Unusual Transformation of the 3-Hydroxy-picolinoyl Residue of Pristinamycin I_A†

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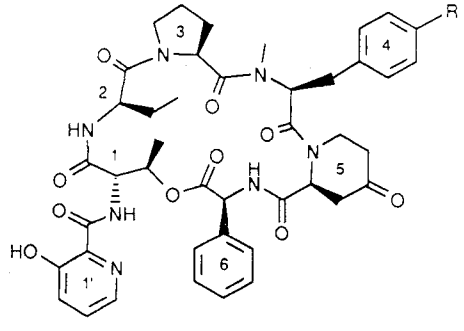
Pristinamycin I_A was modified in a two-step procedure to give original derivatives possessing a tricyclic nucleus (**8a**, **8b**, **8c**) or a substituted pyrrole ring (**10a**, **10b**) in place of the natural exocyclic 3-hydroxy-picolinoyl residue. This transformation involved firstly preparation of pyridinium betaines **5** from pristinamycin I_A and secondly a $1 \sim 3$ dipolar cycloaddition between **5** and *N*-substituted maleimides or diethyl acetylenedicarboxylate. The compounds obtained were evaluated as antibacterial agents alone and in association with pristinamycin II_A.

Among the large number of antibiotics that inhibit protein synthesis in prokaryotic cells, the streptogramins are unique in that they consist of two kinds of structurally unrelated molecules, group A and group B[1,2] (Fig. 1).

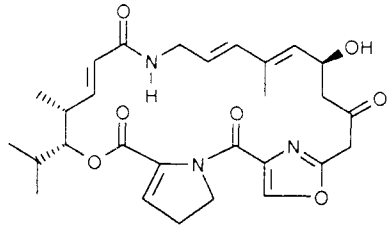
Group A and B streptogramins act in synergy both *in vitro* and *in vivo*. The synergy between them results in a greater potency and a broader spectrum of antibacterial activity than those of each component alone, rendering the mixture bactericidal against a wide variety of Gram-positive bacteria. Since their discovery, intensive efforts have been made to synthesize modified analogues of the two groups of constituents[3,4] and to elucidate the mechanism of action of the streptogramins[5~7]. We recently reported some of our results in the streptogramin field which resolved the problem of the poor water solubility of the two constituents and which led to the selection for clinical trials of the first semi-synthetic injectable streptogramin, derived from pristinamycin I and II (RP 59500)[4,8].

In the course of our continuing program aimed at chemically modified group B streptogramins, we have been particularly interested in the modification of the characteristic 3-hydroxy-picolinoyl exocyclic residue of pristinamycin I_A **1** (Fig. 1). This residue is an unusual feature in natural antibiotics and is probably involved in the mechanism of action of the streptogramins: it is thought to form a salt bridge with suitable acceptor atoms of the ribosome[6]. In this context, we attempted to replace or modify this residue, in order to clarify the role of this nucleus in antibacterial activity. We report in this paper an unusual reaction observed during functionalisation of this residue involving firstly, formation of pyridinium betaines **5** and secondly their transformation *via* a cycloaddition reaction into tricyclic systems **8** or into unexpected substituted pyrrole derivatives **10**.

Fig. 1. Structure of representative streptogramins.



1 : R = NMe_2 pristinamycin I_A
2 : R = H virginiamycin S_1
(group B streptogramins)



3 : pristinamycin II_A = virginiamycin M_1
(group A streptogramins)

† This article is a special contribution in honour of Professor SATOSHI ŌMURA's 60th birthday.

Results

The alkylation of group B streptogramins can lead to 3 products depending on the reaction conditions used[10~14]. Diazomethane alkylates both the hydroxyl group and the pyridyl-nitrogen of the picolinic acid residue, affording a mixture of the betaine (**5a**, R=Me) and, as the minor product, the methyl ether (**6**, R=Me). Pristinamycin I_A reacts with methyl iodide or methylsulfate, yielding selectively the quaternary ammonium salt **4** of the 4-dimethylamino function.

Betaines **5** were successfully obtained in fair to good yields by treating pristinamycin I_A with a reactive diazoalkane or merely by reacting it with an appropriate alkyl halide in DMF in the presence of potassium fluoride (Method A) or caesium fluoride (Method B), by analogy with the results described for virginiamycin S[13] (Scheme 1). The betaines **6** could be purified by flash-chromatography in order to eliminate side-products resulting from the *O*-alkylation of the hydroxyl group and from extended *N*-alkylation resulting in the formation of minor species **7**.

As 3-oxydo-pyridinium betaines are known to act as 1,3-dipoles and to undergo 1,3-dipolar additions with a great variety of dipolarophiles,[15~17] we investigated this type of reaction with pristinamycin I_A betaines using electron-deficient olefins, such as methyl or phenyl maleimide and diethyl acetylenedicarboxylate.

