Glycylcyclines. 1. A New Generation of Potent Antibacterial Agents through Modification of 9-Aminotetracyclines

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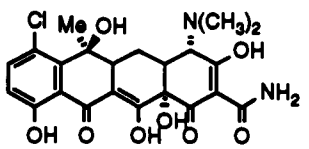
This report describes the discovery of a new generation of tetracycline antibacterial agents, the "glycylcyclines". These agents are notable for their activity against a broad spectrum of tetracycline-susceptible and -resistant Gram-negative and Gram-positive aerobic and anaerobic bacteria possessing various classes of tetracycline-resistant determinants [*tet* B (efflux), *tet* M (ribosomal protection)]. The design and synthesis of a number of 7-substituted 9-substituted-amido 6-demethyl-6-deoxytetracyclines are described.

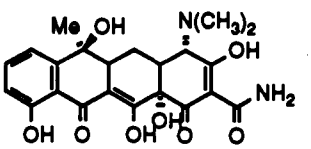
Since the discovery of chlorotetracycline (**1a**) in 1945 and tetracycline (**1b**) in 1953,[1] numerous natural and semisynthetic tetracyclines have been reported with broad-spectrum antibacterial activity.[2] However, no new drugs in this class have been reported since the discovery of minocycline (**2**) in the early 1970's. The widespread use of these agents has caused many bacteria to develop resistance.[3] Similarly, the increasing incidence of multiply-resistant pathogenic bacteria to the currently available antibacterial agents has also become a problem.[4]

There are two resistance mechanisms to the tetracyclines among pathogens: (1) expulsion of antibiotics by tetracycline efflux pumps and (2) ribosome protection.[3] The efflux mechanism (*tet* A–*tet* D), first observed in Gram-negative organisms (*Escherichia coli*), is an active transport mechanism based on a proton-motive force.[5] A similar mechanism has also been identified (*tet* K–*tet* L) in several classes of Gram-positive organisms (*Staphylococcus aureus* and *Streptococcus* spp.).[6] The ribosomal protection mechanism (*tet* M–*tet* O) is mediated by proteins that interact with the ribosome, allowing protein synthesis even in the presence of tetracyclines. This type of resistance mechanism is found in bacteria that cause many sexually transmitted diseases and also in *Staphylococcus* spp.[7]

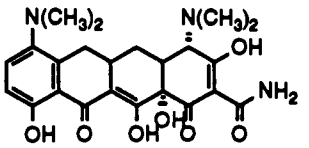
While research in this area has focused exclusively on the nature of the resistance mechanisms, no new structural entities have been reported. Recently, Levy reported that 13-substituted-thio 5-hydroxy-6-deoxytetracyclines **3** inhibited tetracycline efflux in everted vesicles, but the intrinsic antibacterial activity of these analogues is yet to be communicated.[8]



1a



1b



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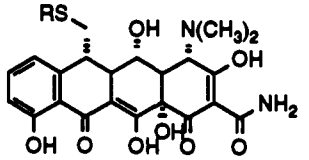

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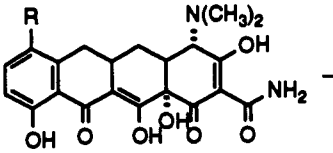
The objective of our program was to design and synthesize new molecular entities with an improved antibacterial spectrum, including activity against bacteria currently resistant to the tetracyclines and other antibiotics. In the search for novel tetracyclines which would meet the above goals, we decided to use minocycline as a model for designing new derivatives since it was the most active tetracycline antibiotic known. Earlier structure–function studies indicate that modifications of the D ring of the tetracycline molecule provide a high probability of retaining antibacterial activity. Accordingly, we focused our efforts on modifying the C-7 and C-9 positions of the 6-demethyl-6-deoxytetracycline nucleus.

This report describes the discovery of a new generation of potent antibacterial agents, the "glycylcyclines". These new compounds are notable for their broad antimicrobial spectrum with potent activity against both Gram-positive and Gram-negative aerobic and anaerobic bacteria possessing various classes of tetracycline-resistant determinants [*tet* B (efflux), *tet* M (ribosomal protection)].[9] The mechanism of action of the enhanced activity is under investigation and will be communicated separately.

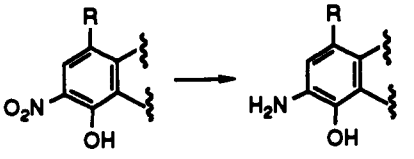
Chemistry

The new derivatives synthesized are shown in Scheme 1. Several literature compounds (**4**, **8**, and **12**)[10] are also included for comparison.

