STUDIES ON ANTIVIRAL AGENTS

V. SYNTHESIS AND *IN VITRO* ANTIVIRAL ACTIVITY OF NEW AMINOGLYCOSIDE DERIVATIVES HAVING PALMITOYL GROUP

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The synthesis and antiviral activity of various aminoglycoside derivatives having a palmitoyl group are described. All of these aminoglycoside derivatives exhibited almost the same excellent antiviral activity against herpes simplex virus type I and influenza virus.

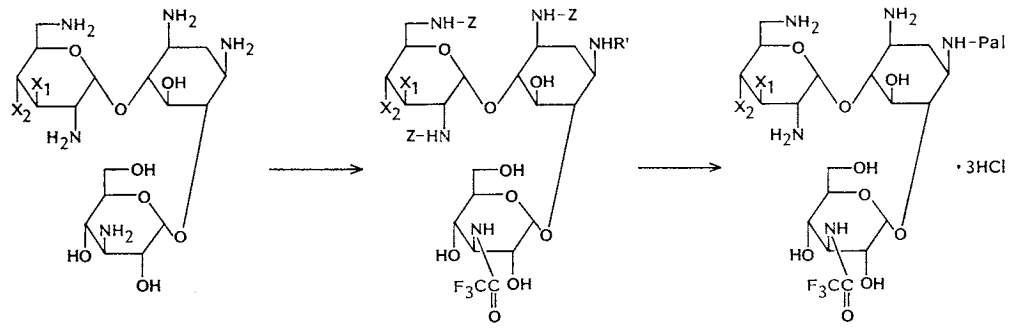
As reported previously[1~3], we have discovered that 1-*N*-higher-acyl-3″-*N*-substituted kanamycin A derivatives exhibited excellent activity against both herpes simplex virus type I Miyama (HSV-I) and influenza virus (A/PR8). Among these derivatives, 1-*N*-palmitoyl-3″-*N*-(trifluoroacetyl)kanamycin A dihydrochloride (PTKA) showed especially potent antiviral activity as well as cytotoxicity. We have also reported that the positional isomers of *N*-palmitoyl- and *N*-palmitoyl-*N*′-(trifluoroacetyl)-kanamycin A exhibited comparable and outstanding activity against HSV-I and influenza virus[4]. These findings indicate to some extent that *N*-acylation of kanamycin A with a higher acyl group is essential for antiviral activity, but it was interesting to speculate that the kanamycin A nucleus could be replaced with other aminoglycoside antibiotics without loss of the antiviral activity.

To test the above assumption, we linked the palmitoyl group to amino groups of other aminoglycoside antibiotics. The results of these experiments are subject to this paper.

Chemistry

The synthesis of pseudotrisaccharide derivatives (**10**, **11**, **12**, **15**, **21**, **25** and **32**) containing the palmitoyl group is summarized in Schemes 1, 2 and 3. 3,2′,6′-Tris-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin B (**4**), -tobramycin (**5**), -dibekacin (**6**), and -gentamicin C (C_1, C_{1a} and C_2 complex) (**19**) were prepared according to the method of TSUCHIYA *et al.*[5]. Acylation of **4**, **5**, **6** and **19** with palmitoyl chloride (PalCl) gave the corresponding 3,2′,6′-tris-*N*-(benzyloxycarbonyl)-1-*N*-palmitoyl-3″-*N*-(trifluoroacetyl)kanamycin B (**7**), -tobramycin (**8**), -dibekacin (**9**), and -gentamicin C (**20**). The yields and spectral data of these derivatives (**7**, **8**, **9** and **20**) are summarized in Table 1. 1-*N*-Palmitoyl-3″-*N*-(trifluoroacetyl)kanamycin B trihydrochloride (**10**), -tobramycin trihydrochloride (**11**), -dibekacin trihydrochloride (**12**), and -gentamicin C trihydrochloride (**21**) were derived from their key intermediates (**7**, **8**, **9** and **20**) in a similar manner to that of PTKA[1,2]. 1-*N*-Palmitoyldibekacin tetrahydrochloride (**15**), -sagamicin tetrahydrochloride (**25**), and -ribostamycin tetrahydrochloride (**32**) were synthesized as follows. Protection of 3,2′,6′-tris-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)dibekacin (**6**) with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON), followed by treatment with concentrated NH_4OH in *N*,*N*-dimethylformamide (DMF), gave the intermediate

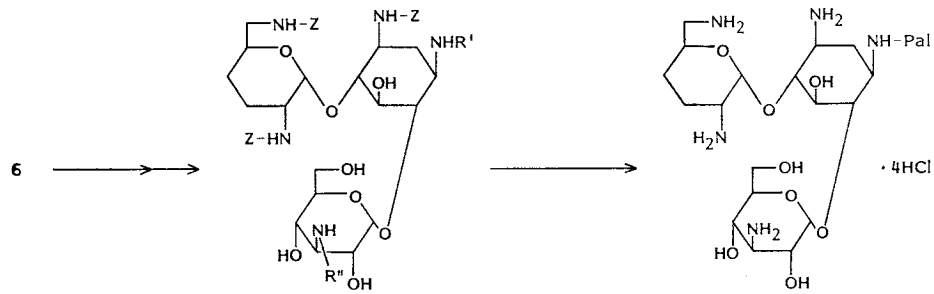
Scheme 1. Synthesis of 1-*N*-palmitoylkanamycin B (**10**), -tobramycin (**11**), and -dibekacin derivatives (**12** and **15**).



