N^{15}-ALKYL AND N^{15},N^{15}-DIALKYL DERIVATIVES OF TEICOPLANIN ANTIBIOTICS

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The synthesis and the biological properties of a series of N^{15}-alkyl and N^{15},N^{15}-dialkyl derivatives of teicoplanin A2, its pseudoaglycones and aglycone are described.

The alkylation of the terminal amino group did not affect the ability of these teicoplanin derivatives to bind with Ac_2-L-Lys-D-Ala-D-Ala, a synthetic model of the antibiotic's target peptide in bacterial cell walls, but influenced their *in vitro* and *in vivo* antimicrobial activities to a different extent, depending on the structure and length of the alkyl chains and the type and number of sugars present.

Teicoplanin,[1] a glycopeptide antibiotic of the vancomycin-ristocetin group (dalbaheptides),[2,3] was recently introduced in therapeutic use for the parenteral treatment of severe infections caused by Gram-positive bacteria.[4] Like the other dalbaheptides, its mechanism of action consists in the inhibition of the biosynthesis of bacterial cell wall through the formation of a complex with terminal peptide D-Ala-D-Ala of peptidoglycan precursors.[5,6]

As a part of the program of chemical transformation of teicoplanin aimed at broadening the antimicrobial spectrum to coagulase-negative Staphylococci[7] and Gram-negative bacteria, and at achieving some oral absorption, a series of amides[8] of teicoplanin A2 (CTA), its acidic hydrolysis pseudoaglycones (TB, TC) and aglycone (TD), a series of peptides[9] of CTA and TD, and some esters[10] of TC and TD were previously prepared. The findings were that the modification of the carboxyl group does not affect the ability of these derivatives to bind to Ac_2-L-Lys-D-Ala-D-Ala, a synthetic analogue of the antibiotic's target peptide,[11] but has an influence on the antimicrobial activity related to their ionic and lipophilic character. On the contrary, the terminal amino group was proved to play an important role as a base in the first step of complex formation with the target peptide, since *N*-acylation[12] and deamination[13] at C-15 lower the binding strength by approximately one order of magnitude. Though to a different extent,† this was reflected in the decreased *in vitro* activity of the resulting acyl and deamino derivatives.

In order to establish a correlation between lipophilicity and biological activity of teicoplanin antibiotics while maintaining substantially unmodified their isoelectric point, and preserving the binding strength to the synthetic tripeptide by retaining the basic character of the nitrogen at C-15, a series of N^{15}-alkyl derivatives carrying different aliphatic chains and functional groups have been synthesized. In this paper, the synthesis and some biological properties of a few N^{15},N^{15}-dialkyl teicoplanins are also reported.

Chemistry

The alkylation of the 15-amino group of CTA, TB, TC and TD (Fig. 1) was carried out following

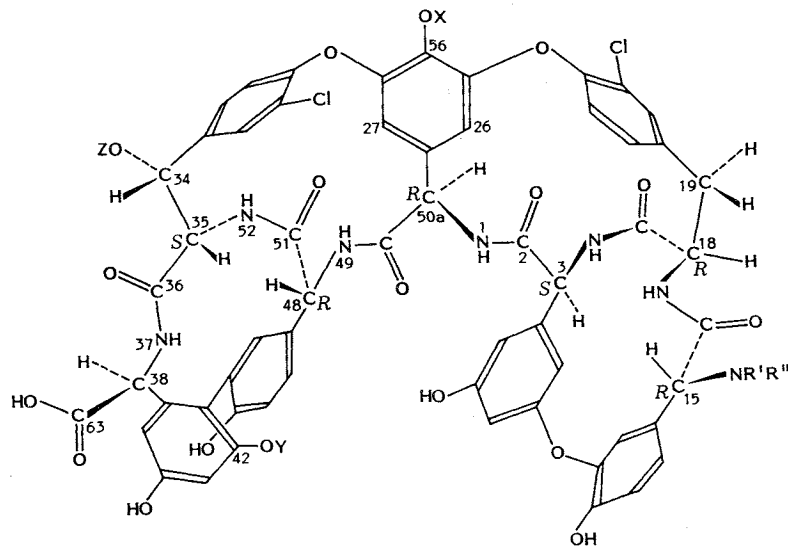
† The *in vitro* activity of the N^{15}-acetyl derivative of TC was about ten times lower (unpublished data; these laboratories) than that of TC, while the loss of the terminal amino group reduced the activity of deamino teicoplanins[13] by only twice to three times with respect to the corresponding unmodified antibiotics.

different procedures depending on the structure of the alkyl chain.

Lower N^{15}-alkyl or N^{15},N^{15}-dialkyl derivatives were selectively prepared according to the "reductive alkylation" method (Scheme 1),[14] by reaction of the above cited teicoplanins with appropriate carbonyl compounds in methanol in the presence of sodium borohydride as the reducing agent.

At basic pH (~9), only monoalkyl teicoplanins were obtained when the aldehyde or ketone was added to a previously prepared methanolic solution (or suspension) of a teicoplanin antibiotic and sodium borohydride (or 0.1 M potassium acetate) and the resulting mixture was then treated with an additional amount of the reducing agent (Method A). This was somewhat surprising with short chain aldehydes since they are usually so reactive that they give mixtures of mono- and dialkylated compounds, especially when the reaction is carried out under basic conditions. In the case of teicoplanins, the high selectivity of monoalkylation at basic pH might be tentatively explained by the particular peptidic environment of terminal NH_2 which could be involved in a five membered hydrogen bonding system **a**, as depicted in Fig. 2. The electron pair on the nitrogen atom would be engaged in the formation of an hydrogen bond with the hydroxyl group of the adjacent amide, which can exist in the enolic form just at basic pH. The dehydration of intermediate hemiaminal **b** to give the corresponding Schiff base **c**† would be favored by the unusual relative acidity of the proton on the nitrogen atom. The impairment of the basicity of the monoalkylated

Fig. 1. Structures of teicoplanin antibiotics (R′=R″=H), and their alkyl derivatives (R′, R″, see Tables 1~4).



