N^{63}-CARBOXAMIDES OF N^{15}-ALKYL AND N^{15},N^{15}-DIALKYL DERIVATIVES OF TEICOPLANIN AND DEGLUCOTEICOPLANIN

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(Received for publication May 15, 1992)

The synthesis and biological properties of a series of N^{63}-carboxamides of 15-*N*-alkylated derivatives of teicoplanin A2 (CTA) and its aglycone (TD) are described. Among the compounds, those carrying hydrophilic groups or ionizable amino functions on the N^{15}-alkyl chain are more soluble in water than parent N^{15}-methylated or unmodified amides.

Selected compounds were more active *in vitro* than CTA or TD, and a few of them were also slightly more efficacious *in vivo* than the parent antibiotics in streptococcal septicemia in the mouse. Their degree of activity varied with the structure and length of the N^{15}-alkyl chains.

As a part of a program of chemical transformation of teicoplanin[1] (Fig. 1) aimed at improving its antimicrobial properties and determining structure-activity relationships, we prepared N^{63}-carboxamides[2,3] and N^{15}-alkyl and N^{15},N^{15}-dialkyl[4] derivatives of teicoplanin A2 (CTA), its acidic hydrolysis pseudoaglycones (TB, TC), and aglycone (TD). Conversion of the carboxyl group into an amide often improved the *in vitro* antimicrobial activity and *in vivo* efficacy when the amide had a net positive charge and was relatively lipophilic. Alkylation of the terminal amino group also influenced the *in vitro* and *in vivo* activities of the resulting N^{15}-alkyl and N^{15},N^{15}-dialkyl derivatives; the activity varied with the structure and length of the alkyl chains.

This paper describes the synthesis and antimicrobial activity of a new series of semisynthetic teicoplanin antibiotics obtained by alkylation of the 15-amino group of some amides of CTA and TD which bear aliphatic alkyl chains with or without hydrophilic functions. The aim of this work was to further improve the antimicrobial activities of amides and *N*-alkyl derivatives of CTA and TD by combining the positive effects of these two types of modification. Our expectation was that the alkylated teicoplanin amides would be more lipophilic than the unmodified amides and at the same time more soluble in water than the corresponding alkyl derivatives.

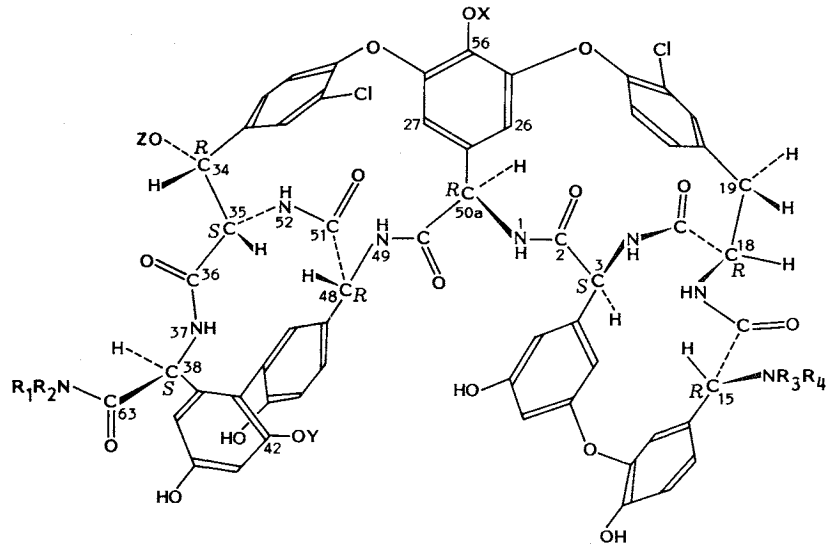
Chemistry

Most of the teicoplanin derivatives listed in Tables 1 and 2 were prepared by alkylation of the 15-NH_2 of the corresponding amides of CTA and TD, following the procedures outlined (Methods A~C; Scheme 1) which mainly depended on the structure of the alkyl chain.

N^{15}-Monoalkyl teicoplanin-amides were obtained by reaction of a suitable CTA- or TD-amide with the appropriate carbonyl compound under basic (pH ~9) conditions in methanol in the presence of sodium borohydride as the reducing agent (Method A).

