(courtesy of Dr. Klaus Biemann), Facility for Biomedical Research, Department of Chemistry, Massachusetts Institute of Technology, Cambridge. The 250.0-MHz ^1H NMR information was supplied by Dr. Robert Bittner (courtesy of Dr. Axel A. Bothner-By), NMR Facility for Biomedical Studies, Carnegie-Mellon University, Pittsburgh, and the 270-MHz ^1H NMR spectra were determined by Dr. David Rubin (courtesy of Dr. Leo J. Neuringer), Molecular Biophysics Group, Francis Bittner National Magnetic Laboratories, Massachusetts Institute of Technology, Cambridge. We are grateful for this help and wish to thank all concerned.

Registry No. **3**, 71041-98-0; **4**, 71041-99-1; **5**, 63318-26-3; **6**, 71042-00-7; **7**, 71042-01-8; **7** bis(tetrahydropyran) derivative, 71042-28-9; **8**, 71042-02-9; **8** tosylate, 71042-03-0; **9**, 71042-04-1; **10**, 5921-73-3; **11**, 10160-28-8; **12**, 10160-26-6; **13**, 71042-05-2; **14**, 71042-06-3; **15**, 71042-07-4; **16**, 71042-08-5; **17**, 929-33-9; **18**, 71042-09-6; **19**, 71042-10-9; **20**, 71042-11-0; **21**, 71042-12-1; **22**, 59014-67-4; **23**, 71042-13-2; **24**, 71042-14-3; **25**, 71042-15-4; **26**, 71042-16-5; **27**, 71042-17-6; **28**, 71042-18-7; **29**, 71042-19-8; **30**, 71042-20-1; **31**, 629-72-1; **32**, 5802-82-4; **33**, 506-46-7; sodium acetylide, 1066-26-8; 1-chloro-6-iodohexane, 34683-73-3; 8-chloro-1-octyne, 24088-97-9; 6-chlorohexyl *p*-toluenesulfonate, 71042-21-2; 6-chlorohexyl alcohol, 2009-83-8; 8-hexadecynedinitrile, 71042-22-3; *cis*-8-hexadecenedioic acid, 71042-23-4; 1,16-dihydroxy-*cis*-8-hexadecene, 71042-24-5; methylene diiodide, 75-11-6; *cis*-8,9-methylenehexadecanedioic acid monomethyl ester, 71042-25-6; 1-hydroxy-8-nonyne, 10160-28-8; 1,7-dichloroheptane, 821-76-1; 1,25-bis(tetrahydropyranyloxy)-8,17-pentacosadiyne, 71042-26-7; undecyl iodide, 4282-44-4; *cis*-8,9-methyleneheptacosyl 4-chloropentanoate, 71042-27-8; 1-(tetrahydropyranyloxy)-16-chlorohexadecane, 71042-29-0; 9,10-methyleneoctacosane, 71042-30-3.

Oxidation of N^6,N^6-Dialkyl-2′,3′,5′-tri-*O*-acyladenosines with Ruthenium Tetroxide and a Novel Selective N-Monodealkylation Sequence

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Oxidation of 2′,3′,5′-tri-*O*-acetyl (benzoyl) derivatives of N^6,N^6-dimethyladenosine (**1d** and **1e**), N^6,N^6-diethyladenosine (**1f** and **1g**), N^6,N^6-dibenzyladenosine (**1h**), 6-(*N*-pyrrolidino)nebularine (**1i**), and 6-(*N*-piperidino)nebularine (**1j**) with RuO_4 in CCl_4 gave the corresponding monoamido derivatives **2a–g**. The reaction is selective—no formation of diamido (imido) compounds was observed—and in conjunction with deacylation effected by alkaline hydrolysis in the case of **2a–e** it represents a monodealkylation sequence of N^6,N^6-dialkyladenosines. A formal similarity of this transformation to the N-monodemethylation step in the metabolism of antibiotic puromycin is discussed. Selectivity of oxidation of the title compounds with RuO_4 can be explained in terms of electronic (inductive and/or resonance) effects of N^6-alkyl groups, the pyrimidine portion of the purine ring, and the carbonyl function in the reaction products.

Nucleosides derived from N^6,N^6-dimethyladenosine play an important role in biological processes. Thus, the antibiotic puromycin (**1a**, Scheme I) is a powerful inhibitor of protein synthesis, exhibiting distinct antibacterial and antitumor activity.[1] A related nucleoside—N^6,N^6-dimethyladenosine (**1b**)—occurs as a part of 16S and 18S ribosomal RNA[2] which is believed to be responsible for the binding of antibiotic kasugamycin.[3] Little is known of the metabolism of the N^6,N^6-dimethyl derivatives **1a** and **1b**. Puromycin (**1a**) is converted in vivo to the highly nephrotoxic aminonucleoside **1c** which is then selectively monodemethylated and phosphorylated to the corresponding 5′-phosphate[4] (Scheme II). The mechanism of demethylation has not yet been elucidated, although it is possible that as in cases of certain xenobiotics[5] the process involves an oxidation of an N^6-methyl group catalyzed by microsomal oxydase followed by cleavage (hydrolysis) of the resultant carbinolamine intermediate **4** to N^6-methylnucleoside **5**. No chemical (nonenzymic) model of this selective N-monodealkylation has been reported to date.