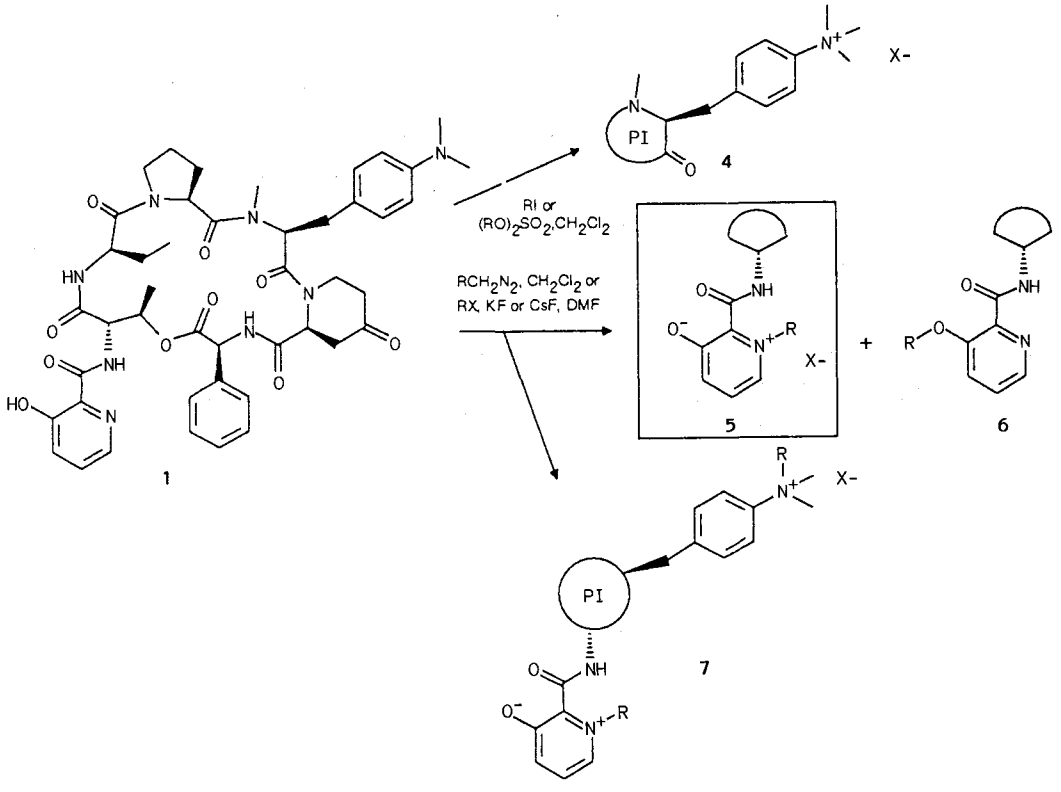
Pristinamycin betaines **5** reacted in the expected way with *N*-methyl and *N*-phenyl maleimide in refluxing 1,2-dichloroethane to give rise, after purification by flash-chromatography, to the exo adduct 8 (Scheme 2).

The thermodynamically stable exo product **8** was obtained (up to 90%) during this concerted cycloaddition; this compound is probably favoured by steric factors and dipole interactions. The evidence for structure **8** (exo) was mainly obtained from the coupling constants obtained by ^1H NMR data and was confirmed by NOE experiments, no NOE interaction occured between the NCH_3 and the bridge head protons of maleimide moities.

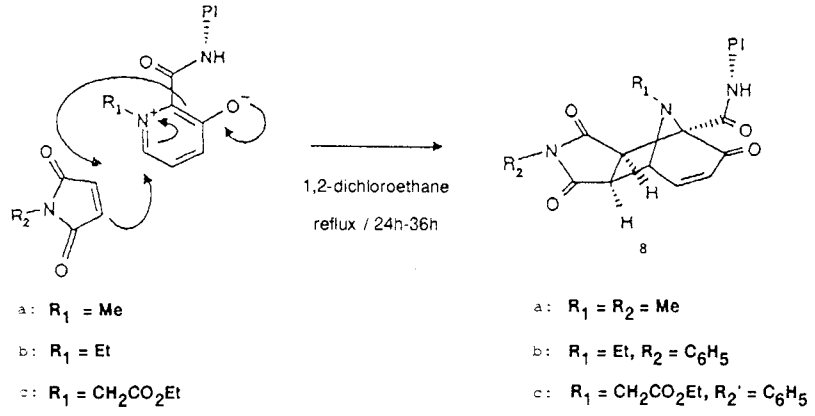
When heated in the presence of diethyl acetylenedicarboxylate, pristinamycin betaines 5 yielded the unexpected pyrroles **10a~10b** *via* an original process (Scheme 3).

Reaction of simple 3-oxydopyridiniums with diethyl acetylenedicarboxylate led generally to the normal 2~6 adduct **9**. Formation of pyrrole **10**, which was the only product characterised in this reaction, is an original reaction and can be viewed as a formal removal of acetylene and CO from **9**. Compounds **5a~5c**, **8a~8c** and **10a~10c** were tested *in vitro* for antimicrobial activity against several genera. None had shown potent activity on any of the strains tested, either alone or

Scheme 1. Alkylation reactions on pristinamycin I_A.



Scheme 2.



Scheme 3.

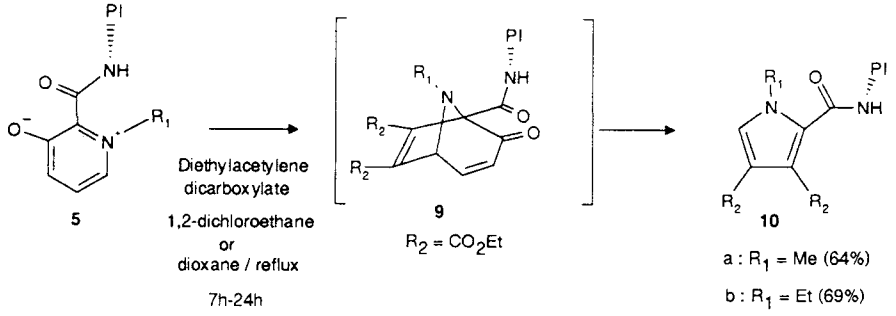


Fig. 2. [a]NOE interactions observed for compound **8a**.
[a] The expected NOE were recorded at 250 MHz in $CDCl_3$.