Compounds **5** and **6** were prepared by the procedure as shown below. Nitration of minocycline gave the nitro compound **22** which was reduced *via* catalytic hydrogenation to give **5** in good yield. Similarly, **6** was prepared



2 R = $N(CH_3)_2$ *minocycline*
21 R = NEt_2



22 R = $N(CH_3)_2$
23 R = NEt_2

5 R = $N(CH_3)_2$
6 R = NEt_2

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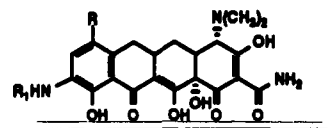
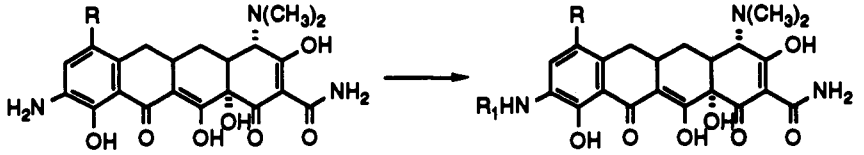
Table 1. In Vitro Antibacterial Activity of 7-Substituted 9-Substituted-Amido 6-Demethyl-6-deoxytetracyclines



compd	types of salt	R	R_1	organism; minimum inhibitory concentration (MIC) (μg/mL)										
				E. coli UBMS 88-1 (*tet* B)	*E. coli* PRP-1 (*tet* A)	*E. coli* J3272 (*tet* C)	*E. coli* J3272 (*tet* D)	*E. coli* UBMS 90-4 (*tet* M)	*E. coli* UBMS 90-5 sensitive	*S. aureus* UBMS 88-7 (*tet* K)	*S. aureus* UBMS 90-1 (*tet* M)	*S. aureus* UBMS 90-3 sensitive	*S. aureus* Smith sensitive	*Enterococcus* ATCC 29212
4	$\cdot H_2SO_4$	H	H	>32	32	>32	NT	16	1	4	8	≤0.015	0.03	0.5
5	HCl	$N(CH_3)_2$	H	32	4	16	8	8	0.5	0.25	0.5	0.06	0.06	0.25
6	·2HCl	NEt_2	H	2	2	4	NT	32	1	0.06	2	0.03	0.03	0.12
7	$\cdot H_2SO_4$	I	H	>32	>32	>32	NT	>32	4	0.5	0.5	0.03	≤0.015	0.12
8	$\cdot H_2SO_4$	H	CHO	32	>32	>32	NT	1	0.25	>32	1	≤0.015	0.03	0.06
9		$N(CH_3)_2$	CHO	>32	16	>32	8	1	0.25	4	0.06	0.03	0.03	0.03
10		NEt_2	CHO	16	32	32	NT	8	0.5	0.25	0.5	0.03	0.03	0.06
11	$\cdot H_2SO_4$	I	CHO	>32	>32	>32	NT	32	0.5	16	4	0.03	0.03	0.25
12	$\cdot H_2SO_4$	H	$COCH_3$	>32	>32	>32	0.5	8	8	32	2	0.12	0.12	0.25
13		$N(CH_3)_2$	$COCH_3$	>32	>32	>32	NT	4	8	8	1	0.25	0.25	0.25
14		NEt_2	$COCH_3$	32	>32	>32	NT	32	32	2	2	0.12	0.12	0.25
15	$\cdot H_2SO_4$	I	$COCH_3$	>128	>128	>128	NT	>128	16	16	1	0.12	0.12	0.25
16	CF_3COOH	$N(CH_3)_2$	$COCH_2NH_2$	2	32	32	2	1	2	>32	2	0.5	1	0.25
17	·2HCl	$N(CH_3)_2$	$COCH_2N(CH_3)_2$	0.25	2	1	0.12	0.25	0.25	2	0.25	0.06	0.12	0.06
18	·2HCl	H	$COCH_2N(CH_3)_2$	0.25	1	0.5	0.12	0.12	0.12	1	0.12	0.06	0.12	0.03
19	2HCl	NEt_2	$COCH_2N(CH_3)_2$	1	4	4	0.5	1	1	1	0.5	0.5	0.5	0.25
20	·2HCl	I	$COCH_2N(CH_3)_2$	1	8	4	0.5	1	2	4	0.5	0.12	0.25	0.06
2			minocycline	16	4	4	8	>32	1	0.25	4	0.12	0.12	1
1b			tetracycline	>32	32	>32	32	>32	1	>32	>32	0.25	0.25	18

