STUDIES ON ANTIVIRAL AGENTS

III. SYNTHESIS AND *IN VITRO* ANTIVIRAL ACTIVITY OF 1-*N*-HIGHER-ACYL-3″-*N*-FUNCTIONALIZED ACYLKANAMYCIN A DERIVATIVES

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The synthesis and antiviral activity of 1-*N*-palmitoyl- or 1-*N*-(3-hydroxytetradecanoyl)-kanamycin A derivatives (**7**, **8**) having various type of acyl substituents at the *N*-3″ position were investigated. The structure-activity relationships between the antiviral activity and the substituent at the *N*-3″ position is described. In this series, 3″-*N*-acetyl-1-*N*-palmitoyl-kanamycin A (**7c**) showed the excellent antiviral activity against HSV-I and influenza virus. Further, we examined the synthesis and the antiviral activity of 3″-*N*-glycylkanamycin A derivatives (**9**) having a higher-acyl group at the *N*-1 position. The 3″-*N*-glycyl-1-*N*-pentadecanoylkanamycin A (**9a**) also exhibited excellent antiviral activity.

In the previous papers[1,2], we have first reported that the 1-*N*-acyl-3″-*N*-trifluoroacetylkanamycin A derivatives had the strong antiviral activity against herpes simplex virus type I (HSV-I) and influenza virus, and have described the structure-activity relationships between the antiviral activity and alkyl chain length in an acyl group at the *N*-1 position. 3″-*N*-Trifluoroacetylkanamycin A derivatives having a higher-acyl group, such as palmitoyl, pentadecanoyl, or hexadecanyloxycarbonyl, were found to have excellent antiviral activity against both HSV-I (ID_{50} 1.0～1.4 μg/ml) and influenza virus (ID_{50} 5.6～22 μg/ml). We have also suggested that the introduction of a higher-acyl group in the kanamycin A molecule was essential for strong antiviral activity. While, 1-*N*-higher-acyl-3″-*N*-trifluoroacetylkanamycin A derivatives[1] exhibited excellent antiviral activity. They were very soluble in water under acidic condition (pH<5.8), but were slightly soluble in water (0.1 mg/ml) at around neutral pH. Based on these facts, we felt that the 1-*N*-higher-acylkanamycin A derivatives bearing various acyl groups in place of the trifluoroacetyl radical at the *N*-3″ position might improve the activity over that of 1-*N*-palmitoyl-3″-*N*-trifluoroacetylkanamycin A (**7u**). Furthermore, the 1-*N*-higher-acylkanamycin A derivatives having a functionalized acyl group, such as amino group and/or hydroxyl group, at the *N*-3″ position could be soluble in water at neutral pH.

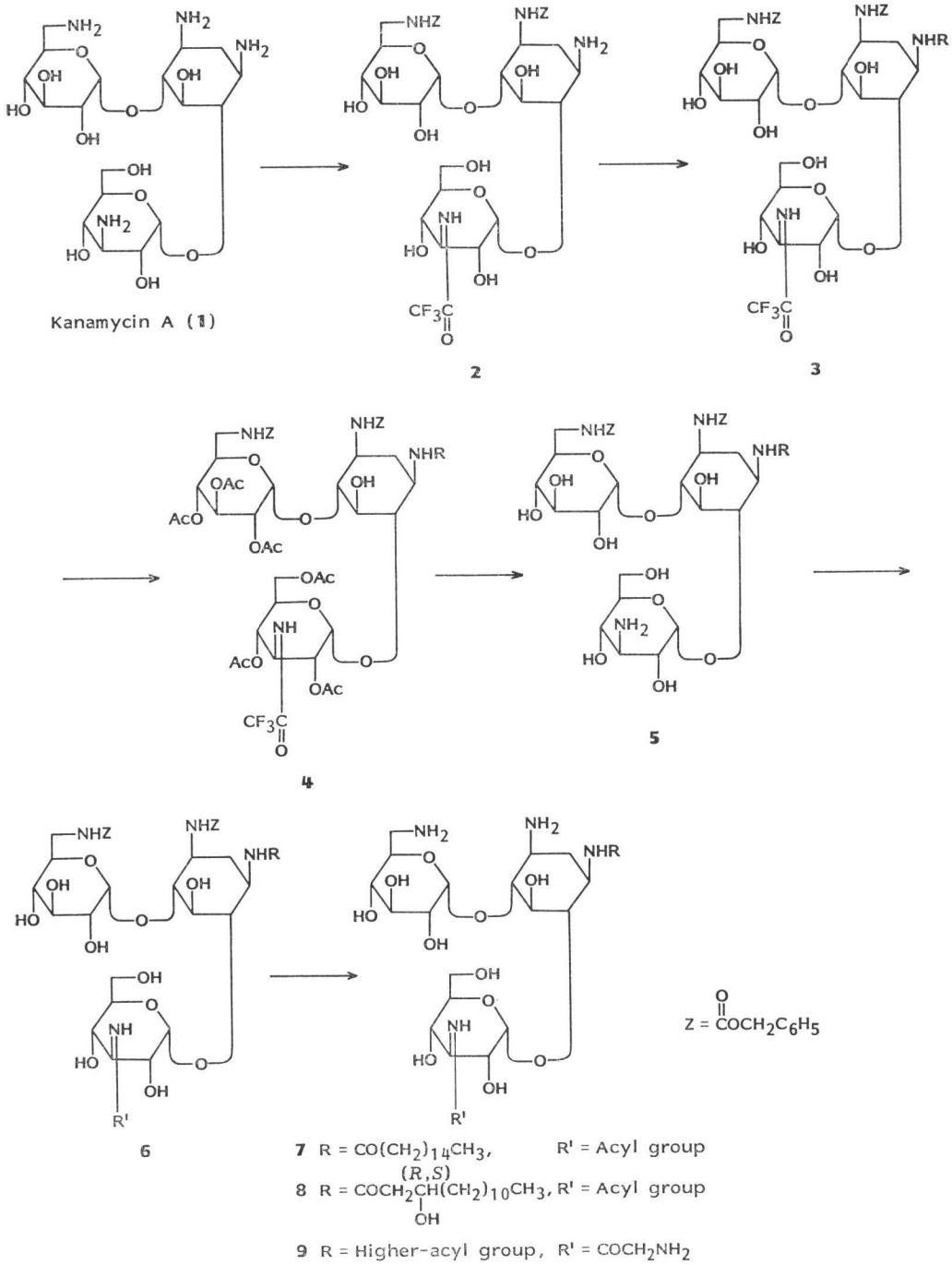
In this paper, we report the synthesis and antiviral activity of the 1-*N*-higher-acylkanamycin A derivatives (**7**, **8**, **9**) having a variety of acyl group at the *N*-3″ position.

Chemistry

We synthesized 1-*N*-higher-acyl-3″-*N*-functionalized acylkanamycin A derivatives (**7**, **8**, **9**) as follows (Schemes 1 and 2).

