SYNTHESIS AND BIOLOGICAL ACTIVITY OF N^{63}-CARBOXYPEPTIDES OF TEICOPLANIN AND TEICOPLANIN AGLYCONE

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A series of peptide derivatives of teicoplanin A2 (CTA) and deglucoteicoplanin (TD) was prepared by condensation of the 63-carboxyl function with the α-amino group of selected amino acids and their derivatives.

The modification of the ionic character of CTA and TD influenced their *in vitro* and *in vivo* antimicrobial properties to a different extent, depending on the structure of the amino acidic moiety at C-63. A certain effect on binding strength to Ac_2-L-Lys-D-Ala-D-Ala, a synthetic model of the antibiotic's target peptide, was also observed.

Teicoplanin,[1] a glycopeptide antibiotic recently introduced in therapeutic use for the parenteral treatment of severe infections caused by Gram-positive bacteria,[2] has been extensively studied clinically in the past few years. It was also submitted to chemical modifications aiming at improving the activity against coagulase-negative Staphylococci (CNST) and at broadening the antibacterial spectrum of activity to Gram-negative organisms. The possibility of obtaining teicoplanin derivatives active by oral route was also investigated.

As a preliminary approach, the sugars of teicoplanin A2 (CTA) were hydrolyzed, under acidic conditions, to give the pseudo-aglycones T-A3-1 (TB) and T-A3-2 (TC), and the aglycone (TD, Fig. 1).[3,4] Though to a different extent, these compounds still possessed antimicrobial activity. In particular, TD was more *in vitro* active than CTA against CNST and had a certain activity against Gram-negative bacteria.[3]

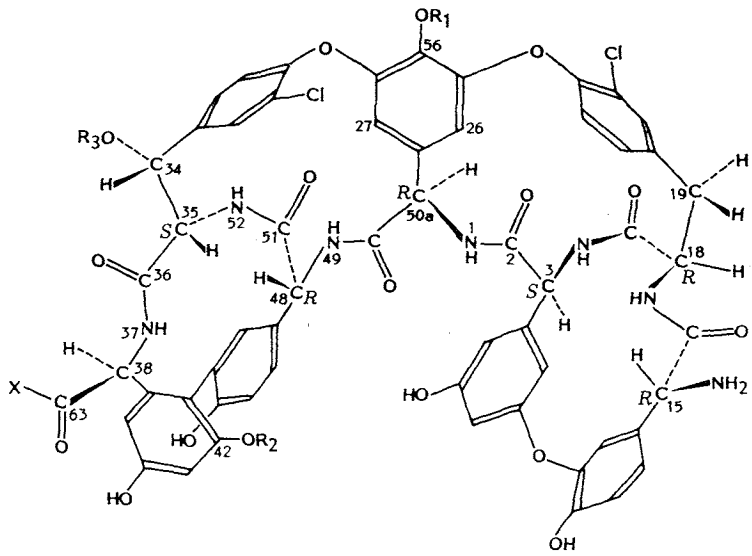
As for the other glycopeptide antibiotics of the vancomycin-ristocetin family, the mechanism of action of teicoplanin consists in the inhibition of the biosynthesis of bacterial peptidoglycan through a complex formation with the terminal D-alanyl-D-alanine of muramyl pentapeptide.[5] The synthesis of some ester derivatives of TC and TD[6] and of a series of amides of CTA, TB, TC and TD[7] indicated that the carboxyl group is not directly involved in the binding with the target peptide. These modifications also led to compounds with better biological properties than those of the corresponding unmodified antibiotics. In particular, some basic amides of TD were more *in vitro* active than TD against Gram-negative organisms, and some basic amides of CTA were more *in vitro* active than CTA against CNST and more *in vivo* effective than CTA in experimental *Streptococcus pyogenes* septicemia in the mouse, by oral route.

In this paper a series of N^{63}-carboxypeptides of CTA and TD with acidic, neutral, basic amino acids and their derivatives is described.

Chemistry

Condensation of the 63-carboxyl group of CTA (Fig. 1) with the α-amino group of selected amino acids was carried out according to the procedures outlined in Schemes 1 and 2.

Fig. 1. Structures of teicoplanin A2 (CTA), its pseudo-aglycones T-A3-1 (TB), T-A3-2 (TC), and aglycone (TD): X=OH, and of CTA and TD peptides: X=NH-CHR'-COR'' (see Tables 1 and 2).



N-Acyl (NHCOR) side chains:

CTA 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*-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 $R_1 = R_2 = R_3 = H$

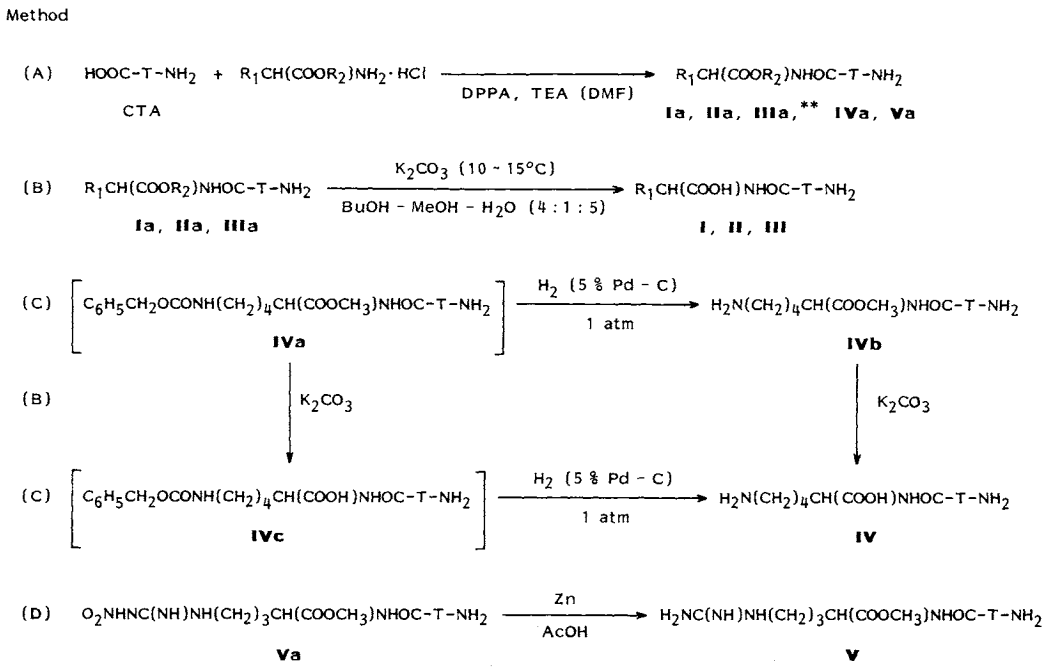
Reaction of CTA with suitably protected amino acids in DMF, in the presence of diphenylphosphorylazide (DPPA) as condensing agent, and triethylamine (TEA) gave the corresponding N^{63}-carboxypeptide derivatives (Method A). Removal of the protecting groups, when present, yielded the compounds listed in Table 1.

The peptides with glycine (**I**), norleucine (**II**), N_ε-benzyloxycarbonyl (CBZ) lysine (**IVc**), and glutamic acid (**III**)† were obtained by saponification of their ethyl ester **Ia**, methyl esters **IIa** and **IVa**, and dimethyl ester **IIIa** with K_2CO_3 in heterogeneous hydroalcoholic BuOH - MeOH - H_2O (4 : 1 : 5) mixture at 10 ~ 15°C (Method B). The peptide with lysine (**IV**) and its methyl ester (**IVb**) were prepared by catalytic hydrogenolysis (1 atm, 5% Pd - C) of **IVc** and **IVa**, respectively (Method C). Compound **IV** was also obtained by basic hydrolysis of **IVb** (Method B). The nitro group of peptide **Va** with N_ω-nitro arginine, methyl ester, was stable to catalytic hydrogenation (1 atm) in the presence of 5 ~ 10% Pd - C. The removal of this protecting function was carried out by reduction with Zn dust at room temperature in 90% aqueous AcOH, thus obtaining **V** (Method D).