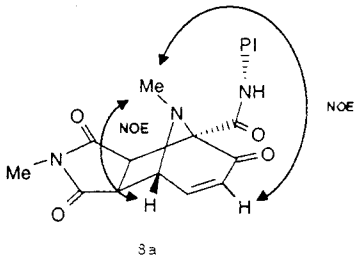


Table 1. Preparation of tricyclic adducts and of pyrrole derivatives from **1**.

Compound No.	R_1	R_2	Method[a] Step 1	Yield (%)[b] Step 1, Step 2
8a	Me	Me	A	71/41
8b	Et	C_6H_5	A	60/56
8c	CH_2CO_2Et	C_6H_5	B	45.9/51
10a	Me	CO_2Et	A	71/64
10b	Et	CO_2Et	A	60/69

[a] Reaction conditions. Step 1 Method A : RX, KF, DMF, Method B : RX, CsF, DMF.
[b] Yields are not optimised.

combined with pristinamycin II_A.

Conclusion

In conclusion, different modifications of the 3-hydroxy-picolinoyl residue of pristinamycin I_A have been performed. From a chemical point of view, the ease of formation of the betaines and their transformation into various cycloadducts can be noted. Noteworthy also is the original formation of the pyrrole moiety **10** obtained during this study. Generalization of this reaction to different substrates is currently underway in our laboratory.

The biological results obtained with modified pristinamycins I_A clearly indicate the importance of the 3-hydroxy pyridine ring for the antimicrobial activity of pristinamycin I_A. Betaines **5** were found to be totally inactive alone or in association with pristinamycin II_A against both erythromycin-sensitive (Ery^s) and resistant (Ery^r) strains of *Staphylococcus aureus*. Replacement of the 3-hydroxy-picolinoyl residue of pristinamycin I_A by a tricyclic ring system or by a pyrrole ring also yielded compounds that had no detectable antibacterial activity.

Experimental

Reagents were used as supplied unless otherwise noted and were purchased from Prolabo or Janssen Chemica.

Melting points were recorded on a Köfler apparatus and were not corrected. ^{1}H NMR spectra were recorded on Bruker AC 250 (250 MHz) or AM 400 (400 MHz) spectrometers. Chemical shifts (δ) are reported in ppm relative to internal tetramethylsilane. The atoms of pristinamycin I_A are numbered according to the method used by ANTEUNIS and co-workers[18] for virginiamycin S. Infrared spectra (IR) were determined with a Perkin-Elmer Model 938G or 580B. Mass spectra (MS) were recorded on a Kratos AEI MS50 spectrometer (FAB, matrice used *m*-nitro benzyl alcohol, reacting gaz Xenon, 8 eV). Unless otherwise noted, all the compounds presented a pseudo molecular ion at $m/z = MH^+$. Satisfactory spectral data were obtained for all new compounds. Crude products were purified by flash column chromatography on silica gel (0.04~0.063 mm; Merck). For thin layer chromatography (TLC), 250 μm E. Merck silica gel 60 F_{254} plates were used. Evaporations were in most cases carried out below 30~35°C. Pristinamycin I_A used in this study was obtained from the bulk pharmaceutical and purified as described[19].

Formation of Pristinamycin I_A Betaines

(*N*-Methyl 3-oxydo 2-pyridinio-1 carbonyl)-1-des(-3-hydroxy-picolinoyl) Pristinamycin I_A **5a**

In a three-necked round-bottomed flask were successively added under N_2 atmosphere, anhydrous DMF (50 ml), KF (1.68 g, 24.9 mmol) and MeI (0.9 ml, 15 mmol). After 2 minutes **1a** (10.6 g, 11.9 mmol) was added and the mixture stirred for 15 hours at room temperature then for 1 hour at 50°C. After cooling, the reaction mixture was partitioned between a saturated $NaHCO_3$ solution (300 ml) containing NaCl (5 g) and ethyl acetate (100 ml). The aqueous layer was separated and washed with ethyl acetate (100 ml). Then the organic layers were pooled, and distilled water (200 ml) was added. The pH of the aqueous layer was adjusted to 3.5 by hydrochloric acid and the organic layer was separated and washed with water (50 ml). The aqueous layers were adjusted to pH 7 with solid $NaHCO_3$ then washed with dichloromethane (3 × 50 ml). The organic layers were pooled, dried over $MgSO_4$, and concentrated to yield 7.2 g (71%) of **5a** as a yellow powder which could be used without further purification in the cycloaddition reaction.

