Articles

"Glycylcyclines". 3. 9-Aminodoxycyclinecarboxamides

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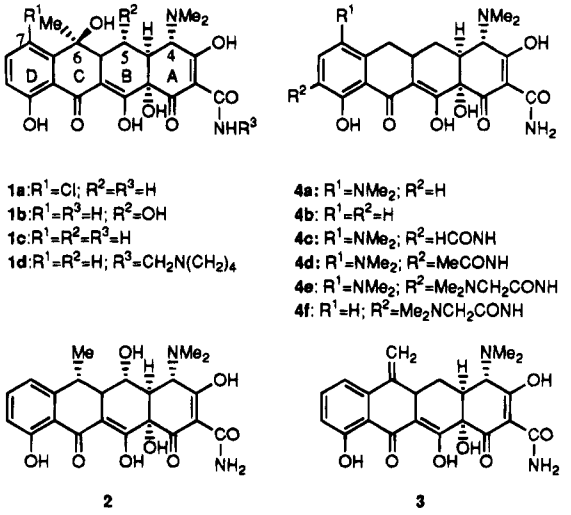
A series of 9-(acylamino)doxycycline derivatives has been prepared. These analogs exhibit good activity against both tetracycline sensitive and tetracycline resistant Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria that are encoded with the efflux and ribosomal resistance gene factors. *N,N*-Dialkylglycylamido derivatives possessed the highest activity. Replacment of glycine moiety with other amino acids did not further enhance the activity.

The isolation and identification of chlortetracycline[1] (**1a**) in 1948 followed shortly thereafter by oxytetracycline[2] (**1b**) accelerated the ongoing revolution in the treatment of infectious diseases. These new "broad spectrum" antibiotics exhibited clinically useful oral activity against a wider spectrum of pathogens than the penicillins, and they were quickly adopted by clinicians. Unlike the discovery of the β-lactam antibiotics, which stimulated the synthesis and isolation of a large number of natural and semisynthetic analogs with varying antibacterial spectra and pharmacokinetic properties, few novel tetracyclines have emerged over the following decades despite valiant efforts by many research groups. The chemical complexity and dense functionality embedded in the tetracyclic nucleus have limited the search for novel analogs. Among the natural and synthetic analogs that have reached the clinic are tetracycline[3] (**1c**), pyrrolidinomethyltetracycline (**1d**), doxycycline (**2**), methacycline (**3**), and minocycline[7] (**4a**). The latter compound was the last novel tetracycline to be introduced (early 1970s) (Scheme 1).

The widespread usage of these compounds, however, has resulted in a significant increase of tetracycline-resistant organisms which has severely limited the utility of these valuable compounds. The existence of multiple plasmid- and transposon-mediated pathways for tetracycline resistance has made the problem of managing antibiotic usage even more complex. Two distinct pathways for resistance have been identified: (a) energy-dependent efflux pumps that limit the intracellular concentration of the antibiotic in both Gram-positive and Gram-negative organisms and (b) proteinaceous ribosomal protection factors that decrease the sensitivity of protein biosynthetic pathways to the antibiotic.[8,9]

The therapeutic potential for a broad spectrum tetracycline with activity against resistant organisms prompted the initiation of a synthetic program to explore new tetracycline derivatives. Previous studies have established that the β-dicarbonyl systems in the A and B/C rings, the 4α-dimethylamino moiety, the

Scheme 1



D-ring phenol and the carboxamide collectively are essential for maximum spectrum and potency. On the other hand, modification of the 5 and 6 positions has produced most of the clinically useful derivatives. Conventional wisdom suggested that electrophilic substitution of the D ring also was permissible, preferably at the 7 position to avoid perturbing the essential phenol.[10,11]

Recently a team at Lederle Laboratories found simple 9-(acylamido)minocycline derivatives, e.g., **4c** and **4d**, exhibited typical tetracycline bioactivity although they were less potent against tet-resistant organisms. However, when the acyl group was modified to include a *N,N*-dialkylamine moiety, good activity was noted against both tetracycline sensitive *and tetracycline resistant Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria that are encoded with the efflux and ribosomal resistance genes factor.* Maximal activity in both the minocycline (**4a**) and DMDOT (**4b**) series occurred with the 9-(*N,N*-dimethylglycinamido) substituent, **4e** and **4f**, respectively.[12,13] Other *N,N*-dialkyl substitution, along with *N-tert*-butylamides, also were effective. We will describe our studies with the doxycycline series along with our efforts on the synthesis of other novel 9-(acylamino)tetracycline derivatives.

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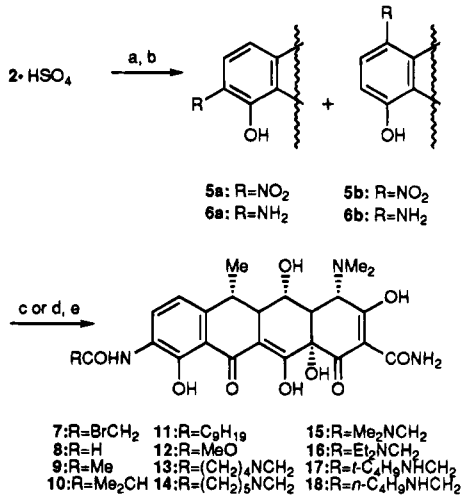
Scheme 2[a]



[a] (a) $NaNO_3$, H_2SO_4; (b) H_2, 10% Pd/C; (c) RCOCl; (d) $BrCH_2COBr$; (e) R_1R_2NH.