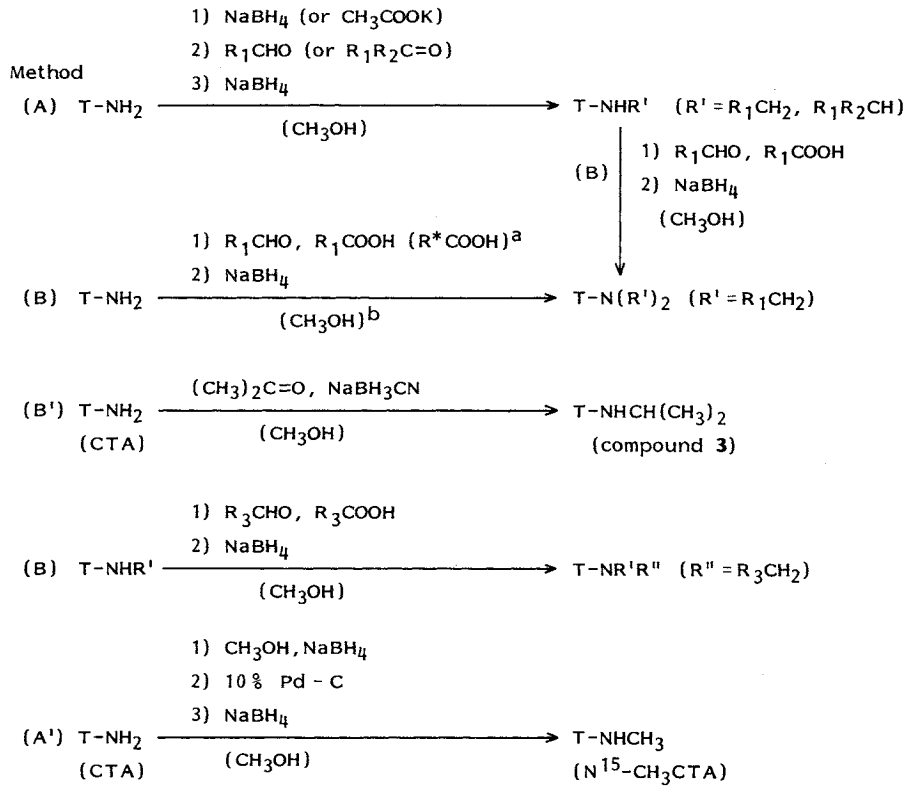
N-Acyl (NH-COR) side chains:

CTA X=*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=$(CH_2)_7CH(CH_3)_2$

Y=α-D-Mannosyl
Z=*N*-Acetyl-β-D-glucosaminyl

TB X=H Y=α-D-Mannosyl Z=*N*-Acetyl-β-D-glucosaminyl
TC X=Y=H Z=*N*-Acetyl-β-D-glucosaminyl
TD X=Y=Z=H

Scheme 1.



$T\text{-}NH_2$: CTA, TB, TC, or TD, R′,R″: See Tables 1~4.
[a] When $R_1 = p\text{-Br-}C_6H_4$, $R^* = CH_3$, in [b] 95% C_2H_5OH.

Fig. 2. Proposed hydrogen bonding systems and mechanism of reductive monoalkylation of teicoplanins at basic pH.

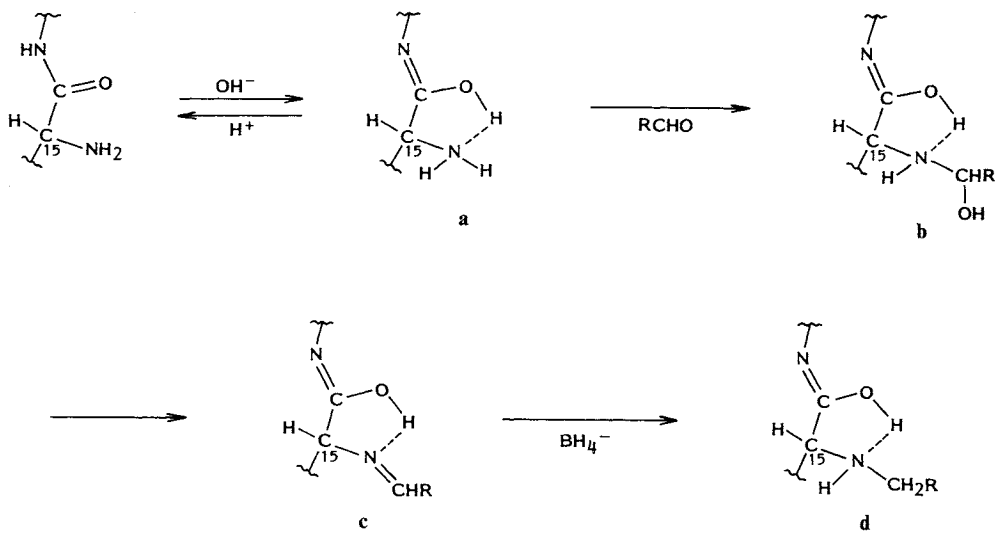
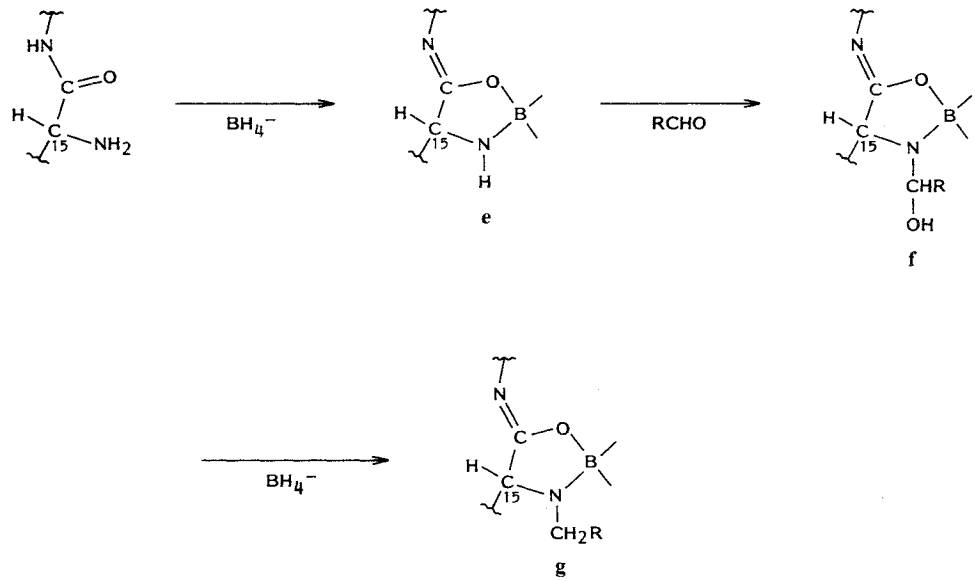


