Synthesis and Antibacterial Activity of a Series of Basic Amides of Teicoplanin and Deglucoteicoplanin with Polyamines

Adriano Malabarba,* Romeo Ciabatti, Jürgen Kettenring, Roberto Scotti, Gianpaolo Candiani, Rosa Pallanza, Marisa Berti, and Beth P. Goldstein

Marion Merrell Dow Research Institute, Lepetit Research Center, Via R. Lepetit 34, 21040 Gerenzano (Varese), Italy

Received May 15, 1992

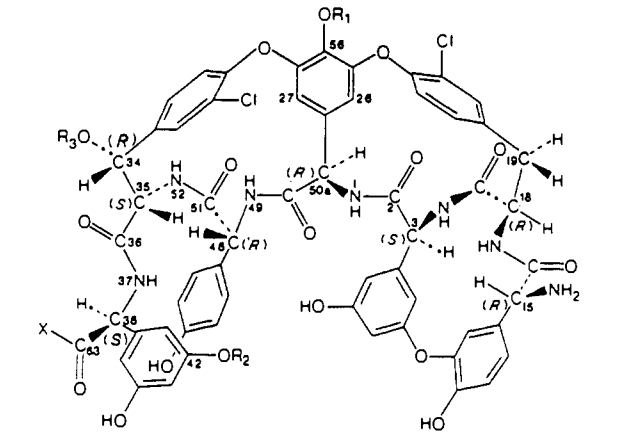
Basic carboxamides of teicoplanin A2 (CTA) and its aglycon (TD) are prepared by condensation of the 63-carboxyl function of these antibiotics with linear or branched polyamines. The antimicrobial activities of some of the resulting compounds were better than those of the unmodified antibiotics. The presence of more than one basic group in the amidic chain enhanced the in vitro activity of some TD-amides against Gram-negative bacteria; two of these derivatives were also effective in vivo against *Escherichia coli* septicemia in the mouse. Among the CTA derivatives, the amide with spermine showed some unexpected in vitro activity against Gram-negatives. Both CTA- and TD-amides with polyamines are very soluble in water over a wide range of pH and are very hydrophilic.

In our program of chemical transformation of teicoplanin,[1] we first modified functional groups, including the carboxyl group, which are not directly involved in binding to the antibiotic's target peptide D-Ala-D-Ala.[2] N^{63}-carboxamides of teicoplanin A2 (CTA), its pseudo-aglycons (TB, TC) and aglycon (TD) (Figure 1), with amines carrying various functional groups and chains were previously prepared[3] in order to improve the activity against less sensitive coagulase-negative staphylococci (CNST) and enterococci and to broaden the antibacterial spectrum to Gram-negative organisms.

As a result of that work, some preliminary structure–activity relationships were established between isoelectric point (p*I*), lipophilicity and hydrosolubility at physiological pH, and antimicrobial activity. It was found that the conversion of the carboxyl group to an amide generally improved in vitro activity and in vivo efficacy; the extent of improvement depended on the ionic and lipophilic character of the resulting derivatives and on the number and structure of the sugars. The majority of the positively charged amides were more active than the respective unmodified antibiotics against some Gram-positive organisms. In particular, most of the basic amides of CTA were markedly more active than teicoplanin against CNST. Moreover, amides of TD and some amides of TC showed a certain activity against Gram-negative bacteria.

In general, the combined effect of a moderate basicity (p*I* 7.9–8.8) and a slightly increased lipophilicity at neutral pH had a positive influence on in vitro activity. In vivo, in the murine model of experimental *Streptococcus pyogenes* septicemia, the most efficacious compounds were teicoplanin amides with pI 8.3–8.5 which, at physiological pH, are more lipophilic and markedly more soluble in water than the parent unmodified antibiotics.

Recently, a series of amides of CTA and TD with polyamides was synthesized to verify the effect on their antimicrobial properties of an additional increase in their hydrosolubility at physiological pH, while maintaining a moderate basicity. A certain decrease in their relative lipophilicity was also expected due to the increase in the number of the ionizable functions at neutral pH.



CTA

N-acyl (NHCOR) side chains

R_1 = *N*-acyl-β-D-glucosaminyl
- component A2-1: $R = (CH_2)_2CH{=}CH(CH_2)_4CH_3$
- component A2-2: $R = (CH_2)_6CH(CH_3)_2$
- component A2-3: $R = n\text{-}C_9H_{19}$
- component A2-4: $R = (CH_2)_6CH(CH_3)CH_2CH_3$
- component A2-5: $R = (CH_2)_7CH(CH_3)_2$

R_2 = α-D-mannosyl; R_3 = *N*-acetyl-β-D-glucosaminyl

TB: R_1 = H; R_2 = α-D-mannosyl; R_3 = *N*-acetyl-β-D-glucosaminyl

TC: $R_1 = R_2$ = H; R_3 = *N*-acetyl-β-D-glucosaminyl

TD: $R_1 = R_2 = R_3$ = H

Compound **29**, $R_1 = R_2 = R_3$ = H; $NR'R'' = N(CH_3)(CH_2)_3NHCH_3$

Figure 1. The structures of teicoplanin antibiotics (X = OH) and their amides (X = NR′R″; see tables).

The structures of these new carboxamide derivatives were determined by ^{1}H-NMR spectroscopy. Their approximate isoelectric points (p*I*'s) were calculated on the basis of the p*K* values of the ionizable functions as drawn from acid–base titration curves.

All the products were tested for their in vitro antibacterial activity. Some of them, selected among those having good in vitro activity, were also tested in vivo in mouse septicemia caused by *Streptococcus pyogenes* and, in some cases, by *Escherichia coli*.

(1) Malabarba, A.; Parenti, F. Semi-synthetic Teicoplanin Antibiotics. *Curr. Antimicrob. Pat.* **1990**, *2*, 263–287.
(2) Barna, J. C. J.; Williams, D. H. The Structure and Mode of Action of Glycopeptide Antibiotics of the Vancomycin Group. *Annu. Rev. Microbiol.* **1984**, *38*, 339–357.
(3) Malabarba, A.; Trani, A.; Strazzolini, P.; Cietto, G.; Ferrari, P.; Tarzia, G.; Pallanza, R.; Berti, M. Synthesis and Biological Properties of N^{63}-Carboxamides of Teicoplanin Antibiotics. Structure-Activity Relationships. *J. Med. Chem.* **1989**, *32*, 2450–2460.