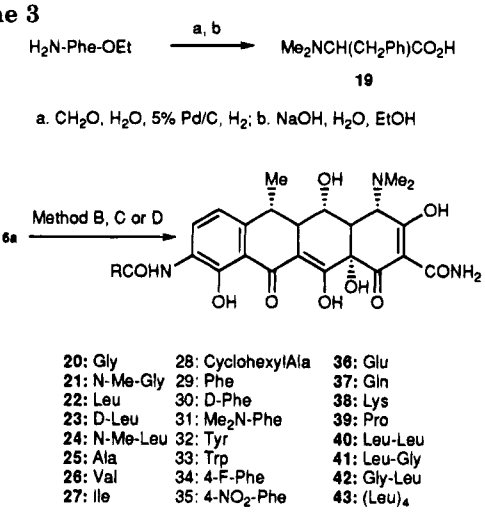
Chemistry

The preparation of 9-aminodoxycycline **6a** was accomplished by well-established procedures.[14] As reported, the 6α-methyl substituent suppresses nitration at C-7. Nitration of the hydrosulfate salt of **2** with $NaNO_3$ and H_2SO_4 produced a 5:1 mixture of **5a** and **5b**, respectively (Scheme 2). The initial conversion of the hydrochloride salt to the hydrosulfate salt was required to prevent side reactions during the nitration. Separation of the two isomers generally was not performed at this stage since the two regioisomers could be separated more easily at the end of the reaction sequence. Catalytic reduction in the presence of 10% Pd/C and MeOH produced a mixture of regioisomers consisting of 90% of **6a** by proton NMR analysis. Condensation of **6a** with bromoacetyl bromide produced **7**, the precursor to N-substituted glycinamide analogs **13**–**18.** The latter were prepared by reacting **7** with an excess of the appropriate amine. The simple amides **8**–**11** and the carbamate **12** also were prepared by direct acylation of **6a**.

Amino acids and peptides were coupled to **6a** using standard peptide protocols (Scheme 3).[15] Most Boc- and Fmoc-protected starting materials were commercially available. Fmoc-protected amino acids were used for all compounds except **20**, **21**, **24**, **29**, **34**, and **35**, which were prepared from the corresponding *N*-Boc-protected amino acids. Three dipeptides, Leu-Leu, Leu-Gly, and Gly-Leu, were N-protected with (Fmoc)-O-succinimide prior to coupling with **6a**. Tetraleucine was prepared on a Wang resin using standard methodology. The terminal amine was protected with (Fmoc)-O-succinimide and cleaved from the resin with TFA. *N,N*-Dimethyl-L-phenylalanine **19** was prepared by reductive alkylation of H-Phe-OEt and subsequent saponification (Scheme 3).[16]

Symmetrical anhydrides were prepared by treating the protected amino acid with dicyclohexylcarbodiimide (DCC) and purified prior to condensation with **6a**. In most cases the reaction proceeded smoothly in DMF. *N,N*-Dimethyl-Phe-OH (**19**) was insoluble in organic solvents so preparation of **31** was carried out in aqueous solution with 1-[3-(diethylamino)propyl]-3-ethylcarbodiimide in place of DCC. The Boc and Fmoc protecting

Scheme 3



groups were equally effective for protection of the α-amino substituent. Side chain functionality was protected as *tert*-butyl ethers or esters, Boc carbamates or trityl groups, as noted in the Experimental Section. Piperidine/*N*-methylpyrrolidin-2-one (1:3) was used to cleave the Fmoc moiety after coupling with **6a**. Side chain *tert*-butyl esters (for Glu), ethers (for Tyr), Boc (for Lys), and α-amino Boc moieties were removed by brief treatment with TFA/anisole (95:5). Detritylation of the Gln side chain amide occurred spontaneously during the workup.

Symmetrical anhydrides from hindered β-branched amino acids, Ile and Val, did not react with **6a**. In these cases, the hydroxybenzotriazole (HOBT) esters, prepared from the amino acid, BOP, and HOBT, produced the desired amide, although substantially longer reaction times were required.

Biology

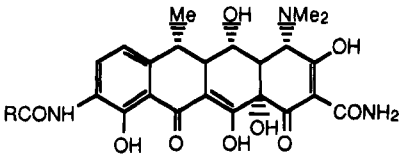
The *in vitro* antibacterial activity is reported as the minumum inhibitory concentration (MIC), the lowest concentration of the analog which inhibits growth of the test organism. It was determined by the agar dilution method using Muller-Hinton II agar (Baltimore Biological Laboratories). An inoculum density of $(1-5) \times 10^5$ CFU/mL and antibiotic concentration range of 32–0.004 μg/mL was used. The plates were incubated for 18 h at 35 °C in a forced air incubator.