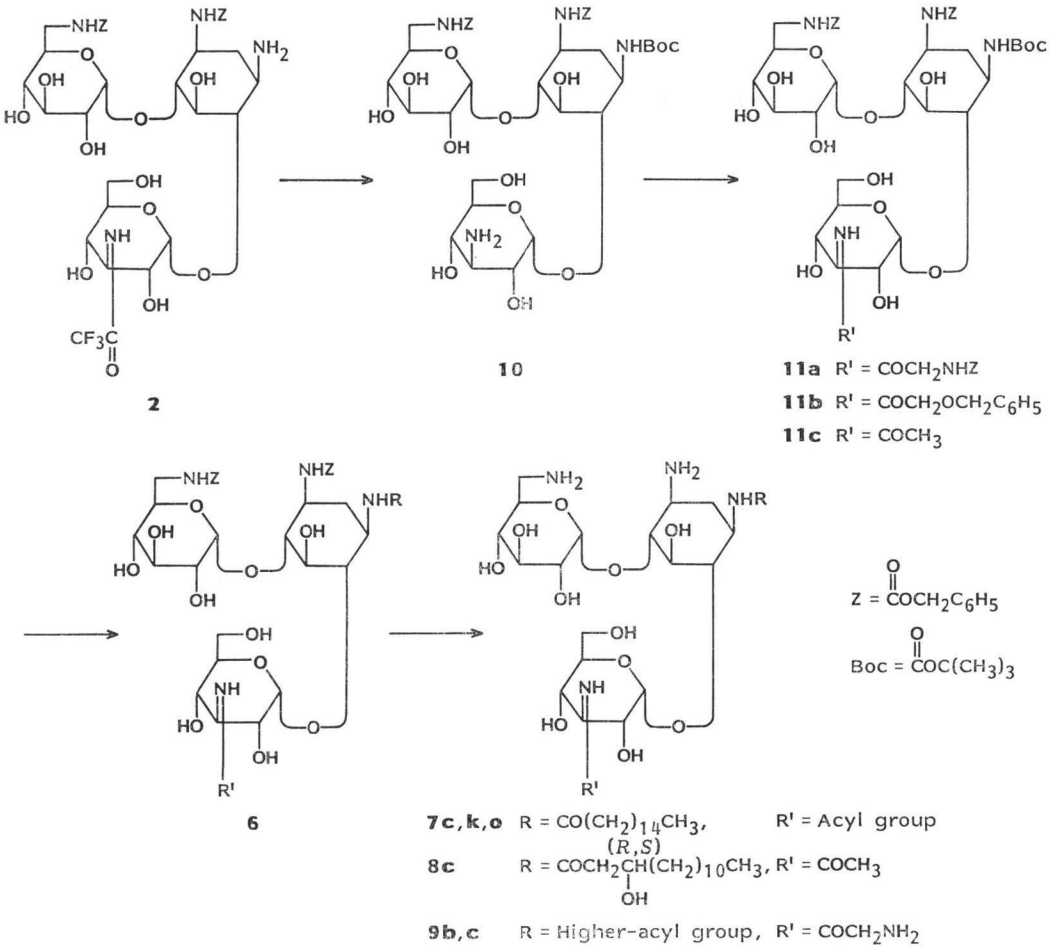
Protection of two amino groups at the C-3 and C-6′ positions of kanamycin A (**1**) by reaction with *N*-benzyloxycarbonyloxy-5-norbornene-2,3-dicarboximide, followed by regiospecific 3″-amino

Scheme 1. Synthesis of 1-*N*-acyl-3″-*N*-functionalized acylkanamycin A derivatives (**7**, **8**, **9**).



protection with ethyl trifluoroacetate, gave 3,6′-bis-*N*-benzyloxycarbonyl-3″-*N*-trifluoroacetylkanamycin A (**2**) by using the method of TSUCHIYA *et al.*[3]. Acylation of **2** with an acyl chloride or an activated ester gave the corresponding 1-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-trifluoroacetylkanamycin

Scheme 2. Synthesis of 1-*N*-acyl-3″-*N*-functionalized acylkanamycin A derivatives (**7c, k, o, 8c, 9b, c**).



A derivatives (**3**) as reported in the previous paper[2]. Physical data and yields of **3** are summarized in Table 1. Removal of the trifluoroacetyl group of **3** under alkaline condition (1 M NH_4OH - aqueous THF or KOH - MeOH) was unsuccessful, because steric hindrance of the higher-acyl group at the *N*-1 position might prevent hydroxide anion from attacking the trifluoroacetyl group at the *N*-3″ position. Therefore, we assumed that acetylation of the hydroxyl groups in **3** could overcome this steric hindrance by changing the conformation of **3**. Acetylation of **3** with acetic anhydride gave the corresponding 2′,3′,4′,2″,4″,6″-hexa-*O*-acetyl-1-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-trifluoroacetylkanamycin A derivatives (**4**), (Table 2). Treatment of **4** with a solution of KOH in methanol afforded the 1-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)kanamycin A derivatives (**5**) in good yield (Table 3). Acylation of **5** with an acyl chloride or an activated ester gave the 1-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-acylkanamycin A derivatives (**6**) as a solid (Tables 4, 5 and 6). Finally, hydrogenation of **6** with 10% palladium on carbon, followed by lyophilization afforded the corresponding 1-*N*-acyl-3″-*N*-functionalized acylkanamycin A di- or trihydrochloride (**7**, **8**, **9**) as a hygroscopic solid (Tables 7, 8 and 9).

On the other hand, reaction of **2** with 2-(*tert*-butoxycarbonyloximino)-2-phenylacetonitrile in

Table 1. Physical data and yields of 1-*N*-higher-acyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-trifluoroacetylkanamycin A derivatives (**3**).

No.	R	Yield (%)	MP (°C, dec)	IR (Nujol, cm^{-1})	NMR (DMSO-d_6, δ)
3a	$CO(CH_2)_{13}CH_3$	84	284	1690, 1640, 1530, 1260	0.93 (3H, m, CH_3), 1.07 (24H, s, CH_2)
3b	$CO(CH_2)_{14}CH_3$	92	270	1690～1680, 1530, 1040～1010	
3c	$COCH_2\underset{\displaystyle OAc}{\underset{\vert}{(R,S)CH}}(CH_2)_6CH_3$	69	283	1705, 1540, 1055	0.90 (3H, m, CH_3), 1.93 (3H, s, CH_3CO)
3d	$COCH_2\underset{\displaystyle OAc}{\underset{\vert}{(R,S)CH}}(CH_2)_{10}CH_3$	74	265	1690, 1640, 1540, 1270	0.88 (3H, m, CH_3), 1.22 (20H, s, CH_2), 1.92 (3H, s, CH_3CO)
3e	$COCH_2\underset{\displaystyle OAc}{\underset{\vert}{(R,S)CH}}(CH_2)_{12}CH_3$	66	—[a]		0.91 (3H, m, CH_3), 1.95 (3H, s, CH_3CO)
3f	$COCH_2\underset{\displaystyle OAc}{\underset{\vert}{(R,S)CH}}(CH_2)_{14}CH_3$	77	272	1690, 1540, 1170	0.90 (3H, m, CH_3), 1.90 (3H, s, CH_3CO)

[a] Not measured.

Table 2. Physical data and yields of 2′,3′,4′,2″,4″,6″-hexa-*O*-acetyl-1-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-trifluoroacetylkanamycin A derivatives (**4**).

No.	R	Yield (%)	IR (Nujol, cm^{-1})	NMR ($CDCl_3$, δ)
4a	$CO(CH_2)_{13}CH_3$	98	1745, 1720, 1690, 1640, 1530	0.92 (3H, m, CH_3), 1.90～2.15 (18H, CH_3CO)
4b	$CO(CH_2)_{14}CH_3$	100		0.93 (3H, m, CH_3), 1.28 (26H, s, CH_2)
4c	$COCH_2\underset{\displaystyle OAc}{\underset{\vert}{(R,S)CH}}(CH_2)_6CH_3$	89	1750, 1645, 1230, 1150	0.95 (3H, m, CH_3)
4d	$COCH_2\underset{\displaystyle OAc}{\underset{\vert}{(R,S)CH}}(CH_2)_{12}CH_3$	76	1740, 1700, 1640, 1540, 1260	0.95 (3H, m, CH_3)
4e	$COCH_2\underset{\displaystyle OAc}{\underset{\vert}{(R,S)CH}}(CH_2)_{14}CH_3$	100	1740, 1650, 1540, 1230, 1150	0.93 (3H, m, CH_3)

aqueous THF, followed by removal of trifluoroacetyl group at the *N*-3″ position with a mixture of conc NH_4OH and DMF gave the desired 3,6′-bis(*N*-benzyloxycarbonyl)-1-*N*-(*tert*-butoxycarbonyl)kanamycin A (**10**). In this procedure, removal of trifluoroacetyl group at the *N*-3″ position was smoothly carried out under alkaline condition. Acylation at the *N*-3″ position with *N*-(benzyloxycarbonyl)-glycine, benzyloxyacetic acid, or acetic acid afforded the 3,6′-bis(*N*-benzyloxycarbonyl)-1-*N*-(*tert*-butoxycarbonyl)-3″-*N*-(*N*-benzyloxycarbonylglycyl)-, -3″-*N*-(benzyloxyacetyl)-, or -3″-*N*-acetylkanamycin A (**11a, b, c**). Treatment of **11** with trifluoroacetic acid and anisole, followed by acylation at the *N*-1 position with a higher-acyl chloride gave the 1-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-functionalized acylkanamycin A derivatives (**6**) (Tables 4, 5 and 6). Finally, hydrogenation with 10%

Table 3. Physical data and yields of 1-*N*-higher-acyl-3,6′-bis(*N*-benzyloxycarbonyl)kanamycin A derivatives (**5**).