Amide derivatives **Ib**, and **IIb**, **IIc** were prepared by condensation of **I** and **II**, respectively, with the appropriate amine, hydrochloride, in the presence of DPPA and TEA (Method A). Compound **Ic** was synthesized by reaction of ester **Ia** in solution in 3,3-diethylamino-1-propylamine, at room temperature

† Compound **III** was also described in a previous paper.[7]

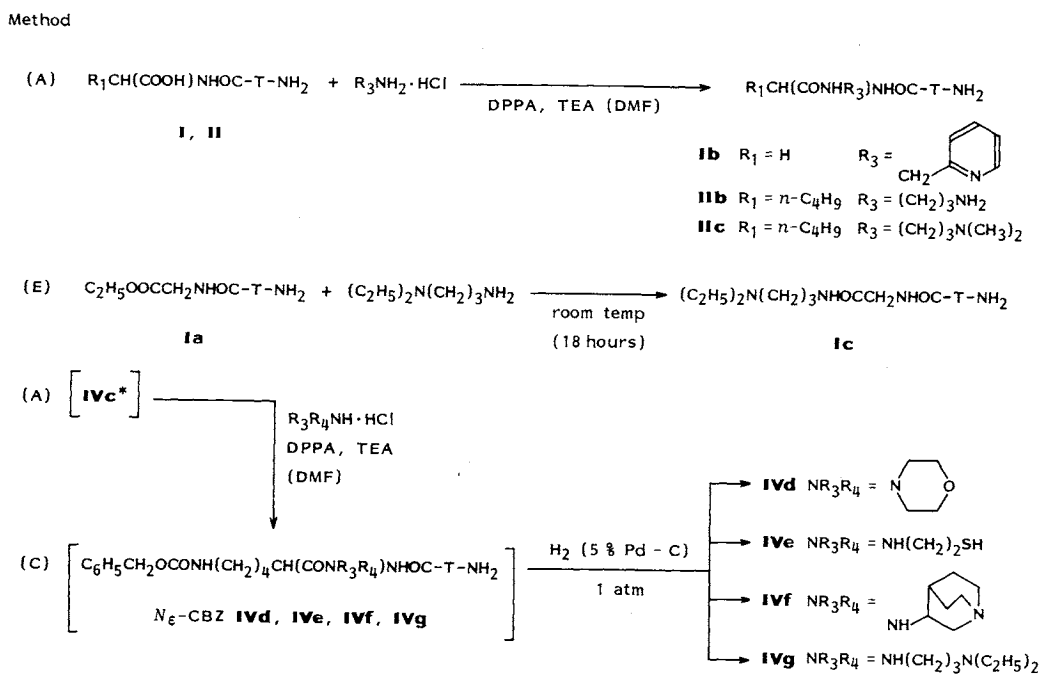
Scheme 1.*



* $R_1=R'$ $OR_2=R''$ in Table 1.

** **IIIa**: $R_1=(CH_2)_2COOCH_3$ $R_2=CH_3$.

Scheme 2.



* See Scheme 1.

Table 1. N^{63}-peptides of CTA (Fig. 1).

Compound	R′	R″	Yield[a] (%)	HPLC[b] t_R (minutes)[e]	pI[c]	MW[d] (found)	Formula[e]	MW[e] (calcd)
I	H	OH	38	15.6	5.5		$C_{90}H_{100}N_{10}O_{34}Cl_2$	1,936.7
Ia	H	OC_2H_5	75	20.8	7.6	2,009	$C_{92}H_{104}N_{10}O_{34}Cl_2$	1,964.8
Ib	H	$NHCH_2(C_5H_4N)$	13	20.4	7.8	1,977	$C_{96}H_{106}N_{12}O_{33}Cl_2$	2,026.9
Ic	H	$NH(CH_2)_3N(C_2H_5)_2$	38	22.4	8.5		$C_{97}H_{116}N_{12}O_{33}Cl_2$	2,049.0
II	n-C_4H_9	OH	27	16.8	5.7		$C_{94}H_{108}N_{10}O_{34}Cl_2$	1,992.8
IIa	n-C_4H_9	OCH_3	45	24.4	7.8		$C_{95}H_{110}N_{10}O_{34}Cl_2$	2,006.9
IIb	n-C_4H_9	$NH(CH_2)_3NH_2$	11	20.2	8.4	2,085	$C_{97}H_{116}N_{12}O_{33}Cl_2$	2,049.0
IIc	n-C_4H_9	$NH(CH_2)_3N(CH_3)_2$	16	29.6	8.6		$C_{99}H_{120}N_{12}O_{33}Cl_2$	2,077.1
III	$(CH_2)_2COOH$	OH	42	14.6	4.3	2,110	$C_{93}H_{104}N_{10}O_{36}Cl_2$	2,008.8
IV[f]	$(CH_2)_4NH_2$	OH	20～25[g]	20.7	7.6	2,082	$C_{94}H_{109}N_{11}O_{34}Cl_2$	2,007.9
IVb[f]	$(CH_2)_4NH_2$	OCH_3	36	24.2	8.8		$C_{95}H_{111}N_{11}O_{34}Cl_2$	2,021.9
IVd[f]	$(CH_2)_4NH_2$	$N(C_4H_8O)$	18	27.2	8.8		$C_{98}H_{116}N_{12}O_{34}Cl_2$	2,077.0
IVe[f]	$(CH_2)_4NH_2$	$NH(CH_2)_2SH$	12	29.3	nd		$C_{96}H_{114}N_{12}O_{33}Cl_2S$	2,067.0
IVf[f]	$(CH_2)_4NH_2$	$NH(C_7H_{12}N)$	23	29.7	8.9	2,256	$C_{101}H_{121}N_{13}O_{33}Cl_2$	2,116.0
IVg[f]	$(CH_2)_4NH_2$	$NH(CH_2)_3N(C_2H_5)_2$	19	26.4	9.0	2,287	$C_{101}H_{125}N_{13}O_{33}Cl_2$	2,120.0
V	$(CH_2)_3NHC(NH)NH_2$	OCH_3	46	26.3	(>9.5)	2,170	$C_{95}H_{111}N_{13}O_{34}Cl_2$	2,049.9
Va	$(CH_2)_3NHC(NH)NHNO_2$	OCH_3	62	22.5	7.8		$C_{95}H_{110}N_{14}O_{36}Cl_2$	2,094.9

[a] Overall yield calculated from CTA.
[b] See Experimental section. Factor A2-2 of CTA: Retention time (t_R) 15.2 minutes.
[c] Isoelectric point determined by IEF (see Experimental section). For CTA, pI 5.8.
[d] Molecular weight determined by acid-base titration (see Experimental section). Values given are not corrected for solvent content.
[e] Of component A2-2.
[f] Component A2-1 free derivatives.
[g] Depending on the procedure followed (see Experimental section).
nd: Not determined.

(Method E). Method A was also applied to **IVc** for the preparation of the N_ε-CBZ derivatives of compounds **IVd**~**IVg**. Hydrogenolysis (Method C) of the N_ε-CBZ group freed the final products (**IVd**~**IVg**).†

Unlike CTA, a preliminary protection of the free amino group of TD was required for the synthesis of the N^{63}-carboxypeptides listed in Table 2. For this purpose, the 15-NH_2 of TD was protected as *N-tert*-butyloxycarbonyl (*N-t*-BOC).[6] Reaction of N^{15}-*t*-BOC-TD with suitably protected amino acids, to give the corresponding N^{15}-*t*-BOC derivatives **VIa**, **VIIa**, **VIIIa** was carried out according to Method A (Scheme 3). The removal of the benzyl(Bz) groups of diester **VIa** and of the N_ε-CBZ of **VIIa**, by catalytic hydrogenolysis (Method C), gave the peptides of N^{15}-*t*-BOC-TD with glutamic acid (**VIb**) and with lysine methyl ester (**VIIb**), respectively. The N^{15}-*t*-BOC group of **VIb** and **VIIb** was then removed with dry TFA at room temperature (Method F), thus obtaining compounds **VI**††and **VIIc**. The peptide with lysine (**VII**) was prepared by basic hydrolysis (Method B) of ester **VIIc**. Treatment of the peptide of N^{15}-*t*-BOC-TD with N_ω-nitro arginine (**VIIIa**) with Zn dust in TFA (Method D') led to a one pot removal of both N_ω- and N^{15}-protecting groups, thus obtaining compound **VIII**.

