Syntheses and Antitumor Activities of N^6,N^6-Dimethyladenosine Carboxylate Analogues[1)]

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Several analogues substituted with fatty acid at the 2′-, 3′-, or 5′-position of the ribose moiety of N^6,N^6-dimethyladenosine were synthesized and tested for antitumor activity against cultured cells of L1210 leukemia and/or Ehrlich ascites. The cytotoxity and increase of life span obtained with congeners in the N^6,N^6-dimethyladenosine 3′- or 5′-substituted series were comparable to *in vitro* or several times better *in vivo* than those of the mother compound.

Keywords antitumor activity; L1210 leukemia; Ehrlich ascites; N^6,N^6-dimethyladenosine; long-chain aliphatic ester; tritylation

N^6,N^6-Dimethyladenosine (**1**) is a component of transfer ribonucleic acid (t-RNA) and the antibiotic puromycin.[2)] The nucleoside is present in 16S and 18S ribosomal RNA, which was suggested to be the binding site of the antibiotic kasugamycin.[3)]

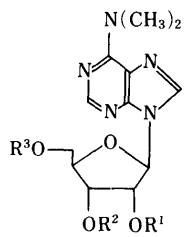
Some free fatty acids such as pentadecanoic acid, elaidic acid, arachidonic acid, γ-linolenic acid, and eicosapentaenoic acid are noteworthy in that they suppress tumor development both *in vitro* and *in vivo*.[4)] The introduction of long-chain aliphatic esters into the ribose moiety of **1** is therefore of interest since the products may include antitumor nucleosides possessing high activity and low toxicity.

We have established a method for the synthesis of 8-substituted N^6,N^6-dimethyladenosines, and we reported their antitumor activities.[5)] The present paper describes the esters prepared by condensation of a variety of carboxylic acids with the ribose moiety in **1**, and their high antitumor activities.

Chemistry

Our synthetic route is based on the esterification of **1** or partially protected **1** with carboxylates by using usual methods. The partially protected N^6,N^6-dimethyladenosines were prepared as shown in Chart 1.

Acetonation of **1** with acetone in the presence of perchloric acid gave 2′,3′-*O*-isopropylidene-N^6,N^6-dimethyladenosine (**2**)[6)] as crystals. Tritylation of **1** with trityl chloride in pyridine at 50 °C gave 5′-*O*-trityl-N^6,N^6-dimethyladenosine (**4**),[6)] which afforded, on further tritylation, two products on thin-layer chromatography. The 3′,5′-(**5**) and 2′,5′-ditritylates (**6**) were isolated by silica gel column chromatography, in 25% and 51% yields from the earlier and latter fractions, respectively. The structure of each of these products was determined by proton magnetic resonance (^{1}H-NMR) spectroscopy in deuterochloroform ($CDCl_3$), in comparison with the parent compound (**1**) and 5′-tritylate (**4**).



1: $R^1=R^2=R^3=H$
2: R^1, $R^2=$  , $R^3=H$
3: $R^1=R^2=H$, $R^3=RCO$
4: $R^1=R^2=H$, $R^3=Tr$
5: $R^1=H$, $R^2=R^3=Tr$
6: $R^1=R^3=Tr$, $R^2=H$
7: $R^1=Tr$, $R^2=R^3=H$
8a—d: $R^1=R^2=RCO$, $R^3=H$
9: $R^1=RCO$, $R^2=R^3=H$
10a—e: $R^1=R^3=H$, $R^2=RCO$
11a—d: $R^1=H$, $R^2=R^3=RCO$
12a, b: $R^1=R^2=R^3=RCO$

Tr = trityl

Chart 1

The resonance due to 1′-H of **6** was shifted to lower field, deshielded by the trityl group at the 2′-position, as compared with those of **1**, **4**, and **5**. On the other hand, the signals due to 3′-H of **6** and 4′-H of **5** were shifted to higher field shielded by the trityl group at the 5′-position as compared with those of **1** and **4**, respectively. The observations are similar to those on 2′,5′-di-*O*-trityluridine reported by Cook and Moffatt.[7)]

Thus, compounds **5** and **6** were determined to be 3′,5′-di-*O*-trityl-N^6,N^6-dimethyladenosine and 2′,5′-di-*O*-trityl-N^6,N^6-dimethyladenosine, respectively.