0022-2623/92/1835-4054$03.00/0

Table I. Amides of CTA

compd	NR′R″	method	yield,[a] %	HPLC[b] t_R, min	p*I*[c]	titration, EW[d]	MW[e]
1	$NH(CH_2)_2NH(CH_2)_2NH_2$	b	56	15.6	8.7	675	1964.9
2	$NH(CH_2)_3NH(CH_2)_3NH_2$	b	48	16.1	8.9	690	1992.9
3	$NH(CH_2)_3NH(CH_2)_4NH_2$[f]	b	57	15.7	9.0	680	2006.9
4	$NH(CH_2)_2NH(CH_2)_3NH(CH_2)_2NH_2$	b	63	16.9	8.9	506	2022.0
5	$NH(CH_2)_3NH(CH_2)_2NH(CH_2)_3NH_2$	b	60	16.8	9.0	511	2036.0
6	$NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$	b	61	16.9	8.9	512	2050.0
7	$NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	b	55	17.1	9.1	510	2064.0
8	$NH(CH_2)_2NH[(CH_2)_2NH]_3(CH_2)_2NH_2$	b	23	11.9	9.0	345	2094.1
9	$NH(CH_2)_2N[(CH_2)_2NH_2]_2$	b	71	15.7	8.9	492	2007.9
10	$NH(CH_2)_3N[(CH_2)_3NH_2]_2$	a′	20	14.4	9.0	500	2050.0
11	$NH(CH_2)_2N[(CH_2)_2]_2NH$	a	15	10.3	9.1	630	1990.9
12	$NH(CH_2)_3N[(CH_2)_2]_2N(CH_2)_3NH_2$	a	53	17.0	9.1	496	2062.0
13	$N[(CH_2)_2]_2N(CH_2)_3N(CH_3)_2$	a	45	12.4	9.1	671	2033.0
14	$N[(CH_2)_2NH(CH_2)_2NH(CH_2)_2]_2NH$	b	19	7.5	9.0	350	2120.1

[a] Overall yields calculated from CTA. [b] Data are referred to component A2-2 of each "complex" compound. See Experimental Section. [c] Approximate values calculated from the pK_a's of the ionizable functions in water by extrapolation of pK_{MCS} values determined (acid–base titration) at various decreasing concentrations of MCS in H_2O. See Chemistry. The calculated values of p*I* are in accordance with those obtained for a few compounds by isoelectrofocusing (IEF).[17] [d] EW = equivalent weight. [e] Molecular weight calculated for the component A2-2 of the compounds of formula: **1**, $C_{92}H_{108}N_{12}O_{32}Cl_2$; **2**, $C_{94}H_{112}N_{12}O_{32}Cl_2$; **3**, $C_{95}H_{114}N_{12}O_{32}Cl_2$; **4**, $C_{95}H_{115}N_{13}O_{32}Cl_2$; **5**, $C_{96}H_{117}N_{13}O_{32}Cl_2$; **6** and **10**, $C_{97}H_{119}N_{13}O_{32}Cl_2$; **7**, $C_{98}H_{121}N_{13}O_{32}Cl_2$; **8**, $C_{98}H_{123}N_{15}O_{32}Cl_2$; **9**, $C_{94}H_{113}N_{13}O_{32}Cl_2$; **11**, $C_{94}H_{110}N_{12}O_{32}Cl_2$; **12**, $C_{96}H_{119}N_{13}O_{32}Cl_2$; **13**, $C_{97}H_{116}N_{12}O_{32}Cl_2$; **14**, $C_{100}H_{125}N_{15}O_{32}Cl_2$. [f] In equimolecular mixture with NR′R″ = $NH(CH_2)_4NH(CH_2)_3NH_2$.

Table II. Amides of TD

compd	NR′R″	method	yield,[a] %	HPLC[b] t_R, min	p*I*[c]	titration, EW[d]	MW[e]
15	$NH(CH_2)_2NH(CH_2)_2NH_2$	b	81	12.4	8.5	441	1284.1
16	$NH(CH_2)_3NH(CH_2)_3NH_2$	b	63	12.7	8.6	442	1312.2
17	$NH(CH_2)_3NH(CH_2)_4NH_2$[f]	b	86	12.8	8.5	456	1326.2
18	$NH(CH_2)_2NH(CH_2)_3NH(CH_2)_2NH_2$	b	84	15.8	8.7	348	1341.2
19	$NH(CH_2)_3NH(CH_2)_2NH(CH_2)_3NH_2$	b	83	15.7	8.7	349	1355.2
20	$NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$	b	86	15.7	8.5	361	1369.3
21	$NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	b	81	15.9	8.8	351	1383.3
22	$NH(CH_2)_2N[(CH_2)_2NH_2]_2$	b	88	12.5	8.7	369	1327.2
23	$NH(CH_2)_3N[(CH_2)_3NH_2]_2$	a′	35	13.6	8.7	335	1369.3
24	$NH(CH_2)_3N[(CH_2)_3NH_2](CH_2)_2N(C_2H_5)_2$	a[g]	15	14.2	8.8	350	1411.3
25	$NH(CH_2)_2N[(CH_2)_2]_2NH$	c[h]	27	8.5	8.9	397	1310.2
26	$NH(CH_2)_3N[(CH_2)_2]_2N(CH_2)_3NH_2$	b	83	15.9	8.9	349	1381.3
27	$N[(CH_2)_2]_2N(CH_2)_3N(CH_3)_2$	c[i]	31	13.1	8.8	415	1352.2
28	$N[(CH_2)_2NH(CH_2)_2NH(CH_2)_2]_2NH$	b	29	7.7	8.8	237	1439.4

[a] Calculated from N^{15}-*t*-BOC-TD. [b] See Experimental Section. [c] Approximate values calculated from the pK_a's of the ionizable functions in water by extrapolation of pK_{MCS} values determined (acid–base titration) at various decreasing concentrations of MCS in H_2O. See Chemistry. The calculated values of p*I* are in accordance with those obtained for a few compounds by isoelectrofocusing (IEF).[17] [d] EW = equivalent weight. [e] Molecular weight of the compounds of formula: **15**, $C_{62}H_{56}N_{10}O_{17}Cl_2$; **16**, $C_{64}H_{60}N_{10}O_{17}Cl_2$; **17**, $C_{65}H_{62}N_{10}O_{17}Cl_2$; **18**, $C_{65}H_{63}N_{11}O_{17}Cl_2$; **19**, $C_{66}H_{65}N_{11}O_{17}Cl_2$; **20** and **23**, $C_{67}H_{67}N_{11}O_{17}Cl_2$; **21**, $C_{68}H_{69}N_{11}O_{17}Cl_2$; **22**, $C_{64}H_{61}N_{11}O_{17}Cl_2$; **24**, $C_{70}H_{73}N_{11}O_{17}Cl_2$; **25**, $C_{64}H_{58}N_{10}O_{17}Cl_2$; **26**, $C_{68}H_{67}N_{11}O_{17}Cl_2$; **27**, $C_{67}H_{64}N_{10}O_{17}Cl_2$; **28**, $C_{70}H_{73}N_{13}O_{17}Cl_2$. [f] In equimolecular mixture with NR′R″ = $NH(CH_2)_4NH(CH_2)_3NH_2$. [g] Obtained according to method a in DMSO in the presence of DPPA and a large excess of TEA. [h] From amide **11**. [i] From amide **13**.