Diamides **VIc** and **VId** were obtained by condensation of the carboxyl groups of **VIb** with thiomorpholine and 3,3-dimethylamino-1-propylamine, respectively (Method A), followed by the

Table 2. N^{63}-peptides of TD (Fig. 1).

Compound	R'	R''	Yield[a] (%)
VI	$(CH_2)_2COOH$	OH	35
VIc	$(CH_2)_2CON(C_4H_8S)$	$N(C_4H_8S)$	19
VId	$(CH_2)_2CONH(CH_2)_3N(CH_3)_2$	$NH(CH_2)_3N(CH_3)_2$	26
VII	$(CH_2)_4NH_2$	OH	15
VIIc	$(CH_2)_4NH_2$	OCH_3	24
VIIh	$(CH_2)_4NH_2$	$NHCH_2COOC_2H_5$	12
VIIi	$(CH_2)_4NH_2$	$N(CH_3)_2$	13
VIIj	$(CH_2)_4NH_2$	$N(C_4H_8O)$	15
VIII	$(CH_2)_3NHC(NH)NH_2$	OCH_3	26

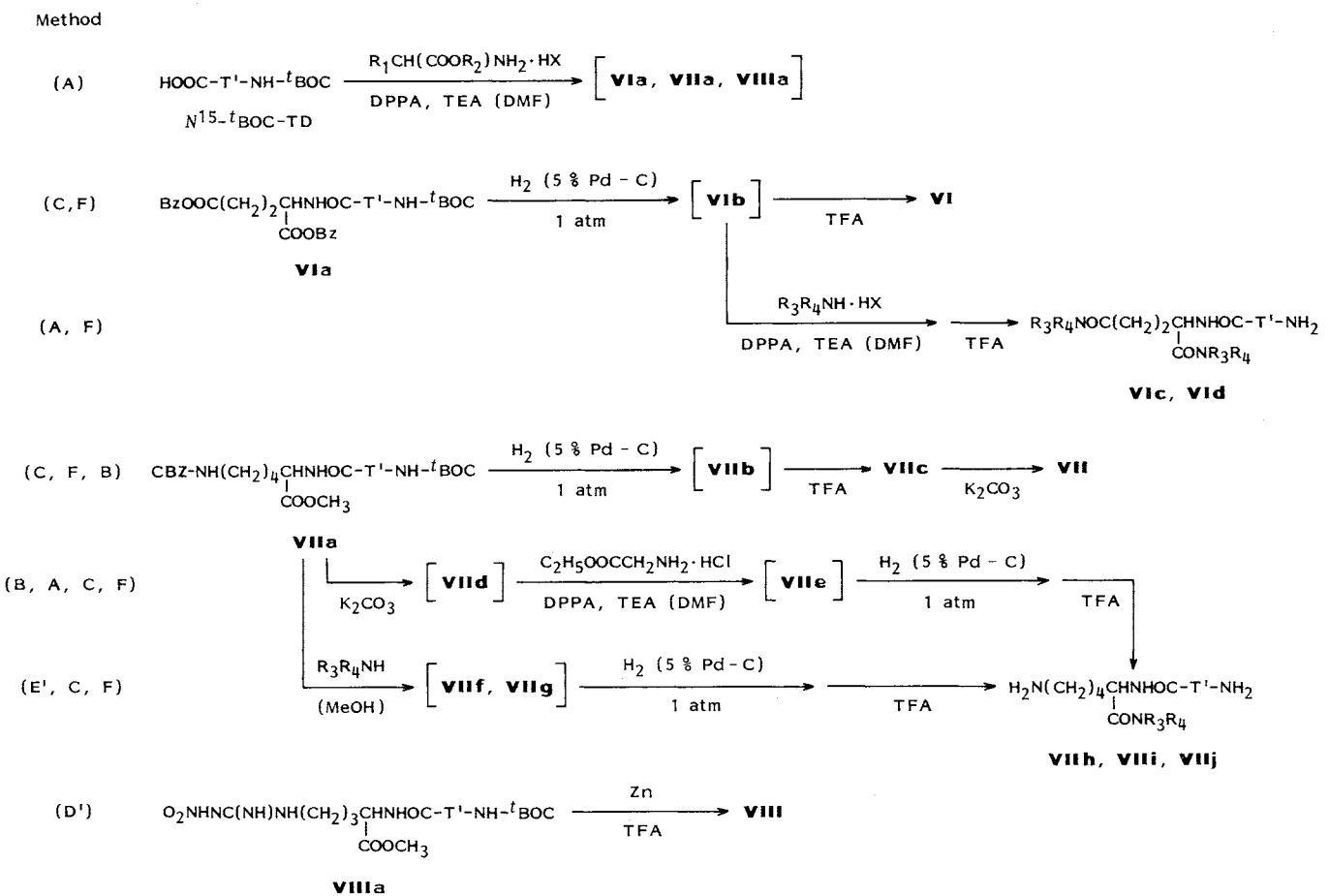
Compound	HPLC[b] t_R (minutes)	pI[c]	MW[d] (found)	Formula	MW (calcd)
VI	9.2	3.6	1,402	$C_{63}H_{52}N_8O_{21}Cl_2$	1,328.1
VIc	23.8	7.4	1,535	$C_{71}H_{66}N_{10}O_{19}Cl_2S_2$	1,498.4
VId	16.7	8.6		$C_{73}H_{76}N_{12}O_{19}Cl_2$	1,496.4
VII	12.8	7.8		$C_{64}H_{57}N_9O_{19}Cl_2$	1,327.1
VIIc	14.2	8.3		$C_{65}H_{59}N_9O_{19}Cl_2$	1,341.1
VIIh	16.8	8.4	1,501	$C_{68}H_{64}N_{10}O_{20}Cl_2$	1,412.2
VIIi	15.1	8.4		$C_{66}H_{62}N_{10}O_{18}Cl_2$	1,354.2
VIIj	15.4	8.3	1,533	$C_{68}H_{64}N_{10}O_{19}Cl_2$	1,396.2
VIII	16.5	(>9.5)	1,514	$C_{65}H_{59}N_{11}O_{19}Cl_2$	1,369.2

[a] Overall yield from TD.
[b] See Experimental section. TD: Retention time (t_R) 9.7 minutes.
[c] Isoelectric point obtained by IEF (see Experimental section). For TD, pI 5.5.
[d] Molecular weight determined by acid-base titration (see Experimental section). Values given are not corrected for solvent content.

† Compounds **IV**, **IVb** and **IVd**~**IVg** are derivatives of factor A2-1 free CTA. In fact, factor A2-1 is invariably transformed into factor A2-3 under hydrogenolytic conditions (U.S. 4,725,668, Feb. 2, 1988).

†† Compound **VI** was also described in a previous paper.[7]

Scheme 3.*



* $NR_3R_4 = R''$ in Table 2.

'BOC: *tert*-Butyloxycarbonyl. HX: HCl or *p*-toluenesulfonic acid.

displacement of N^{15}-*t*-BOC with TFA.

Amides **VIIi** and **VIIj** were prepared by reaction of **VIIa** with dimethylamine and morpholine, respectively, at room temperature in MeOH (Method E'), and subsequent removal of N_ε-CBZ (Method C) and N^{15}-*t*-BOC (Method F) groups from the corresponding protected compounds **VIIf** and **VIIg**.

The dipeptides with lysyl-glycine ethyl ester (**VIIh**) was synthesized following a different procedure. The ester function of **VIIa** was hydrolyzed to give **VIId** (Method B) which was then treated with glycine ethyl ester, hydrochloride, DPPA and TEA in DMF (Method A). The resulting N^{15}-*t*-BOC-N_ε-CBZ derivative (**VIIe**) was submitted to hydrogenation (Method C) and finally to acidic treatment (Method F), thus obtaining **VIIh**.