Compounds **5** and **6** were also obtained by direct tritylation of **1** with trityl bromide in pyridine at 80 °C in yields of 44.9 and 48.2%, respectively.

The partial deblocking of the trityl group of **6** to produce the 2′-tritylate (**7**) was achieved by treatment with aqueous acetic acid in 77.5% yield.

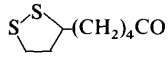
Compounds **1**, **2**, **4**, **5**, **6**, and **7** were esterified by usual methods. After deprotection and purification, we obtained the target compounds, 2′,3′,5′-triesters (**12a, b**) from **1**, 5′-monoesters (**3a—y**) from **2**, 2′,3′-diesters (**8a—d**) from **4**, 3′-monoesters (**10a—e**) from **6**, and 3′,5′-diesters (**11a—d**) from **7**.

2′-Monoesters of **1**, however, could not be isolated, and this may be because of the migration of the ester group from position 2′ to 3′ during the processes of deprotection and purification. The mechanism of the migration is not known in detail, but it seems to involve a general type of acyl migration through the orthoacid intermediate at the 2′,3′-*cis* vicinal glycol moiety.[8)]

Biological Results and Discussion

The antitumor activity of the title compounds was investigated using L1210 leukemia cultured cells and Ehrlich ascites tumor, and the results are shown in Table II. N^6,N^6-Dimethyladenosine (**1**) has an IC_{50} value of 0.5 μg/ml and inhibits the growth of L1210 cells in a dose-dependent manner over the concentration range of 0.2 to 1 μg/ml. The cytotoxic activities of the N^6,N^6-dimethyladenosine esters obtained in this study were the same as or less than those of