We also studied the ability of a few amides of CTA and TD to bind to Ac_2-L-Lys-D-Ala-D-Ala, a synthetic analogue of the antibiotic's target peptide.

Chemistry

The amides of CTA and TD with polyamides (Tables I and II) were synthesized according to three main procedures (Schemes I and II).

Some amides of CTA were prepared (method a) by reaction of N^{15}-benzyloxycarbonyl (CBZ) or N^{15}-*tert*-butyloxycarbonyl (BOC) CTA with a proper polyamine at room temperature in DMF or DMSO in the presence of diphenyl phosphorazidate (DPPA) as the condensing agent and TEA. Deprotection of the resulting N^{15}-CBZ compounds by catalytic hydrogenolysis (1 atm, 5% Pd/C), or of N^{15}-*t*-BOC derivatives by acidolysis with dry TFA, freed the final CTA-amides. In general, the CBZ protecting group was preferred to *t*-BOC since the acidic conditions required to remove the latter were critical for the stability of CTA derivatives which were easily transformed into the corresponding TB-amides by hydrolysis of the *N*-acylglucosamine moiety at the 56-position.[3,4] On the other hand, compounds obtained after hydrogenation of their N^{15}-CBZ protecting group were derivatives of factor A2-1-free CTA (CTA′), since this component was transformed into factor A2-3 under these conditions.[5]

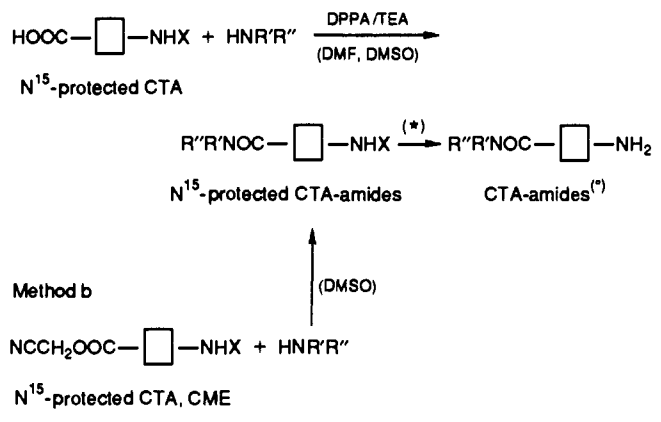
Most of the amides of CTA and TD were obtained (method b) by treatment of their N^{15}-protected derivatives activated as cyanomethyl esters, with a proper polyamine at room temperature in DMSO, followed by the removal of the protecting groups. Some TD-amides were alternatively synthesized (method c) from the corresponding CTA-amides by hydrolysis of the sugars with HCl in trifluoroethanol (TFE) at 70–75 °C.

The progress of the reactions and the homogeneity of the final products were monitored by HPLC. The relative lipophilicity of the polyamine-amides of CTA and TD as compared with CTA or TD was deduced from their HPLC retention times (t_R's). Though still possessing a marked hydrophilic character,[6] all the amides of CTA and TD

(4) Malabarba, A.; Strazzolini, P.; Depaoli, A.; Landi, M.; Berti, M. Cavalleri, B. Teicoplanin, Antibiotics from *Actinoplanes Teichomyceticus* Nov. Sp. VI. Chemical Degradation: Physico-Chemiical and Biological Properties of Acid Hydrolysis Products. *J. Antibiot.* **1984**, *37*, 988–999.

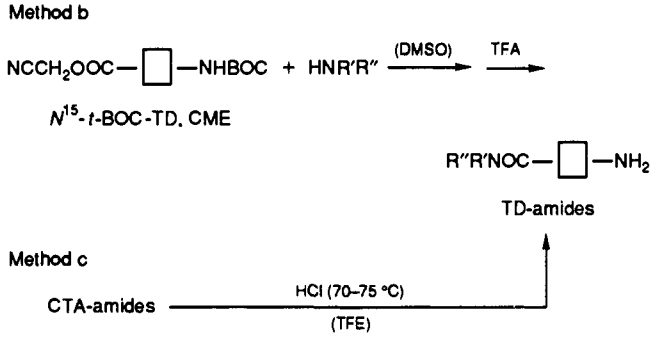
(5) The double bond-containing N-decenoylglucosamine of CTA-factor A2-1 is invariably reduced under hydrogenation conditions to give the saturated N-decanoylglucosamine of CTA-factor A2-3 (U.S. Patent 4,-725,668, 1988).

Scheme I [a]



[a] X = *t*-BOC, (*) = TFA (2 min) (°) CTA-amides; X = CBZ, (*) = H_2 (5% Pd/C) (°) CTA'-amides; CME = cyanomethyl ester.

Scheme II [a]



[a] CME = cyanomethyl ester; TFE = 2,2,2-trifluoroethanol.

described in this paper are more lipophilic than CTA or TD at neutral pH.

Preliminary data[7] showed that the majority of CTA- and TD-amides with polyamines are very soluble in water at both acidic and basic pH and that, at neutral and basic pH, all of them are markedly more soluble than the parent compounds and than most of the previously reported amides.[3]

The ^{1}H NMR spectra of these amide derivatives, obtained at 500 MHz, show the signals of the protons of the polyamine chain and the typical pattern of the teicoplanin structure.[8] As already observed in the ^{1}H NMR spectra of the amides previously described, the presence of a signal between δ 3.7 and 3.3, due to the new CH_2-NH-amidic group at C-38, confirms that the polyamine and teicoplanin moieties are linked together by an amide bond.

Acid–base titrations indicate that the free original carboxyl group is modified. Equivalent weights (EW's) confirm the number of additional amino groups present in the amidic chain.

Approximate isoelectric points (p*I*'s) were calculated on the basis of the values of the dissociation constants (pK_{MCS}'s) determined for each ionizable function in methylcellosove/water (MCS/H_2O) solutions at various decreasing concentrations of MCS.[9] The pK_a values in water were drawn by extrapolation from the resulting titration curves.[10]

All of these polyamine-amides are basic compounds with p*I* ranging from 8.5 to 8.9 for the TD-amides and from 8.7 to 9.1 for the CTA-amides.