Fig. 3. Proposed formation of cyclic boron complexes in the presence of $NaBH_4$, and alternative mechanism of reductive monoalkylation of teicoplanins.



amino group, due to hydrogen bonding **d** formation, is reflected in the very low reactivity of monoalkyl teicoplanins to give dialkyl derivatives under basic conditions. In the presence of sodium borohydride, the formation of a cyclic boron complex **e** can also be hypothesized, as outlined in Fig. 3. Monoalkylation should occur through the reduction of intermediate hemiaminal **f**, and the resulting borate adduct **g** would prevent dialkylation. Most of monoalkyl derivatives listed in Tables 1~4 were prepared by following the above procedure.

The N^{15}-methyl derivative **1a**[††] of CTA was also selectively obtained according to an unusual procedure (Method A′) based on a preliminary *in situ* oxidation of methanol to formaldehyde at basic pH. In this case, a methanolic solution of CTA and sodium borohydride was allowed to react until the reducing agent was completely transformed into methyl borate, and then left overnight to oxidize at room temperature, in the presence of 10% Pd-C as dehydrogenation catalyst, before final treatment with sodium borohydride. The monomethylation of TB, TC, or TD was also achieved by Method A′ but yields were low, essentially due to side epimerization at C-3.[17]

When initial mild acidic conditions were used, N^{15},N^{15}-dialkyl derivative were the only "reductive alkylation" products with formaldehyde or acetaldehyde. In this case, the appropriate teicoplanin was added to a methanolic solution of the aldehyde and its corresponding carboxylic acid, and the resulting suspension was allowed to react at room temperature overnight before adding sodium borohydride (Method B). This procedure, with minor modifications, was also suitable to prepare the di-*p*-bromobenzyl derivative (**31**) of TD, by reaction of TD with *p*-bromobenzaldehyde in 95% ethanolic solution in the presence of sodium acetate and acetic acid (pH 6), and to synthesize lower dialkyl derivatives from monoalkyl teicoplanins with

† These Schiff bases have not yet been isolated, likely due to their instability. Only the N^{15}-salicylidene derivative of TD has been so far obtained which was stable to mild acidic hydrolysis (1 N HCl) and reduction ($NaBH_4$; or H_2, 10% Pd-C). Unpublished results; these laboratories.

†† Compound **1a** is the N^{15}-methyl derivative of component A2-1-free CTA, since factor A2-1 is invariably transformed into factor A2-3 under hydrogenolytic conditions.[15,16]

Table 1. Alkyl derivatives of CTA (Fig. 1).

Compound	R′	R″	Starting product (SP)	Molar ratio RC/SP	Reactant compound (RC)	Method[a] (yield, %)	HPLC[b,d] Rt (minutes)	Titration[c] EW (found)	Formula[d]	MW[d] (calcd)
1	CH_3	H	CTA	7	HCHO	A (95)	21.2	950	$C_{89}H_{99}N_9O_{33}Cl_2$	1,893.7
1a[e]	CH_3	H	CTA	—	(HCHO)	A′ (82)				
2	C_2H_5	H	CTA	10	CH_3CHO	A (93)	22.9	965	$C_{90}H_{101}N_9O_{33}Cl_2$	1,907.8
3	$CH(CH_3)_2$	H	CTA	100	$(CH_3)_2CO$	B (80)	23.8	960	$C_{91}H_{103}N_9O_{33}Cl_2$	1,921.8
			CTA	1.4	$(CH_3)_2CO$	B′ (94)				
4	$CH(CH_3)CH_2OH$	H	CTA	20	$HOCH_2COCH_3$	A (63)	23.4	985	$C_{91}H_{103}N_9O_{34}Cl_2$	1,937.8
5	$CH(CH_3)CH(CH_3)OH$	H	CTA	20	$HOCH(CH_3)COCH_3$	A (82)	24.5	975	$C_{92}H_{105}N_9O_{34}Cl_2$	1,951.8
6	$CH_2CHOHCH_2OH$	H	CTA	20	$HOCH_2CHOHCHO$	A (75)	22.4	980	$C_{91}H_{103}N_9O_{35}Cl_2$	1,953.8
7	$CH_2O(CH_2)_2OCH_3$	H	CTA	4.9	$CH_3O(CH_2)_2OCH_2Cl$	C (23)	26.1	995	$C_{92}H_{105}N_9O_{35}Cl_2$	1,967.8
8[f]	$(CH_2)_2N(CH_3)_2$	H	CTA	9	$(CH_3)_2NCH_2CHO$	A (45)	24.1	670[g]	$C_{92}H_{106}N_{10}O_{33}Cl_2$	1,950.8
9	CH_3	CH_3	CTA	53	HCHO	B (95)	23.3	950	$C_{90}H_{101}N_9O_{33}Cl_2$	1,907.8
			1	45	HCHO	B (98)				
10	CH_3	C_2H_5	**1**	25	CH_3CHO	B (91)	24.2	970	$C_{91}H_{103}N_9O_{33}Cl_2$	1,921.8
			2	20	HCHO	B (93)				
11	C_2H_5	C_2H_5	CTA	110	CH_3CHO	B (91)	25.6	990	$C_{92}H_{105}N_9O_{33}Cl_2$	1,935.8
			2	65	CH_3CHO	B (97)				

[a] See Schemes 1 and 2.

