenine (**9**). The migration of the pivaloyl group occurred virtually quantitatively even in very dilute solution, as followed by ultraviolet spectroscopy, indicating an intramolecular migration.

The formation of N^6-pivaloylated products **9** and **10** during the alkylation of sodium adenide with chloromethyl pivalate (**6**) can thus be explained as arising from rearrangement of initially formed 7-pivaloyloxymethyladenine (**1c**) to N^6-pivaloyladenine (**9**), which can be further alkylated, this time apparently exclusively in the 7 position, giving N^6-pivaloyl-7-pivaloyloxymethyladenine (**10**). It is attractive to consider a cyclic mechanism for the rearrangement in ethanolic sodium hydroxide, including bond formation from N^6 to the carbonyl carbon, liberation of formaldehyde, and formation of the anion of N^6-pivaloyladenine. No N^6-pivaloyl-9-pivaloyloxymethyladenine, synthesized independently by pivaloylation of 9-pivaloyloxymethyladenine (**8**) in pyridine, was detected in the crude product from the reaction described above. Improved conditions for obtaining 7-pivaloyloxymethyladenine (**1c**) in preponderance over the 9 isomer were found by avoiding excess alkalinity during the course of the reaction. Thus, inverse addition of sodium adenide to excess chloromethyl pivalate resulted in an enhanced yield (48%) of 7-pivaloyloxymethyladenine (**1c**), with no N^6-pivaloylated products and a reduced amount (20%) of the 9 isomer **8**.

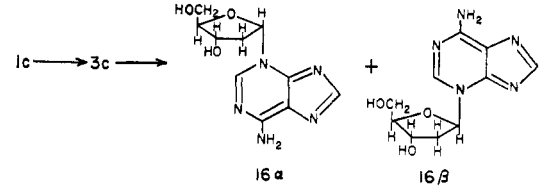
In order to test the alkylation of adenine protected by the 7-Pom group and then removal of the group, we examined this route to the known 3-benzyladenine. Alkylation of **1c** with benzyl bromide proceeded readily in refluxing acetonitrile to give 3-benzyl-7-pivaloyloxymethyladenine hydrobromide (**11a**), which was not



purified but was treated directly with methanolic ammonia to give 3-benzyladenine (**12a**) in 85% yield. The reaction of the intermediate **11a** with 0.1 *M* ethanolic sodium hydroxide gave 3-benzyl-N^6-pivaloyladenine (**13a**) (65%). The advantages of this method of synthesizing 3-substituted adenine derivatives were exemplified further in the facile preparation of 3-benzyloxymethyladenine (**12b**).[12] Analytically pure 3-benzyloxymethyl-7-pivaloyloxymethyladenine hydrochloride (**11b**) (74%) separated directly from the reaction of 7-pivaloyloxymethyladenine (**1c**) with benzyl chloromethyl ether in acetonitrile at 60°. The reaction of this product (**11b**) with methanolic ammonia gave the sensitive 3-benzyloxymethyladenine (**12b**)[12] in 75% yield. In contrast, direct alkylation of adenine with benzyl chloromethyl ether has been reported[12] to give only 6% of 3-benzyloxymethyladenine (**12b**).



The use of the Pom group for the synthesis of sensitive 1-substituted adenine derivatives was exemplified by the reaction of 9-pivaloyloxymethyladenine (**8**) with benzyl chloromethyl ether to give 1-benzyloxymethyl-9-pivaloyloxymethyladenine hydrochloride (**14**) (53%), which was converted by methanolic ammonia to 1-benzyloxymethyladenine (**15**), isolated in 30% yield.



Returning to the problem of the synthesis of 3-(2′-deoxy-D-ribofuranosyl)adenine, which was not readily obtainable by direct alkylation of adenine, we were able to achieve the synthesis by application of the 7-Pom protecting group. The alkylation of 7-pivaloyloxymethyladenine (**1c**) with 1-chloro-3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranoside[28] proceeded rapidly in acetonitrile at 60° to give, following low-temperature work-up, the unstable intermediate **3c** as a paste containing about 10–20% of 7-pivaloyloxymethyladenine hydrochloride. This crude product was treated with methanolic ammonia at room temperature to give a mixture of adenine (**7**) and 3-(2′-deoxy-D-ribofuranosyl)-adenine (**16**). Chromatography[35] of this mixture on a column of Dowex 1-X8 OH^- removed the adenine and effected separation of the α- and β-nucleoside anomers. 3-α-(2′-Deoxy-D-ribofuranosyl)adenine (**16α**) was eluted fairly rapidly and sharply with the initial methanol–water (2:3) fractions. Crystallization from absolute ethanol gave the pure α anomer as colorless needles, mp 184–185°, in 12–17% yield from **1c**. Elution of the column with a large volume of methanol–water (2:3) then gave the β anomer (**16β**) (5%) mixed with a small amount of an easily oxidized contaminant, probably a sugar degradation product. Several recrystallizations from absolute ethanol and decolorization gave pure 3-β-(2′-deoxy-D-ribofuranosyl)adenine as needles, mp 187–188°. The assignment of the structures **16α** and **16β** to the above two compounds followed from their microanalytical data and ultraviolet spectra,[34] which estab-

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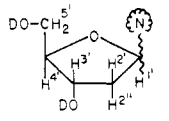
lished them as 3-(2′-deoxyribosyl)adenines. Confirmation of the 2′-deoxyribofuranosyl structure and the assignment of configuration at the anomeric center were accomplished by nmr spectroscopy according to the observations of Robins and Robins[36] and by the direct comparison of the spectra of **16**α and **16**β (see Table I) with the recorded nmr spectra of α- and β-thymidine.[37]

Table I. Nmr Spectral Data[a] for the Anomeric 3-(2′-Deoxy-D-ribofuranosyl)adenines