Antibacterial Activity

In vitro, most of the CTA-amides with polyamines (Table III) were as active as CTA against *Staphylococcus aureus* (*Staph. aureus*), streptococci, and *Enterococcus faecalis* (*Entero. faecalis*). With the exception of compound **14**, they were more active than CTA against *Staphylococcus haemolyticus* (*Staph. haemolyticus*), a species of CNST. Many of these derivatives had 32–64-fold better activity than CTA against this strain. Compounds **3**, **4**, and **6–8** also had slight activity (MIC, 32–128 μg/mL) against *Pseudomonas aeruginosa*. Compound **7** showed additional slight activity against *Escherichia coli* (MIC, 64 μg/mL) and *Proteus vulgaris* (MIC, 128 μg/mL). CTA and all CTA derivatives prepared previously were inactive against these organisms (MIC, >128 μg/mL).[1]

TD-amides had better in vitro activity against Gram-negative bacteria (Table IV). Most of them had MIC values of 0.5–8 μg/mL against *Escherichia coli* (*E. coli*) and 2–16 μg/mL against *Pseudomonas aeruginosa* (*Ps. aerugin.*). Compound **20** was the most active of this series with MIC of 0.5 μg/mL for *E. coli* and 2 μg/mL for *Ps. aeruginosa*. This compound was further tested, in comparison with the most active of the previously described TD-amides (compound **29** in Figure 1; MIC, 4 μg/mL for *E. coli* and 16 μg/mL for *Ps. aeruginosa*),[3] against a larger number of Gram-negative isolates. As shown in Table V, compound **20** was 2–8 times as active as compound **29** against *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and some strains of *Shigella*, *Salmonella*, *Serratia*, and *Pseudomonas fluorescens*. Both compounds had poor activity against *Ps. aeruginosa*, *Bacteroides fragilis* (*B. fragilis*) and *Proteus* species. Against Gram-positive organisms, the amides of TD were as active as those of CTA (Table III), but this did not represent an improvement over TD itself.

In vivo, in *Streptococcus pyogenes* (*Strep. pyogenes*) septicemia in the mouse, the amides of CTA (Table VI) with polyamines were somewhat more efficacious than CTA and much more efficacious than TD-amides (Table VII) upon subcutaneous (sc) administration; the amides of CTA also had some activity by oral (po) route, whereas those of TD were completely inactive.[11] Against *E. coli* septicemia (Table VII), TD-amides **20** and **23** had some activity (ED_{50}, ≤40 mg/kg) after intravenous (iv) administration; compound **23** was also active sc (ED_{50}, 40 mg/kg). Compound **23** was rapidly bactericidal (99.99% within 4 h) for *E. coli* at 2 and 4 μg/mL (Figure 2).

(6) All the polyamine-amides of CTA and TD are very hydrophilic at any pH, as shown by preliminary experiments carried out using a solvent mixiture 1-butanol/water (1/1) for the determination of their lipid–water partition coefficients. The concentration of these compounds in the butanolic phase is negligible with respect to the aqueous layer (unpublished results).

(7) Unpublished results from these laboratories.

(8) Coronelli, C.; Gallo, G. G.; Cavalleri, B. Teicoplanin: Chemical, Physico-Chemical and Biological Aspects. *Il Farmaco, Ed. Sci.* **1987**, *42*, 767–786.

(9) Although almost all polyamine-amides of CTA and TD are very soluble in water at any pH, acid–base titrations were performed in MCS/H_2O mixtures in order to compare the p*K* values of their ionizable functions with those of parent CTA and TD which are soluble in water only at pH ≥ 7.

(10) The pK_a values in water for the most acidic phenolic group of CTA and TD are 9.0 and 8.5, respectively, as determined by extrapolation to 100% of H_2O from their pK_{MCS} values obtained by titration at various decreasing concentrations of MCS in MCS/H_2O solutions.

(11) None of the amides of TD was orally active up to 300 mg/kg.

Table III. In Vitro Activity of CTA-Amides

compd	MIC, μg/mL					
	Staphylococcus aureus L 165	*Staph. epidermidis* ATCC 12228	*Staph. haemolyticus* L 602	*Streptococcus pyogenes* L 49	Strep. *pneumoniae* L 44	*Enterococcus faecalis* ATCC 7080
1	0.25	0.063	0.25	0.063	0.125	0.125
2	1	0.25	2	0.125	0.125	0.5
3	0.125	0.063	0.125	0.063	0.125	0.125
4	0.125	0.063	0.125	0.063	0.125	0.125
5	0.25	0.125	0.125	0.063	0.125	0.125
6	0.125	0.063	0.125	0.063	0.125	0.125
7	0.125	0.063	0.125	0.063	0.125	0.125
8	0.25	0.063	0.125	0.063	0.125	0.125
9	0.125	0.063	0.125	0.063	0.063	0.063
10	0.125	0.125	2	0.063	0.063	0.125
11	0.125	0.063	0.125	0.063	0.125	0.125
12	0.25	0.063	0.125	0.063	0.125	0.125
13	0.125	0.063	0.5	0.063	0.125	0.125
14	0.25	0.125	8	0.063	0.063	0.125
CTA	0.125	0.125	8	0.063	0.063	0.125

Table IV. In Vitro Activity of TD-Amides

compd no.	MIC, μg/mL								
	Staph. aureus L 165	*Staph. epiderm.* ATCC 12228	*Staph. haemolyt.* L 602	*Strep. pyogenes* L 49	*Strep. pneumon.* L 44	*Entero. faecalis* ATCC 7080	*E. coli* L 47	*Proteus vulgaris* ATCC 881	*Ps. aerugin.* ATCC 10145
15	0.063	0.063	0.125	0.125	0.125	0.125	8	64	64
16	0.063	0.032	0.125	0.063	0.125	0.125	1	32	8
17	0.063	0.063	0.063	0.063	0.125	0.125	2	32	8
18	0.063	0.063	0.063	0.063	0.125	0.125	4	128	64
19	0.125	0.063	0.125	0.063	0.125	0.125	2	64	16
20	0.063	0.016	0.063	0.063	0.125	0.25	0.5	64	2
21	0.063	0.063	0.125	0.063	0.125	0.125	2	64	8
22	0.063	0.016	0.063	0.063	0.125	0.125	1	32	8
23	0.125	0.008	0.063	0.063	0.125	0.125	1	128	8
24	0.125	0.032	0.125	0.125	0.125	0.125	32	>128	128
25	0.125	0.063	0.125	0.063	0.125	0.125	8	64	64
26	0.063	0.063	0.125	0.063	0.125	0.125	4	128	16
27	0.063	0.063	0.063	0.063	0.125	0.125	8	128	32
28	0.125	0.125	1	0.25	0.25	0.5	>128	>128	>128
29	0.063	0.063	0.063	0.063	0.125	0.125	2	32	16
TD	0.063	0.016	0.25	0.125	0.125	0.125	64	128	>128

