The Applicant identified below requests the grant of a patent to the
nominated person identified below for an invention described in the
accompanying standard complete patent specification.

[70,71] Applicant and Nominated Person:

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[54] Invention Title:

CHEMICAL MODIFICATION OF BLSAMICIN A AT THE 3' AND/OR 4'OH GROUPS

[72] Actual Inventors:

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[31,33,32]

Details of basic application(s):

707,471 UNITED STATES OF AMERICA US 30 May 1991

Applicant states the following:

1. The nominated person is the assignee of the actual inventor(s)
2. The nominated person is the assignee of the applicant authorized to make this application by the applicant of the basic application.
3. The basic application(s) was/were the first made in a convention country in respect of the invention.

The nominated person is not an opponent or eligible person described in Section 33-36 of the Act.

29 May 1992

Bristol-Myers Squibb Company
By PHILLIPS ORMONDE & FITZPATRICK
Patent Attorneys
By

Our Ref: 287930

David B Fitzpatrick

(11) 17240/92  -2-

2. A compound having the formula:
1. A compound having the formula

![Chemical Structure](image)

wherein Z is alkylidene, cycloalkylidene, arylalkylidene or alkoxyalkylidene.
2. A compound having the formula:

![Chemical Structure](image)

7. A pharmaceutical formulation which comprises as an active ingredient a compound claimed in any one of claims 1-3, associated with one or more pharmaceutical acceptable carriers or diluents.

8. A compound as claimed in anyone of claims 1-3, for use as an antitumor agent.
Name of Applicant:
Bristol-Myers Squibb Company

Actual Inventor(s):
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Invention Title:
CHEMICAL MODIFICATION OF ELSAMICIN A AT THE 3' AND/OR 4'OH GROUPS

Our Ref: 287930
POF Code: 129416/1490

The following statement is a full description of this invention, including the best method of performing it known to applicant(s):
CHEMICAL MODIFICATION OF ELSAMICIN A AT THE 3' AND/OR 4' OH GROUPS

This invention relates to novel elsamicin A derivatives which have improved antitumor activity, to their production, to compositions containing the same as the active ingredient, and a method for therapy using said compositions.

Elsamicin A is an antitumor antibiotic produced by cultivating an elsamicin A-producing strain of actinomycete designated strain J907-21 (ATCC 39417), or a mutant thereof. Elsamicin A exhibits antibacterial activity against aerobic gram-positive bacteria and anaerobic bacteria. It also exerts activity against various murine tumor cells including leukemia P388, lymphoid leukemia L1210, and melanotic melanoma B16 in vitro and in vivo. Konishi, et al,


Interconversion of both compounds by chemical process has never been reported.

In the course of chemical modification of elsamicin A, we found that introduction of alkylidene group on the 3' and 4'-OH groups, or a tetrahydropyranyl group on the 4'-OH group led to an increase of antitumor activity of elsamicin A.

The present invention provides new derivatives of elsamicin A which exhibit improved antitumor activity. More particularly the present invention describes the chemical modification on the 3' and/or 4'-OH groups of elsamicin A.

This invention further provides an antitumor composition comprising, as the active ingredient, at least one member selected from the group consisting of the elsamicin A derivative of the present invention.

This invention further provides a method for therapy of cancer using the above antitumor composition.

Further provided is a process for producing the above-mentioned elsamicin A derivative.

U. S. Patent No. 4,518,589 to Konishi et al, discloses the production and isolation of the antitumor agent designated elsamicin A. (Formula I, above). The above-mentioned elsamicin A compound is the principal component of the fermentation of the elsamicin A-producing strain of actinomycete, designated strain J907-21 (ATCC 39417).
It has now been found according to the present invention that chemical modification on the 3' and/or 4'-OH groups of elsamicin A leads to new derivatives having improved antitumor activity.

The elsamicin A derivatives of the present invention have the general Formula III and IV below:

Formula III

wherein Z is alkylidene, cycloalkylidene, arylalkylidene or alkoxyalkylidene.
As shown in Scheme 1, the 3',4'-O-alkylidenation of elsamicin A (1) was carried out, by treatment of 2''-N-protected elsamicin A (2 or 4) with dimethyl acetals of an appropriate ketone or aldehyde in the presence of acid catalysts to give intermediates, 5 and 7, followed by subsequent deprotection which afforded the 3',4'-O-alkylidene derivatives (6a - 6d). Isopropylidene (6a) and benzylidene (6c) were also prepared from elsamicin A without N-protection. As shown in Scheme 2, reaction of compound 1 with dihydropyran in the presence of an acid catalyst gave a mixture of per-tetrahydropyranyl(THP) derivatives, which was treated with p-toluenesulfonic acid (TsOH) in methanol to afford the mono-O-THP derivative (8). Based on the mass spectrum, the structure of 8 was determined to be the 4'-O-THP derivative.

