New Compounds of the Manumycin Group of Antibiotics and a Facilitated Route for Their Structure Elucidation

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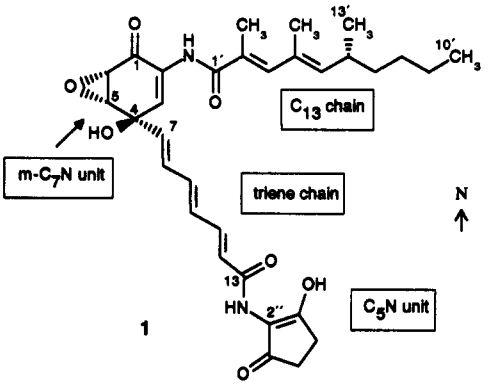
Manumycin B (2) and C (3) are structural analogs of manumycin A (1), the main metabolite of *Streptomyces parvulus* (strain Tü 64). The new minor components vary within the polyketide assembling of the acylamino side chain and the stereochemistry at C-4 in the central mC_7N unit. Apart from unreported stereochemical details 2 and 3 resemble the recently found UCF1-A and -B, inhibitors of ras farnesyltransferase. Manumycin D (4) is the first of the manumycin type compounds without an oxirane in the mC_7N unit. The relative stereochemistry in the mC_7N unit of 2–4 was elucidated with proton NMR spectroscopy using different ASIS (aromatic solvent induced shift) effects on the olefinic 3-H. In combination with the established circular dichroism (CD) spectroscopy the absolute stereochemistry of the whole mC_7N unit was determined without chemical degradation.

The manumycin group is a small and discrete class of antibiotics, which includes about a dozen secondary metabolites, all of them produced by *Streptomyces* species. Manumycin A (1), first reported in 1963, bears two unsaturated carbon chains linked in "southern" and "eastern" orientation to a multifunctional six-membered ring (mC_7N unit, C-1–C-7) and an 2-amino-3-hydroxycyclopent-2-enone (C_5N unit) positioned at the end of the "southern" polyene chain.[1] Similar structural moieties are present in all members of the group, indicating their close structural and biosynthetic relationship. The substances are especially characterized by the mC_7N unit that, although widespread in natural products as variably functionalized building block, is uniquely biosynthesized from a C_3 and a C_4 precursor.[2]



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Most of the manumycin group antibiotics exhibit a biological activity against Gram-positive bacteria, as well as antifungal and cytotoxic activities. In addition, interesting insecticidal effects and an inhibition of polymorphonuclear leucocyte elastase were found with manumycin A (1). A new and promising effect concerns antitumor activities because the analogs UCF1-A, -B, and -C were detected in a screening for ras farnesyltransferase inhibitors.[3] The most potent substance in this novel pharmacological approach of inhibiting oncogenic ras activity is UCF1-C, which is identical to 1. Furthermore, the recently described alisamycin[4] demonstrates that the manumycin group is still a growing class of substances.

In this paper we present the new manumycins B (2), C (3), and D (4) that are analogs of 1 as minor components of *Streptomyces parvulus* (strain Tü 64). With these new substances we developed further methodology for the structural elucidation by NMR spectroscopy, since crystals suitable for X-ray studies have not been obtained yet.

Results and Discussion

Metabolite Pattern of *Streptomyces parvulus*. Detailed chromatographic analysis on reversed-phase systems revealed that *Streptomyces parvulus* (strain Tü 64) produces in addition to manumycin A (1) less lipophilic minor components. They were isolated from the acetone extract of the mycelium obtained from a standard 50-L cultivation (soybean meal 2%, mannitol 2%) by extraction with acetone followed by silica gel chromatography (chloroform/methanol mixtures). The similar substances were separated through multiple runs on Sephadex LH-20 with chloroform as eluent, in which 1 eluted before manumycin B (2) and C (3). According to HPLC analysis 2 and 3 were produced in an amount of approximately 1% of 1.

Like manumycin A (1), they are pale yellow amorphous powders that decomposed slowly upon exposure to light and are unstable in acids and bases. Their solubility in organic solvents of medium polarity like chloroform, methanol, acetone, or acetonitrile was good, whereas they were insoluble in water or *n*-pentane. The absorption spectra of IR and UV/vis indicated the structural similarity of manumycin B (2) and C (3) to 1. The structural elucidation was mainly achieved through ^1H NMR and ^{13}C NMR spectroscopy, whereby spectra were measured in pyridine-d_5 for simplification of the proton spin systems.