TABLE I. Structure and Physical Properties of N^6,N^6-Dimethyladenosine Esters

No.	R^1	R^2	R^3	Yield (%)	mp (°C) (Crystn. solvent)[a]	UV λ_{max}^{MeOH} nm (ε)[b]	Formula[c]
3a	H	H	$CH_3(CH_2)_3CO$	79.1	94—96 (A)	275 (200)	$C_{17}H_{25}N_5O_5 \cdot 1/4\,H_2O$
3b	H	H	$CH_3(CH_2)_4CO$	93.8	118—119 (A)	274 (158)	$C_{18}H_{27}N_5O_5$
3c	H	H	$CH_3(CH_2)_5CO$	67.9	124—125 (A)	275 (152)	$C_{19}H_{29}N_5O_5$
3d	H	H	$CH_3(CH_2)_6CO$	84.9	113—114 (A)	275 (193)	$C_{20}H_{31}N_5O_5$
3e	H	H	$CH_3(CH_2)_7CO$	92.4	93—94 (B)	275 (164)	$C_{21}H_{33}N_5O_5$
3f	H	H	$CH_3(CH_2)_8CO$	88.9	88—89 (A)	275 (195)	$C_{22}H_{35}N_5O_5 \cdot 1/4\,H_2O$
3g	H	H	$CH_3(CH_2)_9CO$	82.5	70—72 (A)	275 (174)	$C_{23}H_{37}N_5O_5 \cdot 1/5\,H_2O$
3h	H	H	$CH_3(CH_2)_{10}CO$	80.4	85—87 (A)	274 (187)	$C_{24}H_{39}N_5O_5 \cdot 1/3\,H_2O$
3i	H	H	$CH_3(CH_2)_{11}CO$	81.9	89—92 (B)	274 (190)	$C_{25}H_{41}N_5O_5 \cdot 3/5\,H_2O$
3j	H	H	$CH_3(CH_2)_{12}CO$	89.7	88—91 (A)	274 (197)	$C_{26}H_{43}N_5O_5 \cdot 5/4\,H_2O$
3k	H	H	$CH_3(CH_2)_{13}CO$	87.8	89—91 (A)	274 (208)	$C_{27}H_{45}N_5O_5$
3l	H	H	$CH_3(CH_2)_{16}CO$	56.7	86—88 (A)	274 (209)	$C_{30}H_{51}N_5O_5 \cdot 6/5\,H_2O$
3m	H	H	$CH_3(CH_2)_{18}CO$	76.4	93—95 (A)	275 (194)	$C_{32}H_{55}N_5O_5 \cdot 11/10\,H_2O$
3n	H	H	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$[d]	63.9	84—86 (A)	274 (140)	$C_{30}H_{49}N_5O_5 \cdot 1/4\,H_2O$
3o	H	H	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$[e]	84.9	69—72 (A)	275 (127)	$C_{30}H_{49}N_5O_5 \cdot 1/2\,H_2O$
3p	H	H	$CH_2{=}CH(CH_2)_8CO$	69.9	74—75 (B)	275 (188)	$C_{23}H_{35}N_5O_5 \cdot H_2O$
3q	H	H	$CH_3(CH_2)_4C{\equiv}CCO$	73.9	124—126 (A)	274 (182)	$C_{20}H_{27}N_5O_5 \cdot 1/4\,H_2O$
3r	H	H	$(CH_3)_3CCH_2CH(CH_3)CH_2CO$	92.0	62—65 (A)	275 (177)	$C_{21}H_{33}N_5O_5 \cdot 1/4\,H_2O$
3s	H	H	$[CH_3(CH_2)_7]_2CHCO$	96.1	Syrup	274 (195)	$C_{28}H_{47}N_5O_5$
3t	H	H		56.0	78—81 (A)	275 (189)	$C_{20}H_{29}N_5O_5S_2 \cdot H_2O$
3u	H	H	$CH_3CH(Br)CO$	73.3	93—96 (A)	275 (191)	$C_{15}H_{20}BrN_5O_5 \cdot 1/5\,H_2O$
3v	H	H	$BrCH_2CH_2CO$	56.3	81—84 (A)	274 (160)	$C_{15}H_{20}BrN_5O_5 \cdot 1/2\,H_2O$
3w	H	H	$BrCH_2(CH_2)_2CO$	75.1	74—77 (A)	274 (206)	$C_{16}H_{22}BrN_5O_5 \cdot 1/5\,H_2O$
3x	H	H	$BrCH_2(CH_2)_3CO$	72.0	75—78 (A)	275 (192)	$C_{17}H_{24}BrN_5O_5$
3y	H	H	$BrCH_2(CH_2)_9CO$	94.4	81—82 (A)	274 (186)	$C_{23}H_{36}BrN_5O_5$
8a	$CH_3(CH_2)_7CO$	$CH_3(CH_2)_7CO$	H	82.2	67—69 (C)	274 (177)	$C_{30}H_{49}N_5O_6$
8b	$CH_3(CH_2)_9CO$	$CH_3(CH_2)_9CO$	H	67.2	66—67 (C)	274 (165)	$C_{34}H_{57}N_5O_6$
8c	$CH_3(CH_2)_{11}CO$	$CH_3(CH_2)_{11}CO$	H	69.2	71—72 (C)	274 (189)	$C_{36}H_{61}N_5O_6$
8d	$CH_3(CH_2)_{11}CO$	$CH_3(CH_2)_{11}CO$	H	46.5	78—79 (C)	274 (200)	$C_{38}H_{65}N_5O_6$
10a	H	$CH_3(CH_2)_7CO$	H	49.0	90—91 (D)	275 (176)	$C_{21}H_{33}N_5O_5 \cdot 1/8\,H_2O$
10b	H	$CH_3(CH_2)_8CO$	H	54.0	93—95 (D)	275 (184)	$C_{22}H_{35}N_5O_5 \cdot 2/9\,H_2O$
10c	H	$CH_3(CH_2)_9CO$	H	55.0	91—92 (D)	275 (186)	$C_{23}H_{37}N_5O_5 \cdot 1/4\,H_2O$
10d	H	$CH_3(CH_2)_{10}CO$	H	49.0	93—94 (D)	275 (175)	$C_{24}H_{39}N_5O_5 \cdot 1/8\,H_2O$
10e	H	$CH_3(CH_2)_{11}CO$	H	55.0	93—94 (D)	275 (183)	$C_{25}H_{41}N_5O_5 \cdot 1/10\,H_2O$
11a	H	$CH_3(CH_2)_8CO$	$CH_3(CH_2)_8CO$	59.0	43—44 (D)	275 (177)	$C_{32}H_{53}N_5O_6 \cdot 1/5\,H_2O$
11b	H	$CH_3(CH_2)_9CO$	$CH_3(CH_2)_9CO$	63.8	64—65 (D)	274 (169)	$C_{34}H_{57}N_5O_6$
11c	H	$CH_3(CH_2)_{10}CO$	$CH_3(CH_2)_{10}CO$	67.7	56—57 (D)	274 (191)	$C_{36}H_{61}N_5O_6$
11d	H	$CH_3(CH_2)_{11}CO$	$CH_3(CH_2)_{11}CO$	59.0	43—44 (D)	274 (194)	$C_{38}H_{65}N_5O_6$
12a	$CH_3(CH_2)_{11}CO$	$CH_3(CH_2)_{11}CO$	$CH_3(CH_2)_{11}CO$	61.8	Syrup	274 (180)	$C_{51}H_{89}N_5O_7$
12b	$CH_3(CH_2)_{16}CO$	$CH_3(CH_2)_{16}CO$	$CH_3(CH_2)_{16}CO$	68.0	42—43 (E)	273 (228)[f]	$C_{66}H_{119}N_5O_7 \cdot 1/2\,H_2O$