Table 1 indicates the compounds of the present application and their respective number.
### Table 1
Compounds of the present invention and their respective number

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Elsamicin A</td>
</tr>
<tr>
<td>2</td>
<td>Chartreusin</td>
</tr>
<tr>
<td>3</td>
<td>2''-N-t-Butoxycarbonylesamicin A</td>
</tr>
<tr>
<td>4</td>
<td>2''-N-Benzylloxy carbonylesamicin A</td>
</tr>
<tr>
<td>5a</td>
<td>2''-N-t-Butoxycarbonyl-3',4''-O-isopropylideneelsamicin A</td>
</tr>
<tr>
<td>5b</td>
<td>2''-N-t-Butoxycarbonyl-3',4''-O-cyclohexylideneelsamicin A</td>
</tr>
<tr>
<td>5c</td>
<td>3',4'-O-Benzylidene-2''-N-t-butoxy-carbonylesamicin A</td>
</tr>
<tr>
<td>5a</td>
<td>3',4'-O-Isopropylideneelsamicin A</td>
</tr>
<tr>
<td>5b</td>
<td>3',4'-O-Cyclohexylideneelsamicin A</td>
</tr>
<tr>
<td>6c</td>
<td>3',4'-O-Benzylideneelsamicin A</td>
</tr>
<tr>
<td>6d</td>
<td>3',4'-O-Methoxymethylideneelsamicin A</td>
</tr>
<tr>
<td>7a</td>
<td>2''-N-Benzylloxy carbonyl-3',4''-O-isopropylideneelsamicin A</td>
</tr>
<tr>
<td>7d</td>
<td>2''-N-Benzylloxy carbonyl-3',4''-O-methoxy-methylideneelsamicin A</td>
</tr>
<tr>
<td>8</td>
<td>4''-O-Tetrahydropyranylesamicin A</td>
</tr>
</tbody>
</table>
Scheme 1
Synthetic Route of 3',4'-O-Alkylidene Derivatives of Elsamicin A

1)  

2)  

3)  

4)  

5)  

6)  

7)  

\[ \text{R} = \text{t-BOC} \]
\[ \text{R} = \text{Cbz} \]
\[ \text{R} = \text{H} \]
\[ \text{R} = \text{Cbz} \]
Scheme 1 (cont'd)

1) (t-BOC)₂O/NEt₃

2) BnOCODN / NEt₃

3) Z-OCH₃ / TsOH

4) TsOH or TFA

5) H₂/Pd-C

Z: (a) CH₃<br><br>(b) \( \text{OCH}_{2} \)<br><br>(c) \( \text{OCH}_{2} \)<br><br>(d) CH₃OCH₃
Scheme 2
Preparation of 4'-O-Tetrahydropyranylelsamicin A

\[ \text{Scheme 2} \]

Preparation of 4'-O-Tetrahydropyranylelsamicin A
Antitumor activity of 3'-and/or 4'-0-modified elsamycin A derivatives

Five 3-and/or 4'-0-modified elsamycin A derivatives were synthesized and comparatively tested with the parent compound for in vitro and in vivo antitumor activities.

For in vitro cytotoxicity experiment, murine melanoma B16-F10 cells were grown and maintained in Eagle's minimum essential medium (Nissui), which contains kanamycin (60 µg/ml), supplemented with heat-inactivated fetal calf serum (10%) and non-essential amino acids (0.6%) at 37°C under a humidified atmosphere in a 5% CO₂ incubator. Exponentially growing B16-F10 cells were harvested, counted and suspended in the culture medium at the concentration of 2.0x10⁶ cells/ml. The cell suspension (180 µl) was planted into wells of a 96-well microtiter plate and incubated for 24 hours. Test compound (20 µl) were added to the wells and the plates were further incubated for 72 hours. The cytotoxic activity was colorimetrically determined at 540 nm after staining viable cells with neutral red solution. All of the 3'-and/or 4'-0 modified elsamycin A derivatives tested showed quite strong cytotoxicity against B16-F10 cells with the IC₅₀ values of 0.025 - 0.07 µg/ml (Table 2).

