# STUDIES ON ANTIVIRAL AGENTS

## II†. SYNTHESIS AND *IN VITRO* ANTIVIRAL ACTIVITY ON NEW KANAMYCIN A DERIVATIVES HAVING HIGHER ACYL GROUP AT *N*-1 POSITION

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(Received for publication March 6, 1985)

The synthesis and antiviral activity of 3″-*N*-trifluoroacetylkanamycin A derivatives (**6**) having higher acyl group at the *N*-1 position are described. On the basis of the structure-activity relationships between antiviral activity and alkyl chain length in an acyl group at the *N*-1 position, analogs (**6f**~**l**) having higher alkylcarbonyl group exhibited antiviral activity against not only HSV-I but also influenza virus. Analogs (**6q**~**v**) having higher alkyloxycarbonyl group showed antiviral activity against HSV-I. In addition, kanamycin A derivatives (**6n, o, y, z**) possessing higher alkylcarbonyl group with a functional group, higher alkylaminocarbonyl group, and higher alkylthiocarbonyl group had antiviral activity against HSV-I. The analog (**6h**) showed a broad antiviral spectrum against both DNA (HSV-I, HSV-II, VZV) and RNA (influenza) viruses.

CARRASCO[1] has recently proposed that one factor of viral infection was the modification of the plasma membrane leading to leakiness and increased permeability. He has also reported that the membrane of infected cells becomes modified during viral infection at the time in which the synthesis of late viral proteins occurs, and has suggested that it may be possible to design specific antiviral agents that interfere with macromolecular synthesis[2]. GHENDON and MIKHAILOVSKAYA[3] have reported that kanamycin A at a concentration of 8 mmol/liter had no effect on the protein synthesis in chick embryo cell, but caused a 2-fold decrease of virus-specific protein synthesis in this cell culture infected with fowl plaque virus, although the dose used here is considerably higher than the permissible single dose of kanamycin A for man.

Based on these reports, kanamycin A presumably might penetrate into the infected cell as a result of virus-induced impairment of cell membrane permeability and inhibit certain stages of the synthesis of virus protein.

These results led us to the idea that specific antiviral agents could be designed to make use of the modification of membranes caused by viral development. Namely, we presumed that the more lipophilic the kanamycin A molecule became, the more it might be capable of penetrating through the membrane of a virus infected cell. For this purpose, we introduced higher acyl group at the *N*-1 position of kanamycin A, because the hydrophilic property of kanamycin A could be altered into the lipophilic one to a certain extent by means of chemical modification.

We here report the synthesis of a new family of antiviral agents, 1-*N*-acyl-3″-*N*-(trifluoroacetyl)-

† Paper I. MATSUDA, K.; N. YASUDA, H. TSUTSUMI & T. TAKAYA: Studies on antiviral agents. I. Synthesis and *in vitro* antiviral activity of new kanamycin A derivatives. J. Antibiotics 38: 547~549, 1985

kanamycin A derivatives (**6**), and the effect of methylene chain length of the acyl group in **6** on antiviral activity against herpes simplex virus type I (HSV-I) and influenza virus. We have reported the effect of other acyl group such as higher alkyloxycarbonyl, higher alkylaminocarbonyl, or higher alkylthiocarbonyl group at the *N*-1 position in kanamycin A on antiviral activity.

## Chemistry

We synthesized 1-*N*-acyl-3″-*N*-(trifluoroacetyl)kanamycin A derivatives (**6**) as follows (Scheme 1).

Regioselective benzyloxycarbonylation at the *N*-3 and *N*-6′ positions of kanamycin A (**1**) with *N*-benzyloxycarbonyloxy-5-norbornene-2,3-dicarboximide, followed by regiospecific *N*-3″ protection with ethyl trifluoroacetate, gave 3,6′-bis-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**2**) by using the method of TSUCHIYA *et al.*[4]. Acylation of **2** with an acyl chloride or an activated ester gave the corresponding 1-*N*-acyl-3,6′-bis-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**4**) as a solid in high yield. Physical data and yields are summarized in Table 1. Hydrogenation of **4** with 10% palladium on carbon, followed by lyophilization afforded 1-*N*-acyl-3″-*N*-(trifluoroacetyl)-kanamycin A dihydrochloride (**6**) as a hygroscopic solid in good yield. Physical data and yields of **6** are shown in Table 2.

In an alternative method, regioselective protection of the amino group at the *N*-3, *N*-6′, and *N*-3″

Scheme 1. Synthesis of 1-*N*-acyl-3″-*N*-(trifluoroacetyl)kanamycin A derivatives (**6**).