a) A, EtOH–H_2O; B, MeOH–H_2O; C, MeOH; D, ether–petroleum ether; E, powder. *b*) Values in parentheses are 1/100 of the measured value. *c*) All compounds were analyzed for C, H, N and the results were within ±0.3% of theoretical values. *d*) 9-*trans*. *e*) 9-*cis*. *f*) Measured in EtOH solution.

the mother compound. The 2′,3′,5′-triesters (**12a**, **b**), 2′,3′-diesters (**8a**—**d**), and 3′,5′-diesters (**11a**—**d**) have reduced activities ($IC_{50} > 1\ \mu g/ml$).

The mother compound has no antitumor activity against Ehrlich ascites tumor, while several 3′-monoesters (**10b**—**e**) and 5′-monoesters (**3b**—**i**) exhibited marked antitumor activities. These compounds produced significant increases of life span with or without long-term survivors in Ehrlich ascites tumor-bearing mice. Among the 5′-monoesters examined, the compounds having esters with less than thirteen carbon atoms have potent antitumor activities, and the compounds with short-chain fatty acid esters ranging from 6 to 10 carbon numbers have higher activities. With increasing number of carbons in the fatty acid, the activity was generally decreased. We could not obtain more active compounds by introducing a Br atom into the fatty acid (**3u**—**y**).

It seems that introducing one carboxylic acid with less than 13 carbon atoms at position 3′ or 5′ of the ribose moiety in **1** yields compounds which might gradually be hydrolyzed enzymatically *in vivo* to release **1** over a prolonged period of time. This conclusion is supported by the fact that free carboxylic acids such as tridecanoic acid showed no antitumor activity *in vivo*. The release of **1** may depend on the size of the aliphatic carboxyl group condensed.