An analytical sample of **5a** can be obtained by flash-chromatography using CH_2Cl_2 - MeOH (96 : 4) as eluent to yield **5a** as a white-yellow solid (mp 254°C dec., lit.[11] 260~270°C dec.).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.60 (dd, J=16 and 5.5 Hz, 1H: 5β); 0.89 (t, J=7.5 Hz, 3H: CH_3 2γ); 1.15 (m, 1H: 3β); from 1.15 to 1.30 (m, 1H: 3γ); 1.25 (d, J=6.5 Hz, 3H: CH_3 1γ); 1.53 (m, 1H: 3γ); 1.65 and 1.75 (2 m, 1H each: CH_2 2β); 1.98 (m, 1H: 3β); 2.19 and 2.32 (respectively m and m, J=16 Hz, 1H each: CH_2 5δ); 2.46 (d, J=16 Hz, 1H: 5β); 2.85 (m, J=13 and 3.5 Hz, 1H: 5ε); 2.90 (s, 6H: $N(CH_3)_2$); 2.92 (dd, J=12 and 4 Hz, 1H: 4β); 3.24 and 3.54 (2m, 1H each: CH_2 3δ); 3.26 (s, 3H: NCH_3 4); 3.31 (t, J=12 Hz, 1H: 4β); 4.53 (s, 3H: N^+CH_3); 4.59 (dd, J=8 and 6.5 Hz, 1H: 3α); 4.75 (br dd, J=13 and 8 Hz, 1H: 5ε); 4.83 (m, J=9 and 7.5 Hz, 1H: 2α); 5.00 (dd, J=9.5 and 1.5 Hz, 1H: 1α); 5.46 (dd, J=12 and 4 Hz, 1H: 4α); 5.84 (m, J=6.5 and 1.5 Hz, 1H: 1β); 5.89 (d, J=9.5 Hz, 1H: 6α); 5.92 (br d , J=5.5 Hz, 1H: 5α); 6.58 (d, J=8.5 Hz, 2H: H Aromatics in 4ε); 6.81 (d, J=9 Hz, 1H: NH 2); 7.09 (d, J=8.5 Hz, 2H: H Aromatics in 4δ); from 7.15 to 7.40 (m, 8H: H Aromatics in 6-1'H_4~1'H_5 and 1'H_6); 8.85 (d, J= 9.5 Hz, 1H: NH 6); 12.88 (d, J=9.5 Hz, 1H: NH 1).

Anal Calcd for $C_{46}H_{56}N_8O_{10}$:
C 62.71 H 6.41, N 12.72, O 18.16
Found: (1,6 H_2O)
C 62.34, H 6.39, N 12.30, O 17.89

(*N*-Ethyl 3-oxydo 2-pyridinio-1 carbonyl)-1-des(-3-hydroxy-picolinoyl) Pristinamycin I_A **5b**

Ethyl iodide (1.2 ml, 15 mmol) was added to potassium fluoride (1.67 g, 24.9 mmol) in anhydrous DMF (50 ml). After 2 minutes, pristinamycin I_A was added. The reaction mixture was stirred for 16 hours at room temperature then for 3 hours at 50°C. The reaction mixture was cooled and then poured into a saturated $NaHCO_3$ solution; The product was extracted with ethyl acetate (2 × 100 ml) then with dichloromethane (3 × 100 ml). The organic layers were dried over $MgSO_4$, then concentrated to dryness below 30°C. The resulting solid was partitioned between ethyl acetate (100 ml) and distilled water. The pH of the aqueous layer was adjusted to 3 by hydrochloric acid. The organic layer was separated and washed with water (50 ml). The aqueous layers were adjusted to pH 7 with solid $NaHCO_3$ then washed with dichloromethane (100 ml then 2 × 50 ml). The organic layers were pooled, dried over $MgSO_4$, and concentrated to give 6.14 g (60%) of **5b** as yellow powder (94% purity containing 6% of *O*-alkyl derivative) which can be used without further purification for the cycloaddition reaction.

This product can be purified by flash-chromatography by using dichloromethane - methanol (94 : 6) as eluant to yield 2.2 g (36% yield) of **5b** as yellow solid (mp 230°C).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.60 (dd, J=16 and 5.5 Hz, 1H: 5β); 0.89 (t, J=7.5 Hz, 3H: CH_3 2γ); 1.14 (m, 1H: 3β); 1.27 (d, J=6.5 Hz, 3H: CH_3 1γ); 1.32 and 1.58 (2m, 1H each: CH_2 3γ); 1.67 (t, J=7.5 Hz, 3H: CH_3 ethyl); 1.67 and 1.78 (2m, 1H each: CH_2 2β); 2.02 (m, 1H: 3β); 2.18 and 2.32 (2m, respectively J=16, 12.5 and 8 Hz and J=16 Hz, 1H each: CH_2 5δ); 2.38 (d, J=16 Hz, 1H: 5β); 2.84 (m, J=12.5 and 4.5 Hz, 1H: 5ε); 2.90 (s, 6H: $N(CH_3)_2$); 2.96 (dd, J=12 and 4 Hz, 1H: 4β); 3.28 (s, 3H: NCH_3 4); 3.32 (t, J=12 Hz, 1H: 4β); 3.32 and 3.58 (2m, 1H each: CH_2 3δ); 4.59 (dd, J=8 and 7 Hz, 1H: 3α); 4.75 (br dd, J=12.5 and 8 Hz, 1H: 5ε); 4.86 (m, J=9.5 and 6.5 Hz, 1H: 2α); 4.92 and 5.12 (m, J=13 and 7.5 Hz, 2H: N^+CH_2 ethyl); 5.04 (br d, J=9.5 Hz, 1H: 1α); 5.51 (dd, J=12 and 4 Hz, 1H: 4α); 5.85 (m, J=6.5 Hz, 1H: 1β); 5.87 (d, J=10 Hz, 1H: 6α);

5.92 (br d, J=5.5 Hz, 1H: 5α); 6.60 (d, J=8.5 Hz, 2H: H Aromatics in 4ε); 6.98 (d, J=9.5 Hz, 1H: NH 2); 7.16 (d, J=8.5 Hz, 2H: H Aromatics in 4δ); from 7.15 to 7.30 (m, 6H: H Aromatics in 6 and 1'H_5); 7.33 (br d, J=5 Hz, 1H: 1'H_6); 7.38 (br d, J=8.5 Hz, 1H: 1'H_4); 8.87 (d, J=10 Hz, 1H: NH 6); 12.76 (d, J=9.5 Hz, 1H: NH 1).