Proton(s)	α Anomer (**16**α)[b] Chemical shift[d]	Coupling constants[e]	β Anomer (**16**β)[c] Chemical shift[d]	Coupling constants[e]
H^2	8.37		8.58	
H^8	7.82		8.01	
$H^{1'}$	6.39		6.56	
$H^{1'}$–$H^{2'}$		6.8		
				6.5 av
$H^{1'}$–$H^{2''}$		2.0		
$H^{2'}$	2.92		2.72	
$H^{2'}$–$H^{2''}$		−15.0		*f*
$H^{2''}$	2.47		2.72	
$H^{2'}$–$H^{3'}$		6.0		
				5.2 av
$H^{2''}$–$H^{3'}$		2.0		
$H^{3'}$	*ca.* 4.6		4.67	
$H^{3'}$–$H^{4'}$		*f*		*ca.* 3.5
$H^{4'}$	*ca.* 4.6		*ca.* 4.3	
$H^{4'}$–$H^{5'}$		*f*		*ca.* 3.6
$2H^{5'}$	3.79		3.94	

[a] Analyzed on a first-order basis. [b] Solvent D_2O at *ca.* 25°. [c] Solvent D_2O at *ca.* 83°. [d] The values quoted are δ values measured from an internal dioxane standard with assigned δ 3.77 ppm. [e] Coupling constants are in cps with an accuracy of ±0.2 cps except where denoted as "*ca.*," in which case the probable error is greater. [f] Not derivable from the spectrum.

Experimental Section[38]

3-(3′,5′-Di-*O*-(*p*-chlorobenzoyl)-2′-deoxy-D-ribofuranosyl)-7-benzyladenine Hydrochloride (3a). 1-Chloro-3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranoside (**2**)[28] (3.980 g) (9.3 mmoles) was added to a stirred solution of 2.025 g (8.9 mmoles) of 7-benzyladenine (**1a**)[9,29] in 450 ml of anhydrous acetonitrile. After 12 min at 53–56°, the reaction mixture was cooled rapidly to 12° and filtered to give 1.770 g (30%) of **3a** as colorless needles, mp 167–169°; λ_{max} 241 mμ ($\epsilon \times 10^{-3}$ 39.7), 279 (15.7), and 291 (sh, 9.5), λ_{min} 218 (20.8) and 264 (12.5); λ_{max} (pH 1) 241 (40.4), 279 (16.4), and 291 (sh, 9.5), λ_{min} 218 (21.2) and 264 (13.0); λ_{max} (pH *ca.* 12) 238 (37.2), 276 (sh, 13.8), and 281 (14.0), λ_{min} 227 (31.6) and 260 (10.0); nmr ($CDCl_3$): 2.90 (2 H, pt, J = 4.0, 2′-H_2), 4.55 (2 H, d, J = 4.6, 5′-H_2), 4.9 (1 H, m, 4′-H), 5.6 (1 H, m, 3′-H), 5.70 (2 H, s, $C_6H_5CH_2$), 6.35 (1 H, pt, J = 4.0, 1′-H), 7.28 (5 H, s, C_6H_5), 7.23–8.01 (*ca.* 10 H, m, C_6H_4ClCO–, 2-H and 8-H).

Anal. Calcd for $C_{31}H_{26}Cl_2N_5O_5$: C, 56.87; H, 4.01; N, 10.74. Found: C, 56.65; H, 3.98; N, 10.78.

7-Benzyloxymethyladenine (1b). Benzyl chloromethyl ether (5.0 ml) (0.036 mole) was added to a stirred suspension of 4.01 g (0.026 mole) of 4,6-diamino-5-formamidopyrimidine and 3.6 g (0.026 mole) of anhydrous potassium carbonate in 450 ml of dry dimethylformamide, and the resulting suspension was stirred vigorously for 19 hr at 25°. A further 3.7 g (0.027 mole) of potassium carbonate was then added, and the reaction mixture was heated slowly to reflux. After cooling, the orange reaction mixture was filtered, and the filtrate was evaporated *in vacuo*. The residue was dissolved in 5:1 chloroform–ethanol and chromatographed on 200 g of silica gel. The crude product (1.46 g, 22%) eluted with 10–20% ethanol in ethyl acetate was recrystallized from ethanol–acetone as nearly colorless needles of **1b**, mp 208–212°; λ_{max} 228 mμ (sh, $\epsilon \times 10^{-3}$ 8.8) and 282 (b, 11.4), λ_{min} 247 (3.2); λ_{max} (pH 1) 277 (16.8), λ_{min} 238 (3.8); λ_{max} (pH 13) 279 (11.3), λ_{min} 247 (3.2); nmr (DMSO): 4.58 (2 H, s, $C_6H_5CH_2O$), 5.87 (2 H, s, NCH_2O), 6.85 (2 H, bs, NH_2), 7.32 (5 H, s, C_6H_5), 8.29 and 8.47 (1 H each, s, s, purine H's).

Anal. Calcd for $C_{13}H_{13}N_5O$: C, 61.16; H, 5.13; N, 27.44. Found: C, 60.91; H, 5.01; N, 27.22.

Hydrogenolytic cleavage of **1b** to form adenine using 10% Pd–C and 60 psi hydrogen atmosphere proceeded to the extent of about 20% in ethanol after 24 hr at 25° and to about 50% in glacial acetic acid after about 3 hr at 25°.