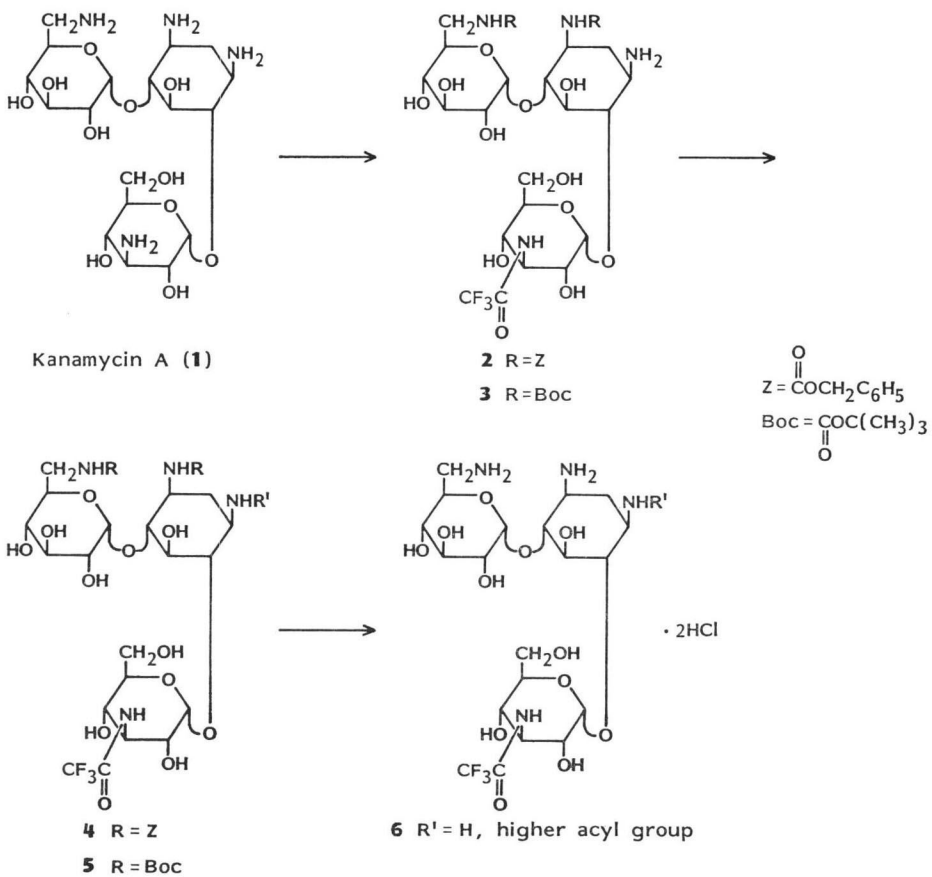

Table 1. Physical data and yields of protected 1-*N*-acyl-3″-*N*-(trifluoroacetyl)kanamycin A derivatives (**4**, **5**).

| No. | R′ | Method* | Yield (%) | MP (°C, dec) | IR (Nujol) $cm^{-1}$ | NMR (DMSO-$d_6$) $\delta$ (ppm) |
|---|---|---|---|---|---|---|
| **4a** | $CO(CH_2)_4CH_3$ | A | 78 | 298 | 1690, 1640, 1530 | 0.83 (3H, m) |
| **4b** | $CO(CH_2)_6CH_3$ | A | 81 | 297 | 1690, 1640, 1530 | 0.90 (3H, m), 1.23 (10H, br s) |
| **4c** | $CO(CH_2)_5CH_3$ | A | 82 | 285 | 1690, 1640, 1540 | 0.90 (3H, m), 1.26 (14H, br s) |
| **4d** | $CO(CH_2)_{10}CH_3$ | A | 83 | 278 | 1680, 1630, 1530 | |
| **4e** | $CO(CH_2)_{12}CH_3$ | A | 81 | 273 | 1690, 1640, 1540 | 0.90 (3H, m) |
| **4f** | $CO(CH_2)_{13}CH_3$ | B | 84 | 284 | 1690, 1640, 1530 | 0.93 (3H, m), 1.07 (24H, br s) |
| **4g** | $CO(CH_2)_{14}CH_3$ | A | 92 | 270 | 1690~1680, 1530 | |
| **4h** | $CO(CH_2)_{15}CH_3$ | B | 88 | 285 | 1680, 1630, 1530 | 0.93 (3H, m) |
| **4i** | $CO(CH_2)_{16}CH_3$ | A | 88 | 264 | 1680, 1630, 1530 | 0.93 (3H, m), 1.24 (30H, br s) |
| **4j** | $CO(CH_2)_{18}CH_3$ | B | 83 | 288 | 1680, 1630, 1530 | 0.93 (3H, m), 1.25 (34H, br s) |
| **4k** | $CO(CH_2)_{11}CH$ (Z) $CH_3(CH_2)_7CH$ | A | 94 | 251 | 1690, 1640, 1540 | 0.90 (3H, m) |
| **4l** | $COCH(CH_2)_7CH_3$ (*R*,*S*) $(CH_2)_5CH_3$ | B | 74 | 276 | 1690, 1640, 1530 | 1.20 (24H, br s) |
| **4m** | $COCH_2CH(CH_2)_{10}CH_3$ (*R*,*S*) $OCOCH_3$ | B | 90 | 188 | 1685, 1630, 1530 | 0.90 (3H, m), 1.92 (3H, s, $COCH_3$) |
| **4n** | $COCH_2CH(CH_2)_{10}CH_3$ (*R*,*S*) OH | B | 74 | 265 | 1690, 1630, 1540 | 0.88 (3H, m), 1.22 (20H, br s) |
| **4o** | $COO(CH_2)_{12}CH_3$ | C | 69 | 268 | 1680, 1530 | 0.90 (3H, m) |
| **4p** | $COO(CH_2)_{13}CH_3$ | C | 27 | 263 | 1700, 1685, 1535 | 0.90 (3H, m) |
| **4q** | $COO(CH_2)_{14}CH_3$ | C | 66 | 263 | 1700, 1540 | 0.95 (3H, m) |
| **4r** | $COO(CH_2)_{15}CH_3$ | C, D | 80 | 268 | 1700, 1680 | 0.92 (3H, m) |
| **4s** | $COO(CH_2)_{16}CH_3$ | C | 69 | 277 | 1700, 1690, 1530 | 0.91 (3H, m) |
| **4t** | $COO(CH_2)_{17}CH_3$ | D | 74 | 296 | 1690, 1545 | 0.92 (3H, m) |
| **4u** | $COO(CH_2)_{18}CH_3$ | C | 83 | 275 | 1690, 1530 | 0.90 (3H, m) |
| **4v** | $COOCH(CH_2)_{11}CH_3$ (*R*,*S*) $CH_3$ | C | 71 | 272 | 1700, 1530 | 0.93 (3H, m) |
| **4w** | $CONH(CH_2)_{14}CH_3$ | — | 63 | 236 | 1690, 1620, 1540 | 0.90 (3H, m) |
| **5a** | $CO(CH_2)_7CH=CH$ (Z) $CH_3(CH_2)_7$ | — | 61 | 219 | | 0.90 (3H, m) |
| **5b** | $COS(CH_2)_{15}CH_3$ | — | 47 | 226 | 1700, 1680, 1530 | 0.90 (3H, m) |