Scheme I

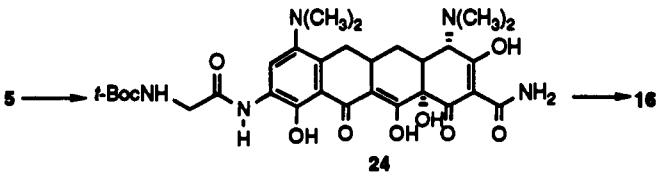


4 R = H
5 R = $N(CH_3)_2$
6 R = NEt_2
7 R = I

8 R = H, R_1 = CHO
9 R = $N(CH_3)_2$, R_1 = CHO
10 R = NEt_2, R_1 = CHO
11 R = I, R_1 = CHO
12 R = H, R_1 = $COCH_3$
13 R = $N(CH_3)_2$, R_1 = $COCH_3$
14 R = NEt_2, R_1 = $COCH_3$

15 R = I, R_1 = $COCH_3$
16 R = $N(CH_3)_2$, R_1 = $COCH_2NH_2$
17 R = $N(CH_3)_2$, R_1 = $COCH_2N(CH_3)_2$
18 R = H, R_1 = $COCH_2N(CH_3)_2$
19 R = NEt_2, R_1 = $COCH_2N(CH_3)_2$
20 R = I, R_1 = $COCH_2N(CH_3)_2$

from **21**[11] *via* **23**. Iodination of **8** and **12** gave the 7-iodo compounds **11** and **15**, respectively. Subsequent treatment of **11** with aqueous acid in methanol afforded **7**. The formamido compounds **8–10** were prepared from the 9-amino compounds **4–6**, respectively, using a mixture of acetic anhydride and 90% formic acid. This method gave cleaner products than the method previously used for the preparation of **8**.[10] Treatment of **4–6** with sodium carbonate and acetyl chloride gave **12–14**, respectively. Coupling of **5** with *N*-*t*-Boc-glycine *N*-hydroxysuccinimide ester[12] gave **24** which was deprotected with trifluoroacetic acid to give **16**.



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Compounds **17–20** were prepared by treating the 9-amino compounds **4–7** with *N*,*N*-dimethylglycyl chloride hydrochloride.[13] The use of an aprotic solvent [*e.g.*, *N*,*N*-dimethylpropyleneurea (DMPU)] in the acylation reactions also minimized epimerization at C-4. The epimer is identified by ^1H NMR with its characteristic H-4 chemical shifts at δ 4.8 *vs* δ 4.3 in the natural tetracyclines.

Biology

The *in vitro* antibacterial activities of compounds **4–20** are shown in Table 1. The minimum inhibitory concentration (MIC), the lowest concentration of the antibiotic which inhibits growth to the test organism, was determined by the agar dilution method using Muller–Hinton II agar (Baltimore Biological Laboratories). An inoculum density of (1–5) × 10^5 CFU/mL and a range of antibiotic concentrations (32–0.004 μg/mL) were used.

The plates were incubated for 18 h at 35 °C in a forced air incubator. The test organisms comprised of both tetracycline-sensitive and tetracycline-resistant strains. The Gram-negative bacteria were represented by *E. coli* strains and the Gram-positive bacteria by *S. aureus* and *Enterococcus faecalis*. Minocycline and tetracycline were included in the assays as standards. Certain derivatives were selected for further evaluation *in vivo* on the basis of their *in vitro* activities, and these results will be discussed in a separate report.[9]

Results and Discussion

As shown in Table 1, the 9-amino derivatives **4–7** are active against both *S. aureus* containing *tet* M (ribosomal protection) and *tet* K (efflux) determinants but are less active against resistant Gram-negative bacteria. Com-

pounds 4–6 were also found to be oxidatively unstable in the standard *in vitro* testing condition (pH 7 or higher); therefore, cysteine was added to the test media to stabilize these compounds. While the formamido compounds 9 and 10 show improved activity against *S. aureus* (*tet* M), they show no improvement in activity against resistant Gram-negative bacteria. All the acetamido compounds 12–15 are less active as compared to the formamido compounds 8–11.

Attachment of a glycine unit to 5 (compound 16) enhances activity against *E. coli* containing the *tet* B (efflux) determinant. On the basis of this finding, the glycyl unit was further modified to give compounds 17–20. These compounds (the glycylcyclines), especially 17 and 18, are active against tetracycline susceptible Gram-positive and -negative bacteria. Notably, they are active against resistant strains of *E. coli* and *S. aureus* containing *tet* M (ribosomal protection) determinants and *E. coli* containing *tet* A, *tet* B, *tet* C, and *tet* D (efflux) determinants. In conclusion, the glycylcyclines described in this report represent a new generation of tetracyclines with potent broad-spectrum antibacterial activities. Their activity against methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) renders them as potential therapeutic agents.[9]