We wish to report on the results of oxidation of a series of N^6,N^6-dialkyl-2′,3′,5′-tri-*O*-acyladenosines, **1d–j**, with ruthenium tetroxide in a nonpolar solvent (CCl_4) to the corresponding monoamido derivatives **2a–g**. In the case of N^6-formyl, -acetyl, or -benzoyl derivatives **2a–e** a simple deacylation with ammonia in methanol afforded the N^6-monoalkylated nucleosides, thus accomplishing a selective removal of a single alkyl group from the starting N^6,N^6-dialkylnucleoside.

Results and Discussion

The results of oxidation of the title series of nucleosides **1d–j** are summarized in Table I. N^6,N^6-Dimethyl-2′,3′,5′-tri-*O*-acetyladenosine (**1d**) was readily oxidized with RuO_4 to give the N^6-formyl-N^6-methyl derivative **2a** in 72% yield along with a small amount (4%) of N^6-methyl-2′,3′,5′-tri-*O*-acetyladenosine (**1m**). Confirmation of the structure of **2a** came from the NMR spectrum which inter alia showed only one three-proton singlet for NCH_3 (δ 3.59) and another low-field one-proton singlet (δ 10.42) for the *N*-formyl group. The electron-impact mass spectrum (MS) indicated, in addition to the corresponding molecular peak (M^+, m/e 435), an ion of m/e 407 derived by decarbonylation of M^+. Compound **2a** on further oxidation with RuO_4 did not afford compound **1m**, which indicated that the formation of the latter did not result from an oxidative removal of the *N*-formyl group. Rather, it was formed in some preceding step, possibly decom-

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(2) J. K. Vass and B. E. H. Maden, *Eur. J. Biochem.*, **85**, 241 (1978).
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(4) (a) H. T. Nagasawa, K. F. Swingle, and C. S. Alexander, *Biochem. Pharmacol.*, **16**, 2211 (1967); (b) R. F. Derr, D. K. Loechler, C. S. Alexander, and H. T. Nagasawa, *ibid.*, **17**, 265 (1968); (c) E. Kmetec and A. Tirpack, *ibid.*, **19**, 1493 (1970); (d) H. T. Nagasawa, F. N. Shirota, and C. S. Alexander, *J. Med. Chem.*, **15**, 177 (1972).
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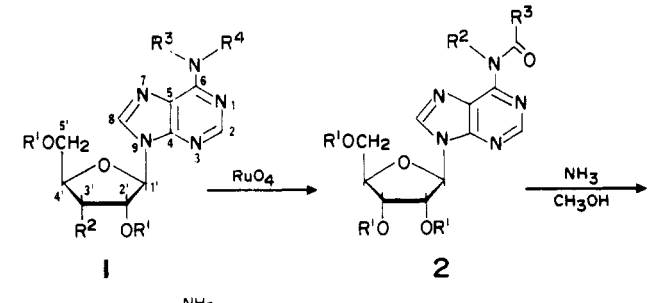
Scheme I

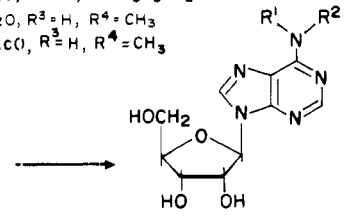


1

2

Ia : R' = H, R² = NHCOCHC₆H₄OCH₃-p, R³ = R⁴ = CH₃
Ib : R' = H, R² = OH, R³ = R⁴ = CH₃
Ic : R' = H, R² = NH₂, R³ = R⁴ = CH₃
Id : R' = Ac, R² = AcO, R³ = R⁴ = CH₃
Ie : R' = Bz, R² = BzO, R³ = R⁴ = CH₃
If : R' = Ac, R² = AcO, R³ = R⁴ = C₂H₅
Ig : R' = Bz, R² = BzO, R³ = R⁴ = C₂H₅
Ih : R' = Bz, R² = BzO, R³ = R⁴ = C₆H₅CH₂
Ii : R' = Bz, R² = BzO, R³ + R⁴ = (CH₂)₄
Ij : R' = Bz, R² = BzO, R³ + R⁴ = (CH₂)₅
Ik : R' = Bz, R² = BzO, R³ = H, R⁴ = C₆H₅CH₂
Il : R' = Bz, R² = BzO, R³ = H, R⁴ = CH₃
Im : R' = Ac, R² = AcO, R³ = H, R⁴ = CH₃

2a : R' = Ac, R² = CH₃, R³ = H
2b : R' = Bz, R² = CH₃, R³ = H
2c : R' = Ac, R² = C₂H₅, R³ = CH₃
2d : R' = Bz, R² = C₂H₅, R³ = CH₃
2e : R' = Bz, R² = C₆H₅CH₂, R³ = C₆H₅
2f : R' = Bz, R² + R³ = (CH₂)₃
2g : R' = Bz, R² + R³ = (CH₂)₄

