CHAPTER III

EXPERIMENTAL

1. Source of animal materials

The nudibranch, *Jorunna funebris*

The nudibranch was identified by E. F. Kelaart 1858 as *Jorunna funebris*, a shell-less marine mollusc. The rhinophores are black with a white base. The body is white with irregular black rings and its mantle is covered in small spiculate papillae (caryophyllidia) (Bidgrain, 2005). *J. funebris* was collected by using SCUBA in the vicinity of Sichang Island at the depth of 3-5 meters in March 2004. Its egg ribbons were obtained in November 2004. The animal materials were frozen at -20 °C until used.

The sponge, *Xestospongia* sp.

The sponge sample was identified as *Xestospongia* sp. #2133 (Family Petrosiidae) by Dr. John N. A. Hooper and the voucher specimens have been deposited at Queensland Museum (serial no. QMG 306998), Australia and the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University. The sponge was collected by SCUBA in the vicinity of Si Chang Island at the depth of 3-5 meters in May 2005 and frozen at -20 °C until used.

2. General techniques

2.1 Chromatography

2.1.1 Analytical thin-layer chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel GF_{254} (E. Merck)
Layer thickness	:	250 μm
Distance	:	5 and 8 cm
Temperature	:	Room temperature (25-32 °C)
Detection	:	1. Visual detection under daylight

Most of the renieramycins appear as yellow to orange spots.

2. Ultraviolet light at wavelength of 254 nm
3. Spraying with anisaldehyde-sulfuric acid reagent (0.5 ml anisaldehyde, 3 ml EtOH, 0.5 ml 97% sulfuric acid, and 0.1 ml glacial acetic acid) and heating until colors develop

2.1.2 Column chromatography

2.1.2.1 Gel filtration chromatography

Adsorbent : Sephadex LH-20 (Pharmacia Biotech AB)

Packing method : The adsorbent was suspended in the eluant and left standing to swollen for 24 hours before using, then poured into the column and allowed to settle tightly.

Sample loading : The sample was dissolved in a small volume of the eluant and loaded on top of the column.

Detection : Fractions were examined by TLC under ultraviolet light at wavelength of 254 nm, and spraying with anisaldehyde-sulfuric reagent.

2.1.2.2 Normal phase flash column chromatography

Adsorbent : Silica gel (No. 7734), particle size 0.040-0.063 mm (230-400 mesh ASTM) (E. Merck)

Packing method : The adsorbent was suspended in the eluant. The slurry of the adsorbent was poured into the column, tapped and pressed down under and air pump, and then allowed to settle for 1 hour.

Sample loading : The sample was dissolved in a small volume of the eluant and loaded on top of the column.

Detection : Fractions were examined in the same manner as described above.

2.1.2.3 Reversed phase column chromatography

Adsorbent : Kieselgel 60 RP-18 (No. 1.10167), partical size 40-63 μm (E. Merck)

Packing method : The adsorbent was suspended in the eluant. The slurry of the adsorbent was poured into the column, tapped and then allowed to settle for 1 hour.

Sample loading : The sample was dissolved in a small volume of the eluant and loaded on top of the column.

Detection : Fractions were examined in the same manner as described above.

2.2 Spectroscopy

2.2.1 Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy

^{1}H and ^{13}C NMR, DEPT 90 and 135, ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY spectra were obtained on a JEOL-JNM-LA 500 FT-NMR spectrometer at 500 and 125 MHz, respectively (Meiji Pharmaceutical University, Japan), and on a Bruker AVANCE DPX-300 FT-NMR spectrometer, operating at 300 MHz and 75 MHz, respectively (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University). Solvents for NMR spectra were deuterated chloroform ($CDCl_3$). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal and TMS as internal standard. Proton-detected heteronuclear correlations were measured using HMQC (optimized for $^nJ_{HC}$ = 145 Hz) and HMBC (optimized for $^nJ_{HC}$ = 8 Hz) pulse sequences. Proton-proton correlations through the space were observed by using NOESY.

2.2.2 Mass spectrometry

Mass spectra were recorded on JMS-DX 302 and JMS-700 mass spectrometers with a direct inlet system operating at 70 eV.

2.2.3 Ultraviolet (UV) absorption spectroscopy

Ultraviolet-visible (UV) absorption spectra were obtained on a Milton Roy Spectronic 3000 Array spectrophotometer and a Shimadzu UV-Visible spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.2.4 Circular dichroism (CD) spectroscopy

CD spectra were obtained using a JASCO-J715 spectropolarimeter (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.2.5 Infrared (IR) absorption spectroscopy

Infrared spectra (IR) were obtained on a Perkin Elmer 2000 FT-IR spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.2.6 Optical rotation

Optical rotations were measured on a Horiba-SEPA polarimeter (Meiji Pharmaceutical University, Japan) and a Perkin-Elmer 341 polarimeter (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

3. Chemicals

Reagents	Distributors
Acetyl chloride	Nacalai tesque
Benzoyl chloride	TCI
n-Butyric anhydride	Nacalai tesque
Chloroform	Nacalai tesque
3-Chloropropionyl chloride	Keisai kogyo
Cyclohexanecarbonyl chloride	Aldrich
Cyclohexanecarboxylic acid	TCI
1-Cyclohexene-1-carboxylic acid	Acros
3-Cyclohexene-1-carboxylic acid	TCI
Cyclopentanecarbonyl chloride	Aldrich
1-Cyclopentenecarboxylic acid	Wako
3-Cyclopentene-1-carboxylic acid	Aldrich
Dichloromethane	Wako
Diethyl ether	Nacalai tesque
3,3-Dimethylacryloyl chloride	Acros
4-Dimethylaminopyridine (DMAP)	Nacalai tesque

Ethyl acetate	Wako
n-Heptanoic anhydride	TCI
n-Hexanoic anhydride	TCI
Isobutyric anhydride	TCI
Isopropyl chlorocarbonate	Nacalai tesque
1.0 M Lithium aluminium hydride in THF	Aldrich
DL-2-Methylbutyric acid	TCI
DL-2-Methylbutyryl chloride	Kanto
2-Methoxybenzoyl chloride	TCI
4-Methoxybenzoyl chloride	TCI
3-Methylcrotonic acid	TCI
Molecular sieve type 4°A	Fluka
4-Nitrobenzoyl chloride	TCI
20% Palladium hydroxide on activated charcoal	Kawaken
Potassium carbonate	Nacalai tesque
Pyridine	Nacalai tesque
4-Quinolinecarboxylic acid	TCI
Sodium formate	Nacalai tesque
Thionyl chloride	Keisai kogyo
Silver nitrate	Nacalai tesque
Sodium sulfate, anhydrous	Merck
Sulfuric acid concentrated	Nacalai tesque
Tetrahydrofuran	Nacalai tesque

4. Solvents

All solvents were either analytical or laboratory grade and were redistilled prior to use. For chemical reactions, ethyl acetate, and dichloromethane were dried over molecular sieve type 4°A. Tetrahydrofuran was refluxed and distilled over sodium metal.

5. Biological activity assay

5.1 Cytotoxic assay

A single-cell suspension of each cell line (1.5-3 x 10^3 cells/well) was added to the serially diluted tested compounds in a microplate. Then, the cells were cultured

for 3 or 4 days. Cell growth was measured with a cell counting kit (DOJINDO, Osaka, Japan) or MTT colorimetric assay (Mosmann, 1983). IC_{50} was expressed as the concentration at which cell growth was inhibited by 50 % compared with the control.

5.2 Oligonucleotide microarray

Drugs and cells culture

Renieramycin M and JorunnamycinC were isolated from KCN-pretreated sponge, *Xestospongia* sp. and nudibranch, *Jorunna funebris*, respectively. Human cancer cell lines HCT116 (colon) and MDA-MB-435 (breast) were grown in RMPI1640 culture medium (Sigma) containing supplements which were 10% (v/v) heat-inactivated fetal bovine serum (Equitech-BIO) and a solution of 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen). Cell culture was performed at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

In vitro cell growth inhibition assay (IC_{50})

Exponentially growing cells (1,500 cells per well for HCT116 and 3,000 cells per well for MDA-MB-435) were seeded into 96-well microtiter plates and pre-cultured for one day. Both of test compounds (renieramycin M and jorunnamycin C) were dissolved at 20 μM in dimethyl sulfoxide (DMSO) and further diluted with the culture medium to prepare 3-fold serial dilutions with the maximum concentration being 100 nM after the following addition into each well. The obtained dilutions were added to the plates, and then incubation was continued for additional three days. The antiproliferative activity was measured by the MTT colorimetric assay (Mosmann, 1983). The absorbance was measured by using a TECAN microplate reader at a test wavelength of 540 nm and a reference wavelength of 660 nm to be taken as an index of the number of viable cells. The IC_{50} value (the drug concentration required for 50% cell growth inhibition) was determined by the least-squares method.

Oligonucleotide microarray-based gene expression analysis (Martinez *et al.*, 2001).

HCT116 and MDA-MB-435 cells were each plated at 2.0×10^6 cells per dish in 10-cm diameter dishes with 10 ml fresh RPMI1640 medium. After 24 h pre-

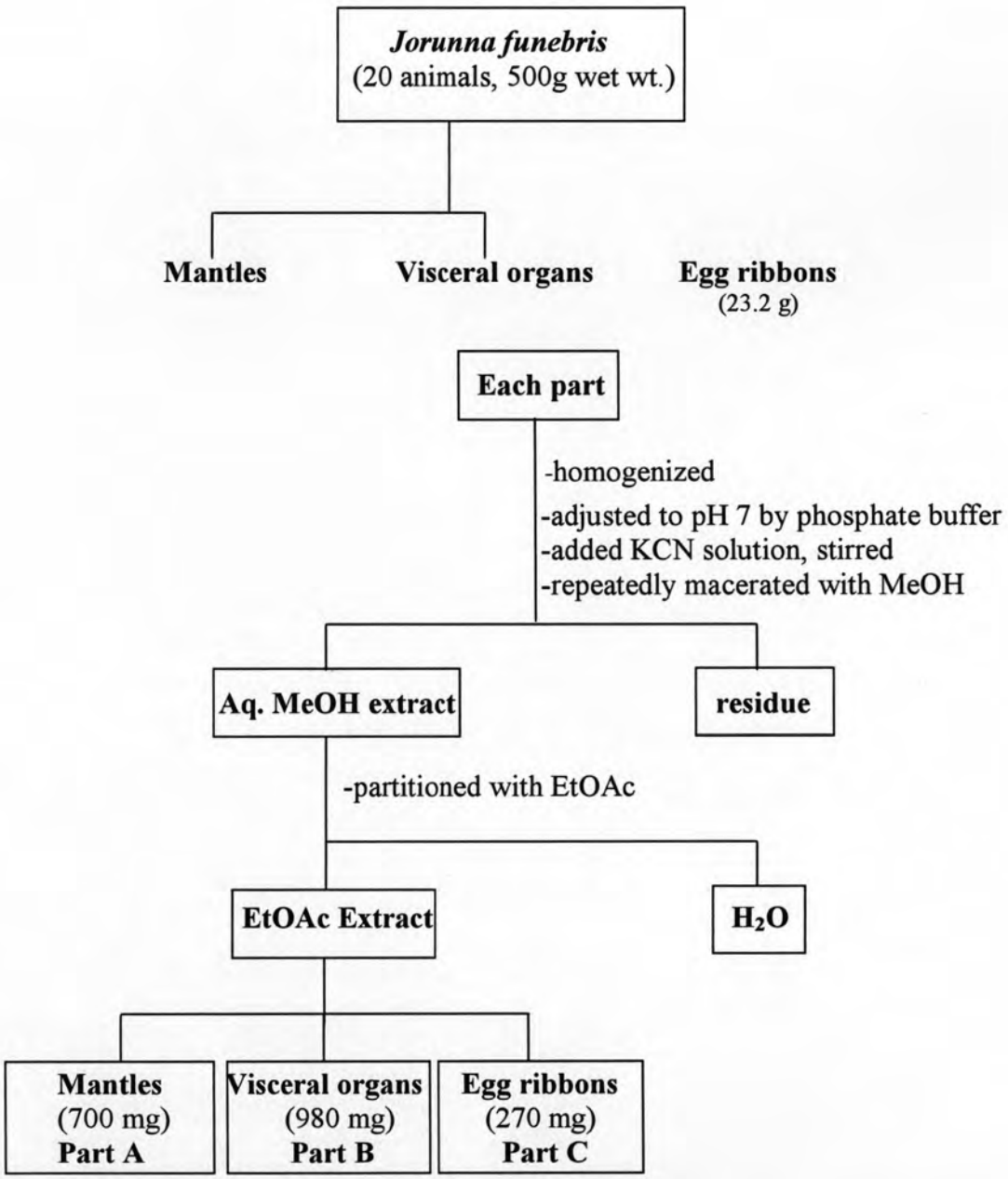
incubation, HCT116 cells were treated with the 2 x IC_{50} concentration of each test compound (33 nM of renieramycin M or 55 nM of jorunnamycin C) for 4 h and 12 h. MDA-MB-453 cells were also treated with the 2 x IC_{50} concentration of each test compound (13 nM renieramycin M or 33 nM Jorunnamycin C) for 4 h and 12 h. DMSO (0.2%) treatment was used as a control. Total RNA was extracted from the cells using Trizol (Invitrogen). The extracted RNA was purified using the RNeasy kit (Qiagen). Double-stranded cDNA was synthesized from 5 μg of total RNA by means of the SuperScript™ double-stranded cDNA synthesis kit (Invitrogen) with T7-$d(T)_{24}$ primer. The cDNA product was purified by phenol/chloroform/isoamyl alcohol extraction. *In vitro* transcription was carried out by means of the GeneChip IVT Labeling kit (Affymetrix). The resulting biotin-labeled cRNA was purified using the RNeasy kit. The cRNA was fragmented at 94°C for 35 min, and then hybridized for 16 h onto the Affymetrix GeneChip Human Genome Focus array capable of probing about 8,500 transcripts. The probe arrays were washed and stained with streptavidin-phycoerythrin and biotinylated goat anti-streptavidin on an Affymetrix fluidics station. Fluorescence intensities were captured with a Hewlett-Packard confocal laser scanner. All quantitative data were processed using the RMA (robust multi-array average) method. Up- and down-regulated genes were selected according to the following criteria: i) at least 2-fold change as compared with the control data; and ii) statistical significance ($p < 0.05$) in triplicate data. Gene ontology analysis was used for illuminating compound-related biological processes, cellular components, and molecular functions.

6. Extraction and isolation of the isoquinolines from *Jorunna funebris*

6.1 Preparation of the crude extracts from *J. funebris*

Twenty animals (500 g, wet weight) were carefully dissected into two parts, the mantles and the visceral organs. The mantles, the visceral organs and the egg ribbons (23.2 g, wet weight) were separately homogenized with phosphate buffer and pH was adjusted to 7. Then, 10% KCN solution was added very slowly to the suspension, and the mixture was macerated three times with methanol at room temperature for 24 h. The combined methanol extracts were filtered, and the filtrate was concentrated *in vacuo* to give the syrupy residue that was further partitioned between ethyl acetate and water. The ethyl acetate layer was concentrated to dryness to give the crude

extracts of the mantles (Part A: 700 mg), the visceral organs (Part B: 980 mg), and the egg ribbons (Part C: 270 mg) as shown in Scheme 3.

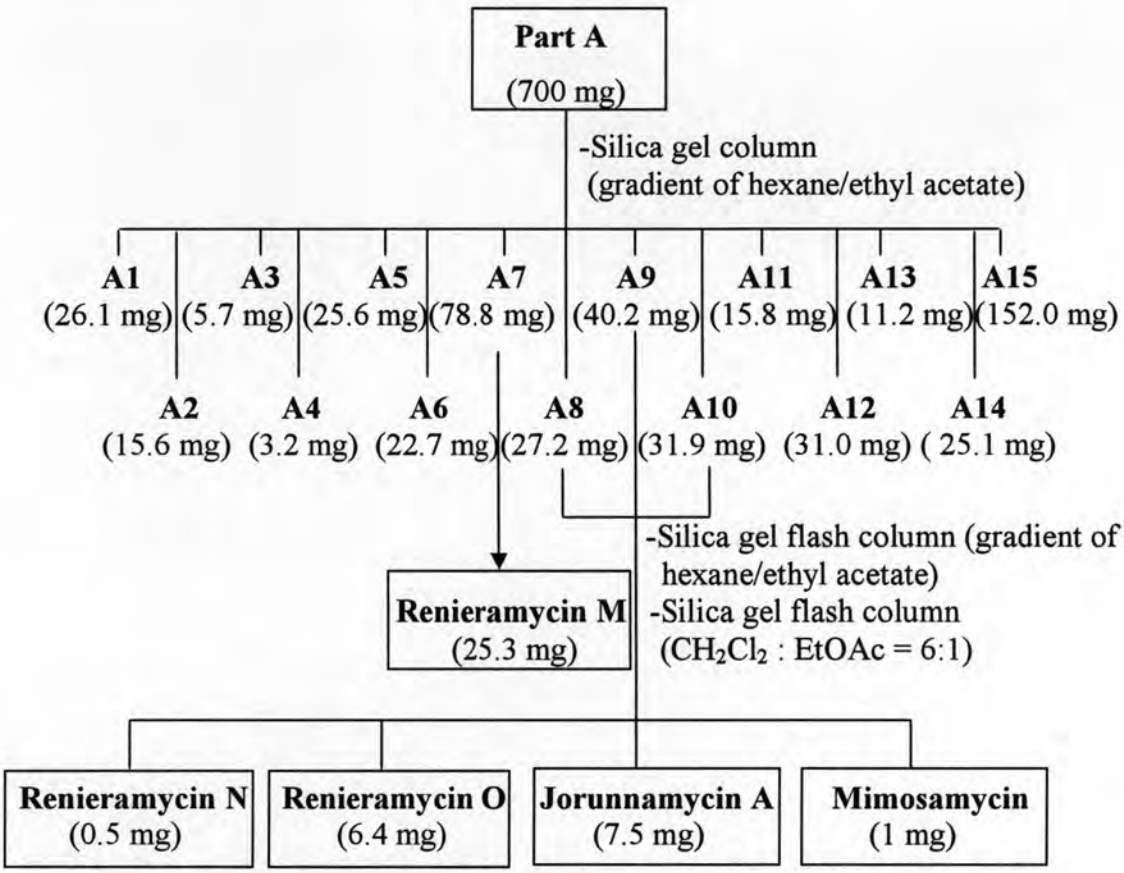


Scheme 3 Preparation crude extracts from *J. funebris*.

6.2 Isolation of the isoquinolines from *J. funebris*

The crude ethyl acetate extract of the mantles (Part A: 700 mg) was chromatographed on a silica gel column (2.5 cm inner diameter) with a step gradient of hexane/ethyl acetate to give fifteen fractions **A1-A15**. Fraction **A7** gave an orange precipitate of renieramycin M (25.3 mg). Fractions **A8-A10** were combined and

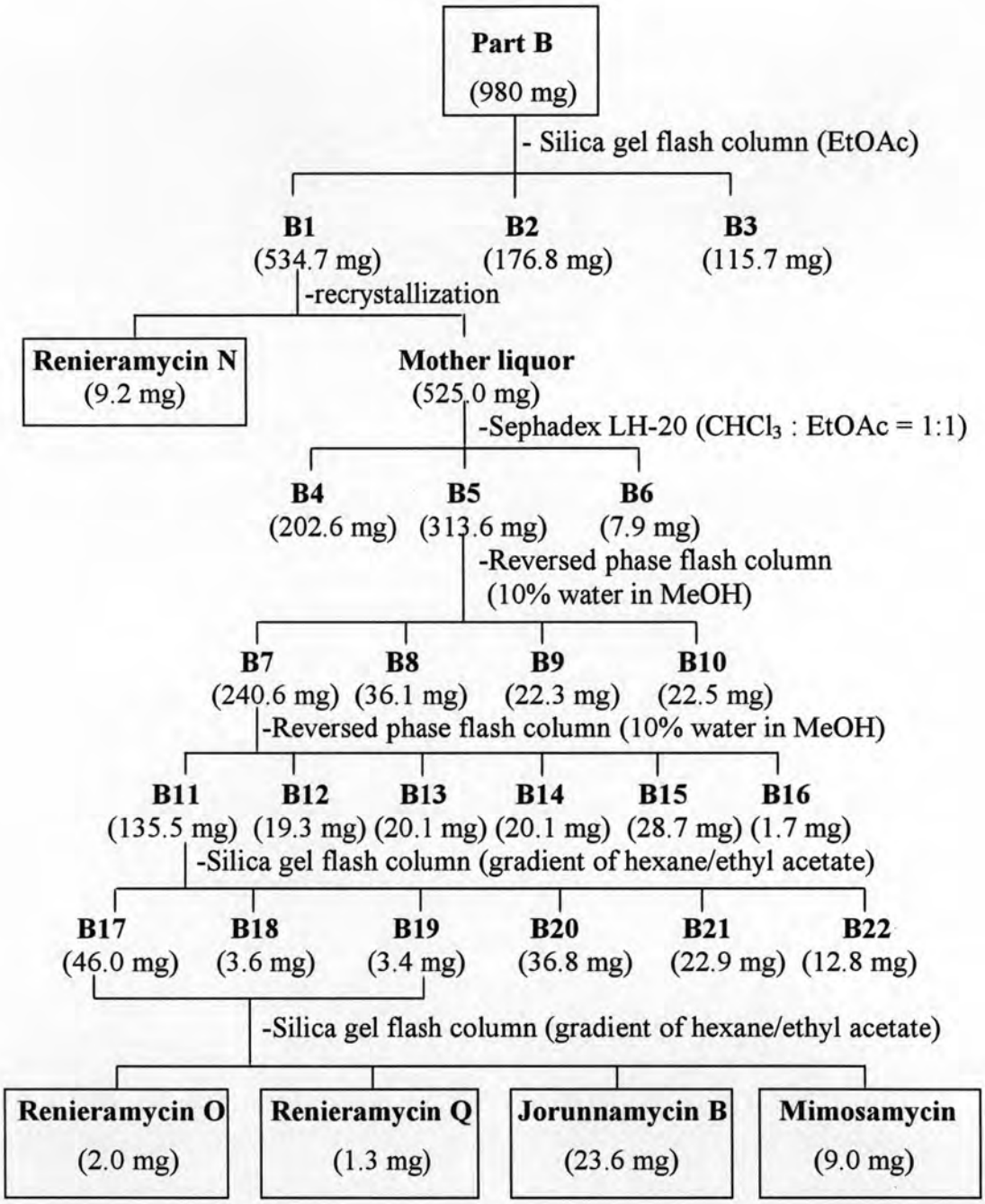
further fractionated on two silica gel flash columns (1.0 cm inner diameter) with step gradient of hexane/ethyl acetate and dichloromethane/ethyl acetate 6:1, respectively, to afford renieramycin N (0.5 mg), renieramycin O (6.4 mg), jorunnamycin A (7.5 mg), and mimosamycin (1.0 mg) as shown in Scheme 4.



Scheme 4 Isolation of the isoquinolines from the mantles of *J. funebris*.