Experimental Section

Unless otherwise stated, the following are implied. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a QE 300 spectrometer with chemical shifts in parts per million (ppm) reported with tetramethylsilane (Me_4Si) as the internal standard, and only the key signals are listed. Infrared spectra (IR) were recorded on a Nicolet FT 7000 spectrometer. Mass spectra were determined on a VG ZAB-SE high-performance mass spectrometer, using 3-nitrobenzyl alcohol as the matrix. High-performance liquid chromatography (HPLC) was carried out on a Waters 4000 liquid chromatograph fitted with a 5-μm PRP-1 poly(styrene–divinylbenzene) column using a mixture of H_2O/CH_3CN with Et_3N·HOAc as the buffer, and the flow rate was 0.75 mL/min. Due to the observed properties (hygroscopic, oxidative, and epimerizable, etc.) of this class of compounds, most purified compounds did not furnish satisfactory elemental analyses, and similar observations were also reported by other investigators.[8] Most of the new compounds in this report were characterized by 300 MHz ^{1}H NMR and high-resolution FAB mass spectroscopy. Purities were checked by reverse-phase HPLC.

[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-1,4,4a,5,5a,6,11,-12a-octahydro-3,10,12,12a-tetrahydroxy-9-nitro-1,11-dioxo-2-naphthacenecarboxamide (22). To an ice-cold solution of minocycline monohydrochloride (20.25 g, 41.07 mmol) in 180 mL of concentrated sulfuric acid was added potassium nitrate (4.92 g, 48.7 mmol). The reaction mixture was stirred at 0 °C for about 1.5 h (or followed by HPLC) and poured slowly into 1500 mL of ice-cold ether. The yellow solid was filtered, washed several times with ether, and dried under vacuum at 40 °C for 24 h. Yield of 22 as disulfate salt was 26.67 g (93%). The product was used in the next reaction without further purification.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.55 (m, 1H), 2.25 (m, 1H), 4.3 (s, 1H), 8.0 (s, 1H), 9.05 (s, 1H), 9.55 (s, 1H), 9.95 (b s, 1H). MS (FAB): m/z 503 (M + H).

[4*S*-(4α,12aα)]-9-Amino-4,7-bis(dimethylamino)-1,4,4a,5,-5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (5). A mixture of the disulfate salt of 22 (10 g, 14.3 mmol), 2 g of 10% palladium on charcoal, and 30 mL of 2 N sulfuric acid in 40 mL of 2-methoxyethanol was hydrogenated in a Parr apparatus at 40 psi for 1.5 h. The catalyst was filtered and the filtrate added dropwise to a mixture of 2-propanol (700 mL) and ether (500 mL). The yellow solid was filtered, washed several times with ether, and dried under vacuum at 40 °C. Yield of disulfate salt of 5 was 9.2 g (96%). The crude product was dissolved in water, the pH was adjusted to ~4.5 by adding dilute NH_4OH, and the solution was extracted with chloroform. The solvent was removed and the residue dissolved in a minimum amount of methanol and added dropwise to 500 mL of ether; 1 equiv of 1.0 M hydrogen chloride in ether was added, and the solid was filtered to give 4.4 g (61%) of 5 as monohydrochloride salt.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.45 (m, 1H), 2.0–2.5 (m, 2H), 2.5 (s, 6H), 2.8 (s, 6H), 2.9 (m, 1H), 3.15 (m, 1H), 4.2 (s, 1H), 6.9 (s, 1H), 9.05 (s, 1H), 9.45 (s, 1H), 11.4 (b s, 1H). MS (FAB): m/z 473 (M + H). Anal. ($C_{23}H_{28}N_4O_7$·HCl) C, H, N, Cl (corrected for 7.3% water).

[4*S*-(4α,12aα)]-7-(Diethylamino)-4-(dimethylamino)-1,4,-4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-9-nitro-1,11-dioxo-2-naphthacenecarboxamide (23). To an ice-cold solution of the disulfate salt of 21[11] (1.0 g, 1.47 mmol) in 10 mL of concentrated sulfuric acid was added 1.2 mL of 10% nitric acid in sulfuric acid. The reaction mixture was stirred at 0 °C for about 2 h (or followed by HPLC) and poured into 400 mL of ice-cold ether. The yellow solid was filtered, washed several times with ether, and dried under vacuum at 45 °C for 24 h. Yield of 23 as disulfate salt was 1.045 g (98%). This compound was used in the next reaction without purification.

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, 6H), 1.5 (m, 1H), 4.25 (s, 1H), 8.2 (b s, 1H), 9.1 (s, 1H), 9.6 (s, 1H), 9.95 (b s, 1H). MS (FAB): m/z 531 (M + H).

[4*S*-(4α,12aα)]-9-Amino-7-(diethylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (6). A mixture of the disulfate salt of 23 (0.82 g, 1.13 mmol), 0.35 g of 10% palladium on charcoal, and 10 mL of 2 N sulfuric acid in 40 mL of 2-methoxyethanol was hydrogenated in a Parr apparatus at 40 psi for 1.5 h. The catalyst was filtered and the filtrate added dropwise to a mixture of 2-propanol (200 mL) and ether (100 mL). The yellow solid was filtered, washed several times with ether and dried under vacuum at 40 °C. Yield of 6 as disulfate salt was 0.68 g (86%). The disulfate salt of 6 was converted to its dihydrochloride salt for *in vitro* testing, using the same method as described in the preparation of 5.

