Notes

STUDIES ON ANTIVIRAL AGENTS

IV. SYNTHESIS AND *IN VITRO* ANTIVIRAL ACTIVITY OF NEW *N*-PALMITOYLKANAMYCIN A DERIVATIVES

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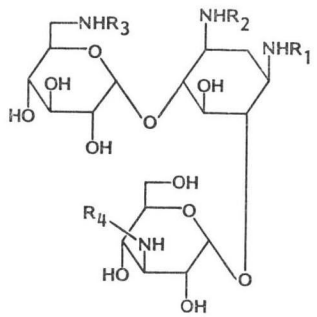
During the initial stages of this program[1~3], we discovered that 1-*N*-(higher-acyl)-3″-*N*-substituted kanamycin A derivatives exhibited the remarkable antiviral activity against not only herpes simplex virus type I Miyama (HSV-I) but also influenza virus A/PR8 (influenza virus). Among these analogs, 1-*N*-palmitoyl-3″-*N*-(trifluoroacetyl)kanamycin A dihydrochloride (**12**) was found to show both antiviral activity and low cytotoxicity. Also, 1-*N*-palmitoylkanamycin A trihydrochloride (**3**), which was unsubstituted at the *N*-3″ position, exhibited this excellent antiviral activity. However, the cytotoxicity on Vero cells of **3** was stronger than that of **12**. Based on these results, we have suggested that the introduction of higher-acyl group at the *N*-1 position of kanamycin A (**1**) was essential for emergence of the antiviral activity, and that the introduction of trifluoroacetyl group at the *N*-3″

Scheme 1. Synthesis of *N*-palmitoylkanamycin A and *N*-palmitoyl-*N*′-(trifluoroacetyl)kanamycin A derivatives.



No.	R_1	R_2	R_3	R_4	
1	H	H	H	H	(Kanamycin A)
2	H	Z	Z	$COCF_3$	
3	Pal	H	H	H	(3HCl)
4	Z	Z	Z	$COCF_3$	
5	H	H	H	Pal	(3HCl)
6	H	H	Z	H	
7	Boc	Boc	Z	Boc	
8	H	H	Pal	H	($3CF_3COOH$)
9	H	Boc	Z	H	
10	Z	Boc	Z	Z	
11	H	Pal	H	H	(3HCl)
12	Pal	H	H	$COCF_3$	(2HCl)
13	Boc	Z	Z	Pal	
14	$COCF_3$	H	H	Pal	(2HCl)
15	Z	Boc	Z	$COCF_3$	
16	H	Pal	H	$COCF_3$	(2HCl)

Z=$COOCH_2C_6H_5$
Boc=$COOC(CH_3)_3$
Pal=$CO(CH_2)_{14}CH_3$

Table 1. Physical data and yields of *N*-palmitoylkanamycin A (**3**, **5**, **8**, **11**) and *N*-palmitoyl-*N'*-(trifluoroacetyl)kanamycin A isomers (**12**, **14**, **16**).

No.	Yield (%)	MP (°C, dec)	$[\alpha]_D^{20}$ (°) (H_2O)	IR (Nujol) cm^{-1}	NMR		FD-MS (*m/z*)
					Solvent	δ (ppm)	
3	87	221	+71.1 (*c* 0.5)	1640~1630, 1540, 1510	D_2O	0.91 (3H, m), 1.26 (26H, br s)	723 (M^+)
5	55	183	+58.0 (*c* 1.0)	1620, 1510	CD_3OD	0.91 (3H, t, J=6 Hz), 1.26 (26H, br s)	723 (M^+)
8	44	193	+57.7 (*c* 1.22)	1670	D_2O	1.27 (26H, br s)	723 (M^+)
11	98	181	+62.9 (*c* 0.12)	1620	CD_3OD	0.80~1.10 (3H, m), 5.13 (1H, d, J=3 Hz), 5.37 (1H, d, J=3 Hz)	723 (M^+)
12	86	230	+59.9 (*c* 1.0)	1705, 1640~1630, 1550	CD_3OD	0.94 (3H, t, J=6 Hz), 5.12 (1H, d, J=3.5 Hz), 5.48 (1H, d, J=3 Hz)	819 (M^+)
14	94	193	—	1700, 1620, 1550	D_2O	0.87~1.07 (3H, m), 5.03 (1H, d, J=3 Hz), 5.50 (1H, d, J=3 Hz)	819 (M^+)
16	96	181	—	1710, 1620, 1550	CD_3OD	0.87~1.03 (3H, m), 5.10 (1H, d, J=3 Hz), 5.40 (1H, d, J=3 Hz)	819 (M^+)

—: Not measured.

position of 1-*N*-(higher-acyl)-kanamycin A derivatives was significant for reduction of the cytotoxicity. However, mode of the action of these antiviral agents is not yet clear.