Manumycin B (2), according to its molecular formula of $C_{28}H_{34}N_2O_7$ from the HR FAB mass spectrum, proved to be a C_3H_4 smaller analog of 1. It showed the characteristic ^1H NMR signals of the oxirane protons (δ = 3.94/

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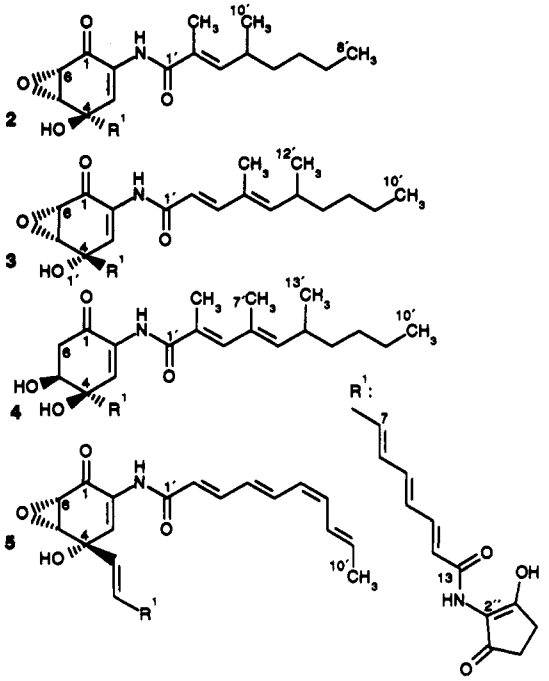
Table I. ^{13}C NMR Chemical Shifts (δ, ppm) of Manumycin A (1), B (2), C (3), and D (4)

carbon	1[a]	2[a]	3[b]	4[a]
C-1	189.0 s	188.9 s	188.6 s	192.5 s
C-2	128.0 s	127.9 s	128.2 s	131.7 s
C-3	126.6 d	126.5 d	126.1 d	127.8 d
C-4	71.2 s	71.2 s	71.2 s	73.3 s
C-5	57.4 d	57.3 d	57.4 d	71.8 d
C-6	52.8 d	52.8 d	53.0 d	40.8 t
C-7	136.6 d	136.5 d	136.2 d	139.0 d
C-8	131.4 d	131.4 d	131.6 d	131.2 d[c]
C-9	139.6 d	139.5 d	139.6 d	139.9 d
C-10	131.7 d	131.7 d	131.7 d	131.2 d[c]
C-11	143.4 d	143.4 d	143.5 d	143.5 d
C-12	121.7 d	121.6 d	121.5 d	121.4 d
C-13	165.6 s	165.5 s	165.4 s[d]	166.0 s
C-1′	168.8 s	168.0 s	165.4 s[d]	169.2 s
C-2′	128.4 s	129.1 s	117.2 d	128.2 s
C-3′	140.2 d	144.8 d	148.6 d	140.4 d
C-4′	129.9 s	33.2 d	130.9 s	130.0 s
C-5′	142.7 d	36.6 t	149.4 d	142.9 d
C-6′	32.9 d	29.6 t	33.3 d	33.0 d
C-7′	37.1 t	22.7 t	36.9 t	37.1 t
C-8′	29.8 t	14.0 q	29.6 t	29.9 t
C-9′	22.8 t	12.6 q	22.7 t	22.9 t
C-10′	14.1 q	20.1 q	14.0 q	14.1 q
C-11′	14.0 q		12.5 q	14.0 q
C-12′	16.5 q		20.4 q	16.6 q
C-13′	20.7 q			20.8 q
C-1″	197.5 s	197.5 s	197.2 s	e
C-2″	115.2 s	115.1 s	117.2 s	115.4 s
C-3″	174.5 s	174.5 s	173.8 s	e
C-4″	32.2 t	32.2 t	32.1 t	e
C-5″	25.7 t	25.6 t	25.6 t	e

[a] 50.3 MHz. [b] 125.6 MHz. [c] Verified through spectral extension. [d] Separable in benzene-d_6 (125.6 MHz): 165.2 (C-13), 165.8 (C-1′). [e] Not detectable because of coalescence phenomena.[9]

4.08) in the mC_7N unit and the multiplet at δ = 2.40 of the two methylene groups in the C_5N unit. The all-(*E*) configuration of the "southern" triene chain was derived from the vicinal coupling constants of the six olefinic protons *via* the double bonds ($^3J_{H,H}$ > 14.5 Hz). In contrast to manumycin A (1) the "eastern" side chain only contained one allylic methyl group (δ = 1.89, d *J* = 1.0 Hz, 9′-H_3). Together with one olefinic proton (δ = 6.37 dd, *J* = 10.0 Hz, 1.5 Hz, 3′-H), two aliphatic methyl groups, and seven aliphatic protons it was identified as a C_{10} chain, which is shorter than the C_{13} chain in 1 by an isopropylene unit. The configuration of the methyl-branched double bond was derived from the coupling constant between the C-1′ carbonyl and 3′-H in a coupled ^{13}C NMR spectrum.[1] The carbonyl signal (δ = 168.0) was simplified to a doublet by H/D exchange of the amide proton and decoupling of the methyl protons at C-9′. The value of 7 Hz for $^3J_{C\text{-}1'/3'\text{-}H}$ indicated the (2′*E*) configuration.[5] The constitution of manumycin B (2) was confirmed by the ^{13}C NMR data (Table I).