No.	R	Yield (%)	IR (Nujol, cm^{-1})	NMR (DMSO-d_6, δ)
5a	$CO(CH_2)_{13}CH_3$	92	1710, 1685, 1640, 1530, 1265	0.86 (3H, m, CH_3)
5b	$CO(CH_2)_{14}CH_3$	85	1680, 1640, 1540, 1275, 1230	0.90 (3H, m, CH_3), 1.62 (26H, s, CH_2)
5c	$COCH_2\underset{\underset{OH}{\vert}}{\overset{(R,S)}{C}}H(CH_2)_6CH_3$	86	1690, 1640, 1540, 1150	0.92 (3H, m, CH_3)
5d	$COCH_2\underset{\underset{OH}{\vert}}{\overset{(R,S)}{C}}H(CH_2)_{10}CH_3$	93	1690, 1640, 1540, 1150	0.95 (3H, m, CH_3)
5e	$COCH_2\underset{\underset{OH}{\vert}}{\overset{(R,S)}{C}}H(CH_2)_{12}CH_3$	93	1690, 1640, 1540, 1270, 1230	0.90 (3H, m, CH_3)
5f	$COCH_2\underset{\underset{OH}{\vert}}{\overset{(R,S)}{C}}H(CH_2)_{14}CH_3$	54	1680, 1640, 1540, 1150	0.90 (3H, m, CH_3)

palladium on carbon, followed by lyophilization, gave the corresponding 1-*N*-acyl-3″-*N*-functionalized acylkanamycin A derivatives (**7c, k, o, 8c, 9b, c**) as summarized in Tables 7, 8 and 9.

Biological Activity

Effect of Acyl Group at the *N*-3″ Position of 1-*N*-Palmitoylkanamycin A

Antiviral activity against HSV-I and influenza virus and cytotoxicity of the 3″-*N*-acylkanamycin A derivatives (**7**) having palmitoyl group at the *N*-1 position are summarized in Table 10.

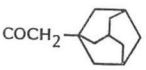
Antiviral Activity against HSV-I:

The 3″-*N*-unsubstituted (**7a**), 3″-*N*-formyl (**7b**), 3″-*N*-acetyl analogs (**7c**) having palmitoyl group at the *N*-1 position exhibited excellent antiviral activity against HSV-I (ID_{50} 1.0~3.2 μg/ml) and were about 30 times more active than virazole[4], though they were 20 times less active than acyclovir[5]. The 1-*N*-palmitoyl analogs (**7d, e, f**) having propionyl, butyryl, or *iso*-butyryl group at the *N*-3″ position also showed remarkable antiviral activity against HSV-I (ID_{50} 1.7~4.8 μg/ml). Further, the analogs (**7g, h, i**) having a bulky acyl group, such as *tert*-butylacetyl, benzoyl, or adamantylacetyl group, exhibited remarkable activity against HSV-I (ID_{50} 2.7~3.2 μg/ml). The 3″-*N*-chloroacetyl analog (**7j**) showed similar activity against HSV-I to the 3″-*N*-trifluoroacetyl analog (**7u**). However, the analogs (**7k, l, m**) having an acyl group with hydroxyl radical, such as hydroxyacetyl, 2-hydroxypropionyl, or 3-hydroxybutyryl group were about 2 times less active (ID_{50} 2.7~7.8 μg/ml) than **7u**. The 3″-*N*-glycyl analog (**7o**) exhibited the strongest antiviral activity against HSV-I (ID_{50} 1.0 μg/ml) in this series and was 30 times more active than virazole, but was 30 times less active than acyclovir. Furthermore, the 3″-*N*-carbamoyl (**7n**), 3″-*N*-((*S*)-3-amino-2-hydroxypropionyl) (**7p**), and 3″-*N*-(*S*)-(2,4-diaminobutyryl) (**7q**) analogs were about 3 times less active than **7u**.

The cytotoxicity of these 3″-*N*-acyl derivatives (**7**) tended to increase as compared with that of 3″-*N*-trifluoroacetyl derivative (**7u**).

Table 4. Physical data and yields of 1-*N*-palmitoyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-acylkanamycin A derivatives (**6A**～**T**).

R=$CO(CH_2)_{14}CH_3$

No.	R′	Method	Yield (%)	MP (°C, dec)	IR (Nujol, cm^{-1})	NMR (DMSO-d_6, δ)
6A (**5b**)	H	—	85	—[a]	1680, 1640, 1540, 1275, 1230	0.90 (3H, m, CH_3), 1.62 (26H, s, CH_2)
6B	CHO	—	85	261	1690, 1640, 1530, 1280, 1230	0.90 (3H, m, CH_3), 8.10 (1H, s, CHO)
6C	$COCH_3$	—	98	—	1680, 1640, 1520, 1270, 1230	0.85 (3H, m, CH_3), 1.83 (3H, s, CH_3CO)
6D	$COCH_2CH_3$	A	84	—	1710, 1690, 1640, 1545, 1270	0.70～1.10 (6H, m, CH_3CH_2)
6E	$COCH{=}CHCH_3$	A	100	228	1685, 1620, 1530, 1260	0.90 (3H, m, CH_3)
6F	$COCH(CH_3)_2$	A	99	255	1690, 1630, 1530, 1270	
6G	$COCH_2C(CH_3)_3$	B	91	256	1720, 1680, 1620, 1540	
6H	COC_6H_5	A	88	284	1710, 1680, 1620, 1540, 1300	0.90 (3H, m, CH_3), 1.20 (26H, s, CH_2)
6I		C	100	254	1680, 1615, 1530, 1260	
6J	$COCH_2Cl$	A	100	238	1690, 1640, 1540, 1270	0.90 (3H, m, CH_3), 1.20 (26H, s, CH_2)
6K	$COCH_2OCH_2C_6H_5$	—	99	—[a]	1690, 1640, 1540	1.20 (26H, s, CH_2), 4.57 (2H, s, CH_2), 7.25 (5H, s), 7.30 (5H, s), 7.33 (5H, s)
6L	$COCH(OH)CH_3$ (*R*,*S*)	C	94	232	1720, 1680, 1640, 1530, 1280	0.90 (3H, m, CH_3)
6M	$COCH_2CH(OH)CH_3$ (*R*,*S*)	C	91	205	1720, 1690, 1640, 1540, 1270	0.90 (3H, m, CH_3)
6N	CONHZ	D	100	—	1785, 1685, 1530, 1300, 1250	0.90 (3H, m, CH_3)
6O	$COCH_2NHZ$	—	87	269	1690, 1650, 1540, 1270	0.90 (3H, m, CH_3), 1.22 (26H, s, CH_2)
6P	$COCH(OH)CH_2NHZ$ (*S*)	C	20	255	1680, 1630, 1520, 1260	0.90 (3H, m, CH_3), 1.25 (26H, s, CH_2)
6Q	$COCH(NHZ)CH_2CH_2NHZ$ (*S*)	C	69	230	1690, 1640, 1300, 1225	0.90 (3H, m, CH_3)
6R	$COCH_2CH_2COOH$	—	99	252	1690, 1640, 1530, 1270	
6S	$COC_6H_4COOH(o)$	—	20	263	1700, 1640, 1530	0.90 (3H, m, CH_3)
6T	$COCH_2NHCHO$	C	58	253	1690, 1640, 1540, 1280, 1230	0.90 (3H, m, CH_3), 1.23 (26H, s, CH_2)

[a] Not measured.

Table 5. Physical data and yields of 3,6′-bis(*N*-benzyloxycarbonyl)-1-*N*-(3-hydroxytetradecanoyl)-3″-*N*-acylkanamycin A derivatives (**6U～Z**).