[b] See Experimental section. CTA: Component A2-2, Rt 19.1 minutes.

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

[d] Values referred to component A2-2.

[e] Component A2-1-free compound **1**.

[f] Obtained as the monoacetate.

[g] The titration curve shows the presence of one additional basic function (pK_{MCS} 8.45) with respect to TD, that is attributable to the alkyl-$N(CH_3)_2$.

Table 2. Alkyl derivatives of TB (Fig. 1).

Compound	R′	R″	Starting product (SP)	Molar ratio RC/SP	Reactant compound (RC)	Method[a] (yield, %)	HPLC[b] Rt (minutes)	Titration[c] EW (found)	Formula	MW (calcd)
12	CH_3	H	TB	8	HCHO	A (91)	9.6	815	$C_{73}H_{70}N_8O_{28}Cl_2$	1,578.3
			1			D (95)				
13	n-$C_{12}H_{25}$	H	TB	1	n-$C_{12}H_{25}Br$	C (45)	32.2	840	$C_{84}H_{92}N_8O_{28}Cl_2$	1,732.6
14	CH_3	CH_3	TB	60	HCHO	B (87)	10.4	830	$C_{74}H_{72}N_8O_{28}Cl_2$	1,592.4
			12		HCHO	B (90)				
			9			D (98)				

[a] See Schemes 1～3.
[b] See Experimental section. TB: Rt 8.7 minutes.
[c] Equivalent weights (EWs) determined by acid-base titration. See Experimental section. Values given are corrected for solvent content.

Table 3. Alkyl derivatives of TC (Fig. 1).

Compound	R′	R″	Starting product (SP)	Molar ratio RC/SP	Reactant compound (RC)	Method[a] (yield, %)	HPLC[b] Rt (minutes)	Titration[c] EW (found)	Formula	MW (calcd)
15	CH_3	H	TC	10	HCHO	A (86)	10.9	730	$C_{67}H_{60}N_8O_{23}Cl_2$	1,416.2
			1			E (52)				
			12			E (66)				
16	n-C_8H_{17}	H	TC	1.2	n-$C_8H_{17}Br$	C (65)	30.6	800	$C_{74}H_{74}N_8O_{23}Cl_2$	1,514.4
17	$CH_2OCO(CH_3)_3$	H	TC	2	$(CH_3)_3COOCH_2Cl$	C (40)	24.4	nd	$C_{71}H_{68}N_8O_{25}Cl_2$	1,504.3
18	CH_2–C_6H_5	H	TC	1.2	C_6H_5-CH_2Br	C (55)	22.7	770	$C_{73}H_{64}N_8O_{23}Cl_2$	1,492.3
19	CH_3	CH_3	TC		HCHO	B (83)	11.8	750	$C_{68}H_{62}N_8O_{23}Cl_2$	1,430.2
			15		HCHO	B (90)				
			9			E (62)				

[a] See Schemes 1～3.
[b] See Experimental section. TC: Rt 9.8 minutes.
[c] Equivalent weights (EWs) determined by acid-base titration. See Experimental section. Values given are corrected for solvent content.
nd: Not determined. Compound **17** was not stable by titration with 0.01 N NaOH.

Table 4. Alkyl derivatives of TD (Fig. 1).

Compound	R′	R″	Starting product (SP)	Molar ratio RC/SP	Reactant compound (RC)	Method[a] (yield, %)	HPLC[b] Rt (minutes)	Titration[c] EW (found)	Formula	MW (calcd)
20	CH_3	H	TD	10	HCHO	A (86)	12.9	635	$C_{59}H_{47}N_7O_{18}Cl_2$	1,213.0
			1			F (34)				
21	C_2H_5	H	TD	10	CH_3CHO	A (96)	13.4	620	$C_{60}H_{49}N_7O_{18}Cl_2$	1,227.0
			2			F (64)				
22	n-C_3H_7	H	TD	30	CH_3CH_2CHO	A (97)	14.9	640	$C_{61}H_{51}N_7O_{18}Cl_2$	1,241.0
23	Cyclo-C_5H_9	H	TD	3	Cyclo-C_5H_8O[d]	B (68)	19.1	660	$C_{63}H_{53}N_7O_{18}Cl_2$	1,267.1
24	CH_2CN	H	TD	55	$NCCH_2Cl$	C (28)	14.0	645	$C_{60}H_{46}N_8O_{18}Cl_2$	1,238.0
25	$CH(CH_3)CH_2OH$	H	TD	20	$HOCH_2COCH_3$	A (65)	13.9	650	$C_{61}H_{51}N_7O_{19}Cl_2$	1,257.0
26	$CH(CH_3)CH(CH_3)OH$	H	TD	20	$HOCH(CH_3)COCH_3$	A (78)	14.9	675	$C_{62}H_{53}N_7O_{19}Cl_2$	1,271.0
27[e]	$(CH_2)_2N(CH_3)_2$	H	TD	8	$(CH_3)_2NCH_2CHO$	A (48)	14.3	450[f]	$C_{62}H_{54}N_8O_{18}Cl_2$	1,270.1
28	CH_3	CH_3	TD	12	HCHO	B (91)	13.7	630	$C_{60}H_{49}N_7O_{18}Cl_2$	1,227.0
29	CH_3	C_2H_5	**20**	10	CH_3CHO	B (89)	15.2	650	$C_{61}H_{51}N_7O_{18}Cl_2$	1,241.0
			21	10	HCHO	B (98)				
30	C_2H_5	C_2H_5	TD	15	CH_3CHO	B (92)	16.3	645	$C_{62}H_{53}N_7O_{18}Cl_2$	1,255.0
			11			F (88)				
31	CH_2–C_6H_4-*p*-Br	CH_2–C_6H_4-*p*-Br	TD	7	*p*-Br–C_6H_4–CHO	B (66)	37.2	nd	$C_{72}H_{55}N_7O_{18}Cl_2Br_2$	1,537.0

[a] See Schemes 1～3.