The crude ethyl acetate extract of the visceral organs (Part B: 980mg) was separated on a silica gel flash column (2.5 cm inner diameter) with 100% ethyl acetate to give 3 fractions, **B1-B3**. Fraction **B1** was recrystallized by ethanol to give a yellow solid of renieramycin N (9.2 mg). The mother liquor of fraction **B1** was subjected to a Sephadex LH-20 column (2.5 cm inner diameter) by using chloroform/ethyl acetate as a solvent to give 3 fractions **B4-B6**. Fraction **B5** was applied to a reversed phase flash column (2.5 cm inner diameter) using 10% H_2O in methanol as a solvent to give 4 fractions **B7-B10**. Fraction **B7** was separated by using a reversed phase flash column (1.5 cm inner diameter) with 10% H_2O in methanol as a solvent to give 6 fractions **B11-B16**. **B11** was subjected to silica gel flash column (1.5 cm inner diameter) with gradient solvent of hexane/ethyl to give 6 fractions **B17-B22**.

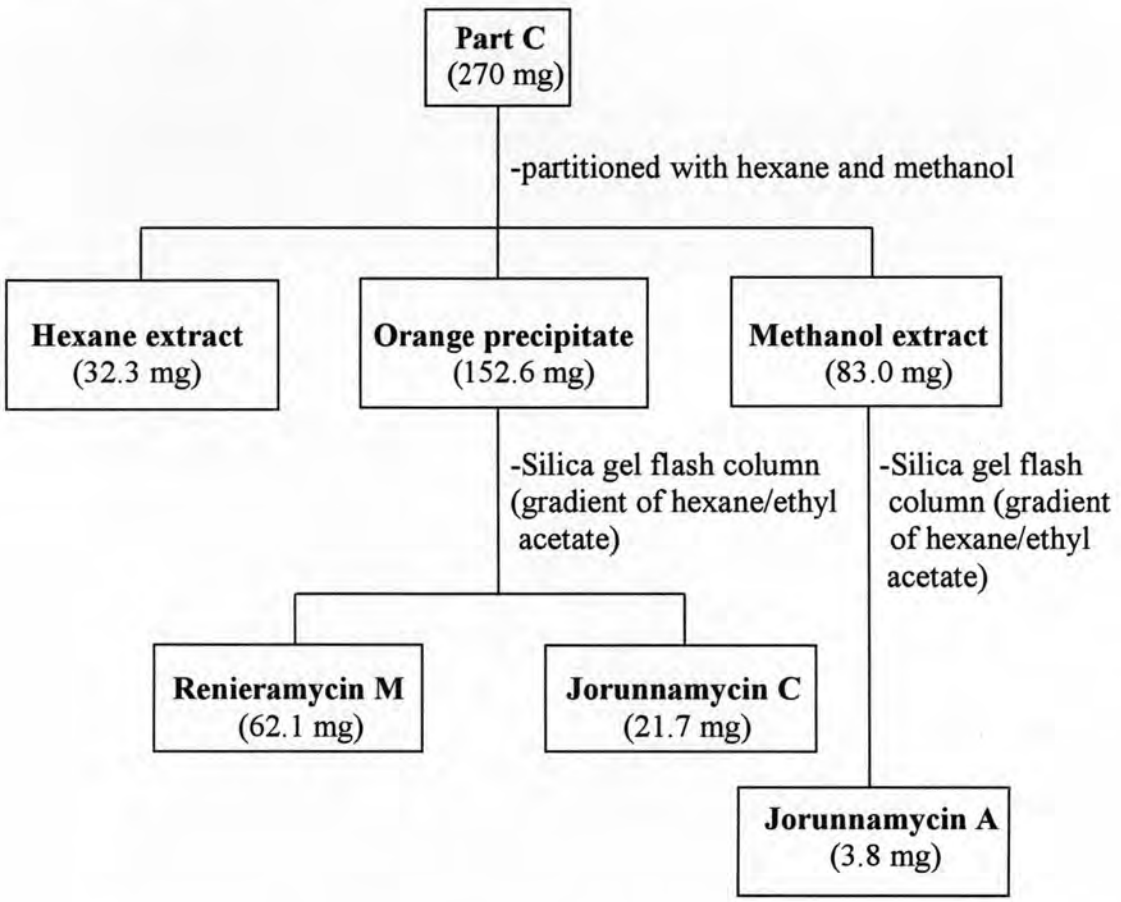
Fractions **B17** and **B19** were combined and rechromatographed in a manner similar to that of fraction **B11** to provide renieramycin O (2.0 mg), renieramycin Q (1.3 mg), jorunnamycin B (23.6 mg), and mimosamysin (9.0 mg) as shown in Scheme 5.



Scheme 5 Isolation of the isoquinolines from the visceral organs of *J. funebris*.

The crude ethyl acetate extract (Part C: 270 mg) of the egg ribbons was suspended in methanol and extracted with hexane to give an orange precipitate (152.6 mg), which was subsequently separated on a silica gel flash column (1.5 cm inner

diameter) with hexane/ethyl acetate as a solvent to give renieramycin M (62.1 mg) and jorunnamycin C (21.7 mg). The methanol extract (83.0 mg) was subjected to silica gel flash column (1.0 cm inner diameter) chromatography to give jorunnamycin A (3.8 mg) as shown in Scheme 6.

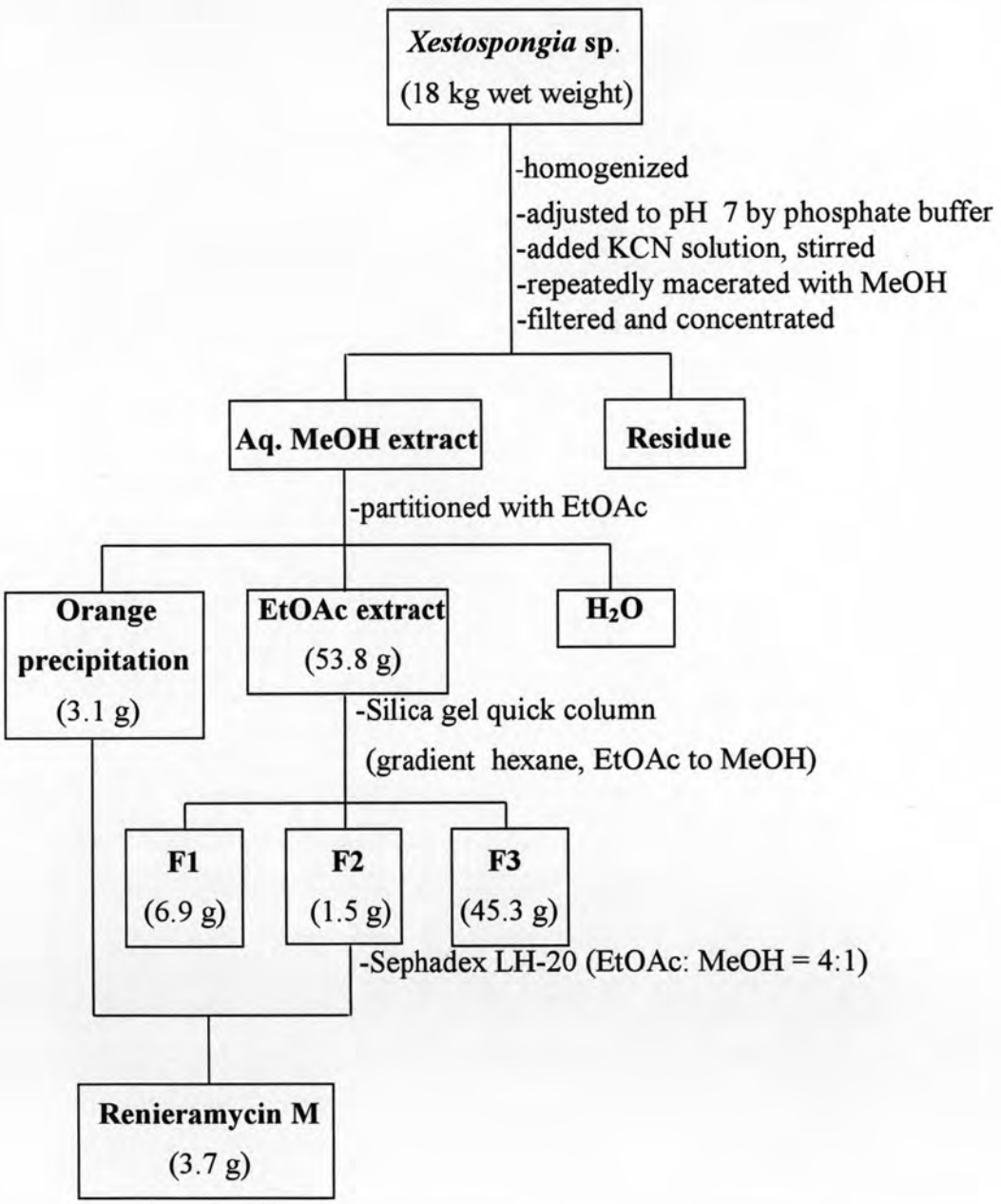


Scheme 6 Isolation of the isoquinolines from the egg ribbons of *J. funebris*.

7. Isolation of renieramycin M from *Xestospongia* sp.

The sponge *Xestospongia* sp. (18 kg. wet weight) was homogenized, and adjusted to pH 7 with phosphate buffer solution. Then, 10% potassium cyanide solution was added to the homogenized solution, and the mixture was stirred for 5 h. After that, the mixture was macerated with methanol for 2 days and was filtered, and the filtrate was concentrated under reduced pressure. The aqueous methanolic solution was partitioned with ethyl acetate, and concentrated to give an ethyl acetate extract (53.8 g, 0.3% yield based on the sponge wet weight) and an orange precipitate of renieramycin M (3.1 g).

The ethyl acetate extract was further chromatographed on a silica gel quick column (10.5 cm inner diameter) with gradients of hexane, ethyl acetate to methanol as mobile solvents to give 3 fractions, **F1-F3**. **F2** (1.5 g) was further separated on a Sephadex LH-20 column (2.5 cm inner diameter) with ethyl acetate/methanol 4:1 as a solvent to provide renieramycin M (600 mg). The total amount of renieramycin M isolated from the ethyl acetate extract was 3.7 g (6.5% yield based on the ethyl acetate extract).



Scheme 7 Isolation of renieramycin M from *Xestospongia* sp.

8. Physical and spectral data of the isolated compounds from *J. funebris* and *Xesospongia* sp.

8.1 Jorunnamycin A or deangeloylrenieramycin M

Jorunnamycin A or deangeloylrenieramycin M was obtained as a pale yellow amorphous solid (7.5 mg (1.07%) and 3.8 mg (1.41%) from the ethyl acetate extracts of the mantles and the egg ribbons, respectively).

$[\alpha]^{25}_{D}$: -270.6 (c = 1.0, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 8
269 (4.26)

CD : Δε nm (c = 103.3 μM, methanol, 24 °C); Figure 9
-2.9 (352), -1.5 (300), -10.2 (280), +3.2 (257), -1.8 (230)

FABMS : *m/z* (% intensity); Figure 7
494 ($[M+H]^+$, 8), 467 (4), 309 (20), 220 (15), 155 (59), 119 (100), 85 (9)

HR-FABMS : *m/z* 494.1910 $[M+H]^+$, (calcd for $C_{26}H_{28}N_3O_7$, 494.1927)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 10
3631, 3368, 3015, 2945, 2840, 1656, 1449, 1375, 1311, 1189

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.15 (1H, d, J = 2.4 Hz, 21-H), 4.07 (1H, d, J = 2.6 Hz, 11-H), 4.03 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 3.89 (1H, ddd, J = 3.7, 3.1, 2.4 Hz, 1-H), 3.71 (1H, dd, J = 11.3, 3.1 Hz, 22-Ha), 3.48 (1H, dd, J = 11.3, 3.7 Hz, 22-Hb), 3.41 (1H, dd, J = 7.6, 2.4 Hz, 13-H), 3.17 (1H, ddd, J = 11.6, 2.6, 2.4 Hz, 3-H), 2.92 (1H, dd, J = 17.4, 2.4 Hz, 4-Hα), 2.82 (1H, dd, J = 21.1, 7.6 Hz, 14-Hα), 2.30 (3H, s, NCH_3), 2.27 (1H, d, J = 21.1 Hz, 14-Hβ), 1.93 (6H, s, 6-CH_3, 16-CH_3), 1.42 (1H, ddd, J = 17.4, 11.6, 2.4 Hz, 4-Hβ); Figure 11

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.3 (C-15), 185.5 (C-5), 182.3 (C-18), 181.4 (C-8), 155.7 (C-7), 155.4 (C-17), 141.7 (C-20), 141.4 (C-10), 136.1 (C-9), 135.6 (C-19), 128.8 (C-6), 128.6 (C-16), 116.9 (21-CN), 64.2 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 59.1 (C-21), 58.0 (C-1), 54.5 (C-13), 54.3 (C-3), 54.2

(C-11), 41.5 (NCH_3), 25.4 (C-4), 21.5 (C-14), 8.7 (6-CH_3), 8.7 (16-CH_3); Figure 13

8.2 Jorunnamycin B

Jorunnamycin B was a yellowish orange amorphous solid (23.6 mg (2.41%) from the ethyl acetate extract of the visceral organs).

$[\alpha]^{20}_D$: +117.6 (*c* = 0.11, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 23
254 (3.96), 275 (4.15), 371 (3.64)

CD : Δε nm (*c* = 86.4 μM, methanol, 25 °C); Figure 24
+3.4 (382), +1.6 (289), -6.7 (271), -4.7 (246), +1.2 (223), -10.7 (206)

FABMS : *m/z* (% intensity); Figure 22
510 ($[M+H]^+$, 27), 483 (14), 307 (20), 289 (13), 236 (85), 235 (81), 154 (100), 136 (71), 107 (23), 89 (21)

HR-FABMS : *m/z* 510.1877 $[M+H]^+$, (calcd for $C_{26}H_{28}N_3O_8$, 510.1876)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 25
3436, 2943, 1737, 1656, 1639, 1462, 1414, 1376, 1233, 755

^1H NMR : δ ppm, 300 MHz, in $CDCl_3$; 11.46 (1H, s, 15-OH), 5.80 (1H, s, 18-OH), 4.37 (1H, br s, 11-H), 4.33 (1H, d, *J* = 2.6 Hz, 21-H), 3.96 (3H, s, 7-OCH_3), 3.90 (1H, overlap, 1-H), 3.85 (3H, s, OCH_3), 3.61 (1H, dd, *J* = 11.5, 3.0 Hz, 22-Ha), 3.35 (1H, dt, *J* = 11.4, 2.7 Hz, 3-H), 3.35 (1H, d, *J* = 11.5 Hz, 22-Hb), 3.44 (1H, br s, 13-H), 3.08 (1H, dd, *J* = 17.9, 2.5 Hz, 4-Hα), 2.44 (3H, s, NCH_3), 2.17 (3H, s, 16-CH_3), 1.90 (3H, s, 6-CH_3), 1.64 (1H, ddd, *J* = 17.9, 11.4, 2.4 Hz, 4-Hβ); Figure 26

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 197.4 (C-14), 185.5 (C-5), 180.9 (C-8), 155.3 (C-7), 154.0 (C-17), 141.1 (C-10), 139.9 (C-18), 135.8 (C-9), 128.5 (C-6), 119.3 (C-16), 116.4 (C-19), 115.4 (21-CN), 111.4 (C-20), 65.9 (C-13), 63.5 (C-22), 61.5 (17-OCH_3), 61.1 (7-OCH_3), 57.6 (C-1), 56.7 (C-11, C-21), 53.7

(C-3), 54.3 (C-3), 42.7 (NCH_3), 24.0 (C-4), 9.1 (16-CH_3), 8.9 (6-CH_3); Figure 28

8.3 Jorunnamycin C

Jorunnamycin C was obtained as a yellow amorphous solid (21.7 mg (8.04%) from the ethyl acetate extract of the egg ribbons).

$[\alpha]^{20}_D$: -91.6 (c = 0.1, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 36
268 (4.25), 347 (3.14)

CD : Δε nm (c = 72.8 μM, methanol, 25 °C); Figure 37
+1.0 (438), -3.2 (353), -10.2 (280), +3.9 (255), -1.5 (227), +4.2 (208)

EIMS : *m/z* (% intensity); Figure 35
549 ($M^{+\cdot}$, 6), 462 (2), 435 (3), 221 (20), 220 (100), 219 (17), 218 (24), 204 (12)

HR-EIMS : *m/z* 549.2112 ($M^{+\cdot}$), (calcd for $C_{29}H_{31}N_3O_8$, 549.2111)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 38
3453, 2943, 2851, 1736, 1655, 1617, 1450, 1411, 1374, 1235, 756

^1H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.40 (1H, dd, J = 11.6, 3.2 Hz, 22-Ha), 4.07 (1H, d, J = 2.4 Hz, 21-H), 4.02 (1H, d, J = 2.7 Hz, 11-H), 4.01 (3H, s, 7-OCH_3), 4.01 (3H, s, 17-OCH_3), 3.99 (1H, d, J = 2.4 Hz, 1-H), 3.89 (1H, dd, J = 11.6, 4.0 Hz, 22-Hb), 3.37 (1H, d, J = 2.2 Hz, 13-H), 3.10 (1H, ddd, J = 11.6, 2.7, 2.5 Hz, 3-H), 2.93 (1H, dd, J = 17.1, 2.4 Hz, 4-Hα), 2.76 (1H, dd, J = 21.1, 7.6 Hz, 14-Hα), 2.31 (1H, d, J = 21.1 Hz, 14-Hβ), 2.29 (3H, s, NCH_3), 2.11 and 2.02 (1H each, dq, J = 16.5, 7.6 Hz, 25-H_2), 1.95 (6H, s, 6-CH_3 and 16-CH_3), 1.31 (1H, ddd, J = 17.1, 11.6, 2.4 Hz, 4-Hβ), 0.95 (3H, t, J = 7.6 Hz, 26-H_3); Figure 39

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.2 (C-15), 185.4 (C-5), 182.4 (C-18), 180.9 (C-8), 173.4 (C-24), 155.6 (C-7), 155.2 (C-17), 142.1 (C-20), 141.7 (C-10), 135.5 (C-9), 135.0 (C-19), 128.6

(C-6), 128.6 (C-16), 116.9 (21-CN), 63.6 (C-22), 61.1 (17-OCH_3), 61.0 (7-OCH_3), 59.0 (C-21), 55.9 (C-1), 54.6 (C-13), 54.5 (C-3), 54.3 (C-11), 41.5 (NCH_3), 27.4 (C-25), 25.3 (C-4), 21.2 (C-14), 8.9 (C-26), 8.8 (16-CH_3), 8.6 (6-CH_3); Figure 41

8.4 Renieramycin M

Renieramycin M was obtained as orange precipitates (25.3 mg (3.61%) and 62.1 mg (23%) from the ethyl acetate extracts of the mantles and the egg ribbons, respectively).

ESI-TOF MS : *m/z* 576.45 $[M+H]^+$; Figure 48

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 5.94 (1H, qq, *J* = 7.2, 1.1 Hz, 26-H), 4.51 (1H, dd, *J* = 11.7, 2.9 Hz, 22-Ha), 4.10 (1H, dd, *J* = 11.7, 2.6 Hz, 22-Hb), 4.05 (1H, d, *J* = 2.3 Hz, 21-H), 4.01 (1H, overlap, 11-H), 4.00 (3H, s, 17-OCH_3), 3.99 (1H, overlap, 1-H), 3.97 (3H, s, 7-OCH_3), 3.37 (1H, d, *J* = 7.5 Hz, 13-H), 3.09 (1H, ddd, *J* = 11.4, 2.7 Hz, 3-H), 2.87 (1H, dd, *J* = 17.4, 2.2 Hz, 4-Hα), 2.73 (1H, dd, *J* = 21.1, 7.5 Hz, 14-Hα), 2.31 (1H, d, *J* = 21.1 Hz, 14-Hβ), 2.26 (3H, s, NCH_3), 1.92 (3H, s, 16-CH_3), 1.88 (3H, s, 6-CH_3), 1.80 (3H, dq, *J* = 7.2, 1.2 Hz, 27-H_3), 1.55 (3H, br s, 28-H_3), 1.35 (1H, ddd, *J* = 17.4, 11.4, 2.6 Hz, 4-Hβ); Figure 49

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 185.5 (C-15), 185.1 (C-5), 182.0 (C-18), 180.7 (C-8), 166.3 (C-24), 155.6 (C-7), 155.0 (C-17), 142.9 (C-20), 141.0 (C-10), 140.4 (C-26), 135.5 (C-9), 134.4 (C-19), 128.5 (C-6), 128.3 (C-16), 126.1 (C-25), 116.7 (21-CN), 62.0 (C-22), 61.1 (17-OCH_3), 61.0 (7-OCH_3), 58.3 (C-21), 56.3 (C-1), 54.6 (C-13), 54.3 (C-11), 54.0 (C-3), 41.5 (NCH_3), 25.5 (C-4), 21.5 (C-14), 20.5 (C-28), 15.8 (C-27), 8.9 (16-CH_3), 8.6 (6-CH_3); Figure 50

8.5 Renieramycin N

Renieramycin N was obtained as a pale yellow amorphous solid (0.5 mg (0.07%) and 9.5 mg (0.94%) from the ethyl acetate extracts of the mantles and the visceral organs, respectively).

ESI-TOF MS : *m/z* 594.44 $[M+H]^+$; Figure 51

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 5.84 (1H, qq, *J* = 7.2, 1.2 Hz, 26-H), 4.58 (1H, d, *J* = 2.8 Hz, 21-H), 4.55 (1H, br s, 14-Hβ), 4.22 (1H, dd, *J* = 11.0, 2.3 Hz, 22-Ha), 4.01 (1H, br s , 11-H), 4.00 (1H, d, *J* = 11 Hz, 22-Hb), 3.98 (3H, s, 7-OCH_3), 3.95 (1H, br s, 1-H), 3.73 (3H, s, 17-OCH_3), 3.44 (1H, br s, 13-H), 3.08 (1H, br d, *J* = 2.6 Hz, 3-H), 3.03 (1H, 4-Hα), 2.46 (3H, s, NCH_3), 2.13 (3H, s, 16-CH_3), 1.91 (3H, s, 6-CH_3), 1.75 (3H, dq, *J* = 7.2, 1.1 Hz, 27-H_3), 1.59 (3H, br s, 28-H_3), 1.30 (1H, 4-Hβ); Figure 52

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 185.6 (C-5), 180.8 (C-8), 166.9 (C-24), 155.6 (C-7), 146.2 (C-15), 144.9 (C-17), 142.2 (C-10), 140.0 (C-26), 139.0 (C-18), 135.2 (C-9), 128.3 (C-6), 126.5 (C-25), 118.9 (C-16), 118.0 (C-19), 116.7 (21-CN), 114.5 (C-20), 64.2 (C-13), 64.1 (C-14), 61.6 (C-22), 61.1 (7-OCH_3), 61.0 (17-OCH_3), 56.7 (C-11), 56.5 (C-21), 56.1 (C-1), 54.4 (C-3), 42.9 (NCH_3), 24.6 (C-4), 20.0 (C-28), 15.7 (C-27), 9.3 (16-CH_3), 8.9 (6-CH_3); Figure 53

8.6 Renieramycin O

Renieramycin O (1o) was obtained as a pale yellow amorphous solid (6.4 mg (0.91%) and 2.0 mg (0.20%) from the ethyl acetate extracts of the mantles and the visceral organs, respectively).