Kanamycin B (**1**) $X_1 = X_2 = OH$
Tobramycin (**2**) $X_1 = H$ $X_2 = OH$
Dibekacin (**3**) $X_1 = X_2 = H$

4 R' = H $X_1 = X_2 = OH$
5 R' = H $X_1 = H$ $X_2 = OH$
6 R' = H $X_1 = X_2 = H$
7 R' = Pal $X_1 = X_2 = OH$
8 R' = Pal $X_1 = H$ $X_2 = OH$
9 R' = Pal $X_1 = X_2 = H$

10 $X_1 = X_2 = OH$
11 $X_1 = H$ $X_2 = OH$
12 $X_1 = X_2 = H$



13 R' = H R'' = Z
14 R' = Pal R'' = Z

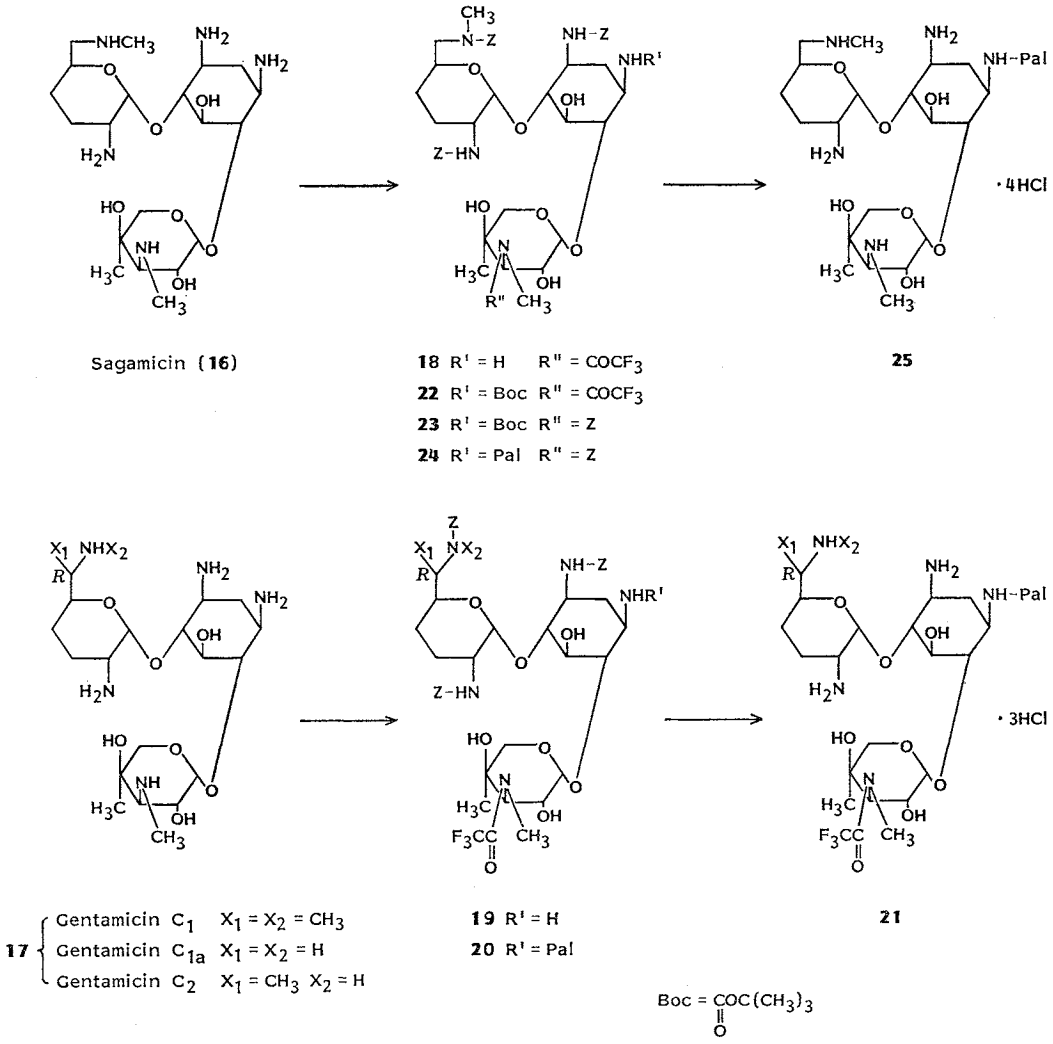
15

Pal = $-\overset{O}{\overset{\|}{C}}(CH_2)_{14}CH_3$

Z = $-\overset{O}{\overset{\|}{C}}OCH_2C_6H_5$

unprotected at the *N*-3'' position. Reaction of the above intermediate with benzyloxycarbonyl chloride (ZCl), followed by removal of *tert*-butoxycarbonyl group with trifluoroacetic acid (TFA) and anisole, afforded the corresponding 3,2',6',3''-tetrakis-*N*-(benzyloxycarbonyl)dibekacin (**13**). Acylation of **13** with PalCl gave a protected 1-*N*-palmitoyldibekacin derivative (**14**). Finally, hydrogenation of **14** in the presence of Pd-black under acidic condition gave the desired **15**. 1-*N*-Palmitoylsagamicin tetrahydrochloride (**25**) was derived from 3,6'-bis-*N*-(benzyloxycarbonyl)-3''-*N*-(trifluoroacetyl)sagamicin (**18**) as described above for **15**. To prepare 1-*N*-palmitoylribostamycin tetrahydrochloride (**32**), protection with the benzyloxycarbonyl group of four amino groups in ribostamycin (**26**) and subsequently tritylation at the 5''-hydroxy group, followed by protection at vicinal hydroxy groups with 1,1-dimethoxycyclohexane, gave the desired intermediate **28**. The selective removal of the

Scheme 2. Synthesis of 1-*N*-palmitoylsagamicin (**25**) and -gentamicin C (C_1, C_{1a} and C_2 complex) derivatives (**21**).