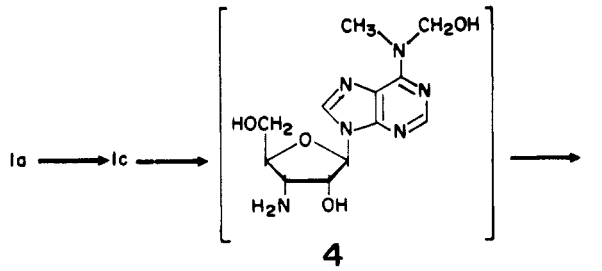


3

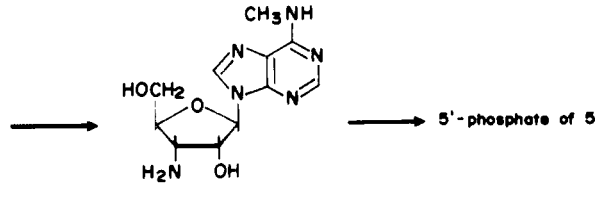
Ac = CH₃CO
Bz = C₆H₅CO

3a : R' = CH₃, R² = H
3b : R' = C₂H₅, R² = H
3c : R' = C₆H₅CH₂, R² = H
3d : R' = R² = C₆H₅CH₂

Scheme II



4



5

position of the carbinolamine intermediate. Deacylation **2a** with methanolic ammonia gave N^6-methyladenosine (**3a**) whose properties were in accord with those of an authentic sample.[6] An analogous oxidation of tribenzoyl

Table I. 2',3',5'-Tri-*O*-acyl-N^6,N^6-dialkyladenosines and Their Oxidation Products[a]

compd	yield, %	calcd/found, % C	H	N
1e	86	63.96[b]	4.93	11.30
		64.04	4.76	11.15
1f	87.9[c]	51.12[c]	5.79	14.80
		51.09	5.82	14.76
1g	88.5	65.76[d]	5.27	10.96
		65.62	5.21	10.94
1h	87.4[e]	71.13	4.91	9.22
		71.02	4.92	9.25
1i	82.4	66.34	4.93	11.05
		66.16	4.97	11.03
1j	88.6[f]	66.75	5.14	10.81
		66.49	5.17	10.83
1k	73.6	67.81[g]	4.67	10.14
		67.75	4.77	10.36
1l	62.6	64.75	4.59	11.80
		64.50	4.60	11.84
1m	69	49.39[h]	5.28	16.94
		49.28	5.11	16.84
2a	72.3	47.91[i]	4.69	15.38
		47.70	4.61	15.11
2b	58.7	63.15[h]	4.44	11.16
		63.09	4.36	10.96
2c	56.8	50.84[g]	5.33	14.82
		50.83	5.38	14.78
2d	38.1	64.12[h]	4.83	10.68
		64.06	4.79	10.62
2e	1.8	69.85	4.56	9.05
		69.43	4.58	8.90
2f	25.9	62.49[j]	4.32	10.34
		62.08	4.15	10.24
2g	14.7	63.67[k]	4.89	10.31
		63.84	4.69	10.22

[a] All compounds were obtained as amorphous materials uniform on TLC, and their molecular formulas were confirmed by MS (the presence of M^+). Compound **2b** did not give M^+ but only $M^+ - CO$ (m/e 593). The MS of **1m** was not run. [b] Contains $^2/_3$ mol of H_2O. [c] Syrup containing $^1/_5$ mol of $CHCl_3$. [d] Contains $^1/_5$ mol of H_2O. [e] Crystalline solid, mp 144–145 °C. [f] Crystallized from methanol, mp 159.5–160.5 °C. [g] Contains $^1/_2$ mol of H_2O. [h] Contains $^1/_3$ mol of H_2O. [i] Contains $^1/_6$ mol of $CHCl_3$. [j] Contains $^1/_4$ mol of $CHCl_3$. [k] Contains 1 mol of H_2O.

derivative **1e** afforded the corresponding N^6-formyl-N^6-methyl derivative **2b** in almost 60% yield.

We have noted that the yields of *N*-acyl derivatives decrease with an increased complexity of the alkyl group. Thus, N^6,N^6-diethyl-2',3',5'-tri-*O*-acetyladenosine (**1f**) and the corresponding tribenzoate **1g** afforded the N^6-ethyl-N^6-acetyl derivatives **2c** and **2d** in 57 and 38% yield, respectively. Again, the structures of **2e** and **2d** were confirmed by NMR spectra (presence of three magnetically different methyl groups) and also by MS (M^+ at m/e 463 and 649, respectively). Compound **2c** was resistant toward further oxidation with RuO_4, and it was readily deacylated with ammonia in methanol to N^6-ethyladenosine (**3b**), identical with an authentic sample.[7]

N^6,N^6-Dibenzyl-2',3',5'-tri-*O*-benzoyladenosine (**1h**) proved to be very unreactive, and the corresponding N^6-benzyl-N^6-benzoyl derivative **2e** was obtained in only 2% yield. It appears that a large portion of the starting material (**1h**) was destroyed in the oxidation (only 17% of **1h** was recovered), which is in accord with reports[8] that the phenyl groups are rapidly oxidized with RuO_4. The structure of **2e** was confirmed by deacylation with am-

(6) A wide range of melting points for **3a** is reported in the literature: (a) I. Wempen and J. J. Fox, *Methods Enzymol.*, **12A**, 89 (1967), 219–221 °C, softening at 206–208 °C; (b) J. A. Johnson, H. J. Thomas, and H. J. Schaeffer, *J. Am. Chem. Soc.*, **80**, 699 (1958), 135–140 °C, resolidification at 160 °C and melting with decomposition at 208 °C.