In vivo antitumor activity of the above five derivatives was tested in the lymphocytic leukemia P388 and melanoma B16 systems. Female CDF₁ (for P388) and male BDF₁ (for B16) mice were inoculated by ip injection at 10⁶ P388 cells and 0.5 ml of a 10% B16 brei per mouse, respectively (day 0). Test compounds were introperitoneally administered to the mice once daily on days 1 to 3 (Q1DX3) in the P388 system or
once a day on days 1, 5 and 9 (Q4Dx3) in the B16 system and animals were observed for 45 days.

The percent increase of median survival time (MST) of treated animals over that of untreated control animals was determined and reported as T/C %. Compounds showing T/C % values of 125 or greater are considered to have significant antitumor activity. As shown in Table 2, among the above five derivatives, 4'-O-tetrahydropyranylelsamicin A, (8) was the most interesting compound in the P388 system. It showed three times more potent minimum effective dose (MED) than elsamicin A and high T/C % values. 3',4'-O-isopropylideneelsamicin A, (6a), 3',4'-O-benzylideneelsamicin A, (6c) and 3',4'-O-methoxymethylideneelsamicin A, (6d) were as active as the parent compounds in terms of MED. In the B16 system, all of five derivatives tested showed good response to the tumor (Table 3). Similar to the results in the P388 system, compound 8 was better than elsamicin A (1) in terms of MED and T/C % values. Some survivors were observed on day 50 in the groups tested with 3-20 mg/kg/day of this compound. Compound 6d also gave extremely potent therapeutic activity with higher T/C % values at 3 and 10 mg/kg/day than elsamicin A (1).
Table 2  *In vitro* cytotoxicity against B16 melanoma and *in vivo* antitumor activity against P388 leukemia in mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Z</th>
<th>IC50(μg/ml)</th>
<th>T/C % of MST(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>CH₃</td>
<td>0.025</td>
<td>210 170 140 120</td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td>0.04</td>
<td>165 175 140 120</td>
</tr>
<tr>
<td>6c</td>
<td>C₄H₅</td>
<td>0.07</td>
<td>Tox 180 165 150 130 110</td>
</tr>
<tr>
<td>6d</td>
<td>OCH₃</td>
<td>0.05</td>
<td>Tox 224 167 167 137 124</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>0.04</td>
<td>Tox 225 190 168 145 130</td>
</tr>
<tr>
<td>Elsamicin A (1)</td>
<td></td>
<td>0.03</td>
<td>Tox 190 180 155 140 123</td>
</tr>
</tbody>
</table>

\(^1\) Median survival time in days
\(^2\) Dose in mg/kg/day, Q1Dx3ip
Table 3  In vivo antitumor activity against B16 melanoma in mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>T/C % of MST*1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20*2</td>
</tr>
<tr>
<td>6a</td>
<td>82</td>
</tr>
<tr>
<td>6b</td>
<td>200</td>
</tr>
<tr>
<td>6c</td>
<td>186</td>
</tr>
<tr>
<td>6d</td>
<td>≥333</td>
</tr>
<tr>
<td>6</td>
<td>≥370</td>
</tr>
<tr>
<td>1</td>
<td>≥298</td>
</tr>
</tbody>
</table>

*1 Median survival time in days
*2 Dose in mg/kg/day, Q4Dx3 ip
*3 No. of survivors/tested on day 50
The present invention includes within its scope a process for producing the elsamicin A derivatives of the present invention.

Another aspect of the invention, there are provided pharmaceutical compositions which comprise an effective tumor-inhibiting amount of the compound of Formula III or IV, in combination with an inert pharmaceutically acceptable carrier or diluent.

According to another aspect of the invention provides a method for therapeutically treating an animal, preferably mammalian, host affected by a tumor which comprises administering to such host an effective tumor-inhibiting dose of the antibiotic of the compound of Formula III or IV.

Examples of suitable compositions include solid compositions for oral administration such as tablets, capsules, pills, powders and granules, liquid compositions for oral administration such as solutions, suspensions, syrups and elixirs and preparations for parenteral administration such as sterile solutions, suspensions or emulsions. They may also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, physiological saline or some other sterile injectable medium immediately before use.