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (m, 6H), 1.5 (m, 1H), 4.3 (s, 1H), 7.25 (s, 1H), 9.05 (s, 1H), 9.55 (s, 1H). MS (FAB): m/z 501 (M + H). HRMS (FAB) calcd for $C_{25}H_{32}N_4O_7$: 501.2349 (M + H). Found: 501.2358 (M + H).

[4*S*-(4α,12aα)]-4-(Dimethylamino)-9-(formylamino)-1,4,-4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-7-iodo-1,11-dioxo-2-naphthacenecarboxamide (11). To an ice-cold solution of the monosulfate salt of 8 (3.33 g, 6.0 mmol) in 50 mL of concentrated sulfuric acid was added *N*-iodosuccinimide (1.48 g, 6.5 mmol), and the reaction was stirred at 0 °C for 1 h. The mixture was then poured slowly into 500 mL of cold ether, and the yellow solid was filtered, washed several times with ether, and dried in vacuum at 40 °C for 24 h. Yield of 11 as monosulfate salt was 3.89 g (95%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.5 (m, 1H), 2.2 (m, 1H), 2.4 (m, 1H), 4.25 (s, 1H), 8.3 (s, 1H), 8.8 (s, 1H), 9.1 (s, 1H), 9.6 (s, 1H), 10.0 (s, 1H), 12.4 (s, 1H). MS (FAB): m/z 584 (M + H). HRMS (FAB) calcd for $C_{22}H_{22}N_3IO_8$: 584.053 (M + H). Found: 584.0541 (M + H).

[4*S*-(4α,12aα)]-9-Amino-4-(dimethylamino)-1,4,4a,5,5a,6,-11,12a-octahydro-3,10,12,12a-tetrahydroxy-7-iodo-1,11-dioxo-2-naphthacenecarboxamide (7). A mixture of the monosulfate salt of 11 (1.5 g, 2.2 mmol), 5 mL of 2 N sulfuric acid, and 50 mL of methanol was stirred at ambient temperature for 4 h. The solvent was reduced to about half of the original volume and the residue triturated with 2-propanol and ether. The resulting solid was filtered, washed several times with ether, and dried under vacuum for 24 h. Yield of 7 as monosulfate salt was 1.33 g (93%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.5 (m, 1H), 2.2–2.4 (m, 2H), 4.25 (s, 1H), 7.6 (s, 1H), 9.05 (s, 1H), 9.55 (s, 1H), 9.9 (s, 1H), 12.1 (b s, 1H). MS (FAB): m/z 556 (M + H). HRMS (FAB) calcd for $C_{21}H_{22}N_3IO_7$: 556.0581 (M + H). Found: 556.0575 (M + H).

General Procedure for Formylation. To an ice-cold solution of the 9-amino compound in 90% formic acid was added excess acetic anhydride. The mixture was then stirred at 0 °C for 5 min and then at ambient temperature for 1 h. It was poured into cold ether, and the solid was filtered, washed several times with ether, and dried under vacuum. The crude material could be purified by dissolving the compound in water, adjusting the pH to *ca.* 4.5–6.5 with dilute ammonium hydroxide solution, and extracting

with either methylene chloride or chloroform. The solvent was removed *in vacuo*, the residue was triturated with ether and hexanes, and the solid was collected. The neutral compound was converted to its salt form by dissolving the compound in a minimum amount of chloroform and methanol, diluting with ether, and adding the appropriate concentrated acid.

[4*S*-(4α,12aα)]-4-(Dimethylamino)-9-(formylamino)-1,4,-4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (8).[10] Compound 8 was prepared according to the general procedure from the monosulfate salt of 4[10] (5 g, 9.49 mmol), 12 mL of acetic anhydride, and 80 mL of 90% formic acid. Yield of 8 as monosulfate salt was 4.51 g (86%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.5 (m, 1H), 2.15 (m, 1H), 4.27 (s, 1H), 6.8 (d, 1H), 8.25 (d, 1H), 8.35 (s, 1H), 9.05 (s, 1H), 9.55 (s, 1H), 9.8 (s, 1H), 9.95 (b s, 1H), 12.05 (b s, 1H). MS (FAB): *m/z* 458 (M + H).