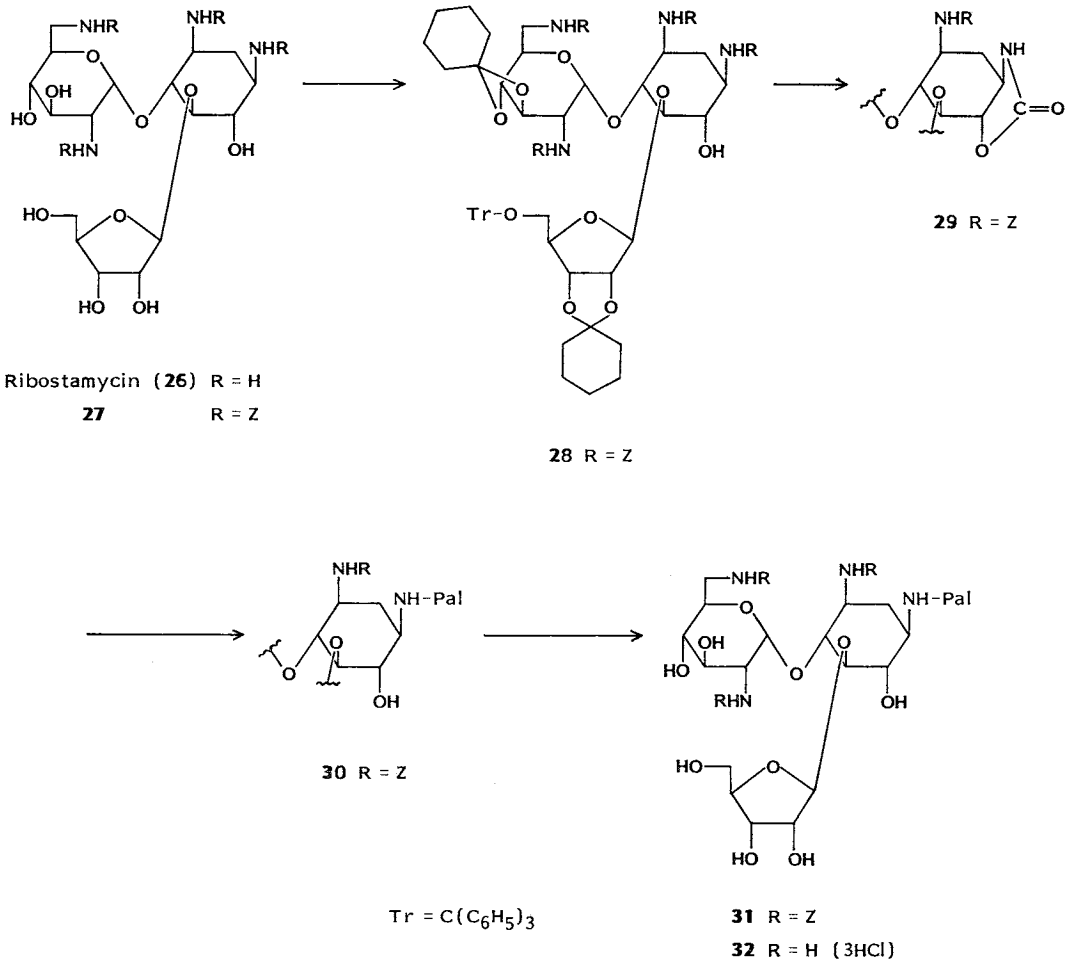


benzyloxycarbonyl group at the *N*-1 position of **28** was accomplished with sodium hydride to give the desired cyclic carbamate **29**[6]. Removal of the cyclic carbamate in **29** under alkaline condition, followed by acylation with PalCl, afforded the protected 1-*N*-palmitoylribostamycin derivative **30**. Finally, deprotection of **30** provided **32**.

4-*N*-Palmitoylfortimicin B trihydrochloride (**36**) and 2'-*N*-palmitoylkasugamycin (**38**) were synthesized as follows (Scheme 4). 1,2',6'-Tris-*N*-(benzyloxycarbonyl)fortimicin B (**34**) was derived from fortimicin B (**33**) according to the method of TADANIER *et al.*[7]. **36** was obtained by acylation of **34** with PalCl and subsequent hydrogenation in the presence of 10% Pd-C. Treatment of kasugamycin (**37**) with *N*-(palmitoyloxy)succinimide gave the desired derivative **38**.

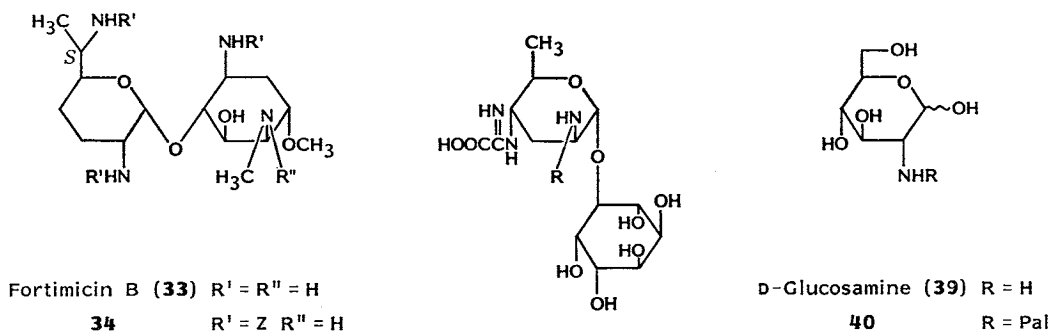
The synthesis of the monosaccharide derivative **40** containing the palmitoyl group is shown in Scheme 4. 2-*N*-Palmitoyl-D-glucosamine (**40**) was prepared from D-glucosamine (**39**) by treatment with PalCl. The yields and spectral data of these derivatives (**10**, **11**, **12**, **15**, **21**, **25**, **32**, **36**, **38** and

Scheme 3. Synthesis of 1-*N*-palmitoylribostamycin derivative (32).