* Refer to experimental section.

positions of **1** with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile and ethyl trifluoroacetate gave 3,6′-bis-*N*-(*tert*-butoxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**3**). Acylation of **3** with an acyl chloride or an activated ester gave the desired 1-*N*-acyl-3,6′-bis-*N*-(*tert*-butoxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**5a**, **b**) as a solid in good yield (Table 1). Deprotection of **5a**,**b** with trifluoroacetic acid, followed by treatment with Amberlite IRA-400 ($Cl^-$ type) and lyophilization gave the corresponding 1-*N*-acyl-3″-*N*-(trifluoroacetyl)kanamycin A dihydrochloride (**6y**,**z**) as a hygroscopic solid in good yield (Table 2).

Table 2. Physical data and yields of 1-*N*-acyl-3″-*N*-(trifluoroacetyl)kanamycin A derivatives (6).

| No. | R′ | Yield (%) | MP (°C, dec) | $[\alpha]_D^{20}$ (°) ($H_2O$) | IR (Nujol) $cm^{-1}$ | NMR | | FD-MS (*m/z*) |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Solvent | $\delta$ (ppm) | |
| **6a** | H | 72 | 129 | +155.9 (*c* 1.0) | 1705, 1620, 1500[b] | $CD_3OD$ | 5.15 (1H, d, *J*=3.5 Hz), 5.55 (1H, d, *J*=3 Hz) | 581 ($M^+$) |
| **6b** | $CO(CH_2)_4CH_3$ | 100 | 133 | +79.4 (*c* 1.0) | 1710, 1640~1620, 1560 | $CD_3OD$ | 0.90 (3H, t, *J*=5 Hz), 5.12 (1H, d, *J*=3 Hz), 5.52 (1H, d, *J*=2.5 Hz) | 701 ($M^+$+Na), 679 ($M^+$) |
| **6c** | $CO(CH_2)_6CH_3$ | 98 | 193 | +66.6 (*c* 1.0) | 1705, 1625, 1560~1540 | $CD_3OD$ | 0.88 (3H, t, *J*=5 Hz), 5.12 (1H, d, *J*=3 Hz), 5.50 (1H, d, *J*=2 Hz) | 729 ($M^+$+Na) 707 ($M^+$) |
| **6d** | $CO(CH_2)_8CH_3$ | 94 | 210 | +75.2 (*c* 1.0) | 1700, 1620, 1560~1540 | $D_2O$ | 0.90 (3H, m), 5.22 (1H, d, *J*=3 Hz), 5.55 (1H, d, *J*=3 Hz) | 757 ($M^+$+Na), 735 ($M^+$) |
| **6e** | $CO(CH_2)_{10}CH_3$ | 88 | 183 | +57.0 (*c* 1.0) | 1700, 1640~1630, 1560~1540 | $D_2O$ | 0.75~0.95 (3H, m), 5.15 (1H, d, *J*=4 Hz), 5.52 (1H, d, *J*=2 Hz) | 763 ($M^+$) |
| **6f** | $CO(CH_2)_{12}CH_3$ | 86 | 194 | +57.4 (*c* 1.0) | 1700, 1640~1620, 1560~1540 | $D_2O$ | 0.80~1.00 (3H, m), 5.18 (1H, d, *J*=4 Hz), 5.56 (1H, br s) | 813 ($M^+$+Na), 791 ($M^+$) |
| **6g** | $CO(CH_2)_{13}CH_3$ | 87 | 176 | +59.8 (*c* 1.0) | 1700, 1630, 1560 | $CD_3OD$ | 5.13 (1H, d, *J*=4 Hz), 5.52 (1H, d, *J*=3 Hz) | 805 ($M^+$) |
| **6h** | $CO(CH_2)_{14}CH_3$ | 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 ($M^+$) |
| **6i** | $CO(CH_2)_{15}CH_3$ | 80 | 225 | +42.1 (*c* 1.0) | 1700, 1640~1620, 1560~1540 | $CD_3OD$ | 0.72 (3H, m), 5.06 (1H, d, *J*=4 Hz), 5.44 (1H, d, *J*=2 Hz) | 833 ($M^+$) |
| **6j** | $CO(CH_2)_{16}CH_3$ | 78 | 233 | +52.9 (*c* 2.0) | 1700, 1635, 1560~1540 | $D_2O$ | 0.70~1.03 (3H, m) | —[a] |
| **6k** | $CO(CH_2)_{18}CH_3$ | 83 | 223 | +55.8 (*c* 0.5) | 1700, 1630, 1550 | $CD_3OD$ | 0.75~1.05 (3H, m), 5.10 (1H, d, *J*=4 Hz), 5.47 (1H, d, *J*=2 Hz) | 875 ($M^+$) |
| **6l** | $CO(CH_2)_{20}CH_3$ | 71 | 223 | +39.3 (*c* 1.0) | 1700, 1630, 1550 | $CD_3OD$ | 0.92 (3H, m), 5.13 (1H, d, *J*=4 Hz) | — |
| **6m** | $COCH(CH_2)_7CH_3$ (*R*,*S*) $(CH_2)_5CH_3$ | 97 | 183 | +82.2 (*c* 1.0) | 1700, 1630 | $CD_3OD$ | 5.17 (1H, d, *J*=3.5 Hz) | 819 ($M^+$) |