[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-9-(formylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (9). Compound 9 was prepared according to the general procedure from the monohydrochloride of 5 (0.254 g, 0.5 mmol), 0.5 mL of acetic anhydride, and 5 mL of 90% formic acid. The product was purified according to the extraction method described in the general procedure; yield of 9 as dihydrochloride salt was 0.143 g (50%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.5 (m, 1H), 2.15–2.3 (m, 2H), 2.85 (s, 6H), 2.95 (s, 6H), 4.3 (s, 1H), 8.35 (s, 1H), 8.65 (d, 1H), 9.05 (s, 1H), 9.55 (s, 1H), 10.2 (s, 1H), 10.55 (b s, 1H), 12.3 (b s, 1H). MS (FAB): *m/z* 501 (M + H). Anal. ($C_{24}H_{28}N_4O_8$) C, H, N (corrected for 2.6% water).

[4*S*-(4α,12aα)]-7-(Diethylamino)-4-(dimethylamino)-9-(formylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (10). The title compound was prepared according to General Procedure for Formylation from the disulfate of 6 (0.238 g, 0.34 mmol), sodium acetate (0.036 g, 0.51 mmol), 0.75 mL of acetic anhydride, and 6 mL of 90% formic acid. Yield of 10 as disulfate salt was 0.2 g (81%). The disulfate was converted to its neutral compound by the extraction method described in the general procedure to give 0.11 g (61%) of product.

^{1}H NMR (300 MHz, DMSO-d_6) of the disulfate salt of 10: δ 0.95 (m, 6H), 1.5 (m, 1H), 2.15–2.4 (m, 2H), 2.85 (s, 6H), 4.28 (s, 1H), 8.4 (s, 1H), 8.5 (s, 1H), 9.1 (s, 1H), 9.6 (s, 1H), 10.15 (b s, 1H), 12.4 (b s, 1H). MS (FAB): *m/z* 529 (M + H). HRMS (FAB) calcd for $C_{26}H_{32}N_4O_8$: 529.2298 (M + H). Found: 529.2296 (M + H).

^{1}H NMR (300 MHz, DMSO-d_6) of the neutral compound: δ 0.85 (t, 6H), 1.5 (m, 1H), 2.1–2.3 (m, 2H), 8.3 (s, 1H), 8.32 (s, 1H), 9.15 (b s, 2H), 9.8 (s, 1H), 12.4 (s, 1H).

General Procedure for Acylation. To a solution of the 9-amino compound in a (1:5) mixture of acetonitrile and *N,N*-dimethylpropyleneurea (DMPU) was added excess anhydrous sodium carbonate. The reaction was stirred at ambient temperature for 5 min, and the acylating agent was added. Stirring was continued for an additional 30 min, a few milliliters of methanol were added, and the solid was filtered off. The filtrate was added dropwise to a mixture of 2-propanol and ether (1:10); concentrated hydrochloric acid or hydrogen chloride (1.0 M in ether) was added until a yellow solid formed. It was then filtered, washed several times with ether, and dried under vacuum at *ca.* 40 °C. If necessary, the compound was purified by dissolving in water, the pH was adjusted by adding dilute ammonium hydroxide to *ca.* 4.5–7.0 and the mixture was extracted with either chloroform or methylene chloride. The solvent was removed, the residue was triturated with a mixture of ether and hexanes, and the solid was collected. The isolated neutral compound was then converted to its salt form by adding the appropriate acid.

[4*S*-(4α,12aα)]-9-(Acetylamino)-4-(dimethylamino)-1,4,-4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (12).[10] The title compound was prepared according to the general acylation procedure from the monosulfate of 4 (0.23 g, 0.44 mmol), acetyl chloride (0.078 g, 1 mmol), 2 mL of acetonitrile, and 8 mL of DMPU. The reaction time was 30 min, and yield of 12 as monosulfate salt was 0.24 g (96%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.48 (m, 1H), 2.1 (s, 3H), 2.15–2.3 (m, 2H), 2.8 (b s, 6H), 4.3 (s, 1H), 6.8 (d, 1H), 8.0 (d, 1H), 9.05 (s, 1H), 9.38 (s, 1H), 9.55 (s, 1H), 10.4 (b s, 1H), 12.0 (s, 1H). MS (FAB): *m/z* 472 (M + H).

[4*S*-(4α,12aα)]-9-(Acetylamino)-4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (13). The title compound was prepared according to General Procedure for Acylation from the disulfate of 5 (0.334, 0.5 mmol), 0.330 g of sodium carbonate, acetyl chloride (0.078 g, 1 mmol), 2 mL of acetonitrile, and 8 mL of DMPU. The product was purified by the extraction method described above to give 0.142 g (48%) of 13 as dihydrochloride salt.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.5 (m, 1H), 2.15 (s, 3H), 2.2–2.45 (m, 2H), 2.85 (s, 6H), 2.95 (s, 6H), 4.4 (s, 1H), 8.45 (s, 1H), 9.1 (s, 1H), 9.6 (s, 1H), 9.7 (s, 1H), 10.55 (b s, 1H), 12.3 (s, 1H). MS (FAB): *m/z* 515 (M + H). HRMS (FAB) calcd for $C_{25}H_{30}N_4O_8$: 515.2142 (M + H). Found: 515.2150 (M + H).