Table V. In Vitro Activity against Gram-Negative Isolates

organism	MIC, μg/mL		organism	MIC, μg/mL	
	20	29		20	29
Escherichia coli L 1153	1	4	*Proteus vulgaris* L 1539	32	128
E. coli L 1347	4	8	*Serratia marcescens* L 1488	8	16
E. coli L 1484	2	16	*S. marcescens* L 1506	8	32
E. coli L 1513	2	8	*Enterobacter cloacae* L 1485	4	32
E. coli L 1536	2	8	*E. cloacae* L 1504	8	32
E. coli L 1572	2	8	*Pseudomonas aeruginosa* L1138	32	64
Salmonella typhimurium L 35	4	16	*Ps. aeruginosa* L1348	4	16
Shigella dysenteriae L 155	1	4	*Ps. aeruginosa* L 1498	64	128
Shigella sonnei L 363	2	8	*Ps. aeruginosa* L 1533	64	128
Klebsiella pneumoniae L 1481	4	16	*Ps. fluorescens* L 1330	8	64
K. pneumoniae L 1499	8	32	*Bacteroides fragilis* L 1010	16	32
Proteus mirabilis L 1157	128	64	*B. fragilis* L 1011	32	32
P. mirabilis L 1356	32	32	*B. fragilis* L 1236	128	64

Peptide Binding Studies

We measured the binding of some compounds of each class of polyamine-amides of CTA and TD to Ac_2-L-Lys-D-Ala-D-Ala, a synthetic analogue of the antibiotic's target peptide, by the differential UV assay.[12] The procedure has been previously described.[3] No significant modification was observed with respect to CTA, TD, or corresponding earlier amide derivatives. In particular, the values of the association constants (determined at pH 9) were 9.5×10^4 to 3.5×10^5 M^{-1} for the polyamine-amides of CTA and 5.0×10^4 to 7.5×10^4 M^{-1} for those of TD.

Conclusions

As previously observed,[3] the conversion of the carboxyl group into an amide does not affect the ability of teicoplanin antibiotics to bind to Ac_2-L-Lys-D-Ala-D-Ala and may improve the antimicrobial activity of the resulting teicoplanin-amides against some organisms.

Introduction of a polycationic side chain provides derivatives with a slightly increased basic character with

(12) Nieto, M.; Perkins, H. R. The Specificity of Combination between Ristocetin and Peptides Related to Bacterial Cell Wall Mucopeptide Precursors. *Biochem. J.* 1971, *124*, 845–852.

Table VI. In Vivo (ED_{50}) Efficacy of CTA-Amides against *Streptococcus pyogenes* L 49 Septicemia in the Mouse

compd	MIC (μg/mL)	ED_{50} (mg/kg) sc	ED_{50} (mg/kg) po
1	0.063	0.09	60
3	0.063	0.05	81
6	0.063	0.06	80
7	0.063	0.03	81
8	0.063	0.08	112
9	0.063	0.04	58
11	0.063	0.08	90
12	0.063	0.06	60
CTA	0.063	0.12	>170

Table VII. In Vivo (ED_{50}) Efficacy of TD-Amides in Mouse Septicemia

	S. pyogenes L 49		*E. coli* L 47		
	MIC	ED_{50}	MIC	ED_{50} (mg/kg)	
compd	(μg/mL)	(mg/kg) sc	(μg/mL)	sc	iv
16	0.13	1.65			
17	0.06	0.31			
19	0.06	2.87			
20	0.06	0.72	0.5	>200	<40*
21	0.06	0.41			
22	0.06	1.25			
23	0.06	0.72	1	40	<40*
29	0.06	1.25			
TD	0.13	0.95			

[a] Survivors/treated: 6/8 with compound **20**, 7/8 with compound **23**.

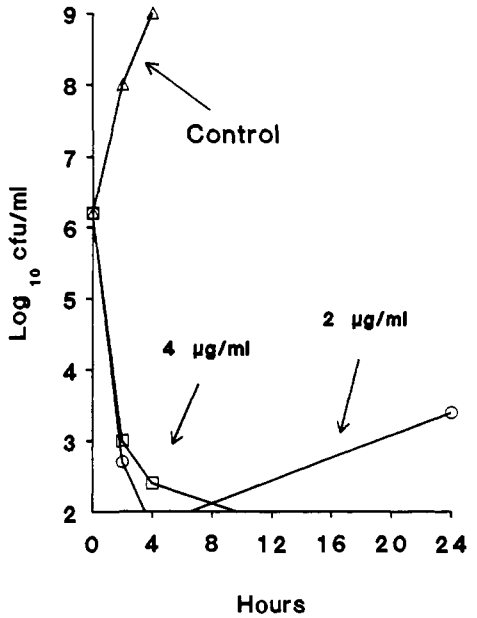


Figure 2. Bactericidal activity of amide **23** against *E. coli* L 47.

respect to teicoplanin-amides with diamines; the polyamine-amides are much more soluble in water at physiological pH than most of the earlier amides. Though this results in a markedly decreased lipophilicity, most of the polyamine-amides of TD had somewhat better activity against Gram-negative bacteria than the previously synthesized TD-amides. The polyamine side chain also confers weak anti-Gram-negative activity on some of the CTA-amides. In spite of their lower lipophilicity, the good in vitro activity of CTA- and TD-amides with polyamines against Gram-positive organisms and their excellent in vivo efficacy against *Strep. pyogenes* septicemia in the mouse might be likely related to the higher hydrosolubility of the polyamine-amides.

The structure of the polyamine chain has little influence on the in vitro and in vivo activity of teicoplanin-amides against Gram-positive bacteria, but seems to play a role in the efficacy of TD-amides **20** and **23** against *E. coli* septicemia. They differ in that compound **20** has a linear and compound **23** a branched triaminic chain; they had the same in vivo activity (ED_{50}, ≤40 mg/kg) against *E. coli* after iv administration, whereas only the latter was somewhat efficacious (ED_{50}, 40 mg/kg) upon sc treatment. As the structure and physicochemical properties of these two TD-amides are similar, and they had the same in vivo efficacy (ED_{50}, 0.72 mg/kg) against *Strep. pyogenes* septicemia by sc route, we have no explanation at present for the difference in sc activity against *E. coli*.

Experimental Section

^1H NMR spectra were recorded at 500 MHz on a Bruker AM 500 NMR spectrometer equipped with an Aspect 3000 computer. The spectra were obtained at 40 °C in DMSO-d_6 solution, using Me_4Si (δ 0.00 ppm) as internal reference.

Acid–base titrations were carried out under the following conditions: the sample was dissolved in MCS/H_2O (4/1). After adding an excess of 0.01 M HCl in the same solvent mixture, the resulting solution was titrated with 0.01 N NaOH.