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Table II. NMR Constants of Starting Materials and Reaction Products[a]

compd	H_8 and H_2[b]	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$ and $H_{5'}$	other signals[c]
1e	8.30 (s, 1) 7.83 (s, 1)[d]	6.46 (d, 1) $J_{1',2'}$ = 4.9	6.43 (t, 1)[e]	6.29 (b t, 1)	4.64-4.93 (m, 3)	3.50 (s, 6, $N(CH_3)_2$)
1f	8.32 (s, 1) 7.87 (s, 1)	6.21 (d, 1) $J_{1',2'}$ = 5.6	5.91 (t, 1)[e]	5.65 (b m, 1)	4.40 (b m, 1)	3.97 (q, 4, CH_2 of C_2H_5), 2.13 and 2.07 (2 s, 9, CH_3CO), 1.29 (t, 6, CH_3 of C_2H_5)
1g	8.30 (s, 1) 7.88 (s, 1)[d]	6.46 (d, 1) $J_{1',2'}$ = 4.9	6.39 (t, 1)[e]	6.25 (b t, 1)	4.80 (m, 3)	3.95 (q, 4, CH_2 of C_2H_5), 1.27 (t, 6, CH_3 of C_2H_5)
1h	8.37 (s, 1) 7.90 (s, 1)[d]	6.4 (m, 2)		6.28 (b t, 1)	4.78 (m, 3)	7.20 (s, 5, C_6H_5),[f] 5.23 (b s, 4, CH_2 of $C_6H_5CH_2$)
1i	8.31 (s, 1) 7.88 (s, 1)[d]	6.46 (d, 1) $J_{1',2'}$ = 5.1	6.39 (b t, 1)[e]	6.25 (b t, 1)	4.80 (m, 3)	3.88 (b m, 4, NCH_2), 2.02 (b s, 4, CCH_2)
1j	8.29 (s, 1) 7.89 (s, 1)[d]	6.47 (d, 1) $J_{1',2'}$ = 4.9	6.38 (t, 1)[e]	6.29 (b t, 1)	4.64-4.96 (m, 3)	4.20 (b s, 4, NCH_2), 1.70 (b s, 6, CCH_2)
1k	8.36 (s, 1) 7.88 (s, 1)[d]	6.33-6.45 (m, 3)			4.62-4.95 (m, 3)	5.95 (b t, 1, NH),[f,g] 4.86 (s,[h] 2, CH_2 of $C_6H_5CH_2$)
1l	8.36 (s, 1) 7.89 (s, 1)[d]	6.3 (m, 3)			4.80 (m, 3)	5.80 (m, 1, NH), 3.20 (d, 2, CH_3, $J_{CH_3,NH}$ = 4.9)
1m	8.41 (s, 1) 7.90 (s, 1)	6.19 (d, 1) $J_{1',2'}$ = 4.9	5.93 (t, 2)[e,i]	5.73 (b t, 1)	4.42 (b s, 3)	3.21 (d, 2, $CH_{3'}$, $J_{CH_{3'}NH}$ = 4.8), 2.14, 2.13, and 2.08 (3 s, 9, CH_3CO)
2a	8.71 (s, 1) 8.15 (s, 1)	6.26 (d, 1) $J_{1',2'}$ = 4.9	5.95 (t, 1)[e]	5.67 (b t, 1)	4.44 (b s, 3)	10.47 (s, 1, CHO), 3.59 (s, 3, NCH_3), 2.17, 2.14, and 2.09 (3 s, 9, CH_3CO)
2b	8.59 (s, 1) 8.12 (s, 1)[d]	6.49 (d, 1) $J_{1',2'}$ = 4.9	6.42 (t, 1)[e]	6.28 (m, 1)	4.65-4.98 (m, 3)	10.42 (s, 1, CHO), 3.57 (s, 3, NCH_3)
2c	8.79 (s, 1) 8.19 (s, 1)	6.24 (d, 1) $J_{1',2'}$ = 5.3	5.97 (b t, 1)	5.69 (b t, 1)	4.45 (b s, 3)	4.27 (q, 2, CH_2 of C_2H_5), 2.29, 2.16, 2.14, and 2.11 (s, 12, CH_3CO), 1.23 (t, 3, CH_3 of C_2H_5)
2d	8.65 (s, 1) 8.19 (s, 1)[d]		6.2-6.4 (m, 3)		4.6-4.9 (m, 3)	4.25 (q, 2, CH_2 of C_2H_5), 2.25 (s, 3, CH_3CO), 1.21 (t, 3, CH_3 of C_2H_5)
2e	8.44 (s, 1) 7.99 (s, 1)[d]		6.28 (m, 3)		4.80 (m, 3)	7.17 (s, 5, C_6H_5),[f] 5.62 (s, 2, CH_2 of $C_6H_5CH_2$)
2f	8.68 (s, 1) 8.19 (s, 1)[d]	6.51 (d, 1) $J_{1',2'}$ = 5.4	6.48 (t, 1)[e]	6.25 (b t, 1)	4.6-4.9 (m, 3)	4.29 (b t, 2, NCH_2), 2.70 (b t, 2, $COCH_2$), 2.24 (qp, 4, CCH_2)
2g	8.70 (s, 1) 8.19 (s, 1)[d]	6.48 (m, 2)		6.26 (m, 1)	4.8 (m, 3)	3.98 (b s or m, 2, NCH_2), 2.71 (m, 2, $COCH_2$), 2.01 (m, 4, CCH_2)