The test organisms comprised strains which were both sensitive and resistant to tetracycline. Gram-negative bacteria were represented by *E. coli* strains and Gram-positive bacteria by *S. aureus*. Tetracycline, minocycline, and doxycycline were included in each assay as standards.

Results and Discussion

The trends in the structure–activity relationships in the doxycycline series (Table 1) paralleled those in the minocyclines *(vide supra)*. Simple amides, 9-(formamido)- (**8**) and 9-(acetamido)doxycycline (**9**), were highly active against tet-sensitive strains but inactive against tet-resistant organisms. Primary amides with larger alkyl chains, **10** and **11**, were significantly less active. Incorporation of a nitrogen atom into the side chain, i.e., **20**, produced the first hint of limited activity against resistant organisms. Substitution of the amine with a methyl group **21** further increased the potency, a trend

Table 1. *In Vitro* Antibacterial Activity[f] of 9-(Glycylamido)tetracycline Derivatives



structure		biological activity									
		E. coli						*S. aureus*			
no.	R	UBMS 90-5 sensitive	PRP-1 tet-A[a]	UBMS 88-1 tet-B[a]	J3272 tet-C[a]	J3272 tet-D[a]	UBMS 90-4 tet-M[b]	Smith	UBMS 90-3 sensitive	UBMS 88-7 tet-K[a]	UBMS 90-1 tet-M[b]
4a[c]	–	1	4	16	2	8	>32	0.12	0.06	4	4
4e[d]	–	0.25	2	0.5	2	0.25	0.25	0.25	0.25	2	0.5
2[e]	–	1	16	32	16	>32	>32	0.12	0.12	8	8
8	H	2	>32	>32	>32	>32	>32	0.12	0.12	>32	>32
9	Me	8	>32	>32	>32	>32	32	0.5	.025	>32	>32
10	Me_2CH	>32	>32	16	>32	>32	>32	1	0.5	>32	>32
11	C_9H_{19}	>32	>32	>32	>32	>32	>32	>32	16	>32	>32
12	MeO	>32	>32	>32	>32	>32	>32	0.06	0.06	4	16
13	$(CH_2)_4NCH_2$	0.5	2	0.5	1	0.5	0.25	0.5	0.25	4	1
14	$(CH_2)_5NCH_2$	0.5	2	1	1	0.25	1	0.25	0.12	1	1
15	Me_2NCH_2	0.5	2	1	8	0.25	0.25	0.25	0.12	4	0.5
16	Et_2NCH_2	0.5	4	1	2	0.25	0.5	0.25	0.12	1	2
17	*t*-Bu-$NHCH_2$	0.5	1	1	1	0.25	0.5	0.5	0.25	2	1
18	*n*-Bu-$NHCH_2$	0.5	4	1	1	0.5	1	0.5	0.25	8	4
21	$MeNHCH_2$	8	>32	8	>32	4	4	8	4	>32	8

[a] Efflux resistance. [b] Ribosomal protection. [c] Minocycline (Scheme 2). [d] DMG–minocycline. [e] Doxycycline. [f] MIC ($\mu g/cm^2$).

which continued as the size of the alkyl group increased, e.g., **17** and **18** *vs* **21**. *N,N*-Dialkylglycinamides exhibited maximal activity, and that activity was retained by a variety of nitrogen substituents **13–16**.

The previously reported series[12] were limited to *N*-alkyl and *N,N*-dialkylglycinamides. The naturally occurring amino acids afford a structurally diverse pool of glycine replacements. Relative to Gly, a wide range of bioactivity was observed. Neutral and aromatic side chains, e.g., **22**, **27**, and **29**, were the most active (Table 2). These compounds were more active than the corresponding Gly analog. Polar amino acids, e.g., **36** and **38**, were less potent. None of the other amino acids, however, produced levels of biological activities comparable to the dialkylated glycinamides. To ascertain whether the lack of nitrogen substitution was responsible for the lower activity, two relatively active amino acids, Leu and Phe, were converted to the corresponding *N*-Me and *N,N*-diMe derivatives and coupled with **6a** to produce **24** and **31**, respectively. Neither modification enhanced the biological activity relative to its unalkylated parent. The Pro derivative **39** was similar to the *N*-Me-Leu analog **24** and to other nonpolar amino acids. The chirality of the side chain had a significant effect on the biological activity: The L-Leu isomer **22** was more active than the corresponding D-Leu derivative **23**. Less difference was noted between D- and L-Phe derivatives **30** and **29**, respectively.

Leu and D-Leu were also coupled with 9-amino-DMDOT **44a** to compare doxycycline with other tetracyclines (Scheme 4). Neither amide **44b** and **44c** was comparable with the previously tested DMG derivative **4f**.

Thus, substitution of the 9-position of doxycycline with *N,N*-dialkylamides produced tetracyclines with substantial activity against organisms resistant to classical tetracyclines. Other nonpolar amino acids were frequently comparable to the Gly analog. In general, modifications in all the tetracycline series produced compounds with improved activity against tet-resistant organisms. The comparable modification in the minocycline and DMDOT series afforded compounds superior to the present series.