In the following report on this series, we attempt to study the effect of the position substituted by acyl group(s) in **1** on antiviral activity and cytotoxicity. We here report the synthesis, antiviral activity, and cytotoxicity of four positional isomers (**3**, **5**, **8**, **11**) of *N*-palmitoylkanamycin A and three positional isomers (**12**, **14**, **16**) of *N*-palmitoyl-*N'*-(trifluoroacetyl)kanamycin A.

Materials and Methods

The synthesis of four positional isomers (**3**, **5**, **8**, **11**) of the *N*-palmitoylkanamycin A is summarized in Scheme 1.

3 was prepared as described in the previous paper[3].

The preparation of 3''-*N*-palmitoylkanamycin A trihydrochloride (**5**) was synthesized as follows. Reaction of 3,6'-bis-*N*-(benzyloxycarbonyl)-3''-*N*-(trifluoroacetyl)kanamycin A[4] (**2**) with benzyloxycarbonyl chloride (ZCl) gave 1,3,6'-tris-*N*-(benzyloxycarbonyl)-3''-*N*-(trifluoroacetyl)-kanamycin A (**4**; 89%): MP >260°C (dec); IR (Nujol) cm^{-1} 1680, 1645, 1520; NMR (DMSO-d_6) δ 7.28 (15H, s). Removal of the trifluoroacetyl group in **4** under alkaline condition, followed by acylation with palmitoyl chloride (PalCl) and subsequently hydrogenation in the presence of 10% palladium on carbon (Pd-C) under acidic condition, gave the desired **5** in good yield.

6'-*N*-Palmitoylkanamycin A tris(trifluoroacetate) (**8**) was synthesized as follows. Reaction of 6'-*N*-(benzyloxycarbonyl)kanamycin A[5,6] (**6**) with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) afforded the corresponding 6'-*N*-(benzyloxycarbonyl)-1,3,3''-tris-*N*-(*tert*-butoxycarbonyl)kanamycin A (**7**; 49%): IR (Nujol) cm^{-1} 1670, 1520; NMR (DMSO-d_6) δ 1.33 (27H, s), 7.33 (5H, s). Hydrogenation of **7** in the presence of Pd-C, followed by acylation with PalCl and subsequently deprotection of *tert*-butoxycarbonyl group with trifluoroacetic acid (TFA) and anisole gave **8** in good yield.

3-*N*-Palmitoylkanamycin A trihydrochloride (**11**) was prepared as follows. Regio-selective *tert*-butoxycarbonylation at the *N*-3 position of **6** with Boc-ON in the presence of $Zn(OAc)_2$ gave 6'-*N*-(benzyloxycarbonyl)-3-*N*-(*tert*-butoxycarbonyl)kanamycin A (**9**; 82%): NMR (DMSO-d_6) δ 1.33 (9H, s), 7.27 (5H, s). Acylation of **9** with *N*-(benzyloxycarbonyl)-5-norbornene-2,3-dicarboximide afforded 1,6',3''-tris-*N*-(benzyloxycar-

Table 2. *In vitro* antiviral activity of *N*-palmitoylkanamycin A and *N*-palmitoyl-*N'*-(trifluoroacetyl)kanamycin A derivatives.

Substance No.	Antiviral activity ID_{50} (μg/ml)		Cytotoxicity (μg/ml)	
	HSV-I (Miyama)	Influenza virus (A/PR8)	Vero cell	MDCK cell
3	2.8	32	10	>100
5	1.1	18	10	>100
8	1.5	—	32	—
11	1.9	22	32	>100
12	1.4	16	>100	>100
14	2.0	68	>100	>100
16	2.6	20	32	100
Virazole	32	10	>100	—
Amantadine	>100	10	—	—
Acyclovir	0.1	—	>10	—

Viral maximal cytopathic effect inhibition method.
Assay system: HSV-I/Vero cell, Influenza/MDCK cell.
—: Not measured.

bonyl)-3-*N*-(*tert*-butoxycarbonyl)kanamycin A (**10**; 96%): IR (Nujol) cm^{-1} 1680, 1520; NMR (DMSO-d_6) δ 1.37 (9H, s), 7.33 (15H, s). Treatment of **10** with TFA and anisole, followed by acylation with PalCl and finally hydrogenation under acidic condition, gave **11** in excellent yield.

Three positional isomers (**12, 14, 16**) of the *N*-palmitoyl-*N'*-(trifluoroacetyl)kanamycin A were synthesized as follows (Scheme 1).

1-*N*-Palmitoyl-3''-*N*-(trifluoroacetyl)kanamycin A dihydrochloride (**12**) was derived from **2**[1,2].

3''-*N*-Palmitoyl-1-*N*-(trifluoroacetyl)kanamycin A dihydrochloride (**14**) was prepared as follows. Reaction of **2** with Boc-ON, followed by treatment with ammonium hydroxide and subsequently acylation with PalCl, gave the desired 3,6'-bis-*N*-(benzyloxycarbonyl)-1-*N*-(*tert*-butoxycarbonyl)-3''-*N*-palmitoylkanamycin A (**13**; 76%): IR (Nujol) cm^{-1} 1670, 1510; NMR (DMSO-d_6) δ 7.33 (15H, s). Removal of the *tert*-butoxycarbonyl group in **13** with TFA and anisole, followed by acylation with ethyl trifluoroacetate and finally hydrogenation under acidic condition, afforded **14** in excellent yield.