The products were purified by reversed-phase column chromatography on silanized silica gel (0.063–0.2 mm; Merck). Reactions, column eluates, and final products were checked by HPLC analyses,[13] which were performed on a column Hibar (120 × 4.5 mm; Merck) prepacked with LiChrosorb RP-8 (10 μm), using a Varian Model 5500 LC pump equipped with a 20-μL loop injector Rheodyne Model 7125 and a Varian Model 2050 UV variable detector. Chromatograms were recorded at 254 nm, using CTA component A2-2 or TD as internal references. Elutions were carried out at a flow rate of 2 mL/min according to a linear step gradient from 20% to 60% of CH_3CN in 0.2% aqueous HCO_2NH_4 in 30 min.

All derivatives were analyzed for N and Cl on samples previously dried at 140 °C under N_2 atmosphere. Weight loss was determined by thermogravimetry (TG) at 140 °C. Inorganic residue was determined after heating the samples at 900 °C in O_2 atmosphere. The analytical results obtained for N and Cl were within ±0.4% of the theoretical values. Solvent content (in general H_2O with traces of 1-BuOH) and inorganic residue were always less than 10% and 0.3%, respectively.

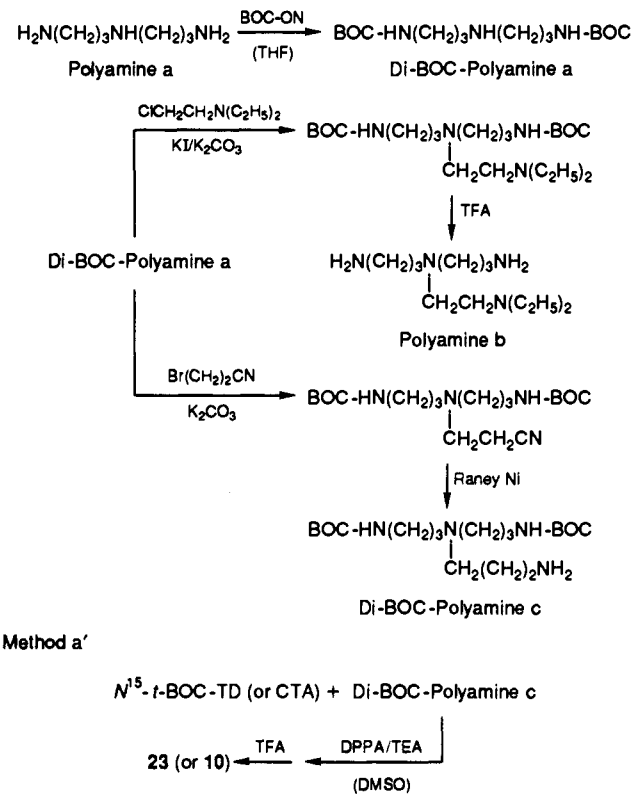
The composition of CTA and its amide derivatives, expressed as percentages of the areas of the peaks (HPLC) for each component of the complex, was approximately as follows: factor A2-1 10% (absent in CTA′-amides), factor A2-2 50%, factor A2-3 15% (25% in CTA′-amides), factor A2-4 12%, and factor A2-5 13%.

Most of the intermediate polyamines are commercially available products which were purchased from Fluka-Chemie AG or Aldrich-Chemie Gmbh & Co. KG, with the exception of branched tetraamines **b** and **c** whose preparation is described here (Scheme III).

3,3′-Di-[(*tert*-butyloxycarbonyl)amino]dipropylamine (Di-BOC-Polyamine a). A solution of 148 g (0.6 mol) of 2-[[(*tert*-butyloxycarbonyl)oxy]imino]-2-phenylacetonitrile (BOC-ON) in 300 mL of THF was added dropwise at 5–10 °C to a stirred solution of 42 mL (0.3 mol) of 3,3′-diaminodipropylamine (Polyamine a) in 400 mL of the same solvent. Then the reaction mixture was stirred at room temperature for 16 h, and the solvent was evaporated. The oily residue was dissolved in 1 L of EtOAc, and the resulting solution was washed with 200 mL of 1 N NaOH and then with H_2O (2 × 500 mL). Afterwards, it was extracted with 500 mL of 0.001 N HCl. The aqueous layer was adjusted at pH 8.2 with 1 N NaOH and extracted with 1 L of 1-BuOH. The organic phase was concentrated at 40 °C under reduced pressure to a small volume (about 100 mL). On standing at room temperature overnight, the title compound separated as needle-shaped crystals which were collected to give 75 g of pure Di-

(13) All derivatives had a HPLC purity ≥95%.

Scheme III



BOC-Polyamine a: ^{1}H NMR δ 6.68 (NH), 2.93 (2 CH_2NH-BOC), 2.44 (2 CH_2NH), 1.47 (CH_2), 1.36 (*N*-BOC) ppm.

N^3-[2,2-(Diethylamino)ethyl]-N^3-(3-aminopropyl)-1,3-diaminopropane (Polyamine b). To a stirred solution of 35 g (about 0.2 mol) of *N,N*-diethyl-2-chloroethylamine hydrochloride in 600 mL of absolute EtOH was added 17 g (about 0.1 mol) of KI at room temperature. After 2 h, 33 g (about 0.1 mol) of di-BOC-polyamine a and 35 g (about 0.25 mol) of K_2CO_3 were added and stirring was continued overnight. The insoluble matter was filtered off, and the filtrate was concentrated to a small volume; afterwards, 600 mL of H_2O was added. The resulting solution was extracted with 1 L of EtOAc, the organic layer was washed with H_2O (2 × 200 mL), and the solvent was evaporated. The oily residue was chromatographed on a column of silica gel 60 (400 g; 0.063–0.2 mm, Merck) in CH_2Cl_2, eluting with 1 L each of the following mixtures of CH_2Cl_2/MeOH: 95/5, 90/10, 85/15, and 70/30. About 11 g of pure di-BOC-polyamine b was obtained: ^{1}H NMR δ 6.76 (NH-BOC), 3.48 (CH_2CH_3), 2.93 (2 CH_2NH-BOC), 2.47 (CH_2N), 2.35 (CH_2N), 1.48 (CH_2CH_2N), 1.36 (*N*-BOC), 0.90 (CH_3CH_2) ppm.

The above product was dissolved in 100 mL of absolute EtOH, and the resulting solution was stirred at room temperature for 20 min while bubbling dry HCl; afterwards the solvent was evaporated to give 10 g of an oily residue which was crystallized from absolute EtOH, yielding 7 g of pure polyamine b as tetrahydrochloride: ^{1}H NMR δ 8.41 (NH_2), 3.46 (CH_2CH_3), 3.39 (CH_2NEt_2), 3.22, 2.97 (CH_2N), 2.15 (CH_2), 1.27 (CH_3CH_2) ppm.