When initial mild acidic conditions were used, in the presence of formic acid and formaldehyde, N^{15},N^{15}-dimethyl derivatives were obtained (Method B). Specific mechanisms of mono- and dialkylation

Fig. 1. Structure of teicoplanin A2 (CTA) and deglucoteicoplanin (TD), and their N^{15}-alkyl-N^{63}-carboxamide derivatives: NR_1R_2, R_3 and R_4 in Tables 1 and 2.



N-Acyl(NH-COR) side chains:

- Teicoplanin A2-1 R = $(CH_2)_2CH{=}CH(CH_2)_4CH_3$
- Teicoplanin A2-2 R = $(CH_2)_6CH(CH_3)_2$
- Teicoplanin A2-3 R = *n*-C_9H_{19}
- Teicoplanin A2-4 R = $(CH_2)_6CH(CH_3)CH_2CH_3$
- Teicoplanin A2-5 R = $(CH_2)_7CH(CH_3)_2$

CTA X = *N*-Acyl-β-D-glucosaminyl

Y = α-D-Mannosyl

Z = *N*-Acetyl-β-D-glucosaminyl

NR_1R_2 = OH $R_3 = R_4 = H$

TD X = Y = Z = H

NR_1R_2 = OH $R_3 = R_4 = H$

Table 1. N^{15}-Alkylated amides of teicoplanin A2 (CTA) (Fig. 1).

Compound	NR_1R_2	R_3	R_4	Starting product	Method (yield, %)	HPLC[a] t_R (minutes)	Titration[b] EW (found)	MW[c] (calcd)
1	$NH(CH_2)_3NH_2$	H	CH_3	Me-CTA[d]	D (44)	12.4	981	1,953
2	$NH(CH_2)_3NH_2$	CH_3	CH_3	DiMe-CTA[e]	D (53)	12.1	1,002	1,967
(I)	$NH(CH_2)_3NH_2$	H	H	—	—	8.3	—	1,939
3	$NH(CH_2)_2N(CH_3)_2$	CH_3	CH_3	**II**	B (93)	12.5	1,008	1,981
(II)	$NH(CH_2)_2N(CH_3)_2$	H	H	—	—	8.5	—	1,953
4	$NH(CH_2)_3N(CH_3)_2$	H	CH_3	**III**	A (89)	13.2	1,015	1,981
5	$NH(CH_2)_3N(CH_3)_2$	CH_3	CH_3	**III**	B (87)	14.9	1,061	1,995
6	$NH(CH_2)_3N(CH_3)_2$	H	$CH(CH_3)CH_2OH$	**III**	A (86)	10.3	988	2,025
7	$NH(CH_2)_3N(CH_3)_2$	H	$CH(CH_3)CH(CH_3)OH$	**III**	A (90)	12.2	1,003	2,039
8	$NH(CH_2)_3N(CH_3)_2$	H	$CH_2CH(OH)CH_2OH$	**III**	A (53)	9.6	1,014	2,041
9	$NH(CH_2)_3N(CH_3)_2$	H	$CH_2O(CH_2)_2OCH_3$	**III**	C (44)	12.1	1,024	2,055
10	$NH(CH_2)_3N(CH_3)_2$	H	$(CH_2)_2N(CH_3)_2$	**III**	A (54)	12.5	689	2,038
(III)	$NH(CH_2)_3N(CH_3)_2$	H	H	—	—	9.0	—	1,967

[a] See Experimental section. Values referred to teicoplanin A2-2.

[b] Equivalent weights (EWs) determined by acid-base titration. See Experimental section. Values given are corrected for solvent content.

[c] Weighted average values, dealing with mixtures of five components (Fig. 1).

[d] N^{15}-Methyl-CTA.

[e] N^{15},N^{15}-Dimethyl-CTA.

Table 2. N^{15}-Alkylated amides of deglucoteicoplanin (TD) (Fig. 1).