3-*N*-Palmitoyl-3''-*N*-(trifluoroacetyl)kanamycin A dihydrochloride (**16**) was synthesized as follows. Reaction of **9** with ethyl trifluoroacetate, followed by treatment with ZCl, gave 1,6'-bis-*N*-(benzyloxycarbonyl)-3-*N*-(*tert*-butoxycabonyl)-3''-*N*-(trifluoroacetyl)kanamycin A (**15**; 83%): IR(Nujol) cm^{-1} 1700, 1680, 1520; NMR(DMSO-d_6) δ 1.37 (9H, s), 7.33 (10H, s). The desired compound (**16**) was prepared from **15** according to the similar synthetic procedure of **11** derived from **10**. The yields and spectral data of these derivatives (**3, 5, 8, 11, 12, 14, 16**) are summarized in Table 1.

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 supplemented with 5% fetal bovine serum. HSV-I (Miyama) and influenza virus (A/PR8) were respectively grown in Vero cells and Madin and Darby canine kidiney cells (MDCK cells). The test compounds were dissolved in H_2O*. The viral maximal cytopathic effect (CPE) was observed microscopically (X40). Antiviral activity was expressed as ID_{50} (50% inhibitory dose), that is, the concentration of the 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.

The cytotoxicity on Vero and MDCK cells was expressed as the minimum concentration of the compound which destroyed the cell monolayer.

Results and Discussion

The antiviral activity and cytotoxicity of the derivatives (**3, 5, 8, 11, 12, 14, 16**) against HSV-I

* The derivatives (**3, 5, 11, 16**) were very soluble in H_2O at around neutral pH. Other derivatives (**8, 12, 14**) were also very soluble in H_2O under acidic condition (pH 5.8), but were slightly soluble in H_2O (0.1 mg/ml) at around neutral pH.

and influenza virus are summarized in Table 2.

All positional isomers (**3, 5, 8, 11**) of the *N*-palmitoylkanamycin A showed almost the same excellent antiviral activity against HSV-I (ID_{50} 1.1~2.8 μg/ml) and influenza virus (ID_{50} 18~32 μg/ml). The antiviral activity of these isomers against HSV-I was about 15 times more than that of virazole, but was about 15 times less than that of acyclovir. The antiviral activity against influenza virus was about 2 times less than that of virazole and amantadine. The cytotoxicity on Vero and MDCK cells of these isomers (**3, 5, 8, 11**) was almost the same level. From these results, the antiviral activity and the cytotoxicity were little affected by the position substituted by the palmitoyl group in kanamycin A. However, the cytotoxicity on Vero cell of these four isomers was stronger than that of **12**.

Therefore, in the next step we attempted to study the antiviral activity and cytotoxicity of three positional isomers (**12, 14, 16**) of the *N*-palmitoyl-*N'*-(trifluoroacetyl)kanamycin A, because the cytotoxicity was found to be reduced by the introduction of trifluoroacetyl group into the *N*-palmitoylkanamycin A[3].

The positional isomers (**12, 14, 16**) of the *N*-palmitoyl-*N'*-(trifluoroacetyl)kanamycin A exhibited almost the same excellent antiviral activity against HSV-I (ID_{50} 1.4~2.6 μg/ml). The activity of these three isomers was about 15 times more than that of virazole, but was about 20 times less than that of acyclovir. The isomers (**12, 16**) also exhibited excellent antiviral activity against influenza virus (ID_{50} 16, 20 μg/ml), but the isomer (**14**) showed weaker activity against influenza virus than **12** and **16**. Particularly, **12** exhibited the strongest activity against not only HSV-I but also influenza virus. The cytotoxicity on Vero and MDCK cells of these isomers (**12, 14, 16**) was almost the same level.

Thus, the antiviral activity of the *N*-palmitoyl-*N'*-(trifluoroacetyl)kanamycin A derivatives was almost similar to that of *N*-palmitoylkanamycin A derivatives. Moreover, the cytotoxicity on Vero cell of these isomers (**12, 14, 16**) was reduced by the introduction of trifluoroacetyl group, as expected. Particularly, the cytotoxicity on Vero cell (100 μg/ml) of the isomers (**12, 14**) was the weakest among the derivatives described above. The cytotoxicity on MDCK cell of **3, 5, 8, 11, 12, 14** and **16** was also more than 100 μg/ml.

Based on these results, we found that the position substituted by the palmitoyl group in kanamycin A was not necessarily significant factor to reveal the excellent antiviral activity and that the introduction of the trifluoroacetyl group into *N*-(higher-acyl)-kanamycin A derivatives was required for the reduction of cytotoxicity on Vero cell.

In vivo antiviral activity of their derivatives will be reported by other researchers elsewhere[7]. Mechanism of the growth inhibition of virus is now under investigation.

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