7-(N,N-Diphenylcarbamoyl)adenine. Sodium hydride (0.361 g, 59% in oil) (8.9 mmoles) and 1.182 g (7.7 mmoles) of 4,6-diamino-5-formamidopyrimidine were heated together in 75 ml of dry dimethylformamide at 50–60° for 30 min. N,N-Diphenylcarbamoyl chloride (2.018 g) (8.7 mmoles) was then added, and the resulting reaction mixture was stirred for 1 hr at 70°. After cooling, the yellow reaction mixture was filtered, and the filtrate was evaporated *in vacuo* to give a yellow paste, which was triturated with acetone–ethyl acetate and the insoluble material removed. The filtrate was chromatographed on 200 g of silica gel. The crude product was eluted with 1:19 ethanol–ethyl acetate and crystallized from methanol as fawn-colored rods (0.215 g, 8.4%). Recrystallization from methanol gave 0.101 g of analytically pure 7-(N,N-diphenylcarbamoyl)adenine as colorless rods, mp 259–260°; ν_{max} 3310 and 3170 cm^{-1} (b, st, NH_2), 1719 (st, >NCON<); λ_{max} 256 mμ ($\epsilon \times 10^{-3}$ 21.8), λ_{min} 226 (15.2); λ_{max} (pH 1) 255 (21.4), λ_{min} 226 (16.5); λ_{max} (pH 12) (very unstable) *ca.* 242 (*ca.* 17.2), *ca.* 257 (*ca.* 16.7), and 278 (sh, 9.6), λ_{min} *ca.* 235 (*ca.* 17.0) and *ca.* 252 (*ca.* 16.5); nmr (DMSO): 7.32 (*ca.* 12 H, s, C_6H_5 and NH_2), 8.15 and 8.34 (1 H each, s, s, purine H's).

Anal. Calcd for $C_{18}H_{14}N_6O$: C, 65.44; H, 4.27; N, 25.44. Found: C, 65.52; H, 4.32; N, 25.21.

Degradation of 7-(N,N-diphenylcarbamoyl)adenine to adenine was complete (tlc) in less than 22 hr at 25° when dissolved in methanol saturated with ammonia.

Chloromethyl Pivalate (6). Anhydrous zinc chloride (*ca.* 0.1 g) was stirred with a solution of 24.3 g (0.81 mole) of paraformaldehyde in 97.0 g (0.81 mole) of pivaloyl chloride (Eastman) for 21 hr at 90–100°. Fractional distillation of the deeply colored reaction mixture gave 54.85 g (45%) of **6** as a colorless liquid, bp 71–75° (*ca.* 50 mm); $n^{22}D$ 1.4518; ν_{max} 1761 and 1110 cm^{-1} (st, ester), 710 (st, C–Cl); nmr (neat): 1.29 (9 H, s, $(CH_3)_3C$), 5.70 (2 H, s, OCH_2Cl).

Anal. Calcd for $C_6H_{11}ClO_2$: C, 47.85; H, 7.36. Found: C, 48.24; H, 7.53.

4,6-Diamino-5-(N-pivaloyloxymethylformamido)pyrimidine. To a stirred suspension of 1.072 g (7.8 mmoles) of anhydrous potassium carbonate and 1.168 g (7.6 mmoles) of 4,6-diamino-5-formamidopyrimidine in 250 ml of dimethylformamide was added 1.2 ml (8.3 mmoles) of chloromethyl pivalate. After 12 days of vigorous stirring at 25°, the reaction mixture was filtered, and the filtrate was evaporated *in vacuo*. The residual white paste was triturated with hot 1:1 chloroform–acetone, and the filtered solution was applied to a column of 100 g of silica gel. Elution with 1:19 ethanol–acetone gave 4,6-diamino-5-(N-pivaloyloxymethylformamido)py-

(36) M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 4934 (1965).

(37) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).

(38) All melting points are corrected. The infrared spectra were recorded as Nujol mulls on a Perkin-Elmer 337 grating infrared spectrophotometer at slow scan. The ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer on samples dissolved in 95% aqueous ethanol and 0.1 *M* hydrochloric acid in 95% aqueous ethanol (pH 1).[39] Since most of the compounds were unstable to basic conditions their spectra in alkaline solution (pH 12–13) was run as soon as possible after adding one drop of 4 *M* sodium hydroxide to an ultraviolet cell containing the compounds dissolved in 3 ml of 95% aqueous ethanol. The proton magnetic resonance spectra were determined with a Varian A-60A spectrometer. Unless otherwise stated chemical shifts are reported in δ units (ppm downfield from internal TMS) and coupling constants in cps with estimated maximum errors of ±0.2 cps. The following conventions and abbreviations are used: m = multiplet, pt = pseudo-triplet, t = triplet, d = doublet, s = singlet, b = broad, D = signal disappears on exchange with D_2O, sh = shoulder, st = strong.

Thin layer chromatography (tlc) was performed on Eastman silica gel strips (with fluorescent indicator) routinely using 1:19 to 1:4 methanol–chloroform solvent systems.

We are indebted to Mr. Josef Nemeth and his associates at the University of Illinois for the microanalyses.

(39) N. J. Leonard, K. L. Carraway, and J. P. Helgeson, *J. Heterocyclic Chem.*, **2**, 291 (1965).

rimidine which crystallized from chloroform–acetone as colorless needles; yield 1.045 g (52%); mp 236° dec; ν_{max} 3470, 3365, and 3200 (st, NH_2), 1727 and 1153 cm^{-1} (st, ester); λ_{max} *ca.* 252 mμ ($\epsilon \times 10^{-3}$ 3.7), λ_{min} *ca.* 245 (3.6); λ_{max} (pH 1) 221 (32.1) and 264 (10.9), λ_{min} 238 (3.4); λ_{max} (pH 12) (unstable) 269 (*ca.* 6.3), λ_{min} 242 (2.7).

Anal. Calcd for $C_{11}H_{17}N_5O_3$: C, 49.42; H, 6.52; N, 26.20. Found: C, 49.48; H, 6.27; N, 26.50.

Although this material possessed a sharp melting point (236° dec), was analytically pure, and appeared homogeneous by tlc in four different solvent systems, its nmr spectrum seemed to indicate the presence of two components in a ratio of about 2:1: nmr (DMSO-d_6): 1.13 (9.00 H, s, $(CH_3)_3C$), 5.41 (1.99 H, bs, NCH_2O), 6.15 (1.66 H, bs, D, NH), 6.35 (0.77 H, bs, D, NH), 7.78 (*ca.* 0.6 H, s), 7.82 (*ca.* 0.4 H, s), 7.91 (0.4 H, s), and 8.35 (0.6 H, s) (pyrimidine H and CHO?). Reaction in DMSO solution is a possibility.