Compound	NR_1R_2	R_3	R_4	Starting product	Method (yield, %)	HPLC[a] t_R (minutes)	Titration[b] EW (found)	MW[c] (calcd)
11	$N[(CH_2)_2]_2O$	H	$CH(CH_3)CH_2OH$	**IV**	A (92)	8.1	1,213	1,326
12	$N[(CH_2)_2]_2O$	H	$(CH_2)_2N(CH_3)_2$	**IV**	A (53)	9.8	671	1,339
(IV)	$N[(CH_2)_2]_2O$	H	H	—	—	7.9	—	1,268
13	$N(CH_3)(CH_2)_3NHCH_3$	H	$CH(CH_3)CH(CH_3)OH$	OBu-TD[d]	D (53)	10.9	687	1,355
14	$N(CH_3)(CH_2)_3NHCH_3$	H	$CH_2CH(OH)CH_2OH$	DiOPr-TD[e]	D (67)	9.8	711	1,357
(V)	$N(CH_3)(CH_2)_3NHCH_3$	H	H	—	—	8.3	—	1,283
15	$NH(CH_2)_3N(CH_3)_2$	H	$CH(CH_3)CH_2OH$	**VI**	A (84)	9.9	743	1,341
16	$NH(CH_2)_3N(CH_3)_2$	H	$CH(CH_3)CH(CH_3)OH$	**VI**	A (86)	11.3	712	1,355
17	$NH(CH_2)_3N(CH_3)_2$	H	$CH_2CH(OH)CH_2OH$	**VI**	A (67)	9.2	759	1,357
18	$NH(CH_2)_3N(CH_3)_2$	H	$CH_2O(CH_2)_2OCH_3$	**VI**	C (36)	14.4	756	1,371
19	$NH(CH_2)_3N(CH_3)_2$	H	$(CH_2)_2N(CH_3)_2$	**VI**	A (37)	11.9	499	1,354
(VI)	$NH(CH_2)_3N(CH_3)_2$	H	H	—	—	9.1	—	1,283

[a] See Experimental section.

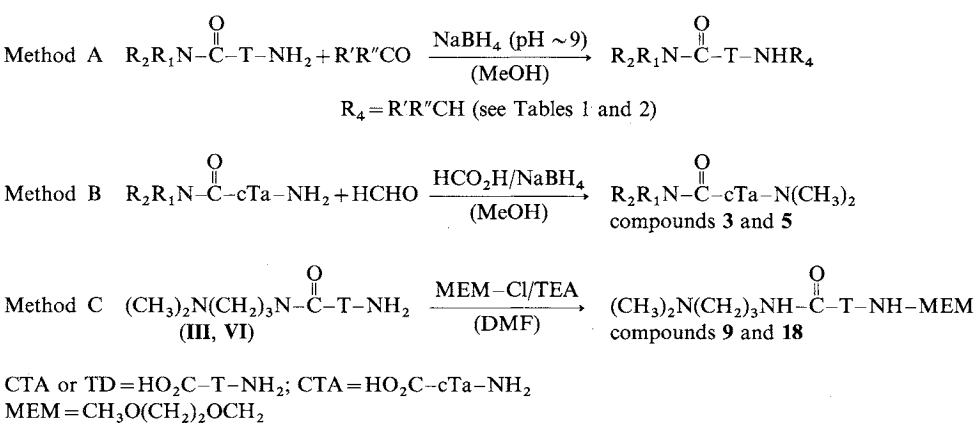
[b] Equivalent weights (EWs) determined by acid-base titration. See Experimental section.

[c] Molecular weight of the compounds of formula: **11**, $C_{65}H_{58}N_8O_{19}Cl_2$; **12**, $C_{66}H_{61}N_9O_{18}Cl_2$; **IV**, $C_{62}H_{52}N_8O_{18}Cl_2$; **13**, $C_{67}H_{65}N_9O_{18}Cl_2$; **14**, $C_{66}H_{63}N_9O_{19}Cl_2$; **V**, $C_{63}H_{57}N_9O_{17}Cl_2$; **15**, $C_{66}H_{63}N_9O_{18}Cl_2$; **16**, $C_{67}H_{65}N_9O_{18}Cl_2$; **17**, $C_{66}H_{63}N_9O_{19}Cl_2$; **18**, $C_{67}H_{65}N_9O_{19}Cl_2$; **19**, $C_{67}H_{66}N_{10}O_{17}Cl_2$; **VI**, $C_{63}H_{57}N_9O_{17}Cl_2$.

[d] N^{15}-[2-(2-Hydroxy)butyl]-TD.

[e] N^{15}-(2,3-Dihydroxy)propyl-TD.