ESI-TOF MS : *m/z* 592.45 $[M+H]^+$; Figue 54

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 5.95 (1H, q, *J* = 7.1 Hz, 26-H), 4.49 (1H, d, *J* = 11.5 Hz, 22-Ha), 4.42 (1H, s, 14-Hβ), 4.27 (1H, br s, 21-H), 4.09 (1H, d, *J* = 11.5 Hz, 22-Hb), 4.02 (1H, overlap, 11-H), 4.01 (3H, s, 17-OCH_3), 4.00 (3H, s, 7-OCH_3), 4.00 (1H, overlap, 1-H), 3.75 (1H, br s, 14-OH), 3.50 (1H, br

s, 13-H), 3.10 (1H, d, J = 11.0 Hz, 3-H), 2.86 (1H, d, J = 17.4 Hz, 4-Hα), 2.50 (3H, s, NCH_3), 1.90 (6H, s, 6-CH_3 and 16-CH_3), 1.79 (3H, d, J = 7.1 Hz, 27-H_3), 1.54 (3H, br s, 28-H_3), 1.26 (1H, 4-Hβ); Figure 55

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 187.1 (C-15), 185.0 (C-5), 182.2 (C-18), 180.5 (C-8), 166.4 (24-C), 155.7 (C-7), 155.3 (C-17), 141.0 (C-20), 140.7 (C-10), 140.4 (C-26), 135.5 (C-9, and C-19), 128.7 (C-6), 128.2 (C-16), 126.1 (C-25), 116.1 (21-CN), 62.5 (C-13), 62.2 (C-14 and C-22), 61.1 (7-OCH_3 and 17-OCH_3), 56.4 (C-21), 56.1 (C-1), 55.2 (C-11), 53.2 (C-3), 42.5 (NCH_3), 25.3 (C-4), 20.4 (C-28), 15.8 (C-27), 8.9 (16-CH_3), 8.6 (6-CH_3); Figure 56

8.7 Renieramycin Q

Renieramycin Q was obtained as a pale yellow amorphous solid (1.3 mg (0.13%) from the ethyl acetate extract of the visceral organs).

ESI-TOF MS : m/z 592.41 $[M+H]^+$; Figure 57

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 5.88 (1H, q, J = 7.3 Hz, 26-H), 4.51 (1H, br s, 21-H), 4.42 (1H, br s, 11-H), 4.08 (1H, br s, 1-H), 4.08 (1H, overlap, 22-Ha), 4.04 (1H, overlap, 22-Hb), 4.00 (3H, s, 7-OCH_3), 3.86 (3H, s, 17-OCH_3), 3.61 (1H, br s, 13-H), 3.44 (1H, d, J = 11.5, 3-H), 3.10 (1H, d, J = 17.3 Hz, 4-Hα), 2.55 (3H, s, NCH_3), 2.14 (3H, s, 16-CH_3), 1.90 (3H, s, 6-CH_3), 1.78 (3H, d, J = 7.3 Hz, 27-H_3), 1.60 (3H, overlap, 28-H_3), 1.60 (1H, ddd, J = 17.3, 11.5, 2.8 Hz, 4-Hβ); Figure 58

8.8 Mimosamycin

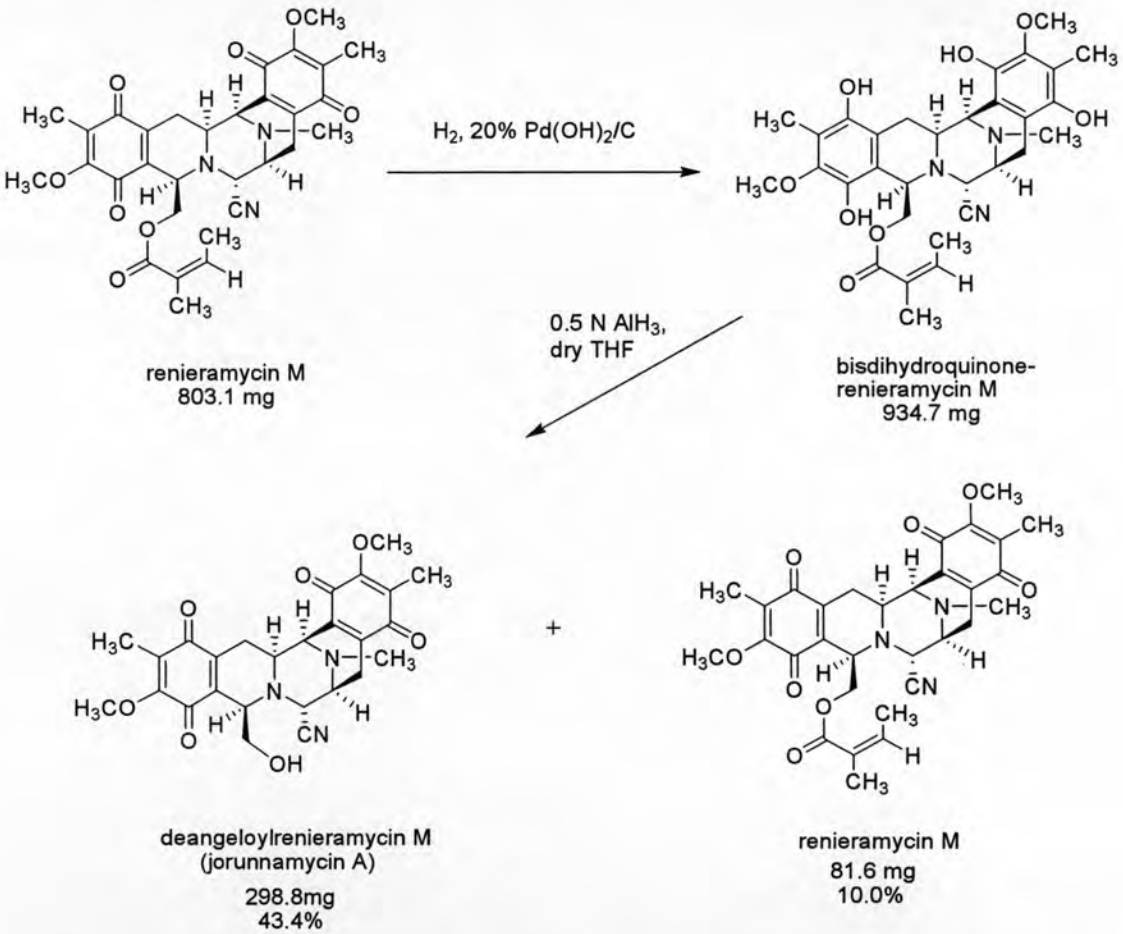
Mimosamycin was obtained as yellow amorphous solids (1.0 mg (0.14%) and 9.0 mg (0.92%) from the ethyl acetate extracts of the mantles and the visceral organs, respectively).

ESI-TOF MS : m/z 234.50 $[M+H]^+$; Figure 59

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 8.24 (1H, s, 1-H), 7.09 (1H, s, 4-H), 4.15 (3H, s, 7-OCH_3), 3.64 (3H, s, NCH_3), 2.04 (3H, s, 6-CH_3); Figure 60

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 183.2 (C-5), 177.1 (C-8), 162.6 (C-3), 159.3 (C-7), 141.1 (C-1), 138.7 (C-10), 133.0 (C-6), 116.6 (C-4), 111.2 (C-9), 61.3 (7-OCH_3), 38.5 (NCH_3), 183.2 (6-CH_3); Figure 61

9. Chemical transformation of renieramycin M to deangeloylrenieramycin M



A solution of renieramycin M (803.1 mg, 1.4 mmol) in ethyl acetate (140 ml) was hydrogenated over 20% $Pd(OH)_2/C$ (400 mg) at 1 atm for 6 h. The catalyst was removed by filtration and washed with ethyl acetate/chloroform (>1000 ml). The combined filtrates were concentrated *in vacuo* to give the leuco compound, bisdihydroquinonerenieramycin M (934.7 mg) as a colorless amorphous powder, which was used in the next step without further purification. A solution of 0.5 M aluminium hydride in THF (11.2 mmol, 8 eq) was added dropwise over 10 min to the

stirred solution of bisdihydroquinonerenieramycin M in dry THF (70 ml) which was cooled at -18 °C. The suspension was stirred at -18 °C for 4 h. After quenching by the addition of water (0.3 ml) and chloroform (16 ml), stirring was continued at room temperature for 16 h. The reaction mixture was diluted with brine (50 ml) and extracted with chloroform (50 ml x 3). The combined extracts were washed with 5% $NaHCO_3$, and extracted with $CHCl_3$ (50 ml x 3), dried over Na_2SO_4, and concentrated *in vacuo* to give a residue (766.9 mg) that was separated by using silica gel flash column chromatography with a gradient of hexane/ethyl acetate as the eluant to give deangeloylrenieramycin M or jorunnamycin A (298.8 mg, 43.4% yield) and renieramycin M (81.6 mg, 10.0% recovery yield).

0.5 M aluminium hydride in THF was freshly prepared before use: conc. sulfuric acid 0.4 ml was added dropwise to a solution of 1.0 M lithium aluminium hydride in THF 15 ml in THF 15 ml and the suspension was stirred for 1 h. After that, the suspension was left to settle for 1 h. The clear solution in upper part was used.

7-Cyano-6,7,9,14,14a,15-hexahydro-9-(hydroxymethyl)-2,11-dimethoxy-3,12,16-trimethyl (6S,7S,9R,14aS,15R) 6,15-imino-4H-isoquino[3,2-b][3]benzazocine-1, 4,10,13(9H)-tetrone **(deangeloylrenieramycin M or jorunnamycin A)**

$[\alpha]^{25}_D$: -270.6 (*c* = 1.0, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 8
269 (4.26)

CD : Δε nm (*c* = 103.3 μM, methanol, 24 °C); Figure 9
-2.9 (352), -1.5 (300), -10.2 (280), +3.2 (257), -1.8 (230), +0.1 (220)

FABMS : *m/z* (% intensity); Figure 7
494 ($[M+H]^+$, 8), 467 (4), 309 (20), 220 (15), 155 (59), 119 (100), 85 (9)

HR-FABMS : *m/z* 494.1910 $[M+H]^+$, (calcd for $C_{26}H_{28}N_3O_7$, 494.1927)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 10
3631, 3368, 3015, 2945, 2840, 1656, 1449, 1375, 1311, 1189

^1H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.15 (1H, d, *J* = 2.4 Hz, 21-H), 4.07 (1H, d, *J* = 2.6 Hz, 11-H), 4.03 (3H, s, OCH_3), 3.98 (3H,

s, OCH_3), 3.89 (1H, ddd, J = 3.7, 3.1, 2.4 Hz, 1-H), 3.71 (1H, dd, J = 11.3, 3.1 Hz, 22-Ha), 3.48 (1H, dd, J = 11.3, 3.7 Hz, 22-Hb), 3.41 (1H, dd, J = 7.6, 2.4 Hz, 13-H), 3.17 (1H, ddd, J = 11.6, 2.6, 2.4 Hz, 3-H), 2.92 (1H, dd, J = 17.4, 2.4 Hz, 4-Hα), 2.82 (1H, dd, J = 21.1, 7.6 Hz, 14-Hα), 2.30 (3H, s, NCH_3), 2.27 (1H, d, J = 21.1 Hz, 14-Hβ), 1.93 (6H, s, 6-CH_3, 16-CH_3), 1.42 (1H, ddd, J = 17.4, 11.6, 2.4 Hz, 4-Hβ); Figure 11

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.3 (C-15), 185.5 (C-5), 182.3 (C-18), 181.4 (C-8), 155.7 (C-7), 155.4 (C-17), 141.7 (C-20), 141.4 (C-10), 136.1 (C-9), 135.6 (C-19), 128.8 (C-6), 128.6 (C-16), 116.9 (21-CN), 64.2 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 59.1 (C-21), 58.0 (C-1), 54.5 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (NCH_3), 25.4 (C-4), 21.5 (C-14), 8.7 (6-CH_3), 8.7 (16-CH_3); Figure 13

10. Chemical transformation of deangeloylrenieramycin M

10.1 Transformation of deangeloylrenieramycin M to acyl renieramycin derivatives

10.1.1 Acyclic acyl derivatives of deangeloylrenieramycin M

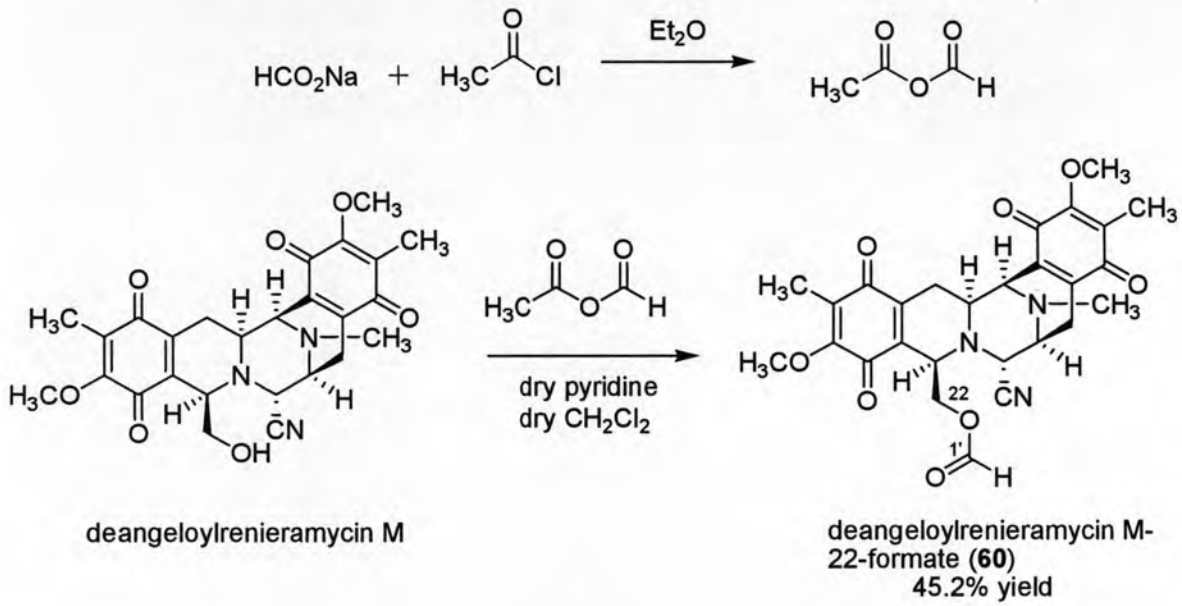
10.1.1.1 Saturated acyclic acyl derivatives of deangeloylrenieramycin M

10.1.1.1.1 Deangeloylrenieramycin M-22-formate (60)

Acetyl chloride, 2.6 ml (0.04 mol), was gradually added to a suspension of sodium formate 3 g (0.04 mol) in dry Et_2O 5 ml and stirred at 23 °C for 5.5 h. The suspension was filtered. The solid residue was rinsed with 100 ml of ether. The ether filtrate was obtained and dried under reduced pressure.

The acetic formic anhydride solution (1.8 M in CH_2Cl_2), 66.7 µl, was added to a stirred solution of deangeloylrenieramycin M (24.7 mg, 0.05 mmol in 2 ml dry CH_2Cl_2). Dry pyridine 20.2 µl (0.25 mmol, 4.95 eq) was added to reaction mixture at room temperature. The reaction mixture was stirred at room temperature for 21 h and then diluted with water (20 ml) and partitioned with chloroform (20 ml x 3). The chloroform layer was subsequently washed with aq. saturated $NaHCO_3$ and aq.

saturated NaCl solutions (Shibuya and Shiratsuchi, 1995). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (27.5 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to give deangeloylrenieramycin M-22-formate (**60**) 11.8 mg (45.2 % yield) as a yellow amorphous solid.



Deangeloylrenieramycin M-22-formate (60)

$[\alpha]^{22}_D$: -92.2 (c = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 63
267 (4.29)

EIMS : *m/z* (% intensity); Figure 62
521 ($M^{+\cdot}$, 10), 495 (0.5), 462 (2), 435 (3), 260 (9), 243 (7), 220 (100), 218 (25), 204 (14), 176 (10)

HR-EIMS : 521.1792 ($M^{+\cdot}$), (calcd for $C_{27}H_{27}N_3O_8$, 521.1798)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 64
3446, 2944, 2852, 2229, 1724, 1654, 1619, 1451, 1374, 1310, 1234, 1079, 1021, 756

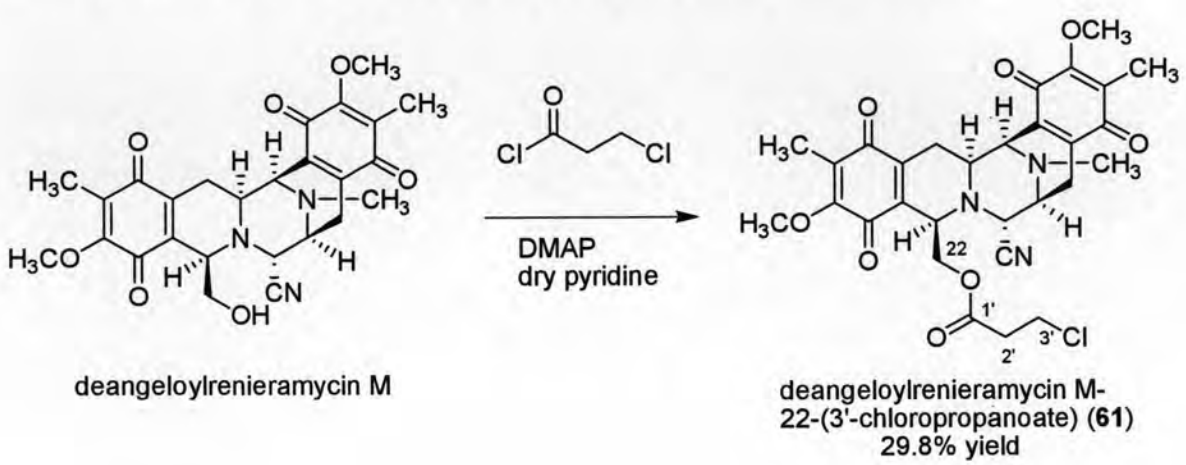
^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 7.80 (1H, s, 1'-H), 4.47 (1H, dd, J = 11.3, 2.9 Hz, 22-Ha), 4.05 (1H, d, J = 2.4 Hz, 21-H), 4.02 (3H, s, OCH_3), 4.02 (1H, overlap, 1-H), 4.01 (1H, overlap, 11-H), 4.01 (3H, s, OCH_3), 3.96 (1H, dd, J = 11.3, 4.3 Hz, 22-

Hb), 3.38 (1H, ddd, J = 7.6, 2.1, 1.7 Hz, 13-H), 3.12 (1H, ddd, J = 11.6, 3.1, 2.7 Hz, 3-H), 2.94 (1H, dd, J = 17.2, 2.5 Hz, 4-Hα), 2.77 (1H, dd, J = 21.1, 7.6 Hz, 14-Hα), 2.31 (3H, s, NCH_3), 2.25 (1H, d, J = 21.1 Hz, 14-Hβ),1.96 (3H, s, 16-CH_3), 1.95 (3H, s, 6-CH_3), 1.36 (1H, ddd, J = 17.2, 11.6, 2.4 Hz, 4-Hβ); Figure 65

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$;186.2 (C-15), 185.3 (C-5), 182.5 (C-18), 181.0 (C-8), 159.8 (C-1'), 155.5 (C-7), 155.2 (C-17), 142.2 (C-20), 142.1 (C-10), 135.0 (C-9), 134.9 (C-19), 128.8 (C-6), 128.7 (C-16), 116.8 (21-CN), 63.5 (C-22), 61.1 (OCH_3), 61.1 (OCH_3), 59.2 (C-21), 55.6 (C-1), 54.6 (C-13), 54.6 (C-3), 54.3 (C-11), 41.5 (NCH_3), 25.2 (C-4), 21.3 (C-14), 8.8 (6-CH_3), 8.7 (16-CH_3); Figure 66

10.1.1.1.2 Deangeloylrenieramycin M-22-(3'-chloropropanoate) (61)

A solution of deangeloylrenieramycin M (37.9 mg, 0.075 mmol) in 1.48 ml dry pyridine was cooled at -17 °C, and 4-dimethylaminopyridine (DMAP, 0.92 mg, 0.1 eq) was added. The mixture was stirred for 5 min at same temperature. 3-chloropropionyl chloride, 105.2 µl (1.10 mmol, 14.7 eq), was added dropwise for 10 min and the reaction mixture was stirred for 3 h at -17 °C. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (170.7 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford to deangeloylrenieramycin M-22-(3'-chloropropanoate) (**61**, 13.4 mg, 29.8% yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(3'-chloropropanoate) (**61**) 29.8% yield

Deangeloylrenieramycin M-22-(3'-chloropropanoate) (61)

$[\alpha]^{22}_{D}$: -79.6 (*c* = 0.7, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 68
268 (4.24)

EIMS : *m/z* (% intensity); Figure 67
583 ($M^{+\cdot}$, 3), 547 (5), 462 (3), 435 (1), 260 (7), 220 (100), 218 (24), 204 (10), 176 (6)

HR-EIMS : 583.1728 ($M^{+\cdot}$), (calcd for $C_{29}H_{30}ClN_3O_8$, 583.1722)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 69
3446, 2944, 2853, 2229, 1937, 1654, 1449, 1374, 1311, 1235, 1150, 1080, 966, 767

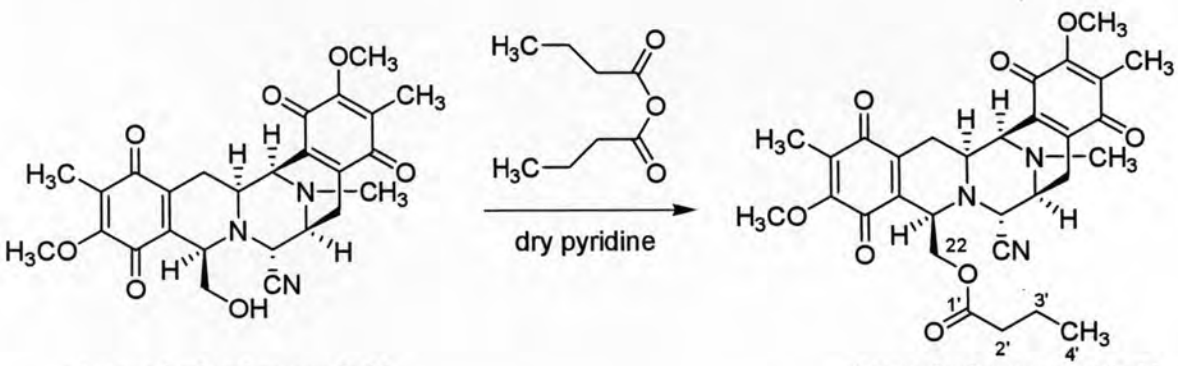
^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.39 (1H, dd, *J* = 11.6, 3.1 Hz, 22-Ha), 4.10 (1H, dd, *J* = 11.6, 3.7 Hz, 22-Hb), 4.07 (1H, d, *J* = 3.1 Hz, 21-H), 4.03 (1H, overlap, 11-H), 4.03 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.01 (1H, overlap, 1-H), 3.53 (2H, m, 3'-H_2), 3.40 (1H, ddd, *J* = 7.3, 1.8, 1.5 Hz, 13-H), 3.11 (1H, ddd, *J* = 11.6, 3.1, 2.7 Hz, 3-H), 2.92 (1H, dd, *J* = 17.4, 2.4 Hz, 4-Hα), 2.76 (1H, dd, *J* = 21.0, 7.5 Hz, 14-Hα), 2.53 (2H, m, 2'-H_2), 2.31 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.30 (3H, s, NCH_3), 1.96 (3H, s, 16-CH_3), 1.95 (3H, s, 6-CH_3), 1.37 (1H, ddd, *J* = 17.4, 11.5, 2.6 Hz, 4-Hβ); Figure 70