The absolute stereochemistry of C-4 in the mC_7N unit was established with circular dichroism (CD) spectroscopy using the exciton chirality method according to certain structural and physicochemical prerequisites.[6,7] The orientation of the CD curve of the intact molecule allows, independently from other centers of chirality, the establishment of the spatial arrangement of the two polyene amide chromophores;[8,9] details have been discussed for 1.[10] The medium strong negative CD couplet ($\Delta\epsilon_{max}$ −9.05 at 325 nm, +10.23 at 283 nm, acetonitrile) was nearly identical to the one of 1 and indicated the (4*R*) configuration.[10] Whereas the configuration of C-5 and C-6 in 1 was determined by CD spectroscopy of a degradation product, here an ASIS (aromatic solvent induced shift) effect in pyridine-d_5 was used for deducing the relative stereochemistry in the mC_7N unit (see below). Manumycin B (2) showed the same (4*R*,5*R*,6*S*) configuration as 1, but not enough material was available for determining the absolute configuration of C-4′ in the "eastern" side chain. Manumycin B (2) resembles the recently described UCF1-A, also of the (4*R*) configuration.[3]



For manumycin C (3) the FD mass spectrum indicated a molecular weight of 536 g/mol that led to a molecular formula of $C_{30}H_{36}N_2O_7$ as a CH_2 smaller homolog of 1. The characteristic ^1H and ^{13}C (Table I) NMR signals of the mC_7N unit, the C_5N unit, and the triene chain were determined to be the same as in manumycin A (1) and B (2). Again, the "eastern" side chain was varied: Instead of the allylic 3′-H as in 1 a new olefinic AX-system (δ = 6.50, 2′-H; δ = 7.64, 3′-H, *J* = 15.0 Hz) and only one allylic methyl group were found. The protonated double bond had to be situated near the amide terminus of the chain, indicated by the strong downfield shift of 3′-H. The remaining chain from C-4′ to C-12′ resembles that of 1. The all-(*E*) configuration of the protonated double bonds was again derived from a value of 15.0 Hz for the $^3J_{H,H}$ coupling constants. A significant NOE (nuclear Overhauser enhancement) effect between 3′-H and 5′-H, shown in a NOESY NMR spectrum, proved the (4′*E*) configuration. The unexpected (4*S*) configuration was deduced from a positive CD couplet ($\Delta\epsilon_{max}$ +6.09 at 324 nm, −11.49

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at 289 nm, acetonitrile) and in combination with a strong ASIS (aromatic solvent induced shift) effect suggested a (4*S*,5*R*,6*S*) configuration (see below). The absolute configuration of C-6′ was not studied due to insufficient amounts of material. Manumycin C (3) resembles UCF1-B, which also has a (4*S*) configuration.[3]

Manumycin D (4) was isolated from a culture of *Streptomyces parvulus* (strain Tü 64) by feeding of *p*-aminobenzoic acid in a precursor-directed biosynthesis (high concentration method).[11] Later it was shown that the new compound was also part of the manumycin complex produced under normal conditions.[12] The HR EI mass spectroscopy led to the molecular formula of $C_{31}H_{38}N_2O_6$ (*m/z* 534), and a characteristic peak at *m/z* 193 indicated the intact "eastern" C_{13} side chain as in 1. In the ^{1}H NMR spectrum (chloroform-d_1) 40 protons were detected, with five of them exchangeable by D_2O. Thus, the ion at *m/z* 534 in the EI mass spectrum might represent a dehydrated product. However, the ^{1}H and ^{13}C (Table I) NMR data confirmed the resemblance of the C_5N unit, the triene chain, and the C_{13} chain to 1. For the C_5N unit, only the characteristic signal of C-2″ at δ = 115.4 turned up as a result of the coalescence between the enol and the keto function.[9] Instead of the characteristic oxirane protons a new ABX system (δ = 4.12, 5-H; 2.92, 6-H_A; 2.75, 6-H_B) and an additional exchangeable proton (δ = 3.78) were found, indicating a hydroxyethylene function, which would be consistent with the proposed dehydration in the mass spectrum. The regiochemistry was deduced from the coupling of 3-H (J = 2.5 Hz) that was not detectable with the sharp double doublets of the methylene protons. Thus, the partner of the long-range coupling was the methine proton, whose multiplet had to be situated at C-5. The appearance of the coupling constant also indicated a quasiequatorial position of 5-H. This was confirmed by the small vicinal coupling constants of the ABX system ($J_{A,X}$ = 3.2 Hz, $J_{B,X}$ = 5.9 Hz) that demonstrated a half-chair conformation of the cyclohexenone. According to the small difference in the shift of 6-H_A and 6-H_B ($\Delta\delta$ = 0.18) 4-OH should be oriented quasiequatorial, leading to a *cis* configuration with the axial 5-OH. A diaxial *trans* location of the hydroxy groups in α and β position should result in a shift difference of about 1 ppm. The proposed relative stereochemistry was supported by a striking NOE effect between 5-H and 7-H in the triene chain, shown in a NOESY NMR spectrum of 4. This is consistent with the position of the methine proton at C-5 and caused us to assume that 5-H and the triene chain are located on the same side of the ring plane. There are only a few conformations of the C_4–C_7 bond in which the distances of 5-H and 7-H become small enough for interactions. Especially in a half-chair conformation of the cyclohexenone only the *cis* configuration of 5-H and the triene chain gives rise to the observed NOE effect.