R=COCH$_2$CH(CH$_2$)$_{10}$CH$_3$ (*R*,*S*), OH

No.	R′	Method	Yield (%)	MP (°C, dec)	IR (Nujol, cm^{-1})	NMR (DMSO-d_6, δ)
6U (**5d**)	H	—	93	—[a]	1690, 1640, 1540, 1150	0.95 (3H, m, CH_3)
6V	CHO	—	91	251	1690, 1645, 1535, 1280	0.90 (3H, m, CH_3)
6W	$COCH_3$	—	80	266	1685, 1640, 1520, 1270	1.84 (3H, s, CH_3CO)
6X	$COCF_3$	—	89	265	1690, 1640, 1540, 1270	0.88 (3H, m, CH_3), 1.22 (20H, s, CH_2)
6Y	$COCH_2NHZ$	B	99	218	1680, 1520, 1260	0.90 (3H, m, CH_3)
6Z	COCHCH$_2$NHZ (*S*), OH	C	77	238	1690, 1640, 1530, 1265	0.92 (3H, m, CH_3), 1.25 (20H, s, CH_2)

[a] Not measured.

Table 6. Physical data and yields of 1-*N*-higher-acyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-[(*N*-benzyloxycarbonyl)glycyl]kanamycin A derivatives (**6a～f**).

R′=$COCH_2NHZ$

No.	R	Method	Yield (%)	MP (°C, dec)	IR (Nujol, cm^{-1})	NMR (DMSO-d_6, δ)
6a	$CO(CH_2)_{13}CH_3$	B	85	217	1690, 1535, 1270	0.92 (3H, m, CH_3)
6b	$COO(CH_2)_{15}CH_3$	—	57	246	1690, 1540, 1270	0.90 (3H, m, CH_3)
6c	$CONH(CH_2)_{14}CH_3$	—	56	251	1690, 1540, 1270	0.91 (3H, m, CH_3)
6d	COCH$_2$CH(CH$_2$)$_6$CH$_3$ (*R*,*S*), OH	B	91	—[a]	1695, 1640, 1540, 1270	0.91 (3H, m, CH_3)
6e	COCH$_2$CH(CH$_2$)$_{12}$CH$_3$ (*R*,*S*), OH	B	100	—	1690, 1640, 1540, 1270	0.91 (3H, m, CH_3)
6f	COCH$_2$CH(CH$_2$)$_{14}$CH$_3$ (*R*,*S*), OH	B	99	—	1690, 1640, 1540	0.90 (3H, m, CH_3)

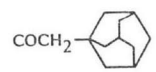
[a] Not measured.

Antiviral Activity against Influenza Virus:

Antiviral activity of the 1-*N*-palmitoyl-3″-*N*-acylkanamycin A derivatives (**7**) against influenza virus tended to be similar to antiviral activity against HSV-I. The 1-*N*-palmitoyl analogs (**7a～d**) exhibited remarkable antiviral activity against influenza virus (ID_{50} 10～32 μg/ml) and showed similar activity to **7u**. However, analogs (**7f, h, i**) having a bulky acyl group at the *N*-3″ position tended to decrease antiviral activity against influenza virus. Further, analogs (**7k～q**) having an acyl group with functional groups (hydroxyl and/or amino group(s)) at the *N*-3″ position exhibited much less antiviral activity against influenza virus, and their activity was about 6 times less than that of amantadine[6].

Table 7. Physical data and yields of 1-*N*-palmitoyl-3''-*N*-acylkanamycin A derivatives (7).

$R = CO(CH_2)_{14}CH_3$

No.	R′	Yield (%)	MP (°C, dec)	$[\alpha]_D^{20}$ (°) (H_2O)	IR (Nujol, cm^{-1})	NMR		FD-MS (*m/z*)
						Solvent	δ (ppm)	
7a	H	87	221	+71.1 (*c* 0.5)	1640~1630, 1540, 1510[b]	D_2O	0.91 (3H, m, CH_3), 1.26 (26H, s, CH_2)	723 (M^+)
7b	CHO	97	197	+68.0 (*c* 1.0)	1640, 1540	CD_3OD	0.95 (3H, m, CH_3), 5.12 (1H, d, J=3 Hz), 5.50 (1H, d, J=3 Hz), 8.23 (1H, s, CHO)	751 $(M)^+$
7c	$COCH_3$	100	193	+74.5 (*c* 1.0)	1630, 1540	D_2O	0.83 (3H, m, CH_3), 1.23 (26H, s, CH_2), 2.02 (3H, s, CH_3CO)	765 (M^+)
7d	$COCH_2CH_3$	85	134	+49.2 (*c* 1.0)	1635, 1550~1540	CD_3OD	0.87 (6H, m, CH_3), 5.10 (1H), 5.58 (1H)	
7e	$COCH_2CH_2CH_3$	69	158	+71.5 (*c* 0.5)	1630, 1540	D_2O	0.96 (6H, m, CH_3)	793 (M^+)
7f	$COCH(CH_3)_2$	64	144	+45.9 (*c* 1.0)	1640, 1550	CD_3OD	5.08 (1H, d, J=4 Hz)	793 (M^+)
7g	$COCH_2C(CH_3)_3$	76	137	+45.3 (*c* 1.0)	1630, 1540	CD_3OD	1.05 (9H, s, CH_3), 5.50 (1H, d, J=3 Hz)	821 (M^+)
7h	COC_6H_5	70	166	+62.5 (*c* 1.0)	1630, 1550, 1140	CD_3OD	5.25 (1H, d, J=4 Hz), 7.35~8.00 (5H, m, COC_6H_5)	828 (M^+)
7i		99	129	+62.7 (*c* 1.0)	1640~1610, 1540, 1140	CD_3OD	0.95 (3H, m, CH_3), 5.31 (1H, d, J=3.5 Hz)	900 (M^++1)
7j	$COCH_2Cl$	100	135	+62.3 (*c* 1.0)	1640, 1540, 1140	CD_3OD	0.95 (3H, m, CH_3), 5.11 (1H, d, J=4 Hz), 5.48 (1H, d, J=3 Hz)	—

7k	$COCH_2OH$	91	159	+73.2 (*c* 0.55)	1640, 1550	CD_3OD	5.10 (1H, d, *J*=3 Hz), 5.47 (1H)	803 (M^++Na)
7l	$\overset{(R,S)}{\underset{\vert}{COCHCH_3}}$ OH	99	122	—[a]	1700, 1630, 1220[b]	CD_3OD	0.90 (3H, m, CH_3)	
7m	$\overset{(R,S)}{\underset{\vert}{COCH_2CHCH_3}}$ OH	76	121	—	1720, 1630, 1240	CD_3OD	0.90 (3H, m, CH_3)	810 (M^++1)
7n	$CONH_2$	70	194	+62.1 (*c* 1.0)	1700, 1630, 1540, 1240	CD_3OD	0.95 (3H, m, CH_3), 5.10 (1H, d, *J*=3 Hz), 5.51 (1H, d, *J*=3 Hz)	
7o	$COCH_2NH_2$	79	163	+47.6 (*c* 1.0)	1620, 1560, 1300, 1140	CD_3OD	0.97 (3H, m, CH_3), 1.30 (26H, s, CH_2)	780 (M^+)
7p	$\overset{(S)}{\underset{\vert}{COCHCH_2NH_2}}$ OH	58	168	+39.7 (*c* 0.5)	1640, 1540, 1140[b]	CD_3OD	0.95 (3H, m, CH_3), 5.08 (1H, d, *J*=3.5 Hz)	810 (M^+)
7q	$\overset{(S)}{\underset{\vert}{COCHCH_2CH_2NH_2}}$ NH_2	12	125	—	1710, 1640, 1540, 1215	CD_3OD	0.92 (3H, m, CH_3)	—
7r	$COCH_2CH_2COOH$	96	145	+54.0 (*c* 1.0)	1720, 1640, 1550, 1140	CD_3OD	2.60 (4H, s), 5.50 (1H, d, *J*=3 Hz)	—
7s	$COC_6H_4COOH(o)$	64	213	—	1770, 1710, 1635, 1510	CD_3OD	0.93 (3H, m, CH_3)	—
7t	$COCH_2NHCHO$	55	138	+60.2 (*c* 1.0)	1665, 1560, 1290	CD_3OD	0.93 (3H, m, CH_3), 5.10 (1H, d, *J*=3 Hz)	—

[a] Not measured, [b] KBr disk method.

Table 8. Physical data and yields of 1-*N*-(3-hydroxytetradecanoyl)-3″-*N*-acylkanamycin A derivatives (8).