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.3 (C-15), 185.3 (C-5), 182.4 (C-18), 181.0 (C-8), 169.5 (C-1'), 155.6 (C-7), 155.4 (C-17), 142.0 (C-20), 141.8 (C-10), 135.1 (C-9), 135.1 (C-19), 128.7 (C-6), 128.5 (C-16), 116.8 (21-CN), 63.8 (C-22), 61.1 (OCH_3), 61.1 (OCH_3), 58.8 (C-21), 55.9 (C-1), 54.6 (C-13), 54.4 (C-3), 54.2 (C-11), 41.5 (NCH_3), 38.5 (C-3'), 37.4 (C-2'), 25.4 (C-4), 21.2 (C-14), 8.8 (6-CH_3), 8.6 (16-CH_3); Figure 71

10.1.1.1.3 Deangeloylrenieramycin M-22-butyrate (62)

Butyric anhydride 24.0 µl (0.147 mmol, 14.7 eq) was added to a stirred solution of deangeloylrenieramycin M, 5 mg (0.01 mmol) in 0.2 ml dry pyridine, at -17 °C and

the reaction mixture was stirred at same temperature for 5 h. The reaction mixture was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (12.2 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford deangeloylrenieramycin M-22-butyrate **(62)** 3.7 mg (64.8% yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-butyrate (**62**)
64.8% yield

Deangeloylrenieramycin M-22-butyrate (62)

$[\alpha]^{23}_{D}$: -79.9 (c = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 73
269 (4.13), 235 (3.65)

CD : Δε nm (c = 75.1μM, methanol, 25 °C); Figure 74
+0.8 (435),-2.3 (355), -4.7 (280), +1.9 (254), -0.9 (236), +1.8 (204)

EIMS : *m/z* (% intensity); Figure 72
563 ($M^{+\cdot}$, 7), 462 (3), 435 (4), 243 (13), 220 (100), 218 (23), 204 (11), 176 (6)

HR-EIMS : 563.2271 ($M^{+\cdot}$), (calcd for $C_{30}H_{33}N_3O_8$, 563.2268)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 75
3445, 3279, 2935, 2854, 2221, 1738, 1654, 1615, 1456, 1412, 1373, 1235, 757

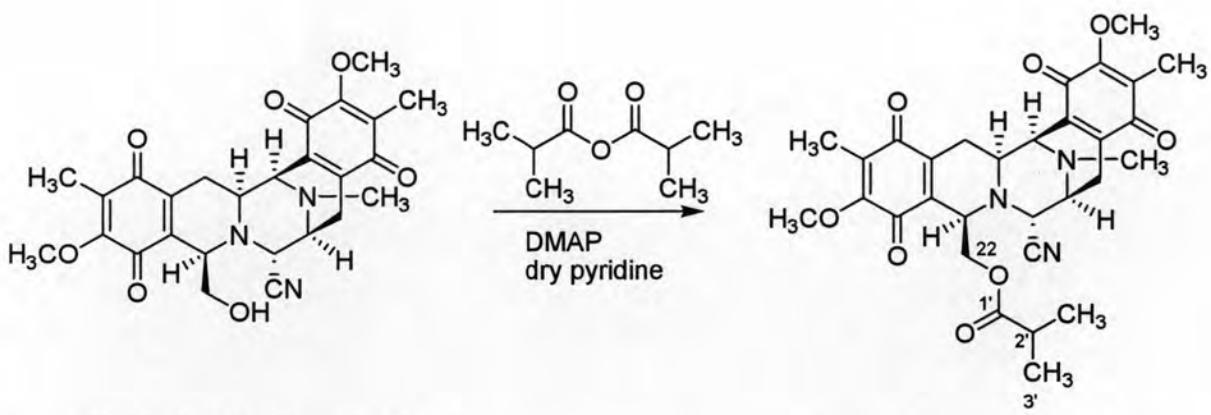
^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.39 (1H, dd, J = 11.5, 3.1 Hz, 22-Ha), 4.06 (1H, d, J = 2.4 Hz, 21-H), 4.02 (3H, s, OCH_3),

4.01 (1H, overlap, 11-H), 4.01 (3H, s, OCH_3), 3.99 (1H, br s, 1-H), 3.93 (1H, dd, J = 11.5, 3.6 Hz, 22-Hb), 3.38 (1H, d, J = 7.3 Hz, 13-H), 3.10 (1H, ddd, J = 11.6, 2.7 Hz, 3-H), 2.93 (1H, dd, J = 17.4, 2.7 Hz, 4-Hα), 2.76 (1H, dd, J = 21.0, 7.3 Hz, 14-Hα), 2.31 (1H, d, J = 21.0 Hz, 14-Hβ), 2.29 (3H, s, NCH_3), 2.01 (2H, t, J= 7.3 Hz, 2'-H_2), 1.95 (3H, s, 16-CH_3), 1.95 (3H, s, 6-CH_3), 1.42 (2H, sept, J= 7.3 Hz, 3'-H_2) 1.32 (1H, ddd, J = 17.4, 11.6, 2.4 Hz, 4-Hβ), 0.82 (3H, t, J = 7.3 Hz, 4'-H_3); Figure 76

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.1 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 172.6 (C-1'), 155.6 (C-7), 155.2 (C-17), 142.1 (C-20), 141.7 (C-10), 135.5 (C-9), 135.0 (C-19), 128.6 (C-6), 128.5 (C-16), 116.9 (21-CN), 63.3 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 58.9 (C-21), 56.0 (C-1), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5 (NCH_3), 36.0 (C-2'), 25.4 (C-4), 21.2 (C-14), 18.3 (C-3'), 13.6 (C-4'), 8.8 (16-CH_3), 8.6 (6-CH_3); Figure 77

10.1.1.1.4 Deangeloylrenieramycin M-22-(2'-methylpropanoate) (63)

A solution of deangeloylrenieramycin M (25.2 mg, 0.05 mmol) in 0.9 ml dry pyridine was cooled at -17 °C, and DMAP (0.61 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. Isobutyric anhydride, 21.9 µl (0.735 mM, 14.7 eq), was added dropwised for 10 min. The reaction mixture was stirred for 1.5 h at -17 °C. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (199.5 mg) was purified silica gel column chromatography with a gradient of hexane/ethyl acetate to afford deangeloylrenieramycin M-22-(2'-methylpropanoate) (**63**, 12.7 mg, 44.1 % yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(2'-methylpropanoate) (**63**)
44.1% yield

Deangeloylrenieramycin M-22-(2'-methylpropanoate) (63)

$[\alpha]^{22}_{D}$: -67.0 (*c* = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 79

268 (4.21)

EIMS : *m/z* (% intensity); Figure 78

563 ($M^{+\cdot}$, 7), 464 (4), 435 (3), 260 (15), 243 (5), 220 (100), 218 (20), 204 (9), 176 (5)

HR-EIMS : 563.2273 ($M^{+\cdot}$), (calcd for $C_{30}H_{33}N_3O_8$, 563.2268)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 80

3445, 2963, 2854, 2228, 1732, 1653, 1455, 1411, 1374, 1261, 1190, 1081, 956, 769

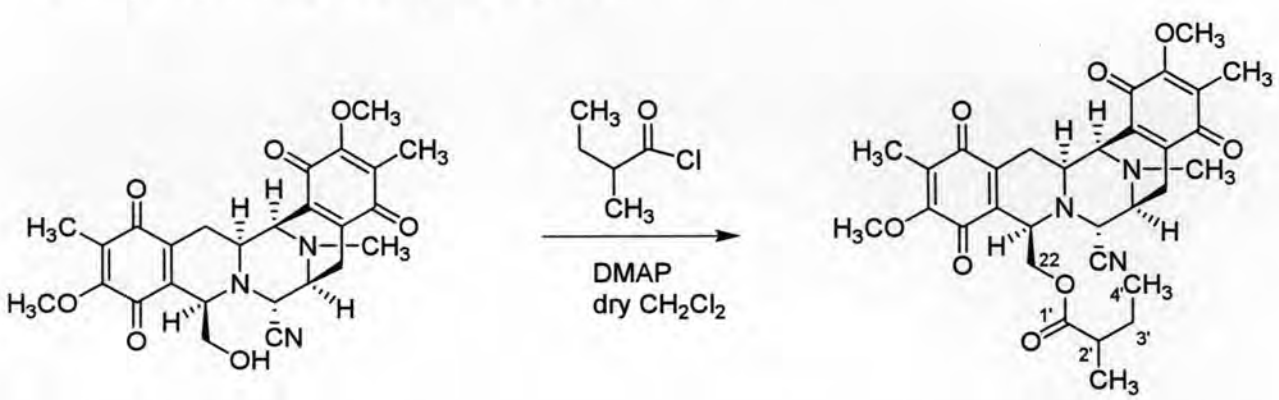
^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.30 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.07 (1H, d, *J* = 2.4 Hz, 21-H), 4.06 (1H, d, *J* = 3.6 Hz, 22-Hb), 4.04 (1H, overlap, 11-H), 4.02 (3H, s, 7-OCH_3), 4.01 (1H, overlap, 1-H), 4.00 (3H, s, 17-OCH_3), 3.40 (1H, ddd, *J* = 7.6, 1.8 Hz, 13-H), 3.10 (1H, ddd, *J* = 11.5, 3.1, 2.7 Hz, 3-H), 2.91 (1H, dd, *J* = 17.4, 2.8 Hz, 4-Hα), 2.78 (1H, dd, *J* = 20.8, 7.6 Hz, 14-Hα), 2.31 (1H, d, *J* = 20.8 Hz, 14-Hβ), 2.30 (1H, overlap, 2'-H), 2.29 (3H, s, NCH_3), 1.94 (6H, s, 6-CH_3 and 16-CH_3), 1.35 (1H, ddd, *J* = 17.4, 11.5, 2.4 Hz, 4-

Hβ), 0.95 (3H, d, J = 7.0 Hz, 3'-H_3), 0.93 (3H, d, J = 10.1 Hz, 2'-CH_3); Figure 81

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.3 (C-15), 185.4 (C-5), 182.5 (C-18), 181.0 (C-8), 176.0 (C-1'), 155.7 (C-7), 155.3 (C-17), 142.0 (C-20), 141.4 (C-10), 135.5 (C-9), 135.1 (C-19), 128.7 (C-6), 128.4 (C-16), 116.8 (21-CN), 63.3 (C-22), 61.1 (7-OCH_3), 61.0 (17-OCH_3), 58.8 (C-21), 56.2 (C-1), 54.6 (C-13), 54.4 (C-3), 54.2 (C-11), 41.5 (NCH_3), 33.8 (C-2'), 25.4 (C-4), 21.2 (C-14), 19.0 (C-3'), 18.5 (2'-CH_3), 8.7 (16-CH_3), 8.6 (6-CH_3); Figure 82

10.1.1.1.5 Deangeloylrenieramycin M-22-(2'-methylbutyrate) (64)

A solution of deangeloylrenieramycin M (16.6 mg, 0.03 mmol) in 3 ml dry CH_2Cl_2 was cooled at 0° C, and DMAP (0.73 mg, 0.2 eq) was added. The mixture was stirred for 5 min at same temperature. *DL*-2-methylbutyryl chloride, 6.6 µl (0.054 mmol, 1.8 eq), was added dropwise for 10 min and the reaction mixture was stirred for 1.3 h. After that *DL*-2methylbutyryl chloride 11.8 µl (0.096 mmol, 3.2 eq) was added in the reaction mixture and stirred at room temperature for 22.6 h. Finally, *DL*-2-methylbutyryl chloride 18.5 µl (0.15 mmol, 5 eq) was added and stirred at room temperature for 6 h. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (19.6 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford a yellow amorphous solid, deangeloylrenieramycin M-22-(2'-methylbutyrate) (**64**, 7.8 mg, 40.1% yield).



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(2'-methylbutyrate) (**64**)
40.1% yield

Deangeloylrenieramycin M-22-(2'-methylbutyrate) (64)

$[\alpha]^{23}_{D}$: -79.1 (c = 0.7, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 84
269 (4.26), 234 (3.73)

CD : Δε nm (c = 67.2 μM, methanol, 25 °C); Figure 85
+1.0 (437),-2.5 (356), -6.5 (279), +2.4 (254), -0.7 (234), +2.2 (212), +3.2 (206)

EIMS : *m/z* (% intensity); Figure 83
577 (M^+, 10), 462 (3), 435 (2), 260 (6), 243 (6), 220 (100), 218 (24), 204 (10), 176 (6)

HR-EIMS : 577.2424 (M^+), (calcd for $C_{31}H_{35}N_3O_8$, 577.2424)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 86
3445, 3281, 2964, 2855, 2229, 1732, 1652, 1615, 1463, 1410, 1373, 1235, 802

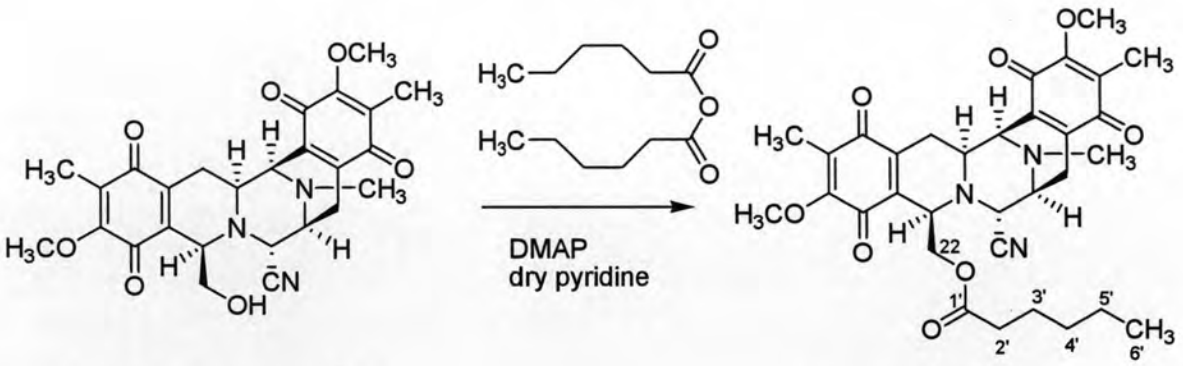
^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **minor isomer**, 4.20 (1H, dd, J = 11.6, 2.7 Hz, 22-Ha), 4.01 (1H, t, J = 2.7 Hz, 21-H), 4.05 (1H, ddd, J = 11.6, 2.7, 0.9 Hz, 22-Hb), 3.96 (1H, br d, J = 2.4 Hz, 11-H), 3.94 (3H, s, 17-OCH_3), 3.94 (3H, s, 7-OCH_3), 3.93 (1H, overlap, 1-H), 3.34 (1H, ddd, J = 7.3, 1.2, 0.9 Hz, 13-H), 3.04 (1H, d, J = 10.7 Hz, 3-H), 2.83 (1H, dt, J = 17.1, 2.4 Hz, 4-Hα), 2.71 (1H, dd, J = 21.0, 7.6 Hz, 14-Hα), 2.23 (1H, dd, J = 21.0,1.2 Hz, 14-Hβ), 2.22 (3H, s, NCH_3), 2.02 (1H, m, 2'-H), 1.87 (3H, s, 16-CH_3), 1.87 (3H, s, 6-CH_3), 1.28 (1H, ddd, J = 17.1, 11.3, 2.7 Hz, 4-Hβ), 1.20 (2H, m, 3'-H_2), 0.84 (3H, d, J = 7.0 Hz, 2'-CH_3), 0.67 (3H, td, J = 7.6, 1.5 Hz, 4'-H_3); **major isomer**, 4.17 (1H, dd, J = 11.6, 2.7 Hz, 22-Ha), 4.05 (1H, ddd, J = 11.5, 2.7, 0.9 Hz, 22-Hb), 4.01 (1H, t, J = 2.7 Hz, 21-H), 3.96 (1H, brd, J = 2.4 Hz, 11-H), 3.94 (3H, s, 17-OCH_3), 3.94 (3H, s, 7-OCH_3), 3.93 (1H, overlap, 1-H), 3.34 (1H, ddd, J = 7.3, 1.2 Hz, 13-H), 3.04 (1H, d, J = 10.7 Hz, 3-H), 2.83 (1H, dt, J = 17.1, 2.4 Hz, 4-Hα), 2.71 (1H, dd, J = 21.0, 7.6 Hz, 14-Hα), 2.23 (1H, dd, J = 21.0, 1.2 Hz, 14-Hβ),

2.22 (3H, s, NCH_3), 2.02 (1H, m, 2'-H), 1.87 (3H, s, 16-CH_3), 1.87 (3H, s, 6-CH_3), 1.28 (1H, ddd, J = 17.1, 11.3, 2.7 Hz, 4-Hβ), 1.23 (2H, overlap, 3'-H_2), 0.82 (3H, d, J = 7.0, 2'-CH_3), 0.81 (3H, td, J = 7.2, 1.2 Hz, 4'-H_3); Figure 87

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **minor isomer**, 186.2 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 175.7 (C-1'), 155.8 (C-7), 155.3 (C-17), 142.0 (C-20), 141.3 (C-10), 135.5 (C-9), 135.1 (C-19), 128.6 (C-6), 128.3 (C-16), 116.8 (21-CN), 63.2 (C-22), 61.0 (17-OCH_3), 61.0 (7-OCH_3), 58.8 (C-21), 56.2 (C-1), 54.6 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (NCH_3), 40.8 (C-2'), 25.4 (C-4), 26.2 (C-3'), 21.2 (C-14), 15.9 (2'-CH_3), 11.4 (C-4'), 8.7 (16-CH_3), 8.6 (6-CH_3); **major isomer**, 186.2 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 175.7 (C-1'), 155.8 (C-7), 155.3 (C-17), 142.0 (C-20), 141.3 (C-10), 135.5 (C-9), 135.2 (C-19), 128.6 (C-6), 128.3 (C-16), 116.8 (21-CN), 63.4 (C-22), 61.0 (17-OCH_3), 61.0 (7-OCH_3), 58.8 (C-21), 56.3 (C-1), 54.6 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (NCH_3), 40.8 (C-2'), 25.4 (C-4), 26.6 (C-3'), 21.2 (C-14), 16.5 (2'-CH_3), 14.1 (C-4'), 8.7 (16-CH_3), 8.6 (6-CH_3); Figure 88

10.1.1.1.6 Deangeloylrenieramycin M-22-hexanoate (65)

A solution of deangeloylrenieramycin M (10 mg, 0.02 mmol) in 0.4 ml dry pyridine was cooled at -17 °C, and DMAP (0.24 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. The hexanoic anhydride, 69.0 µl (0.298 mmol, 14.7 eq), was added dropwise for 10 min and the reaction mixture was stirred for 1.8 h at -17 °C. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (68.8 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford deangeloylrenieramycin M-22-hexanoate (**65**, 7.0 mg, 58.4% yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-hexanoate (**65**)
58.4% yield

Deangeloylrenieramycin M-22-hexanoate (65)

$[\alpha]^{23}_D$: -84.7 (c = 0.5, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 90
269 (4.12), 235 (3.61)

CD : Δε nm (c = 58.9μM, methanol, 25 °C); Figure 91
+1.0 (430),-2.5 (353), -5.0 (279), +2.2 (256), -1.3 (233), +1.2 (217), +2.6 (211)

EIMS : *m/z* (% intensity); Figure 89
591 ($M^{+\cdot}$, 5), 464 (3), 435 (3), 260 (10), 243 (100), 220 (69) 204 (10)

HR-EIMS : 591.2578 ($M^{+\cdot}$), (calcd for $C_{32}H_{37}N_3O_8$, 591.2581)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 92
3446, 2932, 2857, 2229, 1738, 1652, 1615, 1456, 1417, 1373, 1235, 803

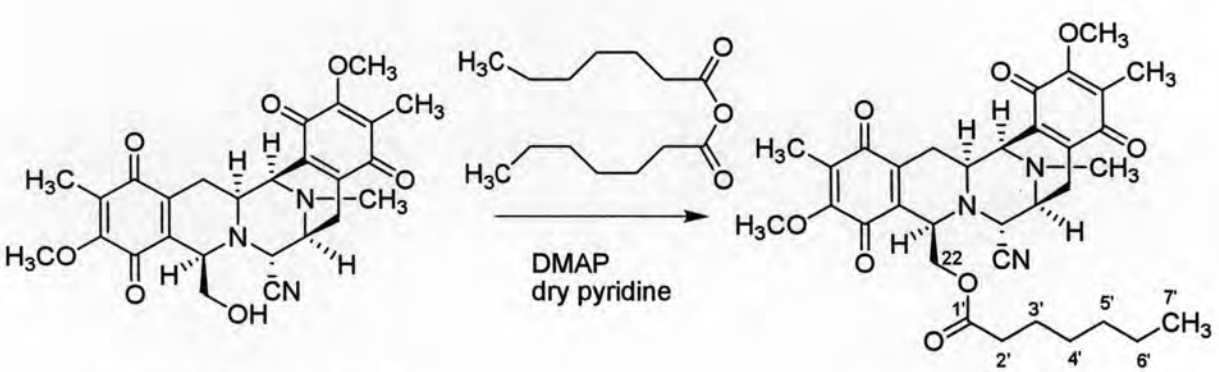
^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.38 (1H, dd, J = 11.6, 3.1 Hz, 22-Ha), 4.06 (1H, d, J = 2.4 Hz, 21-H), 4.02 (1H, d, overlap, 11-H), 4.02 (3H, s, 7-OCH_3), 4.01 (3H, s, 17-OCH_3), 3.99 (1H, br s, 1-H), 3.91 (1H, dd, J = 11.6, 4.0 Hz, 22-Hb), 3.38 (1H, ddd, J = 7.3, 1.5, 0.6 Hz, 13-H), 3.10 (1H, ddd, J = 11.6, 3.1, 2.7 Hz, 3-H), 2.93 (1H, dd, J = 17.1, 2.7 Hz, 4-Hα), 2.76 (1H, dd, J = 20.7, 7.3 Hz, 14-Hα), 2.31 (1H, d, J = 20.7 Hz, 14-Hβ), 2.29 (3H, s, NCH_3), 2.02 (2H, t, J = 8.3 Hz, 2'-H_2), 1.95 (6H, s, 6-CH_3 and 16-CH_3), 1.40 (2H, sextet, J = 8.3 Hz,