[b] See Experimental section. TD: Rt 11.4 minutes.

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

[d] The reaction was carried out in the presence of AcOH and cyclopentanone.

[e] Obtained as the monoacetate.

[f] The titration curve shows the presence of one additional basic function with respect to TD, whose pK_{MCS} (>8.5) attributable to the alkyl-$N(CH_3)_2$ was not determined because of overlapping with the titration of the free 56-phenolic-OH.

nd: Not determined.

good yields. In both procedures (Methods A and B), a large excess of carbonyl compound was generally required to complete the reactions. Only monoalkyl derivatives were obtained with ketones by both methods.

In the case of the N^{15}-isopropyl derivative (**3**) of CTA, the reaction was carried out at neutral pH in the presence of an equimolecular amount of acetone, using sodium cyanoborohydride as the reducing agent (Method B′). Under these conditions, CTA was completely transformed into compound **3** within 6 hours.

The 2-methoxyethoxymethyl (**7**) derivative of CTA, the lauryl (**13**) derivative of TB, the *n*-octyl (**16**), pivaloyloxymethyl (**17**) and benzyl (**18**) derivatives of TC, and the cyanomethyl (**24**) derivative of TD were prepared by reaction of CTA, TB, TC, and TD, respectively, with the appropriate alkyl halide at room temperature in *N*,*N*-dimethylformamide (DMF), in the presence of triethylamine (TEA) or sodium bicarbonate (Method C, Scheme 2). In this case, only monoalkyl compounds were obtained using an equimolecular amount or a small excess of alkyl halide, thus avoiding the esterification of the 38-COOH.

Most of the *N*-alkylated derivatives of TB, TC, and TD were also prepared from the corresponding alkylated derivatives of CTA by selective hydrolysis of the sugar moieties under acidic conditions (Methods D～F, Scheme 3).[18,19]

The course of the reactions and the homogeneity of products were checked by HPLC. All these compounds were less hydrophilic than the parent teicoplanins. In particular, those of TB, TC, and TD possessing aromatic or relatively long aliphatic chains were even more lipophilic than CTA.

The structures of these derivatives were determined by ^{1}H NMR spectroscopy. The chemical shift of protons belonging to teicoplanin structures[18～21] are not modified by the presence of the alkyl chains. The N^{15}-CH_2 and -CH protons of the alkyl chains resonate at 3.7～3.3 ppm.

The basicity of the alkylated terminal amino group, determined by acid-base titration, resulted

Scheme 2.

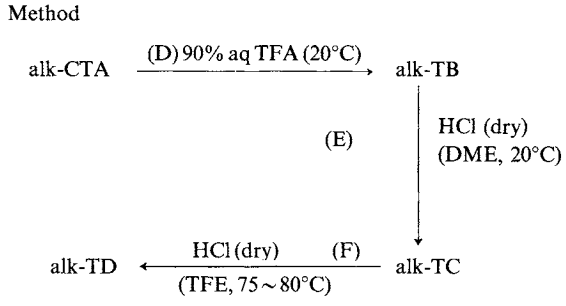
Method

(C) T-NH_2 $\xrightarrow[\text{DMF}]{\text{R′hal, TEA (or NaHCO}_3\text{)}}$ T-NHR′

T-NH_2: CTA, TB, TC, or TD, hal: Cl, Br, R′: See Tables 1～4.

Scheme 3.

Method



alk: N^{15}-Alkyl, N^{15},N^{15}-dialkyl.

substantially unchanged with respect to that of the 15-NH_2 of unmodified teicoplanins.

Except for basic compounds **8** (isoelectric point: pI 7.5) and **27** (pI 7.0), the pIs of the alkylated derivatives, determined by the isoelectrofocusing technique, were almost the same as those of the parent teicoplanins (CTA: 5.8, TB: 5.7, TC: 5.6 and TD: 5.5).

UV and IR spectra were in accordance with the structures of teicoplanin derivatives.

Peptide Binding Studies

The ability of the alkyl compounds to complex with the antibiotic's target peptide (D-Ala-D-Ala), determined by measuring their binding to the synthetic analogue Ac_2-L-Lys-D-Ala-D-Ala according to the differential UV assay,[22] was not influenced by the presence of alkyl chains on the terminal amino group. In fact, the binding constants (K_as, M^{-1}) of most alkylated teicoplanins, obtained at pH 5,[23] were not significantly different from those of CTA (1.6×10^6), TB (1.2×10^6), TC (2.6×10^5) and TD (2.5×10^5), respectively. In general, the dialkyl compounds had binding constants slightly lower than those of monoalkyl derivatives. Only compound **31** showed a binding strength ($7.6\times10^4\,M^{-1}$) significantly lower than that of the unmodified TD, likely due to the steric effect caused by the bulky *p*-bromobenzyl groups which might hinder the approach by the carboxylate anion of synthetic tripeptide to the positively charged dialkylated amino group.

Biological Activity

The majority of the alkylated derivatives maintained the *in vitro* antimicrobial properties of the parent antibiotics (Tables 5~7). A significant improvement or decrease in the antibacterial activity was observed

Table 5. *In vitro* (MIC) and *in vivo* (ED_{50}) activity of alkyl derivatives of CTA.