The CD curve of 4 did not allow an interpretation following the exciton chirality method for deducing the absolute stereochemistry of C-4. Possibly, a weaker conformational stability of the mC_7N unit disturbed the chiral transition moments of the polyene amide chromophores. Due to significant characteristics of the optical rotation values among manumycins A–D (1–4) we assume a (4*S*) configuration leading to (5*R*),[13] but cannot exclude the enantiomer.

Table II. Selected ^{1}H NMR Chemical Shifts (δ, ppm) and ASIS of Manumycin A (1), B (2), C(3), D (4) and Colabomycin A (5)

	type I		type II		
hydrogen[a]	1	2	3	4	5
3-H (C)	7.39	7.39	7.42	7.57	
3-H (P)	7.94	7.94	8.21	8.28	8.15
$\Delta_{3\text{-H}}$	−0.55	−0.55	−0.79	−0.71	
5-H (C)	3.72	3.72	3.72	4.12	
5-H (P)	4.05	4.08	4.04	4.50	4.03
$\Delta_{5\text{-H}}$	−0.33	−0.36	−0.32	−0.38	
6-H (C)	3.65	3.66	3.66	2.75/2.92	
6-H (P)	3.94	3.94	3.91	3.17/3.32	3.88
$\Delta_{6\text{-H}}$	−0.29	−0.28	−0.25	−0.42/−0.40	

[a] Key: C, chloroform-d_1; P, pyridine-d_5; $\Delta_H = \delta^{chloroform} - \delta^{pyridine}$.

Relative Stereochemistry of the mC_7N Unit. The ^{1}H NMR spectra of the manumycins A–D (1–4) in chloroform-d_1 and pyridine-d_5 revealed almost identical ^{1}H chemical shifts and ASIS (aromatic solvent induced shift)[14] effects $\Delta_H = \delta^{chloroform} - \delta^{pyridine}$ for the corresponding structural parts. A striking irregularity concerned 3-H of the mC_7N unit in pyridine-d_5 (Table II). In the case of manumycin A (1) and B (2) a value of Δ_H = −0.55 was found (type I), whereas with type II compounds the ASIS effects were significantly stronger, e. g., Δ_H = −0.79 for manumycin C (3). An extraordinary downfield shift of 3-H in pyridine-d_5 was also found for colabomycin A (5).[9,15]

The magnitude of the ASIS effect depends on the relative configurations in the mC_7N unit, the hydroxy group at C-4, and the neighboring oxygen substitution. Independent determination of the parent compound and a degradation product by CD spectroscopy led to a *trans* configuration in manumycin A (1)[10] and to the *cis* configuration in the type-II compound colabomycin A (5).[9] The latter was also found for manumycin D (4) by detailed analysis of ^{1}H NMR data and a NOE effect. Consequently, the smaller ASIS effect corresponds to a *trans* arrangement of both oxygen functionalities and the larger one with their *cis* position.

Although the ASIS has been widely used and analyzed for different structural moieties, for example, in steroids, it is not fully understood regarding the various physicochemical contributions.[16] Especially, we cannot give a detailed mechanistic explanation that includes the identical ASIS on the adjacent oxirane protons in both configurational types,[13] but however, the determination of the ASIS effect on 3-H can be stated as a new, facile method for the assignment of the relative stereochemistry in the mC_7N unit of manumycin group compounds as 1–3.

Conclusions

The new minor components of *Streptomyces parvulus*, manumycin B (2) and C (3), differed from 1 within the amidically bound "eastern" side chain. In manumycin A (1) the polyene chains are biosynthesized *via* two polyketide pathways with the methyl branches incorpo-

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rated by propionate building blocks instead of acetate.[17] Thus, the terminal propionate building block is missing in 2 and is exchanged for an acetate in 3. Although "variations on a theme" are widely known in late biosynthesis after the polyketide assembling,[18] variations in the skeleton are rarely reported. The minor components B and C of monensin from *Streptomyces cinnamonensis* are built up by replacing a butyrate with a propionate in the fifth or in the thirteenth final elongation step, respectively.[19] The polyketide synthase with its different functional units for each elongation and functionalization step is known to work with high fidelity.[20] The acyl carrier protein (ACP), which carries the acyl chain during elongation and further processing, plays presumably a crucial role in controlling the highly programmed enzyme or multienzyme complex. It is difficult to decide whether the minor components 2 and 3 result out of enzymatic mistakes or whether their side chains are assembled by different polyketide synthases. Strikingly, the UCF1 complex from *Streptomyces* sp. (UOF1, FERM BP-2844) is similar to the manumycins A–C (1–3), even with the same change in the absolute configuration of C-4, but with equal amounts of the three components.