Ribostamycin (26) R = H
27 R = Z
28 R = Z
29 R = Z
30 R = Z
Tr = $C(C_6H_5)_3$
31 R = Z
32 R = H (3HCl)

Scheme 4. Synthesis of 1-*N*-palmitoylfortimicin B (36), 2′-*N*-palmitoylkasugamycin (38), and 2-*N*-palmitoyl-D-glucosamine (40).



Fortimicin B (33) R' = R" = H
34 R' = Z R" = H
35 R' = Z R" = Pal
36 R' = H R" = Pal (3HCl)
Kasugamycin (37) R = H
38 R = Pal
D-Glucosamine (39) R = H
40 R = Pal

Table 1. Physical data and yields of protected 1-*N*-palmitoyl-3″-*N*-trifluoroacetyl aminoglycoside derivatives (**7**, **8**, **9** and **20**).

No.	Yield (%)	MP (°C, dec)	IR (Nujol, cm^{-1})	^1H NMR	
				Solvent	δ (ppm)
7	86	—	—	DMSO-d_6	0.84 (3H, t, J=6 Hz), 1.25 (26H, s), 1.80~2.25 (2H, m)
8	90	284~286	1710, 1690, 1540, 1290, 1170	DMSO-d_6	0.85~1.03 (3H, m), 1.25 (26H, s), 1.50~1.80 (2H, m), 1.75~2.30 (4H, m)
9	47	—	1680, 1520, 1250, 1180	DMSO-d_6	0.80~1.00 (3H, m), 1.28 (26H, s)
20	60	156~157	1700~1665, 1510, 1300, 1240, 1140	$CDCl_3$	2.57 (3H, m), 3.24 (3H, m)

—: Not measured.

40) are summarized in Tables 1 and 2.

Biological Activity and Results

The antiviral activity and the cytotoxicity of various *N*-palmitoyl aminoglycoside derivatives are summarized in Table 3.

N-Palmitoyl Aminoglycoside Derivatives

Pseudotrisaccharide Derivatives

1-*N*-Palmitoyldibekacin tetrahydrochloride (**15**), 1-*N*-palmitoylsagamicin tetrahydrochloride (**25**), and 1-*N*-palmitoylribostamycin tetrahydrochloride (**32**) exhibited excellent antiviral activity against HSV-I (ID_{50} 0.8~1.8 μg/ml). The antiviral activity of these pseudotrisaccharides (**15**, **25** and **32**) was of the same level as that of 1-*N*-palmitoylkanamycin A trihydrochloride (PKA), and was about 20 times more active than that of virazole but less than that of acyclovir. **32** also exhibited remarkable activity against influenza virus (ID_{50} 25 μg/ml). Thus, various 1-*N*-palmitoyl pseudotrisaccharide aminoglycosides are equipotent agents against HSV-I and influenza viruses as the corresponding kanamycin A derivative.

Pseudodisaccharide and Monosaccharide Derivatives

4-*N*-Palmitoylfortimicin B trihydrochloride (**36**) and 2′-*N*-palmitoylkasugamycin (**38**) exhibited excellent antiviral activity against HSV-I (ID_{50} 1.9 and 2.7 μg/ml). **38** showed the strongest antiviral activity against influenza virus (ID_{50} 5.6 μg/ml) in this series and was twice as active than virazole and amantadine. However, 2-*N*-palmitoyl-D-glucosamine (**40**) was about 10 times less active than pseudotrisaccharide and disaccharide derivatives. Thus, *N*-palmitoyl pseudodisaccharide and monosaccharide derivatives were found to exhibit activity against HSV-I and influenza virus. The cytotoxicity on vero cell of these *N*-palmitoyl pseudotri-, pseudodi-, and monosaccharides (**15**, **25**, **32**, **36**, **38** and **40**), however, was stronger than that of PTKA[1]. Since we had previously demonstrated reduced cytotoxicity of PTKA[3,4], it was of interest to prepare other *N*-trifluoroacetylated 1-*N*-palmitoyl pseudotrisaccharide aminoglycosides.

N-Palmitoyl-*N*′-trifluoroacetyl Aminoglycoside Derivatives

1-*N*-Palmitoyl-3″-*N*-(trifluoroacetyl)kanamycin B trihydrochloride (**10**) and -tobramycin trihydrochloride (**11**) exhibited excellent antiviral activity against HSV-I (ID_{50} 1.4~5.2 μg/ml). Both

Table 2. Physical data and yields of *N*-palmitoyl aminoglycoside derivatives (**10**, **11**, **12**, **15**, **21**, **25**, **32**, **36**, **38** and **40**).