Scheme 1.



of teicoplanin antibiotics with carbonyl compounds in the presence of sodium borohydride, under various reaction conditions, have been reported and discussed in a previous paper.[4]

Compounds **9** and **18** were synthesized from the 3,3-dimethylamino-1-propylamides (**III** and **VI**) of CTA and TD, respectively, by reaction with 2-methoxyethoxymethyl chloride (MEM-Cl) at room temperature in *N*,*N*-dimethylformamide (DMF), in the presence of triethylamine (TEA) (Method C).

Those compounds which possess a free primary or secondary amino group on the N^{63}-amidic side chain were obtained (Method D, Scheme 2) by condensation of the 38-carboxyl group of N^{15}-monoalkyl or N^{15},N^{15}-dialkyl-CTA or TD with the appropriate diamine at room temperature in DMF in the presence of diphenylphosphoryl azide (DPPA) as the condensing agent. The protection of the

Scheme 2.

Method D

$$HO_2C\text{-}cTa\text{-}NR_3R_4 + H_2N(CH_2)_3NH_2 \xrightarrow[(DMF)]{DPPA} H_2N(CH_2)_3NH\text{-}\overset{O}{\overset{\|}{C}}\text{-}cTa\text{-}NR_3R_4$$

compounds **1** and **2**

$$HO_2C\text{-}Td\text{-}NR_3R_4 + CH_3NH(CH_2)_3NHCH_3 \xrightarrow[(DMF)]{DPPA} CH_3NH(CH_2)_3N(CH_3)\text{-}\overset{O}{\overset{\|}{C}}\text{-}Td\text{-}NR_3R_4$$

compounds **13** and **14**

CTA = HO_2C-cTa-NH_2; TD = HO_2C-Td-NH_2

N^{15}-monoalkylamino group was unnecessary in both CTA and TD derivatives under the above conditions.*

The structures of these disubstituted teicoplanins were determined by comparison of their ^{1}H NMR spectra with those of the corresponding N^{15}-alkyl and N^{15},N^{15}-dialkyl derivatives[4] and previously synthesized N^{63}-carboxamides of CTA or TD.[2,3] The presence of the N^{15}-alkyl chain does not modify the chemical shifts of protons belonging to the teicoplanin structure. The N^{15}-CH_2 and -CH protons of the alkyl chains resonate at 3.7~3.3 ppm. As already observed in the ^{1}H NMR spectra of the amides previously described, the presence of an additional signal between δ 3.7 and 3.3, due to the CH_2–N–C=O amidic group at C-38, confirms that the amide and teicoplanin moieties are linked together by an amide bond. Furthermore, in TD derivatives bearing a secondary amide bond, the formation of the amide linkage is also confirmed by the downfield shift (~0.2 ppm) of signal due to proton at C-34, likely caused by an anisotropic effect on 34-H due to a change of conformation in the surroundings induced by a hydrogen bond between the amidic NH and 36-C=O. This behavior had already been observed with amide derivatives of TD described previously.[2] In the other N^{15}-alkylated TD-amides, as in N^{15}-alkylated CTA-amides, the chemical shifts of protons belonging to the teicoplanin structure are unchanged compared to those of unmodified TD and CTA, respectively.[1] Their HPLC retention times (t_R's) and equivalent weights (EW's) determined by acid-base titration are given in Tables 1 and 2. Preliminary data** of solubility in water show that the alkylated amides **6**~**10** of CTA are about twice as soluble as the parent N^{15}-methylated amides **4** and **5** and ~1.5 times as soluble as corresponding amide **III** at pH 7. At this pH, the alkylated amides **13**~**19** of TD are also at least twice as soluble in water as the corresponding unmodified amides **V** and **VI**, while compounds **11** and **12** are 1.5 and four times as soluble as amide **IV**.

Biological Activity

The *in vitro* antibacterial activity and the efficacy against *Streptococcus pyogenes* septicemia in the mouse of the N^{15}-alkylated amides of CTA were compared with those of CTA and the corresponding amides **I**~**III**. With the exception of compound **9** (MIC 4 μg/ml), the new derivatives, like the parent unmodified amides, had significantly better activity against a strain of *Staphylococcus haemolyticus* (L 602) against which CTA had relatively poor activity (MIC 8 μg/ml). As expected,[5] none of these new

* As previously reported,[2] the preparation of the N^{63}-carboxamide derivatives of TD required protection of the free terminal amino group to avoid undesired (intermolecular) side-reactions, while CTA-amides can be obtained directly from CTA using DPPA as the condensing agent.