It will be appreciated that the actual preferred dosages of the elsamicin A derivative of the present invention will vary according to the particular compound being used, the particular composition formulated, the mode of application and the particular situs, host and disease being treated. Many factors that modify the action of the drug will be taken into account by those skilled in the art, e.g. age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations,
reaction sensitivities and severity of the disease. Administration can be carried out continuously or periodically within the maximum tolerated dose. Optimal application rates for a given set of conditions can be readily ascertained by those skilled in the art using conventional dosage determination tests.

The present invention is illustrated by the following examples which are not intended to be construed as limiting the scope of the invention.

Specific synthesis examples of the intermediates 3-5c, 7a and 7d are explained below, from which intermediates the compounds of this invention 6a-6d and 8 are synthesized by the above process.

**EXAMPLE 1**

**Synthesis of 2"-N-t-Butoxycarbonylelsamicin A (3)**

A mixture of elsamicin A (653 mg, 1 mmole), di-t-butyldicarbonate (348 mg, 1.6 mmoles) and triethylamine (0.14 ml, 1 mmole) in dioxane (10 ml) was stirred at room temperature overnight. The reaction mixture was evaporated in vacuo to give a semi-crystalline residue, which was recrystallized from CH₂Cl₂/ether to obtain 768 mg (100%) of compound 3 as yellowish crystalline powder.

MP 183-184°C. IR \( v_{\text{max}} \) (KBr) cm⁻¹ 3410, 1700, 1505, 1370, 1255, 1120, 1065, 875, 780. UV \( \lambda_{\text{max}} \) (MeOH nm (ε)) 236 (38300), 266 (37800), 333 (6180), 380 (8770), 400 (14500), 423 (15900). \(^1\)H NMR (CDCl₃) δ 0.73 (9H, br.s.), 1.31 (3H, d, J=7.0 Hz), 1.36 (3H, s), 1.39 (3H, d, J=6 Hz), 2.68 (3H, s), 3.35 (3H, s), 5.37 (1H, d, J=8.0 Hz), 4.66 (1H, d, J=4 Hz), 8.18 (1H, dd, J=8.0 & 1.5 Hz), 11.59 (1H, s).
EXAMPLE 2

Synthesis of 2\textsuperscript{-N-Benzzyloxy carbonylesamicin A (4)}

To a stirred suspension of elsamicin A (653 mg), NEt\textsubscript{3} (0.14 ml) in dioxane (10 ml) was added N-benzzyloxy carbonyloxy-5-norbornene-2,3-dicarboximide (334 mg). The reaction mixture was stirred overnight at room temperature and evaporated in vacuo. The residue was dissolved in CH\textsubscript{2}Cl\textsubscript{2} and the solution was washed with diluted aqueous NaHC\textsubscript{0}\textsubscript{3}, water and saturated aqueous NaCl, successively, dried with MgSO\textsubscript{4} and concentrated under reduced pressure to give a yellow mass. The residue was chromatographed on a silica gel column (Wakogel C-200, 21x80 mm) using 2% MeOH/CHCl\textsubscript{3} as an eluant to give 772 mg (98%) of the title compound.

MP 166-167\degreeC. IR \nu\textsubscript{max} (KBr) cm\textsuperscript{-1} 1730, 1695, 1510, 1380, 1260, 1240, 1150, 1070, 785. UV \lambda\textsubscript{max} (MeOH nm (\epsilon)) 236 (38900), 266 (38500), 335 (6480), 381 (9190), 401 (15300), 423 (16600). \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \delta 1.32 (3H, d, J=6 Hz), 1.38 (3H, s), 2.74 (3H, s), 3.36 (3H, s), 5.39 (1H, d, J=8 Hz), 5.74 (1H, d, J=4 Hz), 8.08 (1H, dd, J=8 \& 1.5 Hz), 11.41 (1H, s).

Anal. Calcd. for C\textsubscript{41}H\textsubscript{41}NO\textsubscript{15} \cdot \textfrac{1}{2}H\textsubscript{2}O:

C 61.80, H 5.31, N 1.76.

Found: C 61.92, H 5.14, N 2.23.
EXAMPLE 3

Synthesis of 2''-N-t-Butoxycarbonyl-3',4'-0-isopropylideneelsamicin A (5a)

To a solution of compound 3 (200 mg) and 2,2-dimethoxypropane (1.2 ml) in dry CH₂Cl₂ (4 ml) was added TsOH (5 mg) and the mixture was kept at room temperature overnight. Saturated aqueous NaHCO₃ was added to the reaction mixture and the organic layer was taken up, dried with MgSO₄ and evaporated in vacuo to give 190 mg (90%) of the title compound as a yellow solid.