Table 2. (continued)

| No. | R′ | Yield (%) | MP (°C, dec) | $[\alpha]_D^{20}$ (°) ($H_2O$) | IR (Nujol) $cm^{-1}$ | NMR | | FD-MS (m/z) |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Solvent | δ (ppm) | |
| **6n** | $COCH_2CH(CH_2)_{10}CH_3$ (R,S)\| $CH_3COO$ | 80 | 163 | +59.4 (c 1.0) | 1700, 1640, 1560 | $CD_3OD$ | 2.03 (3H, s, $CH_3CO$) | — |
| **6o** | $COCH_2CH(CH_2)_{10}CH_3$ (R,S)\| OH | 85 | 116 | +63.8 (c 1.0) | 1700, 1630, 1560 | $CD_3OD$ | 5.13 (1H, d, J=3 Hz), 5.25 (1H, d, J=3 Hz) | 807 ($M^+$) |
| **6p** | $CO(CH_2)_7CH$(Z)$=CH$ \| $CH_3(CH_2)_7$ | 53 | 169 | +53.2 (c 1.0) | 1700, 1630, 1560～1540 | $CD_3OD$ | 0.91 (3H, m) | 855 ($M^+$) |
| **6q** | $COO(CH_2)_{12}CH_3$ | 77 | 132 | +43.8 (c 1.0) | 1700, 1540 | $CD_3OD$ | 0.95 (3H, m) | 808 ($M^+$+1) |
| **6r** | $COO(CH_2)_{13}CH_3$ | 100 | 153 | +53.7 (c 1.0) | 1700, 1560 | $CD_3OD$ | 0.95 (3H, m), 5.18 (1H, d, J=3 Hz) | — |
| **6s** | $COO(CH_2)_{14}CH_3$ | 36 | 179 | +56.5 (c 1.0) | 1700, 1540 | $CD_3OD$ | 0.95 (3H, m), 5.10 (1H, d, J=3 Hz) | — |
| **6t** | $COO(CH_2)_{15}CH_3$ | 86 | 232 | +55.3 (c 1.0) | 1700, 1560～1540, 1510 | $CD_3OD$ | 0.95 (3H, m), 5.12 (1H, d, J=3 Hz), 5.48 (1H, d, J=3 Hz) | — |
| **6u** | $COO(CH_2)_{16}CH_3$ | 64 | 159 | +55.6 (c 1.0) | 1700, 1550 | $CD_3OD$ | 0.95 (3H, m), 5.12 (1H, d, J=4 Hz), 5.48 (1H, d, J=3 Hz) | — |
| **6v** | $COO(CH_2)_{17}CH_3$ | 76 | 219 | +54.4 (c 1.0) | 1700, 1540 | $CD_3OD$ | 0.95 (3H, m), 5.15 (1H, d, J=3 Hz) | 898 ($M^+$+Na), 876 ($M^+$−1) |
| **6w** | $COO(CH_2)_{18}CH_3$ | 26 | 96 | —[a] | 1700 | $CD_3OD$ | 0.96 (3H, m) | — |
| **6x** | $COOCH(CH_2)_{11}CH_3$ (R,S)\| $CH_3$ | 91 | 133 | — | 1700 | $CD_3OD$ | 0.95 (3H, m), 5.15 (1H, d, J=3 Hz) | — |
| **6y** | $CONH(CH_2)_{14}CH_3$ | 91 | 135 | — | 1705, 1570 | $CD_3OD$ | 0.93 (3H, m), 5.12 (1H, d, J=3 Hz), 5.47 (1H, d, J=3 Hz) | — |
| **6z** | $COS(CH_2)_{15}CH_3$ | 74 | 213 | — | 1705, 1635, 1520 | $CD_3OD$ | 0.95 (3H, m), 5.13 (1H, d, J=3 Hz), 5.48 (1H, d, J=3 Hz) | 864 ($M^+$−1) |

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

## Biological Activity and Results

### Effect of Methylene Chain Length in Alkylcarbonyl Group

The antiviral activity of analogs (**6a**~**l**) against HSV-I and influenza virus is summarized in Table 3.

### Antiviral Activity against HSV-I

The 1-*N*-unsubstituted analog (**6a**), 1-*N*-hexanoyl (**6b**) and 1-*N*-octanoyl analogs (**6c**) exhibited no antiviral activity against HSV-I. However, 1-*N*-decanoyl analog (**6d**) was slightly active against HSV-I. The analogs (**6e**~**h**) having lauroyl, myristoyl, pentadecanoyl, or palmitoyl group at the *N*-1 position showed excellent antiviral activity against HSV-I. Particularly, analogs (**6g, h**) showed the strongest *in vitro* antiviral activity and were 15 times more active than virazole[5], although they were 20 times less active than acyclovir[6]. The analogs (**6i**~**l**) having hexadecanoyl, stearoyl, eicosanoyl, or docosanoyl group also exhibited antiviral activity against HSV-I, and were 7 times more active than virazole.

The cytotoxicity in Vero cell of their analogs (**6a**~**l**) was more than 100 $\mu$g/ml.