** Unpublished results, these laboratories.

Table 3. *In vitro* (MIC) and *in vivo* (ED_{50}) antimicrobial activity of N^{15}-alkylated amides of CTA.

Organism	MIC (μg/ml)						
	CTA	**1**	**2**	**I**	**3**	**II**	**4**
Staphylococcus aureus L 165	0.13	0.13	0.13	0.13	0.25	0.13	0.13
S. haemolyticus L 602	8	0.13	0.13	0.13	0.13	0.13	0.13
S. epidermidis ATCC 12228	0.13	0.06	0.06	0.06	0.06	0.06	0.06
Streptococcus pyogenes L 49	0.06	0.06	0.06	0.03	0.06	0.06	0.06
S. pneumoniae L 44	0.06	0.13	0.13	0.13	0.25	0.06	0.13
Enterococcus faecalis ATCC 7080	0.13	0.06	0.13	0.13	0.13	0.13	0.13
ED_{50} (mg/kg)[a] (sc)	0.12	0.13	0.18	0.08	0.31	0.09	0.06

Organism	MIC (μg/ml)						
	5	**6**	**7**	**8**	**9**	**10**	**III**
Staphylococcus aureus L 165	0.5	0.25	0.25	0.25	1	0.13	0.13
S. haemolyticus L 602	1	0.25	0.5	0.5	4	0.25	0.13
S. epidermidis ATCC 12228	0.13	0.13	0.13	0.13	0.13	0.06	0.13
Streptococcus pyogenes L 49	0.13	0.06	0.06	0.13	0.06	0.06	0.06
S. pneumoniae L 44	0.5	0.25	0.13	0.25	0.13	0.13	0.13
Enterococcus faecalis ATCC 7080	0.13	0.13	0.13	0.13	0.25	0.13	0.13
ED_{50} (mg/kg)[a] (sc)	nd[b]	0.23	0.13	0.31	0.06	0.24	0.05

[a] Infecting organism: *S. pyogenes* L 49.
[b] nd: Not determined.

derivatives of CTA had activity against Gram-negative bacteria.*** Among them, compounds **4** and **9** were the most efficacious against *Streptococcus pyogenes* septicemia in the mouse (Table 3); like the corresponding unmodified amide **III**, these two compounds were about twice as active *in vivo* as CTA.

The *in vitro* activity (Table 4) of the N^{15}-alkylated amides of TD against Gram-positive bacteria was comparable to that of TD and corresponding amides **IV**～**VI**. Several of the N^{15}-alkyl amides were more active than TD against *E. coli* but not against the other Gram-negative species tested. Amides **IV**～**VI** were more active against these organisms. Against *S. pyogenes* septicemia in the mouse, only compounds **13** and **18** were more efficacious than TD; the activity of compound **18** was similar to that of the parent amide **VI**, while compound **13** was significantly more active than the corresponding unmodified amide **V**.

Conclusion

The introduction of aliphatic alkyl chains carrying hydrophilic hydroxy, oxy or ionizable basic functions on the free terminal amino group at C-15 of teicoplanin carboxamides further increased the solubility in water of the resulting N^{15}-alkylated amides.

This modification had relatively little effect on the *in vitro* and *in vivo* activity of most of the N^{15}-alkylated amides of CTA, which generally were as active as the N^{63}-carboxamides which had better activity than CTA against *S. haemolyticus* L 602.

The *in vitro* activity of the N^{15}-alkyl-N^{63}-amides of TD against Gram-positive bacteria was also similar to that of TD and the corresponding unmodified N^{63}-carboxamides, but alkylation of the TD-amides reduced the activity against Gram-negative bacteria. The structure of the alkyl chain had little influence on the *in vivo* efficacy of these compounds against streptococcal septicemia in the mouse. However, in one case (compound **13**), the presence of a hydroxybutyl chain resulted in better *in vivo*

*** Data not shown.

Table 4. *In vitro* (MIC) and *in vivo* (ED_{50}) antimicrobial activity of N^{15}-alkylated amides of TD.