No.	Yield[a] (%)	MP (°C, dec)	$[\alpha]_D^{20}$ (°)	IR (Nujol, cm^{-1})	^1H NMR		FD-MS (*m/z*)
					Solvent	δ (ppm)	
10	81	220	+69.8 (*c* 0.65, H_2O)	1700, 1630, 1550	CD_3OD	0.90~1.08 (3H, m), 5.08 (1H, d, *J*=3 Hz)	819 (M^++1)
11	64	224	+58.7 (*c* 1.0, H_2O)	1710, 1645~1620, 1165	CD_3OD	0.80~0.97 (3H, m), 1.28 (26H, s), 1.90~2.50 (6H, m), 5.10 (1H, d, *J*=3 Hz), 5.90 (1H, d, *J*=3.5 Hz)	824 (M^++22), 802 (M^+)
12	69	206	+55.7 (*c* 0.35, H_2O)	1700, 1630		—	808 (M^++22), 787 (M^++1)
15	87	167	+63.5 (*c* 0.16, H_2O)	1640, 1500[b]	D_2O	0.84 (3H, t, *J*=7 Hz), 5.24 (1H, br s), 5.80 (1H, br s)	690 (M^+)
21	83	174	+70.3 (*c* 1.0, H_2O)	1680, 1650~1630, 1245	D_2O	2.78 (3H, s)	812, 798, 784 (M^+)
25	73	154	+98.1 (*c* 1.0, H_2O)	1640, 1540, 1280, 1120[b]	D_2O	2.76 (3H, s), 2.90 (3H, s), 5.30 (1H, d, *J*=4 Hz), 5.81 (1H, d, *J*=3 Hz)	702 (M^+)
32	45	166	—	1640~1620	D_2O	0.88 (3H, m), 1.05~1.50 (26H, s)	693 (M^+)
36	99	208	+71.0 (*c* 0.49, H_2O)	1600	CD_3OD	3.17 (3H, s), 3.47 (3H, s), 5.33 (1H, d, *J*=3 Hz)	587 (M^+)
38	69[c]	162	+113.4 (*c* 0.58, H_2O)	1660, 1540	CD_3OD	0.90 (3H, t, *J*=5 Hz), 1.20 (3H, d, *J*=5 Hz), 5.27 (1H, s)	574 (M^+ − 44)
40	69[d]	129~132	+125.8 (*c* 1.0, DMSO)	1700, 1640, 1550	DMSO-d_6	0.90 (3H, t, *J*=5 Hz), 7.43 (1H, m)	418 (M^+)

[a] Yields of deprotecting process. [b] KBr disc method. [c] Yield from **37**. [d] Yield from **39**.
—: Not measured.

Table 3. *In vitro* antiviral activity of aminoglycoside derivatives having palmitoyl group.

Substance	Antiviral activity ID_{50} (μg/ml)		Cytotoxicity (μg/ml)	
	HSV-I	Influenza virus	Vero cell	MDCK cell
10	5.2	—	>100	—
11	1.4	—	>100	—
12	1.9	24	32	>100
15	0.8	—	10	—
21	1.7	—	10	—
25	1.8	—	32	—
32	1.0	25	10	>100
36	1.9	—	10	—
38	2.7	5.6	10	32
40	28	—	100	—
PTKA	1.4	16	>100	>100
PKA	3.2	32	10	>100
Virazole	32	10	>100	>100
Amantadine	—	10	>100	>100
Acyclovir	0.1	—	>10	—

Assay system: HSV-I/vero cell, influenza virus/MDCK cell, CPE inhibition method.
—: Not measured.

antiviral activity and cytotoxicity of **10** and **11** in vero cell were as low as those of PTKA. Moreover, 1-*N*-palmitoyl-3″-*N*-(trifluoroacetyl)dibekacin trihydrochloride (**12**) and -gentamicin C trihydrochloride (**21**) showed excellent antiviral activity against HSV-I (ID_{50} 1.7 and 1.9 μg/ml) as well as **10** and **11**. The antiviral activity of **10**, **11**, **12** and **21** against HSV-I was about 30 times higher than that of virazole, but was less than that of acyclovir. Compound **12** also showed remarkable antiviral activity against influenza virus (ID_{50} 24 μg/ml). Surprisingly, however, the cytotoxicity of **12** and **21** in vero cell was stronger than that of PTKA, **10** and **11**.

These results show that the chemical modification, originally described for kanamycin A, furnishes equipotent drugs against HSV-I and influenza virus when applied to other aminoglycosides. However, the cytotoxicity in vero cell appears to depend on the structure of the aminoglycoside and tends to become stronger with increasing lipophilic character of the aminoglycoside part. Presumably, the balance between lipophilic and hydrophilic character in these derivatives is an important factor for the expression of antiviral activity and cytotoxicity. Furthermore, it can be expected that *N*-higher acyl derivatives of other aminoglycosides such as streptomycin, neomycin, amikacin, sporaricin B *etc.* will also exhibit some degree of antiviral activities.

In vivo results of the aminoglycosides described in this paper will be reported elsewhere[8]. The mechanism of growth inhibition of virus is now under investigation[9].

Experimental

Melting points were determined with a 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 internal standard. IR spectra were taken on Hitachi 260-10 spectrophotometer. Optical rotations were determined with a Jasco DIP-140 polarimeter. Field desorption mass spectra (FD-MS) were measured on a Jeol-D 300 mass spectrometer. All evaporation were carried out under reduced pressure.

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/PR8 were grown in vero cells and Madin and Darby canine kidney cells (MDCK cells), respectively. The test compounds were dissolved in water* (phosphate buffer solution). The culture medium was changed to 0.5% FBS-MEM. The cell culture were inoculated with about 100 times the virus dose needed to infect 50% 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. Four wells (multi-well trays (96 wells)) were used in each concentrations. They were fixed with 5% TCA 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 and MDCK cells 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.

Materials

3,2′,6′-Tris-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin B (**4**), -tobramycin (**5**), -dibekacin (**6**), -sagamicin (**18**), and -gentamicin C (C_1, C_{1a} and C_2 complex) (**19**).

These compounds (**4**, **5**, **6**, **18** and **19**) were synthesized according to the method of TSUCHIYA *et al.*[5].