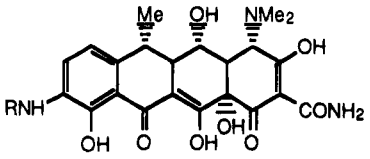
Experimental Section

^{1}H-NMR spectra were measured in CD_3OD with a Varian Associates XL300 spectrometer with characteristic chemical shifts of the parent ring system and diagnostic signals of the side chain given in Table 3. Low resolution mass spectrometry analyses were obtained using a Finnigan MAT TSQ 700 mass spectrometer using an electrospray ionization technique. High resolution mass spectrometry analyses were obtained using a Cyanamid designed and built Fourier transform ion cyclotron mass spectrometer based on a 7 T magnet with an external ion source. Accurate masses were determined for each compound's protonated molecular ion using a minimum resolution of 25 000 at *m/z* 500 (M/Δm at half-height). Final purification of products was accomplished by preparative HPLC using a Hamilton Co. preparative PRP-1 column and a linear gradient of water to acetonitrile. Trifluoroacetic acid (0.1%) was added to maintain acidic conditions and inhibit bacterial growth. The purity of final products was assessed by ^{1}H-NMR and analytical HPLC.

[4S-(4α,12aα)]-4-(Dimethylamino)-9-nitro-1,4,4a,5,5a,6,-11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (5a). Doxycycline hydrochloride (14.5 g, 30.1 mmol) was slowly added to concentrated H_2SO_4 (50 mL). After gas evolution had stopped, the orange solution was slowly dripped into 1 L of ether cooled in an ice bath. The hydrosulfate salt was collected by filtration, washed with ether, and dried under nitrogen. The orange powder was redissolved in H_2SO_4 (70 mL) at room temperature, and $NaNO_3$ (4.0 g, 47.0 mmol) was added over 2 min. The reaction mixture was stirred an additional 3 h and then dripped into ice-cooled, stirred ether (2 L), and the mixture was filtered. The precipitate was washed well with ether and air-dried to give an orange powder which was used without further purificaton.

[4S-(4α,12aα)]-9-Amino-4-(dimethylamino)-1,4,4a,5,5a,6,-11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (6a). Crude **5a** was dissolved in a small amount of methanol and filtered into a 500 mL Paar hydrogenation bottle. The solution was diluted

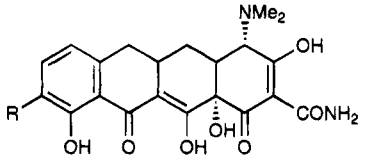
Table 2. *In Vitro* Antibacterial Activity[h] of 9-(Glycylamido)tetracycline Derivatives



structure		*E. coli*						*S. aureus*			
no.	R	UBMS 90-5 sensitive	PRP-1 tet-A[a]	UBMS 88-1 tet-B[a]	J3272 tet-C[a]	J3272 tet-D[a]	UBMS 90-4 tet-M[b]	Smith	UBMS 90-3 sensitive	UBMS 88-7 tet-K[a]	UBMS 90-1 tet-M[b]
2[c]		1	16	32	16	>32	>32	0.12	0.12	8	8
4b[d]		2	8	16	4	16	16	0.25	0.25	4	2
4f[e]		0.25	2	1	2	0.25	0.25	0.12	0.12	1	0.25
20	Gly	8	>32	16	>32	8	8	8	4	>32	16
22	Leu	1	16	2	4	1	1	0.5	0.5	2	1
23	D-Leu	8	>32	8	16	4	4	2	2	16	>32
24	N-Me-Leu	4	16	8	8	2	4	2	1	2	2
39	Pro	2	16	4	8	1	2	2	1	4	4
25	Ala	8	>32	16	>32	8	8	2	2	>32	8
26	Val	2	32	4	8	1	2	1	0.5	8	2
27	Ile	1	16	2	4	0.5	1	1	0.25	2	1
28	Cha	4	8	8	8	2	4	1	0.5	2	8
29	Phe	4	8	4	4	1	2	0.25	0.5	4	1
30	D-Phe	4	16	4	4	1	2	0.5	1	16	8
31	Me_2N-Phe	32	16	32	32	16	16	4	1	2	2
32	Tyr	8	32	16	16	8	8	1	0.5	32	4
33	Trp	8	16	8	32	4	4	1	2	16	4
34	4-F-Phe	8	>32	8	32	4	8	1	0.5	16	8
35	4-NO_2-Phe	16	>32	32	>32	8	8	1	0.5	32	4
36	Glu	>32	>32	>32	>32	>32	>32	>32	32	>32	>32
37	Gln	16	>32	>32	>32	>32	>32	4	1	32	>32
38	Lys	>32	>32	>32	>32	>32	>32	>32	>32	16	>32
40	$(Leu)_2$	>32	>32	>32	>32	>32	>32	8	4	32	4
41	Leu-Gly	>32	>32	>32	>32	>32	>32	16	>32	>32	>32
42	Gly-Leu	>32	>32	>32	>32	>32	>32	16	>32	>32	>32
43	$(Leu)_4$	>32	>32	>32	>32	>32	>32	16	32	>32	>32
44b[f]		2	4	4	4	1	2	2	1	1	1
44c[g]		8	16	8	16	8	8	8	8	16	16

[a] Efflux resistance. [b] Ribosomal resistance. [c] Doxycycline. [d] DMDOT (Scheme 2). [e] DMG–DMDOT. [f] L-Leu-DMDOT. [g] D-Leu-DMDOT. [h] MIC ($\mu g/cm^2$).