The protected intermediates (Schemes 1 ~ 3) that are not reported in Tables 1 and 2 were not isolated in a pure state.

The course of the reactions and the homogeneity of final compounds were checked by HPLC.

The structures of these derivatives were determined by ^{1}H NMR. The spectra of CTA peptides show that the protons of the peptidic moieties are present, and that the chemical shifts of CTA protons are unchanged compared to those of unmodified CTA.[1] This behavior had already been observed with the amides of CTA described previously.[7] The spectra of TD peptides are also in accordance with the structures assigned. In this case, the formation of the peptidic bond is confirmed by the downfield shift (~0.2 ppm) of the signal due to proton at C-34, while the overall pattern of the spectra is unchanged with respect to that of TD.[1,4] This downfield shift of 34-H, also diagnostic for the presence of the amide bond in TD amides,[7] is 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 peptidic NH and 36-C=O. The presence of the acetylglucosamine causes, in CTA, a similar downfield shift of 34-H signal. In fact, it is at δ 5.30 in CTA and at δ 5.10 in TD. This is interpreted as due to both the inductive and anisotropic effects of the sugar on 34-H. In CTA peptides, the deshielding effect on this proton is only exerted by the acetylglucosamine, since the peptidic group is prevented from assuming the suitable conformation to affect the chemical shift of 34-H because of the steric hindrance of the sugar. In order to confirm their structures, CTA derivatives **III**, **IV** and **V** were also transformed into the corresponding TD derivatives **VI**, **VII** and **VIII** by acidic hydrolysis of the sugars with gaseous HCl in trifluoroethanol.[4]

The IR spectra of compounds **Ia**, **IIa**, **IVb**, **V**, **Va**, **VIIc** and **VIII** show the $\nu_{C=O}$ ester band at 1725 cm^{-1}.

Acid-base titrations of some representative compounds are in accordance with the structures assigned (Tables 1 and 2).

Peptide Binding Studies

The ability of some peptide derivatives of CTA and TD to complex with the antibiotic's target peptide

Table 3. Association constants[a] with Ac_2-L-Lys-D-Ala-D-Ala.

Compond	K_a(M^{-1})	Compound	K_a(M^{-1})
I	7.4×10^4	**Va**	7.5×10^5
Ia	1.2×10^5	CTA	2.1×10^5
III	4.6×10^5	**VI**	8.2×10^4
IV	7.7×10^4	**VIIc**	3.6×10^4
IVb	6.0×10^4	TD	2.9×10^4
V	9.2×10^4		

[a] Binding constants (K_a) obtained at pH 9 with a standard deviation of about 20% (see Experimental section).

D-Ala-D-Ala was determined by measuring their binding to the synthetic analogue Ac_2-L-Lys-D-Ala-D-Ala in comparison with CTA and TD. The differential UV assay[8] was used. The values of association constants (K_a's, Table 3) determined at pH 9 show that both peptides **III** and **VI** of CTA and TD with glutamic acid possess a binding strength to the tripeptide 2 to 3 times higher than that of CTA and TD, respectively. Moreover, the K_a value of CTA peptide **Va** with N_ω-nitro arginine results about 3.5 times higher than that of CTA, while the ability of the other peptides to complex with Ac_2-L-Lys-D-Ala-D-Ala is comparable to that of the respective unmodified antibiotics or slightly lower. As a general rule, a difference of 2~3 times

Table 4. *In vitro* (MIC)[a] and *in vivo* (ED_{50}) activity of CTA peptides.

Organism	MIC (μg/ml)								
	Teicoplanin	**I**	**Ia**	**Ib**	**Ic**	**II**	**IIa**	**IIb**	**IIc**
Staphylococcus aureus Tour	0.125	0.125	0.25	0.125	0.125	0.5	0.5	0.25	0.125
S. haemolyticus L 602[b]	4~8	4	4	8	0.25	8	16	2	1
S. epidermidis ATCC 12228	0.25	0.125	0.125	0.125	0.063	0.5	0.5	0.125	0.125
Streptococcus pyogenes C 203	0.063	0.125	0.063	0.125	0.063	0.063	0.063	0.125	0.063
S. pneumoniae UC41	0.063	0.125	0.063	0.125	0.125	0.063	0.125	0.125	0.125
S. faecalis ATCC 7080	0.125	0.25	0.125	0.125	0.125	0.25	0.125	0.25	0.125
ED_{50}[c] (mg/kg)									
sc	0.12	0.23	0.10	0.18	0.08	0.31	nd	nd	0.13
po	>170	>300	>300	300	170	300	nd	nd	300

Table 4. (Continued)

Organism	MIC (μg/ml)								
	III	**IV**	**IVb**	**IVd**	**IVe**	**IVf**	**IVg**	**V**	**Va**
Staphylococcus aureus Tour	0.5	0.125	0.125	0.25	0.5	0.125	0.125	0.125	0.125
S. haemolyticus L 602[b]	4	nd	0.5	1	4	0.125	0.125	1	8
S. epidermidis ATCC 12228	0.125	0.25	0.125	0.125	0.125	0.063	0.125	0.125	0.125
Streptococcus pyogenes C 203	0.063	0.063	0.063	0.063	0.125	0.063	0.125	0.063	0.063
S. pneumoniae UC41	0.125	0.125	0.063	0.125	0.125	0.125	0.063	0.063	0.063
S. faecalis ATCC 7080	0.25	0.125	0.125	0.125	0.5	0.125	0.125	0.125	0.125
ED_{50}[c] (mg/kg)									
sc	nd	0.06	0.08	0.13	0.54	0.08	0.08	0.05	0.13
po	nd	90	72	220	270	90	170	300	>300

[a] CTA peptides were inactive against Gram-negative bacteria up to the concentration of 128 μg/ml.
[b] Clinical isolate.
[c] In mice septicemically infected with *S. pyogenes* C 203 (see Experimental section).
nd: Not determined.

Table 5. *In vitro* (MIC) and *in vivo* (ED_{50})[a] activity of TD peptides.

Organism	MIC (μg/ml)									
	TD	VI	VIc	VId	VII	VIIc	VIIh	VIIi	VIIj	VIII
Staphylococcus aureus Tour	0.063	0.125	0.125	0.125	0.125	0.063	0.125	0.063	0.063	0.25
S. haemolyticus 602[b]	0.25	1	1	nd	0.25	0.063	0.125	0.125	0.125	0.5
S. epidermidis ATCC 12228	0.016	0.125	0.063	0.063	0.063	0.063	0.063	0.063	0.063	0.063
Streptococcus pyogenes C 203	0.125	0.125	0.125	0.063	0.125	0.125	0.125	0.063	0.063	0.125
S. pneumoniae UC41	0.125	0.125	0.125	0.063	0.125	0.063	0.125	0.063	0.125	0.125
S. faecalis ATCC 7080	0.125	0.5	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.25
Escherichia coli SKF 12140	64	>128	>128	8	64	4	8	8	16	64
Proteus vulgaris HX19 ATCC 881	128	>128	>128	>128	>128	32	128	64	128	>128
Pseudomonas aeruginosa ATCC 10145	>128	>128	>128	32	>128	32	32	32	32	>128
ED_{50}[c] (mg/kg) sc	0.95	5	8.7	0.95	1.7	0.81	1.3	0.95	0.41	nd

[a] TD peptides were inactive po up to the dose of 300 mg/kg.
[b] Clinical isolate.
[c] In mice septicemically infected with *S. pyogenes* C 203 (see Experimental section).
nd: Not determined.

in the binding strength is not considered significant to confirm an increase in the affinity for the target peptide. However, in this case, it is meaningful that only peptides (**III** and **Va**) carrying a carboxyl or a nitro group on the amino acidic side chain show a binding strength 6~8 times higher than that of compounds (**IV**, **IVb** and **V**) bearing a free amino or guanidino group. In particular, the difference in their K_a values resulting from the comparison of CTA derivatives **Va** and **V** is noteworthy and cannot be overlooked. A possible interaction of the γ-carboxyl and nitro groups of glutamic acid and N_ω-nitro arginine moieties with the acetylated ε-amino group of Ac_2-L-Lys-D-Ala-D-Ala is hypothesized to enforce the binding of CTA derivatives **III** and **Va** with the tripeptide. This might be due to the respective positions of the side chain of lysyl residue of the tripeptide, in the complex with CTA† peptides, and of the side chains of glutamic acid and N_ω-nitro arginine in **III** and **Va**. The possible different orientation of the side chain of glutamic acid in TD peptide **VI** with respect to that of glutamic acid in CTA peptide **III** might be the reason for a lesser, though similar, interaction with the ε-AcNH of di-Ac-lysine. This different orientation could be an effect of the hydrogen bond between the peptidic NH and 36-C=O that should be present in **VI** but absent in **III**, as gathered from ^{1}H NMR spectroscopy.

