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, 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.

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-(benzyloxyacarbonyl)-3''-N-(trifluoroacetyl)kanamycin B (4), -tobramycin (5), -dibekacin (6), and -gentamicin C (C1a, C1b and C2 complex) (19) were prepared according to the method of Tsuchiya et al. Acylation of 4, 5, 6 and 19 with palmitoyl chloride (PalCl) gave the corresponding 3,2',6'-tris-N-(benzyloxyacarbonyl)-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-N-Palmitoyldibekacin tetrahydrochloride (15), -sagamicin tetrahydrochloride (25), and -ribostamycin tetrahydrochloride (32) were synthesized as follows. Protection of 3,2',6'-tris-N-(benzyloxyacarbonyl)-3''-N-(trifluoroacetyl)dibekacin (6) with 2-(tert-butoxycarbonyloxyimino)-2-phenylacetanitrile (Boc-ON), followed by treatment with concentrated NH4OH 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) \( X_1 = X_2 = \text{OH} \)
Tobramycin (2) \( X_1 = \text{H}, X_2 = \text{OH} \)
Dibekacin (3) \( X_1 = X_2 = \text{H} \)

4 \( R' = \text{H}, X_1 = X_2 = \text{OH} \)  
5 \( R' = \text{H}, X_1 = \text{H}, X_2 = \text{OH} \)  
6 \( R' = \text{H}, X_1 = X_2 = \text{H} \)  
7 \( R' = \text{Pal}, X_1 = X_2 = \text{OH} \)  
8 \( R' = \text{Pal}, X_1 = \text{H}, X_2 = \text{OH} \)  
9 \( R' = \text{Pal}, X_1 = X_2 = \text{H} \)  
10 \( X_1 = X_2 = \text{OH} \)  
11 \( X_1 = \text{H}, X_2 = \text{OH} \)  
12 \( X_1 = X_2 = \text{H} \)

unprotected at the N-3" position. Reaction of the above intermediate with benzoylcarbonyl chloride (ZCl), followed by removal of tert-butoxycarbonyl group with trifluoroacetic acid (TFA) and anisole, afforded the corresponding 3,2',6',3"-tetrakis-N-(benzoylcarbonyl)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',2'-bis-N-(benzoylcarbonyl)-3"-N-(trifluoroacetyl)sagamicin (18) as described above for 15. To prepare 1-N-palmitoylribostamycin tetrahydrochloride (32), protection with the benzoylcarbonyl 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 (C₁, C₁α and C₂ complex) derivatives (21).

benzyloxy carbonyl group at the N-1 position of 28 was accomplished with sodium hydride to give the desired cyclic carbamate 29. 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-(benzyloxy carbonyl)fortimicin B (34) was derived from fortimicin B (33) according to the method of Tadanier et al. 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 4. Synthesis of 1-N-palmitoylfortimicin B (36), 2'-N-palmitoylkasugamycin (38), and 2-N-palmitoyl-D-glucosamine (40).
Table 1. Physical data and yields of protected 1-N-palmitoyl-3'-N-trifluoroacetyl aminoglycoside derivatives (7, 8, 9 and 20).

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield (%)</th>
<th>MP (°C, dec)</th>
<th>IR (Nujol, cm⁻¹)</th>
<th>¹H NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DMSO-d₆</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>86</td>
<td>—</td>
<td>1710, 1690, 1540</td>
<td>0.84 (3H, t, J=6 Hz), 1.25 (26H, s), 1.80~2.25 (2H, m)</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
<td>284~286</td>
<td>1290, 1170</td>
<td>0.85<del>1.03 (3H, m), 1.25 (26H, s), 1.50</del>1.80 (2H, m), 1.75~2.30 (4H, m)</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>—</td>
<td>1680, 1520, 1250</td>
<td>0.80~1.00 (3H, m), 1.28 (26H, s)</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
<td>156~157</td>
<td>1700~1665, 1510</td>
<td>2.57 (3H, m), 3.24 (3H, m)</td>
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</table>