In conclusion, we have found that the introduction of a suitable carboxylic acid at the 3′- or 5′-position of N^6,N^6-dimethyladenosine leads to compounds with potent antitumor activity. Further research on these compounds is in progress.

Experimental

Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded with a JEOL JNM-PMX 60si spectrometer using tetramethylsilane as an internal standard. Chemical shifts are given in the δ scale. Ultraviolet (UV) spectra were recorded with a Hitachi 124 spectrophotometer and a Shimadzu UV-240 UV-visible recording spectrophotometer. Eluants for column chro-

TABLE II. Antitumor Activity of N^6,N^6-Dimethyladenosine Esters

No.[a]	IC_{50} (μg/ml)	% ILS[b] (mg/kg/d)			
		300	200	100	50
3b	1.0	35.4	>113.9 (1/5)[c]	102.5	
3c	0.5	>112.0 (1/5)[c]	>94.0 (2/5)[c]	91.6	32.5
3d	0.9	58.2	148.1	150.6	
3e	0.5	20.2	52.8	107.9	
3f	0.6	174.1	269.0	96.6	
3g	0.6	70.1	88.6	11.7	34.0
3h	0.5	>102.7 (1/5)[c]	53.8	50.7	36.6
3i	0.4	77.2	45.7	>71.3 (1/5)[c]	35.6
3j	1.0		23.0	55.2	23.0
3k	1.0	25.0	47.8	−2.3	37.0
3n	1.0	38.0	27.2	17.2	5.2
3p	0.5	>113.9 (2/5)[c]	58.8	69.7	
3t	0.5	>63.6 (3/5)[c]	>57.6 (1/5)[c]	70.9	
3v	0.4	−16.7	35.2	−22.2	
3y	0.6	>110.9 (1/5)[c]	83.0	81.8	
10b	0.6	58.2	89.9	91.1	
10c	0.5	>67.6 (3/5)[c]	79.5	88.6	
10d	0.6	>80.1 (2/5)[c]	137.5	93.2	
10e	0.4	134.8	>106.8 (1/5)[c]	125.8	
1	0.5	−93.0 (500 mg/kg)		−37.5 (250 mg/kg)	
		38.0 (100 mg/kg)		21.7 (50 mg/kg)	

a) The other compounds showed IC_{50} values of >1 μg/ml. *b*) $(T-C)/C \times 100$; T=median survival time of test animals, C=median survival time of control animals. *c*) Number of cured mice (60-d survivors)/number of tested mice.

matography with Silica gel BW-200 (Fuji Davison Chem. Ltd.) are shown as v/v.

Biological Methods.[9] **A. For *in Vitro* Assay (IC_{50})** L1210 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells were plated at a density of 1×10^5 cells/ml in the medium containing drugs and grown in suspension for 4 d. After culture, the number of cells which excluded trypan blue dye was counted. The IC_{50} value was determined from the dose–response curve obtained for each compound.

B. For *in Vivo* Assay (% ILS, Percentage Increase in Life Span) ICR mice were inoculated i.p. with 1×10^5 cells/mouse of Ehrlich ascites tumor on day 0. The drugs were suspended in 0.5% carboxymethyl cellulose in physiological saline and given i.p. once daily from day 1 through day 5. The evaluation of activity was made in terms of % ILS.