General Procedure for Acylation of **4**, **5**, **6** and **19**

Method A: Acylation with *N*-(palmitoyloxy)succinimide. A mixture of **4** (0.51 mmol) and *N*-(palmitoyloxy)succinimide (0.53 mmol) in DMF (10 ml) was stirred at room temp for 18 hours. The reaction mixture was poured into water (50 ml). The resulting precipitates were collected, washed successively with water and Et_2O, and dried over P_2O_5 *in vacuo* to give 3,2′,6′-tris-*N*-(benzyloxycarbonyl)-1-*N*-palmitoyl-3″-*N*-(trifluoroacetyl)kanamycin B (**7**) (Table 1).

Method B: Acylation with palmitoyl chloride. To a solution of **5**, **6** and **19** (0.32 mmol) in a mixture of THF (20 ml) and water (7 ml) was added PalCl (0.33 mmol) under ice-cooling, keeping pH between 8 and 9 with Et_3N. The mixture was stirred for 30 minutes under the same condition. THF was removed by evaporation. To the resulting residue was added water to give a precipitate. The precipitate was filtered off, washed with water, and dissolved in DMF (2 ml). The solution was dried over $MgSO_4$. The insoluble material was removed by filtration. To the filtrate was added silica gel (0.6 g) and the mixture was evaporated. The mixture was subjected to column chromatography on silica gel and eluted with MeOH - $CHCl_3$ (1 : 10 and 1 : 4, successively). The fractions containing the desired compound were combined and evaporated to give 3,2′,6′-tris-*N*-(benzyloxycarbonyl)-1-*N*-palmitoyl-3″-*N*-(trifluoroacetyl)tobramycin (**8**), -dibekacin (**9**), and -gentamicin C (**20**) (Table 1).

3,2′,6′,3″-Tetrakis-*N*-(benzyloxycarbonyl)dibekacin (**13**)

To a solution of **6** (2.0 g) in a mixture of THF (30 ml) and water (7 ml) was added a mixture of Boc-ON (1.03 g) and Et_3N (0.39 ml) at room temp. The mixture was stirred overnight at room temp. The reaction mixture was evaporated. To the solution of the resulting residue in DMF (30 ml) was added concd NH_4OH (30 ml) at room temp and the mixture was stirred at the same temp overnight. The reaction mixture was evaporated. To the residue, dissolved in DMF (30 ml), was added concd

* The derivatives (**15**, **25**, **32**, **36** and **38**) were very soluble in water at around neutral pH. The derivatives (**10**, **11**, **12** and **21**) were also very soluble in water under acidic condition (pH 5.8), but were slightly soluble in water (0.1 mg/ml) at around neutral pH. Compound **40** was slightly soluble in water.

NH_4OH (10 ml) at room temp. The mixture was stirred again overnight at the same temp and was evaporated. The residue was washed with Et_2O and dissolved in a mixture of THF (40 ml) and water (10 ml). To the resulting solution was added ZCl (0.31 ml) under ice-cooling, keeping pH between 7 and 9 with Et_3N. The reaction mixture was stirred under the same condition for 1 hour. THF was removed by evaporation. The resulting precipitate was collected by filtration, washed with water, and dried over P_2O_5. The suspension of the precipitate in anisole (5.5 ml) was added to TFA (18.3 ml) under ice-cooling. The mixture was stirred for 1 hour under ice-cooling. The mixture was evaporated and completely dried by co-evaporation with toluene. The residue was subjected to column chromatography on silica gel (45 g) eluting with a mixture of $CHCl_3$ - MeOH (10 : 1) and then with a mixture of $CHCl_3$ - MeOH (4 : 1). The fractions containing the desired compound were collected and evaporated to give **13** (0.30 g, 5.3%): IR (Nujol) cm^{-1} 1680, 1510.

3,2′,6′,3″-Tetrakis-*N*-(benzyloxycarbonyl)-1-*N*-palmitoyldibekacin (**14**)

To a solution of **13** (276 mg) in a mixture of THF (20 ml) and water (5 ml) was added PalCl (77 mg) under ice-cooling, keeping pH between 8 and 9 with Et_3N for a period of 30 minutes. THF was removed by evaporation. To the resulting mixture was added water to give a precipitate. The precipitate was collected by filtration, washed with water, and dried over P_2O_5 *in vacuo* to give **14** (333 mg, 97%): IR (Nujol) cm^{-1} 1680, 1540.

3,2′,6′-Tris-*N*-(benzyloxycarbonyl)-1-*N*-(*tert*-butoxycarbonyl)-3″-*N*-(trifluoroacetyl)sagamicin (**22**)

To a solution of **18** (3.1 g) in a mixture of dioxane (45 ml) and water (15 ml) were successively added Boc-ON (1.59 g) and Et_3N (0.9 ml) at room temp. The resulting mixture was stirred for 1 hour and allowed to stand at the same temp overnight. The solution was evaporated and the resulting residue, dissolved in EtOAc (150 ml), was washed successively with aq $NaHCO_3$ and aq NaCl, dried over Na_2SO_4, and evaporated. The residue was subjected to column chromatography on silica gel (70 g) and eluted with a mixture of $CHCl_3$ - MeOH (40 : 1). The fractions containing the desired compound were collected and evaporated to give **22** (3.41 g, 99%): MP 127~128°C; $[\alpha]_D^{20}$ +96.4° (*c* 1.0, $CHCl_3$); IR (Nujol) cm^{-1} 1680, 1500, 1250, 1140; ^1H NMR ($CDCl_3$) δ 1.05 (3H, s, CH_3), 1.40 (9H, s, $C(CH_3)_3$), 2.83 (3H, s, NCH_3).

