"Reduced" Analogues of Puromycin. Synthesis of 3′-*O*-(L-2-Amino-3-phenylpropyl)-N^6,N^6-dimethyladenosine and the Corresponding 2′ Isomer[1a]

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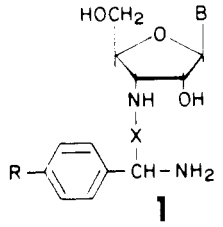
The synthesis and biological investigation of the title compounds **7a** and **7b** are described. The reaction of trityl derivatives **3a** and **3b** with triflate **2b** in the presence of NaH in dioxane gave the isomeric mixtures **4a** plus **5a** and **4b** plus **5b**, respectively. Detritylation in 80% acetic acid led to the product consisting of 80–90% of the 2′ isomer **6b**. The reaction of benzyl derivative **3e** with reagent **2b** led to a 1:1 mixture of 2′ and 3′ isomers **4e** and **5e** which were resolved by combination of column chromatography and preparative thin-layer chromatography on silica gel. The reaction of **2b** with 5′-*O*-acyl derivatives **4c** and **4d** led to a partial removal of 5′ protecting groups whereas alkylations with reagents **2c–e** were not effective. Deblocking of **4e** and **5e** by catalytic hydrogenation and reaction with hydrazine in ethanol gave the target compounds **7a** and **7b**. Compounds **1b**, **7a**, and **7b** failed to participate in the *E. coli* ribosome-catalyzed peptide bond synthesis or to inhibit the puromycin reaction or the cell growth in the murine leukemia L 1210 system. They inhibited the leucine incorporation into protein in the latter system to the extent of 10%. The importance of carbonyl (amide or ester) groups in puromycin and related analogues for the process of ribosome-catalyzed peptide bond synthesis is discussed.

Analogues of the nucleoside antibiotic puromycin (**1a**) with the carbonyl group replaced by a methylene function are of interest as models for investigating the mechanism of protein synthesis[1b] and also as potential antitumor agents. Recently, we have reported the first example[1b] of such a "reduced" analogue of puromycin, compound **1b**.



1

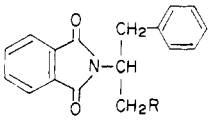
1a: R = CH_3O, X = CO
1b: R = H, X = CH_2

B = N^6,N^6-dimethyladenine

It was, therefore, of interest to prepare a stereoelectronically similar model with N-3′ replaced by an oxygen atom. The latter compound would then resemble 2′(3′)-*O*-L-(phenylalanyl)adenosine, which is a strong inhibitor of ribosome-catalyzed protein synthesis, comparable to puromycin (**1a**).[2]

At the outset, it was clear that the simplest approach to such analogues should include a direct alkylation of the 2′,3′-cis vicinal glycol moiety in N^6,N^6-dimethyladenosine and separation of the resultant mixture of 2′ and 3′ isomers. Monoalkylation of 2′- or 3′-hydroxy groups in ribonucleosides has been the subject of several studies restricted to simple alkylating agents.[3-7] The most complex reagent employed was a very reactive *o*-nitrobenzyl chloride which, in conjuction with NaH, gave modest yields of 2′-*O*-alkyl derivatives.[5b,c]

A method using a stoichiometric amount or a slight excess of alkylating agent and NaOH or NaH was employed for alkylation of the isolated 5′- and 3′-hydroxy groups of suitably protected ribo- or deoxyribonucleosides.[7] However, attempts to apply the reaction to polyfunctional alkylating agents such as 1-bromo-2,2-diethoxypropane failed.[7d] Thus, the currently available methods offer little in terms of a selective 2′/3′ monoalkylation of the cis diol moiety in ribonucleosides with more complex alkylating agents. A successful alkylation[1b] of the 3′-amino group in puromycin aminonucleoside with triflate **2b** and NaH indicated that such a reaction would also be possible with the 2′,3′-cis vicinal glycol grouping of ribonucleosides. The results of such experiments—synthesis of 2′ and 3′ isomers of "reduced" puromycin analogues **7a** and **7b** together with their biochemical investigation—provide the basis of this paper.



2

2a: R = OH **2c**: R = CH_3SO_2O
2b: R = CF_3SO_2O **2d**: R = Br
2e: R = I

The synthesis of reagent **2b** has been described previously.[1b] Because the study of the comparative effectiveness of various alkylating reagents was also of interest, we have prepared the corresponding methylsulfonyl derivative **2c** and halogeno compounds **2d** and **2e**. Reagent **2c** was obtained by a simple methylsulfonylation of the readily available[8] *N*-phthalyl derivative **2a** by using methylsulfonyl

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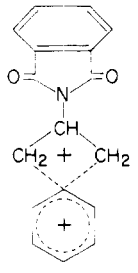
chloride in pyridine. The methylsulfonyl group of **2c** was readily displaced by the reaction with bromide (iodide) ion in dimethylformamide (DMF) to give the corresponding bromo and iodo compounds **2d** and **2e**. The phthalyl group, which is present in compounds **2b–e**, was considered advantageous because it is essentially nonparticipating and it eliminates the possibility of ionization of the N–H bond during the reaction with NaH and an adverse effect of the resultant charged species on alkylation. It may be argued that in peptide chemistry the use of the phthalyl function can lead to racemization, particularly in the presence of base.[9] In our case, however, this possibility is precluded by the absence of an activating carbonyl group in the reagents and reaction products. It may also be argued that participation of the phenyl group in alkylations with compounds **2b–e**, as indicated in formula A, can lead to



