CHEMICAL MODIFICATION OF ANTIBIOTIC STREPTONIGRIN;
SYNTHESIS AND PROPERTIES OF 2'-DECARBOXY-2'-AMINOSTREPTONIGRIN
(STREPTONIGRONE-2'-IMINE)

V. V. Tolstikov, M. N. Preobrazhenskaya,
J. Balzarini and E. De Clercq*

Institute of New Antibiotics of Russian Academy of
Medical Sciences,
Moscow 119867, Russia

Rega Institute for Medical Research,
Katholieke Universiteit Leuven,
B-3000 Leuven, Belgium

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Whereas the cytostatic properties of the antibiotic
streptonigrin (1) and its amides and esters have
been studied1~4), structure-activity relationships for
other derivatives of 1, as well as for derivatives of
antibiotic streptonigrone (2), which is a minor
component of the streptonigrin complex5), remain
to be investigated. In this paper we describe the
synthesis of 2-decarboxy-2-aminostreptonigrin (8),
which can be considered as an analogue of
streptonigrone.

1 was converted to the mixed anhydride (3) (1
equiv CICOOEt; 1.1 equiv Et3N; THF; 0°C; 0.5
hour), which under the action of NaN3 (3 equiv
H2O; 0°C; 1 hour) afforded, after extraction with
EtOAc, the corresponding azide (4), (IR v max
(CHCl3) cm⁻¹ 2250 (N₃), 1750 (C=O)), which was
immediately used in the next step of conversion (see
Scheme 1). All the new compounds were separated
by TLC using Kieselgel 60 (Merck) plates in
chloroform- acetone-methanol, 8 : 1 : 1 mixture.

Decomposition of 4 in anhydrous conditions (dry
toluene, reflux for 2 hours) led via streptonigrin-2'-
decarboxy-2'-isocyanate (5) to the dimer 6 (70%);
MP 164~165°C; RF 0.60; ¹H NMR (Varian VX400
instrument, 400 MHz, CDCl₃) δ 8.78 (1H, d, 
J₃,₄ = 8 Hz, 4-H), 8.77 (1H, d, 4-H), 8.32 (1H, d,
3-H), 8.27 (1H, d, 3-H), 7.05 (1H, d, J₁₁',₁₂' = 8.5 Hz,
12'-H or 11'-H), 6.99 (1H, d, 12'-H or 11'-H), 6.72
(2H, s, 8'-OH, 2-groups), 6.70 (1H, d, 11'-H or
12'-H), 6.65 (1H, d, 11'-H or 12'-H), 6.00 (4H, brs,
7-NH₂, 2-groups), 5.10 (4H, brs, 5'-NH₂, 2-
groups), 4.08 (3H, s, -OCH₃), 4.07 (3H, s, -OCH₃),
3.99 (3H, s, -OCH₃), 3.98 (3H, s, -OCH₃), 3.97
(3H, s, -OCH₃), 3.95 (3H, s, -OCH₃), 2.09 (3H, s,
3'-CH₃), 2.04 (3H, s, 3'-CH₃); CI-MS (Jeol JMS-
DX 300 instrument with NH₃ as a reagent gas)
m/z 504 (3H, C₂₅H₂₂N₅O₇), 475 (3H, C₂₄H₂₁N₅O₆); EI-MS
(Varian-MAT-112 spectrometer at 210~230°C ion source and 70 eV
electron energy, samples being introduced by direct
insertion) m/z 503.1 ([M]⁺, C₂₅H₂₁N₅O₆). Dou-
bling of the signals of all protons in the ¹H NMR
spectrum of 6 demonstrates the absence of symmetry
in the dimer structure (similar to pyridyl-2-
isocyanate dimer6)).