3,2′,6′,3″-Tetrakis-*N*-(benzyloxycarbonyl)-1-*N*-(*tert*-butoxycarbonyl)sagamicin (**23**)

A solution of **22** (3.36 g) in a mixture of THF (40 ml), concd NH_4OH (20 ml), and MeOH (8 ml) was stirred overnight at room temp. The solution was evaporated and the resulting residue, dissolved in EtOAc (100 ml), was washed with aq NaCl, dried over Na_2SO_4, and evaporated to give a residue (2.96 g). To a solution of the residue in a mixture of THF (40 ml) and water (10 ml) was dropwise added ZCl (0.52 ml) at 2~5°C with stirring, keeping pH between 8 and 9 with Et_3N. The solution was stirred at the same temp for 1 hour and evaporated. The resulting residue, dissolved in EtOAc (100 ml), was washed with aq $NaHCO_3$ and aq NaCl, dried over Na_2SO_4, and evaporated to give **23** (3.83 g): MP 91~93°C; $[\alpha]_D^{20}$ +70.3° (*c* 1.0, $CHCl_3$); IR (Nujol) cm^{-1} 1675, 1500, 1250, 1150; ^1H NMR ($CDCl_3$) δ 2.82 (3H, s, NCH_3), 3.06 (3H, s, NCH_3).

3,2′,6′,3″-Tetrakis-*N*-(benzyloxycarbonyl)-1-*N*-palmitoylsagamicin (**24**)

A solution of **23** (1.44 g) in a mixture of anisole (3 ml) and TFA (12 ml) was stirred under ice-cooling for 1 hour. The reaction mixture was evaporated. A solution of the resulting residue in toluene (20 ml) was completely dried by evaporation with toluene. To a solution of the residue in a mixture of THF (40 ml) and water (10 ml) was added dropwise PalCl (0.38 g) at 0~5°C with stirring, keeping pH between 8 and 9 with Et_3N. The mixture was stirred at the same temp for 1 hour. The reaction mixture was evaporated. The residue, dissolved in EtOAc (60 ml), was washed successively with aq $NaHCO_3$, water, and aq NaCl, dried over Na_2SO_4, and evaporated. The resulting residue was chromatographed on silica gel (50 g) and eluted with a mixture of $CHCl_3$ - MeOH (40 : 1). The fractions containing the desired compound were combined and evaporated to give **24** (0.62 g, 38%): MP 124~126°C; $[\alpha]_D^{20}$ +87.5° (*c* 0.5, $CHCl_3$); IR (Nujol) cm^{-1} 1680, 1520, 1140; ^1H NMR ($CDCl_3$) δ 2.82 (3H, s, NCH_3), 3.06 (3H, s, NCH_3).

1,3,2′,6′-Tetrakis-*N*-(benzyloxycarbonyl)ribostamycin (**27**)

The compound (**27**) was synthesized from ribostamycin (**26**) as described[6].

1,3,2′,6′-Tetrakis-*N*-(benzyloxycarbonyl-3′,4′;2″,3″-bis-*O*-(cyclohexylidene)-5″-*O*-tritylribostamycin (**28**)

To a solution of **27** (1.0 g) in pyridine (20 ml) was added trityl chloride (1.4 g) and the mixture was stirred at 60°C for 5 hours. The reaction mixture was evaporated. The resulting residue was washed three times with hexane (20 ml) and the resulting precipitate was collected by filtration. The precipitate was air-dried to give a solid. To a solution of the solid (2.57 g) and *p*-toluenesulfonic acid monohydrate (0.04 g) in DMF (40 ml) was added 1,1-dimethoxycyclohexane (4.8 ml) at 50°C under reduced pressure (30 torr) for 4 hours. The reaction mixture was evaporated. The resulting residue, dissolved in EtOAc (80 ml), was washed with aq $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated. The resulting residue was chromatographed on silica gel (50 g) and eluted with a mixture of $CHCl_3$ - EtOAc (5 : 1). The fractions containing the desired compound were collected and evaporated to give **28** (0.58 g, 41%): IR (Nujol) cm^{-1} 1710~1700, 1530, 1250; ^1H NMR ($CDCl_3$) δ 1.42 (10H, m, CH_2), 1.60 (10H, m, CH_2), 5.10 (8H, s, $CH_2C_6H_5$), 5.28 (1H, d, J=3 Hz), 7.10~7.50 (35H, m, C_6H_5).

3,2′,6′-Tris-*N*-(benzyloxycarbonyl)-1-*N*;6-*O*-carbonyl-3′,4′;2″,3″-bis-*O*-(cyclohexylidene)-5″-*O*-tritylribostamycin (**29**)

To a solution of **28** (19.7 g) in DMF (200 ml) was added NaH (60% in oil) (2.4 g) and the mixture was stirred under nitrogen in an ice-bath for 3 hours. To the reaction mixture was added a solution of NH_4Cl (5.5 g) in water (1 liter) and the mixture was extracted twice with EtOAc (600 ml), which was washed with aq NaCl, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (500 g) and eluted with a mixture of $CHCl_3$ - MeOH (40 : 1). The fractions containing the desired compound were collected and evaporated to give **29** (13.4 g, 74%): IR (Nujol) cm^{-1} 1765, 1715, 1650, 1520, 1250, 1220; ^1H NMR ($CDCl_3$) δ 1.10~1.85 (20H, m), 5.05 (6H, s, CH_2), 7.10~7.50 (30H, m, C_6H_5).