A

a racemization of the reagent. However, it is recognized that such an interaction, as described for the β-phenylethyl (Ar_1-3) and δ-phenylbutyl (Ar_1-5) moieties,[10a] has not yet been reported for the γ-phenylpropyl (Ar_1-4) system[10b,c] which also occurs in compounds **2b–e**. In addition, an absence of Ar_1-4 participation and hence the racemization was confirmed by circular dichroism (CD) spectra. Thus, methylsulfonyl derivative **2c** and the corresponding products of nucleophilic displacement, bromo and iodo compounds **2d** and **2e**, exhibit a weak positive Cotton effect at ca. 308 nm and a strong negative Cotton effect at ca. 230 nm. However, it is interesting to note that hydroxy derivative **2a** exhibits a spectrum which is roughly a mirror image of those of **2c–e**: a weak negative Cotton effect at ca. 310 nm and a strong positive one at ca. 230 nm. Inductive influences probably have little effect on the Cotton effect at 310 nm; this is also evident from the UV_{max} values of **2a–e**, which are very similar. However, it is recognized that formation of the cyclic hydrogen bonded structure of **2a** is, unlike in **2c–e**, a distinct possibility, and it could account for the observed differences in the CD spectra.

A series of 5′-substituted N^6,N^6-dimethyladenosines, **3a–e**, were prepared by conventional methods, and their alkylation with reagents **2b–e** was investigated. The results are summarized in Table I. It is clear that the only effective agent for alkylation of the 2′,3′-cis vicinal glycol function in **3a,b,e** is the triflate **2b** in conjunction with NaH in dioxane, DMF, or dimethyl sulfoxide (Me_2SO). The acyl protecting groups of compounds **3c** and **3d** are not suitable because of their partial removal during the reaction. Addition of 18-crown-6 did not improve the yield, but increasing the reaction time from 18 h to 3 days had a favorable effect. The attempted alkylation with **2b** under the conditions of phase transfer and with bromo derivative **2d** in the presence of NaH or Ag_2O in DMF was not successful. The reaction of 2′,3′-*O*-(dibutylstannylene)-N^6,N^6-dimethyladenosine with iodo compound **2e** or triflate **2b** in DMF also failed.

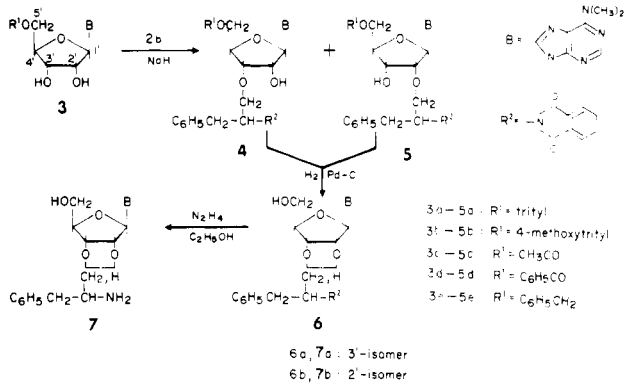
In summary, our results have shown that alkylation of **3a** and **3b** can be achieved in moderate yields to give mixtures of 2′ and 3′ isomers **4a** plus **5a** and **4b** plus **5b** (see Scheme I). NMR spectra of the detritylated compounds **6a** and **6b** indicated that the products contain 80–90% of the 2′ isomer (**6b**) and only 10–20% of the 3′ isomer (**6a**). The assignments are based on the general observation[11] that the $H_{1'}$ signal of a 2′ isomer is at a lower field relative to that of a 3′ isomer. It is also of interest to note that unlike in the case of the corresponding puromycin analogue[1b] we have been unable to detect a molecular ion in the mass spectrum of **6b** (90% 2′ isomer). Attempted separation of isomers **4a**, **5a**, **4b**, **5b**, and **6a**, **6b** by preparative TLC was not successful probably because the subtle differences in polarity between the 2′ and

Table I. Products of the Reaction of 5′-*O*-Protected N^6,N^6-Dimethyladenosines 3a, 3b, and 3c with L-1-Substituted 3-Phenyl-2-Phthalimidopropanes 2b–d at Room Temperature[a,b]

starting nucleoside	alkylating agent	solvent (catalyst)	t, h	products (% yield)
3b	2b	dioxane (NaH)	18	4b, 5b (11)
3b	2b	dioxane (NaH)	72	4b, 5b (40)
3a	2b	dioxane (NaH)	18	4a, 5a (13)
3a	2b	dioxane (NaH)	72	4a, 5a (38)
3b	2b	DMF (NaH)	18	4b, 5b (9)
3b	2b	Me_2SO (NaH)	18	4b, 5b (9)
3b	2b	CH_2Cl_2 (aqueous NaOH $NBu_4^+HSO_4^-$	18	none
3b	2d	DMF (Ag_2O)	18	none
3b	2c	dioxane (NaH)	18	none
3b	2c	Me_2SO (NaH)	18	none
3a	2b	dioxane (NaH, 18-crown-6)	18	4a, 5a (14)
3e	2b	dioxane (NaH)	72	4e, 5e (21)

[a] Bu = *n*-butyl. [b] For further details see the Experimental Section.

Scheme I



6a, **7a**: 3′-isomer
6b, **7b**: 2′-isomer

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3′ isomers are either "swamped" by the presence of lipophilic trityl and phthalyl groups or, in the case of **6a** and **6b**, by the presence of a polar 5′-hydroxy group. The attempted separation of **6a** and **6b** by chromatography on a Dowex 1 (OH^-) column[12] was also fruitless, and some decomposition was observed, probably because of the limited stability of the phthalyl group toward strong aqueous bases.[13]

Predominant formation of the 2′ isomers in the alkylation of 5′-*O*-trityl derivatives is not surprising in view of the report[14] that reaction of 8-bromo-5′-*O*-trityladenosine with 2,4,6-triisopropylbenzenesulfonyl chloride in the presence of NaH in DMF gave 75% of the 2′ isomer and only 25% of the 3′ isomer. By contrast, the corresponding 5′-*O*-benzoyl or -acetyl derivative gave the 2′ and 3′ isomers in an ~1:1 ratio. In our hands, however, the 5′-*O*-acyl derivatives **3c** and **3d** proved to be too unstable (vide infra) to be successfully employed.