Further binding studies with Ac_2-L-Lys-D-Ala-D-Ala, by nuclear Overhauser exchange spectroscopy (NOESY), are planned to verify the above hypotheses.

Biological Activity

Table 4 compares the *in vitro* activity and the efficacy in the murine Streptococcal septicemia of CTA peptides with those of teicoplanin. CTA peptides had generally an *in vitro* activity comparable to that of teicoplanin and, like teicoplanin, were not active against *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* (data not shown). Compounds **Ic**, **IVb**, **IVf** and **IVg** showed a marked improvement in the activity against *Staphylococcus haemolyticus*, a species of CNST generally poorly susceptible to teicoplanin. In addition, these compounds and compounds **IV**, **V** had excellent efficacy in the murine model of Streptococcal septicemia upon sc administration. Peptides **IV**, **IVb** and **IVf** were also somewhat more effective than teicoplanin by oral route.

TD derivatives were generally active against Gram-positive bacteria, including *S. haemolyticus*, reflecting the *in vitro* activity of TD (Table 5). This class of compounds also exhibited somewhat activity against Gram-negatives. In particular, peptides **VIIc** and **VIIi** showed the most interesting activity against *E. coli* (MIC 4~8 μg/ml), *P. vulgaris* and *P. aeruginosa* (MIC 32~64 μg/ml). With few exceptions, this chemical modification did not affect the *in vivo* efficacy, as shown by the sc ED_{50} values in Streptococcal septicemia. Like TD, none of these peptides was effective orally up to 300 mg/kg.

Conclusions

The biological properties of teicoplanin peptides were found to mostly depend on their ionic character. In particular, the most active compounds were those possessing a moderate basicity. In fact, a certain increase in the *in vitro* and *in vivo* activities with respect to those of the corresponding unmodified antibiotics was observed with some derivatives of CTA and TD with pI ranging from 8.2 to 9.0, while no improvement was shown by acidic, neutral or more basic peptides. A similar correlation between pI and biological activity had been already found with CTA and TD amides described in a previous paper.[7]

The higher binding strength to D-Ala-D-Ala of acidic teicoplanin peptides had no positive effect on

† The similarities in their structures of teicoplanin and ristocetin suggest that the position of the lysyl residue of Ac_2-L-Lys-D-Ala-D-Ala in the complex with these glycopeptide antibiotics could be the same.[11,12]

the biological activity, likely due to the physico-chemical characteristics of these derivatives that affect their ability to penetrate through the bacterial cell wall.

Experimental

D,L-Amino acid derivatives were used for the synthesis of CTA and TD peptides.

Evaporation of solvents was carried out, after addition of BuOH to prevent foaming, with a rotary evaporator at 40°C under reduced pressure. If not otherwise stated, the intermediates and the final products were washed with Et_2O and dried at room temperature *in vacuo* overnight.

Some crude products were purified by reverse-phase column chromatography on silanized silica gel 60 (0.06~0.2 mm, Merck).

Reactions, column eluates and final products were checked by HPLC analyses, which were performed on a column Hibar (250 × 4 mm, Merck) pre-packed with Li-Chrosorb 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. Elution was carried out at a flow rate of 2 ml/minute by mixing eluent *a*, 0.2% aqueous $HCOONH_4$, with eluent *b*, CH_3CN, according to a linear step gradient programmed as follows:

Time (minutes):	0	10	20	30	40
% of *b* in *a*:	5	25	30	40	5

All compounds were analyzed for C, H, 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 were in accordance with the theoretical values.

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

IR spectra (Nujol) were recorded with a Perkin-Elmer 580 spectrometer.

^1H NMR spectra were obtained with a Bruker instrument AM 250 equipped with an Aspect 3000 console at 250 MHz. The spectra were recorded at 40°C in DMSO-d_6 solution (internal standard TMS, δ 0.00 ppm).

Isoelectrofocusing (IEF) was made on slabs of 24.5 × 11.5 cm and 1 mm thickness prepared on a sheet of Gel Fix (Serva Fenbiochemica), using a LKB Multiphor 2117 cell and a Bio-Rad Power Supply Model 1420 A.

The composition of CTA peptides, expressed as the percentages of the areas of peaks (HPLC) of the components of the complex, was approximately:

Factor	T-A2-1,	T-A2-2,	T-A2-3,	T-A2-4,	T-A2-5
%	10 (0)	50	15 (25)	12	13

N^{63}-Peptides of CTA with

Glycine Ethyl Ester (**Ia**), Norleucine Methyl Ester (**IIa**), Glutamic Acid Dimethyl Ester (**IIIa**), N_ε-CBZ-Lysine Methyl Ester (**IVa**) and N_ω-Nitro-arginine Methyl Ester (**Va**) (Method A)

To a stirred solution of 10 g (about 5 mmol) of CTA in 100 ml of DMF, 1.5 ml (about 11 mmol) of TEA, 5.5 mmol of the proper amino acid ester hydrochloride derivative and 1.4 ml (about 6.5 mmol) of DPPA were added in the order while cooling to 0~5°C. The reaction mixture was stirred at 5~10°C for 6 hours and at room temperature overnight, afterwards 300 ml of EtOAc was added. A solid separated which was collected by filtration and re-dissolved in 400 ml of a mixture of BuOH - EtOAc - H_2O (3 : 2 : 2). The resulting solution was extracted twice with 300 ml of a 1% aqueous $NaHCO_3$, then with 400 ml (2 × 200 ml) of H_2O. The organic layer was separated and concentrated to a small volume (about 50 ml). By adding Et_2O (200 ml) a solid separated which was collected to yield (Table 1) the title compounds (HPLC titer >95%). Compounds **IIIa** (7.6 g) and **IVa** (8.1 g) (HPLC titer about 80%) were not purified for the next step.

Glycine (I), Norleucine (II) and Glutamic Acid (III) (Method B)

Compounds **Ia**, **IIa** and **IIIa** (about 3 mmol) were dissolved in 300 ml of a mixture of BuOH - MeOH - 2% aqueous K_2CO_3 (4 : 1 : 5) at 10°C under stirring. The reaction mixtures were stirred at 10~15°C overnight, then the organic layers were discarded. The aqueous phases were adjusted at pH 3 with 2 N HCl and extracted with 200 ml of BuOH. The organic layers were washed with 200 ml of H_2O and concentrated to a small volume (about 50 ml). By adding EtOAc (200 ml) the title compounds precipitated from the respective butanolic solutions as solids which were collected by filtration to give pure peptides **I**, **II** and crude **III** (HPLC titer 85%).

Crude product **III** (5 g) was dissolved in 100 ml of a mixture of CH_3CN - H_2O (1 : 1) and 35 g of silanized silica gel was added under stirring followed by 400 ml of H_2O. The resulting suspension was loaded at the top of a column of 500 g of the same silica gel in H_2O. The column was developed with a linear gradient from 10 to 80% of CH_3CN in 0.05 N AcOH in 15 hours at the rate of about 300 ml/hour, while collecting 20 ml fractions. Those containing pure components 1~5 of the desired derivative were pooled and the solvents were evaporated to yield 4.2 g of pure compound **III**.