3'-H_2), 1.31 (1H, ddd, J = 17.1, 11.6, 2.4 Hz, 4-Hβ), 1.23 (2H, m, 5'-H_2), 1.16 (2H, m, 4'-H_2), 0.85 (3H, t, J = 7.3 Hz, 6'-H_3); Figure 93

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.1 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 172.8 (C-1'), 155.6 (C-7), 155.2 (C-17), 142.2 (C-20), 141.7 (C-10), 135.5 (C-9), 135.0 (C-19), 128.6 (C-6), 128.5 (C-16), 116.9 (21-CN), 63. 5 (C-22), 61.1 (17-OCH_3), 61.0 (7-OCH_3), 59.0 (C-21), 55.9 (C-1), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5 (NCH_3), 34.0 (C-2'), 31.2 (C-4'), 25.3 (C-4), 24.4 (C-3'), 22.3 (C-5'), 21.2 (C-14), 13.8 (C-6'), 8.8 (6-CH_3), 8.6 (16-CH_3); Figure 94

10.1.1.1.7 Deangeloylrenieramycin M-22-heptanoate (66)

A solution of deangeloylrenieramycin M (10.7 mg, 0.02 mmol) in 0.4 ml dry pyridine was cooled at -17 °C, and DMAP (0.24 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. The heptanoic anhydride 77.7 µl (0.147 mmol, 14.7 eq) was added dropwise for 10 min and the reaction mixture was stirred for 1.3 h min at -17 °C. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (72.0 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford deangeloylrenieramycin M-22-heptanoate (**66**, 9.0 mg, 68.5% yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-heptanoate (**66**)
68.5% yield

Deangeloylrenieramycin M-22-heptanoate M (66)

$[\alpha]^{23}_{D}$: -68.9 (c = 0.5, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 96
269 (4.11), 235 (3.66)

CD : Δε nm (c = 86.7 μM, methanol, 25 °C); Figure 97
+0.6 (443),-1.5 (353), -2.8 (281), +1.0 (260), -0.9 (234), +1.5 (212)

EIMS : *m/z* (% intensity); Figure 95
605 ($M^{+\cdot}$, 8), 429 (5), 355 (5), 243 (89), 220 (100), 218 (23), 204 (14)

HR-EIMS : 605.2733 ($M^{+\cdot}$), (calcd for $C_{33}H_{39}N_3O_8$, 605.2737)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 98
3446, 2928, 2856, 2221, 1738, 1652, 1615, 1456, 1412, 1374, 1261, 802

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.32 (1H, dd, J = 11.3, 3.1 Hz, 22-Ha), 3.99 (1H, d, J = 2.4 Hz, 21-H), 3.95 (3H, s, 7-OCH_3), 3.94 (1H, overlap, 11-H), 3.94 (3H, s, 17-OCH_3), 3.92 (1H, br s, 1-H), 3.83 (1H, dd, J = 11.3, 4.0 Hz, 22-Hb), 3.31 (1H, ddd, J = 7.6, 1.5, 0.6 Hz, 13-H), 3.03(1H, ddd, J = 11.6, 3.1, 2.7 Hz, 3-H), 2.86 (1H, dd, J = 17.1, 2.4 Hz, 4-Hα), 2.69 (1H, dd, J = 21.0, 7.6 Hz, 14-Hα), 2.24 (1H, d, J = 21.0 Hz, 14-Hβ), 2.22 (3H, s, NCH_3), 1.95 (2H, t, J = 8.9 Hz, 2'-H_2), 1.88 (6H, s, 6-CH_3 and 16-CH_3), 1.32 (2H, m, 3'-H_2), 1.24 (1H, ddd, J = 17.1, 11.3, 2.4 Hz, 4-Hβ), 1.19 (2H, m, 6'-H_2), 1.12 (2H, m, 5'-H_2), 1.11 (2H, m, 4'-H_2), 0.80 (3H, t, J = 7.2 Hz, 7'-H_3); Figure 99

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.1 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 172.8 (C-1'), 155.6 (C-7), 155.2 (C-17), 142.2 (C-20), 141.7 (C-10), 135.5 (C-9), 134.9 (C-19), 128.6 (C-6), 128.5 (C-16), 116.9 (21-CN), 63.5 (C-22), 61.1 (7-OCH_3), 61.0 (17-OCH_3), 59.0 (C-21), 55.9 (C-1), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5 (NCH_3), 34.1 (C-2'), 31.1 (C-5'),

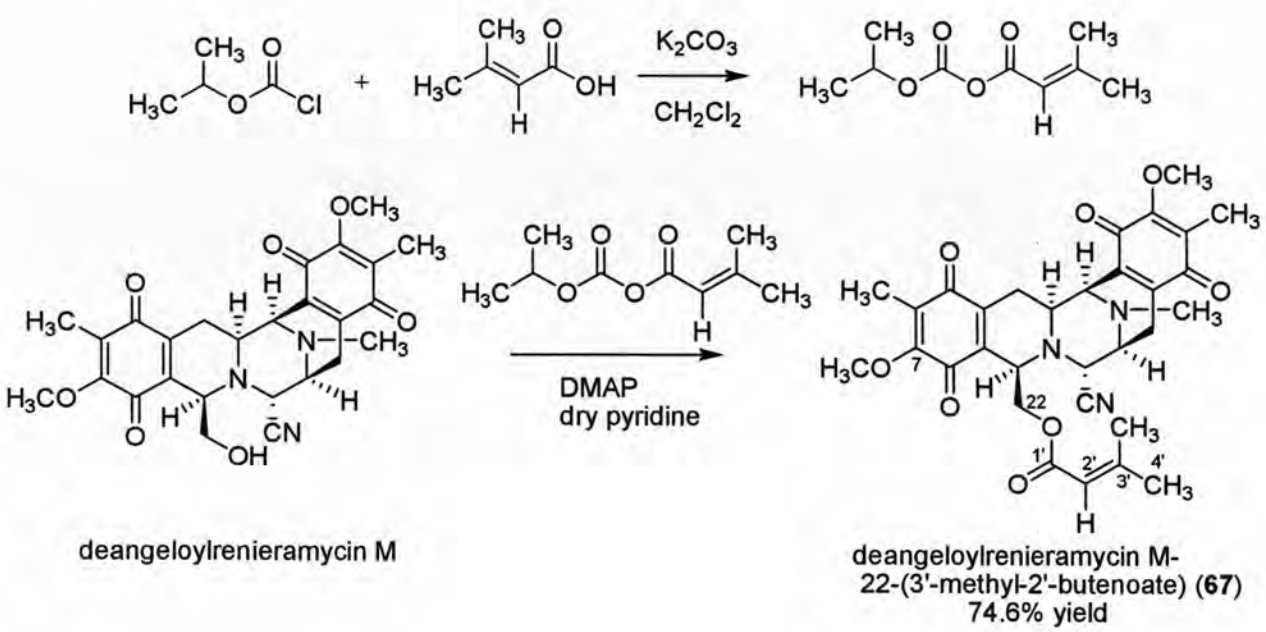
28.7 (C-4'), 25.3 (C-4), 24.7 (C-3'), 22.4 (C-6'), 21.2 (C-14), 13.9 (C-7'), 8.8 (16-CH_3), 8.6 (6-CH_3); Figure 100

10.1.1.2 Unsaturated acyclic acyl derivatives of deangeloylrenieramycin M

10.1.1.2.1 Deangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) (67)

A suspension of 3-methylcrotonic acid 201.3 mg (2.0 mmol) and K_2CO_3 276.4 mg (2.0 mmol) in 16 ml dry CH_2Cl_2 was stirred for 10 min. Isopropyl chlorocarbonate 227.8 µl (2.0 mmol) was added dropwise to the suspension on an ice bath for 5 min. The suspension was stirred at room temperature for 17 h. After that, the suspension was filtered and washed with dry CH_2Cl_2 several times. The combined filtrates were concentrated to afford 3-methylbut-2-enoyl isopropyl carbonate 100 %yield.

A solution of deangeloylrenieramycin M (5.4 mg, 0.01 mmol) in 0.2 ml dry pyridine was cooled at -17 °C, and DMAP (0.13 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. The 3-methylbut-2-enoyl isopropyl carbonate (27.3 mg, 0.147 mmol, 14.7 eq) was added to the reaction mixture. The reaction mixture was stirred for 50 min. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (24.4 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to give a yellow amorphous solid, deangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) (**67**, 4.7 mg, 74.6% yield).



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) (**67**) 74.6% yield

Deangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) (67)

$[\alpha]^{22}_{D}$: -61.6 (c = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 102
269 (3.89), 245 (3.67)

CD : Δε nm (c = 79.1 μM, methanol, 25 °C); Figure 103
+0.8 (443),-2.1 (351), -1.9 (283), +3.7 (258), -1.3 (216), -2.6 (207)

EIMS : *m/z* (% intensity); Figure 101
575 ($M^{+\cdot}$, 8), 464 (5), 260 (9), 220 (100), 218 (24), 204 (10), 176 (6)

HR-EIMS : 575.2268 ($M^{+\cdot}$), (calcd for $C_{31}H_{33}N_3O_8$, 575.2268)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 104
3445, 3281, 2928, 2854, 2228, 1715, 1652, 1615, 1456, 1417, 1374, 1261, 801

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 5.23 (1H, br s, 2'-H), 4.46 (1H, dd, J = 11.4, 3.1 Hz, 22-Ha), 3.99(1H, d, J = 2.4 Hz, 21-H), 3.94 (1H, overlap, 11-H), 3.94 (3H, s, 17-OCH_3), 3.93 (3H, s, 7-OCH_3), 3.93 (1H, overlap, 1-H), 3.80 (1H, dd, J = 11.4, 3.4 Hz, 22-Hb), 3.30 (1H, ddd, J = 7.5, 2.1, 1.8 Hz, 13-H), 3.03 (1H, ddd, J = 11.6, 2.7 Hz, 3-H), 2.84 (1H, dd, J = 17.1, 2.4 Hz, 4-Hα), 2.67 (1H, dd, J = 21.0, 7.5 Hz, 14-Hα), 2.23 (1H, d, J = 21.0 Hz, 14-Hβ), 2.20 (3H, s, NCH_3), 1.94 (3H, s, 4'-H_3), 1.88 (3H, s, 6-CH_3), 1.85 (3H, s, 16-CH_3), 1.74 (3H, s, (3'-CH_3), 1.27 (1H, ddd, J = 17.1, 11.6, 2.4 Hz, 4-Hβ); Figure 105

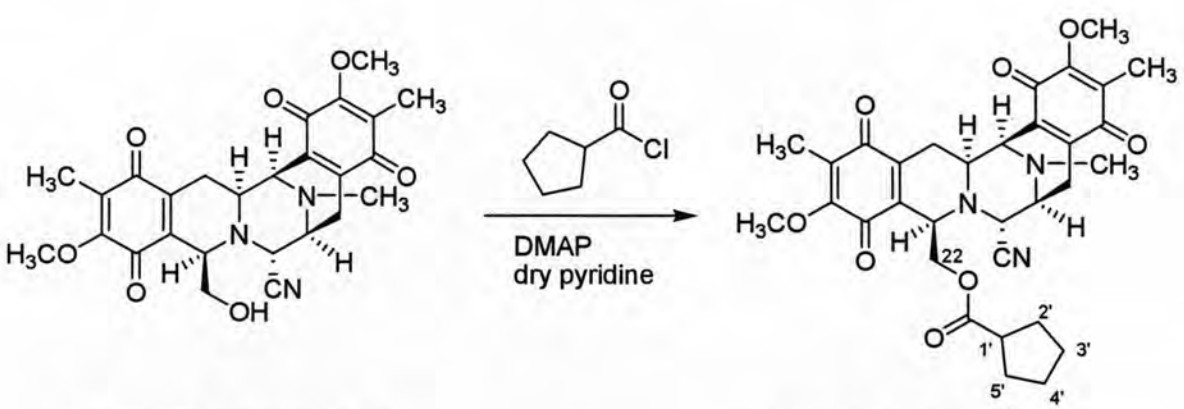
^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 185.9 (C-15), 185.5 (C-5), 182.5 (C-18), 181.0 (C-8), 165.4 (C-1'), 158.7 (C-3'), 155.6 (C-7), 155.2 (C-17), 142.1 (C-20), 141.8 (C-10), 135.7 (C-9), 134.9 (C-19), 128.5 (C-6), 128.4 (C-16), 117.0 (21-CN), 114.8 (C-2'), 62.1 (C-22), 61.1 (17-OCH_3), 61.0 (7-OCH_3), 58.8 (C-21), 56.1 (C-1), 54.6 (C-13), 54.4 (C-3), 54.4 (C-11), 41.5 (NCH_3), 27.4 (3'-CH_3), 25.4 (C-4), 21.1 (C-14), 20.2 (C-4'), 8.7 (6-CH_3), 8.7 (16-CH_3); Figure 106

10.1.2 Alicyclic acyl derivatives of deangeloylrenieramycin M

10.1.2.1 Saturated alicyclic acyl derivatives of deangeloylrenieramycin M

10.1.2.1.1 Deangeloylrenieramycin M-22-(cyclopentane-1'-carboxylate) (68)

The solution of deangeloylrenieramycin M (10.4 mg, 0.02 mmol) in 0.4 ml dry pyridine was cooled at -17 °C, and DMAP (0.24 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. Cyclopentanecarbonyl chloride 35.7 µl (0.294 mmol, 14.7 eq) was added dropwise to the reaction mixture for 10 min and stirred at -17 °C for 50 min. The reaction mixture was partitioned with water (20 ml) and chloroform (20 ml x 3). The chloroform extracts were combined and dried over anhydrous sodium sulfate, filtered, concentrated and dried *in vacuo* to give crude product (41.5 mg). Silica gel column chromatography with a gradient of hexane/ethyl acetate was used to isolate deangeloylrenieramycin M-22-(cyclopentane-1'-carboxylate) (**68**, 9.8 mg, 78.8% yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(cyclopentane-1'-carboxylate)(**68**)
78.8% yield

Deangeloylrenieramycin M-22-(cyclopentane-1'-carboxylate) (68)

$[\alpha]^{22}_D$: -66.6 (c = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 108
270 (4.15), 234 (3.62)

CD : Δε nm (c = 89.1 µM, methanol, 25 °C); Figure 109

+0.9 (436),-2.3 (356), -6.3 (280), +2.2 (253), -0.9 (235), +3.4 (208)

EIMS : *m/z* (% intensity); Figure 107
589 ($M^{+\cdot}$, 9), 462 (4), 260 (11), 243 (47), 220 (100), 218 (24), 204 (12)

HR-EIMS : 589.2427 ($M^{+\cdot}$), (calcd for $C_{32}H_{35}N_3O_8$, 589.2424)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 110
3444, 3277, 2960, 2855, 2228, 1732, 1652, 1615, 1455, 1411, 1373, 1261, 802

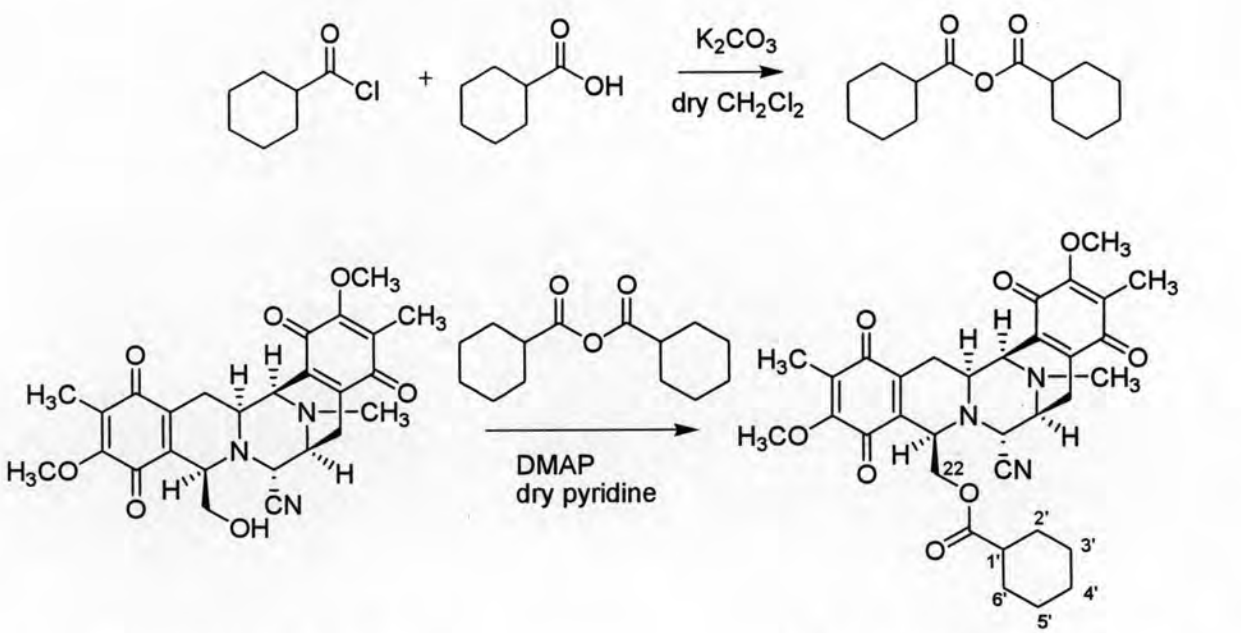
^1H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.23 (1H, dd, *J* = 11.3, 2.7 Hz, 22-Ha), 4.01 (1H, d, *J* = 2.4 Hz, 21-H), 4.00 (1H, d, *J* = 3.7 Hz, 11-H), 3.95 (3H, s, 17-OCH_3), 3.95 (3H, s, 7-OCH_3), 3.95 (1H, overlap, Hz, 22-Hb), 3.92 (1H, d, *J* = 3.7 Hz, 1-H), 3.33 (1H, ddd, *J* = 7.3, 2.1, 1.5 Hz, 13-H), 3.03 (1H, ddd, *J* = 11.6, 3.1, 2.7, Hz, 3-H), 2.84 (1H, dd, *J* = 17.1, 2.4 Hz, 4-Hα), 2.71 (1H, dd, *J* =20.8, 7.5 Hz, 14-Hα), 2.36 (1H, quintet, *J* = 7.5 Hz, 1'-H), 2.23 (1H, d, *J* = 20.8 Hz, 14-Hβ), 2.22 (3H, s, NCH_3), 1.87 (6H, s, 6-CH_3 and 16-CH_3), 1.59 (2H, m, 4'-H_2), 1.58 (2H, m, 2'-H_2), 1.45 (1H, m, 3'-Ha), 1.43 (2H, m, 5'-H_2), 1.39 (1H, m, 3'-Hb) 1.28 (1H, ddd, *J* = 17.1, 11.6, 2.6 Hz, 4-Hβ); Figure 111

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.2 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 175.7 (1'-CO), 155.7 (C-7), 155.3 (C-17), 142.1 (C-20), 141.4 (C-10), 135.5 (C-9), 135.1 (C-19), 128.6 (C-6), 128.3 (C-16), 116.8 (21-CN), 63.3 (C-22), 61.1 (17-OCH_3), 61.0 (7-OCH_3), 58.8 (C-21), 56.3 (C-1), 54.6 (C-13), 54.4 (C-3), 54.2 (C-11), 43.8 (C-1'), 41.5 (NCH_3), 30.1 (C-2'), 29.7 (C-5'), 25.6 (C-3'), 25.5 (C-4'), 25.4 (C-4), 21.2 (C-14), 8.7 (16-CH_3), 8.6 (6-CH_3); Figure 112

10.1.2.1.2 Deangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) (69)

A suspension of cyclohexanecarboxylic acid 248.1 µl (2.0 mmol) and K_2CO_3 276.4 mg (2.0 mmol) in 16 ml dry CH_2Cl_2 was stirred for 10 min. Cyclohexane-carbonyl chloride 272.5 µl (2.0 mmol) was added dropwise to the suspension on ice bath for 5 min. The suspension was stirred at room temperature for 21 h. The suspension was filtered and washed with dry CH_2Cl_2 several times. The combined filtrates were concentrated to afford cyclohexanoic anhydride 100 %yield.