It was possible that the more stable but less bulky 5′-*O*-benzyl group could also offer the advantage of an increased 3′/2′ ratio in the alkylated product. This proved to be the case, and the reaction of 5′-*O*-benzyl derivative **3e** with triflate **2b** in the presence of NaH in dioxane gave an isomeric mixture of **4e** and **5e** in a 1:1 ratio. The presence of the benzyl group probably also favorably influenced the balance of the polar effects in **4e** and **5e** because it was possible to separate isomers **4e** and **5e** by a combination of thin-layer and column chromatography on silica gel. Both derivatives were deprotected by catalytic hydrogenolysis (removal of the *O*-benzyl group) and hydrazinolysis (cleavage of the phthalyl function) to give the desired analogues of puromycin, **7a** and **7b**. The structures were confirmed by NMR spectra which showed an expected[11] downfield shift of the $H_{1'}$ proton for the 2′ isomer **7b** (δ 5.90) relative to the 3′ isomer **7a** (δ 5.82).

Further support for the proposed structures **7a** and **7b** derives from the coupling constants $J_{1',2'}$ (7.8 Hz for the 2′ isomer **7b** vs. 7.1 Hz for the 3′ isomer **7a**) which also reflect the trend observed in similar cases.[11] Compounds **7a** and **7b** are ninhydrin positive, and their electrophoretic mobilities in 1 M acetic acid (3.38 of phenylalanine) are similar to that of N^6,N^6-dimethyl-2′(3′)-*O*-L-(phenylalanyl)adenosine (4.0).[15]

Biological Results

Compounds **1b**, **7a**, and **7b** were tested as inhibitors of protein synthesis in an *E. coli* ribosomal system and in murine leukemia L 1210 assay. It was found that none of them accepted the *N*-acetyl-L-phenylalanyl residue from *N*-acetyl-L-phenylalanyl-tRNA–poly(U)–70S *E. coli* ribosome complex at 10^{-3} M and they also did not inhibit the puromycin reaction in the same system.[16] Compounds **1b**, **7a**, and **7b** inhibited incorporation of leucine into protein to the extent of 10% at 2×10^{-4} M in the murine leukemia L 1210 system, but they did not inhibit cell growth.

The results provide support[17] for the role of the carbonyl group in an acceptor substrate for the protein synthesis catalyzed by ribosomes.[18] It is difficult to rationalize the results in terms of steric hindrance because the CH_2 group is smaller than the CO group. However, differences in hybridization of orbitals at both carbon atoms and a different rotameric population of the aminoacyl moiety in puromycin or 2′(3′)-*O*-(aminoacyl)ribonucleosides and the aminoalkyl portion of **1b**, **7a**, and **7b** can play some role. It is also possible that peptidyl transferase[18] contains a binding locus for the amido or ester carbonyl group of the acceptor substrate. Interestingly, antibiotic lincomycin, a known inhibitor of ribosome-catalyzed protein synthesis, also lost its biological activity when the CO group was replaced by CH_2.[19]

Experimental Section

General Procedures.[20] Thin-layer chromatography (TLC) was performed with precoated silica gel F-254 aluminum foil in the following dichloromethane–methanol mixtures: S_1, 95:5; S_2, 93:7; S_3, 9:1; and S_4, 4:1. For preparative TLC, 4 mm thick, 35 × 15 cm loose layers of silica gel (70–325-mesh ASTM, Merck, Darmstadt, Germany) containing 1% of a fluorescent indicator[20] were used. The same material without the indicator was employed for column chromatography. Paper electrophoresis was carried out at 15 °C on a flat plate[20] in 1 M acetic acid at 40 V/cm for 1 h with Whatman no. 1 paper. NMR spectra were measured on a JEOL FX 100 Fourier transform spectrometer in $CDCl_3$ or CD_3SOCD_3. As internal reference $(CH_3)_4Si$ in $CDCl_3$ or sodium 4,4-dimethyl-4-silapentane-1-sulfonate in CD_3SOCD_3 was used. Electron-impact mass spectra were determined by using a JEOL JMS 01SG-2 spectrometer.

Dioxane was distilled from sodium, and it was kept over sodium wire. Distilled DMF, Me_2SO, and pyridine were stored over Linde molecular sieves 3A. Sodium hydride was employed as a 50% oil dispersion which was washed with benzene immediately before use.

Starting Materials. L-2-Amino-3-phenyl-1-propanol (phenylalaninol, **2a**) and *N*-carbethoxyphthalimide were commercial products. N^6,N^6-Dimethyladenosine and the corresponding 5′-*O*-(4-methoxytrityl) derivative **3b** were prepared as described.[15] Trifluoromethylsulfonyl derivative **2b** was obtained according to the known procedure:[1b,21] mass spectrum, *m/e* (relative intensity) 413 (6.2, M), 322 (13.1, M – $C_6H_5CH_2$), 266 (22.9, M – phthalimide), 263 (8.3, M – CF_3SO_3H), 173 (33.3, M – CF_3SO_3 – $C_6H_5CH_2$), 172 (26.3, M – CF_3SO_3H – $C_6H_5CH_2$), 148 (100.0, phthalimide + H), 117 (26.3, M – CF_3SO_3 – phthalimide), 116 (29.8, M – CF_3SO_3H – phthalimide), 91 (27.7, $C_6H_5CH_2$).