Anal Calcd for $C_{47}H_{58}N_8O_{10}$:
C 63.07, H 6.53, N 12.52, O 17.88
Found: (1.38% H_2O)
C 62.73, H 6.58, N 12.62, O 17.50

(*N*-Methyl carboxyethyl 3-oxydo 2-pyridinio-1 carbonyl)-1-des(-3-hydroxy-picolinoyl) Pristinamycin I_A **5c**

Bromo ethyl acetate (1.034 g, 6.18 mmol) was added to caesium fluoride (1.88 g, 12.3 mmol) in anhydrous DMF (10 ml). After 2 minutes, pristinamycin I_A (4.65 g, 5.24 mmol) was added and the reaction mixture stirred for 17 hours at 80°C. After cooling, the reaction mixture was poured slowly into a saturated $NaHCO_3$ solution (100 ml). The yellow precipitate was filtered then washed with a saturated $NaHCO_3$ solution (2 × 50 ml) and then with hexane (2 × 50 ml). The resulting solid was dissolved in ethyl acetate (70 ml), filtered and washed with water adjusted to pH 3.5 with hydrochloric acid. The organic layer was separated then washed with water (50 ml). The aqueous layers were pooled, washed with ethyl acetate (40 ml), neutralized to pH 7 by addition of an aqueous $NaHCO_3$ solution, and then extracted with dichloromethane (3 × 75 ml). The organic layer was dried over $MgSO_4$ and evaporated to give 2.21 g (45.9% yield) of **5c**, which was used, without further purification, for the cycloaddition reaction.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.70 (dd, J=16 and 6 Hz, 1H: 5β); 0.87 (t, J=7.5 Hz, 3H: CH_3 2γ); 1.14 (m, 1H: 3β); 1.20 (d, J=6.5 Hz, 3H: CH_3 1γ); from 1.20 to 1.35 (m, 1H: 3γ); 1.27 (t, J=7.5 Hz, 3H: CH_3 ethyl); 1.51 (m, 1H: 3γ); 1.58 and 1.73 (2m, 1H each: CH_2 2β); 1.96 (m, 1H: 3β); 2.17 and 2.30 (respectively m and m, J=16 Hz, 1H each: CH_2 5δ); 2.43 (d, J=16 Hz, 1H: 5β); 2.83 (m, J=13.5 and 4 Hz, 1H: 5ε); 2.86 (s, 6H: $N(CH_3)_2$); 2.92 (dd, J=12 and 4.5 Hz, 1H: 4β); 3.20 and 3.51 (2 m, 1H each: CH_2 3δ); 3.22 (s, 3H: NCH_3 4); 3.27 (t, J=12 Hz, 1H: 4β); 4.26 (q, J=7.5 Hz, 2H: $COOCH_2$ ethyl); 4.56 (dd, J=8 and 7 Hz, 1H: 3α); 4.72 (br dd, J=13.5 and 8 Hz, 1H: 5ε); 4.80 (m, J=9 and 7.5 Hz, 1H: 2α); 4.93 (br d, J=10 Hz, 1H: 1α); 5.25 and 5.62 (2d, J=17 Hz, 2H: N^+CH_2COO); 5.49 (dd, J=12 and 4.5 Hz, 1H: 4α); 5.78 (m, J=6.5 Hz, 1H: 1β); 5.84 (d, J=10 Hz, 1H: 6α); 5.96 (br d, J=6 Hz, 1H: 5α); 6.57 (d, J=8.5 Hz, 2H: H Aromatics in 4ε); 6.78 (d, J=9 Hz, 1H: NH 2); 7.08 (d, J=8.5 Hz, 2H: H Aromatics in 4δ); from 7.10 to 7.30 (m, 7H: H Aromatics in 6-1'H_5 and 1'H_6); 7.38 (br d, J=8.5 Hz, 1H: 1'H_4); 8.84 (d, J=10 Hz, 1H: NH 6); 12.87 (d, J=10 Hz, 1H: NH 1).

Anal Calcd for $C_{49}H_{60}N_8O_{12}$:
C 61.75, H 6.35, N 11.76, O 20.14
Found:
C 61.12, H 6.30, N 10.70, O n.d.