N^3,N^3-Di-[3-[(*tert*-butyloxycarbonyl)amino]propyl]-1,3-diaminopropane (Di-*t*-BOC-Polyamine c). To a solution of 33 g (about 0.1 mol) of di-BOC-polyamine a and 15 mL (about 0.18 mol) of 3-bromopropionitrile in 300 mL of absolute EtOH was added 12.5 g (about 0.9 mol) of K_2CO_3. After stirring at room temperature for 20 h, the insoluble matter was filtered off and the solvent was evaporated. The oily residue was redissolved in 500 mL of H_2O, and the resulting solution (pH 8) was extracted with 500 mL of EtOAc. The organic layer was washed with H_2O (2 × 150 mL), and then it was concentrated to a final volume of about 150 mL. The crystalline solid which separated was collected and dried over P_2O_5 in vacuo to give 31 g of 3,3-di-[3-(BOC-amino)propyl]-3-aminopropionitrile: ^{1}H NMR δ 6.75 (NH-BOC), 2.93 (CH_2NH-BOC), 2.63, 2.55 (CH_2N in CH_2CN chain), 2.37 (2 CH_2N), 1.47 (2 CH_2), 1.37 (*N*-BOC) ppm.

The nitrile derivative was dissolved in a solution of 8.5 g of NaOH (drops) in 200 mL of absolute EtOH. After adding 4 g of Raney Ni, the resulting suspension was hydrogenated (2.7 atm, room temperature) for 10 h. The catalyst was filtered off, and the filtrate was concentrated to give 31 g of the title compound (still containing traces of nitrile) pure enough for the next step: ^{1}H NMR δ 6.72 (NH-BOC), 2.92 (2 CH_2NH-BOC), 2.51 (CH_2N), 2.33 (2 CH_2N), 1.46 (2 CH_2), 1.37 (*N*-BOC).

The above reactions and final products were monitored by TLC on silica gel 60 F-254 (0.25 mm; Merck) plates, using a solvent mixture CH_2Cl_2/MeOH 9/1 as the mobile phase (spots detected in I_2 atmosphere).

N^{15}-*t*-BOC-CTA. To a stirred solution of 9.5 g (about 5 mmol) of CTA in 100 mL of DMF were added 1.2 mL (8.5 mmol) of TEA and 2 g (6.7 mmol) of *tert*-butyl-2,4,5-trichlorophenyl carbonate. The reaction mixture was stirred at room temperature overnight, and then it was poured into 200 mL of H_2O. The resulting cloudy solution was adjusted at pH 3 with 1 N HCl and extracted with 700 mL of a mixture EtOAc/1-BuOH 2/1. The organic layer was washed twice with H_2O (2 × 100 mL) and concentrated at 35 °C under reduced pressure. On addition of EtOAc, a solid separated, which was collected, washed with Et_2O, and dried in the air at room temperature overnight to yield 9.3 g (about 93%) of the title compound: HPLC, t_R 13.3 min.[14]

N^{15}-CBZ-CTA. To a stirred solution of 9.5 g (about 5 mmol) of CTA and 1.2 mL (8.5 mmol) of TEA in 75 mL of DMF was added a solution of 0.8 mL (about 5.6 mmol) of benzyl chloroformate in 15 mL of dry DMF dropwise in 30 min, while cooling at 10 °C. After stirring at room temperature for 90 min, the reaction mixture was poured into 200 mL of H_2O and then worked up as described above to yield 9.7 g (about 95%) of the title compound: HPLC, t_R 14.1 min.[14]

N^{15}-Protected CTA, Cyanomethyl Esters. Both N^{15}-*t*-BOC and CBZ-CTA cyanomethyl esters were prepared in one pot from CTA, without isolation of the *t*-BOC and CBZ intermediates, according to the following procedure: To a stirred solution of 1 mmol of CTA in 10 mL of DMF were added 2.5 mmol of TEA and 1.3 mmol of tert-butyl-2,4,5-trichlorophenyl carbonate, or benzyl chloroformate, under the same conditions as those described above for the synthesis of *t*-BOC and CBZ-CTA, respectively, and then the reaction mixture was stirred at room temperature overnight. Afterwards, 2 mL (about 22 mmol) of chloroacetonitrile was added and stirring continued for 24 h. The reaction mixture was poured into 20 mL of H_2O and worked up as described above for the preparation of *t*-BOC-CTA, yielding (about 90%) the title compounds pure enough for the next steps: HPLC, t_R 16.1 (*t*-BOC-CTA),[14] 16.7 (CBZ-CTA)[14] min.

N^{15}-*t*-BOC-TD, Cyanomethyl Ester. The preparation of N^{15}-*t*-BOC-TD was carried out according to a procedure described previously.[15] A solution of 13 g (about 10 mmol) of N^{15}-*t*-BOC-TD and 1.7 mL (about 12.5 mmol) of TEA in 100 mL of a mixture DMF/$ClCH_2CN$ 9/1 was stirred at room temperature for 24 h. Then it was poured into 350 mL of H_2O, and the resulting suspension was extracted with 500 mL of EtOAc. The organic layer was washed with 150 mL of H_2O, with 100 mL of 0.01 N HCl, and then twice with H_2O (2 × 300 mL); afterwards, it was concentrated at 40 °C under reduced pressure. Upon addition of Et_2O, the precipitated solid was collected and dried in vacuo at room temperature overnight to yield 12.5 g (about 95%) of pure title compound: HPLC, t_R 17.4 min.

Amides of CTA (General Procedures). Method a. A solution of 0.24 mL (1.1 mmol) of DPPA in 5 mL of dry DMF was added dropwise at 0–5 °C to a stirred solution of 1 mmol of N^{15}-protected CTA, 1.1 mmol of the appropriate polyamine, and 0.14 mL (1 mmol) of TEA in 30 mL of dry DMF. The reaction mixture was stirred at 5–10 °C for 24 h; afterwards, it was poured into 200 mL of EtOAc. The precipitated solid was collected and then it was chromatographed on 100 g of silanized silica gel. Elution was performed with a linear gradient from 20% to 80% of MeCN in 0.05 N AcOH in 15 h at the rate of 200 mL/h, while

(14) Data referred to component A2-2.