L-3-Phenyl-2-phthalimido-1-propanol (2a). The procedure for the preparation of the racemic compound[8] was used to give compound **2a** in 55% yield: homogeneous on TLC (S_1); mp 106–107 °C [lit.[8] mp (racemic product) 97–98 °C]; UV max (ethanol) 295 nm (ϵ 1800); CD max (ethanol) 308 nm ([Θ] –860), 232 ([Θ] 3960); NMR ($CDCl_3$) δ 7.73 (m, 4, phthalyl), 7.20 (s, 5, phenyl), 4.60 (m, 1, H_2), 3.99 (m, 2, H_1), 3.20 (d, 2, H_3), 2.80 (br m, 1, OH).

L-1-[(Methylsulfonyl)oxy]-3-phenyl-2-phthalimidopropane (2c). A magnetically stirred solution of compound **2a** (1.5 g, 5.3 mmol) in pyridine (40 mL) was treated with methylsulfonyl chloride (0.9 g, 8.4 mmol) at –20 to –25 °C (dry ice bath). The mixture was stirred for 3 h at 0 °C (ice bath) and cooled again to –15 to –10 °C, and additional methylsulfonyl chloride (0.9 g, 8.4 mmol) was added. After the mixture had been allowed to stand at 0 °C overnight, the solvent was evaporated, and the residue was dissolved in chloroform. The resultant solution was washed

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(16) For the details of these assays see: Li, C.; Bhuta, P.; Žemlička, J. *Biochemistry* **1978**, *17*, 2537.
(17) Replacement of the carbonyl group in 2′(3′)-*O*-L-(phenylalanyl)-adenosine with PO(OH) moiety led also to a loss of acceptor activity: (a) Žemlička, J.; Chládek, S. *Collect. Czech. Chem. Commun.* **1969**, *34*, 1007. These results were more recently confirmed (Coutsogeorgopoulos, C., personal communication) by using pure 2′ and 3′ isomers prepared in our laboratory: (a) Bhuta, P.. Chung, H. L., Hwang, J. S., Žemlička, J. *J. Med. Chem.*, in press.
(18) For reviews of ribosome-catalyzed protein synthesis, see: (a) Harris, R. J.; Symons, R. H. *Bioorg. Chem.* **1973**, *2*, 286. (b) Krayevsky, A. A.; Kukhanova, M. K.; Gottikh, B. P. *Nucleic Acids Res.* **1975**, *2*, 2233.
(19) Cheney, B. V. *J. Med. Chem.* **1974**, *17*, 590 (see footnote 1). It has to be stressed that in this paper the antibiotics inhibiting ribosome-catalyzed protein synthesis and containing the carbonyl (amide or ester) group are considered to be analogues of peptidyl tRNA and not aminoacyl tRNA or puromycin.
(20) Žemlička, J.; Owens, J. *J. Org. Chem.* **1977**, *42*, 517.
(21) Inadvertently, the amount of pyridine used (0.13 g, 1.7 mmol) was omitted in the preparation[1b] of **2b**.

with saturated aqueous $NaHCO_3$ and water, dried ($MgSO_4$), and evaporated. The residue crystallized after addition of ether, and it was recrystallized from 2-propanol to give compound **2c**: 1.62 g (84%); homogeneous on TLC (S_1); mp 103–103.5 °C; UV max (ethanol) 295 nm (ϵ 2100); CD max (ethanol) 306 nm ([θ] 840), 226 ([θ] −4730); NMR ($CDCl_3$) δ 7.73 (m, 4, phthalyl), 7.19 (m, 5, phenyl), 4.82 (m, 2, H_1 and H_2), 4.51 (m, 1, H_2), 3.24 (m, 2, H_3), 2.94 (s, 3, CH_3SO_2). Anal. Calcd for $C_{18}H_{17}NO_5S$: C, 60.15; H, 4.77; N, 3.90. Found: C, 59.77; H, 4.80; N, 3.89.

L-1-Bromo- and L-1-Iodo-3-phenyl-2-phthalimidopropanes 2d and 2e. A mixture of methylsulfonyl derivative **2c** (0.36 g, 1 mmol) and tetraethylammonium bromide or tetrabutylammonium iodide (1.5 mmol) in DMF (20 mL) was heated at 140 °C for 4 h. After the mixture cooled, the solvent was evaporated, the residue was dissolved in chloroform, and the resultant solution was washed with 5% HCl, saturated aqueous $NaHCO_3$, and water, dried ($MgSO_4$), and evaporated. The residue crystallized after addition of petroleum ether, and it was recrystallized from methanol to give bromo derivative **2d**: 0.3 g (87%); homogeneous on TLC (S_1); mp 118–119 °C; UV max (ethanol) 294 nm (ϵ 2000); CD max (ethanol) 308 nm ([θ] 960), 232 ([θ] −3950); NMR ($CDCl_3$) δ 7.73, (m, 4, phthalyl), 7.18 (m, 5, phenyl), 4.79 (m, 1, H_2), 4.12 and 3.67 (t and q, 2, H_1), 3.29 (d, 2, H_3). Anal. Calcd for $C_{17}H_{14}BrNO_2$: C, 59.32; H, 4.10; N, 4.07. Found: C, 59.55; H, 4.15; N, 3.99.