Lysine Methyl Ester (**IVb**) (Method C)

Crude product **IVa** (3 mmol) was dissolved in 500 ml of a MeOH - 0.01 N HCl (7 : 3) mixture and hydrogenated (1 atm, 25°C) in the presence of 3 g of 5% Pd - C. The catalyst was filtered off and washed with 100 ml of MeOH. The clear filtrate was adjusted at pH 7.5 with 1 N NaOH and 50 g of silanized silica gel was added under stirring. The solvents were evaporated and the solid residue was suspended in 500 ml of H_2O. The resulting suspension was loaded at the top of a column of 750 g of the same silica gel in H_2O and the elution was carried out as described above for the purification of compound **III**. Fractions containing pure components 2~5 of peptide **IVb** were pooled and the resulting solution was adjusted at pH 8 with 0.1 N NaOH, then it was concentrated to a small volume (about 30 ml). On standing at 6°C overnight, a solid separated which was collected by filtration, washed with 20 ml of H_2O, and dried at 40°C *in vacuo* for 3 days, to give 3.9 g of pure title compound.

Lysine (**IV**)

a) It was prepared from methyl ester **IVb** according to the same procedure (Method B) as previously described for peptides **I**~**III** (overall yield from CTA: 25%).

b) It was also obtained from crude compound **IVa** through the intermediate derivative **IVc**. In the first step, methyl ester **IVa** was hydrolyzed with K_2CO_3, likewise **Ia**~**IIIa** to **I**~**III** (Method B), to give **IVc** which was not isolated in a pure state. Hydrogenolysis (Method C) of **IVc** was then carried out as described above for **IVb** to give the title compound (overall yield from CTA: 20%).

Arginine Methyl Ester (**V**) (Method D)

To a stirred solution of 4.2 g (about 2 mmol) of compound **Va** in 70 ml of 90% aqueous AcOH, 1 g (about 15 g atom) of Zn dust was added at room temperature. The resulting suspension was stirred for 30 minutes, then it was filtered and 230 ml of EtOAc was added to the clear filtrate. A solid separated which was collected by filtration and purified by reverse-phase column chromatography as described above for **III**, except that the elution was carried out with a linear gradient from 5 to 70% of CH_3CN in H_2O. After purification, 3.1 g of the title compound was obtained.

Amides of N^{G3}-Peptides of CTA

N^{G3}-Peptide of CTA with Glycine-*N*-(2-pyridinyl)methyl-amide (**Ib**)

To a stirred solution of 3 g (about 1.5 mmol) of compound **I** in 50 ml of DMF, 0.4 ml (about 4 mmol) of 2-(aminomethyl)pyridine and 0.65 ml (3 mmol) of DPPA were added at 10°C. The reaction mixture was allowed to warm to room temperature and stirring was continued for 4 hours, then 350 ml of 0.5% aqueous $NaHCO_3$ was added and the resulting cloudy solution was extracted with 500 ml of BuOH. The organic layer was separated, washed twice with 250 ml of H_2O and concentrated to a small volume (about 50 ml). By adding Et_2O (150 ml) a solid separated which was collected by centrifugation and then re-dissolved in 10 ml of DMF. By adding 50 ml of H_2O the precipitated solid was collected by filtration, washed with

H_2O and dried at room temperature *in vacuo* for 4 days over P_2O_5, yielding 1.12 g of **Ib**.

N^{63}-Peptide of CTA with Norleucine-*N*-(3-amino)propyl-amide (**IIb**)

To a stirred solution of 2 g (about 1 mmol) of compound **II** in 20 ml of DMF, 0.3 g (about 2 mmol) of 1,3-diaminopropane dihydrochloride was added at room temperature followed by 0.7 ml (about 5 mmol) of TEA. After cooling to $-5°C$, a solution of 0.3 ml (about 1.4 mmol) of DPPA in 5 ml of dry DMF was added dropwise within 30 minutes, while maintaining the temperature at $-5°C$. Stirring was continued at $-5°C$ for 6 hours and at room temperature overnight, afterwards 80 ml of Et_2O was added. The precipitated solid was collected by filtration and purified by reverse-phase column chromatography (on 150 g of silanized silica gel) as for compound **IVb**, to give 0.82 g of the title compound.

N^{63}-Peptide of CTA with Norleucine-*N*-(3,3-dimethylamino)propyl-amide (**IIc**)

To a stirred solution of 2 g (about 1 mmol) of compound **II** in 40 ml of DMF, 0.38 ml (about 3 mmol) of 3,3-dimethylamino-1-propylamine and 0.17 g (about 1 mmol) of the same diamine dihydrochloride were added at 3°C. After 30 minutes, a solution of 0.4 ml (1.87 mmol) of DPPA in 5 ml of dry DMF was added dropwise while cooling to $-5°C$. The reaction mixture was then allowed to warm to room temperature and stirring was continued overnight. By adding 150 ml of EtOAc a solid separated, which was collected by filtration and purified as for **IIb**, yielding 1.2 g of the title compound.

N^{63}-Peptide of CTA with Glycine-*N*-(3,3-diethylamino)propyl-amide (**Ic**)

Method E: A solution of 4 g (about 2 mmol) of compound **Ia** in 30 ml of 3,3-diethylamino-1-propylamine was stirred at room temperature overnight, then 270 ml of Et_2O was added. The precipitated solid was collected and purified on a column of 600 g of silanized silica gel as for **IIb**, giving 2.1 g of compound **Ic**.

N^{63}-Peptides of CTA with Lysine-*N*-morpholinyl (**IVd**), -*N*-(2-Mercaptoethyl) (**IVe**), -*N*-(3-Quinuclidinyl) (**IVf**) and -*N*-(3,3-Diethylamino)propyl (**IVg**)-amides

Crude compound **IVa** (about 2.5 mmol) was dissolved in 250 ml of a mixture of BuOH - MeOH - 2% aqueous K_2CO_3 (4 : 1 : 5) at 10°C under stirring. Stirring was continued at room temperature overnight, then the reaction mixture was worked up as described previously for derivatives **I**～**III**. Crude product **IVc** was thus obtained which was dissolved in DMF (40 ml), then 0.75 ml (about 5.5 mmol) of TEA, 2.8 mmol of the proper amine hydrochloride and 0.7 ml (about 3.3 mmol) of DPPA were added in the order while cooling to 5～10°C. The reaction mixture was stirred at 5～10°C for 6 hours and at room temperature overnight, afterwards 10 ml of Et_2O was added. The precipitated solid was collected by centrifugation and re-dissolved in 400 ml of a MeOH - 0.5 N HCl (7 : 3) mixture. The resulting solution was hydrogenated (1 atm, 25°C) in the presence of 4 g of 5% Pd - C. The catalyst was filtered off, washed with 100 ml of MeOH and the clear filtrate was adjusted at pH 5 with 1 N NaOH. Most of the MeOH was removed at 30°C under reduced pressure, and the resulting aqueous solution was loaded at the top of a column of 500 g of silanized silica gel in H_2O. Chromatography was carried out as for peptide **IVb** and the final products were isolated in the same manner as above, to give the title compounds, as the free bases.

N^{15}-tert-Butyloxycarbonyl(*t*-BOC)-TD

A solution of 12 g (10 mmol) of TD, 5 ml (36 mmol) of TEA and 5.4 g (18 mmol) of 2,4,5-trichlorophenyl-*tert*-butylcarbonate in 200 ml of DMF was stirred at room temperature for 18 hours. By adding 800 ml of Et_2O the precipitated solid was collected by filtration and re-dissolved in 1.5 liters of a mixture MeOH - H_2O (2 : 8). The resulting solution was adjusted at pH 3 with 1 N HCl and extracted with 2 liters of a mixture of EtOAc - BuOH (8 : 2). The organic layer was separated, washed with 1 liter of H_2O, then it was concentrated to a small volume. By adding Et_2O a precipitate formed which was filtered off to give 12.5 g (96%) of the title compound.