Another striking feature concerns the oxygens of the mC_7N unit at C-4 and C-5, which originate in manumycin A (1) from molecular oxygen.[21] Presumably, the oxygenating reaction occurs in two steps as topological considerations rule out a simultaneous introduction of the oxygen atoms by a dioxygenase. Manumycin B (2) shows a similar (4*R*,5*R*,6*S*) configuration as 1, whereas in manumycin C (3) C-4 is oxygenated with the opposite stereospecificity. Whether this is due to the same oxygenating enzyme cannot be decided without studying the biosynthetic enzymes. Possibly, the oxirane oxygen is introduced before that of the hydroxy group; thus, only one oxygenating reaction would be stereospecifically altered. Nevertheless, manumycin C (3) carries the same oxygen pattern in the mC_7N unit as colabomycin A (5) and asukamycin, of which the absolute stereochemistry was recently completed.[22] The stereochemistry of C-4 might be controlled by conformational effects of the "eastern" side chain, since all compounds with a (4*S*) configuration lack a methyl branch at C-2′, which will probably influence its folding. The diastereomeric oxygenating reactions by *Streptomyces parvulus* are an interesting object for enzymatic studies of the complex biosynthesis of the mC_7N unit. The hydroxyethylene unit of manumycin D (4) might be biosynthesized by an attack of a dioxygenase on an earlier intermediate and can be seen as a shunt product. Nevertheless, 4 demonstrates for the first time that the oxirane unit is not a distinctive structural feature of the manumycin group.

The new minor components of *Streptomyces parvulus* show that the biosynthetic machinery is capable of providing natural derivatives of manumycin A (1) that would hardly be available by chemical methods.[23] The approach toward varied polyketide chains is a useful supplement to the precursor-directed biosynthesis, which leads by feeding of benzoic acids to aromatic manumycin A (1) analogs.[11, 24]

Although the ASIS is already a facile method for the structure elucidation of manumycin group compounds, our future interest concerns NOE effects for determining the relative stereochemistry of the mC_7N unit. First results with NOE effects between 5-H and 7-H/8-H in manumycin C (3) turned out not to be diagnostic for this stereochemical problem.[23] We have now focused on NOE's between triene protons and 6-H, which should only be possible in the type-II compounds. Further studies will afford a detailed investigation on parametrization of the NOESY spectra.

Experimental Section

Fermentation. *Streptomyces parvulus* (strain Tü 64) was maintained and cultivated as previously described.[1] The fermentation for isolating manumycins A–C (1–3) was carried out in a 50-L fermentor in the standard medium (2% degreased soybean meal, 2% glucose, 0.05% $CaCO_3$, pH adjusted on 7.2), that was inoculated with 10% of a 48-h-old shaking culture (100 mL of media in a 250-mL Erlenmeyer flasks with three buffles). The fermentation was harvested after 72 h. Manumycin D (4) was produced in a 10-L fermentation of *Streptomyces parvulus* (strain Tü 64) with feeding of 50 mM of *p*-aminobenzoic acid following the described procedure.[11]

Isolation and Characterization. The cultures were harvested as described for manumycin A (1), and the substances were isolated from the filtered mycelium after extraction with ethyl acetate.[1] Purification was performed on silica gel (flash) with appropriate chloroform/methanol mixtures and finally on Sephadex LH-20 columns with chloroform and chloroform/methanol, respectively. The fractions were evaluated by TLC on silica gel and on RP8 silica gel, respectively; the eluents are given with the R_f values of the substances. The compounds were visualized by UV absorption at 254 nm.

Spectroscopy. NMR chemical shifts are given in parts per million (ppm) with TMS or solvent signals as internal standards. ^1H NMR spectra are reported as follows: chemical shift (multiplicity, coupling constants in Hz, integration, interpretation). ^{13}C NMR spectra were assigned with APT (attached proton test) spectra and are reported as follows: chemical shift (multiplicity, interpretation). All data were ascertained by ^1H COSY and ^1H, ^{13}C HETCOR experiments. In the partially decoupled coupled ^{13}C-NMR spectrum of 2 the influence of the decoupling power was examined by lowering the power until $^3J_{C,H}$ showed no more changes.