Iodo derivative **2e** was obtained in the same fashion: 0.35 g (90%); homogeneous on TLC (S_1); mp 138–140 °C; UV max (ethanol) 293 nm (ϵ 2300); CD max (ethanol) 308 nm ([θ] 990), 235 ([θ] −9340); NMR ($CDCl_3$) δ 7.74 (m, 4, phthalyl), 7.18 (m, 5, phenyl), 4.74 (m, 1, H_2), 3.97 and 3.54 (t and q, 2, H_1), 3.30 (d, 2, H_3); mass spectrum, *m/e* (relative intensity) 392 (0.9, M), 391 (3.5, M − H), 301 (6.9, M − $C_6H_5CH_2$), 300 (61.3, M − H − $C_6H_5CH_2$), 265 (12.8, M − I), 264 (65.7, M − HI), 174 (8.0, M − I − $C_6H_5CH_2$), 173 (63.6, M − HI − $C_6H_5CH_2$), 148 (22.7, phthalimide + H), 118 (7.0, M − I −phthalimide), 117 (59.2, M − HI − phthalimide), 91 (100.0, $C_6H_5CH_2$). Anal. Calcd for $C_{17}H_{14}INO_2$: C, 52.19; H, 3.61; N, 3.58. Found: C, 52.43; H, 3.66; N, 3.59.

***N*⁶,*N*⁶-Dimethyl-2′,3′-*O*-isopropylideneadenosine.** A mixture of *N*⁶,*N*⁶-dimethyladenosine[15] (1 g, 3.4 mmol), $HClO_4$ (74%, 1 g, 7.3 mmol), and acetone (300 mL) was magnetically stirred for 3 h at room temperature. After addition of $NaHCO_3$ (5 g), the stirring was continued for 30 min. The inorganic salts were filtered off, the filtrate was evaporated, and the residue was crystallized from methanol: yield 0.64 g (56%); mp 178–180 °C (lit.[22] mp 173–175 °C); NMR ($CDCl_3$) δ 8.26 and 7.72 (2 s, 2, H_8 and H_2), 5.82 (d, 1, H_1, $J_{1',2'}$ = 4.9 Hz), 5.19 (m, 2, $H_{2'}$ and $H_{3'}$), 4.52, 3.87 (d and dq, 1 and 2, $H_{4'}$ and $H_{5'}$), 3.52 (s, 6, $(CH_3)_2N$), 1.64, 1.37 (2 s, 6, isopropylidene).

***N*⁶,*N*⁶-Dimethyl-5′-*O*-trityladenosine (3a).** A mixture of *N*⁶,*N*⁶-dimethyladenosine[15] (0.29 g, 1 mmol) and trityl chloride (0.28 g, 1 mmol) was dried at 0.5 mm at 40 °C for 2.5 h. Pyridine (5 mL) was then added, and the solution was magnetically stirred for 60 h at room temperature. The solvent was evaporated, and ice was added to the residue followed by chloroform. The organic phase was washed with water, dried ($MgSO_4$), and evaporated. The residue was chromatographed on a loose layer of silica gel in solvent S_3, and the major UV-absorbing band was eluted with the same solvent. The eluate was evaporated to give **3a** which solidified after addition of petroleum ether: 0.37 g (73%); homogeneous on TLC (S_3); NMR ($CDCl_3$) δ 8.24 and 8.01 (2 s, 2, H_8 and H_2), 7.23 (m, 15, trityl), 5.94 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.1 Hz), 3.53 (s, overlapped with ribose protons, $(CH_3)_2N$). Anal. Calcd for $C_{31}H_{31}N_5O_4 \cdot {}^1/_2H_2O$: C, 68.11; H, 5.90; N, 12.81. Found: C, 68.43; H, 6.01; N, 12.50.

5′-*O*-Acetyl-*N*⁶,*N*⁶-dimethyladenosine (3c). A mixture of *N*⁶,*N*⁶-dimethyl-2′,3′-*O*-isopropylideneadenosine (0.55 g, 1.6 mmol), acetic anhydride (2 mL), and pyridine (20 mL) was kept overnight at room temperature. The resultant solution was evaporated, and the oily residue was coevaporated several times with ethanol and chromatographed on a silica gel column with chloroform as an eluent. The product-containing fractions were evaporated, the residue was dissolved in 90% HCOOH (20 mL), and the solution was kept for 4 h at room temperature. After evaporation, the crude **3c** was chromatographed on one loose layer of silica gel in solvent S_3. The major UV-absorbing band was eluted with the same solvent, the eluate was evaporated, and the residue was crystallized from methanol to give 0.3 g (51%) of **3c**: homogeneous on TLC (S_2); mp 114–119 °C; NMR ($CDCl_3$ and CD_3SOCD_3) δ 8.24 and 7.96 (2 s, 2, H_8 and H_2), 6.02 (d, 1, H_1, $J_{1',2'}$ = 4.4 Hz), 5.37 and 4.62 (2 d, 2, 2′- and 3′-OH, $J_{OH,2'(3')}$ = 5.1 and 4.9 Hz), 4.54 and 4.33 (2 m, 5, ribose protons), 3.52 (s, 6, $(CH_3)_2N$), 2.07 (s, 3, acetyl); mass spectrum, *m/e* (relative intensity) 337 (12.3, M), 42 (81.3, CH_2=C=O), the rest of ions was identical with those found in *N*⁶,*N*⁶-dimethyladenosine.[23] Anal. Calcd for $C_{14}H_{19}N_5O_5 \cdot {}^5/_4H_2O$: C, 46.73; H, 6.02; N, 19.46. Found: C, 46.94; H, 5.88; N, 19.49.