(15) Malabarba, A.; Trani, A.; Ferrari, P.; Pallanza, R.; Cavalleri, B. Synthesis and Biological Activity of some Esters of the N-Acetylglucosaminyl Aglycone and of the Aglycone of Teicoplanin. *J. Antibiot.* **1987**, *40*, 1572–1587.

collecting 20-mL fractions. The appropriate ones were combined to obtain a solution of the N^{15}-protected amide with the predetermined (see above) composition in its five components. After adding enough 1-BuOH to avoid foaming, the solution was concentrated at 45 °C under reduced pressure to obtain a final dry butanolic solution (or suspension) of about 30 mL. On addition of Et_2O, the precipitated solid was collected, washed with Et_2O, and then dried in the air at room temperature overnight to yield N^{15}-protected CTA-amides.

Method b. A solution of 5 mmol of N^{15}-protected CTA cyanomethyl ester and 75–100 mmol of the appropriate polyamine in 100 mL of DMSO was stirred at room temperature for 2–4 h. On adding EtOAc, the precipitated solid was collected, and washed with EtOAc and then with Et_2O to yield N^{15}-protected CTA-amides.

Deprotection Step. I. The removal of the *t*-BOC group from N^{15}-*t*-BOC-CTA-amides was carried out by dissolving these compounds (1 mmol) in TFA (10 mL) at room temperature. After 2 min, 100 mL of Et_2O was added, and the precipitated solid was readily collected, washed several times with Et_2O, and then dissolved in 100 mL of H_2O. The resulting solution was adjusted at pH 8.5 with 1 N NaOH and loaded on a column of 100 g of silanized silica gel in H_2O. Reversed-phase chromatography was performed as described above to yield pure final compounds (Table I).

II. The CBZ group was removed from N^{15}-CBZ-CTA-amides (1 mmol) by catalytic hydrogenation (1 atm; room temperature) over 5% Pd/C (0.75 g) in MeOH/0.001N HCl 7/3 solution (150 mL). As soon as hydrogenolysis was completed (HPLC), the catalyst was filtered off, and the filtrate was adjusted at pH 8.5 with 1 N NaOH. After addition of 100 mL of 1-BuOH, the solvents were evaporated at 40 °C under reduced pressure. The solid residue was purified by reversed-phase column chromatography as described above to yield final amides of component A2-1-free CTA (Table I).

Amides of TD (General Procedures). Method b. A solution of 7 g (about 5 mmol) of N^{15}-*t*-BOC-TD cyanomethyl ester and 30–50 mmol of the appropriate polyamine in 20 mL of DMSO was stirred at room temperature for 4–8 h; afterwards, 30 mL of MeOH was added. The resulting solution (or suspension) was poured into 250 mL of Et_2O, and the precipitated solid was collected and redissolved in 50 mL of TFA. The solvent was evaporated at room temperature under reduced pressure, and the oily residue was slurried with Et_2O. The solid residue was dissolved in 100 mL of H_2O, and the resulting solution was loaded on a column of 150 g of silanized silica gel. Pure final TD-amides (Table II) were obtained after reversed-phase chromatography was carried out under the same conditions as those described above for CTA-amides.

Method c. A suspension of 1 mmol of a selected amide of CTA in 100 mL of 2,2,2-trifluoroethanol (TFE) was stirred at 70–75 °C, while bubbling dry HCl, for 12–18 h; then the insoluble matter was collected and purified by reversed-phase column chromatography as above to give the product.

N^{63}-[3-[Bis(3-aminopropyl)amino]propyl]teicoplanin A2 Amide (10). To a stirred solution of 10 g (about 5 mmol) of N^{15}-*t*-BOC-CTA, 1.5 mL (about 11 mmol) of TEA, and 4 g (about 21 mmol) of di-*t*-BOC-polyamine c in 70 mL of DMSO was added a solution of 2.5 mL (about 10 mmol) of DPPA in 5 mL of DMSO dropwise in 15 min, while cooling at 15 °C. After stirring at room temperature for 24 h, 500 mL of EtOAc was added. The precipitated solid was collected, washed with EtOAc, and then redissolved in 100 mL of TFA. After 5 min, the solvent was evaporated at room temperature under reduced pressure, and the oily residue was slurried with Et_2O. The solid was collected and purified by reversed-phase column chromatography under the usual above conditions to yield 2.35 g (about 25%) of compound **10**.

N^{63}-[3-[Bis(3-aminopropyl)amino]propyl]deglucoteicoplanin Amide (23). From 6.5 g (about 5 mmol) of N^{15}-*t*-BOC-TD, by following the same procedure as that described above for the preparation of compound **10**, 2.5 g (about 35%) of title compound **23** was obtained.

Antimicrobial Activity. Antibacterial activity, expressed as MIC (minimal inhibitory concentration) in μg/mL, was determined by the microbroth dilution method in Difco Todd-Hewitt broth (streptococci), Difco Wilkins-Chalgren broth (*Bacteroides fragilis*), or Oxoid Iso-Sensitest broth (other bacteria). The final inoculum was 10^4 cfu (colony forming units)/mL, except for *B. fragilis* (10^5 cfu/mL). Incubation was for 24 h at 37 °C in the air, except for *B. fragilis* (48 h in an anaerobic chamber).

Septicemia was induced in mice by intraperitoneal injection of about 10^5 cells of *Strep. pyogenes* L 49 or 10^4 cells of *E. coli* L 47, challenges corresponding to about 100 times the 50% lethal dose. Groups of five to eight mice were treated once, immediately after infection. On the 7th day, the ED_{50} (50% effective dose) in mg/kg was calculated[16] on the basis of the percentage of surviving mice at each dose.

Determination of Bactericidal Activity. A logarithmically growing culture of *E. coli* L 47 in Davies-Mingioli medium supplemented with 2% glucose and 0.25% casamino acids was diluted and inoculated into the same medium at a density of 1.5×10^6 cfu/mL; 5 mL-portions were distributed into flasks with and without antibiotic. At intervals after incubation in a shaking 37 °C water bath, duplicate 0.1 mL-samples of suitable dilutions (at least 10-fold) in saline containing 0.1% peptone were plated by inclusion in 2.5 mL of Iso-Sensitest soft (0.7%) agar on Iso-Sensitest agar plates.

Acknowledgment. We are indebted to G. Cietto for the preparation of most of the teicoplanin-amides and to G. Tamborini and Dr. P. Ferrari for acid–base titrations and determination of isoelectric points.

(16) Finney, D. J. The Spearman-Kärber Method. In *Statistical Method in Biological Assay*; Griffin, G., Ed.: London, 1952; pp 524–530.
(17) Altomare, C.; Carotti, A.; Cellamare, S.; Contento, A.; Ciabatti, R.; Malabarba, A.; Berti, M.; Goldstein, B. P. QSAR of Teicoplanin Antibiotics: Influence of Lipophilicity and Ionic Properties on their *In Vivo* Antimicrobial Activity. *Med. Chem. Res.* **1992**, *1*, 393–398.