Cyclohexanoic anhydride 35.0 µl (0.147 mmol, 14.7 eq) was added dropwise at -17 °C for 10 min to a mixture of deangeloylrenieramycin M (5 mg, 0.01 mmol) in 0.2 ml dry pyridine and DMAP (0.15 mg, 0.1 eq). The reaction mixture was stirred at the same temperature for 1 hr. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (49.3 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford deangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) (**69**, 4.9 mg, 80.1% yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) (**69**) 80.1% yield

Deangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) (69)

$[\alpha]^{25}_{D}$: -61.1 (*c* = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 114
269 (4.11), 235 (3.61)

CD : Δε nm (*c* = 86.2 μM, methanol, 25 °C); Figure 115
+0.8 (444),-2.3 (355), -5.1 (279), -0.9 (234), +0.6 (208)

EIMS : *m/z* (% intensity); Figure 113
603 ($M^{+\cdot}$, 7), 464 (5), 435 (4), 260 (17), 220 (100), 218 (21), 204 (9)

HR-EIMS : 603.2584 ($M^{+\cdot}$), (calcd for $C_{33}H_{37}N_3O_8$, 603.2581)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 116
3432, 3282, 2931, 2855, 2229, 1732, 1652, 1615, 1455, 1410, 1374, 1261, 802

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.29 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.00 (1H, d, *J* = 2.7 Hz, 21-H), 3.98 (1H, d, *J* = 3.4 Hz, 22-Hb), 3.95 (1H, overlap, 11-H), 3.95 (3H, s, 17-OCH_3), 3.95 (3H, s, 7-OCH_3), 3.93 (1H, d, *J* = 4.6 Hz, 1-H), 3.33 (1H, ddd, *J* = 7.6, 2.4, 1.8 Hz, 13-H), 3.02 (1H, ddd, *J* = 11.3, 2.7Hz, 3-H), 2.83 (1H, dd, *J* = 17.2, 2.7 Hz, 4-Hα), 2.71 (1H, dd, *J* = 20.7, 7.6 Hz, 14-Hα), 2.25 (1H, d, *J* = 20.7 Hz, 14-Hβ), 2.21 (3H, s, NCH_3), 1.91 (1H, 1'-H), 1.88 (3H, s, 16-CH_3), 1.87 (3H, s, 6-CH_3), 1.56 (2H, m, 5'-H_2), 1.55 (2H, m, 2'-H_2), 1.52 (2H, m, 4'-H_2), 1.27 (1H, ddd, *J* = 17.2, 11.3, 2.7 Hz, 4-Hβ), 1.06 (2H, m, 6'-H_2), 1.04 (2H, m, 3'-H_2); Figure 117

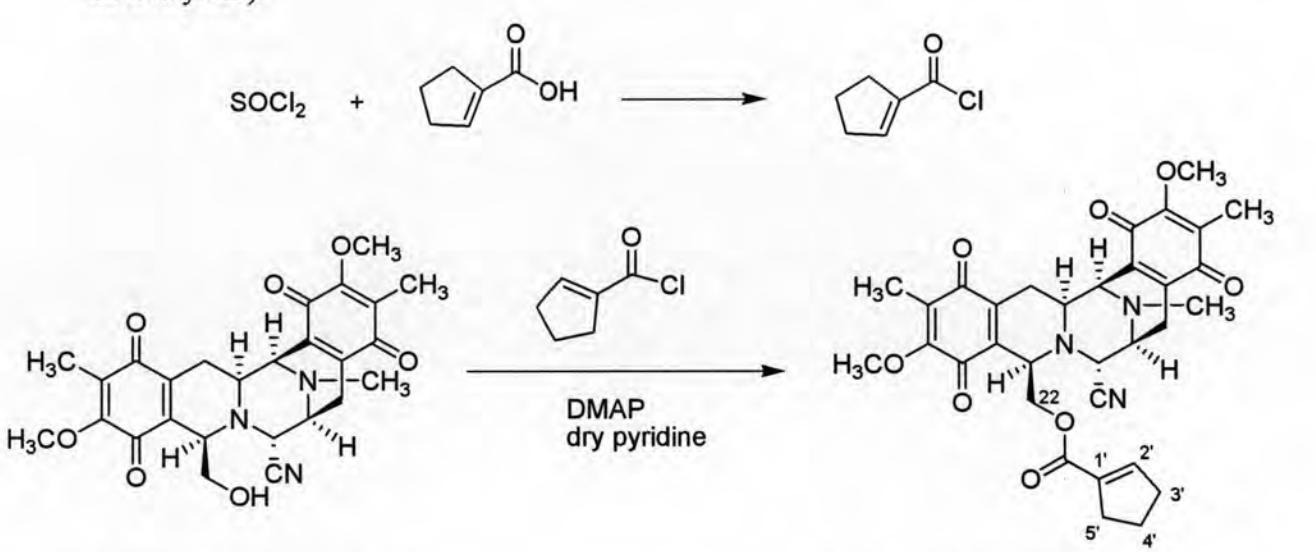
^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.2 (C-15), 185.5 (C-5), 182.5 (C-18), 180.9 (C-8), 175.0 (1'-CO), 155.8 (C-7), 155.3 (C-17), 142.1 (C-20), 141.4 (C-10), 135.5 (C-9), 135.0 (C-19), 128.4 (C-6), 128.3 (C-16), 116.8 (21-CN), 62.6 (C-22), 61.1 (17-OCH_3), 61.0 (7-OCH_3), 58.7 (C-21), 56.5 (C-1), 54.6 (C-13), 54.3 (C-3), 54.2 (C-11), 43.0 (C-1'), 41.5 (NCH_3), 29.3 (C-6'), 28.7 (C-2'), 25.5 (C-5'), 25.5 (C-4'), 25.4 (C-3'), 25.2 (C-4), 21.2 (C-14), 8.7 (6-CH_3), 8.7 (16-CH_3); Figure 118

10.1.2.2 Unsaturated alicyclic acyl derivatives of deangeloylrenieramycin M

10.1.2.2.1 Deangeloylrenieramycin M-22-(1'-cyclopentene-1'-carboxylate) (70)

Thionyl chloride 2.2 ml (30 mmol, 15 eq) was added dropwise over 10 min to 1-cyclopentenecarboxylic acid 224.3 mg (2 mmol) which was cooled on ice bath. The mixture was refluxed under an argon atmosphere for 1 h. After that, the reaction mixture was removed excess thionyl chloride by distillation under low pressure for 2 h. Finally, benzene was added to the mixture and concentrated *in vacuo* resulting in 1-cyclopentenecarbonyl chloride 100% yield.

A solution of the corresponding deangeloylrenieramycin M (16.5 mg, 0.03 mmol) in 0.6 ml of dry pyridine was cooled at -17 °C, and DMAP (0.37 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. The 1-cyclopentenecarbonyl chloride (57.6 mg , 0.44 mmol, 14.7 eq) was added to the reaction mixture. The reaction mixture was stirred for 2.25 h at -17 °C. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude product (66.4 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford a yellow amorphous solid, deangeloylrenieramycin M-22-(1'-cyclopentene-1'-carboxylate) (**70**, 11.9 mg, 60.5% yield).



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(1'-cyclopentene-1'-carboxylate) (**70**) 60.5% yield

Deangeloylrenieramycin M-22-(1'-cyclopentene-1'-carboxylate) (70)

$[\alpha]^{22}_{D}$: -75.5 (*c* = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 120
269 (4.12), 245 (3.87)

CD : Δε nm (*c* = 63.0 μM, methanol, 25 °C); Figure 121
+1.0 (440),-2.7 (357), -5.0 (281), +3.1 (258), -2.3 (235), -1.0 (217), +1.8 (212), +4.7 (208)

EIMS : *m/z* (% intensity); Figure 119
587 ($M^{+\cdot}$, 10), 462 (4), 260 (10), 220 (100), 218 (24), 204 (10), 176 (6)

HR-EIMS : 587.2267 ($M^{+\cdot}$), (calcd for $C_{32}H_{33}N_3O_8$, 587.2268)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 122
3443, 3279, 2961, 2854, 2228, 1714, 1652, 1447, 1373, 1235, 801

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 6.38 (1H, dt, *J* = 6.4, 2.1 Hz, 2'-H), 4.48 (1H, d, *J* = 8.8 Hz, 22-Ha), 3.98 (1H, d, *J* = 2.1 Hz, 21-H), 3.95 (1H, overlap, 1-H), 3.95 (3H, s, 17-OCH_3), 3.94 (1H, d, *J* = 8.8, 22-Hb), 3.93 (1H, overlap, 11-H), 3.93 (3H, s, 7-OCH_3), 3.31 (1H, ddd, *J* = 7.6, 1.8, 1.5 Hz, 13-H), 3.03 (1H, ddd, *J* = 11.3, 3.1, 2.7 Hz, 3-H), 2.84 (1H, dd, *J* = 17.4, 2.7 Hz, 4-Hα), 2.68 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.31 (2H, m, 3'-H_2), 2.24 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.20 (2H, overlap, 5'-H_2), 2.20 (3H, s, NCH_3), 1.88 (3H, s, 6-CH_3), 1.86 (3H, s, 16-CH_3), 1.77 (2H, quintet, *J* = 7.6 Hz, 4'-H_2), 1.26 (1H, ddd, *J* = 17.4, 11.3, 2.1 Hz, 4-Hβ); Figure 123

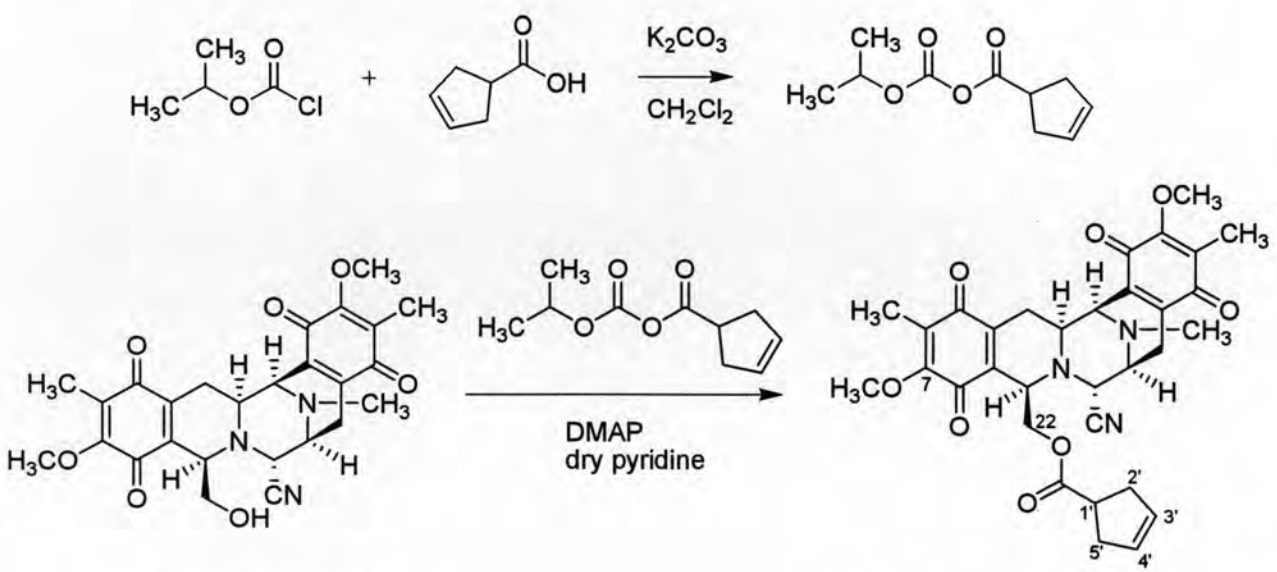
^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.0 (C-15), 185.5 (C-5), 182.5 (C-18), 181.0 (C-8), 164.1 (1'-CO) , 155.7 (C-7), 155.1 (C-17), 144.7 (C-2'), 142.2 (C-20), 141.8 (C-10), 135.8 (C-9), 135.5 (C-19), 134.8 (C-1'), 128.5 (C-6), 128.4 (C-16), 117.0 (21-CN), 62.3 (C-22), 61.1 (17-OCH_3), 61.0 (7-OCH_3), 58.7 (C-21), 56.4 (C-1), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5

(NCH_3), 33.3 (C-3'), 31.2 (C-5'), 25.4 (C-4), 22.9 (C-4'), 21.1 (C-14), 8.8 (16-CH_3), 8.7 (6-CH_3); Figure 124

10.1.2.2.2 Deangeloylrenieramycin M-22-(3'-cyclopentene-1'-carboxylate) (71)

A suspension of 3-cyclopentene-1-carboxylic acid 206.9 µl (2.0 mmol) and K_2CO_3 276.4 mg (2.0 mmol) in 16 ml dry CH_2Cl_2 was stirred for 10 min. Isopropyl chlorocarbonate 227.8 µl (2.0 mmol) was added dropwise to the suspension on ice bath for 5 min. The suspension was stirred at room temperature for 14 h. The suspension was filtered and washed with dry CH_2Cl_2 several times. The combined filtrates were concentrated to afford 3-cyclopentenoyl isopropyl carbonate 64.1 %yield.

A solution of deangeloylrenieramycin M (16.3 mg, 0.03 mmol) in 0.6 ml dry pyridine was cooled at -17 °C, and DMAP (0.37 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. The 3-cyclopentenoyl isopropyl carbonate (87.4 mg, 0.44 mmol, 14.7 eq) was added to the reaction mixture. The reaction mixture was stirred for 1 h at -17 °C. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (84.4 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford a yellow amorphous solid, deangeloylrenieramycin M-22-(3'-cyclopentene-1'-carboxylate) (**71**, 12.5 mg, 64.4% yield).



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(3'-cyclopentene-1'-carboxylate) (**71**) 64.4% yield

Deangeloylrenieramycin M-22-(3'-cyclopentene-1'-carboxylate) (71)

$[\alpha]^{22}_{D}$: -82.5 (*c* = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 126
269 (4.18), 234 (3.65)

CD : Δε nm (*c* = 80.9 μM, methanol, 25 °C); Figure 127
+1.0 (436),-2.7 (352), -5.9 (280), +2.4 (256), -0.7 (236), +6.4 (208)

EIMS : *m/z* (% intensity); Figure 125
587 ($M^{+\cdot}$, 10), 462 (3), 260 (9), 220 (100), 218 (26), 204 (12), 176 (8)

HR-EIMS : 587.2267 ($M^{+\cdot}$), (calcd for $C_{32}H_{33}N_3O_8$, 587.2268)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 128
3446, 3274, 2942, 2855, 2228, 1732, 1652, 1615, 1456, 1410, 1374, 1261, 802

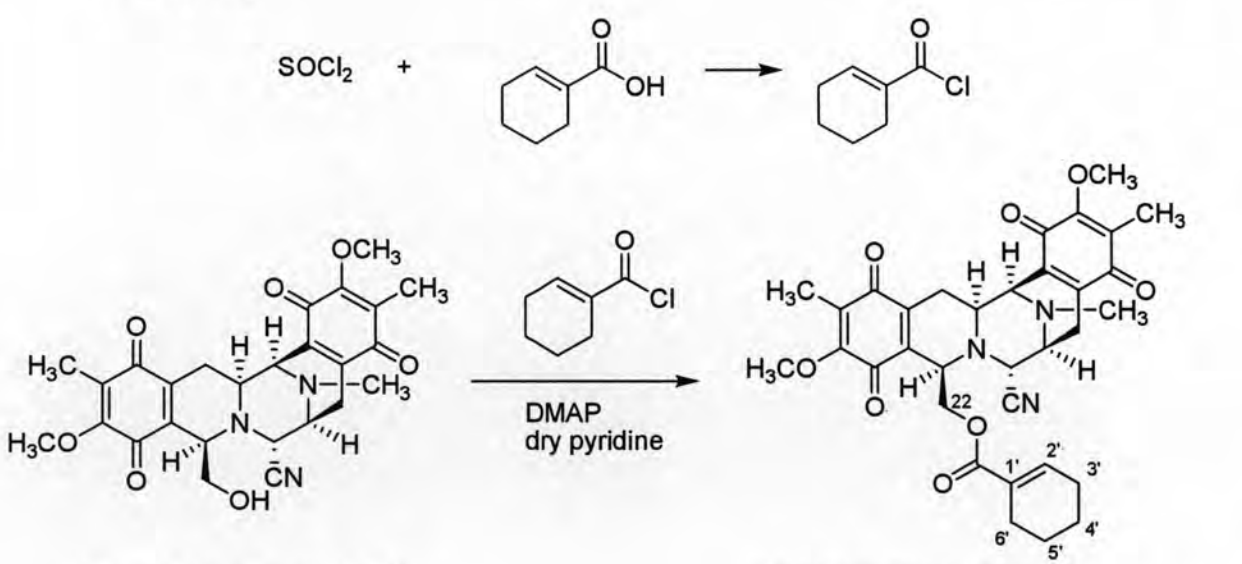
^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 5.46 (2H, m, 3'-H and 4'-H), 4.29 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.01 (1H, d, *J* = 2.4 Hz, 21-H), 3.98 (1H, dd, *J* =11.6, 3.7 Hz, 22-Hb), 3.95 (1H, overlap,11-H), 3.95 (3H, s, 17-OCH_3), 3.93 (3H, s, 7-OCH_3), 3.93 (1H, overlap, 1-H), 3.33 (1H, ddd, *J* = 7.3, 2.1, 1.8 Hz 13-H), 3.03 (1H, ddd, *J* = 11.3, 3.1, 2.7 Hz, 3-H), 2.84 (1H, dd, *J* = 17.3, 2.4 Hz, 4-Hα), 2.77 (1H, quintet, *J* = 7.0 Hz, 1'-H), 2.77 (2H, t, *J* = 7.0 Hz, 2'-H_2), 2.67 (1H, dd, *J* = 21.0, 7.5 Hz, 14-Hα), 2.34 (2H, m, 5'-H_2), 2.24 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.21 (3H, s, NCH_3), 1.88 (3H, s, 6-CH_3), 1.87 (3H, s, 16-CH_3), 1.28 (1H, ddd, *J* = 17.3, 11.3, 2.7 Hz, 4-Hβ); Figure 129

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.2 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 175.0 (1'-CO), 155.7 (C-7), 155.2 (C-17), 142.1 (C-20), 141.6 (C-10), 135.4 (C-9), 135.0 (C-19), 129.1 (C-3'), 128.6 (C-4'), 128.4 (C-6), 128.4 (C-16), 116.8 (21-CN), 63.5 (C-22), 61.0 (17-OCH_3), 61.1 (7-OCH_3), 58.9 (C-21), 56.3 (C-1), 54.6 (C-13), 54.4 (C-3), 54.2 (C-11), 41.6 (C-1'), 41.5 (NCH_3), 36.3 (C-2'), 35.7 (C-5'), 25.4 (C-4), 21.2 (C-14), 8.7 (6-CH_3), 8.7 (16-CH_3); Figure 130

10.1.2.2.3 Deangeloylrenieramycin M-22-(1'-cyclohexene-1'-carboxylate) (72)

Thionyl chloride 1.45 ml (20 mmol, 20 eq) was added dropwise over 10 min to 1-cyclohexenecarboxylic acid 116.2 mg (1 mmol) which was cooled on ice bath. The mixture was refluxed under an argon atmosphere for 1 h. After that, the reaction mixture was removed excess thionyl chloride by distillation under low pressure for 2 h. Finally, benzene was added to the mixture and concentrated *in vacuo* resulting in 1-cyclohexenecarbonyl chloride 100% yield.

A solution of the corresponding deangeloylrenieramycin M (16.7 mg, 0.03 mmol) in 0.6 ml of dry pyridine was cooled at -17 °C, and DMAP (0.37 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the this temperature. The 1-cyclohexenecarbonyl chloride (63.7 mg, 0.44 mmol, 14.7 eq) was added to the reaction mixture. The reaction mixture was stirred for 2 h at -17 °C. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude product (31.3 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford a yellow amorphous solid, deangeloylrenieramycin M-22-(1'-cyclohexene-1'-carboxylate) (**72**, 11.9 mg, 58.4% yield).



Deangeloylrenieramycin M-22-(1'-cyclohexene-1'-carboxylate) (72)

$[\alpha]^{22}_{D}$: -60.9 (*c* = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 132
269 (4.18), 242 (3.88)

CD : Δε nm (*c* = 73.2 μM, methanol, 25 °C); Figure 133
+0.9 (439),-2.0 (357), -3.8 (282), +2.3 (257), -1.7 (234), -1.0 (216), +1.6 (209)

EIMS : *m/z* (% intensity); Figure 131
601 ($M^{+\cdot}$, 10), 464 (6), 260 (12), 220 (100), 218 (27), 204 (12), 176 (8)

HR-EIMS : 601.2427 ($M^{+\cdot}$), (calcd for $C_{33}H_{35}N_3O_8$, 601.2424)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 134
3445, 3281, 2935, 2856, 2228, 1714, 1652, 1615, 1456, 1261, 801

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 6.58 (1H, m, 2'-H), 4.43 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 3.97 (1H, br s, 21-H), 3.95 (1H, overlap, 1-H), 3.95 (3H, s, 7-OCH_3), 3.94 (1H, overlap, 22-Hb), 3.94 (3H, s, 17-OCH_3), 3.93 (1H, overlap, 11-H), 3.31 (1H, ddd, *J* = 7.6, 2.1, 1.8 Hz, 13-H), 3.02 (1H, ddd, *J* = 11.6, 3.1, 2.7 Hz, 3-H), 2.85 (1H, dd, *J* = 17.1, 2.4 Hz, 4-Hα), 2.68 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.24 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.21 (3H, s, NCH_3), 1.99 (2H, m, 3'-H_2), 1.88 (2H, overlap, 6'-H_2), 1.88 (3H, s, 6-CH_3), 1.87 (3H, s, 16-CH_3), 1.87 (2H, overlap, 5'-H_2), 1.44 (2H, m, 4'-H_2), 1.25 (1H, ddd, *J* = 17.1, 11.6, 2.7 Hz, 4-Hβ); Figure 135

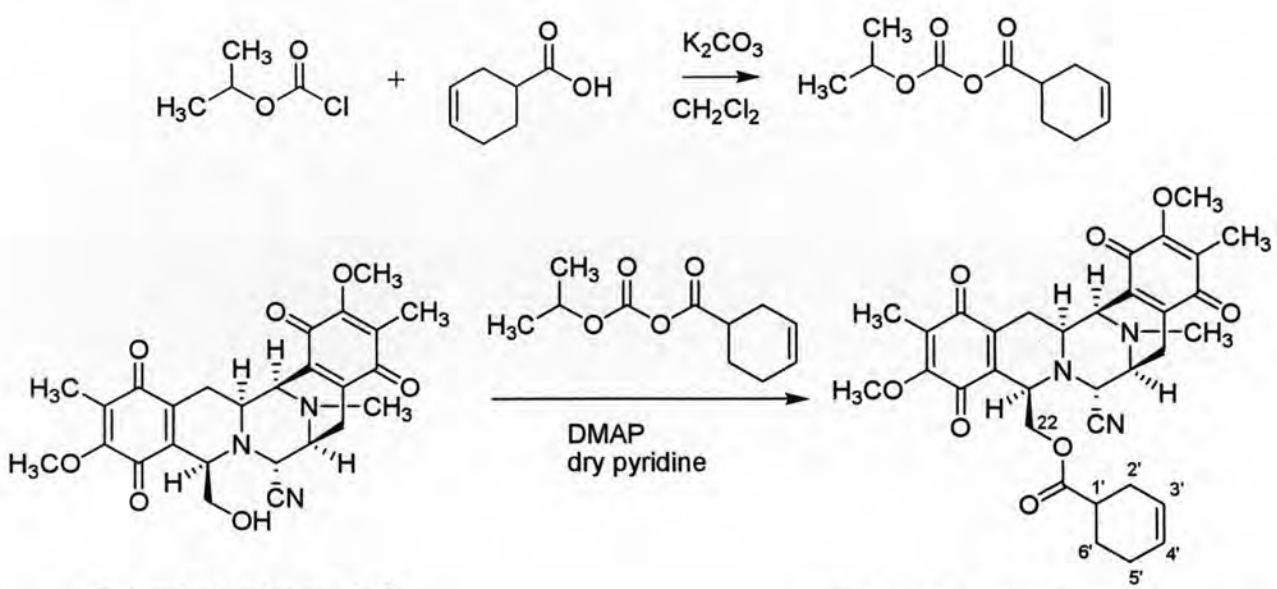
^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.1 (C-15), 185.5 (C-5), 182.5 (C-18), 181.0 (C-8), 166.5 (1'-CO), 155.7 (C-7), 155.1 (C-17), 142.2 (C-20), 141.7 (C-10), 140.6 (C-2'), 135.6 (C-9), 134.8 (C-19), 129.6 (C-1'), 128.4 (C-6), 128.3 (C-16), 116.9 (21-CN), 62.6 (C-22), 61.1 (7-OCH_3), 61.0 (17-OCH_3), 58.8 (C-21), 56.4 (C-1), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5

(NCH_3), 25.7 (C-3'), 25.4 (C-4), 24.0 (C-6'), 21.9 (C-4'), 21.2 (C-5'), 21.1 (C-14), 8.8 (6-CH_3), 8.8 (16-CH_3); Figure 136

10.1.2.2.4 Deangeloylrenieramycin M-22-(3'-cyclohexene-1'-carboxylate) (73)

A suspension of 3-cyclohexene-1-carboxylic acid 233.6 µl (2.0 mmol) and K_2CO_3 276.4 mg (2.0 mmol) in 16 ml dry CH_2Cl_2 was stirred for 10 min. Isopropyl chlorocarbonate 227.8 µl (2.0 mmol) was added dropwise to the suspension on ice bath for 5 min. The suspension was stirred at room temperature for 22 h. The suspension was filtered and washed with dry CH_2Cl_2 several times. The combined filtrates were concentrated to afford 3-cyclohexenoyl isopropyl carbonate 100% yield.