5′-*O*-Benzoyl-*N*⁶,*N*⁶-dimethyl-2′,3′-*O*-isopropylideneadenosine. A mixture of *N*⁶,*N*⁶-dimethyl-2′,3′-*O*-isopropylideneadenosine (0.55 g, 1.6 mmol) and benzoic anhydride (0.41 g, 1.8 mmol) in pyridine (20 mL) was heated at 50 °C for 18 h. The resultant solution was evaporated, the residue was dissolved in chloroform, and the organic layer was washed successively with 3% HCl, saturated aqueous $NaHCO_3$, and water. After the organic layer was dried ($MgSO_4$), the chloroform was removed in vacuo, and the crude product was chromatographed on a silica gel column with chloroform as an eluent to give 0.6 g (83%) of the amorphous title compound:[24] NMR ($CDCl_3$) δ 8.33 and 7.81 (2 s, H_8 and H_2), 7.91 (m, 2, benzoyl, ortho H), 7.39 (m, 3, benzoyl, meta and para H), 6.10 (d, 1, H_1, $J_{1',2'}$ = 2.2 Hz), 5.58 (dd, 1, $H_{2'}$, $J_{2',1'}$ = 2.2 Hz, $J_{2',3'}$ = 6.4 Hz), 5.16 (dd, 1, $H_{3'}$, $J_{3',2'}$ = 6.4 Hz, $J_{3',4'}$ = 2.7 Hz), 4.56 (m, 3, $H_{4'}$ and $H_{5'}$), 3.51 (s, 6, $(CH_3)_2N$), 1.63, 1.42 (2 s, 6, isopropylidene); mass spectrum, *m/e* (relative intensity) 439 (18.0, M), 424 (5.4, M − CH_3), 318 (8.2, M − C_6H_5COO), 247 (9.4, M − 192), 246 (60.2, M − 193), 192 (96.0, B − CHOH), 105 (100.0, C_6H_5CO), 77 (21.1, C_6H_5), the rest of ions corresponded to those found in *N*⁶,*N*⁶-dimethyladenosine.[23] Anal. Calcd for $C_{22}H_{25}N_5O_5 \cdot {}^1/_2H_2O$: C, 58.92; H, 5.84; N, 15.62. Found: C, 59.07; H, 5.59; N, 15.54.

5′-*O*-Benzoyl-*N*⁶,*N*⁶-dimethyladenosine (3d). A solution of 5′-*O*-benzoyl-*N*⁶,*N*⁶-dimethyl-2′,3′-*O*-isopropylideneadenosine prepared as described above from *N*⁶,*N*⁶-dimethyl-2′,3′-*O*-isopropylideneadenosine (0.75 g, 2.2 mmol) in 90% HCOOH (10 mL) was kept for 18 h at room temperature whereupon it was evaporated. The residue was coevaporated several times with ethanol, and it was chromatographed on a silica gel column with chloroform as an eluent. The appropriate fractions were evaporated, and the residue was dissolved in methanol to deposit compound **3d** as a resinous solid which was recrystallized from 60% methanol: yield 0.23 g (26%); homogeneous on TLC (S_3);[25] mp 116–123 °C; NMR (CD_3SOCD_3) δ 8.33 and 8.20 (2 s, H_8 and H_2), 7.96 (m, 2, benzoyl, ortho H), 7.59 (m, 3, benzoyl, meta and para H), 6.00 (d, 1, $H_{1'}$, $J_{1',2'}$ = 4.4 Hz), 5.63 (d, 1, 2′-OH, $J_{OH,2'}$ = 5.4 Hz), 5.45 (d, 1, 3′-OH, $J_{OH,3'}$ = 4.9 Hz), 4.54 (m, 5, ribose H), 3.37 (s, 6, $(CH_3)_2N$). Anal. Calcd for $C_{19}H_{21}N_5O_5 \cdot {}^5/_4H_2O$: C, 54.08; H, 5.61; N, 16.60. Found: C, 54.02; H, 5.41; N, 16.42.

2′,3′-*O*-(Dibutylstannylene)-*N*⁶,*N*⁶-dimethyladenosine. A mixture of *N*⁶,*N*⁶-dimethyladenosine[15] (0.59 g, 2 mmol) and dibutyltin oxide (0.5 g, 2 mmol) was refluxed in methanol (100 mL) for 40 min. The solution was kept overnight at room temperature, the insoluble material was filtered off, and the filtrate was evaporated to ca. 10 mL. Addition of acetone precipitated the product: 0.77 g (73%); mp 223–225.5 °C; mass spectrum, *m/e* (relative intensity) 523–529, 531 (0.4–0.9, M), 192 (7.2, B − CHOH), 163, 148, 134, 120, 93 (typical for *N*⁶,*N*⁶-dimethyladenosine[23]), 57 (18.6, C_4H_9). Anal. Calcd for $C_{20}H_{33}N_5O_4Sn$: C, 45.65; H, 6.32; N, 13.31. Found: C, 45.33; H, 6.24; N, 13.22.

5′-*O*-Benzyl-*N*⁶,*N*⁶-dimethyladenosine (3e). Sodium hydride (69 mg, 3 mmol) was added to a solution of *N*⁶,*N*⁶-dimethyl-2′,3′-*O*-isopropylideneadenosine (0.64 g, 1.7 mmol) in DMF (15 mL) cooled to −5 to −10 °C with magnetic stirring. After

(22) Ikehara, M.; Uno, H.; Ishikawa, F. *Chem. Pharm. Bull.* **1964**, *12*, 267.

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(24) The literature gives a melting point of 136–137 °C for a crystalline compound: (a) Ikehara, M.; Harada, F.; Ohtsuka, E. *Chem. Pharm. Bull.* **1966**, *14*, 1338.