R = $\overset{(R,S)}{\underset{\displaystyle OH}{COCH_2CH(CH_2)_{10}CH_3}}$

No.	R′	Yield (%)	MP (°C, dec)	$[\alpha]_D^{20}$ (°) (H_2O)	IR (Nujol, cm^{-1})	NMR		FD-MS (*m/z*)
						Solvent	δ (ppm)	
8a	H	82	178	+80.8 (*c* 0.5)	1630, 1140	—[a]	—	711 (M^+)
8b	CHO	62	164	+87.6 (*c* 1.0)	1650~1550, 1140	CD_3OD	0.93 (3H, m, CH_3), 8.23 (1H, s, CHO)	—
8c	$COCH_3$	88	113	+71.8 (*c* 1.0)	1630, 1560, 1140	CD_3OD	2.10 (3H, s, CH_3CO), 5.45 (1H, d, J=4 Hz)	—
8d	$COCF_3$	85	116	+63.8 (*c* 1.0)	1700, 1630, 1560, 1150	CD_3OD	5.13 (1H, d, J=3 Hz), 5.23 (1H, d, J=3 Hz)	807 (M^+)
8e	$COCH_2NH_2$	52	146	+58.6 (*c* 1.0)	1630, 1560, 1025[b]	CD_3OD	0.94 (3H, m, CH_3)	768 (M^+)
8f	$\overset{(S)}{\underset{\displaystyle OH}{COCHCH_2NH_2}}$	64	125	+40.7 (*c* 1.0)	1750, 1640, 1140	CD_3OD	0.93 (3H, m, CH_3), 5.13 (1H, d, J=3 Hz)	797 (M^+-1)

[a] Not measured, [b] KBr disk method.

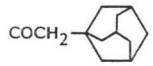
Table 9. Physical data and yields of 1-*N*-higher-acyl-3″-*N*-glycylkanamycin A derivatives (**9**).

$R'=COCH_2NH_2$

No.	R	Yield (%)	MP (°C, dec)	$[\alpha]_D^{20}$ (°) (H_2O)	IR (Nujol, cm^{-1})	NMR		FD-MS (*m/z*)
						Solvent	δ (ppm)	
9a	$CO(CH_2)_{13}CH_3$	100	97	—[a]	1670, 1300	CD_3OD	0.93 (3H, m, CH_3), 5.23 (1H, d, J=4 Hz), 5.55 (1H, d, J=3 Hz)	788 (M^++Na) 766 (M^++1)
9b	$COO(CH_2)_{15}CH_3$	60	197	+58.4 (*c* 1.0)	1690, 1540, 1260	CD_3OD	0.95 (3H, m, CH_3)	—
9c	$CONH(CH_2)_{14}CH_3$	82	169	+41.9 (*c* 1.0)	1680, 1560, 1260, 1140	CD_3OD	0.96 (3H, m, CH_3)	—
9d	(*R*,*S*) $COCH_2CH(CH_2)_6CH_3$ | OH	46	209	+74.7 (*c* 1.0)	1630, 1560, 1140	CD_3OD	0.96 (3H, m, CH_3), 5.52 (1H, d, J=3 Hz)	—
9e	(*R*,*S*) $COCH_2CH(CH_2)_{12}CH_3$ | OH	22	223	+53.3 (*c* 2.0)	1630, 1550, 1140	CD_3OD	0.93 (3H, m, CH_3), 5.50 (1H, d, J=3 Hz)	—
9f	(*R*,*S*) $COCH_2CH(CH_2)_{14}CH_3$ | OH	84	193	+57.2 (*c* 1.0)	1640, 1560, 1140	CD_3OD	0.93 (3H, m, CH_3)	—

[a] Not measured.

Table 10. *In vitro* antiviral activity of 1-*N*-palmitoyl-3″-*N*-acylkanamycin A derivatives (**7**).

No.	R′	Antiviral activity ID_{50} (μg/ml)		Cytotoxicity on Vero cell (μg/ml)
		HSV-I	Influenza virus	
7a	H	3.2	32	10
7b	CHO	2.2	>10	32
7c	$COCH_3$	1.0	22	32
7d	$COCH_2CH_3$	1.8	22	100
7e	$COCH_2CH_2CH_3$	4.8	—[a]	100
7f	$COCH(CH_3)_2$	1.7	63	10
7g	$COCH_2C(CH_3)_3$	3.2	—	32
7h	COC_6H_5	2.8	21	100
7i		2.7	>32	32
7j	$COCH_2Cl$	1.9	—	100
7k	$COCH_2OH$	2.7	>32	32
7l	(*R*,*S*) $COCHCH_3$ | OH	7.8	>32	100
7m	(*R*,*S*) $COCH_2CHCH_3$ | OH	4.4	56	32
7n	$CONH_2$	6.8	24	32
7o	$COCH_2NH_2$	1.0	63	10
7p	(*S*) $COCHCH_2NH_2$ | OH	2.7	—	32
7q	(*S*) $COCHCH_2CH_2NH_2$ | NH_2	8.4	32	100
7r	$COCH_2CH_2COOH$	6.0	—	32
7s	$COC_6H_4COOH(o)$	6.0	—	32
7t	$COCH_2NHCHO$	2.2	—	32
7u	$COCF_3$	1.4	16	>100
	Virazole	32	10	>100
	Amantadine	>100	10	>100
	Acyclovir	0.032	>10	>10

Assay system: Vero cell, CPE inhibition method.
[a] Not measured.

Effect of Acyl Group at the *N*-3″ Position of 1-*N*-(3-Hydroxytetradecanoyl)kanamycin A

The antiviral activity and cytotoxicity of the 3″-*N*-acylkanamycin A derivatives (**8**) having 3-hydroxytetradecanoyl group at the *N*-1 position are summarized in Table 11.

Antiviral Activity against HSV-I:

The 3″-*N*-unsubstituted derivative (**8a**) having 3-hydroxytetradecanoyl group at the *N*-1 position exhibited excellent antiviral activity against HSV-I (ID_{50} 1.7 μg/ml). The activity of **8a** was 4 times more than that of the 3″-*N*-trifluoroacetyl analog (**8d**) and was similar to that of the 1-*N*-palmitoyl-3″-*N*-trifluoroacetylkanamycin A (**7u**). The 3″-*N*-glycyl analog (**8e**) showed remarkable antiviral activity (ID_{50} 3.8 μg/ml) and was 2 times less active than **7u**. However, analogs (**8b, c, f**) having formyl,

Table 11. *In vitro* antiviral activity of 1-*N*-(3-hydroxytetradecanoyl)-3″-*N*-acylkanamycin A derivatives (**8**).

No.	R′	Antiviral activity ID_{50} (μg/ml)		Cytotoxicity on Vero cell (μg/ml)
		HSV-I	Influenza virus	
8a	H	1.7	>100	100
8b	CHO	13	—[a]	>100
8c	$COCH_3$	13	—	>100
8d	$COCF_3$	7.9	—	>100
8e	$COCH_2NH_2$	3.8	>100	100
8f	(*S*) $COCHCH_2NH_2$ | OH	8.8	70	>100
	Virazole	32	10	>100
	Amantadine	>100	10	>100

Assay system: Vero cell, CPE inhibition method.
[a] Not measured.

Table 12. *In vitro* antiviral activity of 1-*N*-higher-acyl-3″-*N*-glycylkanamycin A derivatives (**9**).

No.	R	Antiviral activity ID_{50} (μg/ml)		Cytotoxicity on Vero cell (μg/ml)
		HSV-I	Influenza virus	
9a	$CO(CH_2)_{13}CH_3$	4.0	16	32
9b	$COO(CH_2)_{15}CH_3$	6.2	32	32
9c	$CONH(CH_2)_{14}CH_3$	5.9	60	32
9d	(*R*,*S*) $COCH_2CH(CH_2)_6CH_3$ | OH	>100	>100	>100
(**8e**)	(*R*,*S*) $COCH_2CH(CH_2)_{10}CH_3$ | OH	3.8	>100	100
9e	(*R*,*S*) $COCH_2CH(CH_2)_{12}CH_3$ | OH	3.7	25	32
9f	(*R*,*S*) $COCH_2CH(CH_2)_{14}CH_3$ | OH	2.4	>10	32
	Virazole	32	10	>100
	Amantadine	>100	10	>100

Assay system: Vero cell, CPE inhibition method.

acetyl, or 3-amino-2-hydroxypropionyl group exhibited less weak antiviral activity and were about 10 times less active than that of **7u**.