9-Pivaloyloxymethyladenine (8). Chloromethyl pivalate (3.2 ml) (22.2 mmoles) was added to a stirred suspension of 2.81 g (20.8 mmoles) of adenine and 2.90 g (21 mmoles) of anhydrous potassium carbonate in 150 ml of dry dimethylformamide. The crude mixture was stirred for 94 hr at 25° and filtered; the filtrate was evaporated *in vacuo*. The residue was triturated with 3:1 chloroform–acetone, and the solution, after filtration, was applied to a column of 250 g of silica gel. Elution with acetone gave 9-pivaloyloxymethyladenine, which crystallized from acetone–cyclohexane as colorless needles, mp 197–198°, yield 2.20 g (43%); λ_{max} 210 mμ ($\epsilon \times 10^{-3}$ 21) and 258 (14.8), λ_{min} 225 (2.5); λ_{max} (pH 1) 256 (14.8), λ_{min} 226 (3.0); λ_{max} (pH 12) 259 (*ca.* 14, unstable); nmr ($CDCl_3$): 1.17 (9 H, s, $(CH_3)_3C$), 6.14 (2 H, s, NCH_2O), 6.30 (2 H, bs, NH_2), 8.05 and 8.39 (1 H each, s, s, purine H's). A further crop from the mother liquors raised the yield of **8** to 54%.

Anal. Calcd for $C_{11}H_{15}N_5O_2$: C, 53.00; H, 6.07; N, 28.10. Found: C, 53.04; H, 5.95; N, 27.96.

Further elution of the column with acetone gave a second component which crystallized from acetone as nearly colorless needles, mp 230–232°, of 7-pivaloyloxymethyladenine (**1c**) (see below), yield 0.068 g (1.3%).

7-Pivaloyloxymethyladenine (1c). Sodium hydride (10.21 g, 59% in oil) (0.25 mole) was caused to react with 33.19 g (0.25 mole) of adenine in 1.7 l. of dry dimethylformamide for 4.5 hr at 25°. To the resulting stirred suspension was added dropwise 40 ml (0.28 mole) of chloromethyl pivalate. After stirring for 18 hr at 25°, the reaction mixture was filtered, and the filtrate was evaporated *in vacuo*. The residue was triturated with 1 l. of hot 2:1 chloroform–acetone and, after filtration, the solution was chromatographed on 1 kg of silica gel.

Elution with 3:7 acetone–chloroform gave a yellow mobile gum which crystallized from cyclohexane as nearly colorless solvated needles, yield 12.34 g (15%), of N⁶-pivaloyl-7-pivaloyloxymethyladenine (**10**), mp 102–106°: λ_{max} *ca.* 250 mμ (sh, $\epsilon \times 10^{-3}$ 5.5), 274 (9.4), 307 (1.8), and 319 (1.5), λ_{min} *ca.* 237 (b, 4.7) and 315 (1.45); λ_{max} (pH 1) *ca.* 250 (sh, 5.5), 274 (9.5), 305 (1.6), and 318 (1.24), λ_{min} *ca.* 237 (b, 4.7) and 315 (1.2); λ_{max} (pH 12) (unstable) 310 (14.2), λ_{min} 260 (3.6); nmr ($CDCl_3$): 1.11 (9 H, s, $(CH_3)_3CCOO$), 1.41 (9 H, s, $(CH_3)_3CCON$), 6.26 (2 H, s, NCH_2O), 8.47 and 8.77 (1 H each, s, s, purine H's).

Anal. Calcd for $C_{16}H_{23}N_5O_3$: C, 57.64; H, 6.95; N, 21.01. Found [after drying at 110° (0.1)]: C, 57.59; H, 7.05; N, 20.94.

Elution with acetone gave 9-pivaloyloxymethyladenine (**8**) (see above), which crystallized from acetone as colorless needles, mp 197–199°, yield 17.41 g. A further crop from the mother liquors raised the yield to 31%. The final mother liquors were shown by tlc to contain two components with very close R_f's. The second component was not obtained pure but was identified as N⁶-pivaloyladenine (**9**) (see below) by nmr spectroscopy.

Elution with further quantities of acetone gave 7-pivaloyloxymethyladenine (**1c**), which crystallized from acetone as colorless needles, mp 232–234°, yield 7.55 g; λ_{max} 211 mμ ($\epsilon \times 10^{-3}$ 26), 245 (sh, 7.5), and 272 (11.2), λ_{min} 230 (5.3); λ_{max} (pH 1) 274 (19.1) and 282 (sh, 14.6), λ_{min} 237 (5.4); λ_{max} (pH 12, after 2 min) 280 (*ca.* 16.3, unstable); nmr (DMSO-d_6): 1.11 (9 H, s, $(CH_3)_3C$), 6.39 (2 H, s, NCH_2O), 8.23 and 8.47 (1 H each, s, s, purine H's). Further crops from the mother liquors raised the yield of **1c** to 19%.

Anal. Calcd for $C_{11}H_{15}N_5O_2$: C, 53.00; H, 6.07; N, 28.10. Found: C, 53.10; H, 6.12; N, 28.06.

Slow reverse addition of the sodium adenide suspension to a stirred solution of chloromethyl pivalate in dry dimethylformamide stirring for 16 hr at 25°, and then working up as above, gave 48% of **1c**, 20% of **8**, and none of the N⁶-pivaloylated products **9** and **10**.

N⁶-Pivaloyladenine (9). A solution of 1.75 g (13 mmoles) of adenine and 3.0 ml (20.8 mmoles) of pivaloyl chloride in 70 ml of dry pyridine was heated under reflux for 16 hr. After cooling, the reaction mixture was poured onto ice, and the resulting mixture was basified with saturated aqueous sodium bicarbonate. Chloroform extraction then gave, after evaporation of the dried organic phase, a light brown crystalline mass (2.43 g, 86%), which was recrystallized twice from chloroform to give colorless plates of N⁶-pivaloyladenine, mp 235°; ν_{max} 1680 cm^{-1} (st, amide); λ_{max} 210 mμ ($\epsilon \times 10^{-3}$ 19.9), 258 (sh, 6.5), 276 (sh, 12.3)), 280 (13.0), and 290 (9.0), λ_{min} 232 (3.3) and 288 (8.9); λ_{max} (pH 1) 210 (19.2), 275 (14.1), and 282 (14.0), λ_{min} 232 (3.5) and 279 (13.9); λ_{max} (pH 12) (unstable) 284 (13.6), λ_{min} 241 (2.0); nmr (DMSO-d_6): 1.35 (9 H, s, $(CH_3)_3CCON$), 8.49 and 8.70 (1 H each, s, s, purine H's).