Formation of Tricyclic Adducts

Analogue **8a**

N-Methyl maleimide (0.57 g, 5.1 mmol) was added to a stirred solution of *N*-methylpyridinium pristinamycin I_A betaine **5a** (1.5 g, 1.7 mmol) in 1,2-dichloroethane. The mixture was refluxed for 20 hours then an additional amount of *N*-methyl maleimide (0.38 g, 3.4 mmol) was added. After 5.5 hours, the mixture was evaporated to dryness to yield 2.55 g of a yellow solid which was purified by 2 successive flash-chromatographies by using dichloromethane - methanol (96 : 4) as eluant. This yielded 0.87 g of a solid which was recrystallized in 4 ml of propanol to yield 0.70 g (41% yield) of **8a** as yellow crystals (mp 222°C).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.63 (dd, J=16 and 6 Hz, 1H: 5β); 0.98 (t, J=7.5 Hz, 3H: CH_3 2γ); 1.14 (m, 1H: 3β); 1.25 (d, J=6.5 Hz, 3H: CH_3 1γ); 1.31 and 1.60 (2 m, 1H each: CH_2 3γ); 1.85 and 1.93 (2 m, 1H each: CH_2 2β); 2.03 (m, 1H: 3β); 2.25 and 2.40 (respectively m and m, J=16 Hz, 1H each: CH_2 5δ); 2.42 (s, 3H: NCH_3 9'); 2.45 (d, J=16 Hz, 1H: 5β); from 2.85 to 3.00 (m, 2H: 5ε and 4β); 2.92 (s, 6H: $N(CH_3)_2$); 3.12 (s, 3H: NCH_3 3'); 3.28 (s, 3H: NCH_3 4); 3.30 (t, J=12 Hz, 1H: 4β); 3.37 and 3.47 (2d, J=7.5 Hz, 1H each: H3'a and H8'a); 3.67 (m, 2H: CH_2 3δ); 4.43 (d, J=5 Hz, 1H: H8'); 4.56 (dd, J=7.5 and 7 Hz, 1H: 3α); 4.78 (br dd, J=13 and 8 Hz, 1H: 5ε); 4.95 (m, J=9.5 and 7.5 Hz, 1H: 2α); 5.05 (dd, J=10 and 1.5 Hz, 1H: 1α); 5.34 (dd, J=12 and 4 Hz, 1H: 4α); 5.67 (br d, J=6 Hz, 1H: 5α); 5.93 (m, J=6.5 Hz, 1H: 1β); 5.98 (d, J=10 Hz, 1H: 6α); 6.17 (d, J=9 Hz, 1H: H6'); 6.57 (d, J=8.5 Hz, 2H: H Aromatics in 4ε); 7.02 (d, J=8.5 Hz, 2H: H Aromatics in 4δ); from 7.25 to 7.40 (m, 6H: H Aromatics in 6 and H7'); 8.09 (d, J=9.5 Hz, 1H: NH 2); 8.84 (d, J=10 Hz, 1H: NH 6); 8.97 (d, J=10 Hz, 1H: NH 1).

Anal Calcd for $C_{51}H_{61}N_9O_{12}$:
C 61.74, H 6.20, N 12.71, O 19.35
Found: (propanol 3.4%, H_2O 1.8%)
C 61.72, H 6.31, N 12.48, O 19.25

Analogue **8b**

N-Phenyl maleimide (5.8 g, 3.4 mmol) was added to a stirred solution of *N*-ethyl pyridinium pristinamycin I_A betaine **5b** (6 g, 6.7 mmol) in 1,2-dichloroethane (100 ml). The mixture was refluxed for 20 hours then evaporated to dryness to give a beige solid which was stirred in diethylether (250 ml), filtered, then purified by flash-chromatography by using dichloromethane - methanol (97 : 3) as eluant. This yielded 4 g (56% yield) of **8b** as a yellow solid (mp 200°C).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.65 (dd, J=16 and 6 Hz, 1H: 5β); 0.83 (t, J=7.5 Hz, 3H: CH_3 2γ); from 1.00 to 1.20 (m, 1H: 3β); 1.08 (t, J=7 Hz, 3H: CH_3 ethyl); from 1.15 to 1.35 (m, 1H: 3γ); 1.22 (d, J=6.5 Hz, 3H: CH_3 1γ); 1.55 (m, 1H: 3γ); from 1.60 to 1.85 (m, 2H: CH_2 2β); 1.97 (m, 1H: 3β); 2.20 and 2.35 (respectively m and m, J=16 Hz, 1H each: CH_2 5δ); 2.40 (d, J=16 Hz, 1H: 5β); 2.44 and 2.72 (2m, J_{AB}=12 Hz, 1H each: NCH_2

ethyl); from 2.80 to 2.95 (m, 1H: 5ε); 2.89 (s, 6H: $N(CH_3)_2$); 2.92 (dd, J=12 and 4 Hz, 1H: 4β); 3.24 (s, 3H: NCH_3 4); 3.25 (t, J=12 Hz, 1H: 4β); 3.50 and 3.57 (2d, J=7.5 Hz, 1H each: H3'a and H8'a); 3.60 (m, 2H: CH_2 3δ); 4.53 (dd, J=7 and 6.5 Hz, 1H: 3α); 4.64 (d, J=5 Hz, 1H: H8'); 4.73 (brdd, J=13 and 7.5 Hz, 1H: 5ε); 4.85 (m, J=9.5 and 7.5 Hz, 1H: 2α); 5.02 (dd, J=10 and 1.5 Hz, 1H: 1α); 5.35 (dd, J=12 and 4 Hz, 1H: 4α); 5.67 (br d, J=6 Hz, 1H: 5α); 5.88 (m, J=6.5 and 1.5 Hz, 1H: 1β); 5.95 (d, J=10 Hz, 1H: 6α); 6.13 (d, J=10 Hz, 1H: H6'); 6.56 (d, J=8.5 Hz, 2H: H Aromatics in 4ε); 7.01 (d, J=8.5 Hz, 2H: H Aromatics in 4δ); from 7.20 to 7.35 (m, 6H: H Aromatics in 6 and H7'); 7.35 (d, J=8 Hz, 2H: H in *ortho* of Aromatics in 2'); 7.42 (t, J=8 Hz, 1H: H in *para* of Aromatics in 2'); 7.48 (t, J=8 Hz, 2H: H in *meta* of Aromatics in 2'); 7.88 (d, J=9.5 Hz, 1H: NH 2); 8.82 (d, J=10 Hz, 1H: NH 6); 9.10 (d, J=10 Hz, 1H: NH 1).