[a] In $CDCl_3$, chemical shifts are in δ units, multiplicity and number of protons are in parentheses; s singlet, d doublet, t triplet, q quadruplet, qp quintuplet, b broad; coupling constants J are in Hz. [b] A rigorous assignment of H_8 and H_2 resonances was not performed. It is likely that the proton at lower field is H_8.[22] [c] In all tribenzoates the benzoyl group exhibited two multiplets at δ 7.8-8.0 (ortho H, 2 protons) and 7.3-7.4 (meta and para H, 3 protons). [d] In all tribenzoates one heterocyclic proton, presumably H_2 was overlapped with the benzoyl envelope. The H_8 and H_2 signals were invariably the sharpest in the spectra. Therefore, somewhat arbitrarily, the narrowest signal in the envelope was assigned to be H_2. [e] $J_{2',3'} = J_{2',3'} = J_{1',2'}$. [f] Sharp singlet overlapped with meta and para H signals of C_6H_5CO. [g] The peak at δ 7.29-7.25 (s, 6, C_6H_5) is also overlapped with the $CHCl_3$ signal. [h] After addition of D_2O. [i] Overlapped with NH.

monia in methanol to the known[9,10] N^6-benzyladenosine (**3c**). In addition, compound **2e** was found to be identical (TLC, NMR) with a sample prepared by acylation of **3c** with benzoyl chloride in pyridine.

Although a successful oxidation of aliphatic amines with RuO_4 has not yet been described,[11] attempts to oxidize some cyclic amines (imines) led to the formation of lactams or cyclic imides,[11,12] depending on the type of starting imine. It was therefore of interest to examine oxidation of some purine derivatives substituted at position 6 with a cyclic amine (pyrrolidine and piperidine). Such a reaction could lead to novel 6-substituted purine derivatives of potential biological interest. The pyrrolidino and piperidino derivatives[13] **1i** and **1j** gave on oxidation with RuO_4, under the conditions described above, the corresponding lactams **2f** and **2g** in 26 and 15% yield, respectively.

The mass spectra confirmed that both **2f** and **2g** are monoamido derivatives—M^+ at m/e 647 and 661, respectively. The corroboration of the structure came from the NMR spectra (Table II) and spin-decoupling experiments. Thus, irradiation of the high-field two-proton quintuplet (CCH_2C) in the pyrrolidinone derivative **2f** led to collapse of both triplets at δ 2.70 and 4.29 to the corresponding singlets, indicating a coupling of the former signal (δ 2.24) to both neighboring methylene groups. On the other hand, irradiation at δ 2.70 did not affect the signal at δ 4.29 whereas the quintuplet at δ 2.24 collapsed to a triplet. Similarly, irradiation at δ 4.29 did not change the pattern of the signal at δ 2.70, but the quintuplet at δ 2.24 was again transformed to a triplet. Assignments for the piperidinone derivative **2g** were made in the same fashion. Irradiation of the four-proton multiplet ($C(CH_2)_2C$) at δ 2.01 led to collapse of both lower field triplets δ 2.71 and 3.98 to the corresponding singlets. Thus, it was conclusively proved that only one methylene group of **1i** and **1j** in the α position to the exocyclic nitrogen atom was oxidized. Therefore, the reaction of **1i** and **1j** with RuO_4

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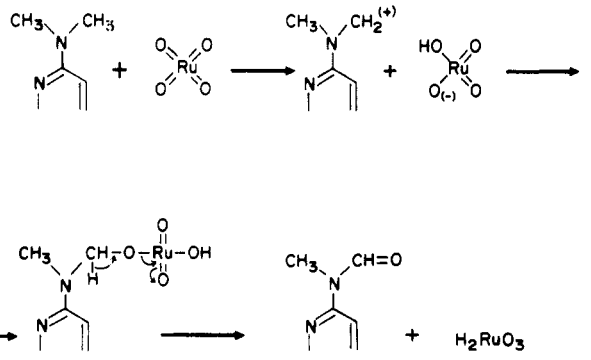
(11) L. M. Berkowitz and P. N. Rylander, *J. Am. Chem. Soc.*, **80**, 6682 (1958).
(12) (a) J. C. Sheehan and R. W. Tulis, *J. Org. Chem.*, **39**, 2264 (1974); (b) N. Tangari and V. Tortorella, *J. Chem. Soc., Chem. Commun.*, 71 (1975); (c) G. Bettoni, C. Franchini, F. Morlacchi, N. Tangari, and V. Tortorella, *J. Org. Chem.*, **41**, 2780 (1976); (d) F. Morlacchi, V. Losacco, and V. Tortorella, *J. Heterocycl. Chem.*, **16**, 297 (1979).
(13) The corresponding free nucleosides have been described: H. Vorbrüggen and K. Krolikiewicz, *Justus Liebigs Ann. Chem.*, 745 (1976).

Scheme III



displayed a selectivity comparable to that of simple N^6,N^6-dialkyladenosine derivatives; the formation of cyclic imides was not observed.