Organism	MIC (μg/ml)[a]					
	Teicoplanin	**1**[b]	**2**	**3**	**4**	**5**
Staphylococcus aureus Tour	0.125	0.125	0.5	0.125	0.25	0.5
S. haemolyticus L 602[c]	8	nd	16	8	16	16
S. epidermidis ATCC 12228	0.25	0.25	0.5	0.125	0.5	0.25
Streptococcus pyogenes C203	0.063	0.063	0.063	0.063	0.125	0.063
S. pneumoniae UC41	0.063	0.063	0.125	0.125	0.25	0.25
S. faecalis ATCC 7080	0.125	0.125	0.125	0.063	0.125	0.125
ED_{50} (mg/kg)[d] (sc)	0.18	0.17	0.31	0.31	nd	nd

Organism	MIC (μg/ml)					
	6	**7**	**8**	**9**	**10**	**11**
S. aureus Tour	0.5	0.125	0.125	0.25	0.25	0.25
S. haemolyticus L 602[c]	16	8	2	nd	8	4
S. epidermidis ATCC 12228	0.25	0.125	0.125	0.25	0.125	0.125
S. pyogenes C203	0.063	0.063	0.125	0.063	0.063	0.063
S. pneumoniae UC41	0.25	0.125	0.125	0.063	0.125	0.125
S. faecalis ATCC 7080	0.125	0.125	0.125	0.125	0.125	0.25
ED_{50} (mg/kg)[d] (sc)	nd	nd	0.41	0.23	nd	0.41

[a] All these compounds were inactive against Gram-negative bacteria up to concentration of 128 μg/ml.
[b] Compound **1a** was substantially as active as compound **1**.
[c] Clinical isolate.
[d] In mice septicemically infected with *S. pyogenes* C203. See Experimental section. None of these derivatives was effective by oral route up to dose of 170 mg/kg.
nd: Not determined.

Table 6. *In vitro* (MIC) and *in vivo* (ED_{50}) activity of alkyl derivatives of TB and TC.

Organism	MIC (μg/ml)[a]									
	TB	**12**	**13**	**14**	TC	**15**	**16**[a]	**17**	**18**	**19**
Staphylococcus aureus Tour	0.25	0.5	2	0.125	0.5	0.125	0.063	2	0.25	0.25
S. haemolyticus L 602[b]	8	nd	nd	nd	0.5	0.5	0.125	nd	nd	nd
S. epidermidis ATCC 12228	0.25	0.5	0.125	0.125	0.125	0.063	0.063	0.063	0.063	0.063
Streptococcus pyogenes C203	0.5	1	0.063	0.5	0.5	0.5	0.063	2	0.5	1
S. pneumoniae UC41	0.5	1	0.063	2	1	2	0.063	2	1	2
S. faecalis ATCC 7080	2	4	2	4	1	1	0.063	4	1	2
ED_{50} (mg/kg)[c] (sc)	2.64	nd	0.95	nd	2.46	nd	1.25	6.6	2.2	6.6

[a] Except compound **16**, which was active against *Proteus vulgaris* HX19 (ATCC 881) at the concentration of 64 μg/ml, these derivatives were inactive against Gram-negative bacteria up to 128 μg/ml.

[b] Clinical isolate.

[c] In mice septicemically infected with *S. pyogenes* C203. See Experimental section. None of these compounds was effective by po route up to dose of 300 mg/kg.

nd: Not determined.

Table 7. *In vitro* (MIC) and *in vivo* (ED_{50}) activity of alkyl derivatives of TD.

Organism	MIC (μg/ml)[a]						
	TD[b]	**20**[b]	**21**	**22**	**23**[c]	**24**	**25**
Staphylococcus aureus Tour	0.06	0.125	0.063	0.063	0.063	0.125	0.063
S. haemolyticus L 602[d]	0.25	0.125	0.25	nd	nd	1	0.5
S. epidermidis ATCC 12228	0.016	0.016	0.032	0.016	0.032	0.125	0.063
Streptococcus pyogenes C203	0.125	0.125	0.063	0.125	0.125	0.125	0.125
S. pneumoniae UC41	0.125	0.125	0.125	0.125	0.063	0.125	0.125
S. faecalis ATCC 7080	0.125	0.125	0.125	0.25	0.25	1	0.25
Escherichia coli SKF 12140	64	64	128	64	128	>128	>128
ED_{50} (mg/kg)[e] (sc)	0.95	1.25	1.65	5	5	nd	nd

Organism	MIC (μg/ml)					
	26	**27**	**28**	**29**	**30**	**31**
S. aureus Tour	0.125	0.125	0.125	0.125	0.125	1
S. haemolyticus L 602[d]	0.5	0.5	0.5	0.25	0.25	nd
S. epidermidis ATCC 12228	0.063	0.063	0.032	0.063	0.063	0.125
S. pyogenes C203	0.125	0.5	0.125	0.063	0.125	1
S. pneumoniae UC41	0.25	0.25	0.125	0.125	0.125	0.25
S. faecalis ATCC 7080	0.125	0.125	0.25	0.125	0.125	1
E. coli SKF 12140	>128	>128	>128	128	>128	>128
ED_{50} (mg/kg)[e] (sc)	nd	1.25	6.6	nd	5	nd

[a] See Experimental section.

[b] Against *Proteus vulgaris* HX19 (ATCC 881), MIC 128 μg/ml. All the other derivatives were inactive against this organism up to concentration of 128 μg/ml.

[c] Against *Pseudomonas aeruginosa* ATCC 10145, MIC 128 μg/ml. All the other derivatives were inactive against this organism up to concentration of 128 μg/ml.

[d] Clinical isolate.