Decomposition of 4 in boiling tert-BuOH (4
hours, evaporation, isolation by preparative TLC)
gave 2-decarboxy-2'-(tert-butoxycarbonylaminio)-
streptonigrin (7), amine 8 and dimer 6. Compound
7 was obtained in 40% yield (MP 162~164°C (dec);
RF 0.85; ¹H NMR (400 MHz, DMSO-d₆) δ 8.65 (1H,
d, J₃,₄ = 8 Hz, 4-H), 8.15 (1H, d, 3-H), 6.67 (1H, d,
J₁₁',₁₂' = 8.5 Hz, 12'-H or 11'-H), 6.45 (1H, d, 11'-H
or 12'-H), 3.71 (3H, s, -OCH₃), 3.69 (3H, s, -OCH₃),
3.67 (3H, s, -OCH₃), 1.92 (9H, s, O-(CH₂)₃), 1.86
(3H, s, 3'-CH₃)). Amine 8 was obtained in 30% yield
(MP 192~194°C; RF 0.40; ¹H NMR (400 MHz
CDCl₃) δ 8.77 (1H, d, J₃,₄ = 8 Hz, 4-H), 8.32 (1H,
d, 3-H), 6.81 (1H, d, J₁₁',₁₂' = 8.5 Hz, 12'-H), 6.63
(1H, d, 11'-H), 5.04 (2H, brs, 5'-NH₂), 4.05 (3H, s,
-OCH₃), 3.96 (3H, s, -OCH₃), 3.93 (3H, s, -OCH₃),
1.93 (3H, s, 3'-CH₃); ¹³C NMR (Varian XL 100,
100 MHz, CDCl₃) δ 179.8 (C-8), 177.1 (C-5), 161.1
(C-8a), 152.6 (C-10'), 148.4 (C-2'), 147.3 (C-8'), 143.9
(C-5'), 140.3 (C-2), 137.2 (C-7), 136.9 (C-3), 136.4
(C-9'), 132.5 (C-6), 128.3 (C-6'), 124.9 (C-4'), 125.4
(C-4a), 124.3 (C-3), 121.9 (C-12), 119.6 (C-4') 115.1
(C-7), 103.6 (C-11'), 59.8 (-OCH₃), 59.1 (-OCH₃),
54.9 (-OCH₃), 13.7 (3'-CH₃); CI-MS m/z 477 (M,
C₂₄H₂₂N₂O₆). Dimer 6 was isolated in 10% yield.

Treatment of 4 with a mixture of CF₃COOH - H₂O
(1 : 5, steam bath; 1 hour) with subsequent evap-
oration and neutralization (1.5 equiv Et₃N in
CHCl₃; 0°C; 0.5 hour), extraction with EtOAc and
washing (5% aq NaHCO₃) afforded the amine 8
in 65% yield. Amine 8 was obtained also by
cleavage of 6 in CF₃COOH-CH₂Cl₂ (1 : 1; 0°C to
20°C; 2 hours) in 80% yield. By using diphenylphos-
sphoryl azide (DPPA)⁷ for the direct conversion of
1, we prepared amine 8 in 40% yield (1.5 equiv
DPPA; 1.5 equiv Et₃N; dioxane - tert-BuOH (5 : 1);
80°C; 36 hours) (see Scheme 1). Amine 8, under the
action of 0.5m HCl in MeOH, afforded the
hydrochloride (9) (95%; MP 235°C (dec)).

The inhibitory effects of compound 8, its
hydrochloride 9 and streptonigrone 2 in comparison
Scheme 1.

\[ \text{STN-COON}_3 \]

\[ \text{STN-N}(\text{CH}_3)_3 \]

\[ \text{STN-NH}_2 \]

Scheme 2.

\[ \text{2} \]

\[ \text{8} \]
Table 1. Inhibitory effects of streptonigrin (1), streptonigrone (2) and derivatives 8 and 9 on the proliferation of murine leukemia (L1210), human T-lymphoblast (MOLT-4F) and human T-lymphocyte (MT-4) cells.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition of tumor cell proliferation IC$_{50}$ * (µM/ml)</th>
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<tbody>
<tr>
<td></td>
<td>L1210</td>
</tr>
<tr>
<td>1</td>
<td>0.044±0.009</td>
</tr>
<tr>
<td>2</td>
<td>2.57±0.28</td>
</tr>
<tr>
<td>8</td>
<td>2.46±0.24</td>
</tr>
<tr>
<td>9</td>
<td>2.91±0.08</td>
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</tbody>
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* 50% inhibitory concentrations, or concentrations required to inhibit cell proliferation by 50%.

with compound 1, which can be considered as 2’-imine of streptonigrone (see Scheme 2), on the proliferation of murine leukemia (L1210), human T-lymphoblast (MOLT-4F) and human T-lymphocyte (MT-4) cells are shown in the Table 1. Compounds 8 and 9 were less cytotoxic than 1, the IC$_{50}$ values for 2, 8 and 9 being similar. Compounds 8, 9 and 2 did not prove effective against HIV-1 or HIV-2 induced cytopathogenicity in MT-4 cells at subtoxic concentrations. The assays for measuring inhibition of tumor cell growth L1210, MOLT-4F, MT-4 and anti-HIV activity in MT-4 cells were performed as previously described.

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