Chemical Methods. A. 3′,5′-Di-*O*-trityl-N^6,N^6-dimethyladenosine (5) and 2′,5′-Di-*O*-trityl-N^6,N^6-dimethyladenosine (6) Trityl chloride (11 g, 39 mmol) was added to a solution of **1** (10 g, 34 mmol) in dry pyridine (60 ml), and the mixture was stirred for 1 h at 50 °C. Then, trityl bromide (13 g, 40 mmol) was added and stirring was continued for a further 2 h at 70 °C. After evaporation of the solvent, the residue was dissolved in $CHCl_3$, and the solution was washed with 1 N HCl, H_2O, aqueous $NaHCO_3$, and H_2O, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH (199 : 1). From the earlier fractions, **5** was isolated as a syrup, which was crystallized from ether–petroleum ether as white crystals (6.7 g, 25%), mp 136—137 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.39—2.77 (1H, m, 4′-H), 3.03—3.50 (2H, m, 5′-H_2), 3.50 [6H, s, N^6-$(CH_3)_2$], 4.20—4.40 (1H, m, 3′-H), 4.45—4.83 (1H, m, 2′-H), 5.32—5.50 (1H, m, exchangeable with D_2O, 2′-OH), 6.12 (1H, d, J=6.0 Hz, 1′-H), 6.83—7.70 [30H, m, $(C_6H_5)_3 \times 2$], 7.96, 8.27 (2H, each s, 2-H, 8-H). *Anal.* Calcd for $C_{50}H_{45}N_5O_4$: C, 77.00; H, 5.82; N, 8.98. Found: C, 77.06; H, 5.52; N, 9.22.

From the later fractions with the same solvent, **6** was isolated as a syrup, which was crystallized from ether–petroleum ether as white crystals (13.2 g, 51%), mp 140—141 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.17 (1H, s, exchangeable with D_2O, 3′-OH), 2.77—3.13 (3H, m, 3′-H, 5′-H_2), 3.56 [6H, s, N^6-$(CH_3)_2$], 3.94—4.14 (1H, m, 4′-H), 4.90—5.13 (1H, m, 2′-H), 6.47 (1H, d, J=7.5 Hz, 1′-H), 6.80—7.53 [30H, m, $(C_6H_5)_3 \times 2$], 7.88, 8.93 (2H, each s, 2-H, 8-H). *Anal.* Calcd for $C_{50}H_{45}N_5O_4 \cdot 1/2\ H_2O$: C, 76.12; H, 5.88; N, 8.88. Found: C, 76.33; H, 5.68; N, 9.13.

B. 2′-*O*-Trityl-N^6,N^6-dimethyladenosine (7) A solution of **6** (10 g, 12.8 mmol) in 20 ml of $CHCl_3$–AcOH–H_2O (1 : 4 : 1) was stirred for 3 h at 60 °C. After evaporation of the solvent, the residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH (49 : 1). From the earlier fractions, unreacted **6** (1.6 g) was recovered. From the later fractions, **7** was isolated as a syrup, which was crystallized from ether as white crystals (4.49 g, 77.5%), mp 115—117 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.99 (1H, d, J=4.0 Hz, 3′-H), 3.40—3.80 [2H, m, overlapped with N^6-$(CH_3)_2$, 5′-H_2], 3.56 [6H, s, N^6-$(CH_3)_2$], 4.02—4.18 (1H, m, 4′-H), 5.17 (1H, dd, J=4.0, 8.0 Hz, 2′-H), 6.05 (1H, d, J=8.0 Hz, 1′-H), 6.52—6.93 (2H, m, exchangeable with D_2O, 2′-OH, 5′-OH), 6.93—7.50 [15H, m, $(C_6H_5)_3$], 7.88, 8.04 (2H, each s, 2-H, 8-H). *Anal.* Calcd for $C_{31}H_{31}N_5O_4 \cdot 1/2\ H_2O$: C, 68.12; H, 5.90; N, 12.81. Found: C, 67.86; H, 5.64; N, 12.82.