[e] In mice septicemically infected with *S. pyogenes* C203. See Experimental section. None of these compounds was effective by po route up to dose of 300 mg/kg.

nd: Not determined.

with those compounds having a marked increased lipophilicity. The lauryl derivative (**13**) of TB was more active than TB against *Streptococcus pyogenes* and *Streptococcus pneumoniae* but less active than TB against *Staphylococcus aureus*. The *n*-octyl derivative (**16**) of TC was markedly more active than TC against both Staphylococci and Streptococci, attaining an activity higher than or comparable to that of teicoplanin; moreover it also showed a weak activity against *Proteus vulgaris* (MIC 64 μg/ml). In contrast, the di-*p*-bromobenzyl derivative (**31**) of TD was remarkably less active than TD, probably due to the decreased affinity for target peptide D-Ala-D-Ala observed in binding experiments. Against *Escherichia coli* only few derivatives of TD maintained the weak activity of the parent compound.

In the murine model of Streptococcal septicemia, most derivatives had efficacy comparable to that of the unmodified antibiotics; only compounds **17**, **19**, **22** and **23** were somewhat less effective (Tables 6 and 7).

Experimental

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

Pure 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) 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 TD as internal reference. Elutions were 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	50
% of B in A:	5	20	30	45	75	5

All compounds were analyzed for C, H, N, Cl on samples previously dried at 140°C under N_2 atmosphere. Weight loss 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 Methyl Cellosolve (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.

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

Isoelectrofocusing 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 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
(Compound **1a**, %)	(0)	(50)	(25)	(12)	(13)

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

Almost all of products were obtained in the form of internal salts. Only compounds **8** and **27** were isolated as monoacetates.

Sodium borohydride, pellets, diameter 0.8 cm, 98% (Aldrich-Chemie), unless otherwise indicated, was used as the reducing agent. Sodium cyanoborohydride powder (Aldrich-Chemie) was only used in the alternative synthesis (Method B′) of compound **3**.

N^{15}-Alkyl Derivatives of Teicoplanin Antibiotics (Method A)

General Procedure

To a stirred solution (or suspension) of 10 mmol of CTA, TB, TC, or TD in 500 ml of MeOH, 18.5 g (500 mmol) of $NaBH_4$, or 5 g (about 50 mmol) of CH_3COOK, was added in aliquots while maintaining the temperature at 40~45℃. After 2 hours, a large molar excess (see Tables 1~4) 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℃ and 11 g (about 300 mmol) of $NaBH_4$ was added at 10~15℃. After 2 hours, 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 800 g of silanized silica gel in H_2O. The column was developed with a linear step gradient from 5 to 70% of CH_3CN in 0.01 N AcOH, in 15 hours at a flow rate of 200~300 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 monoalkylated teicoplanins.

Preparation of N^{15}-Methyl CTA (Components A2-2~A2-5) (**1a**) (Method A')

To a stirred suspension of 10 g (about 5 mmol) of CTA in 750 ml of MeOH, 11 g (about 300 mmol) of $NaBH_4$ powder was added in aliquots over 1 hour, while maintaining the temperature at 35~40℃. After 2 hours (a clear solution formed meanwhile), 5 g of 10% Pd-C was added under N_2 atmosphere and the suspension was stirred in the air at room temperature overnight, afterwards additional 22 g (about 600 mmol) of $NaBH_4$ powder was added under N_2 atmosphere. The temperature rose to 55℃ within 20 minutes. Then the mixture was allowed to cool at room temperature, afterwards it was filtered and the filtrate was poured into a solution of 60 ml of AcOH in 400 ml of H_2O. The solid obtained after evaporation of the solvents (in the presence of BuOH to avoid foaming) was chromatographed as described above, yielding 8.2 g of the title compound.

Preparation of N^{15}-Isopropyl CTA (**3**) (Method B')

To a stirred suspension of 2 g (about 1 mmol) of CTA in 25 ml of MeOH, 1 ml (about 1.4 mmol) of Me_2CO was added at room temperature followed by 70 mg (about 1.1 mmol) of $NaBH_3CN$. After 6 hours, the solvent was evaporated and the solid residue was re-dissolved in 50 ml of H_2O. The resulting solution was adjusted to pH 2 with 1 N HCl and stirred at room temperature for 30 minutes, afterwards it was adjusted to pH 5.8 with 0.1 N NaOH and concentrated to a small volume. The solid which separated was collected, yielding 1.9 g of the title compound.

Preparation of N^{15}-2-Methoxyethoxymethyl CTA (**7**) (Method C)

To a stirred solution of 2 g (about 1 mmol) of CTA in 100 ml of DMF, 1.2 ml (8.6 mmol) of TEA and 0.56 ml (4.9 mmol) of 2-methoxyethoxymethyl chloride were added. After 24 hours, the reaction mixture was concentrated to a small volume and then it was diluted with 200 ml of a BuOH-H_2O (2:1) mixture. The organic layer was separated, washed with H_2O (2×30 ml), and then the solvent was evaporated. The solid residue was dissolved in 200 ml of a CH_3CN-H_2O (1:1) mixture, and then 25 g of silanized silica gel was added. The resulting suspension was stirred at room temperature for 1 hour, after which the solvents were evaporated. The solid residue was suspended in 200 ml of H_2O and loaded on a column of 60 g of silanized silica gel. Chromatography was carried out as described above, yielding 0.5 g of the title compound.

Preparation of N^{15}-Lauryl TB (**13**) (Method C)

A solution of 4 g (about 2.5 mmol) of TB, 0.5 ml (about 3.5 mmol) of TEA, and 0.65 ml (about 2.6 mmol) of lauryl bromide in 40 ml of DMF was stirred at room temperature for 4 days. On adding 300 ml of Et_2O the precipitated solid was collected and purified by reverse-phase column chromatography as described above for compound **7**, obtaining 1.95 g of the title compound.