Manumycin A (1): $C_{31}H_{38}N_2O_7$ (550.77); R_f 0.41 (silica gel, $CHCl_3$/MeOH (9:1)), 0.30 (RP8 silica gel, MeOH/H_2O (85:15)); $[\alpha]^{20}_D$ −164.9 (c = 0.51, $CHCl_3$); CD (AcCN) λ_{max} ($[\theta]^{20}$) 320 (−32 500), 283 (+30 790), 255 (+12 930) nm; ^1H NMR (500 MHz, pyridine-d_5) δ 0.86 (t, J = 7 Hz, 3H, 10′-H_3), 0.93 (d, J = 6.8 Hz, 3H, 13′-H_3), 1.76 (d, J = 1.2 Hz, 3H, 12′-H_3), 1.1–1.4 (m, 6H, 7′-H_2, 8′-H_2, 9′-H_2), 2.12 (d, J = 1.2 Hz, 3H, 11′-H_3), 2.40 (m, 1H, 6′-H), 2.43 (s, 4H, 4″-H_2, 5″-H_2), 3.94 (d, J = 4.0 Hz, 1H, 6-H), 4.05 (dd, J = 4.0, 2.8 Hz, 1H, 5-H), 5.30 (d, J = 9.5 Hz, 1H, 5′-H), 6.22 (d, J = 15.0 Hz, 1H, 7-H), 6.47 (dd, J = 14.5, 11.2 Hz, 1H, 10-H), 6.67 (d, J = 14.5 Hz, 1H, 12-H), 6.74 (dd, J = 14.5, 11.2 Hz, 1H, 9-H), 7.00 (s br, 1H, 3′-H), 7.01 (dd, J = 15.0, 11 Hz, 1H, 8-H), 7.63 (dd, J = 14.5, 11.2 Hz, 1H, 11-H), 7.94 (d, J = 2.8 Hz, 1H, 3-H), 8.84 (s, 1H, NH) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) see Table I.

Manumycin B (2): $C_{28}H_{34}N_2O_7$ (510.58); mp 94 °C; R_f 0.41 (silica gel, $CHCl_3$/MeOH (9:1)), 0.41 (RP8 silica gel, MeOH/H_2O (85:15)); $[\alpha]^{20}_D$ −140.4 (c = 0.26, $CHCl_3$); IR (KBr) ν 3405, 2960, 2930, 1675, 1630, 1515, 1365, 1005 cm^{-1}; UV (MeOH) λ_{max} (ε) 311

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(33 310), 278 (34 060), 263 (33 430) nm; (MeOH, HCl) λ_{max} (ϵ) 329 (32 280); 270 (25 810) nm; (MeOH, NaOH) λ_{max} (ϵ) 296 (39 940), 263 (34 260), 215 (16 430) nm; UV (AcCN) λ_{max} (ϵ) 327 (30 410), 278 (21 610) nm ; CD (AcCN) λ_{max} ($[\theta]^{20}$) 325 (−29 880), 283 (+35 370), 262 (+15 240) nm; FAB-MS (positive) *m/e* = 511 ([M + H]$^+$, calcd for $C_{28}H_{35}N_2O_7$ (511.246) and found 511.244); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (d, 3H, 8′-H_3), 1.01 (d, 3H, 10′-H_3), 1.22–1.39 (m, 6H, 5′-H_2, 6′-H_2, 7′-H_2,), 1.89 (d, 3H, 9′-H_3), 2.48 (m, 1H, 4′-H), 2.58 (m, 4H, 4″-H_2, 5″-H_2), 3.68 (d, *J* = 4.0 Hz, 1H, 6-H), 3.72 (dd, *J* = 4.0, 2.5 Hz, 1H, 5-H), 3.83 (4-OH), 5.86 (m, 1H, 7-H), 6.16 (d, *J* = 15.0 Hz, 1H, 12-H), 6.21 (dd, *J* = 10.0, 1.5 Hz, 1H, 3′-H), 6.42 (m, 1H, 10-H), 6.60 (m, 2H, 8-H, 9-H), 7.33 (dd, *J* = 15.0, 11.2 Hz, 1H, 11-H), 7.39 (d, 1H, 3-H), 7.92 (s, 1H, NH), 7.97 (s, 1H, NH), 13.70 (3″-OH) ppm; ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.76 (t, *J* = 7.0 Hz, 3H, 8′-H_3), 0.83 (d, *J* = 6.5 Hz, 3H, 10′-H_3), 1.09–1.20 (m, 6H, 5′-H_2, 6′-H_2, 7′-H_2), 1.91 (d, *J* = 1.0 Hz, 3H, 9′-H_3), 2.36 (m, 1H, 4′-H), 2.40 (s, 4H, 4″-H_2, 5″-H_2), 3.94 (d, *J* = 4.0 Hz, 1H, 6-H), 4.08 (dd, *J* = 4.0, 2.5 Hz, 1H, 5-H), 6.22 (d, *J* = 15.0 Hz, 1H, 7-H), 6.37 (dd, *J* = 10.0, 1.5 Hz, 1H, 3′-H), 6.46 (dd, *J* = 14.5, 11.2 Hz, 1H, 10-H), 6.70 (d, *J* = 15.0 Hz, 1H, 12-H), 6.73 (dd, *J* = 14.5, 11.2 Hz, 1H, 9-H), 7.01 (dd, *J* = 15.0, 11.2 Hz, 1H, 8-H), 7.64 (dd, *J* = 15.0, 11.2 Hz, 1H, 11-H), 7.94 (d, *J* = 2.5 Hz, 1H, 3-H), 10.7 (s br, 3″-OH), 8.80 (2H) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) see Table I; ^{13}C NMR (125 MHz, pyridine-d_5) δ 12.8 (C-9′), 14.1 (C-8′), 20.1 (C-10′), 22.9 (C-7′), 29.7 (C-6′), 33.2 (C-4′), 36.7 (C-5′), 53.5 (C-6), 58.2 (C-5), 71.5 (C-4), 116.0 (C-2″), 122.8 (C-12), 128.8 (C-2), 129.6 (C-3), 130.2 (C-2′), 131.2 (C-8), 131.8 (C-10), 139.5 (C-7), 140.0 (C-9), 143.1 (C-11), 143.7 (C-3′), 166.8 (C-13), 168.1 (C-1′), 190.2 (C-1) ppm.