### Antiviral Activity against Influenza Virus

The analogs (**6b**~**d**) exhibited no antiviral activity against influenza virus. However, the 1-*N*-

Table 3. Antiviral activity of 1-*N*-alkylcarbonyl-3″-*N*-(trifluoroacetyl)kanamycin A derivatives (**6a**~**p**).

| No. | R′ | Antiviral activity $ID_{50}$ ($\mu$g/ml) | | Cytotoxicity vs. Vero cell ($\mu$g/ml) |
|---|---|---|---|---|
| | | HSV-I | Influenza virus | |
| **6a** | H | >100 | —[a] | >100 |
| **6b** | $CO(CH_2)_4CH_3$ | >100 | >100 | >100 |
| **6c** | $CO(CH_2)_6CH_3$ | >100 | >100 | >100 |
| **6d** | $CO(CH_2)_8CH_3$ | 57 | >100 | >100 |
| **6e** | $CO(CH_2)_{10}CH_3$ | 3.2 | 40 | >100 |
| **6f** | $CO(CH_2)_{12}CH_3$ | 1.6 | 10 | >100 |
| **6g** | $CO(CH_2)_{13}CH_3$ | 1.4 | 5.6 | >100 |
| **6h** | $CO(CH_2)_{14}CH_3$ | 1.4 | 16 | >100 |
| **6i** | $CO(CH_2)_{15}CH_3$ | 2.5 | — | >100 |
| **6j** | $CO(CH_2)_{16}CH_3$ | 4.6 | — | >100 |
| **6k** | $CO(CH_2)_{18}CH_3$ | 2.5 | 32 | >100 |
| **6l** | $CO(CH_2)_{20}CH_3$ | 5.2 | 42 | >100 |
| **6m** | $COCH(CH_2)_7CH_3$ (*R*,*S*) $(CH_2)_5CH_3$ | 32 | — | >100 |
| **6n** | $COCH_2CH(CH_2)_{10}CH_3$ (*R*,*S*) $CH_3COO$ | 5.5 | — | 100 |
| **6o** | $COCH_2CH(CH_2)_{10}CH_3$ (*R*,*S*) OH | 7.9 | — | >100 |
| **6p** | $CO(CH_2)_7CH{=}CH$ (Z) $CH_3(CH_2)_7$ | 1.6 | — | 32 |
| Acyclovir | | 0.032 | >100 | >10 |
| Virazole | | 32 | 10 | >100 |
| Amantadine | | >100 | 10 | — |

[a] Not measured.

lauroyl analog (**6e**) was slightly active against influenza virus. The analogs (**6f**~**h**) showed excellent antiviral activity against influenza virus similar to that of virazole and amantadine[7]. The 1-*N*-eicosanoyl (**6k**) and 1-*N*-docosanoyl analogs (**6l**) also exhibited antiviral activity against influenza virus. On the other hand, acyclovir had no activity against influenza virus.

Effect of Introducing Functional Group into Alkylcarbonyl Group

The antiviral activity of the analogs (**6m**~**p**) against HSV-I is summarized in Table 3.

The 1-*N*-(2-hexyldecanoyl) analog (**6m**) showed weak antiviral activity against HSV-I compared with **6h** having the same carbon number in the alkylcarbonyl group. However, the 1-*N*-((*Z*)-9-octadecenoyl) analog (**6p**) exhibited excellent antiviral activity against HSV-I. The activity was similar to that of **6h** and weaker than that of **6j** having the same carbon number in the alkylcarbonyl group. The analogs (**6n, o**) having 3-hydroxytetradecanoyl or 3-acetoxytetradecanoyl group exhibited remarkable antiviral activity against HSV-I and were about 6 times less than 1-*N*-myristoyl analog (**6f**) having the same chain length in the alkylcarbonyl group.

Effect of Methylene Chain Length in Alkyloxycarbonyl Group

In Table 4 is summarized the antiviral activity of **6q**~**x** against HSV-I and influenza virus.

Antiviral Activity against HSV-I

The analogs (**6q**~**s**) having dodecanyloxycarbonyl, tetradecanyloxycarbonyl, or pentadecanyloxycarbonyl group exhibited antiviral activity against HSV-I. The 1-*N*-hexadecanyloxycarbonyl (**6t**), 1-*N*-heptadecanyloxycarbonyl (**6u**), or 1-*N*-octadecanyloxycarbonyl analogs (**6v**) showed excellent antiviral activity and their activities were similar to that of the 1-*N*-palmitoyl analog (**6h**). The activity of **6t**~**v** was 15 times more active than that of virazole. The analogs (**6w, x**) having nonadecanyloxycarbonyl or 1-methyltridecanyloxycarbonyl group showed weaker activity than **6h**, but their activities were about 7 times more than that of virazole.

Antiviral Activity against Influenza Virus

The analogs (**6q**~**x**) exhibited weak antiviral activity against influenza virus. However, the 1-*N*-hexadecanyloxycarbonyl (**6t**) and 1-*N*-octadecanyloxycarbonyl analogs (**6v**) showed higher antiviral activity against influenza virus and were as active as **6h**.

Table 4. Antiviral activity of 1-*N*-alkyloxycarbonyl and 1-*N*-substituted-3″-*N*-(trifluoroacetyl)kanamycin A derivatives (**6q**~**z**).

| No. | R′ | Antiviral activity $ID_{50}$ (μg/ml) | | Cytotoxicity vs. Vero cell (μg/ml) |
|---|---|---|---|---|
| | | HSV-I | Influenza virus | |
| **6q** | $COO(CH_2)_{12}CH_3$ | 4.0 | 10 | 100 |
| **6r** | $COO(CH_2)_{13}CH_3$ | 6.1 | 100 | >100 |
| **6s** | $COO(CH_2)_{14}CH_3$ | 6.4 | 32 | 100 |
| **6t** | $COO(CH_2)_{15}CH_3$ | 1.0 | 22 | >100 |
| **6u** | $COO(CH_2)_{16}CH_3$ | 2.6 | 100 | >100 |
| **6v** | $COO(CH_2)_{17}CH_3$ | 2.2 | 32 | >100 |
| **6w** | $COO(CH_2)_{18}CH_3$ | 5.6 | 100 | >100 |
| **6x** | $COOCH(CH_2)_{11}CH_3$ (*R,S*) $\vert$ $CH_3$ | 5.7 | 32 | 100 |
| **6y** | $CONH(CH_2)_{14}CH_3$ | 3.2 | 72 | 32 |
| **6z** | $COS(CH_2)_{15}CH_3$ | 3.2 | 100 | 100 |

Effect of Other Substituents at the *N*-1 Position of 3″-*N*-(Trifluoroacetyl)kanamycin A

In Table 4 is summarized the antiviral activity of **6t, y, z** against HSV-I and influenza virus.