Scheme 4



44a: R=NH_2
44b: R=Leu-NH
44c: R=D-Leu-NH

to 200 mL with methanol, 10% Pd/C (1.0 g) was added, the system was charged with 50 psi of H_2, and the bottle was shaken for 2 h. After filtration of the catalyst, the solution was diluted to 300 mL with methanol and rapidly dripped into stirred ether (4 L) to give a light tan powder (16.4 g). Portions were purified by preparative HPLC as needed. ^1H NMR analysis showed the ratio of 9-amino:7-amino derivative to be about 9:1. The remaining 9-aminated material was removed by careful HPLC purification of all final products.

[4S-(4α,12aα)]-9-[(Bromoacetyl)amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (7). 9-Aminodoxycycline **6a** (3.43 g, 7.47 mmol) was mixed in *N*-methylpyrrolidin-2-one (40 mL) with $NaHCO_3$ (2.0 g, 23.8 mmol) at room temperature. Bromoacetyl bromide (0.75 mL, 8.62 mmol) was added, and the solution was stirred for 30 min. More acid bromide (0.4 mL, 4.6 mmol) and $NaHCO_3$ (1.0 g, 11.9 mmol) were added, and stirring was continued another 2 h until no starting material remained (HPLC). The reaction mixture was then filtered into stirred ether (1.5 L) and the crude product (4.04 g, yellow powder) collected by filtration. The sample was purified by HPLC.

Method A: General Procedure for Preparing (Fmoc)-Protected Peptides. The preparation of (Fmoc)-Gly-L-Leu-OH is representative. The peptide H-Gly-L-Leu-OH (1.0 g, 5.3 mmol) was dissolved in a solution of $NaHCO_3$ (0.45 g, 5.36 mmol) in water (20 mL). A solution of (Fmoc)-O-succinimide (1.75 g, 5.19 mmol) in acetone (25 mL) was added. The reaction mixture was stirred at room temperature overnight and adjusted to pH 3 with 2 M aqueous HCl, and the solution was extracted with EtOAc. The organic solution was washed sequentially with water and brine and then dried over Na_2SO_4. The drying agent was filtered off, and the solvent was removed by rotary evaporation. Recrystallization from ethyl acetate/hexanes gave the product as white needles (1.78 g, 82%), mp 133–135 °C.

(Fmoc)-Leu-Leu-OH (method A): white foam.

(Fmoc)-Leu-Gly-OH (method A): mp 157–158 °C.

(Fmoc)-Gly-Leu-OH (method A): mp 133–135 °C.

(Fmoc)-Leu-Leu-Leu-Leu-OH. Fmoc-Leu-Wang resin (5.0 g, 0.85 mmol Leu/g) was placed in a 300 mL Buchner funnel with a coarse glass frit which had been clamped to a 1-L sidearmed flask. The resin was covered with dimethylformamide (DMF, 30 mL), and nitrogen was bubbled through the solution from below by means of a nitrogen inlet connected to the sidearm. After 2 min the DMF was removed by vacuum filtration and replaced with CH_2Cl_2 (30 mL) and nitrogen again bubbled through the solution for 2 min. The CH_2Cl_2 was filtered off, and the resin was treated in succession with the following reagents in the amounts and for the times specified in parentheses, each time removing the previous solution by vacuum filtration and using a nitrogen flow to agitate the mixture: (1) piperidine/DMF (3:7, 30 mL, 5 min); (2) DMF (2 × 30 mL, 1 min); (3) piperidine/DMF (3:7, 30 mL, 5 min); (4) DMF (3 × 30 mL, 2 min); (5) a mixture containing HOBT (3.90