3,2′,6′-Tris-*N*-(benzyloxycarbonyl)-3′,4′;2″,3″-bis-*O*-(cyclohexylidene)-1-*N*-palmitoyl-5″-*O*-tritylribostamycin (**30**)

To a solution of **29** (13.4 g) in a mixture of dioxane (210 ml) and water (70 ml) was added barium hydroxide octahydrate (4.1 g) and the mixture was stirred at 80°C under nitrogen for 4 hours. To the reaction mixture was added barium hydroxide octahydroxide (2 g) and the mixture was stirred at the same temp for 1 hour. The reaction mixture was neutralized with dry-ice and the resulting precipitate was filtered off and filtrate was evaporated. The residue was chromatographed on silica gel (350 g) and eluted with a mixture of $CHCl_3$ - MeOH - concd NH_4OH (10 : 1 : 0.1). The fractions containing the desired compound were collected and evaporated to give 1-*N*-unprotected intermediate (12.6 g). This compound was dissolved in a mixture of THF (200 ml) and water (40 ml). To the solution was added dropwise PalCl (3.0 g) keeping pH between 8 and 9 with Et_3N under ice-cooling with stirring and the mixture was stirred at the same temp for 1 hour. The reaction mixture was evaporated and the residue was dissolved in EtOAc (400 ml). The solution was washed successively with water and aq NaCl, dried over $MgSO_4$, and evaporated. The resulting residue was chromatographed on silica gel (500 g) and eluted with a mixture of $CHCl_3$ - MeOH (100 : 1). The fractions containing the desired compound were collected and evaporated to give **30** (7.3 g, 49%): IR (Nujol) cm^{-1} 1700, 1650, 1535, 1255; ^1H NMR ($CDCl_3$) δ 0.93 (3H, m, CH_3), 1.37 (26H, s, CH_2), 1.42 (10H, m), 1.60 (10H, m), 7.20~7.50 (30H, m, C_6H_5).

3,2′,6′-Tris-*N*-(benzyloxycarbonyl)-1-*N*-palmitoylribostamycin (**31**)

A solution of **30** (7.2 g) in a mixture of AcOH (120 ml) and water (30 ml) was stirred at 80°C for 10 hours. The reaction mixture was cooled and evaporated. The resulting residue was dissolved in a mixture of ethanol (100 ml) and water (20 ml), and evaporated. The resulting residue was three times washed with hexane (100 ml) and air-dried to give **31** (3.8 g, 71%): IR (Nujol) cm^{-1} 1700, 1690,

1640, 1540, 1290, 1230; ^{1}H NMR (DMSO-d_6) δ 0.89 (3H, m, CH_3), 1.22 (26H, s, CH_2), 5.00 (6H, m), 7.25~7.40 (15H, m, C_6H_5).

1,2′,6′-Tris-*N*-(benzyloxycarbonyl)fortimicin B (**34**)

34 was synthesized from fortimicin B (**33**) according to the method of TADANIER *et al.*[7].

1,2′,6′-Tris-*N*-(benzyloxycarbonyl)-4-*N*-palmitoylfortimicin B (**35**)

To a solution of **34** (0.52 g) in a mixture of THF (20 ml) and water (5 ml) was added PalCl (0.21 g) under ice-cooling, keeping pH between 8 and 9 with Et_3N. The mixture was stirred for 30 minutes under the same condition. The mixture was evaporated and dissolved in EtOAc (50 ml). The solution was washed with water and aq NaCl successively, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (20 g) and eluted with a mixture of $CHCl_3$ - MeOH (100 : 1). The fractions containing the desired compound were collected and evaporated to give **35** (0.48 g, 70%): IR (Nujol) cm^{-1} 1700, 1500; ^{1}H NMR ($CDCl_3$) δ 2.87 (3H, s, NCH_3), 3.33 (3H, s, OCH_3).

General Procedure for Hydrogenation of **7**, **8**, **9**, **14**, **20**, **24**, **31** and **35**

A suspension of **7**, **8**, **9**, **14**, **20**, **24**, **31** or **35** (0.42 mmol) in a mixture of MeOH (10 ml) and 1 N HCl (1.5 ml) was hydrogenated under H_2 atmospheric pressure in the presence of Pd-black or 10% Pd-C (0.5 g) at room temp for 5 hours. The catalyst was filtered off and washed with water. The filtrate and washings were combined and lyophilized to give **10**, **11**, **12**, **15**, **21**, **25**, **32** or **36** as a hygroscopic solid. These physical data and yields are summarized in Table 2.

2′-*N*-Palmitoylkasugamycin (**38**)

To a suspension of kasugamycin hemisulfate (**37**) (0.43 g) in a mixture of THF (20 ml) and water (20 ml) was added $NaHCO_3$ (0.09 g) and *N*-(palmitoyloxy)succinimide (0.39 g). The mixture was stirred at room temp overnight and then refluxed for 4 hours. The organic layer was removed from the reaction mixture and the resulting solution was adjusted to pH 2 with 1 N HCl. The insoluble material was filtered off and washed with water. The filtrate and washings were combined, adjusted to pH 6.8 with aq $NaHCO_3$, and lyophilized. The solid was dissolved in water (5 ml) and the aqueous solution was chromatographed on Sephadex G-15 (500 ml) and eluted with water. The fractions containing the desired compound were combined and lyophilized to give **38** as a hygroscopic solid (0.43 g). The physical data and yields are summarized in Table 2.

2-*N*-Palmitoyl-D-glucosamine (**40**)

To a solution of D-glucosamine monohydrochloride (**39**: 2.0 g) in water (40 ml) on ice-cooling, PalCl (3.06 g) was dropwise added with stirring, keeping pH 8~9 with Et_3N. The mixture was stirred at the same temp for 1 hour, and then at room temp for 2 hours. The resulting precipitate was collected by filtration, washed successively with 1 N HCl, Et_2O, and water, and air-dried to give **40** (2.66 g) (Table 2).

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