MP 168-169°C. IR νₓ (KBr) cm⁻¹ 3420, 1740, 1690, 1505, 1375, 1250, 1065, 780. UV λₓ max (MeOH nm (ε)) 236 (40600), 266 (39100), 333 (6370), 380 (8850), 399 (14200), 422 (15500). ¹H NMR (CDCl₃) δ 1.34 (3H, d, J=6 Hz), 1.37 (3H, d, J=7 Hz), 1.37 (3H, s), 1.42 (3H, s), 1.68 (3H, s), 3.35 (3H, s), 5.23 (1H, d, J=8 Hz), 5.8 (1H, d, J=4 Hz), 8.33 (1H, dd, J=8 & 1.5 Hz), 11.63 (1H, br.).

Anal. Calcd. for C₄H₇NO₁₂·H₂O:
C 60.66, H 6.08, N 1.73.
Found: C 60.92, H 6.03, N 2.00.

EXAMPLE 4

Synthesis of 2''-N-Benzylloxycarbonyl-3',4'-0-isopropylideneelsamicin A (7a)

To a solution of compound 4 (507 mg) and 2,2-dimethoxypropane (2.9 ml) in dry dichloromethane (10 ml) was added p-toluenesulfonic acid (10 mg) and the mixture was kept at room temperature overnight. Saturated aqueous NaHCO₃ (10 ml) was added to the reaction mixture and the organic layer was separated, dried over MgSO₄ and evaporated under reduced pressure.
The residue was triturated with a mixture of CH₂Cl₂-ether-n-hexane to give 540 mg (100%) the title compound.

MP 160-162°C. IR ν_max (KBr) cm⁻¹ 1730, 1690, 1500, 1375, 1250, 1140, 1065, 780. UV λ_max (MeOH nm (ε) 236 (39100), 267 (37600), 333 (6440), 380 (9020), 400 (14400), 422 (15500). ¹H NMR (CDCl₃) δ 1.28 (3H, d, J=6 Hz), 1.40 (3H, d, J=7 Hz), 1.41 (3H, s), 1.47 (3H, s), 1.70 (3H, s), 2.88 (3H, s), 3.45 (3H, s), 5.28 (1H, d, J=8 Hz), 5.66 (1H, d, J=4 Hz), 8.32 (1H, dd, J=8 & 2 Hz), 11.53 (1H, br.).

Anal. Calcd. for C₄₄H₄₅NO₁₅:
C 63.84, H 5.48, N 1.69.
Found: C 63.59, H 5.64, N 1.63.

EXAMPLE 5

Synthesis of 3',4'-O-Isopropylideneelsamicin A (6a)

Method A

A solution of compound 7a (67 mg) in 80% aqueous tetrahydrofuran (2.5 ml), was hydrogenated in the presence of 10% Pd-C (30 mg) for 1.5 hour. The reaction mixture was filtered to remove the catalyst and then concentrated to dryness. The residue was purified by column chromatography using 5% MeOH in CHCl₃ as an eluant to give 47 mg (83%) of the title compound.

MP 189-191°C (dec.). IR ν_max (KBr) cm⁻¹ 1710, 1610, 1370, 1250, 1070, 780. UV λ_max (MeOH nm (ε) 236 (34500), 266 (29700), 332 (4980), 399 (10600), 420 (11300). ¹H NMR (CDCl₃ + CD₃OD) δ 1.18 (3H, d, J=6 Hz), 1.29 (3H, d, J=7 Hz), 1.39 (3H, s), 1.46 (3H, s), 1.67 (3H, s), 2.87 (3H, s), 3.52 (3H, s), 5.22 (1H, d, J=8 Hz), 6.02 (1H, br.).
Anal. Calcd. for \( \text{C}_{39}\text{H}_{39}\text{NO}_{13}\cdot3/2\text{H}_{2}\text{O} \):

- C 59.99, H 5.87, N 1.94.
- Found: C 60.09, H 5.76, N 2.13.

MS (SIMS) \( M/Z \) 695 (\( M + H \)^+), 360, 334, 160.