The 1-*N*-hexadecanyloxycarbonyl (**6t**), 1-*N*-pentadecanylaminocarbonyl (**6y**), and 1-*N*-hexadecanyloxythiocarbonyl analogs (**6z**) showed excellent antiviral activity against HSV-1 and were similarly active to the analogs (**6h, i**) having the same methylene chain length in the alkylcarbonyl group. However, the analog (**6y**) exhibited weaker antiviral activity against influenza virus in comparison with **6t**, and the analog (**6z**) showed no antiviral activity against influenza virus. The cytotoxicity of **6y** and **6z** was 32 $\mu$g/ml and 100 $\mu$g/ml, respectively.

Thus, we newly found these 1-*N*-higher acylkanamycin A derivatives (**6**) exhibited the antiviral activity against not only HSV-I but also influenza virus. However, the derivatives (**6**) described in this paper completely lacked the antibacterial activity against typical Gram-positive bacteria strains such as *Staphylococcus aureus* 209P JC-1 and Gram-negative bacteria strains such as *Escherichia coli* JC-2, *Klebsiella pneumoniae*, and *Proteus mirabilis*. Further, the antiviral activity of the methylene homologs tends to enhance by increasing the number of the methylene chain of higher acyl group, and reaches a plateau level when the number of the methylene chain exceeds 10 for 1-*N*-(alkylcarbonyl) analogs. Among their analogs, 1-*N*-pentadecanoyl (**6g**), 1-*N*-palmitoyl (**6h**), and 1-*N*-hexadecanyloxycarbonyl analogs (**6t**) exhibited the strongest antiviral activity against not only HSV-I but also influenza virus. The analog (**6h**) showed the broad antiviral spectrum against both DNA virus (HSV-I, herpes simplex virus type II (HSV-II), acyclovir-resistant mutants of HSV, varicella-zoster virus (VZV)) and RNA virus (influenza virus, hemagglutinating virus of Japan (HVJ), vesicular stomatitis virus (VSV)) (Table 5). Further, the analogs (**6h, k**) exhibited the antiviral activity independently of the timing and term of treatment with them[3] and were not activated by a virus-specified pyrimidine deoxynucleoside (thymidine) kinase[3].

From these results, we assume that the antiviral activity cannot be explained solely by the CARRASCO's suggestion[1,2]. Therefore, we suppose that the analogs have direct inactivation and inhibit viral-specific protein synthesis at certain stages of replication of virus. However, a more detailed study of the mechanism of the action are now under investigation by another researchers in

Table 5. *In vitro* antiviral spectrum of 1-*N*-palmitoyl-3″-*N*-(trifluoroacetyl)kanamycin A (**6h**).

| | Virus | Antiviral activity $ID_{50}$ ($\mu$g/ml) | | |
|---|---|---|---|---|
| | | **6h** | Acyclovir | Amantadine |
| DNA | HSV-I (Miyama)[a] | 1.3 | 0.045 | >100 |
| | HSV-I (Miyama)* ACV-Resistant[a] | 1.2 | 15 | — |
| | HSV-II (UW-268)[a] | 1.6 | 0.015 | — |
| | HSV-II (YS-4) ACV-Resistant[a] | 2.8 | 4.0 | — |
| | VZV (Kawaguchi)[b] | — | 0.45 | — |
| | Vaccinia[a] | 57 | >1.0 | — |
| RNA | Influenza A/PR8[a] | 10 | — | 20 |
| | HVJ[a] | 47 | — | — |
| | Rhino Ia[a] | 56 | — | — |
| | VSV[a] | 32 | — | — |

[a] Viral maximal cytopathic effect inhibition, [b] plaque reduction.
Assay system: HSV, HVJ, VSV/Vero cell, VZV/HEL cell, Vaccinia/RK13 cell, Influenza/MDCK cell, Rhino/FS7 cell.
* Mutant strain.

this laboratory and will be reported elsewhere.

## 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

HSV-I Miyama and influenza virus A/PR8 were respectively grown in Vero cells and MDCK cells. The test compounds were dissolved in water.

The culture medium was changed to 0.5% fetal bovin serum MEM. The cell cultures were inoculated with about 100 $TCID_{50}$ (the virus dose needs to infect 50% of cells) 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% trichloroacetic acid and stained with 0.1% crystal violet. The viral maximal cytopathic effect was observed microscopically (×40). Antiviral activity was expressed as $ID_{50}$ (50% inhibitory dose), that is, the concentration of compound required to reduce viral maximal cytopathic effect by 50% (within the well), when it had reached completion (100% cell destruction) in the control virus-infected cell cultures.

Cytotoxicity was expressed as minimum cytotoxic dose of the test compound in virus-uninfected Vero cell cultures.

### Materials

3,6′-Bis-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**2**): The compound **2** was synthesized according to a method of TSUCHIYA *et al.*[4].