Table 3. Spectral Data for Selected Compounds

	representative ^{1}H NMR shifts							mass spectra		
no.	C(8)H	C(7)H	C(6)Me			side chain		formula (M + H)	calcd	found
5a	8.04	7.01	1.53	3.52	4.35	–	–			–
6a	7.50	7.04	1.52	3.54	4.40	–	–	$C_{22}H_{26}N_3O_8$		460
7	8.21	6.92	1.50	3.52	4.37	4.09 s	–	$C_{24}H_{27}BrN_3O_9$	580.0931	580.0923
13	8.24	6.94	1.51	3.52	4.37	4.37 s	2.01 br m	$C_{28}H_{35}N_4O_9$	571.2404	571.2399
14	8.23	6.92	1.51	3.53	4.37	4.13 s	1.90 br m	$C_{29}H_{37}N_4O_9$	585.2560	585.2569
15	8.23	6.95	1.51	3.32	4.37	4.18 s	2.96 s	$C_{26}H_{33}N_4O_9$	545.2248	545.2256
16	8.23	6.95	1.52	3.52	4.37	4.18 s	3.30 q, 1.33 t	$C_{28}H_{37}N_4O_9$	573.2560	573.2573
17	8.27	6.94	1.51	3.51	4.37	4.03 s	1.38 s	$C_{28}H_{33}N_4O_9$	573.2560	573.2562
18	8.24	6.94	1.51	3.52	4.37	4.03 s	1.68 t, 0.96 t	$C_{26}H_{33}N_4O_9$	573.2560	573.2556
8	8.36	6.92	1.51	3.51	4.37	–	8.33 s	$C_{23}H_{26}N_3O_9$		588
9	8.10	6.87	1.47	3.49	4.34	–	2.12 s	$C_{24}H_{28}N_3O_9$	502.1826	502.1829
10	8.07	6.88	1.47	3.46	4.34	–	1.15 d	$C_{26}H_{32}N_3O_9$	530.2138	530.2143
11	8.07	6.88	1.47	3.48	4.34	–	3.72 s	$C_{32}H_{44}N_3O_9$		613
12	8.04	6.90	1.50	3.51	4.37	–		$C_{24}H_{28}N_3O_{10}$	518.1775	518.1775
20	8.24	6.92	1.50	3.52	4.35	3.92 s		$C_{24}H_{29}N_4O_9$	517.1934	517.1932
21	8.25	6.99	1.52	3.52	4.37	4.02 s	2.60 s	$C_{25}H_{31}N_4O_9$	531.2091	531.2092
22	8.01	6.95	1.51	3.52	4.38	4.18 t	1.01 dd	$C_{28}H_{37}N_4O_9$		573
23	8.12	6.95	1.51	3.53	4.37	4.17 t	1.01 t	$C_{28}H_{37}N_4O_9$	573.2560	573.2562
24	8.11	6.97	1.52	3.58	4.37	4.08 dd	2.71 s, 1.01 dd	$C_{29}H_{39}N_4O_9$	587.2717	587.2702
25	8.15	6.94	1.57	3.53	4.37	4.19(q)	1.57(d)	$C_{25}H_{31}N_4O_9$	531.2091	531.2083
26	8.20	7.02	1.53	3.52	4.42	4.01 d	1.18 m	$C_{27}H_{35}N_4O_9$	559.2404	559.2405
27	8.19	7.01	1.51	3.51	4.34	3.98 s	1.01 m	$C_{28}H_{37}N_4O_9$		573
28	8.10	6.95	1.51	3.51	4.37	4.19 t	1.90 br m	$C_{31}H_{41}N_4O_9$	613.2874	613.2885
29	8.13	6.94	1.51	3.52	4.37	4.40 t	7.30 s	$C_{31}H_{35}N_4O_9$	607.2404	607.2406
30	8.10	6.93	1.21	3.50	4.37	4.40 t	7.30	$C_{31}H_{35}N_4O_9$	607.2404	607.2402
31	7.78	6.88	1.49	3.46	4.36	4.30 dd	7.26 s	$C_{33}H_{39}N_4O_9$	635.2717	635.2726
32	8.13	6.94	1.51	3.53	4.37	4.31 t	7.10 d, 6.73 d	$C_{31}H_{35}N_4O_{10}$	623.2353	623.2352
33	8.05	9.92	1.50	3.52	4.41	4.43 t	7.21 s, 7.07 t	$C_{33}H_{36}N_5O_9$	646.2513	646.2507
34	8.13	6.93	1.51	3.52	4.38	4.38 t	7.30 m, 7.04 m	$C_{31}H_{33}FN_4O_9$	625.2310	625.2310
35	8.14	6.93	1.51	3.52	4.37	4.46 t	8.16 d, 7.53 d	$C_{31}H_{35}N_5O_{11}$	652.2255	625.2267
36	8.11	6.95	1.51	3.52	4.37	4.20 t	2.10 m	$C_{27}H_{33}N_4O_{11}$	589.2146	589.2150
37	8.11	6.94	1.51	3.53	4.38	4.18	2.00 m, 1.70 m	$C_{27}H_{34}N_5O_{10}$		588
38	8.11	6.95	1.52	3.51	4.38	4.17 t	2.00 m, 1.70 m	$C_{28}H_{38}N_5O_9$	588.2670	588.2672
39	8.14	6.95	1.51	3.52	4.37	4.51 t	2.01 m	$C_{27}H_{33}N_4O_9$	557.2248	557.2250
40	8.11	6.92	1.51	3.52	4.37	3.90 t	1.60 m, 0.90 m	$C_{34}H_{48}N_5O_{10}$	686.3401	686.3404
41	8.12	6.94	1.52	3.54	4.39	4.17 s	3.93 t, 1.00 m	$C_{30}H_{40}N_5O_{10}$	630.2775	630.2792
42	8.07	6.92	1.51	3.53	4.37	4.64 t	3.71 s	$C_{30}H_{40}N_5O_{10}$	630.2775	630.2792
43	8.18	6.92	–	3.52	4.37	3.83 t	1.60 m, 0.90 m	$C_{46}H_{70}N_7O_{12}$	912.5082	912.5075
44b	8.00	6.76	–	–	4.15	4.02	1.01 d	$C_{27}H_{35}N_4O_8$	543.2455	543.2464
44c	8.00	6.75	–	–	4.15	4.02	1.01 d	$C_{27}H_{35}N_4O_8$	543.2455	543.2458