Antiviral Activity against Influenza Virus:

The analogs (**8a**~**f**) described above exhibited little or no antiviral activity against influenza virus in contrast with antiviral activity against HSV-I.

The cytotoxicity of **8** was more than 100 μg/ml similarly to that of **7u**.

Effect of Higher-acyl Group at the *N*-1 Position of 3″-*N*-Glycylkanamycin A

The antiviral activity and cytotoxicity of the 3″-*N*-glycylkanamycin A derivatives (**9**) having a higher acyl group at the *N*-1 position are summarized in Table 12.

Antiviral Activity against HSV-I:

The 1-*N*-pentadecanoyl (**9a**), 1-*N*-hexadecanyloxycarbonyl (**9b**), and 1-*N*-pentadecanylaminocarbonyl derivatives (**9c**) having glycyl group at the *N*-3″ position exhibited strong antiviral activity against HSV-I (ID_{50} 1.2~1.8 μg/ml) and were about 30 times more active than virazole. The 1-*N*-(3-hydroxytetradecanoyl) (**8e**), 1-*N*-(3-hydroxyhexadecanoyl) (**9e**), and 1-*N*-(3-hydroxyoctadecanoyl) (**9f**) derivatives also showed excellent antiviral activity against HSV-I (ID_{50} 1.1~2.7 μg/ml). However, the 1-*N*-(3-hydroxydecanoyl) derivative (**9d**) exhibited no antiviral activity.

The cytotoxicity of these derivatives (**9**) was slightly stronger than that of **7u**.

Antiviral Activity against Influenza Virus:

The 1-*N*-pentadecanoyl derivative (**9a**) having glycyl group at the *N*-3″ position exhibited strong antiviral activity against influenza virus (ID_{50} 5 μg/ml). The derivative (**9a**) was 2 times more active than amantadine and virazole and was 3 times more active than the 1-*N*-palmitoyl-3″-*N*-trifluoroacetylkanamycin A (**7u**). The 3″-*N*-glycylkanamycin A derivatives (**9e, f**) having 3-hydroxyhexadecanoyl or 3-hydroxyoctadecanoyl group at the *N*-1 position showed similar antiviral activity against influenza virus to the derivative (**7u**).

Discussion

In this series, the 1-*N*-palmitoyl-3″-*N*-unsubstituted (**7a**) and 1-*N*-pentadecanoyl-3″-*N*-glycyl (**9a**) derivatives exhibited excellent antiviral activity against not only HSV-I but also influenza virus, and were also soluble in water (>0.1 mg/ml) at around neutral pH. Further, the derivatives (**7g~i**) having a bulky acyl group at the *N*-3″ position showed remarkable antiviral activity against HSV-I, but tended to decrease antiviral activity against influenza virus. On the basis of these results, the acyl group at the *N*-3″ position was not essential to give rise to the strong antiviral activity. The balance between lipophilic and hydrophilic character in the kanamycin A molecule might be assumed to be one of important factors for the emergence of antiviral activity, because the lipophilic kanamycin A molecule could penetrate through the cell membrane infected by virus and inhibit certain stages of virus protein synthesis. Mechanism of the growth inhibition of virus is now under investigation.

Experimental

The spectrometric data were obtained by the following instruments. Melting points were determined using Yanagimoto micro melting point apparatus and are uncorrected. NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spectrometer and at 100 MHz on a Jeol-MH 100 NMR spectrometer using Me_4Si as an internal standard. IR spectra were taken on a Hitachi 260-10 spectrophotometer. Optical rotations were determined with Jasco DIP-140 polarimeter. FD-Mass spectra were measured on Jeol-D 300 mass spectrometer.

Assay

Assays were carried out in confluent Vero cell cultures in multi-well trays (96 wells). The cell cultures were grown to confluence in EAGLE's minimal essential medium (MEM) supplemented with 5% fetal bovine serum (FBS). HSV-I Miyama and influenza virus A/PR 8 were respectively grown in Vero cells and Madin and Darby canine kidney cells (MDCK cells). The culture medium was changed to 0.5% FBS-MEM. The cell cultures were inoculated with about 100 times the virus dose needed to infect 50 percent of cells ($TCID_{50}$) of virus, and immediately thereafter, exposed to varying concentrations of the test compound and incubated for 2 days at 37°C in humidified 5% CO_2-95% air. 4 wells (multi-well trays (96 wells)) were used in each concentrations. They were fixed with 5% trichloroacetic acid stained with 0.1% crystal violet. The viral maximal cytopathic effect (CPE) was observed microscopically (×40). Antiviral activity was expressed as ID_{50} (50% inhibitory dose),

that is, the concentration of compound required to reduce viral CPE by 50% (within the well), when it had reached completion (100% cell destruction) in the control virus-infected cell cultures.

Cytotoxicity

In tests which were run in parallel with the antiviral assays in confluent Vero cell cultures (which had not been infected), the compounds were examined for their effects on normal cell morphology. The cytotoxicity was expressed as the minimum concentration of drug which destroyed the cell monolayer.

Material

3,6′-Bis(*N*-benzyloxycarbonyl)-3″-*N*-trifluoroacetylkanamycin A (**2**): The compound (**2**) was synthesized according to the method of TSUCHIYA *et al.*[3].

General Procedure for Acylation of **2**

1-*N*-Acyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-trifluoroacetylkanamycin A Derivatives (**3**): These derivatives (**3**) were obtained according to a similar manner to that of acylation of **2** described in the previous paper[2] (Table 1).

General Procedure for Acylation of **3**

To a solution of **3** (1.97 mmol) in pyridine (40 ml) was added acetic anhydride (25.6 mmol) at room temperature. The mixture was stirred at 50°C for 5 hours and allowed to stand at room temperature overnight. The reaction mixture was concentrated under reduced pressure. A solution of the residue in EtOAc (100 ml) was washed with 1 N HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl successively, dried over anhydrous $MgSO_4$, and evaporated *in vacuo* to afford 2′,3′,4′,2″,4″,6″-hexa-*O*-acetyl-1-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-trifluoroacetylkanamycin A derivatives (**4**) as a glass in good yields (Table 2).

General Procedure for Deacetylation and Detrifluoroacetylation of **4**

To a solution of potassium hydroxide (KOH) (24.9 mmol) in MeOH (50 ml) was added the derivative (**4**, 1.78 mmol) at room temperature and the mixture was stirred at the same temperature overnight. The reaction mixture was poured into ice-water (250 ml). The resultant precipitates were collected by filtration, washed with water, and dried to give the 1-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)-kanamycin A derivatives (**5**) in good yields (Table 3).

General Procedure for Acylation of **5**

Method A; Acylation with Acid Chloride: To a solution of **5** (32.0 mmol) in a mixture of tetrahydrofuran (THF) (600 ml) and water (150 ml) was added carboxylic acid chloride (37.4 mmol) with stirring under ice-cooling. The mixture was stirred at the same condition for 1 hour. The reaction mixture was concentrated under reduced pressure to give a suspension. The resultant precipitates were collected by filtration, washed in turn with 1 N HCl (500 ml), Et_2O, *iso*-propyl alcohol (IPA), and water, and dried to give the 1-*N*-higher-acyl-3″-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)kanamycin A derivatives (**6D**～**F**, **H**, **J**) as a solid in good yields (Table 4).

Method B; Acylation with Carboxylic Acid: Phosphorous oxychloride (3.16 mmol) was added to a mixture of dimethylformamide (DMF) (3.16 mmol) and THF (0.5 ml) at −10～0°C and the suspension was stirred at −5～0°C for 10 minutes. To the above suspension were added a solution of carboxylic acid (3.16 mmol) in dry THF (5 ml) at the same temperature under stirring and the mixture was stirred for 30 minutes to prepare an activated acid solution. To a solution of **5** (1.59 mmol) in a mixture of THF (40 ml) and water (10 ml) was dropwise added the activated acid solution obtained above, keeping the pH 8～9 with Et_3N under ice-cooling. The reaction mixture was stirred for 1 hour at the same condition and concentrated under reduced pressure to give a solid. The solid was washed successively with 1 N HCl, Et_2O, and water and dried to give the 1-*N*-higher-acyl-3″-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)kanamycin A (**6G**, **Y**, **a**, **d**, **e**, **f**) as a solid in good yields (Tables 4, 5 and 6).