A solution of deangeloylrenieramycin M (5.1 mg, 0.01 mmol) in 0.2 ml dry pyridine was cooled at -17 °C, and DMAP (0.12 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. The 3-cyclohexenoyl isopropyl carbonate (31. 2 mg, 0.147 mmol, 14.7 eq) was added to the reaction mixture. The reaction mixture was stirred for 1 h at -17 °C. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (53.4 mg) was purified by silica gel column chromatography with a gradient hexane/ethyl acetate to afford a yellow amorphous solid, deangeloylrenieramycin M-22-(3'-cyclohexene-1'-carboxylate) (**73**, 5 mg, 80.4% yield).



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(3'-cyclohexene-1'-carboxylate)(**73**) 80.4% yield

Deangeloylrenieramycin M-22-(3'-cyclohexene-1'-carboxylate) (73)

$[\alpha]^{22}_{D}$: -68.8 (*c* = 0.4, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 138
270 (4.18), 235 (3.67), 205 (4.31)

CD : Δε nm (*c* = 81.5 μM, methanol, 25 °C); Figure 139
+1.0 (445),-2.5 (357), -5.7 (281), +2.3 (256), -0.5 (237), +3.4 (210), +6.0 (205)

EIMS : *m/z* (% intensity); Figure 137
601 ($M^{+\cdot}$, 9), 462 (3), 260 (11), 220 (100), 218 (22), 204 (10), 176 (5)

HR-EIMS : 601.2420 ($M^{+\cdot}$), (calcd for $C_{33}H_{35}N_3O_8$, 601.2424)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 140
3432, 3277, 3018, 2929, 2841, 2229, 1731, 1646, 1613, 1451, 1374, 1233, 798

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; **minor isomer**, 5.53 (1H, m, 3'-H), 5.44 (1H, m, 4'-H), 4.28 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.06 (1H, dd, *J* = 11.6, 3.1 Hz, 22-Hb), 4.01 (1H, d, *J* = 2.7 Hz, 21-H), 3.95 (3H, s, 17-OCH_3), 3.94 (1H, overlap, 11-H), 3.93 (3H, s, 7-OCH_3), 3.93 (1H, overlap, 1-H), 3.33 (1H, ddd, *J* = 7.6, 2.1, 1.8 Hz, 13-H), 3.03 (1H, ddd, *J* = 11.6, 3.1, 2.7 Hz, 3-H), 2.83 (1H, d, *J* = 16.5 Hz, 4-Hα), 2.70 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.24 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.21 (3H, s, NCH_3), 2.19 (1H, m, 1'-H), 1.97 (1H, m, 2'-Ha), 1.94 (1H, m, 6'-Ha), 1.88 (3H, s, 6-CH_3), 1.87 (1H, m, 2'-Hb), 1.86 (3H, s, 16-CH_3), 1.80 (1H, m, 6'-Hb), 1.28 (2H, m, 5'-H_2), 1.27 (1H, m, 4-Hβ); **major isomer**, 5.71 (2H, br s, 3'-H and 4'-H), 4.35 (1H, dd, *J* = 11.6, 2.7 Hz, 22-Ha), 4.01 (1H, d, *J* = 2.7 Hz, 21-H), 4.00 (1H, dd, *J* = 11.6, 3.1 Hz, 22-Hb), 3.95 (3H, s, 17-OCH_3), 3.94 (1H, overlap, 11-H), 3.94 (3H, s, 7-OCH_3), 3.93 (1H, overlap, 1-H), 3.33 (1H, ddd, *J* = 7.6, 2.1, 1.8 Hz,13-H), 3.03 (1H, ddd, *J* = 11.6, 3.1, 2.7 Hz, 3-H), 2.83 (1H, d, *J* = 16.5 Hz, 4-Hα), 2.70 (1H, dd, *J* = 21.0, 7.6 Hz, 14-Hα), 2.24 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.21 (3H, s, NCH_3),

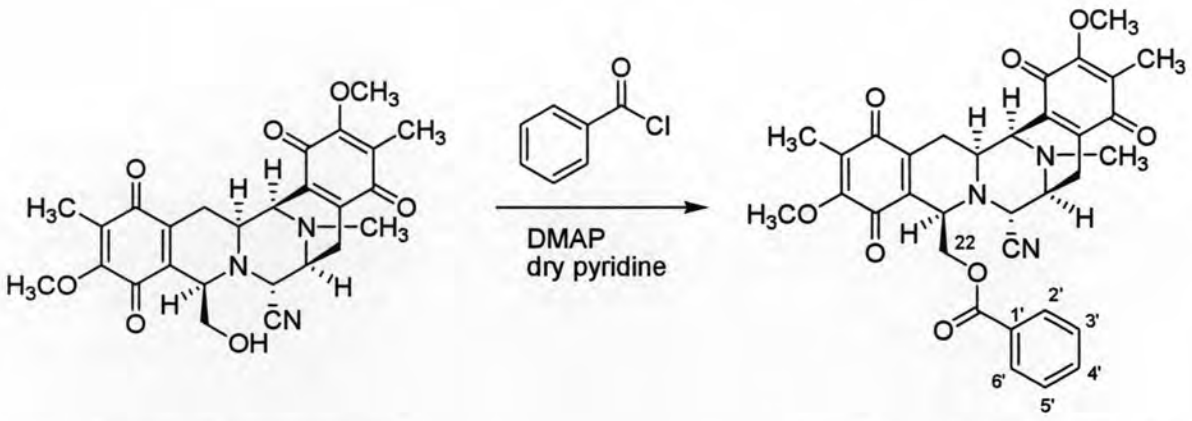
2.19 (1H, m, 1'-H), 1.97 (1H, m, 2'-Ha), 1.94 (1H, m, 6'-Ha), 1.88 (3H, s, 6-CH_3), 1.87 (1H, m, 2'-Hb),1.86 (3H, s, 16-CH_3), 1.80 (1H, m, 6'-Hb), 1.28 (2H, m, 5'-H_2), 1.27 (1H, m, 4-Hβ); Figure 141

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; **minor isomer**, 186.2 (C-15), 185.5 (C-5), 182.4 (C-18), 181.0 (C-8), 174.8 (1'-CO), 155.8 (C-7), 155.3 (C-17), 142.1 (C-20), 141.4 (C-10), 135.5 (C-9), 135.1 (C-19), 128.6 (C-6), 128.4 (C-16), 126.6 (C-3'), 124.6 (C-4'), 116.8 (21-CN), 62.8 (C-22), 61.1 (17-OCH_3), 61.0 (7-OCH_3), 58.6 (C-21), 56.4 (C-1), 54.6 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (NCH_3), 39.1 (C-1'), 27.7 (C-6'), 25.5 (C-4), 25.2 (C-5'), 24.3 (C-2'), 21.2 (C-14), 8.6 (6-CH_3), 8.7 (16-CH_3); **major isomer**, 186.2 (C-15), 185.5 (C-5), 182.4 (C-18), 180.9 (C-8), 174.8 (1'-CO), 155.8 (C-7), 155.3 (C-17), 142.1 (C-20), 141.4 (C-10), 135.5 (C-9), 135.1 (C-19), 128.6 (C-6), 128.3 (C-16), 126.9 (C-3'), 124.8 (C-4'), 116.8 (21-CN), 62.7 (C-22), 61.1 (17-OCH_3), 61.0 (7-OCH_3), 58.6 (C-21), 56.4 (C-1), 54.6 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (NCH_3), 39.2 (C-1'), 27.7 (C-6'), 25.5 (C-4), 25.2 (C-5'), 24.3 (C-2'), 21.1 (C-14), 8.7 (6-CH_3), 8.7 (16-CH_3); Figure 142

10.1.3 Aromatic acyl derivatives of deangeloylrenieramycin M

10.1.3.1 Deangeloylrenieramycin M-22-phenylcarboxylate (74)

The solution of deangeloylrenieramycin M (21.4 mg 0.043 mmol) in 0.8 ml dry pyridine was cooled at -17 °C, and DMAP (0.49 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. Benzoyl chloride (72.8 µl, 0.63 mmol, 14.7 eq) was added dropwise for 10 min and the reaction mixture was stirred at -17 °C for 1.5 h. The reaction mixture was partitioned with water (20 ml) and chloroform (20 ml x 3). The chloroform extracts were combined and dried over anhydrous sodium sulfate, filtered, concentrated and dried *in vacuo* to give crude product (103.7 mg). Silica gel column chromatography with a gradient of hexane/ethyl acetate was used to isolate deangeloylrenieramycin M-22-phenylcarboxylate (**74**, 10. 2 mg, 39.4% yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-phenylcarboxylate (**74**)
39.4% yield

Deangeloylrenieramycin M-22-phenylcarboxylate (74)

UV : λ_{max} nm (log ε), in methanol; Figure 144
269 (4.23), 248 (4.06)

EIMS : *m/z* (% intensity); Figure 143
597 ($M^{+\cdot}$, 10), 464 (3), 435 (3), 368 (5), 260 (13), 243 (19), 220 (100), 218 (25), 204 (11), 105 (24)

HR-EIMS : 597.2115 ($M^{+\cdot}$), (calcd for $C_{33}H_{31}N_3O_8$, 597.2111)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 145
3445, 2926, 2853, 2339, 1721, 1652, 1616, 1452, 1373, 1272, 1117, 956, 757

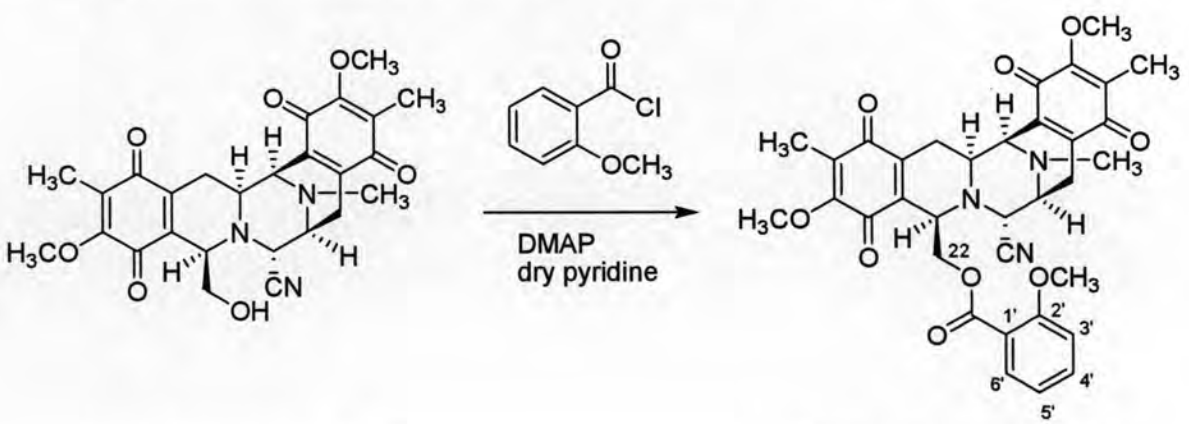
^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 7.60 (2H, dd, *J* = 7.7, 1.2 Hz, 2'-H and 6'-H), 7.50 (1H, dt, *J* = 7.7, 1.6 Hz, 4'-H), 7.31 (2H, t, *J* = 7.7 Hz, 3-H' and 5'-H), 5.00 (1H, dd, *J* = 12.0, 3.1 Hz, 22-Ha), 4.12 (1H, br s, 21-H), 4.06 (1H, d, *J* = 12.0 Hz, 22-Hb), 4.06 (1H, overlap, 1-H), 4.03 (3H, s, 7-OCH_3), 3.67 (3H, s, 17-OCH_3), 3.97 (1H, br d, *J* = 2.0 Hz, 11-H), 3.44 (1H, d, *J* = 7.2 Hz,13-H), 3.12 (1H, ddd, *J* = 11.4, 2.7, 2.5 Hz, 3-H), 2.88 (1H, dd, *J* = 17.4, 2.3 Hz, 4-Hα), 2.74 (1H, dd, *J* = 21.1, 7.2 Hz, 14-Hα), 2.40 (1H, d, *J* = 21.1 Hz, 14-Hβ), 2.25 (3H, s,

NCH_3), 1.97 (3H, s, 6-CH_3), 1.69 (3H, s, 16- OCH_3), 1.27 (1H, ddd, J= 17.4, 11.4, 2.4 Hz, 4-Hβ); Figure 146

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 185.4 (C-15), 185.1 (C-5), 181.6 (C-18), 180.8 (C-8), 165.2 (1'-CO), 155.5 (C-7), 154.7 (C-17), 142.0 (C-20), 141.7 (C-10), 135.3 (C-9), 135.3 (C-19), 133.1 (C-4'), 130.0 (C-1'), 129.2 (C-2' and C-6'), 129.0 (C-6), 128.6 (C-16), 128.4 (C-3' and C-5'), 116.6 (21-CN), 61. 9 (C-22), 61.2 (7-OCH_3), 60.9 (17-OCH_3), 58.0 (C-21), 56.8 (C-1), 54.7 (C-13), 54.3 (C-11), 53.9 (C-3), 41.4 (NCH_3), 25.5 (C-4), 21.8 (C-14), 9.0 (6-CH_3), 9.0 (16-CH_3); Figure 147

10.1.3.2 Deangeloylrenieramycin M-22-(2'-methoxyphenylcarboxylate) (75)

The solution of deangeloylrenieramycin M (20.6 mg 0.042 mmol) in 0.8 ml dry pyridine was cooled at -17 °C, and DMAP (0.49 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. 2-Methoxybenzoyl chloride (91.9 µl, 0.62 mmol, 14.7 eq) was added dropwise for 10 min and the reaction mixture was stirred at -17 °C for 1.3 h. The reaction mixture was partitioned with water (20 ml) and chloroform (20 ml x 3). The chloroform extracts were combined and dried over anhydrous sodium sulfate, filtered, concentrated and dried *in vacuo* to give crude product (158.9 mg). Silica gel column chromatography with a gradient of hexane/ethyl acetate was used to isolate deangeloylrenieramycin M-22-(2'-methoxyphenylcarboxylate) (**75**, 14.3 mg, 54.9% yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(2'-methoxyphenylcarboxylate) (**75**) 54.9% yield

Deangeloylrenieramycin M-22-(2'-methoxyphenylcarboxylate) (75)

UV : λ_{max} nm (log ε), in methanol; Figure 149
269 (4.17), 248 (3.98)

FABMS : *m/z* (% intensity); Figure 148
628 ($[M+H]^+$, 12), 601 (7), 391 (8), 307 (23), 220 (30), 154 (100), 136 (71), 107 (20)

HR-FABMS : 628.2298 $[M+H]^+$, (calcd for $C_{34}H_{34}N_3O_9$, 628.2295)

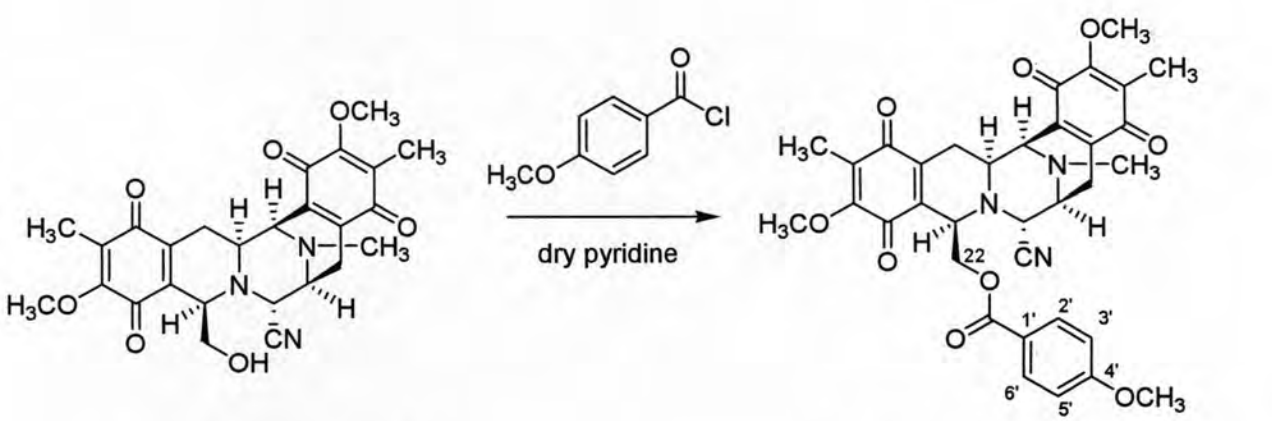
IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 150
3467, 2944, 2850, 2342, 2229, 1704, 1654, 1618, 1491, 1461, 1373, 1236, 1148, 759

^1H NMR : δ ppm, 300 MHz, in $CDCl_3$; 7.50 (1H, dd, *J* = 7.7, 1.6 Hz, 6'-H), 7.41 (1H, dt, *J* = 7.7, 1.6 Hz, 4'-H), 6.88 (1H, t, *J* = 7.7 Hz, 5'-H), 6.85 (1H, d, *J* = 7.7 Hz, 3'-H), 5.00 (1H, dd, *J* = 11.4, 2.1 Hz, 22-Ha), 4.26 (1H, br s, 21-H), 4.06 (2H, br s, 1-H and 11-H), 4.00 (3H, s, 7-OCH_3), 3.98 (1H, d, *J* = 11.4 Hz, 22-Hb), 3.80 (3H, s, 17-OCH_3), 3.62 (3H, s, 2'- OCH_3), 3.59 (1H, overlap, 13-H), 3.22 (1H, d, *J* = 11.5 Hz, 3-H), 2.90 (1H, dd, *J* = 17.1, 2.3 Hz, 4-Hα), 2.76 (1H, br d, *J* = 21.0 Hz, 14-Hα), 2.50 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.35 (3H, s, NCH_3), 1.95 (3H, s, 6-CH_3) 1.59 (1H, ddd, *J* = 17.1, 11.5, 3.6 Hz, 4-Hβ), 1.48 (3H, s, 16-CH_3); Figure 151

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 185.5 (C-15), 185.2 (C-5), 182.0 (C-18), 180.9 (C-8), 165.7 (1'-CO), 158.5 (C-2'), 155.4 (C-7), 154.4 (C-17), 142.1 (C-20), 141.8 (C-10), 135.4 (C-9), 135.4 (C-19), 133.9 (C-4'), 132.3 (C-6'), 128.6 (C-6), 128.4 (C-16), 120.2 (C-5'), 118.5 (C-1'), 116.8 (21-CN), 112.0 (C-3'), 62. 4 (C-22), 61.2 (7-OCH_3), 60.9 (17-OCH_3), 58.0 (C-21), 56.4 (C-1), 55.6 (2'- OCH_3), 54.7 (C-13), 54.5 (C-11), 54.0 (C-3), 41.5 (NCH_3), 25.3 (C-4), 21.2 (C-14), 9.0 (6-CH_3), 8.6 (16-CH_3); Figure 152

10.1.3.3 Deangeloylrenieramycin M-22-(4'-methoxyphenylcarboxylate) (76)

The solution of deangeloylrenieramycin M (5.0 mg 0.01 mmol) in 0.2 ml dry pyridine was cooled at -17 °C. The mixture was stirred for 5 min at the same temperature. 4-Methoxybenzoyl chloride (20.2 µl, 0.15 mmol, 14.7 eq) was added dropwise for 10 min and the reaction mixture was stirred at -17 °C for 2.5 h. The reaction mixture was partitioned with water (20 ml) and chloroform (20 ml x 3). The chloroform extracts were combined and dried over anhydrous sodium sulfate, filtered, concentrated and dried *in vacuo* to give crude product (158.9 mg). Silica gel column chromatography with a gradient of hexane/ethyl acetate was used to isolate deangeloylrenieramycin M-22-(4'-methoxyphenylcarboxylate) (**76**, 2.8 mg, 44.0% yield) as a yellow amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(4'-methoxyphenylcarboxylate) (**76**)
44.0% yield

Deangeloylrenieramycin M-22-(4'-methoxyphenylcarboxylate) (76)

UV : λ_{max} nm (log ε), in methanol; Figure 154
258 (4.36), 230 (3.84)

EIMS : *m/z* (% intensity); Figure 153
627 ($M^{+\cdot}$, 12), 464 (7), 435 (2), 368 (10), 260 (17), 243 (20), 220 (100), 218 (25), 204 (11), 152 (27), 135 (56)

HR-EIMS : 627.2220 ($M^{+\cdot}$), (calcd for $C_{34}H_{33}N_3O_9$, 627.2217)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 155

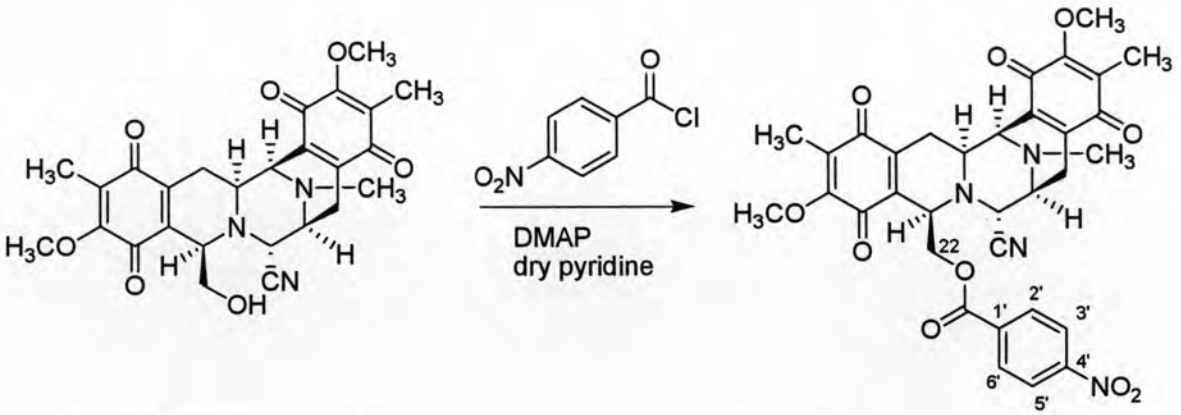
3445, 2927, 2854, 2229, 2045, 1924, 1870, 1831, 1715, 1652, 1512, 1455, 1372, 1258, 1167, 1027, 802, 768

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; 7.55 (2H, d, J = 8.8 Hz, 2'-H and 6'-H), 6.77 (2H, d, J = 8.8 Hz, 3'-H and 5'-H), 4.97 (1H, dd, J = 12.1, 3.1 Hz, 22-Ha), 4.07 (1H, d, J = 2.2 Hz, 21-H), 4.03 (5H, overlap, 22-Hb, 7-OCH_3, 1-H), 3.91 (1H, br s,1-H), 3.81 (3H, s, 4'-OCH_3), 3.71 (3H, s, 17-OCH_3), 3.36 (1H, d, J = 7.4 Hz, 13-H), 3.06 (1H, dt, J = 11.4, 2.6 Hz, 3-H), 2.86 (1H, dd, J = 17.2, 1.9 Hz, 4-Hα), 2.69 (1H, dd, J = 21.0, 7.6 Hz, 14-Hα), 2.34 (1H, d, J = 21.0 Hz, 14-Hβ), 2.19 (3H, s, NCH_3), 1.97 (3H, s, 6-CH_3), 1.72 (3H, s, 16-CH_3), 1.30 (1H, ddd, J = 17.2, 11.7, 2.2 Hz, 4-Hβ); Figure 156