**Method B**

A solution of compound 5a (238 mg) and p-toluene-
sulfonic acid monohydrate (TsOH) (285 mg) in acetone
(5 ml) was stirred at room temperature for 2 hours and
then concentrated in vacuo. The residue was dissolved
in a mixture of methanol and chloroform (1:10, 40 ml).
The solution was successively washed with 10% aqueous
\( \text{NaHCO}_{3} \), water and brine, and dried over \( \text{MgSO}_{4} \) and
concentrated in vacuo. The residue was purified by
column chromatography on silica gel to give 142 mg
(68%) of the title compound. MP 189-191°C. The
spectral and HPLC data of compound 6a obtained here
was completely identical with those obtained in method
A.

**Method C**

A mixture of compound 1 (262 mg), TsOH (80 mg) and
2,2-dimethoxypropane (1 ml) in dry \( \text{CH}_{2}\text{Cl}_{2} \) (5 ml) was
stirred at room temperature for 17 hours. A saturated
aqueous \( \text{NaHCO}_{3} \) was added to the reaction mixture and
the organic layer was separated, dried over \( \text{Na}_{2}\text{SO}_{4} \) and
evaporated. The yellow residue was triturated with
ether to afford 280 mg (100%) of 6a. MP 189-191°C.
The spectral and HPLC data of compound 6a obtained
here was completely identical with those obtained in
method A.
EXAMPLE 6

Synthesis of 2\"-N-t-Butoxycarbonyl-3',4'-O-cyclohexylideneelamsicin A (5b)

A solution of compound 3 (151 mg), 1,1-dimethoxycyclohexane (1.2 ml) and anhydrous p-toluenesulfonic acid (3 mg) in dry CH₂Cl₂ (3 ml) was stirred at room temperature for 2 hours. The reaction mixture was diluted with CH₂Cl₂, washed with saturated aqueous NaHCO₃, dried over MgSO₄ and evaporated to give a crystalline residue, which was washed with ether to afford 165 mg (100%) of compound 5b.

MP 173-175°C. IR ν max (KBr) cm⁻¹ 2910, 1730, 1710, 1680 (sh), 1500. UV λ max (MeOH nm (ε)) 236 (35300), 266 (34200), 333 (4660), 379 (7820), 399 (12500), 422 (13700). ¹H NMR (CDCl₃) δ 0.69 (9H, s), 1.10-2.5 (19H, m), 2.90 (3H, s), 3.36 (3H, s), 5.22 (1H, d, J=8 Hz), 5.83 (1H, d, J=4 Hz), 11.69 (1H, s).

Anal. Calcd. for C₄₄H₅₁NO₁₅: C 63.38, H 6.16, N 1.68.

Found: C 64.38, H 6.63, N 1.60.

EXAMPLE 7

Synthesis of 3',4'-O-Cyclohexylideneelamsicin A (6b)

A solution of compound 5b (83.3 mg) and TsOH (95 mg) in cyclohexanone (1.6 ml) was stirred at room temperature overnight. An aqueous 10% NaHCO₃ (5 ml) and CHCl₃ (10 ml) were added to the reaction mixture and the organic layer was taken up, washed with brine (5 ml), dried over MgSO₄ and evaporated in vacuo. The viscous residue was triturated with isopropyl ether and filtered off to afford 32 mg (44%) of compound 6b.

MP 182-190°C (dec.). IR ν max (KBr) cm⁻¹ 3400, 1605, 1445, 1370, 1230, 1170, 1070. UV λ max (MeOH nm (ε)) 236 (33000), 266 (28900), 331 (5340), 399 (10100), 420
(10500). $^1$H NMR (CDCl$_3$ + CD$_3$OD) $\delta$ 1.1-1.2 (19H, m), 2.87 (3H, s), 3.45 (3H, s), 5.23 (1H, d, J=8 Hz), 5.94 (1H, d, J=4 Hz).

Anal. Calcd. for C$_{39}$H$_{43}$NO$_{13}$·H$_2$O:
C 62.31, H 6.03, N 1.86.
Found: C 62.23, H 5.92, N 1.83.

EXAMPLE 8
Synthesis of 3'-4'-O-Benzylidene-2''-N-t-butoxycarbonyl-elsamicin A (5c)

To a solution of compound 3 (77 mg) and benzaldehyde dimethylacetal (0.5 ml) in CH$_2$Cl$_2$ (2 ml) was added TsOH (5 mg), and the mixture was kept at room temperature for 2 days. An aqueous saturated NaHCO$_3$ solution (ca. 10 ml) was added to the reaction mixture and the mixture was extracted with CHC$_3$. The extract was washed with water, dried over Na$_2$SO$_4$ and evaporated in vacuo to give a yellow solid, which was purified by silica gel column chromatography using MeOH in CHC$_3$ (1-3%) as eluants to afford 70 mg (81%) of the title compound.