Anal Calcd for $C_{57}H_{65}N_9O_{12}$:
C 64.10, H 6.13, N 11.80, O 17.97
Found: (H_2O 1.2%)
C 64.08, H 5.92, N 11.75, O n.d.

Analogue **8c**

N-Phenyl maleimide (1.70 g, 10.1 mmol) was added to a stirred solution of *N*-methyl carboxymethyl pyridinium betaine **5c** (2 g, 2.05 mmol) in 1,2-dichloroethane (50 ml). The mixture was refluxed for 20 hours then evaporated to dryness to yield 1.86 g of a beige solid which was purified by flash-chromatography by using dichloromethane - methanol (95 : 5) as eluant. This yielded 1.8 g (51% yield) of **8c** as a yellow solid (mp 190 ~ 200°C).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.65 (dd, J=16 and 6 Hz, 1H: 5β); 0.83 (t, J=7.5 Hz, 3H: CH_3 2γ); 1.10 (m, 1H: 3β); 1.19 (t, J=7 Hz, 3H: CH_3 ethyl); 1.23 (d, J=6.5 Hz, 3H: CH_3 1γ); 1.25 and 1.53 (2m, 1H each: CH_2 3γ); from 1.60 to 1.80 (m, 2H: CH_2 2β); 1.97 (m, 1H: 3β); 2.21 and 2.37 (respectively m and m, J=16 Hz, 1H each: CH_2 5δ); 2.42 (d, J=16 Hz, 1H: 5β); from 2.80 to 2.95 (m, 1H: 5ε); 2.88 (s, 6H: $N(CH_3)_2$); 2.93 (dd, J=12 and 4 Hz, 1H: 4β); 3.26 (s, 3H: NCH_3 4); 3.27 (t, J=12 Hz, 1H: 4β); 3.44 and 3.49 (2d, J=7.5 Hz, 1H each: H3'a and H8'a); 3.50 and 3.54 (2d, J=16 Hz, 1H each: NCH_2); 3.60 (m, 2H: CH_2 3δ); 4.10 (m, 2H: $COOCH_2$ ethyl); 4.53 (dd, J=7 and 6 Hz, 1H: 3α); 4.73 (brdd, J=13 and 8 Hz, 1H: 5ε); 4.81 (d, J=5 Hz, 1H: H8'); 4.85 (m, J=9.5 and 7.5 Hz, 1H: 2α); 4.99 (dd, J=10 and 1.5 Hz, 1H: 1α); 5.37 (dd, J=12 and 4 Hz, 1H: 4α); 5.67 (br d, J=6 Hz, 1H: 5α); 5.89 (m, J=6.5 and 1.5 Hz, 1H: 1β); 5.97 (d, J=10 Hz, 1H: 6α); 6.13 (d, J=10 Hz, 1H: H6'); 6.55 (d, J=8.5 Hz, 2H: H Aromatics in 4ε); 6.98 (d, J=8.5 Hz, 2H: H Aromatics in 4δ); from 7.15 to 7.35 (m, 5H: H Aromatics in 6); 7.34 (d, J=8 Hz, 2H: H in *ortho* of Aromatics in 2'); 7.40 (dd, J=10 and 5 Hz, 1H: H7'); 7.41 (t, J=8 Hz, 1H: H in *para* of Aromatics in 2'); 7.47 (t, J=8 Hz, 2H: H in *meta* of Aromatics in 2'); 8.08 (d, J=9.5 Hz, 1H: NH 2); 8.82 (d, J=10 Hz, 1H: NH 6); 9.06 (d, J=10 Hz, 1H: NH 1).

Anal Calcd for $C_{59}H_{67}N_9O_{14}$:
C 62.92, H 6.00, N 11.19, O 19.89
Found: (propanol 3.4%)
C 63.22, H 6.00, N 11.09, O n.d.