Preparation of the N^{15}-*n*-Octyl (**16**) and Benzyl (**18**) Derivatives of TC (Method C)

To a stirred solution of 3 g (about 2 mmol) of TC and 2.4 mmol of *n*-octyl or benzyl bromide in 150 ml of DMF, 250 mg (about 3 mmol) of $NaHCO_3$ was added at room temperature. After 2 days, the reaction mixture was filtered and then it was concentrated to a small volume. On adding Et_2O the precipitated solid was collected and purified by reverse-phase column chromatography, as described above, yielding pure title compounds.

Preparation of N^{15}-Pivaloyloxymethyl TC (**17**) (Method C)

A solution of 3.7 g (about 2.5 mmol) of TC, 0.5 ml (about 3.5 mmol) of TEA and 0.74 ml (about 5 mmol) of pivaloyloxymethyl chloride (POM-Cl) in 20 ml of dry DMF was stirred at room temperature overnight, after which further 0.15 ml of TEA and 0.4 ml of POM-Cl were added. After 18 hours, the reaction mixture was diluted with 500 ml of H_2O and extracted with 1 liter of a EtOAc - BuOH (9 : 1) mixture. The organic layer was discarded and the aqueous phase was extracted with 800 ml of BuOH. The butanolic layer was separated, washed with 300 ml of 0.01 N HCl and then with 300 ml of H_2O, afterwards it was concentrated to a small volume. On standing at room temperature overnight the precipitated solid was collected and chromatographed under the usual conditions, to give 1.6 g of the title compound.

Preparation of N^{15}-Cyanomethyl TD (**24**) (Method C)

A solution of 1.2 g (1 mmol) of TD, 3.5 ml (about 55 mmol) of chloroacetonitrile and 0.35 ml (about 2.5 mmol) of TEA in 30 ml of DMF was stirred at room temperature overnight. The solvents were evaporated and the oily residue was slurried with 3 ml of H_2O, afterwards it was collected (0.96 g, identified as the cyanomethyl ester of the title compound: IR $\nu_{C=O}$ cm^{-1} 1750) and then it was re-dissolved in 30 ml of a THF - H_2O (2 : 1) mixture containing 0.5 ml of TEA. After stirring at room temperature overnight, 30 ml of BuOH was added followed by 5 ml of 1 N HCl. The solvents were evaporated and the solid residue was chromatographed to give 0.35 g of the title compound.

N^{15},N^{15}-Dialkyl Derivatives of Teicoplanin Antibiotics (Method B)

General Procedure

To a stirred suspension of 10 mmol of CTA, TB, TC, TD, or the appropriate monomethyl or -ethyl derivative in 500 ml of MeOH, a large molar excess (see Tables 1～4) of formaldehyde or acetaldehyde was added followed by 100 mmol of formic or acetic acid, respectively. After stirring overnight, 7.5 g (about 200 mmol) of $NaBH_4$ was added in aliquots while maintaining the temperature at 35～40℃. The reaction mixture was allowed to cool to room temperature and, after 1～2 hours, 12 ml (about 200 mmol) of AcOH was added at room temperature and solvents were evaporated. Crude products thus obtained were purified by reverse-phase column chromatography using the same procedure as that previously described for the purification of monoalkyl derivatives.

Preparation of N^{15},N^{15}-Di-*p*-bromobenzyl TD (**31**) (Method B)

To a stirred solution of 1 g (about 0.8 mmol) of TD and 50 mg (0.6 mmol) of CH_3COONa in 300 ml of 95% EtOH, 1 g (about 5.5 mmol) of *p*-bromobenzaldehyde was added. The resulting solution was adjusted at pH 6 with glacial AcOH and then it was stirred at 50℃ for 3 hours while adding 111 mg (3 mmol) of $NaBH_4$ powder in 3 portions (37 mg/hour). After stirring at room temperature overnight, the solvent was evaporated and the crude product was purified by reverse-phase column chromatography (see above) to give 0.85 g of the title compound.

Preparation of Mono- and Dialkyl-TB from the Corresponding Derivatives of CTA (Method D)

A solution of 1 mmol of the appropriate derivative of CTA in 25 ml of a TFA - H_2O (9 : 1) mixture was stirred at room temperature for 2 hours, and then the solvents were evaporated. Afterwards, the oily or solid residue was chromatographed to give the title compounds.

Preparation of Mono- and Dialkyl-TC from the Corresponding Derivatives of CTA or TB (Method E)

A suspension of 1 mmol of the appropriate derivative of CTA or TB in 75 ml of 1,2-dimethoxyethane

(DME) was stirred at room temperature overnight while bubbling dry HCl. After evaporation of the solvent, the crude solid product was purified by reverse-phase column chromatography.

Preparation of Mono- and Dialkyl-TD from the Corresponding Derivatives of CTA, TB, or TC (Method F)

A suspension of 1 mmol of the appropriate derivative of CTA, TB, or TC in 30 ml of a 0.5 M HCl solution in 2,2,2-trifluoroethanol (TFE) was stirred at 75~80℃ for 12~18 hours, while bubbling dry HCl. Afterwards it was cooled to room temperature and the insoluble matter was collected by filtration, washed with Et_2O, and then chromatographed to yield the title compounds.

Peptide Binding Assays

The interaction of Ac_2-L-Lys-D-Ala-D-Ala with teicoplanin antibiotics and their alkylated derivatives was determined at pH 5 according to the procedure described in a previous paper.[23]

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℃.

Experimental septicemia was induced in groups of five mice by intraperitoneal injection of about 10^5 cells of *S. pyogenes* C203, a challenge corresponding to about 100 times the lethal dose for 50% infected animals. Mice were treated once immediately after infection by sc or po route. On the 7th day, ED_{50} (infected animals, expressed in mg/kg) was calculated on the basis of the percentage of surviving mice at each dose, by the Spearman-Kärber method.[24]

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