[4*S*-(4α,12aα)]-9-(Acetylamino)-7-(diethylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (14). The title compound was prepared according to General Procedure for Acylation from the disulfate salt of 6 (0.139 g, 0.2 mmol), 0.14 g of sodium carbonate, acetyl chloride (0.031 g, 0.4 mmol), 1 mL of acetonitrile, and 4 mL of DMPU. The crude product was purified by the the method described above to give 0.082 g (67%) of 14 as dihydrochloride salt.

^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, 6H), 1.5 (m, 1H), 2.15 (s, 3H), 2.2–2.45 (m, 2H), 2.85 (s, 6H), 4.3 (s, 1H), 8.35 (s, 1H), 9.05 (s, 1H), 9.55 (s, 1H), 9.78 (b s, 1H), 10.4 (b s, 1H), 11.45 (b s, 1H), 12.6 (b s, 1H). MS (FAB): *m/z* 543 (M + H). HRMS (FAB) calcd for $C_{27}H_{34}N_4O_8$: 543.2455 (M + H). Found: 543.2441 (M + H).

[4*S*-(4α,12aα)]-4-(Dimethylamino)-9-(acetylamino)-1,4,-4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-7-iodo-1,11-dioxo-2-naphthacenecarboxamide (15). To an ice-cold solution of the monosulfate of 12 (0.143 g, 0.25 mmol) in 10 mL of concentrated sulfuric acid was added *N*-iodosuccinimide (0.056 g, 0.25 mmol), the reaction was stirred at 0 °C for 30 min, and work up was the same as described in the preparation of 11. Yield of 15 as monosulfate salt was 0.13 g (75%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.5 (m, 1H), 2.12 (s, 3H), 2.15–2.45 (m, 2H), 2.85 (s, 6H), 4.25 (s, 1H), 8.6 (s, 1H), 9.05 (s, 1H), 9.5 (s, 1H), 9.58 (s, 1H), 9.95 (b s, 1H), 12.4 (s, 1H). MS (FAB): *m/z* 598 (M + H). HRMS (FAB) calcd for $C_{23}H_{34}N_3IO_8$: 598.0686 (M + H). Found: 598.0681 (M + H).

[4*S*-(4α,12aα)]-9-[(Aminoacetyl)amino)-4,7-bis(dimethylamino]-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (16). To a solution of the monosulfate salt of 5 (0.85 g, 1.27 mmol) in a mixture of H_2O and THF (5 mL:25 mL) was added 0.68 g of sodium acetate. The mixture was stirred for 5 min, and *N*-*t*-Boc-glycine *N*-hydroxysuccinimide ester (0.359 g, 1.32 mmol) was added; stirring was maintained for an additional 2 h, and the mixture was extracted with chloroform. The combined chloroform extracts were dried over sodium sulfate, and the solvent was removed to give 0.5 g of crude compound 24. A 240-mg sample of the crude compound was purified by reverse-phase HPLC using acetonitrile and water (1:1) to give 65 mg of 24 [MS (FAB): *m/z* 630 (M + H)]. The pure 24 obtained was treated with 2 mL of trifluoroacetic acid at ambient temperature for 5 h and poured into 10 mL of ether, and the solid was collected to give 65 mg of 16 monotrifluoroacetate salt.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.5 (m, 1H), 2.15–2.4 (m, 2H), 3.85 (b s, 2H), 4.25 (s, 1H), 8.12 (s, 1H), 8.15 (b s, 2H), 9.05 (s, 1H), 9.55 (s, 1H), 10.0 (s, 1H), 11.8 (s, 1H). MS (FAB): *m/z* 530 (M + H).

***N,N*-Dimethylglycyl Chloride Hydrochloride.**[18] A mixture of *N,N*-dimethylglycine hydrochloride (pulverized and dried in a vacuum oven at 45–50 °C for 24 h) (15 g, 107 mmol) and thionyl chloride (19.02 g, 161 mmol) was heated slowly to *ca.* 78 °C and kept at this temperature for 0.5 h (gas evolution observed) or until crystallization occurred. Toluene was added to the mixture and the yellow liquid (excess $SOCl_2$) pipetted out; this process was repeated several times. The pale solid was then transferred to a Buchner funnel (covered with CH_2Cl_2 and ether), washed with a mixture of CH_2Cl_2 and ether, and dried at 50 °C under vacuum for 24 h. Yield of the title compound was 14.2 g (84%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 2.85 (s, 6H), 4.15 (s, 2H). IR 1732 cm^{-1}(C=O). MS (CI): *m/z* 121 (M^+ − HCl).