Manumycin C (3): $C_{30}H_{36}N_2O_7$ (536.63); mp 188–196 °C dec; R_f = 0.41 (silica gel, $CHCl_3$/MeOH (9:1)), 0.39 (RP8 silica gel, MeOH/H_2O (85:15)); $[\alpha]^{20}_D$ +32.4 (*c* = 0.36, $CHCl_3$); IR (KBr) ν 3395, 2960, 2930, 2860, 1675, 1620, 1365, 1005 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 307 (33 080), 278 (42 700) nm; (MeOH, HCl) λ_{max} (ϵ) 333 (25 260), 273 (32 780) nm; (MeOH, NaOH) λ_{max} (ϵ) 265 (43 570) nm; UV (AcCN) λ_{max} (ϵ) 329 (21 090), 277 (22 250) nm; CD (AcCN) λ_{max} ($[\theta]^{20}$) 324 (+22 870), 289 (−37 930) nm; FD-MS *m/e* (abundance) 536 (100); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 3H, 10′-H_3), 0.99 (d, *J* = 6.5 Hz , 3H, 12′-H_3), 1.20–1.40 (m, 6H, 7′-H_2, 8′-H_2, 9′-H_2), 1.80 (d, *J* = 1 Hz, 3H, 11′-H_3), 2.52 (m, 1H, 6′-H), 2.58 (m, 4H, 4″-H_2 , 5″-H_2), 3.0 (br, 1H, OH), 3.66 (d, *J* = 4 Hz, 1H, 6-H), 3.72 (dd, *J* = 4, 2.5 Hz, 1H, 5-H), 5.71 (d, *J* = 10 Hz, 1H, 5′-H), 5.81 (d, *J* = 15 Hz, 1H, 2′-H), 5.87 (m, 1H, 7-H), 6.06 (d, *J* = 14.5 Hz, 1H, 12-H), 6.42 (m, 1H, 10-H), 6.59 (m, 2H, 8-H, 9-H), 7.28 (d, *J* = 15 Hz, 1H, 3′-H), 7.33 (dd, *J* = 14.5, 11.2 Hz, 1H, 11-H), 7.42 (d, *J* = 2.5 Hz, 1H, 3-H), 7.60 (s br, 2H, NH), 13.60 (s br, 1H, OH) ppm; ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.78 (t, *J* = 7.5 Hz, 3H, 10′-H_3), 0.86 (d, *J* = 6.8 Hz, 3H, 12′-H_3), 1.10–1.20 (m, 6H, 7′-H_2, 8′-H_2, 9′-H_2,), 1.65 (d, *J* = 1.0 Hz, 3H, 11′-H_3), 2.35 (m, 1H, 6′-H), 2.40 (s, 4H, 4″-H_2, 5″-H_2), 3.0 (4-OH), 3.91 (d, *J* = 4.0 Hz, 1H, 6-H), 4.04 (dd, *J* = 4.0, 2.8 Hz, 1H, 5-H), 5.56 (d, *J* = 10.0 Hz, 1H, 5′-H), 6.18 (d, *J* = 15.0 Hz, 1H, 7-H), 6.46 (dd, *J* = 15.0, 11.5 Hz, 1H, 10-H), 6.50 (d, *J* = 15.0 Hz, 1H, 2′-H), 6.71 (d, *J* = 15.0 Hz, 1H, 12-H), 6.72 (dd, *J* = 15.0, 11.0 Hz, 1H, 9-H), 7.03 (dd, *J* = 15.0, 11.0 Hz, 1H, 8-H), 7.64 (d, *J* = 15.0 Hz, 1H, 3′-H), 7.65 (dd, *J* = 15, 11.5 Hz, 1H, 11-H), 8.21 (d, *J* = 2.8 Hz, 1H, 3-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) see Table I.