Organism	MIC (μg/ml)						
	TD	**11**	**12**	**IV**	**13**	**14**	**V**
Staphylococcus aureus L 165	0.06	0.06	0.06	0.06	0.13	0.06	0.06
S. haemolyticus L 602	0.25	0.25	0.25	nd[a]	0.13	0.13	0.06
S. epidermidis ATCC 12228	0.01	0.06	0.06	0.01	0.06	0.06	0.06
Streptococcus pyogenes L 49	0.13	0.13	0.13	0.13	0.13	0.13	0.06
S. pneumoniae L 44	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Enterococcus faecalis ATCC 7080	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Escherichia coli L 47	64	64	32	16	16	16	2
Proteus vulgaris ATCC 881	128	>128	>128	64	128	64	32
Pseudomonas aeruginosa ATCC 10145	>128	>128	>128	64	>128	128	16
ED_{50} (mg/kg)[b] (sc)	0.95	nd[a]	nd[a]	1.02	0.31	1.25	0.95

Organism	MIC (μg/ml)					
	15	**16**	**17**	**18**	**19**	**VI**
Staphylococcus aureus L 165	0.13	0.13	0.13	0.06	0.13	0.06
S. haemolyticus L 602	0.13	0.13	0.25	0.13	0.13	nd[a]
S. epidermidis ATCC 12228	0.06	0.06	0.06	0.06	0.06	0.03
Streptococcus pyogenes L 49	0.13	0.13	0.13	0.06	0.13	0.06
S. pneumoniae L 44	0.25	0.25	0.5	0.13	0.13	0.13
Enterococcus faecalis ATCC 7080	0.25	0.25	0.25	0.13	0.25	0.13
Escherichia coli L47	16	16	64	8	16	8
Proteus vulgaris ATCC 881	>128	>128	>128	>128	>128	16
Pseudomonas aeruginosa ATCC 10145	128	>128	>128	128	128	32
ED_{50} (mg/kg)[b] (sc)	2.18	1.25	2.87	0.31	nd[a]	0.31

[a] nd: Not determined.
[b] Infecting organism: *S. pyogenes* L 49.

efficacy as compared with TD and the corresponding amide **V**. This may be due to its higher solubility in water at physiological pH and its lipophilicity.

Experimental

Evaporation of solvents was carried out with a rotary evaporator at 40°C under reduced pressure.

Pure final products were obtained by reverse-phase column chromatography using silanized silica gel (0.06~0.2 mm; Merck) as the stationary phase. The compounds were dried *in vacuo* at room temperature overnight.

Reactions, column eluates and final products were checked by HPLC analyses, which were performed on a column Hibar (250×4 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 reference. Elutions were carried out at a flow rate of 2 ml/minute by mixing Eluent A, 0.2% aqueous HCO_2NH_4, with Eluent B, MeCN, according to a linear step gradient from 20 to 60% of B in A in 30 minutes.

All compounds were analyzed for N and Cl on samples previously dried at 140°C under N_2 atmosphere. Weight loss was determined after heating samples at 900°C in O_2 atmosphere. The analytical results were in accordance with the theoretical values.

Acid-base titrations were carried out under the following conditions: The sample was dissolved in

Methyl Cellosolve (MCS)-H_2O (4:1), then an excess of 0.01 N HCl in the same solvent mixture was added and the resulting solution was titrated with 0.01 N NaOH.

^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 tetramethylsilane (TMS, δ 0.00 ppm) as internal reference.

The composition of CTA-derivatives, expressed as the percentage of the areas of peaks (HPLC) of the components of the complex, was approximately the same as that of CTA used as starting material:

Factor	A2-1	A2-2	A2-3	A2-4	A2-5
%	10	50	15	12	13

The solvent content (essentially H_2O) in CTA, TD and their derivatives was always within 10~12% by weight.

Sodium borohydride ($NaBH_4$), pellets, diameter 0.8 cm, 98% (Aldrich-Chemie), was used as the reducing agent.