Anal. Calcd for $C_{10}H_{13}N_5O$: C, 53.78; H 5.98; N, 31.95. Found: C, 54.67; H, 6.01; N, 32.23.

N⁶-Pivaloyl-7-pivaloyloxymethyladenine (10). To a solution of 0.256 g (1.03 mmoles) of 7-pivaloyloxymethyladenine (**1c**) in 5 ml of pyridine was added 0.13 ml (0.9 mmole) of pivaloyl chloride. The reaction mixture was refluxed for 2 hr, then cooled slowly to room temperature and poured into ice-cold aqueous sodium bicarbonate. The resulting mixture was extracted with chloroform which gave, after evaporation of the dried organic phase, a semisolid gum. This crude poduct was chromatographed on 20 g of silica gel. Elution with 1:24 ethanol–chloroform gave N⁶-pivaloyl-7-pivaloyloxymethyladenine (**10**), which crystallized from cyclohexane as colorless solvated needles, mp 103–107°, yield 0.325 g (<95%). This material had the same spectral characteristics as, and gave an undepressed mixture melting point, mmp 103–107°, with, the compound **10**, mp 102–106°, isolated from the alkylation of sodium adenide with chloromethyl pivalate (see above).

Anal. Calcd for $C_{16}H_{23}N_5O_3$: C, 57.64; H, 6.95; N, 21.01. Found [on the desolvated glass left after drying at 110° (0.1 mm) for 18 hr]: C, 57.35; H, 6.84; N, 20.81.

N⁶-Pivaloyl-9-pivaloyloxymethyladenine. To a solution of 0.229 g (0.92 mmole) of 9-pivaloyloxymethyladenine (**8**) in 5 ml of dry pyridine was added 0.20 ml (1.4 mmoles) of pivaloyl chloride. After refluxing for 5 hr, the reaction mixture was poured into cold aqueous sodium bicarbonate–carbonate buffer. Extraction of this alkaline solution with dichloromethane gave, following evaporation of the dried organic extract, N⁶-pivaloyl-9-pivaloyloxymethyladenine as a pale yellow mobile oil which crystallized on the addition of cyclohexane. Recrystallization of the crude product (0.253 g, 83%) from cyclohexane gave a colorless microcrystalline solid, mp 106–107°; ν_{max} 1745 and 1147 cm^{-1} (st, ester), 1700 (st, amide); λ_{max} 211 mμ ($\epsilon \times 10^{-3}$ 20.8), 257 (sh, 11.5), and 271 (16.8), λ_{min} 229 (3.3); λ_{max} (pH 1) 212 (21.0), 259 (sh, 9.6), and 278 (15.3), λ_{min} 234 (3.9); λ_{max} (pH 12) (unstable) *ca.* 204 (13.8), λ_{min} *ca.* 247 (*ca.* 3.6); nmr ($CDCl_3$): 1.17 (9 H, s, $(CH_3)_3CCOO$), 1.42 (9 H, s, $(CH_3)_3CCON$), 6.21 (2 H, s, NCH_2O), 8.48 (1 H, vbs, NH), 8.25 and 8.78 (1 H each, s, s, purine H's).

Anal. Calcd for $C_{16}H_{23}N_5O_3$: C, 57.64; H, 6.95; N, 21.01. Found: C, 57.46; H, 6.96; N, 20.83.

Alkaline Degradation of 9-Pivaloyloxymethyladenine (8). 9-Pivaloyloxymethyladenine (**8**) was dissolved in ammonia-saturated methanol at 25°, and the reaction was monitored by tlc. Adenine, identified by ultraviolet spectroscopy and by tlc, was the sole ultraviolet-absorbing product, and the reaction was observed to be half complete in *ca.* 1 hr, with complete reaction in less than 10 hr.

The degradation of **8** in 0.1 *M* ethanolic sodium hydroxide at 25° also gave adenine as the sole ultraviolet-absorbing product, with the reaction half-complete in *ca.* 30 sec and complete within 5 min.

Alkaline Degradation of 7-Pivaloyloxymethyladenine (1c). The reaction of 7-pivaloyloxymethyladenine (**1c**) in ammonia-saturated methanol at 25° was shown by tlc to give adenine and a trace of N⁶-pivaloyladenine and was half-complete in *ca.* 3 min and complete within 30 min.

To a solution of 0.128 g (0.51 mmole) of 7-pivaloyloxymethyladenine (**1c**) in 20 ml of ethanol was added 0.6 ml of 4 *M* sodium hydroxide. After 2 min at 25°, the solution was poured into excess aqueous sodium bicarbonate, and the resulting solution was extracted with dichloromethane. Evaporation of the dried organic extract gave a solid which was recrystallized from chloroform–cyclohexane as colorless plates, mp 235–236°, identified as N⁶-pivaloyladenine (**9**) (see above) (0.069 g, 61%) by comparison of infrared and ultraviolet spectra and by an undepressed mixture melting point, mmp 235–236°, with an authentic sample of **9**, mp 235°.