C. Esterification Methods Method (a): 5′-*O*-Tridecanoyl-N^6,N^6-dimethyladenosine (**3i**): A mixture of **2** (2 g, 6.0 mmol), tridecanoic acid (1.53 g, 7.1 mmol), and triisopropylbenzenesulfonyl chloride (2.17 g, 7.2 mmol) in dry pyridine (50 ml) was stirred for 5 h at 70 °C. After evaporation of the solvent, the residue was dissolved in $CHCl_3$, and the solution was washed with 2.2 N HCl, H_2O, and aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. To the residue, 90% CF_3COOH (10 ml) was added and the mixture was stirred for 2 h at room temperature, then evaporated. The residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH (97 : 3). Removal of the solvent afforded **3i** as an amorphous powder, which was crystallized from EtOH–H_2O. Recrystallization from MeOH–H_2O gave colorless needles (2.4 g, 81.9%). ^{1}H-NMR (DMSO-d_6) δ: 0.57—1.70 [23H, m, $CH_3(CH_2)_{10}CH_2CO$–], 2.28 [2H, t, J=6.0 Hz, $CH_3(CH_2)_{10}CH_2CO$–], 3.46 [6H, s, N^6-$(CH_3)_2$], 4.00—4.43 (4H, m, 3′-H, 4′-H, 5′-H_2), 4.43—4.80 (1H, m, 2′-H), 5.32 (1H, d, J=5.5 Hz, exchangeable with D_2O, 2′-OH or 3′-OH), 5.53 (1H, d, J=5.5 Hz, exchangeable with D_2O, 3′-OH or 2′-OH), 5.95 (1H, d, J=4.5 Hz, 1′-H), 8.23, 8.28 (2H, each s, 2-H, 8-H).

Compounds **3g**, **3k—n**, **3p**, **3q**, **3s**, **3t**, **3v**, **3x**, **8b**, **8d**, **10e**, and **11d** were obtained by the same method as described for **3i**. The physical data are given in Table I.

Method (b): 5′-*O*-(11-Bromoundecanoyl)-N^6,N^6-dimethyladenosine (**3y**): A mixture of **2** (3 g, 8.9 mmol), 11-bromoundecanoic acid (2.4 g, 9 mmol), 1,3-dicyclohexylcarbodiimide (5.5 g, 26.7 mmol), and 4-dimethylaminopyridine (1 g, 8.2 mmol) in dry CH_2Cl_2 (80 ml) was refluxed for 2 h with stirring. Then, three further portions of 11-bromoundecanoic acid (2 g, 1 g, and 1 g) was added at 1 h intervals. After filtration to remove the crystalline precipitates, the filtrate was evaporated. The residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH (100 : 1) to afford an amorphous powder. The product was treated as described for **3i** to give **3y**, which was crystallized from EtOH–H_2O as colorless needles (4.58 g, 94.4%). ^{1}H-NMR (DMSO-d_6) δ: 0.77—2.03 [16H, m, $BrCH_2(CH_2)_8CH_2CO$–], 2.30 [2H, t, J=6.5 Hz, $BrCH_2(CH_2)_8CH_2CO$–], 3.48 [6H, s, N^6-$(CH_3)_2$], 3.63 [2H, t, J=6.5 Hz, $BrCH_2(CH_2)_8CH_2CO$–], 3.93–4.53 (4H, m, 3′-H, 4′-H, 5′-H_2), 4.53—4.83 (1H, m, 2′-H), 5.35 (1H, d, J=5.5 Hz, exchangeable with D_2O, 2′-OH or 3′-OH), 5.58 (1H, d, J=5.5 Hz, exchangeable with D_2O, 3′-OH or 2′-OH), 6.00 (1H, d, J=4.5 Hz, 1′-H), 8.28, 8.33 (2H, each s, 2-H, 8-H).