N^{63}-Peptides of TD with

Glutamic Acid (**VI**)

To a stirred solution of 6.5 g (5 mmol) of N^{15}-*t*-BOC-TD in 100 ml of DMF, 2.8 g (6 mmol) of glutamic

acid dibenzyl ester *p*-toluenesulfonate, 1.5 ml (11 mmol) of TEA and 1.4 ml (about 6.5 mmol) of DPPA were added while cooling to 5~10°C. After 6 hours at 10°C and overnight at room temperature, 400 ml of EtOAc was added and the precipitated solid was collected by centrifugation, then it was re-dissolved in 150 ml of a mixture MeOH - H_2O (2 : 3). The resulting solution was adjusted at pH 3 with 1 N HCl and 1 liter of a mixture of EtOAc - BuOH - H_2O (4 : 2 : 4) was added under stirring. The organic layer was separated, washed with 300 ml of H_2O, then with 300 ml of 1% aqueous $NaHCO_3$, and finally twice with 200 ml of H_2O; afterwards it was concentrated to a small volume. By adding Et_2O a solid separated which was collected by filtration, yielding 6.8 g of crude (HPLC titer 75%) N^{15}-*t*-BOC derivative of **VI** dibenzyl ester (**VIa**).

Crude compound **VIa** was dissolved in 400 ml of a mixture of MeOH - 0.05 N HCl (9 : 1) and the resulting solution was hydrogenated (1 atm, room temperature) over 7 g of 5% Pd - C. The suspension was diluted with 400 ml of H_2O and then adjusted at pH 8.5 with 2.5 N NaOH. The catalyst was then removed by filtration through a panel of Celite BDH-545 filter aid. After adding 400 ml of BuOH, most of the MeOH was evaporated, then the organic layer was separated and discarded. The remaining aqueous solution was adjusted at pH 3.5 with 1 N HCl and concentrated to a small volume. After standing at 6°C overnight a solid separated which was collected to give 3.2 g of the crude (HPLC titer about 85%) N^{15}-*t*-BOC derivative (**VIb**) of the title compound.

Crude compound **VIb** was dissolved in 30 ml of dry TFA at room temperature under stirring, then the solvent was evaporated. The solid residue was suspended in 300 ml of H_2O and 2 N NaOH was added under stirring at 10°C to obtain a clear solution at pH 7.9 which was loaded on a column of 300 g of silanized silica gel in H_2O. The column was developed with a linear gradient from 5 to 50% of CH_3CN in 0.001 N HCl in 20 hours at the rate of about 150 ml/hour, while collecting 15 ml fractions. Those containing pure title compound were pooled and most of the CH_3CN was evaporated. The resulting aqueous solution was adjusted at pH 4.2 with 0.5 N NaOH and the precipitated solid was collected, washed with H_2O and dried at room temperature *in vacuo* for 4 days over P_2O_5, yielding 2.4 g of **VI**.

Lysine Methyl Ester (**VIIc**) and Arginine Methyl Ester (**VIII**)

1) N^{G3}-Peptides of N^{15}-*t*-BOC-TD with N_ε-CBZ-Lysine Methyl Ester (**VIIa**) and N_ω-Nitroarginine Methyl Ester (**VIIIa**): To a stirred solution of 6.5 g (5 mmol) of N^{15}-*t*-BOC-TD in 100 ml of DMF, 1.5 ml (about 11 mmol) of TEA, 5.5 mmol of the proper *N*-protected amino acid methyl ester hydrochloride and 1.4 ml (about 6.5 mmol) of DPPA were added in the order at 5~10°C. The reaction mixture was stirred at room temperature for 2 days, then it was poured into 1.2 liters of a mixture of H_2O - EtOAc - BuOH (5 : 2 : 5) under stirring. The organic layer was separated and extracted with 0.01 N HCl (300 ml), then with H_2O (2 × 400 ml), with 2.5% aqueous $NaHCO_3$ (3 × 300 ml), and finally with H_2O (2 × 300 ml), afterwards it was concentrated to a small volume (about 50 ml). By adding Et_2O (250 ml) a solid separated which was collected and dried to give the title compounds **VIIa** (6.8 g, HPLC titer 80%) and **VIIIa** (6.6 g, HPLC titer 83%). These products were not purified for the next step.

2) Preparation of Compound **VIIc**: Crude product **VIIa** (1 mmol) was dissolved in 400 ml of a MeOH - 0.01 N HCl (8 : 2) mixture and hydrogenated (1 atm, 25°C) in the presence of 2 g of 5% Pd - C. The catalyst was filtered off and washed with 200 ml of a mixture of MeOH - H_2O (1 : 1). The clear filtrate was adjusted at pH 8 with 2 N NaOH and concentrated to a small volume to give a cloudy aqueous solution of about 50 ml. On standing at 6°C overnight, a solid separated which was collected by centrifugation, washed with 50 ml of H_2O, and dried *in vacuo* at room temperature overnight over P_2O_5, yielding 1.2 g of crude (HPLC titer 75%) N^{15}-*t*-BOC derivative (**VIIb**) of **VIIc**.

This product was dissolved in 15 ml of dry TFA at room temperature under stirring, then the solvent was evaporated. The oily residue was dissolved in 150 ml of a mixture of H_2O - CH_3CN (9 : 1) and the resulting solution was loaded on a column of 150 g of silanized silica gel in H_2O. Elution was performed by a linear gradient from 10 to 50% of CH_3CN in 0.005 N HCl in 15 hours at the rate of 150 ml/hour, while collecting 20 ml fractions. Those containing pure title compounds were pooled and most of the CH_3CN was evaporated. The concentrated cloudy aqueous solution was adjusted at pH 8 with 1 N NaOH and the solid which separated was collected, washed with 10 ml of H_2O and dried *in vacuo* at room temperature overnight over P_2O_5 to give 0.35 g of **VIIc**.

3) Preparation of Compound **VIII** (Method D'): To a stirred solution of 6 g (about 3.5 mmol)

of crude **VIIIa** in 150 ml of TFA, 2 g (about 30 g atom) of Zn dust was added at room temperature and the suspension was stirred for 1 hour. The insoluble matter was filtered off and the solvent was evaporated. The oily residue was purified by column chromatography as above described for **VIIc**, yielding 1.9 g of the title compound.

Lysine (**VII**)

It was prepared from **VIIc** according to the same procedure (Method B) as previously described for CTA-peptides **I**~**IV**.

Diamides of TD-Peptide **VI** with

Thiomorpholine (**VIc**)

To a stirred solution of 3.2 g of crude (HPLC titer about 85%) **VIb** (see the preparation of compound **VI**) in 30 ml of DMF, 0.56 g (4 mmol) of thiomorpholine hydrochloride, 0.83 ml (6 mmol) of TEA and 0.97 ml (4.5 mmol) of DPPA were added at 0~5°C. After 4 hours at 5°C, 50 ml of MeOH was added and the resulting solution was poured into 400 ml of Et_2O. The precipitated solid was collected and dissolved in 50 ml of dry TFA at room temperature under stirring. The solvent was evaporated and the oily residue was dissolved in 300 ml of a mixture of H_2O-CH_3CN (9:1). The resulting solution was loaded on a column of 750 g of silanized silica gel in H_2O. The column was developed with a linear gradient from 10 to 70% of CH_3CN in H_2O in 20 hours at the rate of 200 ml/hour, while collecting 20 ml fractions. Those containing pure title compound were pooled and most of the CH_3CN was evaporated. The resulting cloudy aqueous solution was adjusted at pH 8 with 1 N NaOH and the precipitated solid was collected by centrifugation, washed with H_2O and dried at 30°C *in vacuo* overnight over P_2O_5, to give 1.6 g of **VIc**.

3,3-Dimethylamino-1-propylamine (**VId**)

To a stirred solution of 3.2 g of crude **VIb** (see above) in 45 ml of DMF, 0.55 ml (4 mmol) of TEA, 0.25 ml (2 mmol) of 3,3-dimethylamino-1-propylamine and 0.7 g (4 mmol) of the same diamine dihydrochloride were added at room temperature. After 30 minutes, 0.97 ml (4.5 mmol) of DPPA were added at 5~10°C and stirring was continued at 10°C for 4 hours and at room temperature overnight. On adding 250 ml of EtOAc a solid separated which was collected by filtration and dissolved in 75 ml of dry TFA at room temperature under stirring. The solvent was evaporated and the solid residue was dissolved in 300 ml of H_2O. The resulting solution was loaded on a column of 750 g of silanized silica gel in H_2O. The chromatography and recovery of pure title compound were carried out as described above for **VIc**, yielding 1.9 g of **VId**.