---: 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 (ID₅₀ 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 (ID₅₀ 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 (ID₅₀ 1.9 and 2.7 μg/ml). 38 showed the strongest antiviral activity against influenza virus (ID₅₀ 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. Since we had previously demonstrated reduced cytotoxicity of PTKA, 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 (ID₅₀ 1.4~5.2 μg/ml). Both
<table>
<thead>
<tr>
<th>No.</th>
<th>Yield* (%)</th>
<th>MP (°C, dec)</th>
<th>[α]D° (°)</th>
<th>IR (Nujol, cm⁻¹)</th>
<th>¹H NMR Solvent</th>
<th>δ (ppm)</th>
<th>FD-MS (m/z)</th>
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<tbody>
<tr>
<td>10</td>
<td>81</td>
<td>220</td>
<td>+69.8</td>
<td>1700, 1630, 1550</td>
<td>CD₂OD</td>
<td>0.90~1.08 (3H, m), 5.08 (1H, d, J=3 Hz)</td>
<td>819 (M⁺⁺⁺)</td>
</tr>
<tr>
<td>11</td>
<td>64</td>
<td>224</td>
<td>+58.7</td>
<td>1710, 1645~1620, 1165</td>
<td>CD₂OD</td>
<td>0.80<del>0.97 (3H, m), 1.28 (26H, s), 1.90</del>2.50 (6H, m), 5.10 (1H, d, J=3 Hz), 5.90 (1H, d, J=3.5 Hz)</td>
<td>824 (M⁺⁺⁺⁺)</td>
</tr>
<tr>
<td>12</td>
<td>69</td>
<td>206</td>
<td>+55.7</td>
<td>1700, 1630</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>87</td>
<td>167</td>
<td>+63.5</td>
<td>1640, 1500b</td>
<td>D₂O</td>
<td>0.84 (3H, t, J=7 Hz), 5.24 (1H, br s), 5.80 (1H, br s)</td>
<td>808 (M⁺⁺⁺⁺)</td>
</tr>
<tr>
<td>21</td>
<td>83</td>
<td>174</td>
<td>+70.3</td>
<td>1680, 1650~1630, 1245</td>
<td>D₂O</td>
<td>2.78 (3H, s)</td>
<td>690 (M⁺⁺)</td>
</tr>
<tr>
<td>25</td>
<td>73</td>
<td>154</td>
<td>+98.1</td>
<td>1640, 1540, 1280, 1120b</td>
<td>D₂O</td>
<td>2.76 (3H, s), 2.90 (3H, s), 5.30 (1H, d, J=4 Hz), 5.81 (1H, d, J=3 Hz)</td>
<td>702 (M⁺⁺)</td>
</tr>
<tr>
<td>32</td>
<td>45</td>
<td>166</td>
<td>—</td>
<td>1640~1620</td>
<td>D₂O</td>
<td>0.88 (3H, m), 1.05~1.50 (26H, s)</td>
<td>693 (M⁺⁺)</td>
</tr>
<tr>
<td>36</td>
<td>99</td>
<td>208</td>
<td>+71.0</td>
<td>1600</td>
<td>CD₂OD</td>
<td>3.17 (3H, s), 3.47 (3H, s), 5.33 (1H, d, J=3 Hz)</td>
<td>587 (M⁺⁺)</td>
</tr>
<tr>
<td>38</td>
<td>69a</td>
<td>162</td>
<td>+113.4</td>
<td>1660, 1540</td>
<td>CD₂OD</td>
<td>0.90 (3H, t, J=5 Hz), 1.20 (3H, d, J=5 Hz), 5.27 (1H, s)</td>
<td>574 (M⁺⁺⁺⁻)</td>
</tr>
<tr>
<td>40</td>
<td>69a</td>
<td>129~132</td>
<td>+125.8</td>
<td>1700, 1640, 1550</td>
<td>DMSO-d6</td>
<td>0.90 (3H, t, J=5 Hz), 7.43 (1H, m)</td>
<td>418 (M⁺⁺)</td>
</tr>
</tbody>
</table>

* Yields of deprotecting process.  
* KBr disc method.  
* Yield from 37.  
* Yield from 39.  
—: Not measured.
Table 3. *In vitro* antiviral activity of aminoglycoside derivatives having palmitoyl group.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Antiviral activity ID$_{50}$ (µg/ml)</th>
<th>Cytotoxicity (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSV-I</td>
<td>Influenza virus</td>
</tr>
<tr>
<td>10</td>
<td>5.2</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>1.4</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>1.9</td>
<td>24</td>
</tr>
<tr>
<td>15</td>
<td>0.8</td>
<td>—</td>
</tr>
<tr>
<td>21</td>
<td>1.7</td>
<td>—</td>
</tr>
<tr>
<td>25</td>
<td>1.8</td>
<td>—</td>
</tr>
<tr>
<td>32</td>
<td>1.0</td>
<td>25</td>
</tr>
<tr>
<td>36</td>
<td>1.9</td>
<td>—</td>
</tr>
<tr>
<td>38</td>
<td>2.7</td>
<td>5.6</td>
</tr>
<tr>
<td>40</td>
<td>28</td>
<td>—</td>
</tr>
<tr>
<td>PTKA</td>
<td>1.4</td>
<td>16</td>
</tr>
<tr>
<td>PKA</td>
<td>3.2</td>
<td>32</td>
</tr>
<tr>
<td>Virazole</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>Amantadine</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>0.1</td>
<td>—</td>
</tr>
</tbody>
</table>

**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$^9$. 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$_4$Si 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-1 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 (TCID50) of virus and immediately thereafter exposed to varying concentrations of the test compound and incubated for 2 days at 37°C in humidified 5% CO2 - 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 ID50 (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 (C1, C1a, and C2 complex) (19).

These compounds (4, 5, 6, 18 and 19) were synthesized according to the method of Tsuchiya et al.\(^4\).