Method (c): 5′-*O*-Nonanoyl-N^6,N^6-dimethyladenosine (**3e**): Nonanoyl chloride (1.6 ml, 8.9 mmol) was added dropwise to a solution of **2** (2.5 g, 7.5 mmol) in dry pyridine (50 ml) with stirring. The mixture was stirred for 3 h at 60 °C, and evaporated. The residue was treated as described as **3i** to afford **3e**, which was crystallized from MeOH–H_2O as colorless needles (3 g, 92.4%). ^{1}H-NMR (DMSO-d_6) δ: 0.53—1.70 [15H, m, $CH_3(CH_2)_6CH_2CO$–], 2.28 [2H, t, J=6.5 Hz, $CH_3(CH_2)_6CH_2CO$–], 3.46 [6H, s, N^6-$(CH_3)_2$], 3.90—4.43 (4H, m, 3′-H, 4′-H, 5′-H_2), 4.43—4.86 (1H, m, 2′-H), 5.33 (1H, d, J=5.5 Hz, exchangeable with D_2O, 2′-OH or 3′-OH), 5.55 (1H, d, J=5.5 Hz, exchangeable with D_2O, 3′-OH or 2′-OH), 5.98 (1H, d, J=4.5 Hz, 1′-H), 8.25, 8.30 (2H, each s, 2-H, 8-H).

Compounds **3b**, **3d**, **3f**, **3h**, **3j**, **3o**, **3r**, **3u**, **3w**, **8a**, **8c**, **10a—d**, and **11a—c** were obtained by the same method as described for **3e**. The physical data are given in Table I.

Method (d): 5′-*O*-Heptanoyl-N^6,N^6-dimethyladenosine (**3c**): Heptanoic anhydride (4 ml, 15.2 mmol) was added dropwise to a solution of **2** (4 g, 11.9 mmol) in dry pyridine (50 ml) with stirring. The mixture was stirred for 3 h at 60 °C, and evaporated. The residue was treated as described for **3i** to afford **3c**, which was crystallized from EtOH–H_2O as colorless needles (3.3 g, 67.9%). ^{1}H-NMR (DMSO-d_6) δ: 0.57—1.87 [11H, m, $CH_3(CH_2)_4CH_2CO$–], 2.30 [2H, t, J=6.5 Hz, $CH_3(CH_2)_4CH_2CO$–], 3.50 [6H, s, N^6-$(CH_3)_2$], 3.97—4.53 (4H, m, 3′-H, 4′-H, 5′-H_2), 4.53—4.83 (1H, m, 2′-H), 5.37 (1H, d, J=5.5 Hz, exchangeable with D_2O, 2′-OH or 3′-OH), 5.58 (1H, d, J=5.5 Hz, exchangeable with D_2O, 3′-OH or 2′-OH), 6.00 (1H, d, J=4.5 Hz, 1′-H), 8.27, 8.33 (2H, each s, 2-H, 8-H).

Compound **3a** was obtained by the same method as described for **3c**. The physical data are given in Table I.

D. 2′,3′,5′-Tri-*O*-tridecanoyl-N^6,N^6-dimethyladenosine (12a) A mixture of **1** (2 g, 6.8 mmol), tridecanoic acid (5.6 g, 26.1 mmol), and tri-

isopropylbenzenesulfonyl chloride (8 g, 26.4 mmol) in dry pyridine (100 ml) was stirred for 6 h at 90 °C, and then evaporated. The residue was dissolved in $CHCl_3$, and the solution was washed with 1 N HCl, H_2O, aqueous $NaHCO_3$, and H_2O, dried ($MgSO_4$), and evaporated. The residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH (200 : 1) to afford **12a** as a syrup (3.7 g, 61.8%). ^{1}H-NMR ($CDCl_3$) δ: 0.63—1.97 [69H, m, $CH_3(CH_2)_{10}CH_2CO-\times 3$], 2.10—2.57 [6H, m, $CH_3(CH_2)_{10}CH_2CO-\times 3$], 3.53 [6H, s, N^6-$(CH_3)_2$], 4.12 (3H, br s, 4′-H, 5′-H_2), 5.57—5.80 (1H, m, 3′-H), 5.90 (1H, dd, J=5.5, 5.5 Hz, 2′-H), 6.24 (1H, d, J=5.5 Hz, 1′-H), 7.90, 8.34 (2H, each s, 2-H, 8-H).

Compound **12b** was obtained by the same method as described for **12a**. The physical data are given in Table I.

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References and Notes

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