Formation of Pyrrole Adducts

N-Methyl Pyrrole **10a**

To a solution of *N*-methyl 3-pyridinium pristinamycin I_A betaine **5a** (7 g, 7.5 mmol) in 1,2-dichloroethane (70 ml) diethyl acetylenedicarboxylate (13 g, 75.5 mmol) was added. The mixture was refluxed for 21 hours, evaporated to dryness then purified by two successive flash-chromatographies for which first dichloromethane - methanol (97 : 3), then toluene - ethanol (90 : 10) was used as eluant. After concentration, the resulting solid was stirred in pentane (2 × 20 ml), filtered, then dried to yield 1.53 g (20% yield) of **10a** as beige solid (mp 190°C).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.25 (dd, J=16 and 6 Hz, 1H: 5β); 0.91 (t, J=7.5 Hz, 3H: CH_3 2γ); from 1.10 to 1.40 (m, 2H: 3β and 3γ); 1.25 (d, J=7 Hz, 3H: CH_3 1γ); 1.31 and 1.36 (2t, J=7.5 Hz, 3H each: CH_3 ethyl); 1.54 (m, 1H: 3γ); from 1.55 to 1.80 (m, 2H: CH_2 2β); 2.03 (m, 1H: 3β); 2.11 and 2.20 (respectively m and m, J=16 Hz, 1H each: CH_2 5δ); 2.20 (d, J=16 Hz, 1H: 5β); 2.75 (m, J=13 and 4 Hz, 1H: 5ε); 2.87 (s, 6H: $N(CH_3)_2$); 2.93 (dd, J=12.5 and 4.5 Hz, 1H: 4β); 3.18 and 3.45 (2m, 1H each: CH_2 3δ); 3.20 (t, J=12.5 Hz, 1H: 4β); 3.25 (s, 3H: NCH_3 4); 3.97 (s, 3H; NCH_3); from 4.15 to 4.40 (m, 4H: $COOCH_2$ ethyl); 4.62 (dd, J=9 and 6 Hz, 1H: 3α); 4.67 (br dd, J=13 and 7.5 Hz, 1H: 5ε); 4.79 (m, J=9 and 7.5 Hz, 1H: 2α); 4.93 (dd, J=10 and 1.5 Hz, 1H: 1α); 5.15 (br d, J=6 Hz, 1H: 5α); 5.34 (dd, J=12.5 and 4.5 Hz, 1H: 4α); 5.88 (m, J=7 and 2.5 Hz, 1H: 1β); 5.95 (d, J=10 Hz, 1H: 6α); 6.60 (d, J=9 Hz, 1H: NH 2); 6.71 (d, J=8.5 Hz, 2H: H Aromatics in 4ε); 7.03 (d, J=8.5 Hz, 2H: H Aromatics in 4δ); from 7.10 to 7.30 (m, 5H: H Aromatics in 6); 7.37 (s, 1H: H5 of pyrrolyl); 8.03 (d, J=10 Hz, 1H: NH 1); 8.84 (d, J=10 Hz, 1H: NH 6).

Anal Calcd for $C_{51}H_{64}N_8O_{13}$:
C 61.43, H 6.47, N 11.24, O 20.86
Found: (toluene, 1.5%, water 2.1%)
C 61.88 , H 6.50 , N 11.06, O 20.20

N-Ethyl Pyrrole **10b**

To a solution of *N*-ethyl 3-pyridinium pristinamycin I_A betaine **5b** (5.5 g, 6.14 mmol) in dichloroethane (40 ml), diethyl acetylenedicarboxylate (10.5 g, 61.5 mmol) was added. The mixture was refluxed for 21 hours, evaporated to dryness then purified by flash-chromatography by using toluene - ethanol (90 : 10) as eluant to yield 4.3 g (69% yield) of **10b** as beige solid (mp 162°C).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.23 (dd, J=16 and 6 Hz, 1H: 5β); 0.90 (t, J=7.5 Hz, 3H: CH_3 2γ); from 1.15 to 1.40 (m, 2H: 3β and 3γ); 1.25 (d, J=6.5 Hz, 3H: CH_3 1γ); 1.33 and 1.37 (2t, J=7 Hz, 3H each: CH_3 diethoxy); 1.53 (m, 1H: 3γ); 1.53 (t, J=7 Hz, 3H: CH_3 ethyl); 1.65 and 1.75 (2 m, 1H each: CH_2 2β); 2.02 (m, 1H: 3β); 2.09 (m, 1H: 5δ); 2.20 (d, J=16 Hz, 1H: 5β); 2.22 (m,

$J=16$ Hz, 1H: 5δ); 2.73 (m, $J=13$ and 4 Hz, 1H: 5ε); 2.87 (s, 6H: $N(CH_3)_2$); 2.94 (dd, $J=12$ and 4 Hz, 1H: 4β); 3.18 and 3.45 (2m, 1H each: CH_2 3δ); 3.20 (t, $J=12$ Hz, 1H: 4β); 3.25 (s, 3H: NCH_3 4); from 4.15 to 4.35 (m, 4H: $COOCH_2$ of diethoxy); 4.35 (q, $J=7$ Hz, 2H; NCH_2 ethyl); 4.60 (dd, $J=8$ and 5 Hz, 1H: 3α); 4.65 (bdd, $J=13$ and 7.5 Hz, 1H: 5ε); 4.78 (m, $J=9$ and 7.5 Hz, 1H: 2α); 4.95 (dd, $J=9.5$ and 1.5 Hz, 1H: 1α); 5.15 (brd, $J=6$ Hz, 1H: 5α); 5.33 (dd, $J=12$ and 4 Hz, 1H: 4α); 5.88 (m, $J=6.5$ and 1.5 Hz, 1H: 1β); 6.02 (d, $J=10$ Hz, 1H: 6α); 6.67 (d, $J=9$ Hz, 1H: NH 2); 6.70 (d, $J=8.5$ Hz, 2H: H Aromatics in 4ε); 7.04 (d, $J=8.5$ Hz, 2H: H Aromatics in 4δ); from 7.10 to 7.30 (m, 5H: H Aromatics in 6); 7.42 (s, 1H: H 5 of pyrrolyl); 8.07 (d, $J=9.5$ Hz, 1H: NH 1); 8.84 (d, $J=10$ Hz, 1H: NH 6).

Anal Calcd for $C_{52}H_{66}N_8O_{13}$:
C 61.77, H 6.58, N 11.08, O 20.57
Found: (H_2O 1.5%)
C 61.75, H 6.80, N 10.45, O 20.30

Antibacterial Activity

The *in vitro* activities were determined as described by ERICSSON and SHERRIS[19]. Strains tested were IP 8302: *Staphylococcus aureus* sensitive, *S. aureus* Faur.: MLS_B inducibly resistant (erythromycin resistant: Ery^r), *S. aureus* Duc.: MLS_B constitutively resistant (Ery^r).

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