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 185.6 (C-15), 185.3 (C-5), 182.0 (C-18), 180.9 (C-8), 164.9 (1'-CO), 163.2 (C-4'), 155.5 (C-7), 154.7 (C-17), 142.1 (C-20), 141.9 (C-10), 135.5 (C-9), 134.3 (C-19), 131.3 (C-2' and C-6'), 128.5 (C-6), 128.2 (C-16), 121.4 (C-1'), 116.9 (21-CN), 113.7 (C-3' and C-5'), 61.6 (C-22), 61.2 (7-OCH_3), 60.7 (17-OCH_3), 58.4 (C-21), 56.9 (C-1), 55.5 (4'-OCH_3), 54.6 (C-13), 54.4 (C-3), 54.3 (C-11), 41.5 (NCH_3), 25.7 (C-4), 21.2 (C-14), 9.0 (6-CH_3 and 16-CH_3); Figure 157

10.1.3.4 Deangeloylrenieramycin M-22-(4'-nitrophenylcarboxylate) (77)

The solution of deangeloylrenieramycin M (20.4 mg, 0.04 mmol) in 0.8 ml dry pyridine was cooled at -17 °C, and DMAP (0.49 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. 4-Nitrobenzoyl chloride (109.10 µl, 0.59 mmol, 14.7 eq) was added dropwise for 10 min and the reaction mixture was stirred at -17 °C for 45 min. The reaction mixture was partitioned with water (20 ml) and chloroform (20 ml x 3). The chloroform extracts were combined and dried over anhydrous sodium sulfate, filtered, concentrated and dried *in vacuo* to give crude product (158.9 mg). Silica gel column chromatography with a gradient of hexane/ethyl acetate was used to isolate deangeloylrenieramycin M-22-(4'-nitrophenylcarboxylate) (**77**, 13.0 mg, 48.6% yield) as a yellowish-orange amorphous solid.



deangeloylrenieramycin M

deangeloylrenieramycin M-22-(4'-nitrophenylcarboxylate) (**77**)
48.6% yield

Deangeloylrenieramycin M-22-(4'-nitrophenylcarboxylate) (77)

UV : λ_{max} nm (log ε), in methanol; Figure 160
264 (4.33), 232 (4.04)

FABMS : *m/z* (% intensity); Figure 159
643 ($[M+H]^+$, 6), 485 (4), 368 (25), 299 (13), 232 (10), 220 (66), 135 (45), 119 (96), 55 (78)

HR-FABMS : *m/z* 643.2037 $[M+H]^+$, (calcd for $C_{33}H_{31}N_4O_{10}$, 643.2030)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 161
3446, 2929, 2853, 2351, 1727, 1654, 1613, 1528, 1449, 1374, 1348, 1273, 1149, 1016, 956, 720

^1H NMR : δ ppm, 300 MHz, in $CDCl_3$; 8.17 (2H, d, *J* = 8.5 Hz, 3'-H and 5'-H), 7.80 (2H, d, *J* = 8.5 Hz, 2'-H and 6'-H), 4.94 (1H, dd, *J* = 11.7, 2.4 Hz, 22-Ha), 4.19 (1H, d, *J* = 11.7 Hz, 22-Hb), 4.11 (2H, overlap, 1-H and 21-H), 4.02 (3H, s, 7-OCH_3), 3.96 (1H, br s, 11-H), 3.77 (3H, s, 17-OCH_3), 3.47 (1H, d, *J* = 6.0 Hz, 13-H), 3.14 (1H, d, *J* = 11.2 Hz, 3-H), 2.91 (1H, dd, *J* = 17.3, 1.5 Hz, 4-Hα), 2.73 (1H, dd, *J* = 21.2, 7.3 Hz, 14-Hα), 2.33 (1H, d, *J* = 21.2 Hz, 14-Hβ), 2.26 (3H, s, NCH_3), 1.97 (3H, s, 6-CH_3), 1.69 (3H, s, 16-CH_3), 1.27 (1H, 4-Hβ); Figure 162

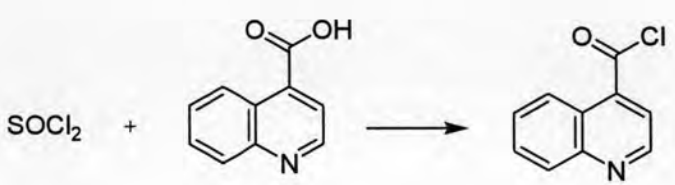
^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; 185.3 (C-15), 185.0 (C-5), 181.5 (C-18), 180.7 (C-8), 163.6 (1'-CO), 155.5 (C-7), 154.6 (C-17),

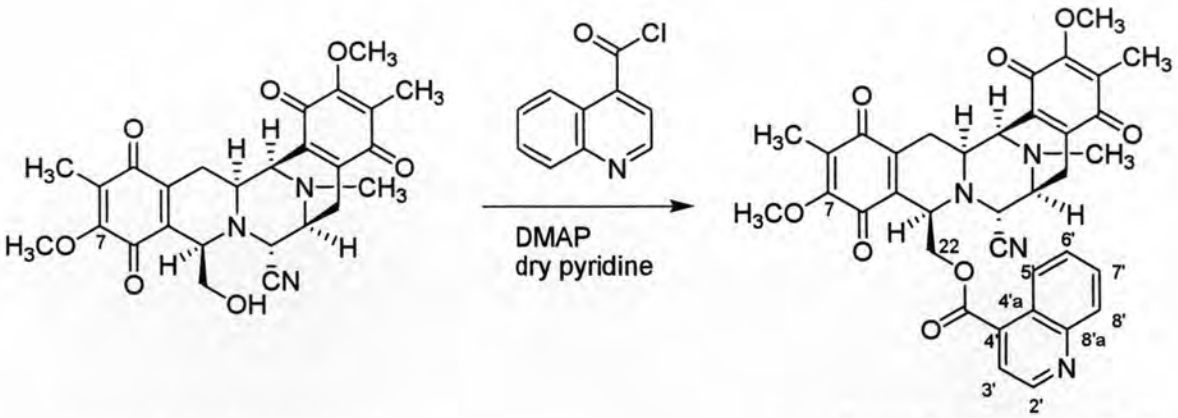
150.5 (C-4'), 142.0 (C-20), 141.5 (C-10), 135.0 (C-9), 134.2 (C-19 and C-1'), 130.4 (C-2' and C-6'), 128.8 (C-6), 127.9 (C-16), 123.6 (C-3' and C-5'), 116.4 (21-CN), 63.3 (C-22), 61.3 (7-OCH_3), 61.0 (17-OCH_3), 58.0 (C-21), 56.5 (C-1), 54.6 (C-13), 54.4 (C-11), 54.0 (C-3), 41.4 (NCH_3), 25.5 (C-4), 21.9 (C-14), 9.1 (6-CH_3), 8.9 (16-CH_3); Figure 163

10.1.3.5 Deangeloylrenieramycin M-22-(4'-quinolinylcarboxylate) (78)

Thionyl chloride 1.45 ml (20 mmol, 20 eq) was added dropwise over 10 mins to 4-quinolinecarboxylic acid 173.17 mg (1 mmol) which was cooled on ice bath. The mixture was refluxed under an argon atmosphere for 1 h. After that, the reaction mixture was removed excess thionyl chloride by distillation under low pressure for 5 h. Finally, benzene was added in the mixture and concentrated *in vacuo* resulting in 4-quinolinecarbonyl chloride 138 %yield.

A solution of deangeloylrenieramycin M (30 mg, 0.06 mmol) in 1.2 ml dry pyridine was cooled at -17 °C, and DMAP (0.73 mg, 0.1 eq) was added. The mixture was stirred for 5 min at the same temperature. 4-quinolinecarbonyl chloride (169 mg ,0.88 mmol, 14.7 eq) was added dropwise for 10 min and the reaction mixture was stirred for 3 h at -17 °C. The reaction was quenched with water (20 ml) and partitioned with chloroform (20 ml x 3). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue (170.4 mg) was purified by silica gel column chromatography with a gradient of hexane/ethyl acetate to afford deangeloylrenieramycin M-22-(4'-quinolinylcarboxylate) (**78**, 26.1 mg, 67.1% yield) as a brown amorphous solid.





deangeloylrenieramycin M

deangeloylrenieramycin M-22-(4'-quinolinylcarboxylate) (**78**) 67.1% yield

Deangeloylrenieramycin M-22-(4'-quinolinylcarboxylate) (78)

$[\alpha]^{18}_{D}$: -88.1 (*c* = 0.6, $CHCl_3$)

UV : λ_{max} nm (log ε), in methanol; Figure 165
270 (4.32), 252 (4.16)

FABMS : *m/z* (% intensity); Figure 164
649 ($[M+ H]^+$, 28), 622 (6), 437 (1), 309 (16), 220 (34), 155 (68), 119 (100), 85 (74), 73 (28)

HR-FABMS : 649.2296 $[M+H]^+$, (calcd for $C_{36}H_{33}N_4O_8$, 649.2299)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 166
3467, 2928, 2850, 2351, 1725, 1655, 1618, 1459, 1374, 1236, 1148, 1019, 768

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; 8.90 (1H, d, *J* = 4.3 Hz, 2'-H), 8.58 (1H, d, *J* = 8.3 Hz, 5'-H), 8.16 (1H, d, *J* = 8.3 Hz, 8'-H), 7.77 (1H, t, *J* = 8.3 Hz, 7'-H), 7.57 (1H, t, *J* = 8.3 Hz, 6'-H), 7.49 (1H, d, *J* = 4.3 Hz, 3'-H), 5.10 (1H, dd, *J* = 11.7, 2.9 Hz, 22-Ha), 4.30 (1H, dd, *J* = 11.7, 2.0 Hz, 22-Hb), 4.17 (1H, overlap, 21-H), 4.16 (1H, 1-H), 4.05 (3H, s, 7-OCH_3), 3.97 (1H, br s, 11-H), 3.65 (3H, s, 17-OCH_3), 3.40 (1H, br d, *J* =7.4 Hz, 13-H), 3.16 (1H, td, *J* = 11.3, 2.4 Hz, 3-H), 2.95 (1H, dd, *J* = 17.7, 2.3 Hz, 4-Hα), 2.67 (1H, dd, *J* = 21.0, 7.4 Hz, 14-Hα), 2.38 (1H, d, *J* = 21.0 Hz, 14-Hβ), 2.22 (3H, s, NCH_3),

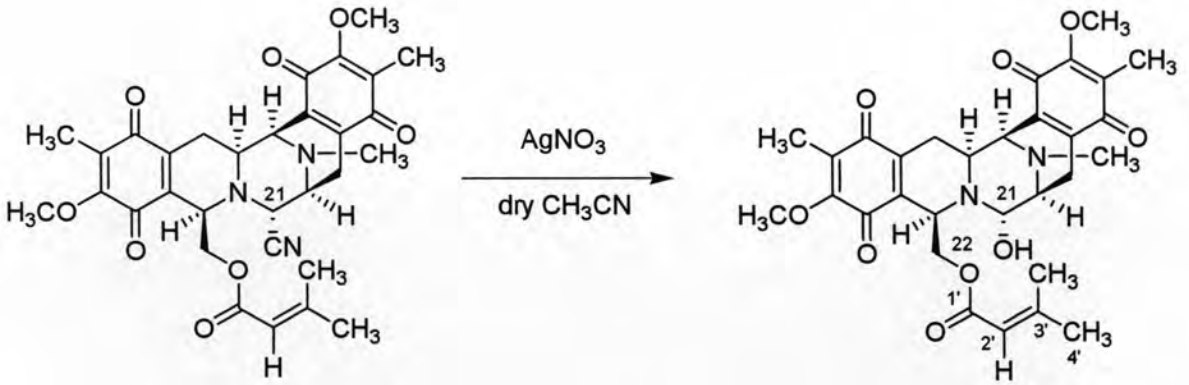
2.02 (3H, s, 6-CH_3), 1.16 (3H, s, 16-CH_3), 1.41 (1H, ddd, J = 17.7, 11.3, 2.7 Hz, 4-Hβ); Figure 167

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 185.5 (C-15), 185.4 (C-5), 182.2 (C-18), 181.1 (C-8), 165.1 (4'-CO), 155.8 (C-7), 154.7 (C-17), 149.4 (C-2'), 149.1 (C-8'a), 142.0 (C-20), 141.9 (C-10), 135.5 (C-9), 134.5 (C-19), 133.4 (C-4'), 130.2 (C-7'), 130.1 (C-8'), 128.8 (C-6), 128.6 (C-6'), 128.1 (C-16), 125.4 (C-5'), 124.7 (C-4'a), 122.0 (C-3'), 116.8 (21-CN), 62. 9 (C-22), 61.2 (7-OCH_3), 60.7 (17-OCH_3), 58.4 (C-21), 56.6 (C-1), 54.6 (C-13), 54.2 (C-3), 54.1 (C-11), 41.4 (NCH_3), 25.7 (C-4), 21.0 (C-14), 8.9 (6-CH_3), 7.8 (16-CH_3); Figure 168

10.2 Conversion 21-CN to 21-OH of acyl renieramycin derivatives

10.2.1 21-hydroxydeangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) (79)

Deangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) 20 mg (0.035 mmol) was dissolved in a mixture of acetonitrile and water [4:3 (v/v), 7 ml], and solid silver nitrate 206.8 mg (1.2 mmol, 35 eq) was added to this solution. The suspension was stirred at 40 °C for 3 h. Futher, the suspension was stirred 60 °C for 1 h. After that the temperature of the reaction was decreased to at 40 °C and stirred at the same temperature for 14 h. Next, solid silver nitrate 29.5 mg (0.173 mmol, 5 eq) was dissolved in a mixture of acetonitrile and water [1:1 (v/v), 1 ml], and this mixture was added into the suspension and stirred for 2 h at 80 °C. Finally, solid silver nitrate 29.5 mg (0.1735 mmol, 5 eq) was dissolved in a mixture of acetonitrile and water [1:1 (v/v), 1 ml], and this mixture was added into the suspension and stirred for 1 h at 80° C. Finally, a mixture of saturated aqueous sodium chloride solution and saturated aqueous sodium carbonate solution [1:1 (v/v), 20 ml] was added and the suspension was extracted with chloroform (20 ml x 3). The combined organic layers were dried with sodium sulfate anhydrous and filtered through a pad of celite. The filtrate was concentrated to give 21-hydroxydeangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) (**79**, 19.1 mg, 97.0% yield) as a yellowish-black solid.



deangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) (**67**)

21-hydroxydeangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) (**79**)
97.0% yield

21-hydroxydeangeloylrenieramycin M-22-(3'-methyl-2'-butenoate) (79)

FABMS : *m/z* (% intensity); Figure 170
549 ($[M+H-H_2O]^+$, 16), 307 (24), 220 (14), 218 (12), 154 (100), 136 (73), 83 (35)

HR-FABMS :549.2241 $[M+H-H_2O]^+$, (calcd for $C_{30}H_{33}N_2O_8$, 549.2237).

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 171
3468, 2929, 2854, 1716, 1652, 1618, 1448, 1364, 1234, 801

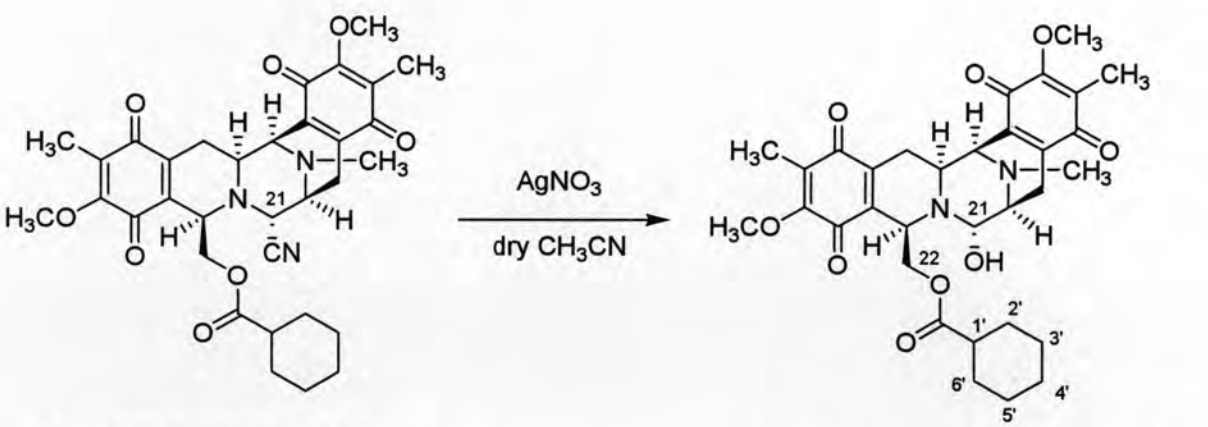
^1H NMR : δ ppm, 500 MHz, in $CDCl_3$; 5.21 (1H, br s, 2'-H), 4.45 (1H, dd, *J* = 2.7, 11.4 Hz, 22-Ha), 4.37 (1H, br s, 21-H), 4.31 (1H, br s, 1-H), 3.92 (3H, s, 17-OCH_3), 3.92 (3H, s, 7-OCH_3), 3.84 (1H, br s, 11-H), 3.80 (1H, d, *J* = 11.4 Hz, 22-Hb), 3.11 (1H, overlap, 13-H), 3.08 (1H, overlap, 3-H), 2.74 (1H, d, *J* = 16.8 Hz, 4-Hα), 2.57 (1H, dd, *J* = 21.0, 7.3 Hz, 14-Hα), 2.18 (3H, br s, NCH_3), 2.11 (1H, 14-Hβ), 1.92 (3H, s, 4'-H_3), 1.86 (3H, s, 6-CH_3), 1.85 (3H, s, 16-CH_3), 1.71 (3H, s, 3'-CH_3) 1.26 (1H, m, 4-Hβ); Figure 172

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.3 (C-15), 185.9 (C-5), 182.6 (C-18), 181.3 (C-8), 165.7 (C-1'), 157.7 (C-3'), 155.8 (C-7), 155.3 (C-17), 141.9 (C-20), 141.8 (C-10), 137.5 (C-9), 137.5 (C-19), 128.5 (C-6), 128.2 (C-16), 115.2 (C-2'), 82.8 (C-21), 62.9 (C-22), 60.9 (17-OCH_3), 60.9 (7-OCH_3), 57.6 (C-13), 54.3 (C-11), 53.0 (C-1), 51.0 (C-3), 41.4 (NCH_3), 27.3 (3'-

CH_3), 25.6 (C-4), 20.2 (C-14), 20.1 (C-4'), 8.7 (16-CH_3), 8.7 (6-CH_3); Figure 173

10.2.2 21-hydroxydeangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) (80)

Deangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) 23.6 mg (0.048 mmol) was dissolved in a mixture of acetonitrile and water [3:2 (v/v), 5 ml], and solid silver nitrate 203.8 mg (1.2 mmol, 25 eq) was added to this solution. The suspension was stirred at 40 °C for 6 h and then solid silver nitrate 81.5 mg (0.48 mmol, 10 eq) was dissolved in a mixture of acetonitrile and water [1:1 (v/v), 2 ml], and this mixture was added to the suspension and stirred for 2 h. at 40 °C. After that, the suspension was stirred at 60 °C for 3 h and cooled down the temperature to 40 °C. Further, it was stirred for 14 h at 40 °C. Later, the temperature of the reaction was increased to 80 °C and the suspension also was stirred for 1 h at this temperature. Finally, a mixture of saturated aqueous sodium chloride solution and saturated aqueous sodium carbonate solution [1:1 (v/v), 20 ml] was added and the suspension was extracted with chloroform (20 ml x 3). The combined organic layers were dried with sodium sulfate anhydrous and filtered through a pad of celite. The filtrate was concentrated to give 21-hydroxydeangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) 22.2 mg (**80**, 95.6% yield) as a yellowish-black solid.



deangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) (**69**)

21-hydroxydeangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) (**80**) 95.6% yield

21-hydroxydeangeloylrenieramycin M-22-(cyclohexane-1'-carboxylate) (80)

FABMS : *m/z* (% intensity); Figure 174
577 ($[M+H-H_2O]^+$, 25), 307 (22), 220 (30), 218 (24), 154 (100), 136 (71), 83 (31)

HR-FABMS : 577.2553 $[M+H-H_2O]^+$, (calcd for $C_{32}H_{37}N_2O_8$, 577.2550)

IR : ν_{max} cm^{-1}, $CHCl_3$; Figure 175
3468, 2931, 2855, 1731, 1655, 1617, 1235, 773

^1H NMR : δ ppm, 500 MHz, in $CDCl_3$; 4.38 (1H, br d, *J* =2.4 Hz, 21-H), 4.32 (1H, br d, *J* = 2.1 Hz, 1-H), 4.25 (1H, dd, *J* = 11.3, 2.7 Hz, 22-Ha), 4.02 (1H, dd, *J* = 11.3, 2.6 Hz, 22-Hb), 3.94 (3H, s, 17-OCH_3), 3.93 (3H, s, 7-OCH_3), 3.85 (1H, br d, *J* = 1.8 Hz, 11-H), 3.14 (1H, d, *J* = 7.7 Hz, 13-H), 3.07 (1H, ddd, *J* = 11.3, 2.7 Hz, 3-H), 2.72 (1H, dd, *J* = 17.0, 1.7 Hz, 4-Hα), 2.61 (1H, dd, *J* = 21.1, 7.5 Hz, 14-Hα) , 2.19 (3H, s, NCH_3) 2.16 (1H, d, *J* = 21.1 Hz, 14-Hβ), 1.88 (1H, overlap, 1'-H), 1.88 (3H, s, 16-CH_3), 1.85 (3H, s, 6-CH_3), 1.55 (2H, m, 5'-H_2), 1.52 (2H, m, 2'-H_2) 1.50 (2H, m, 4'-H_2), 1.25 (1H, 4-Hβ), 1.01 (2H, m, 6'-H_2), 1.00 (2H, m, 3'-H_2); Figure 176

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$; 186.6 (C-15), 185.9 (C-5), 182.6 (C-18), 181.3 (C-8), 175.2 (1'-CO), 156.0 (C-7), 155.4 (C-17), 141.8 (C-20), 141.3 (C-10), 137.4 (C-9), 134.6 (C-19), 128.5 (C-6), 127.9 (C-16), 82.4 (C21), 63.4 (C-22), 61.0 (17-OCH_3), 60.9 (7-OCH_3), 57.5 (C-13), 54.1 (C-11), 53.1 (C-1), 51.0 (C-3), 43.1 (C-1'), 41.4 (NCH_3), 29.2 (C-2'), 28.8 (C-6'), 25.8 (C-4), 25.6 (C-5'), 25.4 (C-4'), 25.3 (C-3'), 20.5 (C-14), 8.7 (16-CH_3), 8.6 (6-CH_3); Figure 177