(25) This compound was characterized previously[24a] only by paper chromatography.

addition of freshly distilled benzyl bromide (0.26 g, 1.5 mmol) the mixture was stirred for 18 h at room temperature. The solvent was evaporated, cold water (20 mL) was added to the residue, and the resultant mixture was extracted with chloroform. The organic layer was dried ($MgSO_4$) and evaporated, and the crude product was chromatographed on a column of silica gel with chloroform as the eluent. The first (major) UV-absorbing fraction containing 5′-*O*-benzyl-N^6,N^6-dimethyl-2′,3′-*O*-isopropylideneadenosine was evaporated, and the resultant syrup was dissolved in 90% formic acid (8 mL). The solution was kept overnight at room temperature whereupon it was evaporated, and the residue was partitioned between saturated aqueous $NaHCO_3$ and chloroform. The organic layer was dried ($MgSO_4$) and evaporated, and the crude **3e** was chromatographed on a 4 mm thick loose layer of silica gel in solvent S_2. The main UV-absorbing band was eluted with the same solvent, the eluate was evaporated, and the residue was crystallized twice from benzene to give **3e**: homogeneous on TLC (S_1 and S_2); mp 142–145 °C; 0.11 g (32%); UV max (ethanol) 275 nm (ϵ 17 700); NMR (CD_3SOCD_3) δ 8.30 and 8.24 (2 s, 2, H_8 and H_2), 7.34 (s, 5, phenyl), 5.97 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.4 Hz), 5.52 and 5.29 (2 d, 2, 2′- and 3′-OH, $J_{OH,2'(3')}$ = 5.9 and 5.4 Hz), 4.59 (m, overlapped with CH_2 of benzyl, 1, $H_{2'}$), 4.56 (s, overlapped with $H_{2'}$, 2, CH_2 of benzyl), 4.19 (m, 2, $H_{3'}$ and $H_{4'}$), 3.68 (m, 2, $H_{5'}$), 3.47 (s, 6, $(CH_3)_2N$). Anal. Calcd for $C_{19}H_{23}N_5O_4 \cdot {}^1/_3H_2O$: C, 58.30; H, 6.09; N, 17.89. Found: C, 58.43; H, 6.07; N, 17.89.

Alkylation of Trityl Derivatives 3a and 3b with Trifluoromethylsulfonyl Derivative 2b. A solution of trityl derivative **3a** (108 mg, 0.2 mmol) in dioxane (15 mL) was cooled to 10–15 °C, and NaH (7 mg, 0.3 mmol) was added with magnetic stirring. After 30 min triflate **2b** (83 mg, 0.2 mmol) was added, and the reaction mixture was stirred for 3 days at room temperature. The solvent was evaporated, and the residue was applied on a 4 mm thick loose layer of silica gel which was developed twice in solvent S_2. Three UV-absorbing bands were obtained, the slowest being the starting material **3a** followed by the products **4a** and **5a** (unresolved mixture of 2′ and 3′ isomers) and reagent **2b**. The middle band was eluted with the same solvent, the eluate was evaporated, and the residue was dissolved in chloroform. A mixture of products **4a** and **5a** precipitated as an amorphous solid after addition of petroleum ether: 60 mg (38%); moving on TLC (S_1 and S_2) as a single spot; UV max (ethanol) 275 nm (ϵ 17 200).

Trityl derivative **3b** was alkylated in the same fashion to give 65 mg (40%) of the isomeric mixture of **4b** and **5b** which moved on TLC (S_1 and S_2) as a single spot; UV max (ethanol) 275 nm (ϵ 17 400). The results of other alkylations are summarized in Table I.

Alkylation of Benzyl Derivative 3e with Trifluoromethylsulfonyl Derivative 2b and Separation of Isomers 4e and 5e. A mixture of 5′-*O*-benzyl-N^6,N^6-dimethyladenosine (**3e**; 0.39 g, 1 mmol), dried over P_2O_5 at 70 °C for 3 days at 0.01 mm, and dioxane (20 mL) was cooled to 10–15 °C, and NaH (28 mg, 1.2 mmol) was added with magnetic stirring. Reagent **2b** (0.41 g, 1 mmol) was then added and the stirring continued for 3 days at room temperature. The mixture was lyophilized, and the residue was chromatographed on a loose layer of silica gel in solvent S_2. The mixture of **4e** and **5e** which traveled as a single band between the bands for **3e** and **2b** (see the previous experiment) was eluted with the same solvent, and the eluate was evaporated. TLC of the crude mixture showed **4e** and **5e** to be present in a 1:1 ratio as determined spectrophotometrically. The residue was rechromatographed on a column of silica gel (30 g) with chloroform as an eluent. Partially separated isomers **4e** (first peak) and **5e** (second peak) were rechromatographed after evaporation of the appropriate fractions (each on one loose layer of silica gel in solvent S_1). The faster moving band of **4e** was eluted. The eluate was evaporated to give 71 mg (11%) of **4e**: homogeneous on TLC (S_2 and S_3); UV max (ethanol) 274 nm (ϵ 17 300). The slower moving band afforded 64 mg (10%) of **5e**: homogeneous on TLC (S_2 and S_3); UV max (ethanol) 275 nm (ϵ 17 700); NMR ($CDCl_3$) δ 8.23 and 7.99 (2 s, 2, H_8 and H_2), 7.72 (m, 4, phthalyl), 7.23 (m, 10, phenyl), 5.93 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.1 Hz), 4.51 (s, 2, CH_2 of benzyl), 3.54 (s, 6, $(CH_3)_2N$).