Two factors may account for a high selectivity of oxidation with RuO_4: (i) the inductive effect of the N^6-alkyl groups and (ii) the strongly electronegative character of the pyrimidine portion of the purine ring.[14] It is of interest to note that an electron-impact study has shown a high reactivity of one methyl group in N^6,N^6-dimethyladenosine vs. N^6-methyladenosine which was explained in terms of the inductive effect.[15] It is also clear that oxidation of an N^6-alkyl group will decrease the reactivity of the remaining alkyl group owing to the electronegativity of the resultant carbonyl function. Thus, in the series pyrrolidine,[11] *N*-benzylpyrrolidine,[12c] *N*-(*p*-toluenesulfonyl)pyrrolidine,[12a] *N*-acetyl-3-phenylpyrrolidine,[12b] and pyrrolidine derivative **1i**, the formation of an imido derivative was observed only in the case of pyrrolidine and *N*-benzylpyrrolidine. A similar trend can be recognized in the piperidine series.[12d] Examples of imide formation using other electronegative but less stable N-protecting groups[12a] can most likely be explained in terms of partial removal of those blocking groups under the reaction conditions.

Little is known of the mechanism of oxidation with RuO_4. It is generally assumed[16] that the rate-determining step involves an abstraction of hydride ion from the substrate (alcohol or ether derivative). Subsequent steps can include the formation of an ester intermediate which then undergoes a β-elimination to give the corresponding carbonyl compound, e.g., butyrolactone from tetrahydrofuran.[16] Therefore, a similar mechanism can be anticipated in the case of N^6,N^6-dialkyl derivatives **1d–j** (Scheme III). It has been noted that oxidation of the nitrogen atom of cyclic amines does not take place[12a] despite the fact that N-oxidation with other reagents (peracids) proceeds successfully. This can be attributed to differences in the reaction mechanism. However, it is not possible to rule out entirely the possibility of formation of *N*-oxides as reaction intermediates in the case of heteroaromatic amines of suitable structure (e.g., N^6,N^6-dialkyladenosines) on the basis of the present evidence.

A formal comparison of the enzymatic demethylation of puromycin aminonucleoside **1c** with the demethylation (dealkylation) sequence described above is also of interest. Remarkable selectivity is a salient feature of both transformations—only a single methyl group is removed. It may be presumed in analogy to dealkylation of xenobiotic compounds[5] that carbinolamine intermediate **4** formed in an enzyme-catalyzed reaction is not further oxidized but is decomposed directly to give the monodemethylated product **5**. Very recently, however, an oxidation of a similar carbinolamine to the corresponding *N*-formyl derivative was postulated in the metabolic pathway of the carcinogen azoxymethane.[17] It appears that regardless of the enzymatic mechanism involved the enzyme effectively utilizes the difference in reactivity of both methyl groups in puromycin aminonucleoside **1c**.

The surprisingly selective reactivity of alkyl groups in N^6,N^6-dialkyladenosine derivatives in oxidation with RuO_4 raises the question of behavior of these models toward a wide spectrum of other oxidizing agents. This possibility is being currently explored in our laboratory. Dealkylation is an important step in metabolism of many xenobiotic compounds of appropriate structure and in the activation of some carcinogens. Clearly, it is of interest to determine whether oxidation with RuO_4 could simulate these transformations at least in some instances.

Experimental Section

General Procedures.[7] Thin-layer chromatography (TLC) was performed on 6 × 2 cm, precoated, silica gel F-254 aluminum foil (Merck, Darmstadt, Germany) in solvents S_1, benzene–ethyl acetate (9:1), S_2, chloroform–methanol (37:3), S_3, chloroform–methanol (95:3), and S_4, chloroform–methanol (7:1). For column chromatography silica gel 60, 70–230 mesh ASTM (Merck, Darmstadt, Germany), was used. NMR spectra were obtained with an FX 100 Fourier transform NMR spectrometer (JEOL Ltd., Tokyo, Japan) using $CDCl_3$ as solvent and $Si(CH_3)_4$ as an internal reference. Electron-impact mass spectra (MS) were determined with a JMS 01SG-2 mass spectrometer (JEOL Ltd., Tokyo, Japan). For characterization (yields, analyses, and NMR spectra) cf. Tables I and II unless specified otherwise.

Starting Materials. Ruthenium dioxide containing 55–58% RuO_2 was a product of Engelhardt Industries, Newark, NJ. 6-Chloro-9-(β-D-2′,3′,5′-tri-*O*-benzoylribofuranosyl)purine and N^6,N^6-dimethyl-2′,3′,5′-tri-*O*-acetyladenosine (**1d**) were prepared as described.[18]

***N*⁶,*N*⁶-Dibenzyladenosine (3d).** A mixture of 6-chloro-9-β-D-ribofuranosylpurine[20] (1 g, 3.5 mmol), dibenzylamine (2 g, 10.15 mmol), and DMF (50 mL) was stirred for 24 h at 50 °C (bath temperature). The resultant solution was evaporated in vacuo, the residue was dissolved in water (30 mL), and the pH was adjusted to 9 with Na_2CO_3. After evaporation, the crude **3d** was purified by column chromatography on silica gel (20 g, 2 × 20 cm) with 3% ethanol in chloroform as eluent. The fraction containing **3d** was evaporated and the product crystallized from ethanol: 0.87 g (56%); mp 184–185 °C; UV max (ethanol) 278 nm (ε 20 400); NMR (CD_3SOCD_3 + D_2O, sodium 4,4-dimethyl-4-silapentane-1-sulfonate as internal reference) δ 8.43 and 8.33 (2 s, 2, H_8 and H_2), 7.30 (b s, 10, C_6H_5), 5.98 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.9 Hz), 5.2 (m, 7, CH_2 of $C_6H_5CH_2$ and OH), 4.66 (q, 1, $H_{2'}$), 4.2 (m, 1, $H_{3'}$), 4.0 (m, 1, $H_{4'}$), 3.6 (m, 2, $H_{5'}$). Anal. Calcd for $C_{24}H_{25}N_5O_4$: C, 64.41; H, 5.63; N, 15.65. Found: C, 64.13; H, 5.62; N, 15.57.