Reductive-monoalkylation of CTA- and TD-amides (Method A)

General Procedure

To a stirred solution of 10 mmol of the appropriate teicoplanin-N^{63}-carboxamide in 500 ml of MeOH, 20 g (~0.55 mol) of $NaBH_4$ is added in aliquots while maintaining the temperature at 40~45°C. After 2 hours, a large molar excess (50~100 mmol) of the relevant carbonyl compound was added at room temperature and stirring was continued for 1 hour. Then the reaction mixture was cooled to 5°C and 10 g (~0.27 mol) of $NaBH_4$ was added in one portion at 10~15°C. After 1 hour, 50 ml of glacial AcOH was dropped at room temperature and solvents were evaporated. The solid residue was dissolved in 500 ml of H_2O and the resulting solution was loaded on a column of 1.5 kg of silanized silica gel in H_2O. The column was developed with a linear step gradient from 5 to 70% of MeCN in 0.01 N AcOH, in 20 hours at a flow rate of 250 ml/hour, while collecting 25 ml-fractions. Those fractions containing pure products were pooled and enough BuOH was added to obtain, after concentration of the resulting mixture, a dry butanolic suspension. On adding Et_2O the precipitated solid was collected to give the relevant monoalkylated teicoplanin-amide. (Compounds **4**, and **6~10**, Table 1; **11**, **12**, **15~17** and **19**, Table 2).

Reductive-dimethylation of CTA-amides with the 2,2-Dimethylamino-1-ethylamine (**II**) and 3,3-Dimethylamino-1-propylamine (**III**) (Method B)

Preparation of compounds **3** and **5**

To a stirred suspension of amide **II** or **III** in 100 ml of MeOH, 4 ml (~53 mmol) of 40% aqueous HCHO and 0.4 ml (~7 mmol) of HCO_2H were added at room temperature. The clear solution which formed was warmed to 30°C and 0.4 g (~11 mmol) of $NaBH_4$ was added in aliquots while maintaining the temperature at 35~40°C. After 1 hour, 1.25 ml of glacial AcOH was added at room temperature and solvents were evaporated. The residue was dissolved in 100 ml of H_2O and purified by reversed-phase column chromatography on silanized silica gel as described above, yielding pure title compounds. (See Table 1)

Alkylation of CTA- and TD-amides (**III** and **VI**, respectively) with the 3,3-Dimethylamino-1-propylamine by Reaction with MEM-Cl (Method C)

Preparation of N^{15}-(2-Methoxyethoxymethyl) Derivatives **9** and **18**

A solution of 5 mmol of amide **III** or **VI**, 6 ml (~43 mmol) of TEA and 2.8 ml of 2-methoxyethoxymethyl (MEM) chloride in 100 ml of DMF was stirred at room temperature for 1 hour. Then 50 ml of absolute EtOH was added and the resulting solution was poured into 550 ml of EtOAc. The precipitated solid was collected, washed with Et_2O and purified in the usual manner by reversed-phase column chromatography on silanized silica gel, yielding pure compound **9** (44%) or **18** (36%).

Coupling of N^{15}-Alkyl and N^{15},N^{15}-Dialkyl Derivatives of CTA and TD with Amines (Method D)

Preparation of Compounds **1**, **2** and **13**, **14**

To a stirred solution of 2 mmol of the proper N^{15}-alkylated teicoplanin derivative and 5 mmol of the appropriate diamine in 50 ml of DMF, 0.6 ml (~2.8 mmol) of diphenylphosphoryl azide (DPPA) were added dropwise at 5°C. The reaction mixture was stirred at 5°C for 1 hour and at room temperature overnight, then it was poured in 500 ml of Et_2O. The precipitated solid was collected and re-dissolved in 150 ml of a H_2O-MeCN (9:1) mixture. The resulting solution was adjusted to pH 3 with glacial AcOH and then it was loaded on a column of 350 g of silanized silica gel in the same above solvent mixture. Chromatography was carried out as described above, yielding pure title compounds. (Compounds **1** and **2**, Table 1; **13** and **14**, Table 2).

Determination of Antibacterial Activity

MIC's were determined by the microbroth dilution method in Difco Todd-Hewitt broth (Streptococci) or Oxoid Iso-Sensitest broth (other bacteria). The inoculum was ~10^4 cfu/ml.

Experimental septicemia was induced by intraperitoneal injection of ~10^5 cells of *S. pyogenes* L 49, a challenge corresponding to ~100 times the lethal dose for 50% infected animals. Groups of five mice were treated sc once, immediately after infection. On the 7th day, the ED_{50} was calculated by the Spearman-Kärber method,[6] from the percentage of surviving mice at each dose.

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