Detritylation of Isomeric Mixtures of 4a, 5a and 4b, 5b. A solution of the mixture **4a** and **5a** (79 mg, 0.1 mmol) in 80% acetic acid (7 mL) was heated at 100 °C for 10 min. After the mixture cooled, the solvent was evaporated, and the residue was chromatographed on a 4 mm thick loose layer of silica gel in solvent S_2. The major UV-absorbing band was eluted with the same solvent, the eluate was evaporated, and the residue was dissolved in chloroform. Addition of petroleum ether precipitated **6a** and **6b**: 44 mg (82%); one spot on TLC (S_1); NMR ($CDCl_3$) δ 8.21 (s, 1, H_8), 7.67 (s, 4, phthalyl), 7.48 (s, 1, H_2), 7.12 (br s, 5, phenyl), 5.70 (d, 1, $H_{1'}$, $J_{1',2'}$ = 7.6 Hz, 2′ isomer), 3.51 (s, 6, $(CH_3)_2N$). The isomeric content was determined from the height of the $H_{1'}$ signals of **6a** and **6b**. The product contained 90% of **6b** (2′ isomer). Anal. Calcd for $C_{29}H_{30}N_6O_6 \cdot {}^9/_8H_2O$: C, 60.17; H, 5.62; N, 14.52. Found: C, 60.50; H, 5.28; N, 14.13.

The same product as shown by TLC and NMR was obtained by detritylation of the mixture of **4b** and **5b** with 80% acetic acid (15 mL) for 24 h at room temperature: yield 41 mg (76%); isomeric content (see above) 80% of **6b** (2′ isomer).

3′-*O*-(L-2-Amino-3-phenylpropyl)-N^6,N^6-dimethyladenosine (7a) and 2′-*O*-(L-2-Amino-3-phenylpropyl)-N^6,N^6-dimethyladenosine (7b). A mixture of compound **4e** (0.13 g, 0.2 mmol), palladium on charcoal (10%, 0.2 g), NH_4OH (0.1 mL), and methanol (20 mL) was hydrogenated in a Parr apparatus at 20 psi of H_2 for 4 h. The catalyst was filtered off, the filtrate was evaporated, and the residue was dissolved in ethanol (10 mL). After addition of hydrazine hydrate (0.6 mL), the solution was allowed to stand for 18 h at room temperature. The solvent was evaporated, and the residue was chromatographed on a loose layer of silica gel in solvent S_3. The major UV-absorbing band was eluted with the same solvent, and the eluate was evaporated. Compound **7a** was obtained as an amorphous solid after addition of petroleum ether to the solution of the residue in chloroform: 39 mg (45%); homogeneous on TLC (S_3 and S_4); mobility on paper electrophoresis 3.38 of phenylalanine, ninhydrin positive; UV max (ethanol) 275 nm (ϵ 17 300); NMR ($CDCl_3$) δ 8.22 and 7.86 (2 s, 2, H_8 and H_2), 7.22 (m, 5, phenyl), 5.82 (d, 1, $H_{1'}$, $J_{1',2'}$ = 7.1 Hz), 3.52 (s, overlapped with ribose and/or aminopropyl signals, $(CH_3)_2N$). Anal. Calcd for $C_{21}H_{28}N_6O_4 \cdot {}^3/_4H_2CO_3$: C, 56.06; H, 6.38; N, 18.04. Found: C, 56.19; H, 6.19; N, 18.14.

Isomer **7b** was obtained from **5e** in the same fashion: 32 mg (38%); homogeneous on TLC (S_3 and S_4); mobility on paper electrophoresis 3.38 of phenylalanine, ninhydrin positive; UV max (ethanol) 275 nm (ϵ 17 100); NMR ($CDCl_3$) δ 8.27 and 7.79 (2 s, 2, H_8 and H_2), 7.25 (m, 5, phenyl), 5.90 (d, 1, $H_{1'}$, $J_{1',2'}$ = 7.8 Hz), 3.54 (s, overlapped with ribose and/or aminopropyl signals, $(CH_3)_2N$). Anal. Calcd for $C_{21}H_{28}N_6O_4 \cdot {}^3/_4H_2CO_3$: C, 56.06; H, 6.38; N, 18.04. Found: C, 55.91; H, 5.99; N, 17.97.

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Registry No. 2a, 70451-01-3; **2b**, 70451-00-2; **2c**, 74397-33-4; **2d**, 74397-34-5; **2e**, 74397-35-6; **3a**, 74397-36-7; **3b**, 57881-34-2; **3c**, 74397-37-8; **3d**, 15087-42-0; **3e**, 74397-38-9; **4a**, 74397-39-0; **4b**, 74397-40-3; **4e**, 74397-41-4; **5a**, 74397-42-5; **5b**, 74397-43-6; **5e**, 74397-44-7; **6a**, 74397-45-8; **6b**, 74397-46-9; **7a**, 74397-47-0; **7b**, 74397-48-1; methanesulfonyl chloride, 124-63-0; tetraethylammonium bromide, 71-91-0; tetrabutylammonium iodide, 311-28-4; N^6,N^6-dimethyl-2′,3′-*O*-isopropylideneadenosine, 19083-21-7; N^6,N^6-dimethyladenosine, 2620-62-4; acetone, 67-64-1; trityl chloride, 76-83-5; acetic anhydride, 108-24-7; 5′-*O*-benzoyl-N^6,N^6-dimethyl-2′,3′-*O*-isopropylideneadenosine, 15502-55-3; benzoic anhydride, 93-97-0; 2′,3′-*O*-(dibutylstannylene)-N^6,N^6-dimethyladenosine, 74397-49-2; dibutyltin oxide, 818-08-6; benzyl bromide, 100-39-0.