3-Benzyladenine (12a). To a solution of 0.146 g (0.59 mmole) of 7-pivaloyloxymethyladenine (**1c**) in 30 ml of acetonitrile was added 0.15 ml (1.3 mmoles) of benzyl bromide. After heating at reflux for 4.5 hr, the reaction mixture was cooled and evaporated *in vacuo* to give crude 3-benzyl-7-pivaloyloxymethyladenine hydrobromide (**11a**). This residue was dissolved in 5 ml of ammonia-saturated methanol. After 20 hr at 25°, the reaction mixture was diluted with 20 ml of ethanol, and the resulting solution was poured into a sodium bicarbonate–carbonate buffer solution. Extraction with chloroform then gave, after evaporation of the dried organic extract, a colorless product which was washed with acetone leaving 0.112 g (85%) of colorless crystals, mp 271–275°. Two recrystallizations from ethanol gave glistening plates, mp 277–279°, identified as 3-benzyladenine (**12a**)[3,9,12] by spectral characteristics and by an undepressed mixture melting point, mmp 279–281°, with an authentic sample of **12a**, mp 280–282°.

3-Benzyl-N⁶-pivaloyladenine (13a). To a refluxing solution of 0.150 g (0.60 mmole) of 7-pivaloyloxymethyladenine (**1c**) in 35 ml of anhydrous acetonitrile was added 0.20 ml (1.7 mmoles) of benzyl bromide. After 4 hr at reflux, the reaction mixture was evaporated *in vacuo* to give crude 3-benzyl-7-pivaloyloxymethyladenine hydrobromide (**11a**), which was dissolved in 20 ml of ethanol. To this solution, 1.0 ml of 3 *M* sodium hydroxide was added, and after 15 min the alkaline solution was poured into excess aqueous sodium bicarbonate. Extraction with chloroform then gave, after evaporation of the dried organic extract, a colorless gum which gradually crystallized. Recrystallizations from chloroform–ether, then acetonitrile, gave 0.120 g (65%) of 3-benzyl-N⁶-pivaloyladenine (**13a**) as colorless needles, mp 184–185°; ν_{max} 1716 cm^{-1} (st, amide); λ_{max} 222 mμ (sh, $\epsilon \times 10^{-3}$ 18.0) and 289 (12.8), λ_{min} 250 (3.4); λ_{max} (pH 1) 217 (18.5), 293 (18.8), and 302 (sh, 15.9), λ_{min} 243 (3.1); λ_{max} (pH 12) (unstable), 273–290 (<8.3), 328 (>9.7), and *ca.* 340 (sh), λ_{min} *ca.* 250 (<5.4) and *ca.* 308 (<6.9); nmr ($CDCl_3$): 1.40 (9 H, s, $(CH_3)_3CCON$), 5.83 (2 H, s, $C_6H_5CH_2N$), 6.6–7.1 (1 H, vbs, NH), *ca.* 7.37 (5 H, m, C_6H_5), 8.26 and 8.92 (1 H each, s, s, purine H's).

Anal. Calcd for $C_{17}H_{19}N_5O$: C, 65.99; H, 6.19; N, 22.64. Found [after drying at 100° (0.05 mm)]: C, 65.70; H, 6.32; N, 22.41.

3-Benzyloxymethyl-7-pivaloyloxymethyladenine Hydrochloride (11b). To a solution of 0.55 g (2.2 mmoles) of 7-pivaloyloxymethyladenine in 80 ml of anhydrous acetonitrile at 60° was added 0.75 ml (5.4 mmoles) of benzyl chloromethyl ether. After 20 min at 60° the reaction mixture was cooled slowly to 0°. Filtration gave **11b** as glistening plates, mp 179–180°, yield 0.674 g (76%); λ_{max} 223 mμ (sh, $\epsilon \times 10^{-3}$ 15.6), 280 (15.0), 287 (sh, 13.8), and *ca.* 305 (sh, 6.4), λ_{min} 248 (5.5); λ_{max} (pH 1) 222 (sh, 12.4), 278 (16.9), 285 (sh, 14.7), and *ca.* 305 (sh, 2.0), λ_{min} 241 (4.8); λ_{max} (pH 12) (after 2 min) (unstable) *ca.* 329 (*ca.* 15–16), λ_{min} 260 (*ca.* 7–8); nmr (DMSO-d_6): 1.14 (9 H, s, $(CH_3)_3C$), 4.79 (2 H, s, $C_6H_5CH_2O$), 6.00 (2 H, s, N^9CH_2O), 6.65 (2 H, s, N^7CH_2O), 7.26 (5 H, s, C_6H_5), 8.97 and 9.15 (1 H each, s, s, purine H's).

Anal. Calcd for $C_{19}H_{24}ClN_5O_3$: C, 56.22; H, 5.96; N, 17.26. Found: C, 56.54; H, 6.12; N, 17.43.

3-Benzyloxymethyladenine (12b). 3-Benzyloxymethyl-7-pivaloyloxymethyladenine hydrochloride (**11b**) (0.623 g) (1.53 mmoles) was dissolved in 20 ml of ammonia-saturated methanol. After 2 hr at 25°, the reaction mixture was evaporated *in vacuo*, and the residue was dissolved in 1 *M* hydrochloric acid. After washing with ether, the acidic solution was basified with aqueous sodium carbonate. Extraction with dichloromethane then gave, after evaporation of the dried organic extract, crude 3-benzyloxymethyladenine (**12b**) which was recrystallized from chloroform as glistening colorless plates, mp 206–209°, yield 0.224 g (57%); λ_{max} 210 mμ ($\epsilon \times 10^{-3}$ 24.3) and *ca.* 282 (b, 11.7), λ_{min} 247 (3.2); λ_{max} (pH 1) 277 (17.3), λ_{min} 239 (4.3); λ_{max} (pH 12) *ca.* 280 (b, 11.7), λ_{min} 247 (3.4); nmr (DMSO-d_6): 4.73 (2 H, s, $C_6H_5CH_2O$), 5.85 (2 H, s, NCH_2O), 7.30 (5 H, s, C_6H_5), 8.30 (2 H, bs, NH_2), 7.87 and 8.49 (1 H each, s, s, purine H's). A further crop from the mother liquors raised the yield to 75%.

Anal. Calcd for $C_{13}H_{13}N_5O$: C, 61.16; H, 5.13; N, 27.44. Found: C, 61.07; H, 5.15; N, 27.17.