g, 25.5 mmol), BOP (11.3 g, 25.5 mmol), *N*-methylmorpholine (3.0 mL, 27.3 mmol), (Fmoc)-L-Leu-OH (9.0 g, 25.5 mmol) in DMF (130 mL, 2 h); (6) DMF (3 × 30 mL, 2 min); (7) CH_2Cl_2 (30 mL, 2 min). Steps 1–7 were repeated were repeated twice more, and the product was finally washed with CH_2Cl_2 (2 × 30 mL) and dried under nitrogen to give 5.56 g (86%) of white powder. A portion of the product resin (1.5 g, 0.85 mmol/g) was stirred in TFA (5 mL) for 1 h. The TFA solution was filtered into stirred ether (200 mL) whereupon a precipitate slowly formed over the course of 2 h. Fmoc-$(Leu)_4$-OH, a white powder, was collected by filtration (0.40 g, 45%).

Method B: General Acylation Procedure Using (Fmoc)-Protected Symmetrical Anhydrides. The preparation of **22** is representative. The (Fmoc)-protected amino acid was dissolved in ethyl acetate or tetrahydrofuran instead of dichloromethane when necessary. (Fmoc)-Leu-OH (361 mg, 1.02 mmol) was dissolved in CH_2Cl_2 (5 mL) at room temperature. DCC (92 mg, 0.45 mmol) was added, and the reaction mixture was stirred 45 min. The precipitated urea was filtered off, and the solvent was removed from the filtrate *in vacuo* to leave the anhydride as a white foam. The anhydride was dissolved in *N*-methylpyrrolidin-2-one (1 mL) and added to **6a** (172 mg, 0.37 mmol) in *N*-methylpyrrolidin-2-one (2 mL). The solution was stirred for 4.5 h at room temperature, and then piperidine (2 mL) was added to cleave the Fmoc group. After another 20 min of stirring, the reaction mixture was dripped into rapidly stirred ether (200 mL) and the crude product was collected by filtration (light yellow powder, 198 mg). Purification gave 46 mg of **22** as an orange glass.

[4*S*-(4α,12aα)]-9-(L-Leucylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (22) (Method B).

[4*S*-(4α,12aα)]-9-(D-Leucylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (23) (Method B).

[4*S*-(4α,12aα)]-9-(L-Prolylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (39) (Method B).

[4*S*-(4α,12aα)]-9-(L-Alanylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (25) (Method B).

[4*S*-(4α,12aα)]-9-(D-Phenylalanylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (30) (Method B).

[4*S*-(4α,12aα)]-9-[(2-L-(Cyclohexylmethyl)glycyl)amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,-12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (28) (Method B).

[4*S*-(4α,12aα)]-9-(2-L-Tryptophanylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (33) (Method B).

[4*S*-(4α,12aα)]-9-[[2-L-(3-Propanoyl)glycyl]amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,-12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (36) (Method B). In this case (Fmoc)-Glu(O-*t*-Bu)-OH was used. The powder from piperidine treatment was stirred in trifluoroacetic acid (2 mL) for 10 min and dripped into ether (150 mL) to remove the *tert*-butyl group.

[4*S*-(4α,12aα)]-9-(L-Glutamylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-

6-methyl-1,11-dioxo-2-naphthacenecarboxamide (37) (Method B). In this case (Fmoc)Gln(O-Trt)-OH was used. The trityl protecting group was spontaneously cleaved upon work-up.

[4S-(4α,12aα)]-9-(L-Lysylamino)-4-(dimethylamino)-1,4,-4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (38) (Method B). In this case (Fmoc)-Lys(Boc)-OH was used. The powder from piperidine treatment was stirred in TFA (2 mL) for 10 min and dripped into ether (150 mL) to remove the Boc group.

[4S-(4α,12aα)]-9-(L-Tyrosylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (32) (Method B). In this case (Fmoc)-Tyr(*t*-Bu)-OH was used. The powder from piperidine treatment was stirred in TFA (2 mL) for 10 min and dripped into ether (150 mL) to remove the *tert*-butyl group.

[4S-(4α,12aα)]-9-(L-Leucyl-L-leucyl-L-leucyl-L-leucylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (43) (Method B).

[4S-(4α,12aα)]-9-(L-Leucyl-L-leucylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (40) (Method B).

[4S-(4α,12aα)]-9-(L-Leucylglycylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (41) (Method B).

[4S-(4α,12aα)]-9-(Glycyl-L-leucylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (42) (Method B).

[4S-(4α,12aα)]-9-(L-Leucylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (44b) (Method B). In this case 9-amino-DMDOT **44a** was the starting material.

[4S-(4α,12aα)]-9-(D-Leucylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide (44c) (Method B). In this case 9-amino-DMDOT **44a** was the starting material.