[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-9-[[(dimethylamino)acetyl]amino]-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (17). The title compound 17 was prepared according to General Procedure for Acylation from the disulfate of 5 (6.68 g, 10 mmol), 6.57 g of sodium carbonate, *N,N*-dimethylglycyl chloride hydrochloride (3.14 g, 20 mmol), 40 mL of acetonitrile, and 120 mL of DMPU. The crude product (5.75 g) was purified using the extraction method described above to give 4.8 g (76%) of 17 as dihydrochloride salt.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.5 (m, 1H), 2.15–2.4 (m, 2H), 2.7 (b s, 6H), 2.85 (s, 12H), 4.22 (s, 2H), 4.35 (s, 1H), 8.2 (s, 1H), 9.1 (s, 1H), 9.6 (s, 1H), 9.95 (b s, 1H), 10.25 (b s, 1H), 10.3 (s, 1H), 12.0 (b s, 1H). MS (FAB): *m/z* 558 (M + H). Anal. ($C_{27}H_{35}N_5O_9$·HCl) C, H, N, Cl (corrected for 3.1% water).

[4*S*-(4α,12aα)]-4-(Dimethylamino)-9-[[(dimethylamino)acetyl]amino]-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (18). The title compound was prepared according to General Method for Acylation from the monosulfate of 4 [10] (0.527 g, 1 mmol), 0.5 g of sodium carbonate, *N,N*-dimethylglycyl chloride hydrochloride (0.314 g, 2 mmol), 5 mL of acetonitrile, and 20 mL of DMPU. The crude product was purified using the extraction method described above to give 0.42 g (72%) of 18 as dihydrochloride salt.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.45 (m, 1H), 2.05–2.25 (m, 2H), 2.85 (s, 12H), 4.2 (b s, 2H), 4.3 (s, 1H), 6.8 (d, 1H), 8.0 (d, 1H), 9.05 (s, 1H), 9.55 (s, 1H), 10.2 (s, 1H), 12.1 (s, 1H). MS (FAB): *m/z* 515 (M + H). Anal. ($C_{25}H_{30}N_4O_8$·HCl) C, H, N, Cl (corrected for 1.1% water).

[4*S*-(4α,12aα)]-7-(Diethylamino)-4-(dimethylamino)-9-[[(dimethylamino)acetyl]amino]-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (19). The title compound was prepared according to General Procedure for Acylation from the disulfate salt of 6 (0.1392 g, 0.2 mmol), 0.14 g of sodium carbonate, *N,N*-dimethylglycyl chloride hydrochloride (0.063 g, 0.4 mmol), 1 mL of acetonitrile, and 4 mL of DMPU. The crude product was purified by the extraction method described in the general procedure to give 0.1 g (76%) of 19 as dihydrochloride salt.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.0 (m, 6H), 1.5 (m, 1H), 2.15–2.4 (m, 2H), 2.9 (s, 12H), 4.25 (s, 2H), 4.38 (s, 1H), 8.3 (b s, 1H), 9.1 (s, 1H), 9.6 (s, 1H), 10.4 (b s, 1H), 10.6 (b s, 1H), 12.6 (b s, 1H). MS (FAB): *m/z* 586 (M + H). HRMS (FAB) calcd for $C_{23}H_{39}N_5O_8$: 586.2877 (M + H). Found: 586.2883 (M + H).

[4*S*-(4α,12aα)]-4-(Dimethylamino)-9-[[(dimethylamino)acetyl]amino]-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-7-iodo-1,11-dioxo-2-naphthacenecarboxamide (20). The title compound was prepared according to General Procedure for Acylation from the monosulfate salt of 7 (0.163 g, 0.25 mmol), 0.16 g of sodium carbonate, *N,N*-dimethylglycyl chloride hydrochloride (0.078 g, 0.5 mmol), 1 mL of acetonitrile, and 5 mL of DMPU. The crude product was purified by the extraction method described in the general procedure to give 0.157 g (88%) of 20 as dihydrochloride salt.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.5 (m, 1H), 2.15–2.4 (m, 2H), 2.85 (s, 12H), 4.2 (s, 2H), 4.32 (s, 1H), 8.58 (s, 1H), 9.05 (s, 1H), 9.6 (s, 1H), 10.22 (b s, 1H), 10.35 (s, 1H), 10.55 (b s, 1H), 12.4 (b s, 1H). MS (FAB): *m/z* 641 (M + H). HRMS (FAB) calcd for $C_{25}H_{29}N_4IO_8$: 641.1108 (M + H). Found: 641.1110 (M + H).

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