3,6′-Bis-*N*-(*tert*-butoxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**3**): To a solution of kanamycin A (**1**: 20 g) and $Zn(OAc)_2 \cdot 2H_2O$ (27 g) in DMSO (200 ml) was added a mixture of 2-(*tert*-butoxycarbonyloximino)-2-phenylacetonitrile (18.9 g) and triethylamine (8 ml) at room temperature and the solution was stirred at the same temperature for 1 hour. The reaction mixture was poured into diethyl ether (2 liters) to give a syrup. The syrup was subjected on a column of cation exchange resin, Amberlite IRC-50 ($H^+$ form, 1 liter) and eluted a mixture of dioxane - water (1 : 1). The fractions were concentrated to give a solid (17.2 g, 82%); IR (Nujol) 1680 $cm^{-1}$; NMR (DMSO-$d_6$) $\delta$ 1.43 (18H, s, $CH_3$).

Ethyl trifluoroacetate (0.31 g) was added to a solution of the solid (1 g) obtained above in DMSO (10 ml) and the mixture was stirred for 1 hour at room temperature. The reaction mixture was poured into diethyl ether (200 ml), triturated, collected by filtration, and dried to give a crude solid (**3**: 1.9 g); mp 215~217°C; IR (Nujol) 1710, 1680 $cm^{-1}$; NMR (DMSO-$d_6$) $\delta$ 1.40 (18H, s, $CH_3$).

### General Procedure for Acylation of **2**

Method A; Acylation with Acyl Chloride: To a solution of **2** (1.77 mmol) in a mixture of THF (40 ml) and water (10 ml) was dropwise added an acyl chloride (1.86 mmol) under ice-cooling, keeping the pH 8~9 with triethylamine and the mixture was stirred for 1 hour at the same condition. Water (30 ml) was added to the reaction mixture. The precipitates were collected by filtration, washed with water and diethyl ether, and dried to give 1-*N*-(alkylcarbonyl)-3,6′-bis-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**4a~e, g, i, k**) as a solid in good yield.

Method B; Acylation with Carboxylic Acid: Phosphorus oxychloride (2.53 mmol) was added to a mixture of DMF (2.53 mmol) and THF (0.4 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 (1.95 mmol) in dry THF (4 ml) at the same temperature under stirring for 30 minutes to prepare an activated acid solution. To a solution of **2** (1.77 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 triethylamine 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 (20 ml), and a mixture of isopropyl alcohol (30 ml) and diethyl ether (30 ml), and finally dried to give 1-*N*-(alkylcarbonyl)-3,6′-bis-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**4f, j, l, m, n**) as a solid in high yield.

Method C; Acylation with Alkyl *p*-Nitrophenyl Carbonate: To a solution of a higher alcohol (4.12 mmol) in dry dichloromethane (20 ml) were successively added pyridine (0.67 ml) and a solution of *p*-nitrophenyl chloroformate (4.12 mmol) in dry dichloromethane (2 ml) under ice-cooling and the mixture was stirred for 30 minutes at the same condition. The solution was washed with 0.5 N HCl and aqueous NaCl, dried over sodium sulfate, and concentrated under reduced pressure to give alkyl *p*-nitrophenyl carbonate (IR (Nujol) 1760, 1520, 1350 $cm^{-1}$) as a solid in high yield.

To a solution of **2** (1.18 mmol) and alkyl *p*-nitrophenylcarbonate (1.30 mmol) obtained above in DMF (20 ml) were added triethylamine (1.30 mmol) under stirring at room temperature for 2 days. The reaction mixture was poured into water (200 ml) to give the precipitates. The precipitates were collected by filtration, washed with water and diethyl ether, and dried to give 1-*N*-(alkyloxycarbonyl)-3,6′-bis-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**4o~s, u, v**) as a solid in good yield.

Method D; Acylation with Alkyloxycarbonyl Chloride: To a solution of **2** (1.18 mmol) in a mixture of THF (40 ml) and water (10 ml) was dropwise added alkyloxycarbonyl chloride (1.30 mmol), keeping the pH 8~9 with triethylamine under ice-cooling and the solution was stirred for 1 hour at the same condition. The reaction mixture was concentrated under reduced pressure to give a solid. The solid were collected by filtration, washed with water and diethyl ether, and dried to give 1-*N*-(alkyloxycarbonyl)-3,6′-bis-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**4r, t**) as a solid. Their physical data and yields are shown in Table 1.

3,6′-Bis-*N*-(benzyloxycarbonyl)-1-*N*-(pentadecanylaminocarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**4w**)

To a solution of palmitoyl chloride (5 g) in dry THF (40 ml) was dropwise added a solution of sodium azide (1.2 g) in water (10 ml) at −20~0°C and the mixture was stirred at 0°C for 2 hours. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in ethyl acetate (100 ml). The solution was washed with water (50 ml), dried over magnesium sulfate, and concentrated under reduced pressure to give a crude palmitoyl azide as a solid; IR (Nujol) 2140, 1725 $cm^{-1}$. A solution of the palmitoyl azide obtained above in ethyl acetate (40 ml) was refluxed for 2 hours. The reaction mixture was concentrated *in vacuo* to give pentadecanyl isocyanate as a solid (4.9 g, 100%); IR (Nujol) 2270 $cm^{-1}$.

To a solution of **2** (1.0 g) in DMF (20 ml) was added pentadecanyl isocyanate (0.45 g) under stirring at room temperature and the mixture was stirred at the same temperature overnight. Further, the solution was stirred at 50°C for 4 hours. The reaction mixture was poured into ice-water (200 ml) to give the precipitates. The precipitates were collected by filtration, washed with water and diethyl ether, and dried to give **4w** (0.82 g), (Table 1).