1-Benzyloxymethyl-9-pivaloyloxymethyladenine Hydrochloride (14). To a solution of 0.369 g (1.48 mmoles) of 9-pivaloyloxymethyladenine (**8**) in 50 ml of anhydrous acetonitrile at 60° was added 0.40 ml (3.0 mmoles) of benzyl chloromethyl ether. After 21 min at 60°, the reaction mixture was cooled slowly to 0°. Filtration then gave **14** as colorless plates, mp 176–177°, yield 0.316 g (53%); λ_{max} 252 mμ (sh, $\epsilon \times 10^{-3}$ 10.8), 258 (12.8), and 265 (11.0), λ_{min} 229 (3.9); λ_{max} (pH 1) 258 (13.5), λ_{min} 231 (4.4); λ_{max} (pH 12) (unstable) 252 (sh, 10.6), 258 (12.2), 265 (sh, 10.2), and *ca.* 295 (vbsh, *ca.* 3.7), λ_{min} 234 (5.0); nmr (DMSO-d_6): 1.14 (9 H, s, $(CH_3)_3C$), 4.85 (2 H, s, $C_6H_5CH_2O$), 6.18 and 6.25 (2 H each, s, s, N^1CH_2O and N^9CH_2O), 7.27 (5 H, s, C_6H_5), 8.69 and 9.14 (1 H each, s, s, purine H's).

Anal. Calcd for $C_{19}H_{24}ClN_5O_3$: C, 56.22; H, 5.96; N, 17.26. Found: C, 56.41; H, 6.17; N, 17.27.

1-Benzyloxymethyladenine (15). 1-Benzyloxymethyl-9-pivaloyloxymethyladenine hydrochloride (**14**) (0.516 g) (1.27 mmoles) was dissolved in 20 ml of ammonia-saturated methanol. After 4 hr at 25°, the solution was evaporated *in vacuo* and the residue was washed with ether and acetone. The crude product was then dissolved in 1:1 aqueous methanol and chromatographed on *ca.* 10 g of Dowex 1-X8 HCO_3^-. Elution with 1:1 aqueous methanol gave crude 1-benzyloxymethyladenine (**15**), which was recrystallized from ethanol–chloroform as nearly colorless needles, mp 203–206° dec, yield 0.096 g (30%); λ_{max} 228 mμ ($\epsilon \times 10^{-3}$ 21.6) and 275 (12.0), λ_{min} 247 (4.2); λ_{max} (pH 1) 260 (12.2), λ_{min} 233 (3.8); λ_{max} (pH 12) 262 (sh, 10.8), 267 (13.9), and 274 (14.1), λ_{min} 252 (4.0) and 270.5 (13.5); nmr (DMSO-d_6): 4.65 (2 H, s, $C_6H_5CH_2O$), 5.71 (2 H, s, NCH_2O), 7.33 (5 H, s, C_6H_5), 7.87 and 8.22 (1 H each, s, s, purine H's).

Anal. Calcd for $C_{13}H_{13}N_5O$: C, 61.16; H, 5.13; N, 27.44. Found: C, 61.12; H, 5.15; N, 27.21.

3-(2′-Deoxy-D-ribofuranosyl)adenine (16). To a solution of 0.992 g (3.98 mmoles) of 7-pivaloyloxymethyladenine (**1c**) in 170 ml of anhydrous acetonitrile at 60° was added 1.895 g (4.4 mmoles) of 1-chloro-3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranoside (**2**).[28] After 16 min at 60°, the reaction mixture was cooled rapidly to 0° and then partially evaporated *in vacuo* (*ca.* 20°) to about 120 ml. Crude 7-pivaloyloxymethyladenine hydrochloride (0.286 g), mp 223–226°, was removed by filtration, and the filtrate was evaporated *in vacuo* (*ca.* 20°) to give a gum which was nearly colorless. Trituration with ether then gave 1.757 g of crude 3-(3′,5′-di-*O*-(*p*-chlorobenzoyl)-2′-deoxy-D-ribofuranosyl)-7-pivaloyloxymethyladenine hydrochloride (**3c**) as colorless crystals, mp 100–102° (resolidified and remelted at 186–189° with decomposition and evolution of gas), which was shown by tlc to contain about 10–20% of 7-pivaloyloxymethyladenine hydrochloride.

This crude product (**3c**) (2.272 g) was dissolved in 40 ml of ammonia-saturated methanol. After 11 hr at 25°, the solution was evaporated *in vacuo* (<30°), the residue was dissolved in 3 ml of methanol, and the resulting solution was diluted with 70 ml of water. The resulting aqueous solution was extracted with dichloromethane, and the aqueous phase remaining was concentrated *in vacuo* to *ca.* 10 ml. This concentrated solution was chromatographed on a 19 × 1.5 cm column of Dowex 1-X8 OH^- (200–400 mesh) in 2:3 methanol–water. The initial fractions eluted with the solvent mixture contained the 3-α-(2′-deoxy-D-ribofuranosyl)-adenine (**16α**), which crystallized from ethanol as colorless needles, mp 174–175° dec, yield 0.234 g (28% from **3c**); λ_{max} 214 mμ ($\epsilon \times 10^{-3}$ 17.8) and 277 (12.8), λ_{min} 245 (2.8); λ_{max} (pH 1) 271 (13.9), λ_{min} 235 (4.0); λ_{max} (pH 13) 276 (13.2), λ_{min} 245 (3.9); nmr (D_2O, 25°): 2.47 (1 H, d of pt, J = 2.0 and 15.0, 2′′-H), 2.92 (1 H, d of (d of d), J = 6.8, 6.0, and 15.0 2′-H), 3.77 (dioxane, internal standard), 3.82 (1 H, s, downfield part of 5′-H_2 signal), *ca.* 4.6 (2 H, m, 3′-H and 4′-H), 4.78 (HDO), 6.39 (1 H, d of d, J = 6.8 and 2.0, 1′-H), 7.82 and 8.37 (1 H each, s, s, purine H's). Samples of **16α** with melting points as high as 184–185° were obtained from some fractions, $[\alpha]^{25}D$ 11 ± 2° (water).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.22; N, 27.87. Found: C, 47.88, 47.85; H, 5.58, 5.34; N, 27.86, 27.70.