Amides of TD-Peptide **VII** with

Glycine Ethyl Ester (**VIIh**)

Crude methyl ester **VIIa** (6.5 g, about 3.5 mmol) was dissolved in 500 ml of a mixture of BuOH-MeOH-2% aqueous K_2CO_3 (5:1:4) at room temperature under stirring. After 48 hours, the aqueous phase was adjusted at pH 4 with 2 N HCl and the organic layer was separated, washed with 200 ml of H_2O, and then concentrated to a small volume (about 50 ml). By adding Et_2O (250 ml) a solid, which separated, was collected to give crude acid **VIId** (5.8 g, HPLC titer 82%).

A solution of 5.8 g of crude compound **VIId**, 0.56 g (4 mmol) of glycine ethyl ester hydrochloride, 1 ml (about 7 mmol) of TEA, and 0.75 ml (3.5 mmol) of DPPA in 75 ml of DMF was stirred at room temperature for 4 hours, afterwards 75 ml of MeOH was added. By adding 350 ml of Et_2O a solid separated which was collected by centrifugation to yield 5.9 g of the crude N^{15}-*t*-BOC-N_ε-CBZ derivative **VIIe** of the title compound (HPLC titer about 65%).

It was dissolved in 500 ml of a mixture of MeOH-DMF-0.04 N HCl (5:3:2) and hydrogenated (1 atm, room temperature) over 5 g of 5% Pd-C. The catalyst was filtered off and the filtrate was concentrated to a final volume of about 150 ml. By adding 450 ml of Et_2O the precipitated solid was collected and re-dissolved in 100 ml of dry TFA. The solvent was evaporated and the oily residue was purified by column chromatography as described above for **VIIc**, yielding 0.94 g of **VIIh**.

Dimethylamine (**VIIi**) and Morpholine (**VIIj**) (Method E')

To a stirred suspension of 6.5 g (about 3.5 mmol) of crude methyl ester **VIIa** in 100 ml of MeOH, a solution of 0.5 mmol of the proper amine in 200 ml of MeOH was added at room temperature. The reaction mixture was stirred at room temperature for 24 hours, then it was concentrated to a final volume of about 100 ml. By adding 400 ml of Et_2O the precipitated solid was collected to give crude (HPLC titer about 80%) N^{15}-*t*-BOC-N_ε-CBZ amides **VIIf** (6.4 g) and **VIIg** (6.6 g) which were hydrogenated (1 atm, room temperature) in solution of 600 ml of a mixture MeOH - 0.05 N HCl (8 : 2) in the presence of 4 g of 5% Pd - C. After filtration of the catalyst, the solvents were evaporated and the solid residue was dissolved in 120 ml of dry TFA at room temperature. Evaporation of the solvent gave an oily residue which was purified by column chromatography as described above for **VIIc**, to give the title compounds (**VIIi**, 0.95 g; **VIIj**, 1.1 g).

Binding Assays

The interaction of Ac_2-L-Lys-D-Ala-D-Ala with CTA, TD, and their selected peptides was determined by UV differential spectroscopy.[8] Experiments were run on a Perkin-Elmer 320 double-beam spectrophotometer with 4 cm pathlength not thermostated cells. The temperature was 24 ± 2°C. The initial volume of antibiotic solution was 10 ml at 30 μM concentration in 10% MeOH in sodium phosphate buffer (pH 9). The difference in absorbance (ΔA) developed on adding the test tripeptide was monitored at wavelength (294 nm) that showed the maximum change. The K_a for complex formation of each derivative was obtained from the slope of the straight line resulting from a SCATCHARD's plot, $\Delta A/(\Delta A_{max} \times C)$ vs. $\Delta A/\Delta A_{max}$, of the data. Binding constants were obtained with a standard deviation of about 20%.[9]

Determination of Antibacterial Activity

MIC was determined using microdilution method in Difco Todd-Hewitt broth (Streptococci) or Oxoid Iso-Sensitest broth (Staphylococci and Gram-negative organisms). The final inoculum was about 10^4 cfu/ml. MIC was read as the lowest concentration (expressed in μg/ml) which showed no visible growth after 18 ~ 24 hours incubation at 37°C.

Experimental septicemia was induced in groups of five mice by intraperitoneal injection of about 10^5 cells of *S. pyogenes* C 203, a challenge corresponding to about 100 times the lethal does for 50% infected animals. Mice were treated once immediately after infection by sc or po route. On the 7th day, ED_{50} (effective dose for 50% infected animals, expressed in mg/kg) was calculated on the basis of the percentage of surviving mice at each dose, by the Spearmann and Kärber method.[10]

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References

1) CORONELLI, C.; G. G. GALLO & B. CAVALLERI: Teicoplanin: Chemical, physico-chemical and biological aspects. Il Farmaco, Ed. Sci. 10: 767 ~ 786, 1987
2) DURRANDE, J. B.; Y. DUMAS & P. DANGLAS: Teicoplanin: A new glycopeptide antibiotic. J. Pharm. Clin. 7: 225 ~ 243, 1988
3) MALABARBA, A.; P. STRAZZOLINI, A. DEPAOLI, M. LANDI, M. BERTI & B. CAVALLERI: Teicoplanin, antibiotics from *Actinoplanes teichomyceticus* nov. sp. VI. Chemical degradation: Physico-chemical and biological properties of acid hydrolysis products. J. Antibiotics 37: 988 ~ 999, 1984.
4) MALABARBA, A.; P. FERRARI, G. G. GALLO, J. KETTENRING & B. CAVALLERI: Teicoplanin, antibiotics from *Actinoplanes teichomyceticus* nov. sp. VII. Preparation and NMR characteristics of the aglycone of teicoplanin. J. Antibiotics 39: 1430 ~ 1442, 1986
5) SOMMA, S.; L. GASTALDO & A. CORTI: Teicoplanin, a new antibiotic from *Actinoplanes teichomyceticus* nov. sp. Antimicrob. Agents Chemother. 26: 917 ~ 923, 1984
6) MALABARBA, A.; A. TRANI, P. FERRARI, R. PALLANZA & B. CAVALLERI: Synthesis and biological activity of some esters of the *N*-acetylglucosaminyl aglycone and of the aglycone of teicoplanin. J. Antibiotics 40: 1572 ~ 1587, 1987
7) MALABARBA, A.; A. TRANI, P. STRAZZOLINI, G. CIETTO, P. FERRARI, G. TARZIA, R. PALLANZA & M. BERTI: Synthesis and biological properties of N^{63}-carboxamides of teicoplanin antibiotics. Structure-activity relationships. J. Med.

Chem. 32: 2450~2460, 1989

8) NIETO, M. & H. R. PERKINS: The specificity of combination between ristocetin and peptides related to bacterial cell wall mucopeptide precursors. Biochem. J. 124: 845~852, 1971

9) HARRIS, C. M.; S. W. FESIK, A. M. THOMAS, R. KANNAN & T. M. HARRIS: Iodination of vancomycin, ristocetin A, and ristocetin pseudoaglycon. J. Org. Chem. 51: 1509~1513, 1986

10) FINNEY, D. J.: Statistical Method in Biological Assay. pp. 524~530, Charles Griffin & Co., Ltd., 1952

11) WILLIAMSON, M. P. & D. H. WILLIAMS: ^{1}H N.M.R. studies of the structure of ristocetin A and of its complexes with bacterial cell wall analogues in aqueous solution. J. Chem. Soc. Perkin Trans. I 1985: 949~956, 1985

12) MUELLER, L.; S. L. HEALD, J. C. HEMPEL & P. W. JEFFS: Determination of the conformation of molecular complexes of the aridicin aglycon with Ac_2-L-Lys-D-Ala-D-Ala and Ac-L-Ala-γ-D-Gln-L-Lys(Ac)-D-Ala-D-Ala: An application of nuclear magnetic resonance spectroscopy and distance geometry in the modeling of peptides. J. Am. Chem. Soc. 111: 496~505, 1989