Method C; Acylation with *N*-(Acyloxy)succinimide: To a solution of **5** (1.00 mmol) in dimethyl

sulfoxide (DMSO) (20 ml) were added *N*-(acyloxy)succinimide (2.04 mmol) and Et_3N (0.3 ml) at room temperature and the mixture was stirred at the same temperature overnight. The reaction mixture was dropwise added to Et_2O (200 ml). The resultant precipitates were collected by filtration, washed with Et_2O and water successively, and dried to give 1-*N*-higher-acyl-3″-*N*-acyl-3,6′-bis(*N*-benzyloxycarbonyl)kanamycin A (**6I, L, M, P, Q, T, Z**) as a solid in good yields. Their physical data and yields are summarized in Tables 4 and 5.

3,6′-Bis(*N*-benzyloxycarbonyl)-3″-*N*-(benzyloxycarbonyl)aminocarbonyl-1-*N*-palmitoylkanamycin A (**6N**)

To a solution of **5b** (1.0 g) in dry THF (20 ml) was added benzyloxycarbonyl isocyanate (0.21 g) at room temperature overnight. The reaction mixture was concentrated under reduced pressure to give a solid. The solid was collected by filtration, washed with water and Et_2O successively, and dried to give **6N** as a solid in a good yield (Table 4).

General Procedure for Formylation of **5** with *p*-Nitrophenyl Formate

To a solution of **5** (0.5 mmol) in a mixture of DMF (10 ml) and water (2 ml) were added *p*-nitrophenyl formate (1.5 mmol) and Et_3N (0.08 ml) and the mixture was stirred for 2 hours. The reaction mixture was dropwise added to Et_2O (200 ml). The resultant precipitates were collected by filtration, washed with Et_2O, and dried to give 3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-formyl-1-*N*-palmitoyl-, or -1-*N*-(3-hydroxytetradecanoyl)kanamycin A (**6B, V**) as a solid in good yields (Tables 4 and 5).

3,6′-Bis(*N*-benzyloxycarbonyl)-1-*N*-palmitoyl-3″-*N*-phthalylkanamycin A (**6S**)

To a solution of **5b** (0.5 g) in DMSO (10 ml) were successively added *N*-(ethoxycarbonyl)phthalimide (122 mg) and Et_3N (0.08 ml) at room temperature. The mixture was stirred at the same temperature for 3 hours. The reaction mixture was poured into Et_2O (200 ml) to give a residue, which was twice washed with Et_2O (200 ml). The resultant precipitates were collected by filtration and dried. The solid was subjected to column chromatography on silica gel (30 g) and eluted with a mixture of $CHCl_3$-MeOH (10: 1). The fractions containing the desired compound were combined and concentrated under reduced pressure to give **6S** (0.12 g) as a solid (Table 4).

3,6′-Bis(*N*-benzyloxycarbonyl)-1-*N*-(3-hydroxytetradecanoyl)-3″-*N*-trifluoroacetylkanamycin A (**6X**)

To a solution of **5d** (800 mg) in DMF (8 ml) was added a solution of ethyl trifluoroacetate (127 mg) in DMSO (1 ml) at room temperature and the mixture was stirred at the same temperature for 2 hours. The reaction mixture was poured into ice-water (80 ml). The resultant precipitates were collected by filtration, washed successively with water (60 ml) and a mixture of IPA (20 ml) and Et_2O (20 ml), and dried to give **6X** as a solid (777 mg) (Table 5).

3,6′-Bis(*N*-benzyloxycarbonyl)-3″-*N*-(3-carboxypropionyl)-1-*N*-palmitoylkanamycin A (**6R**)

To a solution of **5b** (0.5 g) in a mixture of THF (40 ml) and water (10 ml) was added succinic anhydride (0.06 g) and the mixture was stirred at room temperature for 3.5 hours. The reaction mixture was concentrated under reduced pressure to give a solid, which was collected by filtration, washed with water and Et_2O successively, and dried to give **6R** (0.55 g) as a solid (Table 4).

3,6′-Bis(*N*-benzyloxycarbonyl)-1-*N*-(*tert*-butoxycarbonyl)kanamycin A (**10**)

To a solution of **2** (3 g) in a mixture of THF (48 ml) and water (12 ml) were added 2-*tert*-butoxycarbonyloximino-2-phenylacetonitrile (1.04 g) and Et_3N (0.59 ml) at room temperature and the mixture was stirred at the same temperature for 2 hours. Furthermore, 2-*tert*-butoxycarbonyloximino-2-phenylacetonitrile (1.04 g) and Et_3N (0.59 ml) were added to the solution and the mixture was stirred at room temperature overnight. The solution was concentrated under reduced pressure to give a crude solid (12 g).

A solution of the solid (12 g) in a mixture of DMF (50 ml) and conc NH_4OH (25 ml) was stirred at room temperature overnight. Further, conc NH_4OH (30 ml) was added to the solution and the mixture was stirred at room temperature for 8 hours. The reaction mixture was poured into Et_2O

(500 ml) and resultant precipitates were collected by filtration, washed with Et_2O (100 ml) and water (100 ml) successively, and dried to give **10** (2.5 g, 82.9%) as a solid. MP 246~247°C; IR (Nujol) 1685, 1670 (sh), 1650, 1520, 1270 cm^{-1}; NMR (DMSO-d_6, δ) 1.36 (9H, s, $(CH_3)_3C$).

3,6′-Bis(*N*-benzyloxycarbonyl)-3″-*N*-[*N*-(benzyloxycarbonyl)glycyl]-1-*N*-(*tert*-butoxycarbonyl)-kanamycin A (**11a**)

Phosphorous oxychloride (0.56 ml) was added to a mixture of DMF (0.47 ml) and THF (0.9 ml) and the suspension was stirred at −5~0°C for 10 minutes. To the above suspension was added a solution of *N*-(benzyloxycarbonyl)glycine (0.98 g) in THF (9 ml) at the same temperature under stirring and the solution was stirred for 30 minutes to prepare an activated acid solution. To a solution of **10** (2 g) in a mixture of THF (60 ml) and water (15 ml) was dropwise added the activated acid solution obtained above, keeping the pH 8~9 with Et_3N. The reaction mixture was stirred at the same temperature for 30 minutes and was concentrated under reduced pressure to give a suspension. The resultant solid was collected by filtration, washed in turn with 1 N HCl, Et_2O, water, and a mixture of Et_2O - IPA (1: 1), and dried to give **11a** (1.91 g, 78%) as a solid. MP 148~150°C; IR (Nujol) 1680, 1520, 1260, 1150 cm^{-1}; NMR (DMSO-d_6, δ) 1.35 (9H, s, $(CH_3)_3C$).

3″-*N*-(Benzyloxyacetyl)-3,6′-bis(*N*-benzyloxycarbonyl)-1-*N*-(*tert*-butoxycarbonyl)kanamycin A (**11b**)

The compound (**11b**) was obtained according to a similar manner to that of **11a**.

IR (Nujol) 1680, 1520 cm^{-1}; NMR (DMSO-d_6, δ) 1.37 (9H, s, $(CH_3)_3C$), 4.57 (2H, s, CH_2).

3″-*N*-Acetyl-3,6′-bis(*N*-benzyloxycarbonyl)-1-*N*-(*tert*-butoxycarbonyl)kanamycin A (**11c**)

To a solution of **10** (2 g) in a mixture of THF (40 ml) and water (10 ml), acetyl chloride (0.22 g) was dropwise added with stirring at room temperature, keeping the pH 8~8.5 with Et_3N. The solution was stirred under the same condition for 1 hour. The resultant precipitates were collected by filtration, washed three times with Et_2O (20 ml) and water (60 ml) in turn, and dried to give **11c** as a solid (1.86 g, 89%). MP 236°C (dec); IR (Nujol) 1680, 1510, 1500, 1140, 1030 cm^{-1}; NMR (DMSO-d_6, δ) 1.85 (3H, s, CH_3CO).