2′,3′,5′-Tri-*O*-acetyl-*N*⁶,*N*⁶-diethyladenosine (1f). A mixture of N^6,N^6-diethyladenosine[19] (0.45 g, 1.4 mmol), acetic anhydride (3 mL), and pyridine (2 mL) was stirred for 24 h at room temperature. The solution was evaporated, and the residue was partitioned between chloroform (40 mL) and aqueous, saturated Na_2CO_3 (30 mL). After the organic phase was dried ($MgSO_4$), chloroform was evaporated and the residue chromatographed on a column of silica gel (2 × 10 cm) with CCl_4 and chloroform as eluents to give 0.45 g (88%) of **1f** as a syrup homogeneous in solvents S_2 and S_3.

2′,3′,5′-Tri-*O*-benzoyl-*N*⁶,*N*⁶-dialkyladenosines 1e, 1g, 1i, and 1j. The described procedure[18] was modified as follows:

(14) A. Albert, *Synth. Proced. Nucleic Acid Chem.*, **2**, 1, (1973), loc. cit. p 26.
(15) S. J. Shaw, D. M. Desiderio, K. Tsuboyama, and J. A. McCloskey, *J. Am. Chem. Soc.*, **92**, 2510 (1970).
(16) D. G. Lee and M. van den Engh, *Oxid. Org. Chem.*, 177 (1973).
(17) E. S. Fiala, C. Kulakis, G. Christiansen, and J. H. Weisburger, *Cancer Res.*, **38**, 4515 (1978).
(18) J. Žemlička and F. Šorm, *Collect. Czech. Chem. Commun.*, **30**, 1880 (1965).
(19) H. Tuppy and E. Küchler, *Monatsh. Chem.*, **95**, 1677 (1964).
(20) J. Žemlička and J. Owens in "Nucleic Acid Chemistry—Improved and New Synthetic Procedures, Methods and Techniques", L. B. Townsend and R. S. Tipson, Eds., Wiley, New York, 1978, Part 2, p 611.

Alkylamine (3–6 mmol) was added to the solution of 6-chloro-9-(β-D-2,3,5-tri-*O*-benzoylribofuranosyl)purine (0.8–1.67 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was stirred for 20–24 h at room temperature whereupon it was washed with aqueous saturated Na_2CO_3 (20 mL). The organic phase was dried ($MgSO_4$) and evaporated, and the residue was chromatographed on a column of silica gel as in the case of compound **1f**. All products were homogeneous on TLC (S_2 and S_3).

2′,3′,5′-Tri-*O*-benzoyl-N^6,N^6-dibenzyladenosine (1h). A solution of N^6,N^6-dibenzyladenosine (**3d**; 1 g, 2.2 mmol) in CH_2Cl_2 (40 mL) and pyridine (3 mL) was treated dropwise with benzoyl chloride (1.4 g, 10 mmol) in CH_2Cl_2 (40 mL) with stirring and external ice cooling. The stirring was then continued for 20 h at room temperature, the reaction mixture was washed with aqueous, saturated Na_2CO_3 (40 mL), the organic phase was dried ($MgSO_4$) and evaporated, and the residue was chromatographed as above with a $CHCl_3$–CCl_4 mixture (1:1) as eluent to give a TLC-homogeneous (S_1–S_3) foam, **1h** (1.4 g, 82%), which was crystallized from ethanol.

2′,3′,5′-Tri-*O*-benzoyl-N^6-benzyladenosine (1k). The procedure described above for the preparation of 2′,3′,5′-tri-*O*-benzoyl-N^6,N^6-dialkyladenosines was utilized with benzylamine instead of a dialkylamine. The reaction time was 3 days. After chromatography, the product **1k** was obtained as a TLC-homogeneous (S_1 and S_2) foam in 74% yield.

2′,3′,5′-Tri-*O*-benzoyl-N^6-methyladenosine (1l). A mixture of 6-chloro-9-(β-D-2,3,5-tri-*O*-benzoylribofuranosyl)purine (1 g, 1.67 mmol), methylamine hydrochloride (0.34 g, 5.1 mmol), triethylamine (2 mL), and CH_2Cl_2 (20 mL) was stirred for 2 days at room temperature whereupon it was worked up as given for compounds **1e**, **1g**, **1i**, and **1j** to give **1l** (0.62 g, 63%) as a TLC-homogeneous (S_2 and S_3) foam.