Further elution with a large volume of 1:1 aqueous methanol gave 3-β-(2′-deoxy-D-ribofuranosyl)adenine (**16β**) which crystallized from ethanol as pale violet needles, mp *ca.* 191°, yield 0.095 g (11% from **3c**). Two recrystallizations from ethanol and decolorization with charcoal gave colorless needles, mp 188–189°; λ_{max} 214 mμ ($\epsilon \times 10^{-3}$ 18.0) and 278 (12.9), λ_{min} 245 (2.9); λ_{max} (pH 1) 271 (14.3), λ_{min} 235 (4.4); λ_{max} (pH 13) 277 (13.1), λ_{min} 246 (4.0); nmr (D_2O, 83°): 2.72 (2 H, d of d, J = 6.5 and 5.2, 2′- and 2′′-H), 3.94 (2 H, s and d, $J \cong$ 3.6 av, 5′-H_2), 4.32 (HDO and 4′-H), 4.67 (1 H, t of d, $J \cong$ 3.5 and 5.2, 3′-H), 6.56 (1 H, t, 1′-H), 8.01 and 8.58 (1 H each, s, s, purine H's); $[\alpha]^{25}D$ 19 ± 2° (water).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.22; N, 27.87. Found: C, 47.70; H, 5.22; N, 27.75.

Optical Rotatory Dispersion.[40] The ORD curve of the α anomer

(40) We wish to express our appreciation to Professor Robert W. Woody for these measurements.

of 2′-deoxy-3-isoadenosine (**16**α) exhibited a positive Cotton effect with a peak at 300 mμ, $[M]^{25}_{300}$ 2060 ± 200°, and a trough at 272 mμ, $[M]^{25}_{272}$ −6700 ± 700°. Measurement of the ORD of the β anomer could not be extended below 320 mμ owing to a prohibitively high $\epsilon/[M]$ ratio in the short-wavelength region. However, the ORD curve was plain positive from 589 to *ca.* 370 mμ, with a broad peak at 350 mμ, $[M]^{25}_{350}$ 123 ± 10°, and indication of an incipient negative Cotton effect in the lower wavelength region. The molecular amplitude of 88 observed for the α anomer at the peak lies within the range of 50–120° observed for the usual purine nucleosides.[41]

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The Conformation of Gramicidin S in Solution

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Abstract: The conformation of the homodetic cyclic decapeptide antibiotic, Gramicidin S, has been investigated. Measurements were made of the ultraviolet and infrared spectra, peptide hydrogen–deuterium exchange, optical rotatory dispersion, and the circular dichroism of the molecule in solution. The results indicate that most of the models that have been proposed so far do not explain the solution-state properties of the molecule. However, the structure proposed by Scheraga and co-workers, "a structure similar to the β type, with two intramolecular hydrogen bonds," and the mixed α,β structure proposed by Hodgkin and Oughton cannot be ruled out.

The conformation of the cyclic homodetic decapeptide, Gramicidin S, has received renewed attention due to the recent potential energy calculations of Liquori's group[1] and Scheraga's group.[2,3] On the basis of these calculations, Liquori and co-workers have suggested that Gramicidin S (GS, formula I) possesses

$$\left[\begin{matrix}\text{L-Pro-L-Val-L-Orn-L-Leu-D-Phe}\\ \text{D-Phe-L-Leu-L-Orn-L-Val-L-Pro}\end{matrix}\right]$$

I

two single turns of the right-handed α-helical fold, each one facing the other, imparting a twofold axis of symmetry to the molecule. Similar calculations by Scheraga and co-workers have, however, preferred a structure similar to the antiparallel intramolecular β structure for the molecule, with two hydrogen bonds.

Earlier to these two papers, several other workers had also proposed various models for GS. Abbott and Ambrose,[4] on the basis of infrared dichroic studies in the 4500–5000-cm^{-1} region, have suggested an α-ribbon type of conformation for GS. However, X-ray diffraction analysis of GS and possible models proposed by Hodgkin and Oughton[5] to explain the data argue against this suggestion on general grounds, such as the symmetry of the crystal. Thin film dialysis work of Craig and collaborators[6] also argues against this type of α or any other compact structure, and suggests a rather open structure for GS.

Another possible model is the intramolecular antiparallel β form with four hydrogen bonds. This has been favored by Hodgkin and Oughton to explain the X-ray analysis data best. This model has also been favored by Schwyzer.[7] In this structure, out of the possible eight peptide bonds, four are engaged in hydrogen bonding within the molecule, and because of primary structural restrictions, the β form is antiparallel. This model has a truly twofold axis of symmetry. The objections to this model are the infrared work of Abbott and Ambrose, and also the preliminary deuterium exchange studies of Jaffee and Craig,[8] that suggest the easy removal of the peptide hydrogens. It is noteworthy here that the calculations of Scheraga's group predict a structure similar to the β form with only two intramolecular hydrogen bonds. This might make the molecular conformation more flexible.

It is to be pointed out that in both of the above models, the side chains are not rigidly fixed in space, even though Abbott and Ambrose talk about a parallel stacking of the side chains in the crystal.

The third possible model was proposed again by Hodgkin and Oughton, and this model was a little more involved. This is the mixed α,β structure, where the valine to ornithine is an α-type move, ornithine to leucine a β-type move, and leucine to phenylalanine is an α move. This model packs like an α-helix, has a true twofold axis, four intramolecular hydrogen bonds, and is strain-free.

The last model has been proposed by Warner[9] on the basis of molecular model studies. This is simply a model of two fused hexagons to form a naphthalene-type conformation for GS, with each peptide oxygen forming a corner of the hexagon. It has been claimed that this model maximizes the hydrophobic interactions among the side chains, a point that has not been given consideration in the previous models. No mention has been made of intramolecular hydrogen bonds. There is a hole in the center of each of the hexagons

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