3″-*N*-Acetyl-3,6′-bis(*N*-benzyloxycarbonyl)-1-*N*-palmitoylkanamycin A (**6C**)

A solution of **11c** (1.8 g) in a mixture of TFA (20 ml) and anisole (5 ml) was stirred under ice-cooling for 1 hour. The solution was concentrated under reduced pressure to give a residue, which was dissolved in a mixture of THF (60 ml) and water (15 ml). To the resultant solution was dropwise added palmitoyl chloride (0.58 g) with stirring at 0~5°C, keeping the pH 8~8.5 with Et_3N. The mixture was stirred under the same condition for 1 hour and the reaction mixture was concentrated under reduced pressure. The resultant solid was washed in turn with water, 1 N HCl, Et_2O, and water. The solid was dried to give **6C** (1.96 g, 98%) (Table 4).

3″-*N*-(Benzyloxyacetyl)-3,6′-bis(*N*-benzyloxycarbonyl)-1-*N*-palmitoylkanamycin A (**6K**)

To a suspension of **11b** (0.95 g) in anisole (2.9 ml) was added TFA (9.5 ml) under ice-cooling. The mixture was stirred at the same temperature for 2 hours and concentrated under reduced pressure to give a syrup. The syrup was dissolved in a mixture of THF (30 ml) and water (7 ml), and to the mixture was added palmitoyl chloride (265 mg) under ice-cooling, keeping the pH 8~9 with Et_3N. The mixture was stirred under the same condition for 2 hours. The reaction mixture was concentrated under reduced pressure. The resultant precipitates were collected by filtration, washed with water, 1 N HCl, water, and Et_2O in turn, and dried to give **6K** (1.05 g, 99%) (Table 4).

3,6′-Bis(*N*-benzyloxycarbonyl)-3″-*N*-[*N*-(benzyloxycarbonyl)glycyl]-1-*N*-palmitoylkanamycin A (**6O**)

A solution of **11a** (0.92 g) in a mixture of TFA (10 ml) and anisole (3 ml) was stirred under ice-cooling for 1 hour. The solution was concentrated under reduced pressure. To a solution of the resultant residue in a mixture of THF (40 ml) and water (10 ml) was dropwise added a solution of palmitoyl chloride (254 mg) in THF (10 ml) at 0~5°C with stirring, keeping the pH 8~9 with Et_3N.

The mixture was stirred at the same temperature for 1 hour and the reaction mixture was concentrated under reduced pressure to give a solid. The solid was washed with 1 N HCl (20 ml), IPA (20 ml), Et_2O (10 ml), and water in turn and dried to give **6O** (0.91 g, 87%) (Table 4).

3″-*N*-Acetyl-3,6′-bis(*N*-benzyloxycarbonyl)-1-*N*-(3-hydroxytetradecanoyl)kanamycin A (**6W**)

To a solution of **10** (2 g) in pyridine (40 ml) was added acetic anhydride (3.1 ml). The mixture was stirred at room temperature for 3 hours and allowed to stand at the same temperature overnight. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (100 ml) and the solution was washed with aqueous $NaHCO_3$ and water successively, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give 2′,3′,4′,2″,4″,6″-hexa-*O*-acetyl-3″-*N*-acetyl-3,6′-bis(*N*-benzyloxycarbonyl)-1-*N*-(*tert*-butoxycarbonyl)kanamycin A (2.54 g, 97.1%) as an amorphous solid. IR (Nujol) 1740, 1520, 1240, 1170 cm^{-1}; NMR ($CDCl_3$, δ) 1.32 (9H, s, $(CH_3)_3C$), 1.90~2.20 (21H, m, CH_3CO).

A solution of the compound obtained above (2.5 g) in a mixture of TFA (30 ml) and anisole (7 ml) was stirred under ice-cooling for 1 hour. The reaction mixture was concentrated *in vacuo* to give a crude 2′,3′,4′,2″,4″,6″-hexa-*O*-acetyl-3″-*N*-acetyl-3,6′-bis(*N*-benzyloxycarbonyl)kanamycin A monotrifluoroacetate. Phosphorous oxychloride (0.29 ml) was added to a mixture of DMF (0.25 ml) and THF (0.5 ml) at −5~0°C. To the resultant suspension were added successively THF (5 ml) and 3-acetoxytetradecanoic acid (706 mg) at −5~0°C under stirring. The mixture was stirred at the same temperature for 30 minutes to prepare an activated acid solution. To a solution of the compound obtained above in a mixture of THF (40 ml) and water (10 ml) was dropwise added the activated acid solution, keeping the pH 8~9 with Et_3N. The mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (120 ml). The solution was washed with aqueous $NaHCO_3$ and water in turn, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (50 g) and eluted with a mixture of $CHCl_3$-MeOH (30: 1) to give 1-*N*-(3-acetoxytetradecanoyl)-2′,3′,4′,2″,4″,6″-hexa-*O*-acetyl-3″-*N*-acetyl-3,6′-bis(*N*-benzyloxycarbonyl)kanamycin A (1.95 g, 67.9%). IR (Nujol) 3270, 1720~1700, 1650, 1530~1510 cm^{-1}; NMR ($CDCl_3$, δ) 0.88 (3H, br s, CH_3). Furthermore, to a solution of KOH (1.16 g) in MeOH (40 ml) was added the compound (1.9 g) and the mixture was stirred at room temperature for 3 hours. The resultant suspension was poured into ice-water (200 ml). The resultant precipitates were collected by filtration, washed with water, and dried to give **6W** as a solid (1.20 g) (Table 5).

3,6′-Bis(*N*-benzyloxycarbonyl)-3″-*N*-[(*N*-benzyloxycarbonyl)glycyl]-1-*N*-(hexadecanyloxycarbonyl)-kanamycin A (**6b**)

A solution of **11a** (1.0 g) in a mixture of TFA (10 ml) and anisole (3 ml) was stirred under ice-cooling for 1 hour. The solution was concentrated under reduced pressure to give a residue. To a solution of the residue (1 g) in a mixture of THF (25 ml) and water (5 ml) was dropwise added hexadecanyloxycarbonyl chloride (532 mg) with stirring under ice-cooling, keeping the pH 8~9 with Et_3N. The mixture was stirred at the same temperature for 1 hour. The reaction mixture was evaporated *in vacuo* to give a solid. The solid was collected by filtration, washed with 1 N HCl, water, and Et_2O, successively, and dried to give **6b** (0.65 g, 57%) as a solid (Table 6).

3,6′-Bis(*N*-benzyloxycarbonyl)-3″-*N*-[(*N*-benzyloxycarbonyl)glycyl]-1-*N*-(pentadecanylaminocarbonyl)kanamycin A (**6c**)

A solution of **11a** (1.9 g) in a mixture of TFA (20 ml) and anisole (6 ml) was stirred under ice-cooling for 1 hour. The solution was evaporated *in vacuo* and a solution of the residue in MeOH (20 ml) was adjusted to pH 8~9 with aqueous solution of Na_2CO_3. The mixture was poured into water (50 ml). The resultant precipitates were collected by filtration, washed with water, and dried. To a solution of the solid (1.0 g) in DMF (20 ml) was added pentadecanyl isocyanate (400 mg) at room temperature and the mixture was stirred at the same temperature overnight. Furthermore, to the mixture were added Et_3N (0.2 ml) and pentadecanyl isocyanate (400 mg) and the mixture was stirred at 30~40°C for 6 hours. The reaction mixture was filtered off and the filtrate was poured into Et_2O (1 liter). The resultant precipitates were collected by filtration, washed with water (30 ml), and dried

to give **6c** (0.69 g) as a solid (Table 6).

General Procedure for Deprotection of **6**

A solution of the derivatives (**6A**~**Z**, **a**~**f**: 1.3 mmol) in a mixture of THF (30 ml), MeOH (10 ml), and 1 N HCl (10 ml) was hydrogenated under atomospheric pressure of hydrogen at room temperature for 6 hours in the presence of 10% palladium on carbon (1.5 g). The catalyst was filtered off and the filtrate was evaporated *in vacuo* to give a residue. The solution of the residue in water (40 ml) was filtered off and lyophilized to give 1-*N*-higher-acyl-3″-*N*-acylkanamycin A derivative hydrochlorides (**7**, **8**, **9**) as a hygroscopic solid. Their physical data and yields are summarized in Tables 7, 8 and 9.

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