3,6′-Bis-*N*-(*tert*-butoxycarbonyl)-1-*N*-[(*Z*)-9-octadecenoyl]-3″-*N*-(trifluoroacetyl)kanamycin A (**5a**)

To a solution of **3** (3.92 g) in a mixture of THF (40 ml) and water (10 ml) was dropwise added a solution of (*Z*)-9-octadecenoyl chloride (0.34 g) in THF (2 ml) under ice-cooling, keeping the pH 8~9 with triethylamine and the mixture was stirred for 1 hour at the same condition. The reaction mixture was concentrated *in vacuo* to give a solid. The solid was washed successively with 1 N HCl (20 ml), diethyl ether (20 ml), and water (100 ml), and dried to give **5a** (0.65 g) as a solid, (Table 1).

3,6′-Bis-*N*-(*tert*-butoxycarbonyl)-1-*N*-(hexadecanylthiocarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**5b**)

To a solution of hexadecanethiol (3 g) in dry dichloromethane (50 ml) were successively added pyridine (1.9 ml) and a solution of *p*-nitrophenyl chloroformate (2.57 g) in dry dichloromethane (5 ml)

under ice-cooling and the mixture was stirred for 30 minutes at the same condition. The solution was washed with 0.5 N HCl and aqueous NaCl, dried over sodium sulfate, and concentrated under reduced pressure to give *S*-hexadecanyl *p*-nitrophenyl thiocarbonate as a solid (5.0 g, 100%); IR (Nujol) 1705, 1520, 1350 $cm^{-1}$.

To a solution of **3** (1 g) in a mixture of DMSO (20 ml) and DMF (20 ml) were added *S*-hexadecanyl *p*-nitrophenyl thiocarbonate (1.09 g) and triethylamine (0.4 ml) and the mixture was allowed to stand at room temperature for 12 hours. The reaction mixture was poured into ice-water (250 ml) and the resultant precipitates were collected by filtration, washed with water and diethyl ether, and dried to give **5b** (0.64 g) as a solid, (Table 1).

General Procedure for Deprotection of 1-*N*-Acyl-3,6′-bis-*N*-(benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**4a**~**w**)

A solution of the derivatives (**4a**~**w**: 0.90 mmol) in a mixture of MeOH (20 ml) and 10% HCl (0.5 ml) was hydrogenated under atmospheric pressure of hydrogen over 10% palladium on carbon (1.0 g) at room temperature for 6 hours. The catalyst was filtered off and the filtrate was concentrated *in vacuo* and dissolved in water (30 ml). The solution was lyophilized to give 1-*N*-acyl-3″-*N*-(trifluoroacetyl)kanamycin A dihydrochloride (**6a**~**o**, **q**~**y**) as a hygroscopic solid, (Table 2).

General Procedure for Deprotection of 1-*N*-Acyl-3,6′-bis-*N*-(*tert*-butoxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A (**5a, b**)

A solution of the derivatives (**5a, b**: 0.56 mmol) in a mixture of TFA (10 ml) and anisole (2 ml) was stirred under ice-cooling for 1 hour. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in water (40 ml). The solution was washed twice with ethyl acetate (10 ml). The aqueous layer was subjected to a column of anion exchange resin, Amberlite IRA-400 ($Cl^-$ type, 30 ml) and eluted with water. The eluate was lyophilized to give 1-*N*-acyl-3″-*N*-(trifluoroacetyl)-kanamycin A dihydrochloride (**6p**, **z**) as a hygroscopic solid. Their physical data and yields are shown in Table 2.

Acknowledgment

We are grateful to Drs. Y. MINE and Y. WATANABE for providing the biological data and their suggestion.

## References

1) CARRASCO, L.: Membrane leakiness after viral infection and a new approach to the development of antiviral agents. Nature 272: 694~699, 1978
2) CONTRERAS, A. & L. CARRASCO: Selective inhibition of protein synthesis in virus-infected mammalian cells. J. Virol. 29: 114~122, 1979
3) GHENDON, Y. Z. & T. A. MIKHAILOVSKAYA: Effect of kanamycin on the reproduction of orthomyxoviruses. Acta Virol. 26: 241~246, 1982
4) TSUCHIYA, T.; Y. TAKAGI & S. UMEZAWA: 1-*N*-Acylation of aminocyclitol antibiotics *via* zinc chelation and regiospecific *N*-trifluoroacetylation. Tetrahedron Lett. 1975: 4951~4954, 1975
5) SIDWELL, R. W.; J. H. HUFFMAN, G. P. KHARE, L. B. ALLEN, J. T. WITKOWSKI & R. K. ROBINS: Broad spectrum antiviral activity of virazole: 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide. Science 177: 705~706, 1972
6) ELION, G. B.; P. A. FURMAN, J. A. FYFE, P. MIRANDA, L. BEAUCHAMP & H. J. SCHAEFFER: Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. U.S.A. 74: 5716~5720, 1977
7) DAVIES, W. L.; R. R. GRUNERT, R. F. HAFF, J. W. MCGAHEN, E. M. NEUMAYER, M. PAULSHOCK, J. C. WATTS, R. T. WOOD, E. C. HERMANN & C. E. HOFFMANN: Antiviral activity of 1-adamantanamine (Amantadine). Science 144: 862~863, 1964
8) WATANABE, Y.; Y. MINE, Y. YOKOTA, H. KIKUCHI, S. GOTO & S. KUWAHARA: FR55060, a new antiviral agent. II. *In vitro* and *in vivo* antiviral activities. Program and Abstracts of the 24th Intersci. Conf. Antimicrob. Agents Chemother., No. 1,091, Washington, D.C., 1984