Method C: General Acylation Procedure Using Boc-Protected Symmetrical Anhydrides. The following preparation of the 9-(*N*-Me-L-Leucine)doxycycline analog **24** is representative. DCC (91 mg, 0.44 mmol) was added to *N*-Boc-*N*-Me-L-leucine (240 mg, 0.98 mmol) in dichloromethane (3 mL) at room temperature, and the mixture was stirred for 30 min. The precipitate was filtered off, and the filtrate was concentrated *in vacuo*. The syrupy filtrate residue was dissolved in *N*-methylpyrrolidin-2-one (1 mL) and added to **6a** (142 mg, 0.31 mmol) in *N*-methylpyrrolidin-2-one (2 mL). The reaction mixture was stirred 2 h and the solvent was removed under vacuum. The residue was taken up in TFA/anisole (4 mL, 9:1), stirred 45 min, and then dripped into stirred ether (150 mL), and the precipitate was collected by filtration to give crude product (118 mg) as a yellow powder. The purified product was a golden glass.

[4S-(4α,12aα)]-9-[(*N*-Methyl-L-leucyl)amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (24) (Method C).

[4S-(4α,12aα)]-9-(L-Phenylalanylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (29) (Method C).

[4S-(4α,12aα)]-9-[[L-(4-Fluorophenyl)alanyl]amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,-12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (34) (Method C).

[4S-(4α,12aα)]-9-[[L-(4-Nitrophenyl)alanyl]amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,-12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (35) (Method C).

[4S-(4α,12aα)]-9-(Glycylamino)-4-(dimethylamino)-1,4,-4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (20) (Method C).

[4S-(4α,12aα)]-9-(Sarcosylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (21) (Method C).

Method D: General Procedure for Direct Coupling of Sterically Hindered Amino Acids to 9-Aminodoxycycline As Illustrated by the Preparation of the 9-L-Valine Derivative 26. (Fmoc)-Val-OH (110 mg, 0.32 mmol), HOBT (50 mg, 0.33 mmol), BOP (140 mg, 0.32 mmol), and *N*-methylmorpholine (40 μL, 0.32 mmol) were combined in dimethylformamide (DMF, 1.5 mL). This solution was added to **6a** (101 mg, 0.22 mmol) in DMF (2 mL) at room temperature, and the reaction mixture was stirred for 3 days. Piperidine (2 mL) was added, and stirring was continued another 30 min. The mixture was dripped into stirred ether (200 mL) to form a viscous yellow residue which settled to the bottom of the flask. The ether was decanted, and the residue was redissolved in methanol (2 mL) and dripped into stirred ether (200 mL) to give a dark yellow powder which became a yellow glass after purification.

[4S-(4α,12aα)]-9-(L-Valylamino)-4-(dimethylamino)-1,4,-4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (26) (Method D).

[4S-(4α,12aα)]-9-(L-Isoleucylamino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (27) (Method D). In this case the coupling reaction required 6 days.

Method E: General Alkylation Procedure To Prepare Substituted 9-(Alkylamino)glycine Doxycycline Analogs. Bromide **7** (108 mg, 0.19 mmol) was dissolved in *N*-methylpyrrolidin-2-one (2 mL) at room temperature, and piperidine (1 mL) was added. The reaction was stirred for 30 min, and the solvent was stripped *in vacuo*. The residue was taken up in MeOH (1 mL) and dripped into stirred ether (100 mL), and the precipitate was collected by filtration. The powder obtained was purified by preparative HPLC to give the product (45 mg) as a yellow glass.

[4S-(4α,12aα)]-9-[[(Pyrrolidin-1-yl)acetyl]amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (13) (Method E).

[4S-(4α,12aα)]-9-[[(Piperidin-1-yl)acetyl]amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,-12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (14) (Method E).

[4S-(4α,12aα)]-9-[[(Dimethylamino)acetyl]amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,-12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (10) (Method E).

[4S-(4α,12aα)]-9-[[(Diethylamino)acetyl]amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,-12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (16) (Method E).

[4S-(4α,12aα)]-9-[[(*tert*-Butylamino)acetyl]amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,-12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (17) (Method E).

[4S-(4α,12aα)]-9-[[(*n*-Butylamino)acetyl]amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (18) (Method E).

[4S-(4α,12aα)]-9-[(*N*,*N*-Dimethyl-L-phenylalanyl)amino]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,-12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (31). Amine **6a** (115 mg, 0.25 mmol) was dissolved in water (3 mL) at room temperature. Ethyl [3-(dimethylamino)propyl]carbodiimide hydrochloride (50 mg, 0.26 mmol) and *N*,*N*-dimethyl-L-phenylalanine (48 mg, 0.25 mmol) were added, and the mixture was stirred for 10 min.

More of the carbodiimide and phenylalanine reagents (0.1 g each) were added followed by 10 min of stirring and finally a third batch of carbodiimide and phenylalanine reagents (0.1 g each), and the reaction mixture was stirred until no starting material was detected by HPLC (1 h). The solvent was removed *in vacuo*, and the residue was taken up in MeOH (2 mL) and dripped into 150 mL of stirred ether. The precipitated material was allowed to settle. The ether was decanted off, and the viscous residue was purified by HPLC to give **30** as a light yellow powder.

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