Manumycin D (4): $C_{31}H_{40}N_2O_7$ (552.28); mp 96 °C; R_f = 0.32 (silica gel, $CHCl_3$/MeOH (9:1)), 0.31 (RP8 silica gel, MeOH/H_2O, (85:15)); IR (KBr) ν 3440, 3230, 2960, 2930, 1690, 1670, 1620, 1550, 1505, 1380 (br), 1000 cm^{-1}; $[\alpha]^{20}_D$ −25.3 (*c* = 0.32, $CHCl_3$/MeOH (9:1)); UV(MeOH) λ_{max} (ϵ) 309 (43 690), 245 (38 900), 205 (52 690) nm; (MeOH, HCl) λ_{max} (ϵ) 325 (24 150), 207 (32 080) nm; (MeOH, NaOH) λ_{max} (ϵ) 302 (24 570), 261 (25 580), 211 (24 870) nm; UV(AcCN) λ_{max} (ϵ) 313 (25 650), 269 (21 350) nm. CD (AcCN) λ_{max} ($[\theta]^{20}$) 314 (+5620), 293 (−2150), 268 (+9890), 227 (−3003) nm; EI-MS (70 eV) *m/e* (abundance) 534 ($M^+ - H_2O$, 20, calcd for $C_{31}H_{40}N_2O_7$ and found 534.2730), 421 (20), 368 (30), 193 (100), 123 (26), 109 (77); ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 0.89 (t, *J* = 7.0, 3H, 10′-H_3), 0.97 (d, *J* = 6.8 Hz, 3H, 13′-H_3), 1.2–1.4 (m, 6H, 7′-H_2, 8′-H_2, 9′-H_2), 1.82 (d, *J* = 1.8 Hz, 3H, 12′-H_3), 2.06 (d, *J* = 1.1 Hz, 3H, 11′-H_3), 2.45 (m, 1H, 6′-H), 2.57 (m, 4H, 4″-H_2, 5″-H_2), 2.75 (dd, *J* = 16.2, 3.2 Hz, 1H, 6-H_A), 2.92 (dd, *J* = 16.2, 5.9 Hz, 1H, 6-H_B), 3.10 (s, 1H, OH), 3.78 (s, 1H, OH), 4.12 (m, 1H, 5-H), 5.34 (d, *J* = 10.0 Hz, 1H, 5′-H), 6.06 (d, *J* = 14.5 Hz, 1H, 7-H), 6.08 (d, *J* = 15.0 Hz, 1H, 12-H), 6.37 (dd, *J* = 14.5, 11.0 Hz, 1H, 10-H), 6.48–6.64 (m, 2H, 8-H, 9-H), 6.78 (s, 1H, 3′-H), 7.32 (dd, *J* = 15.0, 11.0 Hz, 1H, 11-H), 7.57 (d, *J* = 1.5 Hz, 1H, 3-H), 7.70 (s, 1H, NH), 8.30 (s, 1H, NH), 13.60 (s, 1H, 3″-OH) ppm; ^{1}H NMR (200 MHz, pyridine-d_5, TMS) δ 0.88 (t, *J* = 7.0 Hz, 3H, 10′-H_3), 0.93 (d, *J* = 6.8 Hz, 3H, 13′-H_3), 1.1–1.4 (m, 6H, 7′-H_2, 8′-H_2, 9′-H_2), 1.76 (d, *J* = 1.2 Hz, 3H, 12′-H_3), 2.12 (d, *J* = 1.2 Hz, 3H, 11′-H_3), 2.40 (m, 1H, 6′-H), 2.43 (s, 4H, 4″-H_2, 5″-H_2), 3.17 (dd, *J* = 16.2 Hz, 3.5 Hz, 1H, 6-H_A), 3.32 (dd, *J* = 16.2 Hz, 5.9 Hz, 1H, 6-H_B), 4.50 (m, 1H, 5-H), 5.31 (d, *J* = 9.5 Hz, 1H, 5′-H), 6.42 (dd, *J* = 15.0 Hz, 11.2 Hz, 1H, 10-H), 6.53 (d, *J* = 15.0 Hz, 1H, 7-H), 6.67 (d, *J* = 15.0 Hz, 1H, 12-H), 6.74 (dd, *J* = 14.5, 11.2 Hz, 1H, 9-H), 7.01 (dd, *J* = 15.0, 11.0 Hz, 1H, 8-H), 7.02 (s br, 1H, 3′-H), 7.66 (dd, *J* = 15.0 Hz, 11.5 Hz, 1H, 11-H), 8.28 (d, *J* = 1.5 Hz, 1H, 3-H), 8.84 (s, 1H, NH), 10.6 (br, 1H, OH) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$/MeOH-d_4 (9:1)) see Table I.

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Supplementary Material Available: ^{1}H-NMR of compound 3 recorded in pyridine-d_5 (1 page). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.