2′,3′,5′-Tri-*O*-acetyl-N^6-methyladenosine (1m). A solution of N^6-methyladenosine[6b] (**3a**; 0.2 g, 0.71 mmol), acetic anhydride (4 mL), and pyridine (1 mL) was stirred at room temperature for 12 h. After evaporation, the residue was chromatographed on a silica gel column (1.1 × 28 cm) with 1% methanol in chloroform as the solvent (total 250 mL). From the first fractions, N^6-methyl-N^6,2′,3′,5′-*O*-tetraacetyladenosine was obtained as a TLC-homogeneous (S_2 and S_3) foam: 63 mg (20%); NMR ($CDCl_3$) δ 8.78 and 8.23 (2 s, 2, H_8 and H_2), 6.26 (d, 1, $J_{1',2'}$ = 4.9 Hz), 5.99 (t, 1, $H_{2'}$), 5.70 (b t, 1, $H_{3'}$), 4.45 (b s, 3, $H_{4'}$ and $H_{5'}$), 3.64 (s, 3, NCH_3), 2.35 (s, 3, $NCOCH_3$), 2.17, 2.13, and 2.10 (3 s, 9, $OCOCH_3$).

From the following fractions, compound **1m** was obtained as a TLC-homogeneous (S_2 and S_3) foam, 0.2 g (69%); for the NMR data see Table II.

2′,3′,5′-Tri-*O*-benzoyl-N^6-methyl-N^6-formyladenosine (2b). A mixture of N^6-methyl-2′,3′,5′-tri-*O*-benzoyladenosine (**1l**; 57 mg, 0.096 mmol), sodium acetate (100 mg), and formic acetic anhydride[21] (1 mL) was stirred for 20 h at room temperature. After evaporation, the residue was partitioned between chloroform (30 mL) and aqueous, saturated Na_2CO_3 (10 mL). The aqueous portion was extracted with chloroform (20 mL), and the combined organic phases were dried ($MgSO_4$) and evaporated. The residue was chromatographed on a silica gel column (1.5 g, 0.7 × 9 cm) with CCl_4 and chloroform as eluents. Product **2b** was obtained as a TLC-homogeneous (S_2 and S_3) foam, 30 mg (51%), identical (TLC, NMR) with a sample prepared by oxidation of **1e** with RuO_4. In addition to **2b**, 12 mg (21%) of the starting material **1l** was recovered from the column.

N^6-Benzyl-N^6,2′,3′,5′-*O*-tetrabenzoyladenosine (2e). The procedure for the preparation of compound **1h** was followed starting from derivative **1k** (0.4 g, 0.6 mmol) and benzoyl chloride (0.13 g, 0.9 mmol). Chromatography of the crude product on silica gel column (2 × 13 cm) afforded **2e** as a TLC-homogeneous (S_1–S_3) foam (0.34 g, 74%). In addition, 80 mg (20%) of the starting material **1k** was recovered.

Oxidation N^6,N^6-Dialkyl-2′,3′,5′-tri-*O*-acyladenosines 1d–j with RuO_4 (General Procedure). N^6,N^6-Dialkyl-2′,3′,5′-tri-*O*-acyladenosine (1–3 mmol) in CCl_4 (20 mL) was treated dropwise with a freshly prepared solution of RuO_4 (from 3 equiv of RuO_2 with $NaIO_4$ as oxidizing agent) in CCl_4 with vigorous magnetic stirring at room temperature. After 30 min to 1 h 2-propanol (1 mL) was added to the dark suspension to destroy the excess reagent. The solids were filtered, the filtrate was evaporated, and the residue was chromatographed on a silica gel column as mentioned above.

Ammonolysis of Tri-*O*-acyl Derivatives 2a, 2b, and 2c. A solution of the tri-*O*-acyl derivative (0.05 mmol) in methanolic ammonia saturated at 0 °C (7 mL) was kept for 24 h at room temperature. The reaction mixture was evaporated, and the residue was subjected to preparative TLC in solvent S_4. The appropriate bands were eluted to give compounds **3a** [87%, mp 160–190 °C (foaming[6])], **3b** [94%, mp 195–196 °C (lit.[7] mp 191–193 °C)], and **3c** [92%, mp 168–169 °C (lit.[9,10] mp 167 °C and 177–179 °C)]. All products were identical by TLC (S_4) with appropriate authentic samples.

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Registry No. 1d, 31199-61-8; **1e**, 70230-70-5; **1f**, 71138-53-9; **1g**, 71118-13-3; **1h**, 71138-54-0; **1i**, 71118-14-4; **1j**, 71118-15-5; **1k**, 51549-20-3; **1l**, 71118-16-6; **1m**, 56787-20-3; **2a**, 71118-17-7; **2b**, 71118-18-8; **2c**, 71118-19-9; **2d**, 71118-20-2; **2e**, 71118-21-3; **2f**, 71138-55-1; **2g**, 71118-22-4; **3a**, 2620-62-4; **3b**, 14357-08-5; **3c**, 4294-16-0; **3d**, 71118-23-5; ruthenium tetroxide, 20427-56-9; 6-chloro-9-β-D-ribofuranosylpurine, 5399-87-1; N^6,N^6-diethyladenosine, 2139-60-8; 6-chloro-9-(β-D-2,3,5-tri-*O*-benzoylribofuranosyl)purine, 3510-73-4; N^6-methyladenosine, 1867-73-8; N^6-methyl-N^6,2′,3′,5′-*O*-tetraacetyladenosine, 71118-24-6.

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