MP 168-170°C. IR $\nu_{max}$ (KBr) cm$^{-1}$ 1735 sh, 1695, 1505, 1375, 1255, 1235, 1145, 1070, 780. UV $\lambda_{max}$ (MeOH mm ($\epsilon$) 235 (40600), 266 (39300), 332 (6410), 399 (39900), 421 (15000). $^1$H NMR (CDCl$_3$) $\delta$ 0.71 (9H, s), 1.35 (3H, d, J=6 Hz), 1.58 (3H, s), 2.91 (3H, s), 3.40 (3H, s), 5.32 (1H, d, J=8 Hz), 5.69 (1H, d, J=4 Hz), 5.98 (1H, s).

Anal. Calcd. for C$_{45}$H$_{47}$NO$_{15}$·H$_2$O:
C 62.86, H 5.74, N 1.63.
Found: C 63.17, H 5.42, N 1.53.
EXAMPLE 9

Synthesis of 3',4'-0-Benzylideneelsamicin A (6c)

Method A

Compound 5c (60 mg) was dissolved in TFA (0.3 ml), and the mixture was immediately concentrated in vacuo. The residue was dissolved in a mixture of saturated aqueous NaHCO₃ and CHCl₃. The organic layer was separated, washed with water and evaporated in vacuo. The yellow residue was chromatographed on a silica gel column to afford 24 mg (45%) of compound 6c.

MP 183-189°C. IR ν max (KBr) cm⁻¹ 1710, 1610, 1510, 1375, 1250, 1235, 1070, 780. UV λ max (MeOH nm (ε) 236 (40700), 266 (34400), 331 (5890), 379 (8180), 398 (12200), 420 (13000). ¹H NMR (CDCl₃ + CD₃OD) δ 0.82 (1.5H, d, J=6 Hz), 1.22 (3H, d, J=6 Hz), 1.36 (1.5H, d, J=6 Hz), 1.58 (1.5H, s), 1.61 (1.5H, s), 2.92 (3H, s), 3.53 (3H, s), 5.29 (1H, d, J=8 Hz), 5.95 (0.5H, s), 6.01 (0.5H, br.), 6.11 (0.5H, br.), 6.28 (0.5H, s).

MS (SIMS) M/Z 742 (M + H)⁺, 408, 334, 160.

Method B

A mixture of elsamicin A (66 mg), benzaldehyde dimethylacetal (185 mg) and TsOH (25 mg) in CH₂Cl₂ (5 ml) was kept at room temperature for 2 hours. A saturated aqueous NaHCO₃ solution (ca. 10 ml) and CH₂Cl₂ (10 ml) were added to the reaction mixture. The organic layer was separated and evaporated under reduced pressure to give a yellow solid, which was purified by silica gel column chromatography using MeOH in CHCl₃ as an eluant to give 45 mg (60%) of 6c. The HPLC and spectral data of compound 6c obtained here was indistinguishable to those obtained in Method A.
EXAMPLE 10

Synthesis of 2”-N-Benzylxycarbonyl-3',4'-0-methoxy-methylideneelsamicin A (7d)

A solution of compound 4 (62 mg), orthoformic acid trimethyl ester (0.2 ml) and p-toluenesulfonic acid (5 mg) in CH$_2$Cl$_2$ (3 ml) was stirred at room temperature for 2 hours, and the reaction mixture was diluted with CH$_2$Cl$_2$ (10 ml), washed with saturated aqueous NaHCO$_3$, dried over Na$_2$SO$_4$, and evaporated in vacuo to give 65 mg (100%) of compound 7d.

MP 139-141°C. IR $\nu_{\text{max}}$ (KBr) cm$^{-1}$ 3400, 1725, 1510, 1375, 1255, 1240, 1070. UV $\lambda_{\text{max}}$ (MeOH nm ($\epsilon$) 236 (35700), 267 (34000), 333 (5800), 380 (8090), 399 (13000), 422 (13900). $^1$H NMR (CDCl$_3$ + D$_2$O) $\delta$ 1.32 (3H, d, J=6 Hz), 1.39 (3H, d, J=6.5 Hz), 1.56 (3H, s), 2.88 (3H, s), 3.92 (3H, s), 3.96 (3H, s), 6.25 (1H, d, J=8 Hz), 5.65 (1H, d, J=4 Hz), 5.93 (2H, s).