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 Et2O, and dried over P2O5 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 Et3N. 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 MgSO4. 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 - CHCl3 (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 Et3N (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 NH4OH (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

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* 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.
NH₄OH (10 ml) at room temp. The mixture was stirred again overnight at the same temp and was evaporated. The residue was washed with Et₂O 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 Et₃N. 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 P₂O₅. 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 CHCl₃ - MeOH (10 : 1) and then with a mixture of CHCl₃ - 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',6',3''-Tetrakis-[(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 Et₃N 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 P₂O₅ in vacuo to give 14 (333 mg, 97%): IR (Nujol) cm⁻¹ 1680, 1540.

3',2',6'/ 3''/ -Tris-[(benzyloxycarbonyl)-1-(tert-butoxycarbonyl)-3''/-(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 Et₃N (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 NaHCO₃ and aq NaCl, dried over Na₂SO₄, and evaporated. The residue was subjected to column chromatography on silica gel (70 g) and eluted with a mixture of CHCl₃ - MeOH (40 : 1). The fractions containing the desired compound were collected and evaporated to give 22 (3.41 g, 99%): MP 127~128°C; [α]_D^0 +96.4° (c 1.0, CHCl₃); IR (Nujol) cm⁻¹ 1680, 1500, 1250, 1140; ¹H NMR (CDCl₃) δ 1.05 (3H, s, CH₃), 1.40 (9H, s, C(CH₃)₃), 2.83 (3H, s, NCH₃).

3',2',6'/ 3''/ -Tetrakis-(benzyloxycarbonyl)-1-(tert-butoxycarbonyl)sagamicin (23)

A solution of 22 (3.36 g) in a mixture of THF (40 ml), coned NH₄OH (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 Na₂SO₄, 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 Et₃N. 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 NaHCO₃, water, and aq NaCl, dried over Na₂SO₄, and evaporated to give 23 (3.83 g): MP 91~93°C; [α]_D^0 +70.3° (c 1.0, CHCl₃); IR (Nujol) cm⁻¹ 1675, 1500, 1250, 1150; ¹H NMR (CDCl₃) δ 2.82 (3H, s, NCH₃), 3.06 (3H, s, NCH₃).

3',2',6'/ 3''/ -Tetrakis-(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 Et₃N. 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 NaHCO₃, water, and aq NaCl, dried over Na₂SO₄, and evaporated. The residue was chromatographed on silica gel (50 g) and eluted with a mixture of CHCl₃ - MeOH (40 : 1). The fractions containing the desired compound were combined and evaporated to give 24 (0.62 g, 38%): MP 124~126°C; [α]_D^0 +87.5° (c 0.5, CHCl₃); IR (Nujol) cm⁻¹ 1680, 1520, 1140; ¹H NMR (CDCl₃) δ 2.82 (3H, s, NCH₃), 3.06 (3H, s, NCH₃).
1,3',6'-Tetrakis-N-(benzylxoycarbonyl)ribostamycin (27)

The compound (27) was synthesized from ribostamycin (26) as described.*0.

1,3',6'-Tetrakis-N-(benzylxoycarbonyl)-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-toluene sulfonic 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 NaHCO₃ and water, dried over Na₂SO₄, and evaporated. The resulting residue was chromatographed on silica gel (50 g) and eluted with a mixture of CHCl₃ - 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 (CDCl₃) δ 1.42 (10H, m, CH₂), 1.59 (10H, m, CH₂), 5.10 (8H, s, C₁₂C₆H₅), 5.28 (1H, d, J=3 Hz), 7.10–7.50 (35H, m, C₆H₅).

3,2',6'-Tris-N-(benzylxoycarbonyl)-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 NH₄Cl (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 Na₂SO₄, and evaporated. The residue was chromatographed on silica gel (500 g) and eluted with a mixture of CHCl₃ - 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 (CDCl₃) δ 1.10–1.85 (20H, m), 5.05 (6H, s, CH₂), 5.05 (6H, s, CH₂), 7.10–7.50 (30H, m, C₆H₅).

3,2',6'-Tris-N-(benzylxoycarbonyl)-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 CHCl₃ - MeOH - concd NH₄OH (10:1:0.1). The fractions containing the desired compound were collected and evaporated to give 1₁-N-protected 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 Et₃N 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 MgSO₄, and evaporated. The resulting residue was chromatographed on silica gel (500 g) and eluted with a mixture of CHCl₃ - MeOH - concd NH₄OH (10:1:0.1). 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 (CDCl₃) δ 0.93 (3H, m, CH₃), 1.37 (26H, s, CH₂), 1.42 (10H, m), 1.60 (10H, m), 7.20–7.50 (30H, m, C₆H₅).

3',2',6'-Tris-N-(benzylxoycarbonyl)-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; 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, QH$_5$).

1',2',6'-Tris-N-(benzoyloxy carbonyl)fortimicin B (34)

34 was synthesized from fortimicin B (33) according to the method of Tadanier et al. 7.

1',2',6'-Tris-N-(benzoyloxy carbonyl)-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$_3$N. The mixture was stirred for 30 minutes under the same condition. The mixture 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; 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-Palmitoyl kasugamycin (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$_3$N. 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$_2$O, and water, and air-dried to give 40 (2.66 g) (Table 2).

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References

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