EXAMPLE 11

Synthesis of 3',4'-0-Methoxymethylideneelsamicin A (6d)

A solution of compound 7d (57 mg) in 75% aqueous tetrahydrofuran (4 ml) was hydrogenated in the presence of 10% Pd-C (30 mg) for 1.5 hours at room temperature. The reaction mixture was filtered off and the filtrate was concentrated to dryness. The residue was purified by column chromatography on silica gel to give 35.5 mg (73%) of compound 6d.

MP 209-211°C (dec.). IR $\nu_{\text{max}}$ (KBr) cm$^{-1}$ 3400, 1720, 1680, 1375, 1255, 1205, 1120, 1075. UV $\lambda_{\text{max}}$ (MeOH nm ($\epsilon$) 236 (33900), 266 (30800), 331 (5200), 398 (10800), 419 (11200). $^1$H NMR (CDCl$_3$ + CD$_2$OD) $\delta$ 1.0-1.4 (6H, m), 1.57 (3H, s), 2.62 (3H, s), 3.38 (3H, s), 3.50 (3H, s), 5.44 (1H, d, J=8 Hz), 5.87 (2H, m).

MS (SIMS) M/2 697 (M + 2H)$^+$, 363, 334, 202, 160.
EXAMPLE 12

Synthesis of 4'-0-Tetrahydropyranylelsamicin A (8)

A solution of elsamicin A (110 mg) and dihydropyran (0.5 ml) in dimethylformamide (5 ml) was acidified by a small excess molar equivalents of TsOH and kept at room temperature overnight. A saturated aqueous NaHCO₃ solution (20 ml) was added to the reaction mixture and the mixture was subjected to a column of Diaion HP-20 (ca. 100 ml). The column was washed with water and eluted with aqueous CH₃CN. The yellowish fractions of the eluate were pooled and evaporated in vacuo to give a yellow amorphous solid, which was dissolved in 0.05N TsOH in MeOH (5 ml). After one hour, NaHCO₃ was added to the reaction mixture and the mixture was filtrated. The filtrate was concentrated and the residue was purified by preparative TLC to give 16 mg (13%) of the title compound.

MP 202-205°C. IR ν_max (KBr) cm⁻¹ 1695, 1610, 1375, 1255, 1075, 780. UV λ_max (MeOH nm (ε)) 236 (34000), 266 (29500), 333 (5250), 379 (6850), 399 (10500), 421 (11300). ¹H NMR (CDCl₃ + CD₃OD) δ 1.44 (3H, s), 2.73 (3H, s), 3.48 (2H, s), 5.62 (1H, d, J=8 Hz), 5.93 (1H, d, J=4 Hz).

MS (SIMS); M/Z 738 (M + H)⁺, 404, 334, 160, 85.
The claims defining the invention are as follows:

1. A compound having the formula

![Chemical Structure Image]

wherein Z is alkylidene, cycloalkylidene, arylalkylidene or alkoxyalkylidene.
2. A compound having the formula:

![Chemical Structure](image)

3. The compound of Claim 1 which is 3',4'-O-isopropylideneelsamicin A; 3',4'-O-cyclohexylideneelsamicin A; 3',4'-O-benzylideneelsamicin A; or 3',4'-O-methoxymethylideneelsamicin A.

4. A process for producing the compounds of Claim 1 which comprises treating 2"-N-protected elsamicin A with dimethyl acetals in the presence of an acid catalyst and subsequent deprotection.

5. A process for producing the compounds of Claim 1 which comprises treating elsamicin A with dimethyl acetals in the presence of an acid catalyst.

6. A process for producing the compounds of Claim 2 which comprises treating elsamicin A with dihydropyran in the presence of an acid catalyst, then treating with p-toluenedisulfonic acid in methanol.
7. A pharmaceutical formulation which comprises as an active ingredient a compound claimed in any one of claims 1-3, associated with one or more pharmaceutical acceptable carriers or diluents.

8. A compound as claimed in anyone of claims 1-3, for use as an antitumor agent.
9. A compound of claim 1 substantially as hereinbefore described with reference to any one of Examples 5, 7, 9 or 11.
10. A compound of claim 2 substantially as hereinbefore described with reference to Example 12.
11. A process substantially as hereinbefore described with reference to any one of the Examples.

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